Masarykova univerzita

Lékařská fakulta

Úloha oxidačného stresu v patogenéze septických stavov, respiračného zlyhania na podklade vírusovej infekcie a bolestivých syndrómov

Habilitačná práca podľa § 72 odst. 3 písm. b) zákona o vysokých školách

2024 **MUDr. Ladislav Kočan, PhD.**

Poďakovanie

Na tomto mieste by som sa rád poďakoval všetkým, ktorí ma podporovali a vytvorili vhodné podmienky pre realizáciu tejto práce.

Ďakujem mojím školiteľom doc. MUDr. Jozefovi Firmentovi, PhD. prednostovi I. kliniky anestéziológie a intenzívnej medicíny Univerzitnej nemocnice Louisa Pasteura v Košiciach, MUDr. Róbertovi Rapčanovi PhD., FIPP medicínskemu riaditeľovi EuroPainClinics, doc. MUDr. Igorovi Martuliakovi PhD. prednostovi Kliniky Algeziológie Fakultnej nemocnice s poliklinikou F.D. Roosevelta v Banskej Bystrici, doc. MUDr. Pavlovi Törökovi CSc. emeritnému prednostovi Kliniky anestéziológie a intenzívnej medicíny VÚSCH a.s. z môjho domovského pracoviska, za ich podporu a odborné vedenie v mojej klinickej praxi, ako aj za ich cenné rady a pripomienky pri vypracovaní tejto práce.

Zvlášť ďakujem Prof. RNDr. Janke Vaškovej, PhD. a doc. MVDr. Ladislavovi Vaškovi CSc. z Ústavu lekárskej a klinickej biochémie LF UPJŠ za ich dlhoročné priateľstvo a úzku spoluprácu a konzultácie pri písaní habilitačnej práce.

Moja vďaka patrí aj mojím kolegom z pracovísk z KAIM VÚSCH a.s. a EuroPainClinics, ktorí mi počas mojej klinickej praxe vytvárali príjemné pracovné prostredie.

V neposlednom rade by som sa rád poďakoval svojej rodine, rodičom a manželke Hanke za ich lásku, pochopenie a podporu.

OBSAH

1 ÚVOD

Predkladaná habilitačná práca podľa § 72 ods. 3 písmena b) zákona o vysokých školách je súhrnom štúdií a odborných textov venujúcich sa aktuálnym témam v oblasti anestéziológie, intenzívnej medicíny a algeziológie. Pre rozsiahlosť súboru publikácií a ich tém je práca rozdelená do 5 celkov, ktoré korešpondujú s hlavnými cieľmi mojej klinicko-výskumnej činnosti.

Reaktívne formy kyslíka a dusíka sú atómy, molekuly alebo ich fragmenty, ktoré majú jeden alebo viac nespárených elektrónov a sú schopné, hoci aj krátkej samostatnej existencie. Častokrát zohrávajú dôležitú úlohu pri rôznych fyziologických dejoch, akými sú procesy súvisiace s fagocytózou, podieľajú sa na širokom spektre signalizačných funkcií a mnohých mechanizmov, ktoré variabilným pôsobením v živých sústavách udržiavajú stav homeostázy.

Na druhej strane nežiadúci efekt nadprodukcie týchto reaktívnych foriem, patrí medzi široko skúmané procesy v súvislosti s ich podielom na patogenetickom rozvoji rozličných ochorení. Dôsledok ich negatívneho pôsobenia sa označuje ako oxidačný stres, ktorý je definovaný ako nerovnováha medzi tvorbou a odstraňovaním voľných radikálov a ďalších reaktívnych látok.

Oxidačný stres stojí na pozadí mnohých ťažkých chorobných stavov a zohráva dôležitú úlohu pri amplifikácii imunitných dejov. U kriticky chorých je známa jeho aktivačná úloha v patogenéze syndrómov SIRS, sepsa, septický šok a širokého spektra patologických stavov, akými sú akútna pankreatitída, neurozápalové procesy, infekčné pneumónie a ďalšie závažné diagnózy. Liečba týchto ochorení je veľmi komplexná a prístup je od pacienta k pacientovi individuálny.

Svetovou zdravotníckou iniciatívou bola v rokoch 2002 - 2003 snaha o zvýšenie prežívania pacientov so sepsou. Táto iniciatíva viedla k vypracovaniu a prijatiu spoločného programu za prežitie sepsy pod názvom „Surviving Sepsis Campaign, ktorý pozostával z komplexných a jednotných odporúčaných postupov v diagnostike a liečbe ťažkej sepsy. Odporúčania boli prvýkrát publikované v roku 2004 a následne revidované podľa nových dôkazov Evidence Based Medicine (EBM -medicíny založenej na dôkazoch) v rokoch 2008, 2012, 2017 a 2021 (1, 2). V priebehu týchto časových období boli prehodnocované rôzne postupy, vrátane antioxidačnej suplementačnej terapie formou enterálnej a parenterálnej aplikácie vitamínov, stopových prvkov (zinok a selén) a podávania glutamínu. Medzi dôležité

štúdie, zaoberajúcimi sa touto problematikou v kľúčovom období kritického hodnotenia dôkazov, sa radia štúdie autorov Valenta a kol. a Andrews a kol. (3, 4). Jednou z dôležitých prác nášho lekársko-výskumného kolektívu, ktoré podporili negatívne závery liečby selénom, patrí prospektívna observačná klinická štúdia Se-AOX, ktorej výsledky nepotvrdili významný benefit po suplementácii selénu vzhľadom na sledovanú mortalitu pacientov s ťažkou sepsou a septickým šokom.

V období 2019 - 2022 svetová verejnosť čelila vírusovej pandémii COVID-19. V tomto období náš lekársko-výskumný tím pracoval na zlepšení ventilačných možností u pacientov s nehomogénnym poškodením pľúc v dvoch významných projektoch realizovaných v rámci „Operačného programu Integrovaná infraštruktúra", ktoré boli spolufinancované zo zdrojov Európskeho fondu regionálneho rozvoja v spolupráci s Ministerstvom školstva, výskumu, vývoja a mládeže Slovenskej republiky. Molekulárne princípy prestavby pľúcneho tkaniva na menejcenné fibrózne tkanivo úzko súvisia s excesívnou zápalovou reakciou a rozvojom oxidačného stresu. V rámci prognózy a terapeutickej stratégie u pacientov s ťažkou vírusovou pneumóniou sme mohli v tomto období využiť nadobudnuté poznatky aj zo štúdie Se-AOX. Zo získaných skúseností počas pandémie H1N1 v roku 2009, ako aj skúseností s ventiláciou u septických pacientov s ARDS, bolo možné ďalej rozvíjať metódy viac-hladinovej umelej pľúcnej ventilácie. Jej včasné nasadenie zlepšovalo prežívanie pacientov a poukazovalo na nižšiu úroveň postpneumonickej fibrotickej prestavby pľúcneho tkaniva.

Pôsobenie oxidačného stresu je nešpecifické s potenciálom zasiahnuť rôzne tkanivá a orgánové systémy. Z doterajších výskumov vyplýva, že oxidačný stres zohráva významnú úlohu aj pri chronifikácii bolesti. Sprievodné kaskádové reakcie sprevádzajúce oxidačný stres vyvolávajú negatívne zmeny v centrálnom nervovom systéme na neurónoch ako aj na bunkách glie, vzhľadom na ich vysokú citlivosť voči oxidačným vplyvom a metabolickým zmenám, ktoré prebiehajú v rámci neurozápalu. Táto téma bola rovnako predmetom záujmu našej výskumnej práce. Vplyv oxidačného stresu a možnosti jeho supresie pri rozvoji neurozápalu, neuropatickej bolesti a pooperačnej kognitívnej dysfunkcie u pacientov po kardiochirurgických a nekardiochirurgických výkonoch aktuálne skúmame s naším vedecko-lekárskym tímom v prebiehajúcej prospektívnej observačnej klinickej štúdii NeuOX-postSurg Trial. Z experimentálnych výskumov vyplýva, že oxidačný stres hrá dôležitú úlohu v rámci viacerých mechanizmov, ktoré sú zapojené do nociceptívnej modulácie a centrálnej senzitizácie. Zároveň pretrvávanie prooxidačného stavu umocňuje terapeutické používanie opiátov. Doterajšie dôkazy klinických a experimentálnych štúdií poukazujú na zvýšenú tvorbu reaktívnych foriem kyslíka a dusíka pri metabolizácii morfínu, ktoré sú výsledkom

katalyzovaných reakcií aktivovanými enzýmami NOS (syntázou oxidu dusnatého) a fosfolipázou D2. Predpokladá sa podobný prooxidačný efekt aj pri metabolizácii ďalších silný opiátov z iných farmakologických skupín. Tieto teórie overuje náš lekársko-vedecký tím v prospektívnej observačnej klinickej štúdii Opioid-Redox Study.

Dôležitou súčasťou lekárskej a vedeckej práce, ktorá nadväzuje na náš doterajší výskum je štúdium akútnej a chronickej bolesti v nemocničnom a ambulantnom prostredí. Medzi dôležité klinické štúdie o ktoré sa opiera náš ďalší výskum patrí štúdium pooperačnej bolesti sledovaný v klinickej štúdii VUSCH/POPT1study, ktorý porovnáva skupiny kardiochirurgických pacientov po sternotómiach a torakotómiach, ktorí sú na konvenčnej analgetickej farmakoterapii a skupiny pacientov po regionálnych nervových blokádach a kryoablačných výkonoch zameraných na interkostálne nervy.

Sledovanie klinického stavu a hodnotenie zmien v kvalite života u pacientov s chronickou bolesťou v ambulantnom prostredí sme realizovali vo viacerých klinických štúdiách. Tieto klinické štúdie boli zamerané prevažne na pacientov s bolesťou dolnej časti chrbta tzv. „low back pain" a pacientov s kritickou končatinovou ischémiou. Klinický stav pacientov s postlaminektomickým syndrómom hodnotila po epiduroskopickom terapeutickom výkone multicentrická randomizovaná dvojito zaslepená klinická štúdia EPCS II. Klinická štúdia sledovala efekt samotnej mechanickej lýzy verzus mechanickej lýzy vrátane aplikácie liečiv do epidurálneho priestoru. Terapiu lumbálneho fazetového syndrómu hodnotí aktuálne prebiehajúca prospektívna multicentrická randomizovaná klinická štúdia EPCS XI porovnávajúca 3 skupiny pacientov, ktorí podstupujú rádiofrekvenčnú abláciu, kryoabláciu mediálnych nervových ramienok dorzálnej vetvy spinálneho nervu a endoskopický debridement puzdra fazetového kĺbu. Ďalšou ukončenou štúdiou zameranou na chirurgickú liečbu akútneho radikulárneho syndrómu bola prospektívna observačná štúdia EPCS V, ktorá hodnotila pooperačný stav pacientov a komplikácie po endoskopickej diskektómii. V týchto štúdiách bola hodnotená kvalita života prostredníctvom dotazníka EQ-5D-5L výskumnej skupiny EuroQuol, pričom v spolupráci so Slovenskou Akadémiou Vied boli stanovené koeficienty štatistických váh pre región Slovenskej a Českej republiky.

Aktuálne realizujeme prospektívnu observačnú klinickú štúdiu: Tevi-LuSy-Study v spolupráci Východoslovenským ústavom srdcových a cievnych chorôb a s Technickou Univerzitou v Košiciach, ktorá sa zoberá klinickým hodnotením stavu pacientov a hodnotením úrovne perfúzie dolných končatín prostredníctvom termálnych zmien u pacientov s kritickou ischémiou dolných končatín po sympatikolytických intervenciách. Tento projekt je finančne podporený grantom Slovenskej spoločnosti pre štúdium a liečbu bolesti.

2 OXIDAČNÝ STRES V PATOGENÉZE SEPTICKÝCH STAVOV

2.1 Patofyziológia oxidačného stresu

Reaktívne formy odvodené od kyslíka (ROS, reactive oxygen species) vznikajú za aeróbnych podmienok v regulovaných aj neregulovaných procesoch. Medzi ROS patria atómy alebo molekuly obsahujúce jeden alebo viac nespárených elektrónov. Sú produktami látkového metabolizmus a v živých organizmoch participujú na širokom spektre biologických procesov. Patria medzi ne radikálové formy ako superoxidový radikál ($O_2^{\bullet-}$), hydroxylový radikál ($HO^{\bullet}$), oxid dusnatý (NO) alebo neradikálové formy, napr. peroxid vodíka (H_2O_2) (5). ROS zohrávajú dôležitú úlohu v patogenéze širokého spektra chorobných stavov, vrátane závažných ochorení u kriticky chorých pacientov. Charakteristická je pre nich vysoká reaktivita a veľmi krátky polčas rozpadu. Medzi ich najvýznamnejšie funkcie patria antimikrobiálna ochrana a signalizačné funkcie (6).

Tvorba ROS prebieha prevažne štyrmi hlavnými cestami, a to:

- V dýchacom reťazci prebiehajúcom v mitochondriách každej bunky, kde $O_2^{\bullet-}$ vzniká ako vedľajší produkt reakcie molekulárneho kyslíka a semichinónu. Významná je ale aj produkcia $O_2^{\bullet-}$ v polymorfonukleárnych leukocytoch, kde majú antimikrobiálnu funkciu.
- Druhý spôsob je zabezpečený prostredníctvom, reakcií katalyzovaných enzýmami rodiny NOX, NADPH-oxidázami, ktorými disponujú najmä makrofágy a neutrofilné leukocyty. Touto reakciou vzniká excesívne množstvo $O_2^{\bullet-}$, ktorý zohráva dôležitú baktericídnu úlohu.
- Tretia cesta tvorby ROS je katalyzovaná enzýmom xantínoxidázou, ktorá je aktivovaná napríklad počas ischemických podmienok spojených s následnou reperfúziou tkanív. Rovnako aj pri tejto reakcii vzniká masívne množstvo $O_2^{\bullet-}$ v čase reperfúzie. Jej najväčšia aktivita sa predpokladá najmä pri polytraume a pri kardiochirurgických výkonoch.
- Štvrtou variantou je tvorba ROS pri poškodení buniek. Procesy sú spojené s uvoľňovaním intracelulárnych redoxne aktívnych iónov (Fe, Cu), ktoré amplifikujú tvorbu voľných radikálov v Haber-Weissovej a Fentonovej reakcii, kedy dochádza ku konverzii peroxidu vodíka na hydroxylový radikál (7).

ROS majú schopnosť poškodzovať proteíny, polysacharidy, nukleové kyseliny, nenasýtené mastné kyseliny, čo vedie k dramatickému poškodeniu buniek. Navyše ROS môžu iniciovať uvoľňovanie cytokínov z imunokompetentných buniek, aktivovať zápalovú kaskádu a

zvyšovať expresiu adhéznych molekúl na povrchu buniek. Voľné radikály spúšťajú kaskádu intercelulárnych udalostí, spôsobujúcich uvoľnenie nukleárneho transkripčného faktora NF-κB z jeho inhibičného proteínu IκB. NF-κB faktor sa translokuje do bunkového jadra a naviaže sa na DNA, čo spúšťa iniciáciu transkripcie génov, ktoré sú dôležité z hľadiska zápalu. NF-κB kontroluje produkciu mediátorov akútnej fázy ako TNF-α, IL-2 a IL-2 receptorov, ktoré opäť aktivujú NF-κB, čím amplifikujú zápalovú kaskádu . Poškodenie tkanív a zápal vedú ku akumulácii granulocytov v danom tkanive, čo vedie k zvýšenej produkcii ROS a bludnému kruhu ďalšieho oxidačného poškodenia tkaniva (7, 8).

2.2 Kriticky chorý pacient

Kritické ochorenia sa vyznačujú komplexom porúch vrátane excesívnej zápalovej odpovede, dysfunkcie mitochondrií, bunkovej imunity a rozvojom oxidačného stresu.
Oxidačný stres nemusí byť len dôsledkom, ale čoraz viac sa uznáva ako kľúčový prispievateľ iniciačných patofyziologických procesov, ktoré sú základom vzniku kritického ochorenia (9) (10). Príkladom sledu týchto kaskádových reakcií je syndróm systémovej zápalovej odpovede (SIRS). Z klinického hľadiska je medicínsky manažment kriticky chorých pacientov na intenzivistickom lôžku zameraný na liečbu širokého spektra závažných klinických stavov, ktoré ohrozujú jedinca na živote, a to zlyhávaním jedného alebo viacerých orgánových systémov s možnými prejavmi kardiogénneho, hypovolemického, distribučného, obštrukčného šoku alebo ich kombinácie (11). Medzi najčastejšie príčiny hospitalizácie na oddeleniach intenzívnej medicíny patria stavy spojené s respiračným a kardiálnym zlyhaním. Ďalšími závažnými ochoreniami vyžadujúcimi intenzívnu terapiu sú septické stavy, ťažké traumy, malígne poruchy srdcového rytmu, poranenia CNS, cievne mozgové príhody, rozsiahle nekardiochirurgické a kardiochirurgické operačné výkony, popáleniny a ďalšie stavy vyžadujúce rozsiahly monitoring vitálnych funkcií, ventilačnú podporu, farmakologickú vazopresorickú a inotropnú podporu, mechanické podpory obehu a srdca, mimotelovú eliminačnú liečbu, vrátane komplexnej nutričnej podpory. Pacienti sú vystavení noxam, pri ktorých dochádza k nadmernej tvorbe voľných radikálov, zároveň sú ohrození malnutríciou, deficitom mikronutrientov a vyčerpaním endogénnych zásob molekúl s antioxidačnými vlastnosťami (12, 13).
V klinických štúdiách sledujúcich závažné klinické stavy bol hodnotený terapeutický efekt viacerých látok, ktoré disponujú antioxidačnými vlastnosťami. Medzi významné látky, ktoré

sú súčasťou antioxidačných systémov, patria vitamíny C, E, β-karotén, kyselina eikozapentaénová, zinok, selén a glutamín (5, 12 -14).

2.3 Terapeutické využitie selénu

Organizmus disponuje súborom komplexných obranných mechanizmov, zahŕňajúcich intra- a extracelulárne zložky, ktoré dokážu za fyziologických podmienok efektívne eliminovať tvorbu reaktívnych foriem kyslíka a dusíka. Týmto spôsobom je ich celková kvantita v určitom priestore regulovaná, čo bráni rozvoju nekontrolovaných kaskádových oxidačných reakcií. Esenciálne stopové prvky ako meď, mangán, zinok, železo a selén sú kľúčové pre aktivitu enzýmov, ako je superoxiddismutáza (SOD), kataláza a glutatiónperoxidáza (GPx). Okrem toho neenzymatické obranné mechanizmy zahŕňajú molekuly ako glutatión, albumín, vitamíny E, C a β-karotén. Na pozadí SIRS môže nedostatok týchto endogénnych antioxidantov vzniknúť v dôsledku faktorov, ako je presakovanie kapilár, hemodilúcia, neadekvátny príjem stravy a lekárske zásahy, ako je kontinuálna renálna substitučná terapia. Najzávažnejšie prípady SIRS sa často zhodujú s najvýraznejším úbytkom antioxidantov (9, 15). Medzi ďalšie kritické stavy patrí sepsa a septický šok. Charakteristickým prejavom sepsy je život ohrozujúce zlyhávanie orgánov, ktoré je spôsobené neprimeranou odpoveďou hostiteľa na infekciu (16). Progresiou sepsy do septického šoku dochádza k poruchám krvného obehu a k bunkovým či metabolickým zmenám, ktoré zvyšujú riziko úmrtia.

Sepsa napriek enormne vynaloženému úsiliu predstavuje závažný medicínsky a socio-ekonomický problém. Od roku 2002 bola formou Barcelonskej výzvy odštartovaná kampaň za prežitie sepsy „Surviving Sepsis Campaign“ (SSC), zaoberajúca sa možnosťami liečby ťažkých septických stavov s cieľom zníženia mortality. Tento program zahŕňal vytváranie jednotných odporúčaní zameraných na včasnú diagnostiku a liečbu sepsy na podklade medicíny založenej na dôkazoch. Okrem včasnej identifikácie vyvolávajúcich agensov, empirickej a cielenej antimikrobiálnej liečby, včasnej tekutinovej resuscitácie obehu a udržania cieľových hodnôt stredného arteriálneho tlaku, boli posudzované ďalšie predpokladané terapeutické postupy a aplikácia liečiv, ktoré by mohli pozitívne ovplyvniť priebeh ochorenia (1). Medzi sledovanými látkami boli aj v tomto čase látky s antioxidačnými vlastnosťami ako vitamín C, E, alebo látky, ktoré k syntéze antioxidačne pôsobiacich molekúl sú potrebné, ako glutamín a mikroprvky zinok a selén (17).

Práve selén ako kofaktor významných antioxidačných selenoenzýmov sa javil na základe výsledkov vtedajších veľkých klinických štúdií ako tzv "magic bullet" alebo "game changer", ktorý by mohol zvrátiť nepriaznivý priebeh septických ochorení. Avšak na vyslovenie jednoznačných účinkov chýbali ďalšie dôkazy.

2.4 Klinické štúdie sledujúce efektivitu selénu

Terapeutické ovplyvnenie oxidačného stresu u kriticky chorých pacientov je dlhodobo predmetom klinického výskumu. Pozitívny terapeutický efekt suplementovaného selénu bol na prelome 20 a 21 storočia zaznamenaný v niekoľkých významných štúdiách.

V roku 1994 boli publikované výsledky klinickej štúdie zaoberajúcej sa prospektívnym sledovaním pacientov s diagnostikovanou akútnou pankreatitídou. U pacientov bol počas liečby parenterálne suplementovaný pentahydrát seleničitanu sodného. Štúdia prebiehala na jednotkách intenzívnej starostlivosti v Nemeckých mestách v Rostocku a v Drážďanoch. Celkovo bolo do štúdie zaradených 330 pacientov. Po stanovení diagnózy bol pacientom podaný bolusovou formou 200 µg pentahydrátu seleničitanu sodného a následne 800 µg kontinuálne počas nasledujúcich 24 hodín. Od druhého dňa sa podávalo 500 µg pentahydrátu seleničitanu sodného denne. S dobre načasovanou terapiou selénom miera mortality, komplikácií a počet následných operácií dramaticky poklesol. Mortalita v skupine 245 pacientov z Rostocku klesla na nulu. V Drážďanoch po suplementácii selénu bolo v sledovanej skupine zaznamenaných 8 úmrtí z celkového súboru 85 pacientov, ktorí boli zaradení do štúdie. Komplikácie sa pridružili, až keď sa terapia začala príliš neskoro alebo ak sa jednalo o biliárne formy pankreatitídy (18).

Ďalšie pozitívne výsledky parenterálnej suplementácie selénu publikoval Angstwurm v roku 2007 v časopise Critical Care zhrnutím záverov prospektívnej randomizovanej, placebom kontrolovanej multicentrickej štúdie. Celkovo bolo v tejto štúdii zaradených 249 pacientov so SIRS, sepsou a septickým šokom, ktorých APACHE III bol viac ako 70 bodov. Pacientom bol po stanovení diagnózy podaný bolus 1000 µg pentahydrátu seleničitanu sodného počas 30 minút v kontinuálnej infúzii a nasledujúcich 14 dní denne bola aplikovaná kontinuálna infúzia 1000 µg pentahydrátu seleničitanu sodného. Počas štúdie neboli zistené nežiaduce účinky alebo známky predávkovania selénom. Po analýze podľa pôvodného liečebného zámeru bola stanovená mortalita v placebo skupine (50,0 %) versus skupina liečená selénom (39,7 %) ($p = 0,109$; odds ratio, 0,66; interval spoľahlivosti, 0,39-1,1). Ďalšie analýzy podskupín odhalili významné zníženie úmrtnosti medzi pacientmi so septickým

šokom komplikovaným diseminovanou intravaskulárnou koaguláciou (n = 82, p = 0,018), ako aj medzi najkritickejšie chorými pacientmi so skóre APACHE III ≥ 102 (horný kvartil, n = 54, p = 0,040) alebo u pacientov, ktorí mali dysfunkciu viac ako troch orgánov (n = 83, p = 0,039) (19).

Sakr v roku 2007 v British Journal of Anaesthesia publikoval výsledky prospektívnej observačnej štúdie, do ktorej bolo zaradených 60 pacientov rozdelených do skupín podľa závažnosti klinického stavu. Jednalo sa o pacientov s nekomplikovaným priebehom SIRS, ťažkým priebehom SIRS, ťažkou sepsou a septickým šokom. U všetkých týchto pacientov boli počas hospitalizácie dokumentované znížené hladiny plazmatického selénu oproti referenčnej hladine zdravých jedincov. Zistením bolo, že koncentrácia plazmatických koncentrácií selénu klesala v závislosti od dĺžky hospitalizácie, pričom výraznejší pokles bol pozorovaný u pacientov s orgánovým zlyhaním, najmä v dôsledku infekcií. Nižšie hladiny selénu v plazme boli spojené so zvýšeným poškodením tkaniva, prítomnosťou infekcie alebo orgánovej dysfunkcie/zlyhania a zvýšenou mortalitou na JIS (20).

Optimizmus v odborných kruhoch začal upadať s pribúdaním ďalších štúdií, ktorých výsledky neboli natoľko presvedčivé a nepriniesli očakávaný benefit. Príkladom je multicentrická, dvojito zaslepená štúdia, s kontrolnou placebo skupinou, ktorá sa uskutočnila na 11 klinikách intenzívnej starostlivosti vo Francúzsku. Táto štúdia bola zameraná na podávanie selénu u pacientov v septickom šoku. V štúdii sa podávalo 4000 μg pentahydrátu seleničitanu sodného ako bolus v úvodnej dávke a 1000 μg na deň v kontinuálnej infúzii počas 9 dní. V závere nebol zaznamenaný žiadny signifikantný rozdiel skrátenia času liečby medzi oboma skupinami po vysadení vazopresorov. Nebol zaznamenaný žiadny pozitívny efekt na dobu pripojenia pacienta na mechanickú ventiláciu, ani dĺžku hospitalizácie, pobytu na JIS, vplyvu na výskyt nozokomiálnych pneumónií, alebo zlepšenia renálnych funkcií u pacientov s renálnou insuficienciou. Nebola zistená toxicita, ale ani sa nepotvrdil pozitívny klinický výsledok u pacientov v septickom šoku (21).

2.5 Štúdia SE-AOX

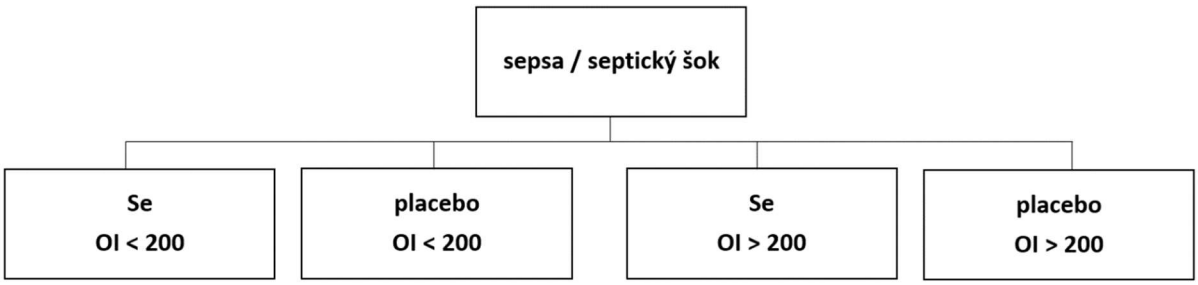
2.5.1 Dizajn klinickej štúdie Se-AOX

Vzhľadom na rozporuplné výsledky doterajších klinických štúdií zaoberajúcich sa suplementáciou selénu a jeho perspektívny terapeutický potenciál v teoretických modeloch, náš vedecko-lekársky tím pripravil dizajn prospektívnej observačnej klinickej štúdie zaoberajúcej sa hypotézou účinnosti liečby selénom u kriticky chorých pacientov s ťažkou sepsou alebo septickým šokom.

V roku 2008 bola schválená Prospektívna observačná klinická štúdia: Therapeutic Effect of Sodium Selenite on Oxidative Stress in Patients With Severe Sepsis, Akronym: Se-AOX, ktorá bola registrovaná v databáze clinicaltrials.gov pod registračným číslom NCT02026856. Cieľom tejto štúdie bolo: porovnanie klinických výsledkov u dvoch skupín pacientov so sepsou, pričom prvej skupine bol suplementovaný pentahydrát seleničitanu sodného v kontinuálnej dávke 750 μg/24 hod počas 6 dní. Druhú skupinu tvorila placebo skupina pacientov. Primárne boli pacienti rozdelení do 4 podskupín, podľa toho, či podstúpili alebo nepodstúpili chirurgický výkon, selén versus placebo.

Obrázok č.1: Flow diagram štúdie SE-AOX - prospektívnej analýzy.

Po zozbieraní a vyhodnotení údajov tejto časti štúdie, sme ďalej hodnotili efektivitu liečby vzhľadom na úroveň oxygenácie arteriálnej krvi v pľúcach. Pacienti boli sekundárne retrospektívne rozdelení do 4 podskupín podľa hodnôt oxygenačného indexu (pomeru parciálneho tlaku kyslíka v arteriálnej krvi a frakcie inspirovaného kyslíka) Skupina OI < 200, OI > 200, selén versus placebo.



Obrázok č.2: Flow diagram štúdie SE-AOX - retrospektívnej analýzy.

V jednotlivých skupinách a podskupinách bola monitorovaná intenzita oxidačného stresu prostredníctvom aktivity antioxidačných enzýmov, a to GPx, glutatiónreduktázy (GR) a SOD v krvnej plazme.

Hodnotené boli dynamické zmeny vybraných laboratórnych parametrov, a to C-reaktívneho proteínu, laktátu, urey, kreatinínu, fibrinogénu, albumínu a celkových bielkovín, celkového počtu leukocytov a ich populácií, pomeru neutrofilov k lymfocytom a počtu trombocytov. Ďalším cieľom štúdie bolo hodnotenie klinických výsledkov v jednotlivých skupinách a podskupinách, a to výskyt infekčných komplikácií, respiračných a renálnych dysfunkcií a mortalita pacientov.

2.5.2 Kazuistiky v súvislosti so štúdiou SE-AOX

Počas fázy klinickej štúdie, v ktorej sme realizovali zber údajov od pacientov, bolo zaznamenaných niekoľko pozoruhodných prípadov, v ktorých pozitívny zvrat kritického stavu nastal po aplikovaní komplexnej intenzivistickej liečby vrátane suplementácie pentahydrátu seleničitanu sodného a glutamínu.

Prvá kazuistika popisuje úspešnú liečbu pacienta s ťažkou akútnou nekrotizujúcou pankreatitídou a s pridruženým multiorgánovým zlyhaním, kvalitatívnou poruchou vedomia, rozvinutým šokovým stavom, s úvodným APACHE skóre 19 bodov a SOFA skóre 6 bodov. V úvode liečby bol pacient napojený na umelú pľúcnu ventiláciu pre závažnú respiračnú insuficienciu (PaO2/FiO2 = 70) s klinickým a RTG obrazom ARDS. Pre potvrdenú difúznu peritonitídu bola pacientovi po prijatí vykonaná abdominálna laparotómia s následnou lavážou brušnej dutiny. Nasledujúca liečba ďalej zahŕňala podporu respiračných funkcií formou umelej pľúcnej ventilácie, kombinovanej cielenej antimikrobiálnej terapie podľa zistených aktuálnych kultivácii a ich citlivostí. Pre pridružené obličkové zlyhanie pacient bol dialyzovaný formou CVVHD. Pacientovi bola aplikovaná komplexná parenterálna výživa

spolu s adjuvantnou terapiou, ktorá zahŕňala suplementáciu selénu intravenóznou aplikáciou pentahydrátu seleničitanu sodného v dávke 750 µg/deň v kontinuálnej infúzii počas šiestich dní. Zároveň bol v infúznom roztoku parenterálne podávaný alanylglutamín v dennej dávke 100 ml počas šiestich dní v tom istom čase. Počas obdobia suplementácie bola monitorovaná dynamika zápalových buniek (leukocyty, pomer neutrofilov a lymfocytov, trombocyty) a zmena dynamiky biochemických markerov a antioxidačných enzýmov (prokalcitonín, fibrinogén, CRP, laktát, GPx, GR). Priame meranie dynamiky antioxidačných enzýmov u pacienta z kazuistiky poukázalo na ich aktiváciu suplementovanými mikronutrientmi. Zároveň bol tento jav sprevádzaný poklesom plazmatických hladín prokalcitonínu a CRP. Dynamické trendy zvýšenia aktivít GPx a GR od aplikácie prvej dávky selénu a glutamínu dokazovala zvýšenú aktivitu antioxidačného systému. (Aktivita GPx pred suplementáciou - 0,55 µkat/l, 2. deň suplementácie - 2,66 µkat/l, 7. deň od začatia liečby - 3 µkat/l. Aktivita GR pred suplementáciou - 0,1813 µkat/l, 2. deň suplementácie - 0,3 µkat/l, 7. deň od začatia liečby - 1,65 µkat/l.) Pomer neutrofilov a lymfocytov vykazoval priaznivú klesajúcu tendenciu aj napriek stúpajúcej hodnote leukocytov. Stanovenie tohto pomeru korelovalo s intenzitou závažnosti systémového zápalu. V tejto kazuistike bola pozorovaná súvislosť medzi závažnosťou klinického stavu a mierou neutrofílie a lymfocytopénie. Pacient bol po 10 dňoch extubovaný, s GCS 15b, cirkulačne stabilný, bez vazopresorickej podpory a s negatívnymi zápalovými parametrami bol preložený na oddelenie chirurgie.

Druhá kazuistika popisuje prípad 66-ročnej ženy, u ktorej sa po kolonoskopickom vyšetrení s polypektómiou rozvinul septický šok s cirkulačnou nestabilitou a multiorgánovým zlyhaním. U pacientky bola realizovaná exploratívna abdominálna laparotómia, ďalšia intenzivistická liečba zahŕňala cielenú antibiotickú liečbu, protektívnu umelú pľúcnu ventiláciu, mimotelovú eliminačnú liečbu, komplexnú parenterálnu a enterálnu výživu, vrátane suplementácie selénu vo forme pentahydrátu seleničitanu sodného v dávke 750 µg/deň počas šiestich dní, čo zodpovedá 250 µg selénu na deň. Parenterálna výživa bola obohatená o glutamín a jeho prekurzory, a to podávaním alanylglutamínu v dennej dávke 2 g. Pacientkin stav sa postupne zlepšoval, vrátane hemodynamických parametrov, bola vysadená vazopresorická podpora, operatívne realizovaná deeskalácia antibiotickej liečby pri poklese zápalových parametrov CRP a PCT, počtu leukocytov, renálnych a hepatálnych parametrov. Pacientka bola postupne odpojená od umelej pľúcnej ventilácie. Šestnásty deň hospitalizácie bola pacientka v zlepšenom stave preložená do spádovej nemocnice.

Tretia kazuistika popisuje prípad 31 - ročnej pacientky s exacerbovanou Stillovou chorobou na dlhodobej imunosupresívnej liečbe. Pacientka bola zaradená do štúdie po náhlom

rozvoji syndrómu systémovej zápalovej odpovede s multiorgánovým zlyhávaním s manifestným hepatorenálnym syndrómom, respiračným zlyhaním, diseminovanou intravaskulárnou koagulopatiou v obraze prebiehajúceho distribučného šoku. Pacientke bola poskytnutá komplexná intenzivistická liečba vrátane enterálnej a parenterálnej výživy so suplementáciou pentahydrátu seleničitanu sodného v dávke 750 μg/deň počas šiestich dní. Nutričné prípravky obsahovali vyšší podiel rozvetvených esenciálnych aminokyselín (valín, leucín, izoleucín) a prekurzorov glutatiónu (cysteín, glutamín). Počas liečby sme zaznamenali zvýšenie aktivít antioxidačných enzýmov a to aktivity GPx z východiskovej hodnoty 0,117μkat/l na 0,27 μkat/l, aktivity GR z úvodnej 0,267μkat/l na 0,683 μkat/l a aktivity SOD z 3,98 μkat/l na 4,45 μkat/l. Tento pozitívny trend korešpondoval s postupne sa zlepšujúcim klinickým stavom až do úplného vysadenia vazopresorickej podpory, zlepšenia respiračných, hepatálnych a renálnych parametrov.

Suplementácia selénu u pacientov s ťažkou akútnou pankreatitídou

Kočan L., Firment J., Šimonová J., Vašková J., Guzy J.

I. klinika anesteziológie a intenzívnej medicíny, Fakultná nemocnica L. Pasteura, Košice, Slovenská republika, prednosta kliniky: doc. MUDr. Jozef Firment, Ph.D.

Súhrn

Kočan L., Firment J., Šimonová J., Vašková J., Guzy J.: Suplementácia selénu u pacientov s ťažkou akútnou pankreatitídou

Suplementácia selénu zlepšuje antioxidačný status u kriticky chorých pacientov s ťažkou akútnou pankreatitídou v závislosti od dávky a doby podávania selénu. Cieľom práce je poukázať na prínos antioxidačnej terapie hradením selénu.

Metóda: Pacient s akútnou pankreatitídou a rozvíjajúcim sa septickým šokom bol prijatý na oddelenie intenzívnej medicíny. V rámci adjuvantnej terapie bola zahájená suplementácia selénu v kontinuálnej infúzii v dávke 750 @g/24 hodín počas šiestich dní. Meraním bolo zistené zvýšenie aktivity antioxidačného enzýmu glutatión peroxidázy a zníženie zápalových markerov v čase suplementácie.

Práca poukazuje na možnosti ovplyvnenia patogenézy syndrómu systémovej zápalovej odpovede v jeho počiatočnej fáze a ovplyvnenie vývoja chorobného stavu u pacientov s ťažkou akútnou pankreatitídou.

Kľúčové slová: ťažká akútna pankreatitída – sepsa – syndróm systémovej zápalovej odpovede – selén – glutatión peroxidáza

Summary

Kočan L., Firment J., Šimonová J., Vašková J., Guzy J.: Selenium Supplementation in Patients with Severe Acute Pancreatitis

Selenium supplementation improves antioxidant status in critically ill patients with severe acute pancreatitis. It depends on quantum of dosage and supplementation time. The aim of this analysis is point out on benefit of antioxidant therapy by supplementing selenium.

Methods: Patient with severe acute pancreatitis and developing septic shock was admitted on anesthesiology and intensive care department. Adjuvant supplementation therapy with selenium was started in continual infusion 750 μg/24 h during next six days. Activity of antioxidant enzyme glutathione peroxidase and others inflammatory markers were decrease.

A case report presents the possibility to affect on systemic inflammatory response syndrome pathogenesis in initial phase. It has to improve therapeutic progress in patients with severe acute pancreatitis.

Key words: severe acute pancreatitis – sepsis – systemic inflammatory response syndrome – selenium – glutathione peroxidase

Rozhl. Chir., 2010, roč. 89, č. 1, s.

ÚVOD

Ťažká akútna pankreatítída predstavuje závažný medicínsky problém z hľadiska vážnosti stavu, rapídnej progresie a vysokej mortality [1, 2, 3]. Stratégia liečby vyžaduje komplexný multidisplinárny prístup. Pacientov je nutné hospitalizovať na oddeleniach, ktoré túto problematiku zvládajú, a to chirurgického alebo interného typu. Najťažšie formy akútnej pankreatitídy spojené s respiračným zlyhaním a multiorgánovou dysfunkciou patria na lôžkové časti oddelení anestéziológie a intenzívnej medicíny [4].

Ochorenie sa spája s rozvojom syndrómu systémovej zápalovej odpovede (SIRS), v patogenéze ktorého dochádza k nadmernej aktivácii imunitných dejov, zápalových mediátorov a vzniku oxidačného stresu, ktorý sa v úvodnej fáze významne podieľa na amplifikácii zápalových dejov [5]. Vážnou komplikáciou ťažkej akútnej pankreatitídy je multiorgánové zlyhanie, ktoré vzniká na podklade SIRS a to vo včasnej fáze priebehu ochorenia [6]. Antioxidačná terapia má potenciál zasiahnuť prostredníctvom vychytávania voľných radikálov do priebehu počiatočnej fázy rozvoja SIRS, čo môže výrazne ovplyvniť prežívanie pacientov [7].

Klinicky významný benefit bol dokázaný substitúciou troch živín s antioxidačnými vlastnosťami. Pri podávaní selénu sa zlepšujú klinické výsledky pri infekciách a orgánovom zlyhaní, pri podávaní glutamínu v rozsiahlych štúdiách bolo zistené zníženie infekčných komplikácií u kriticky chorých pacientov. V súvislosti s kyselinou eikosapentaenovou a mikronutrientmi bol zaznamenaný výrazný protizápalový efekt a otupená odozva endotoxínu [8]. Z podávaných mikronutiientov, sá zdá byť selén najviac účinnou antioxidačnou látkou v klinickom merítku, po ňom nasleduje zinok, vitamíny C a E a ß-karotén.

Cieľom práce je poukázať prostreníctvom kazuistiky na prínos suplementačnej adjuvantnej terapie vhodnými mikronutrientmi u kriticky chorých pacientov [9].

METODIKA

Pacientovi bol počas adjuvantnej terapie v dennej dávke 750 @g v kontinuálnej infúzii počas šiestich dní suplementovaný selén vo forme pentahydrátu seleničitanu sodného. Zároveň bol v infúznom roztoku podávaný alanylglutamín do centrálneho venózneho katétra v dennej dávke 100 ml počas šiestich dní v tom istom čase. Počas obdobia suplementácie bola monitorovaná dynamika zápalových buniek (leukocyty, pomer neutrofilov a lymfocytov, trombocyty) a zmena dynamiky biochemických markerov a antioxidačných enzýmov (prokalcitonín, fibrinogén, CRP, laktát, glutatiónperoxidáza, glutatión reduktáza).

KAZUISTIKA

Pacient vo veku 50 rokov s akútnou hemoragicko-nekrotizujúcou pankreatitídou a pridruženým multiorgánovým zlyhávaním bol preložený na I. KAIM FNLP z OAIM NsP Michalovce, kde bol operovaný pre difúznu peritonitídu. Bola vykonaná drenáž brušnej dutiny s lavážou a zavedením preplachových drénov. Pri prijatí bol pacient v komatóznom stave, APACHE 19 bodov a SOFA skóre 6 bodov na agresívnej umelej pľúcnej ventilácii (UPV) s cirkuláciou podporovanou noradrenalínom. Bolo realizované vstupné CT vyšetrenia abdomenu, pľúc a mozgu.

Po desiatich dňoch hospitalizácie pre anemizáciu a pridružený septický šok chirurg indikuje revíznu operáciu. Bola realizovaná drenáž omentálnej burzy so zavedením preplachovej drenáže a súčasne bola vykonaná punkčná jejunostómia. Ihneď po operácii sa realizovaná adjuvantná antioxidačná terapia kontinuálnou suplementáciou selénu v dávke 750 @g/24 hodín počas nasledujúcich šiestich dní. Pravidelne boli merané aktivity antioxidačných enzýmov ako aj štandardné zápalové parametre (Tab. 1). Počas hospitalizácie sa rozvíja obojstranný fluidothorax viac vľavo, preto boli realizované opakovane evakuačné punkcie aj v spolupráci s hrudníkovým chirurgom. Bola aplikovaná kombinovaná ATB liečba a bolo pridávané antimykotikum (fluconazol) vzhľadom na kandidovú infekciu. Pre pridružené akútne obličkové poškodenie je pacient hemodialyzovaný, v úvode bola aplikovaná mimotelová eliminačná liečba (MEL) formou CVVHD a neskôr MEL formou intermitentnej hemodialýzy. Pacient sa v ďalšom priebehu cirkulačne stabilizuje a už nevyžaduje vazopresorickú podporu.

Po týždni opätovne dochádza k anemizácii pacienta, kontrolné CT vyšetrenie abdomenu odhalilo subkapsulárneho hematóm sleziny. Konziliárny chirurg odporučil konzervatívny postup. Vzhľadom na vzostup zápalových markerov je napokon pacient revidovaný, retroperitoneálny absces bol vydrénovaný a bola ponechaná preplachová drenáž s Betadinovým roztokom. Na oddelení intenzívnej jednotky pokračujeme v ATB liečbe a podpornej UPV. Z hľadiska ďalšej prognózy dlhodobej a potreby podpornej UPV je vykonaná perkutánna dilatačná tracheostómia. Priebeh hospitalizácie bol komplikovaný hematemézou a melénou, pri endoskopickom náleze bol zistený vred v bulbe duodéna a erozívna ezofagitída. Krvácanie z horného GIT-u bolo zvládnuté konzervatívne. Pri kontrolnom CT vyšetrení pretrváva septovaný fluidotorax vľavo, preto konzultovaný hrudníkový chirurg, ktorý odporúča dekortikáciu pľúc. Po štyroch týždňoch hospitalizácie na I. KAIM je celkový stav pacienta stabilný, pacient je pri vedomí s GCS 15 b, na tlakovej podpornej ventilácii, je odpájaný od UPV cez tracheostómiu (netoleruje záťaž), pretrváva oligúria s potrebou pokračovania v MEL, je kardiálne kompenzovaný, pre sínusovú tachykradiu v liečbe ponechaný metoprolol. Postupne pacient dýcha spontánne cez Ayrovo T s následnou dekanyláciou tracheostómie. Počas rehabilitačnej liečby pacienta vertikalizujeme a začína chodiť. Dochádza k miernej úprave azotémie, preto nefrológ neindikuje dlhodobý dialyzačný program. Kombinovaná antihypertenzná liečba bola postupne redukovaná. Po šiestich týždňoch hospitalizácie je pacient pri vedomí, dýcha spontánne cez O_2 masku, je hemodynamicky kompenzovaný, preto po dohode s chirurgom bol preložený na chirurgickú JIS v stabilizovanom stave.

Tab. 1. Laboratórne výsledky pacienta z kazuistiky pred začatím a v priebehu suplementácie selénu
Tab. 1. Laboratory results from casuistic research before start selenium supplementation and within therapy

	Pred podaním selénu	2. deň terapie	7. deň terapie
Leukocyty (10^9/l)	6,6	12,9	20,9
Neutrofily/Lymfocyty	9,9	11	12
Trombocyty (10^9/l)	233	138	313
Fibrinogén (g/l)	5,76	6	5,33
Laktát (mmol/l)	1,01	2,24	1,85
CRP (mg/l)	171	163	132
Prokalcitonín (μg/l)	100	20,3	10,6
GPx (μkat/l)	0,55	2,66	3
GR (μkat/l)	0,1813	0,3	1,65

DISKUSIA

Voľné radikály zohrávajú dôležitú úlohu v patogenéze syndrómu systémovej zápalovej odpovede, sepsy, ťažkej sepsy, septického šoku a akútnej pankreatitídy [5]. Samotné voľné radikály, ako produkt leukocytov majú v prvotnej fáze sepsy dôležitú antimikrobiálnu úlohu pri ničení patogénnych mikroorganizmov. Problémom sa stávajú až vtedy, keď ich tvorba je nekontrolovateľná a ďaleko prevyšuje vychytávaciu schopnosť antioxidantov, a tak dochádza k vzniku oxidačného stresu a aktivácii patologických kaskád [9].

Vznik a účinok voľných radikálov je v rovnováhe s pôsobením antioxidačného obranného systému organizmu [5]. Exhaustná tvorba kyslíkových radikálov indukuje tvorbu cytokínov imunokompetentnými bunkami, ktoré ďalej aktivujú zápalovú kaskádu a vplývajú na expresiu adhéznych molekúl na povrchu buniek. Voľné radikály ovplyvňujú kaskádu intercelulárnych dejov, čoho výsledkom je aktivácia génov dôležitých z hľadiska zápalu. Princípom tejto suplementačnej antioxidačnej terapie je ovplyvnenie zápalovej kaskády ešte v počiatku SIRS a tým priaznivo ovplyvniť klinický priebeh [5, 10].

V skorom štádiu ochorenia u pacientov s ťažkou pankreatitídou bola experimentálne zistená vysoká tvorba voľných radikálov. Takisto príjem a plazmatické hladiny selénu sú v tejto skupine znížené. V niektorých medicínskych centrách využívajú adjuvantnú terapiu selénom pri akútnej pankreatitíde dlhší čas a boli zaznamenané sľubné výsledky. Príkladom je výsledok retrospektívnej štúdie, kde bolo zistené v priebehu desiatich rokov zníženie mortality u pacientov s akútnou pankreatitídou z 35 % na 16 % [8]. Pri experimentálnych meraniach hladiny selénu u kriticky chorých pacientov so sepsou a SIRS bol zistený markantný pokles plazmatického selénu a bola zaznamenaná signifikantná negatívna korelácia medzi plazmatickou hladinou selénu, APACHE II a SAPS II [11].

Referenčná hodnota plazmatického selénu je v rozpätí 89–114 Ķg/l. Tieto ťažké stavy sa spájajú s presunom vitamínov a stopových prvkov do tkanív, v ktorých sa syntetizujú proteíny teraz v oveľa väčšej miere ako za fyziologických podmienok. Tento presun spôsobuje relatívny deficit cirkulujúcich antioxidantov. Klinické štúdie u pacientov so SIRS, sepsou, ARDS a pankreatitídou poukazujú na to, že suplementácia selénu priaznivo ovplyvňuje prežívanie [8]. Podávanie selén obsahujúcich doplnkov sa zdá byť prospešným z hľadiska zníženia mortality [5]. Problémom pri tejto terapii je správne načasovanie, odhad dávky, a doby podávania.

Suplementácia v neskorých fázach SIRS a ťažkej sepsy sa zdá byť neúčinnou terapiou [12].

Selén je dôležitý mikronutrient. Tvorí nebielkovinovú súčasťou dôležitého antioxidačného enzýmu glutatiónperoxidázy (GPx), od ktorej úzko závisí integrita bunkových a subcelulárnych membrán. Antioxidačné vlastnosti enzýmu sú bytostne závislé od prítomnosti selénu [13]. Prísun vysokých dávok selénu u zdravých ľudí počas dlhého obdobia niekoľkých rokov sa spája s ochorením selenózou. Intoxikácie selénom u kriticky chorých pacientov pozorované doteraz neboli, a to ani pri štúdiách kde podávali vyššie dávky selénu [10].

Dávka 1000 @g/deň podaná intravenózne bola dobre tolerovaná pacientmi na JIS [5]. Dávkovanie selénu pri ťažkej akútnej pankreatitíde nie je striktne odporúčané, preto sme používali priemerné dávkovanie vychádzajúc z výsledkov viacerých randomizovaných štúdií [7, 14]. Selén sa uplatňuje ako kofaktor enzýmu gluthationperoxidázy, ktorý patrí do skupiny enzýmov vykazujúcich peroxidázovú aktivitu, jej hlavnou úlohou je ochrana bunkových štruktúr pred oxidačným poškodením. Biochemická funkcia GPx je redukovať lipidové peroxidy na im korenšpodujúce alkoholy a redukovať voľný peroxid vodíka na vodu. Substrátom pre túto reakciu je glutatión [15, 16]. Suplementáciou glutamínu sa zvyšuje efektivita účinku GPx. Regenerácia GSH z oxidovanej formy GSSG je katalyzovaná glutatión reduktázou. To že u pacienta z kazuistiky nedochádza k zvýšeniu aktivity GR, je možné vysvetliť z tohto hľadiska, že regenerácia GSSG na GSH nie je zatiaľ nutná, keďže zároveň podávame aj alanylglutamín, prekurzor GSH [13].

Priame meranie dynamiky antioxidačných enzýmov u pacienta z kazuistiky poukazuje na ich aktiváciu suplementovanými mikronutrientmi. Zároveň je tento jav sprevádzaný poklesom plazmatickej hladiny prokalcitonínu, čo je známkou efektívnej terapie sepsy. Taktiež v priebehu liečby dochádza k významnému poklesu hladiny CRP. Laktát sa výrazne nemení. Zvýšená hodnota GPx v siedmy deň od aplikácie prvej dávky selénu a glutamínu svedčí o zvýšenej aktivite antioxidačného systému. Pomer neutrofilov a lymfocytov má priaznivú klesajúcu tendenciu aj napriek stúpajúcej hodnote leukocytov. Pomer neutrofilov a lymfocytov jednoducho a spoľahlivo vystihuje mieru závažnosti oxidačného stresu a systémového zápalu. Bola zistená súvislosť medzi závažnosťou klinického stavu a mierou neutrofílie a lymfocytopénie [17]. Výsledky tejto kazuistiky potvrdzujú priaznivý vplyv suplementácie selénu na ťažkú formu akútnej pankreatitídy a jej septické komplikácie.

ZÁVER

Podpora antioxidačných mechanizmov sa zdá byť prínosná pre pacientov s diagnostikovanou ťažkou akútnou pankreatitídou. Načasovanie a dávkovanie antioxidantov a kofaktorov antioxidačných enzýmov je pre terapiu zásadné. Táto špecifickosť dávkovania je veľmi významná najmä pri terapii selénom. Optimálna terapeutická dávka, ku ktorej sme dospeli na podklade výsledkov viacerých štúdií bola určená na 750 mg/deň a doba suplementácie šiestich dní. So suplementáciou je nutné začať ihneď po stanovení diagnózy.

Zoznam skratiek:

APACHE II	Acute Physiology and Chronic Health Evaluation II
ARDS	akútny syndróm systémovej zápalovej odpovede acute respiratory
CVVHD	
CRP	C-reaktívny proteín
GCS	Glasgow coma scale
GPx	glutatión peroxidáza
GSH	redukovaná forma glutatiónu
GSSG	oxidovaná forma glutatiónu
MEL	mimotelová eliminačná liečba
SAPS II	Simplified Acute Physiology Score
SIRS	syndróm systémovej zápalovej odpovede systemic inflammatory response syndrome

LITERATÚRA

1. **Rau, B M., Krüger, C. M., Hasel, C., Oliveira, V., Rubie, C., Beger, H. G., a kol.** Effects of immunosuppressive and immunostimulative treatment on pancreatic injury and mortality in severe acute experimental pancreatitis. Pancreas, 2006; č. 33, s. 174–183.
2. **Rau, B. M.,, Bothe, A., Kron, M., Beger H. G.** Role of early multisystem organ failure as major risk factor for pancreatic infections and death in severe acute pancreatitis. Clin. Gastroenterol. Hepatol., 2006; č. 4: s. 1053–1061.
3. **Lakyová L., Toncr, Radoňak J., Šimon, R., Toporcer, T., Vajó, J., Belák, J.** Spontánna perforácia ductus choledochus pri akútnej pankreatitíde – kazuistika. Rozhl. Chir., 2008, roč. 87, č. 2, s. 92–95.
4. **Balthazar, E. J**: Staging of acute pancreatitis. Radiologic clinics of North America, 2002; 40 (6): s. 1199–1209.
5. **Geoghegan, M., McAuley, D., Eaton, S., Powel-Tuck, J.** Selenium in critical illness. Critical Care, 2006, 12, s. 136–141.
6. **Nathens, B. A., Curtis, R. J., Beale, J. R., et al.** Management of the critically ill patient with severe acute pankreatitis. Critical Care Medicine, 2004, roč. 32, č. 12, s. 2524–2536.
7. **Heyland, D., Dhaliwal, R., Suchner, U., Berger, M. M.** Antioxidant nutrients: a systematic review of trace elements and vitamins in the critically ill patient. Intensive Care Medicine, 2005, roč. 31, str. 327–337.
8. **Heyland, D., Dhaliwal, R., Day, A., Drover, J., Cote, H., Wischmeyer, P.** Optimizing the dose of glutamine dipeptides and antioxidants in critically ill patients: a phase I dose-finding study. J. Parenteral Enteral Nutrition, 2007, roč. 31, s. 109–118.
9. **Berger, M., Chiolero, R.** Antioxidant supplementation in sepsis and systematic inflammatory response syndrome. Critical Care Medicine, 2007; roč. 35, s. 584–590.
10. **Angstwurm, M. W. A., Schottdorf, J., Schopohl, J., Gaertner, R.** Selenium replacement in patients with severe systemic inflammatory response syndrome improves clinical outcome. Critical Care Medicine, 1999, roč. 27, s. 1807–1813.
11. **Sakr, Y., Reinhart, K., Bloos, F., Marx, G., Russwurm, S., Bauer, M., Brunkhorst, F.** Time course and relationship between plasma selenium concentrations, systemic inflammantory response, sepsis, and multiorgan failure. Br. J. Anaesth., 2007, roč. 98, s. 775–784.
12. **Forceville, X., Vitoux, D., Gauzit, R., Combes, A., Lahilaire, P., Chappuis, P.** Selenium, systemic immune response syndrome, sepsis, and outcome in critically ill patients. Critical Care Medicine, 1998, roč. 26, s. 1536–1544.
13. **Salama, A., Sakr, Y., Reinhart, K.** The role of selenium in critical illness: Basic science and clinical implications. Critical Care Medicine, 2007, roč. 7, s. 127–138.
14. **Kuklinski, B., Buchner, M., Schweder, R., Nagel, R.** Akute Pancreatitis-eine „Free Radical Disease: Letalitatssenkung durch Natriumselenit (Na2SeO3)-Therapie. Z Gestame Inn. Med., 1991, 46: s. 145–149.
15. **Forceville, X.** Seleno-enzymes and seleno-compounds: the two faces of selenium. Critical Care, 2006, roč. 10, s. 180.
16. **Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., van Remmen, H.** Trends in oxidative aging theories. Free Radic. Biol. Med., 2007, roč. 43, s. 477–503.
17. **Záhorec, R.** Pomer neutrofilov a lymfocytov - rýchly a jednoduchý ukazovateľ systémového zápalu a stresu u pacientov v kritických stavoch; Bratislavské Lekárske Listy, 2001, č. 102: s. 5–14.

MUDr. Ladislav Kočan
I. klinika anesteziológie a intenzívnej medicíny
Trieda SNP 1
040 01 Košice
Slovenská republika
e-mail: kocanladislav@yahoo.com

Původní práce

Akútne multiorgánové zlyhanie po kolonoskopii s polypektómiou

Ladislav Kočan, Janka Vašková, Ladislav Vaško, Lucia Lakyová, Hana Kočanová, Jana Šimonová, Róbert Šimon, Jozef Firment

I. klinika anestéziológie a intenzívnej medicíny, Košice, Slovenská republika

SOUHRN

Východisko. Závažné intraabdominálne infekcie patria medzi život ohrozujúce ochorenia. Vznikajú na podklade šírenia lokalizovaných zápalov alebo narušenia integrity črevnej steny.
Metódy a výsledky. Liečebnou stratégiou je chirurgická intervencia, antimikrobiálna terapia, liečba distribučného šoku a cielená nutričná podpora (1). Predpokladá sa, že suplementácia glutamínu a selénu zlepšuje funkciu črevnej bariéry a regeneruje antioxidačnú obranu (2). U pacientky po endoskopickej polypektómii sa rozvinul septický šok s multiorgánovým zlyhávaním a rozvojom katabolizmu s výrazným poklesom albumínu. Origo sepsy zobrazovacími metódami a probatórnou laparotómiou nebolo zistené. Liečbou distribučného šoku, podávaním širokospektrálnych antibiotík, enterálnym a parenterálnym hradením nutrientov došlo k výraznému zlepšeniu zdravotného stavu. V rámci adjuvantnej terapie boli intavenózne suplementované: glutamín v dennej dávke 2 g a pentahydrát seleničitanu sodného v kontinuálnej infúzii v dávke 750 μg/24 hodín v priebehu 6 dní. Počas terapie došlo k poklesu zápalových markerov: C-reaktívny proteín, prokalcitonínu, leukocytov, neutrofilov. Došlo k zvýšeniu hladín albumínu.
Závěry. Práca poukazuje na terapeutické možnosti pri liečbe septického šoku a možnosťami reverzie katabolickej fázy ochorenia.
Kľúčové slová: kolonoskopia, septický šok, multiorgánové zlyhanie, enterálna výživa, parenterálna výživa.

SUMMARY

Kočan L, Vašková J, Vaško L, Lakyová L, Kočanová H, Šimonová J, Šimon R, Firment J. Acute multiple organ failure after endoscopic polypectomy
Background. Serious intraabdominal infections belong among life treating diseases. They are based on spreading infections from focal sources of inflammation in abdomen or damaged intestinal wall.
Methods and results. Treatment strategies are surgical intervention, antimicrobial therapy, distributional shock treatment and accurate nutritional support (1). Glutamine and selenium supplementation may improve intestinal functions and restore antioxidant defence (2). Septic shock with multiple organ failure accompanied by serious catabolism and decrease of albumin had developed in a patient after endoscopic polypectomy. Infection source was not discovered by medical imaging examinations non surgical laparotomy. After distributive shock treatment, wide spectral antibiotics and enteral and parenteral nutrition the patient's health improved. As adjuvant therapy intravenous supplementation was administered: glutamine in daily dose 2g and sodium selenite in continual infusion in daily dose 750 μg over 6 days. During intensive therapy, inflammatory markers decreased: C-reactive protein, procalcitonin, leukocyte count and neutrophils. Albumin levels increased.
Conclusions. The paper describes therapeutic options during septic shock treatment and reversion possibilities in the catabolic phase of disease.
Key words: colonoscopy, septic shock, multiorgan failure, enteral nutrition, parenteral nutrition. *Ko.*

Čas. Lék. čes. 2012; 151: 568–578

ÚVOD

Ťažká intraabdominálna infekcia ako aj generalizovaná peritonitída sú život ohrozujúce stavy vyžadujúce si okamžitú chirurgickú intervenciu vzhľadom na vysokú mortalitu. Najčastejšou príčinou vzniku komplikovanej intraabdominálnej infekcie býva divertikulitída, apendicitída, či perforácia gastrointestinálneho traktu (3). Vznik kolitídy po kolonoskopii je veľmi zriedkavá komplikácia. Býva sprevádzaná tenezmami a krvavými hnačkami, objavuje sa najčastejšie do 48 hodín po kolonoskopii, alebo sigmoidoskopii (4). V literatúre sú dokumentované zriedkavé prípady vzniku ischemickej kolitídy po endoskopickom vyšetrení. Ako mechanizmus vzniku sa popisuje nadmerná distenzia hrubého čreva po jeho naplnení plynom pri vyšetrení, čo znižuje prietok krvi črevom z mukózy do serózy, alebo je to mechanický tlak endoskopu po zavedení. Ischémia s nekrózou črevnej steny môže zasiahnuť hlboko do črevnej steny, a porušiť tak bariéru a spôsobiť následnú translokáciu baktérií. Pridružené komorbidity u pacienta zhoršujú následne morbiditu a mortalitu (6). Ako jedna z ďalších možných vyvolávajúcich príčin kolitídy po kolonoskopii je používanie glutaraldehydu pri dezifekcii endoskopu. Po vyšetrení vzniká difúzny edém mukózy čreva, pričom klinická prezentácia a morfologický vzhľad sliznice môže mimikovať ischemickú kolitídu, alebo infekčnú kolitídu

ADRESA PRO KORESPONDENCI:
MUDr. Ladislav Kočan, PhD.
I. klinika anestéziológie a intenzívnej medicíny
Trieda SNP 1, 041 90 Košice, Slovenská republika
e-mail: kocanladislav@yahoo.com

(5). Hlavnou liečbou je podávanie antibiotík, prednizonu (steroidov) a mesalazínu (4, 5). Prognóza pacientov je zvyčajne dobrá, pokiaľ nedôjde k perforácii čreva. Vtedy dochádza k vysokej mortalite vzhľadom na to, že infekcia sa dostane do dutiny brušnej. V nízkom percente prípadov môže tento vnútrobrušný zdroj infekcie vyústiť až do multiorgánového zlyhania (MOFS) (3).

Optimálna liečba a pooperačná starostlivosť je stále kontroverzná. Od šesťdesiatych rokov sa preferuje antibiotická liečba proti gram-negatívnym a anaeróbnym baktériám. Táto kombinácia zvyšuje prežívanie u septických pacientov (7). V rámci intenzívnej starostlivosti je dôležité zvolenie vhodnej nutričnej podpory, jej načasovanie, výber, dávkovanie vhodných nutrientov a spôsob ich aplikácie. Podporou metabolizmu sa zabezpečia energetické požiadavky ale aj zvýšená potreba už chýbajúcich (resp. vyčerpaných zdrojov) aminokyselín a antioxidačných molekúl. So stavom imunosupresie a multiorgánového zlyhania korelujú nízke hladiny glutamínu, preto obzvlášť dôležitú úlohu zohráva práve doplnenie glutamínu a jeho prekurzorov. Taktiež suplementácia selénu kriticky chorým pacientom zlepšuje antioxidačný obranný systém organizmu. Nemenej dôležitú úlohu predstavuje včasná enterálna výživa v boji voči atrofii klkov tenkého čreva a následnej translokácie baktérií z čreva do krvného obehu (8).

V prezentovanom prípade ide o zriedkavý prípad vnútrobrušnej infekcie spôsobenej kolitídou v koincidencii s obojstrannou pneumóniou, čo vyústilo až do syndrómu systémovej zápalovej odpovede (SIRS) a popis vlastných skúseností s úspešne zvládnutou pooperačnou liečbou špeciálnymi parenterálnymi prípravkami, širokospektrálnym antibiotickým krytím ako aj enterálnou výživou.

SOUBOR NEMOCNÝCH A POUŽITÉ METODY

Vykonané terapeutické postupy sú na našej klinike rutinne používané. Pacienti, ktorým bol suplementovaný selén a glutamín, boli zaradení do štúdie Se-AOX schválenej Etickou komisiou Univerzitnej nemocnice L. Pasteura.

KAZUISTIKA

Žena, 66 rokov, podstúpila ambulantnou formou preventívne kolonoskopické vyšetrenie s polypektómiou v celkovej anestézii. V nasledujúci deň sa rozvinul kolapsový stav s hypotenziou TK 60/40 v domácom prostredí. Pacientka bola prvotne ošetrená posádkou rýchlej lekárskej pomoci. Vzhľadom na cirkulačnú nestabilitu pacientky bola jej podaná katecholamínová podpora (efedrín v bolusovej forme). Následne bola hospitalizovaná v okresnej nemocnici na internom oddelení. Hospitalizácia trvala dva dni. Vstupné laboratórne výsledky poukazovali na systémovú zápalovú reakciu (tab. 1). U pacientky naďalej pretrvávala závažná hypotenzia vyžadujúca katecholamínovú podporu. Pre podozrenie na infekciu neznámeho pôvodu bola začatá empirická liečba širokospektrálnymi antibiotikami (Cefotaxim, Metronidazol, Ampicilín-Sulbaktam, Ciprofloxacin) a až následne boli odobraté vzorky krvi na hemokultiváciu. RTG vyšetrenie pľúc odhalilo bilaterálnu bronchopneumóniu, viac vpravo. RTG vyšetrením brucha bol zistený ľahko distendovaný, pneumatizovaný žalúdok s primeranou plynatosťou čriev bez jasných známok pneumoperitonea. Konziliárny chirurg vyjadril podozrenie na perforáciu hrubého čreva vzhľadom na predchádzajúcu anamnézu polypektómie. Následne indikoval chirurgickú exploráciu. Pri revízii bolo črevo vitálne, bez známok perforácie a bez prítomnosti voľnej tekutiny. V pooperačnom období sa rozvinula akútna renálna insuficiencia kombinovanej etiológie s nutnosťou zahájenia mimotelovej eliminačnej liečby s pretrvávaním zvýšených zápalových markerov. Zistené kultivácie z odobraných krvných vzoriek nepotvrdili prítomnosť mikroorganizmov. U pacientky postupne dochádzalo k respiračnému zlyhaniu, čo si vyžadovalo zaistiť dýchacie cesty orotracheálnou kanylou a napojiť pacientku na umelú ventiláciu pľúc (UVP). Pre rozvinutý syndróm systémovej zápalovej odpovede (SIRS) bez zisteného infekčného fokusu, bola pacientka preložená na vyššie odborné pracovisko za účelom ďalšieho dodiferencovania klinického nálezu a liečby septického šoku.

Po príjme na I. kliniku anestéziológie a intenzívnej medicíny Univerzitnej nemocnice Louisa Pasteura v Košiciach sme znova odobrali krvné vzorky, obsahy brušných drénov a materiál z dolných dýchacích ciest na mikrobiologické vyšetrenie. Eskalovali sme antibiotickú liečbu proti G+ (linezolid) a G– (imipenem) a anaeróbnym baktériám (metronidazol), do liečby sme zaradili aj antimykotiká (flukonazol) a imunomodulanciá (polyoxidónium a gamaglobulíny).

Realizovali sme echokardiografické vyšetrenie srdca, ktoré vylúčilo infekčnú endokarditídu ako i kontrolné CT vyšetrenie hrudníka a brucha. CT vyšetrenie hrudníka odhalilo infiltratívne zmeny v ľavom laloku pľúc v hrotovom segmente, v laterobazálnom a posterobazálnom segmente splývali do pomerne homogénneho nevzdušného infiltrátu s pozitívnym bronchogramom. Vpravo bola zvýšená denzita pľúcneho parenchýmu v rozsahu celého dolného laloka a čiastočne v zadnom segmente horného laloka. CT vyšetrenie brucha odhalilo malé abscesové ložisko v pravom laloku pečene o veľkosti 0,5 cm, a zväčšený žlčník o šírke 4,25 cm.

Intrahepatálne žlčové cesty boli bez dilatácie a a perihepatálny priestor bol bez prítomnosti voľnej tekutiny. Menšie množstvo voľnej tekutiny bolo zobrazené v perisplenickom a v ľavom subfrenickom priestore, taktiež obojstranne peri-

Tab. 1. Dynamický vývoj hematologických a biochemických parametrov počas hospitalizácie v okresnej nemocnici

Čas hospitalizácie	Začiatok ochorenia	24 hodín	48 hodín
leukocyty ($10^9.l^{-1}$)	25,8	27,6	29,8
neutrofily (%)	95,5	92,3	93,4
lymfocyty (%)	2,7	3,1	3,5
trombocyty (10^3 l^{-1})	165	94	69
albumín ($g.l^{-1}$)	41,4	27,2	25,2
fibrinogén ($g.l^{-1}$)	1,9	1,11	1,72
urea (mmol/l)	14,4	14,7	21,1
kreatinín ($\mu mol.l^{-1}$)	180	333	447
laktát ($mmol.l^{-1}$)	2,9	3,1	3,4
CRP ($mg.l^{-1}$)	127,9	210,9	213,2
prokalcitonín ($\mu g.l^{-1}$)	100	216	70
AST ($\mu kat.l^{-1}$)	3,92	5,15	4,12
ALT ($\mu kat.l^{-1}$)	2,2	2,89	3,12
GGT ($\mu kat.l^{-1}$)	1,99	1,1	1,13

Tab. 2. Dynamický vývoj hematologických a biochemických parametrov počas hospitalizácie na I. KAIM

	1. deň pred začatím terapie	8. deň	16. deň
leukocyty ($10^9.l^{-1}$)	16,94 15,8 12,84		
neutrofily (%)	94	86	85
lymfocyty (%)	3,8	3,9	3,8
trombocyty ($10^3.l^{-1}$)	100	170	419
albumín ($g.l^{-1}$)	22,8	30,1	41,2
fibrinogén ($g.l^{-1}$)	2,03	2,06	2,12
urea (mmol/l)	25	15,91	15,13
kreatinín ($\mu mol.l^{-1}$)	517	235,7	105
laktát ($mmol.l^{-1}$)	3,6	2,1	2
CRP ($mg.l^{-1}$)	196	267,1	75
prokalcitonin ($\mu g.l^{-1}$)	9,55	1,11	0,78
AST ($\mu kat.l^{-1}$)	3	2,12	1,76
ALT ($\mu kat.l^{-1}$)	2,6	1,12	1,01
GGT ($\mu kat.l^{-1}$)	1,09	0,89	0,9

renálne, ale bez príznakov abscesu. V pravom hypogastriu, v tesnej blízkosti drénu, bol zreteľný konvolut vzájomne fixovaných črevných kľučiek, ktoré naliehali na brušnú stenu ako aj v ľavom mezogastriu s nálezom konvolutu fixovaných črevných kľučiek svedčiacich pre prítomnosť kolitídy bez príznakov abscesového ložiska. Konziliárny chirurg vzhľadom na CT nález odporúčal pokračovať v doterajšom konzervatívnom postupe. Pre akútne obličkové poškodenie v štádiu F, podľa klasifikácie RIFLE, bola zahájená mimotelová eliminačná liečba (MEL) formou kontinuálnej veno-venóznej hemodialýzy (CVVHD) iniciálne s citrátovou (Ci-Ca) antikoaguláciou.

Pre cirkulačnú instabilitu boli do liečby zaradené katecholamíny v kontinuálnej infúzii (noradrenalín 0,5 μg/kg/min). Pacientka bola naďalej napojená na umelú ventiláciu pľúc s režimom tlakovej podpory so zaradením vyšších hodnôt pozitívneho endexpiračného tlaku. Príjem živín bol zabezpečený enterálnou výživou cez nazogastrickú sondu (Nutrison-Multifibre) a podporený parenterálnymi výživovými roztokmi podávanými intravenózne (Smofkabiven a Aminomix II). Výživa bola obohatená o glutamín a jeho prekurzory (alanylglutamín do centrálneho venózneho katétra v dennej dávke 2 g). Do liečby boli pridané liečivá s antioxidačnými účinkami. Vitamin C, vitamín E a v kontinuálnej infúzii selén v dennej dávke 750 μg počas šiestich dní (vo forme pentahydrátu seleničitanu sodného, čo zodpovedá 250 μg selénu na deň). Pre výraznú expektoráciu a opakovaný vzostup zápalových parametrov na základe mikrobiologických výsledkov z dýchacích ciest bola antibiotická liečba deeskalovaná na Cefoperazón/Sulbaktám (Sulperazon) v dávke 2 g každých 8 hodín. Opakované RTG vyšetrenia hrudníka v nasledujúcich dňoch hospitalizácie potvrdili regresiu infiltratívnych zmien.

Kontrolné CT vyšetrenie brucha s aplikáciou kontrastnej látky Ultravist 370/100 ml v dvojfázovom režime bolo realizované po siedmich dňoch. V pečeni v pôvodnej lokalizácii pretrvávala pôvodná cystoidná lézia bez signifikantného postkontrastného zvýraznenia a bez evidentnej progresie veľkosti. Perisplenicky, perihepatálne a v malej panve bolo zistené menšie množstvo tekutinových kolekcií. Dutina brušná bola bez známok pneumoperitonea. V porovnaní s predchádzajúcim CT vyšetrením došlo k ústupu prejavov kolitídy, s pretrvávaním edematóznych prejavov v oblasti steny colon descendes a sigmy. Na laterálnej stene colon descendes sa zobrazoval divertikel o priemere 13 mm. Nález na čreve poukazoval na možné reaktívne zmeny pri divertikulitíde.

Klinický stav pacientky sa postupne zlepšoval, hodnoty zápalových parametrov postupne vykazovali klesajúcu tendenciu, cirkulácia sa stabilizovala, čo umožňovalo postupne znižovať vysoké dávky noradrenalínu až po jeho vysadenie z liečby. Pre pretrvávajúcu renálnu insuficienciu musela byť naďalej vedená MEL formou intermitentnej hemodialýzy s nutnou prísnou tekutinovou bilanciou.

Pacientka bola postupne odpájaná od UVP a extubovaná. Pri sporadických poklesoch saturácie krvi kyslíkom bola napojená na dýchací režim neinvazívnej ventilácie pomocou tvárovej masky. Antibiotická liečba bola postupne vysadená. Vzhľadom na iniciálne vysoké dávky noradrenalínu pre cirkulačnú instabilitu ako aj napojenie pacientky na kontinuálnu elimináciu bolo polohovanie pacientky problematické s následnou poruchou celistvosti kože v sakrálnej oblasti, ktorá bola ošetrovaná polyvidonjodidom (Betadine) a kyslíkom.

Pacientka bola po dohovore preložená do spádovej nemocnice v zlepšenom zdravotnom stave, s potrebou pokračovania intermitentnej hemodialýzy.

DISKUSIA

U pacientky z kazuistiky, ktorá podstúpila diagnostickú kolonoskopiu s polypektómiou, došlo k rozvinutiu symptómov distribučného šoku, ktorý je charakteristický pre septický šok. Podľa názoru Wellsa a Erlandsena (9), aj keď dostupné sú len nepriame dôkazy, je za rozvojom bakteriémie a sepsy translokácia baktérií z čreva do krvného obehu. Niektoré novšie štúdie (10) však klinický význam translokácie baktérií spochybňujú. Röntgenovým zobrazením brucha nebola zistená prítomnosť pneumoperitonea, ktoré by poukazovalo na perforáciu v gastrointestinálnom trakte, ale konziliárny chirurg nevylúčil poškodenie črevnej steny a indikoval revíznu laparotómiu brucha, nakoľko perforácia hrubého čreva predstavuje závažnú komplikáciu, ktorá môže vyústiť do vzniku sterkorálnej peritonitídy so septickým šokom. Poškodenie fyziologickej črevnej bariéry umožňuje priľnavosť, alebo internalizáciu črevných baktérií bunkami čreva (9, 10). Už translokácia baktérií alebo toxínov do črevnej steny môže vyvolať SIRS a dysfunkciu vzdialených orgánov tým, že aktivuje črevnú zápalovú odpoveď, aj keď sú baktérie odstránené imunologickými bunkami čreva. Za týchto podmienok sa v čreve masívne tvoria cytokíny a ďalšie prozápalové faktory, čím sa mezenteriálna mikrocirkulácia stáva miestom aktivácie cirkulujúcich neutrofilov (10). Na druhej strane, narušenie intestinálnej steny a následná translokácia baktérií je u imunodeficientných pacientov vyvolaná mnohými faktormi a môže viesť k bakteriémii, sepse a MOFS (11).

Sterkorálna peritonitída však revíziou potvrdená nebola, neboli zachytené mikroorganizmy z odobratých vzoriek krvi, preto sa nedal určiť septický šok ako príčina daného stavu pacienta. Uvažovali sme teda o rozvinutom syndróme zápalovej odpovede s multiorgánovým zlyhávaním, ktorý vznikol na podklade ťažkej bilaterálnej pneumónie, prítomnej divertikulitíde

a dekompenzácii po celkovej anestézii, ktorý vzniká po uvoľnení prozápalových cytokínov do krvného obehu.

Črevo patrí k najcitlivejším a zároveň svojou anatómiou, spektrom funkcií a prebiehajúcimi metabolickými pochodmi k najkomplikovanejším orgánom v tele. Vedľa dôležitej tráviacej funkcie a absorpcie živín, plní funkciu endokrinného orgánu, ktorý produkuje gastrointestinálne hormóny. Je bohaté na obsah lymfatického tkaniva, má podstatnú funkciu v imunitnom systéme a tvorí vitálne dôležitú veľkoplošnú bariéru medzi vonkajším a vnútorným prostredím v organizme. Terapeutická stratégia ďalšej liečby bola založená na chirurgickom konzervatívnom postupe, a to preplachovaním brušnej dutiny polyvidonjodidovými roztokmi cez brušné drény, podávaním širokospektrálnych intravenóznych antibiotík a voľbou kombinácie enterálnej a parenterálnej výživy. Z hľadiska udržania štrukturálnej a funkčnej integrity črevnej sliznice je preferovaná enterálna výživa. Doplnková parenterálna výživa je vhodná na substitúciu tých živín, ktoré sa obtiažne suplementujú enterálnou formou. Vplyvom parenterálnej výživy bohatej na aminokyseliny sa ovplyvňujú proteosyntetické procesy, čo má pozitívny vplyv na bilanciu dusíka. Odporúčaná dávka aminokyselín je 1,5 g/kg telesnej hmotnosti za deň. Toto množstvo by malo pozitívne ovplyvniť endogénnu proteosyntézu, kým na úroveň katabolizmu nemá zásadný vplyv. Kriticky chorí pacienti majú ťažký deficit glutamínu. Koncentrácia glutamínu výrazne klesá pri ťažkých katabolických stavoch, ako sú veľké traumy, ťažká sepsa, veľké operačné výkony. Výrazný úbytok glutamínu sa prejavuje zhoršením regeneračných a imunitných procesov, ako aj narušením integrity črevnej bariéry. Ten je významným zdrojom energie pre enterocyty a imunokompetentné bunky. Nízke hladiny glutamínu korelujú so vznikom multiorgánového zlyhania. Suplementácia glutamínu jednoznačne zlepšuje antioxidačný stav pacienta, znižuje výskyt infekčných komplikácií a skracuje dobu hospitalizácie. Pre nestabilitu sa v roztokoch pripravuje zakomponovaný vo forme dipeptidov, ako je napríklad kombinácia glutamín-alanín alebo glutamín-glycín (11, 12). Počiatočná fáza metabolizmu glutamínu je rovnaká pre všetky imunitné bunky, syntetizuje sa v nej redukovaný nikotínamidadeníndinukleotidfosfát (NADPH) a arginín. Čím sa vysvetľujú aj vysoké nároky na množstvo glutamínu a samotnú účinnosť týchto buniek obzvlášť u ťažko chorých, ktoré práve NADPH využívajú na tvorbu aktívnych zlúčenín voči mikroorganizmom. NADPH vzniká z malátu pri premene na pyruvát a je východiskovou látkou pre syntézu superoxidového radikálu NADPH oxidázou obzvlášť u fagocytujúcich buniek, ktorými sú neutrofily, makrofágy ako aj lymfocyty, a v kombinácii s reakciou katalyzovanou myeloperoxidázou vytvára najsilnejší fyziologický oxidant a mikrobicíd, kyselinu chlórnu. Z arginínu vzniká účinkom syntázy oxidu dusného NO, ktorý vytvára so superoxidom ďalšiu účinnú látku peroxinitril.

Aplikácia aj malého množstva glutamínu výrazne napomáha udržiavať bariérovú funkciu čreva, indukuje tvorbu imunoglobulínov v čreve a zlepšuje perfúziu splanchnickej oblasti (12).

Príčinou vzniku lymfocytopénie u pacientky z kazuistiky môže byť reakcia na vysoké hladiny katecholamínov, kortizolu a prolaktínu pri záťažových situáciách, ako je ich marginácia v RES (retikulo-endotelový systém) tkanivách, lymfatických štruktúrach a slizničnom imunitnom systéme asociovanom s črevom (GALT) alebo ich urýchlená apoptóza. Príčiny neutrofílie sú viaceré. Hypotézy založené na experimentálnych výsledkoch poukazujú na možnosť zvýšenia počtu neutrofilov ich demargináciou z cievnej steny ako aj indukciou tvorby zápalovými mediátormi (13).

U pacientky došlo počas terapie k úprave trombocytopénie, ktorá je častým nálezom u septických pacientov. Pokles množstva trombocytov u septických pacientov pod 150 × 10^9 l^{-1} sa vyskytuje v 35–44 %. Mechanizmus trombocytopénie u septických pacientov je multifaktoriálny (14). Úprava trombocytov a populácií bielych krviniek do referenčného rozmedzia svedčí pre pokles zápalovej odpovede (15). Aj zvýšený prísun selénu zvyšuje antioxidačnú ochranu a napomáha zvládnuť oxidačný stres pri SIRS, a to dvojako, prooxidačným účinkom pred zabudovaním a výrazne po zabudovaní do selenoproteínov. Môže tým zvýšiť prežívanie pacientov so sepsou (9). Podľa meta-analýzy Finley (1999) môžu byť aj trombocyty ukazovateľom adekvátneho príjmu selénu. V priebehu 7 dní po zahájení liečby (tab. 2), ich početnosť výrazne stúpol (16).

U kriticky chorých pacientov sa črevo považuje nielen za cieľové miesto pôsobenia, ale aj za miesto produkcie mediátorov zápalu, ktoré prispievajú k vzniku SIRS ako aj bakteriémie, sepsy alebo septického šoku s multiorgánovým zlyhávaním. Črevo sa môže stať zdrojom zápalových mediátorov. Nebakteriálne faktory produkované v čreve a nachádzajúce sa v mezenteriálnej lymfe vedú následne k poškodeniu vzdialenejších orgánov. Táto interpretácia úlohy čreva ako hlavného orgánu zodpovedného za produkciu zápalových mediátorov SIRS a pre vznik MOFS (17) sa teda javí ako vhodné vysvetlenie aj napriek negatívnym výsledkom hemokultivácie. Keďže pacientke bola nasadená empirická liečba širokospektrálnymi antibiotikami už v perifernej nemocnici, prítomnosť mikroorganizmov skutočne nemusela byť preukázaná. Aj na základe vysokých hodnôt prokalcitonínu (PCT) (viď tab. 1) predpokladáme generalizovanú infekciu, ktorú organizmus zvládol, čo potvrdzuje aj vývoj hodnôt C-reaktívneho proteínu (CRP) (viď tab. 2).

Poškodenie pečene sa prejavilo poklesom syntetickej činnosti a to znížením albumínu a fibrinogénu. Silný pokles albumínu mohol participovať na výskyte tekutín v brušnej dutine zistených pri CT vyšetrení. Podporná liečba, prísun aminokyselín a ďalších živín enterálnou a parenterálnou výživou zabezpečili obnovu funkcií pečene, čo sa prejavilo návratom uvedených bielkovín do rozmedzia normálnych hodnôt. Aktivity enzýmov ALT, AST, GGT tiež poklesli, ale ich zvýšenie nemusí odzrkadliť len poškodenie pečene, nakoľko nie sú orgánovo špecifické. Pokles hodnôt urey a kreatinínu sú vyvolané hemodialýzou, ale poukazujú na stále nedostatočnú funkčnosť obličiek.

ZÁVER

Zvládnutie septického šoku je komplexný problém vyžadujúci si multidisciplinárny prístup. Základom liečby je nájdenie origa infekcie a cielená chirurgická liečba v prípade vnútrobrušnej infekcie. Pri rozvoji MOFS si vyžaduje kritický stav pacienta nielen širokospektrálnu ATB liečbu, parenterálnu a enterálnu výživu. V ťažkom katablickom stave a oxidačnom strese prináša benefit aj suplementácia glutamínu a selénu. Udržiava sa tým bariérová funkcia čreva, zlepšuje perfúzia splanchnickej oblasti, indukuje sa tvorba imunoglobulínov v čreve a podporí sa antioxidačná obrana organizmu, čo zvyšuje pravdepodobnosť prežitia pacienta.

Skratky

ATB	– antibiotiká
Ci-Ca	– citrátový – kalciový modul
CRP	– C-reaktívny proteín
CT	– počítačová tomografia
CVVHD	– kontinuálna veno-venózna hemodialýza
GALT	– s črevom asociovaný slizničný imunitný systém
MEL	– mimotelová eliminačná liečba
MOFS	– syndróm multiorgánoveho zlyhania
NADPH	– redukovaný nikotínamidadeníndinukleotidfosfát
NO	– oxid dusnatý
PCT	– prokalcitonín
RES	– retikulárny endoteliálny systém
RIFLE	– klasifikačný systém pre akútne poškodenie obličiek
SIRS	– syndróm systémovej zápalovej odpovede
UVP	– umelá ventilácia pľúc

LITERATURA

1. **Záhorec R, Firment J, Straková J, Mikula J, Malík P, Novák I, Zeman J, Chlebo P.** Epidemiology of severe sepsis in intensive care units in the Slovak Republic. Infection 2005; 33(3): 122–128.
2. **Andrews P, Avenell A, Noble D, Campbell M, Croal B, Simpson W, Vale L, Battison C, Jenkinson D, Cook J.** Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. British Medical Journal 2011; 342: d1542.
3. Spirt MJ**.** Complicated intra-abdominal infections: a focus on appendicitis and diverticulitis. Postgrad Med 2010; 122(1): 39–51.
4. **Caprilli R, Viscido A, Frieri G, Latella G.** Acute colitis following colonoscopy.Endoscopy 1998; 30(4): 428–431.
5. **Yüksel, Osman MD; Demirezer Bolat, Aylin MD; Köklü, Seyfettin MD; Altparmak, Emin MDı; Albayrak, Murat MD; Caner, Sedat MD.** Ischemic Colitis, An Unusual Complication of Colonoscopy Southern. Medical Journal 2008; 101(9): 972–973.
6. **Ferzoco LB, Raptopoulos V, Silen W.** Acute Diverticulitis. N Engl J Med 1998; 338: 1521–1526.
7. **Grendel T, Hudák V, Firment J.** Low-dose corticosteroids and septic shock. Rozhl Chir 2008; 87(3): 158–164.
8. **Kočan L, Vašková J, Vaško L, Hoková H, Majerník M, Krištofová B, Šimonová J, Firment J.** Ťažký priebeh Stillovej choroby s multiorgánovým zlyhávaním a závažnou pečeňovou dysfunkciou. Anest intenziv Med 2011; 22(6): 337–342.
9. **Wells CL, Erlandsen SL.** Bacterial translocation: Intestinal epithelial permeability. In: Rombeau JL, Takala J. (eds.) Gut Dysfunction in critical illness. Berlin: Springer-Verlag 1996; 131–149.
10. **Deitch EA.** Bacterial translocation or lymphatic drainage of toxic products from the gut: What is important in human beings? Surgery 2002; 131: 241–244.
11. **Zadák Z.** Umělá výživa při poškození čreva. In: Zadák Z. Výživa v intenzivní péči. Praha: Grada Publishing 2008; 383–391.
12. **Pachl J, Roubík K.** Základy anesteziológie a resuscitační péče dospělých i dětí. Praha: Karolinum 2005; 253–267.
13. **Zahorec R.** Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy 2001; 102(1): 5–14.
14. **Kirschenbaum L, Mckevitt D, Rullan M, Reisbeck B, Fujii T.** Importance of platelets and fibrinogen in neutrophil-endothelial cell interactions in septic shock. Critical Care Medicine 2004; 32: 1904–1909.
15. **Spaková T, Rosocha J, Lacko M, Harvanová D, Gharaibeh A.** Treatment of knee joint osteoarthritis with autologous platelet-rich plasma in comparison with hyaluronic acid. Am J Phys Med Rehabil 2012; 91(5): 411–417.
16. **Finley JW, Duffield A, Ha P, Vanderpool RA, Thomson CD.** Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium. Br J Nutr 1999; 82: 357–360.
17. **De-Souza DA, Greene LJ.** Intestinal permeability and systemic infections in critically ill patients: Effect of glutamine. Crit Care Med 2005; 33: 1125–1135.

KAZUISTIKA

Ťažký priebeh Stillovej choroby s multiorgánovým zlyhávaním a závažnou pečeňovou dysfunkciou

Kočan Ladislav[1], Vašková Janka[4], Vaško Ladislav[4], Hoková Hana[3], Majerník Miloš[1], Krištofová Beáta[2], Šimonová Jana[1], Firment Jozef[1]

[1]I. klinika anestéziológie a intenzívnej medicíny, UN LP, Košice, SR
[2]I. interná klinika, UN LP, Košice, SR
[3]Klinika anestéziológie, resuscitácie a intenzívnej medicíny ÚVN SNP Ružomberok – FN, SR
[4]Ústav lekárskej chémie, biochémie a klinickej biochémie, LF UPJŠ, Košice, SR

SÚHRN

Stillova choroba je ochorenie neznámej etiológie a patogenézy. Je charakterizovaná febrilitami, artritídou, exantémom a možným postihnutím ďalších orgánov. Kazuistika sa zaoberá popisom akútnej exacerbácie a liečby Stillovej choroby u 31-ročnej pacientky dlhodobo liečenej kortikoidmi. Pacientka bola prijatá na I. kliniku anestéziológie a intenzívnej medicíny pre febrility do 40 °C, s kvantitatívnou poruchou vedomia (sopor) a s prejavmi multiorgánového zlyhávania (pečene, akútneho zlyhávania obličiek, kardiovaskulárneho systému, dýchania a disseminovanej intravaskulárnej koagulopatie). Komplexnými vyšetreniami sa však zdroj sepsy, malignita, hematologické ochorenia a ani infekčné agens nezistili (opakované vyšetrenia vzoriek krvi boli negatívne). Stav bol definovaný ako akútna exacerbácia Stillovej choroby dospelých v. s. komplikovaná syndrómom nadmernej aktivácie makrofágov so sekundárnym multiorgánovým zlyhaním. Do liečby bolo zahrnuté podávanie pulznej kortikoterapie, hepatoprotektív, doplnkovej umelej výživy vrátane parenterálnej suplementácie selénu, širokospektrálnych antibiotík (ATB), umelej ventilácie pľúc a mimotelovej eliminačnej liečby, v ktorej sa po stabilizácii stavu pokračovalo na Internej klinike. Komplexný terapeutický prístup viedol k významnej regresii hepatálnej insuficiencie. Pacientka bola po 28 dňoch hospitalizácie vo významne zlepšenom stave prepustená do ambulantnej starostlivosti.
Kľúčové slová: **Stillova choroba – syndróm systémovej zápalovej odpovede – selén – enterálna výživa – parenterálna výživa – glutatión peroxidáza**

Abstract

Severe course of Still's disease with multiple organ failure with predominant liver failure

Still's Disease is a disease of unknown aetiology and pathogenesis. It is characterized by fever, arthritis, salmon-coloured rash and organ failure in severe cases. This is a case report of a 31-year old female patient on long-term steroid treatment who suffered from adult Still's disease. She was admitted to the Clinic of Anaesthesiology and Intensive Care with fever up to 40 °C, unconsciousness and signs of multi-organ failure (liver, acute kidney failure, cardiovascular system, lungs, and disseminated intravascular coagulation). A complex of examinations did not confirm the cause to be sepsis, malignancy, a haematological disease or an infectious disease (repeated blood cultures were negative). We concluded this was an acute exacerbation of adult Still's disease complicated by the syndrome of oversize macrophage activation with secondary multi-organ failure. The patient's condition required pulse steroid therapy, hepato-protective therapy and supplementary nutrition inclusive of parenteral selenium supplementation, antibiotics, mechanical ventilation and extra-corporeal elimination therapy. The therapy continued at the Clinic of Internal Medicine. The complex therapy resulted in great regression of the liver insufficiency. After 28 days of hospitalization the patient was discharged in an improved condition.
Keywords: **Still's disease – systemic inflammatory response syndrome – selenium – enteral nutrition – parenteral nutrition – glutathione peroxidase**

Anest. intenziv. Med., 22, 2011, č. 6, s. 337–342

Úvod

Kriticky chorí pacienti sú zaťažení dysfunkciou rôznych orgánových systémov. Rozdielnosti v metabolických pochodoch si vyžadujú špecifický terapeutický prístup adekvátne reagujúci na metabolické požiadavky organizmu. Existencia širokého spektra enterálnych a parenterálnych nutričných prípravkov poskytuje účinné terapeutické možnosti zvrátenia nepriaznivej metabolickej situácie. Obzvlášť závažná situácia nastáva pri stavoch spojených so zlyhávaním pečene. Ide o chorobné stavy, akými sú napríklad systémové, hematologické ochorenia, sepsa a Stillova choroba. Stillova choroba dospelých je akútny febrilný syndróm dospelých, typicky postihujúci niekoľko orgánov s klinickými a laboratórnymi abnormalitami, pričom k plnému rozvoju príznakov môže dôjsť až v priebehu niekoľkých týždňov či mesiacov. Stanovenie diagnózy je obtiažne a nasleduje až po vylúčení ostatných príčin systémových prejavov, ako sú horúčky, vyrážky, hepatosplenomegália, lymfadenopatia a sérozitída. Artritída, ktorá sa môže objaviť kedykoľvek v priebehu ochorenia, má rôzny charakter. Asi v polovici prípadov prebieha ako ťažká, deštruktívna polyartritída. Medzi ostatné príčiny systémových prejavov patria infekčné a systémové afekcie a malignita. Liečba spočíva v podávaní nesteroidných antireumatík, glukokortikoidov, liekov modifikujúcich ochorenie a u ťažších foriem je indikovaná aj biologická liečba. Toto ochorenie bolo po prvýkrát popísané anglickým pediatrom Ericom Bywatersem v r. 1971 [1], ktorý zaznamenal 14 prípadov Stillovej choroby.

Dôležitou úlohu v patogenéze chorobných stavov u kriticky chorých pacientov (syndróm systémovej zápalovej odpovede, sepsa a hepatálne zlyhávanie) zohrávajú voľné kyslíkové radikály a reaktívne kyslíkové častice (ROS, reactive oxygen species). Za normálnych okolností vzniká superoxidový radikál ($O_2^{\cdot -}$) monovalentnou redukciou molekulárneho kyslíka v každom živom systéme, najmä v reakciách spojených s mitochondriálnym elektrónovým transportom. Dizmutáciou $O_2^{\cdot -}$ sa tvorí peroxid vodíka (H_2O_2), ktorý nie je voľným radikálom, avšak vedie k tvorbe hydroxylových radikálov (.OH), ktoré sú považované vôbec za najreaktívnejšie [2]. Produkcia ROS však v organizme môže byť zámerná a mohutná ako v prípade aktivácie neutrofilov a je súčasťou antimikrobiálnej ochrany. Ovplyvnenie ale aj zvládnutie oxidačného stresu je teda významné z hľadiska ovplyvnenia patofyziológie daných ochorení [3].

Parenterálne sa na podporu antioxidačného systému využíva suplementácia selénu [4], ktorý je kofaktorom antioxidačného enzýmu glutatión peroxidázy (GPx). Mnohé štúdie potvrdzujú priamu súvislosť medzi hladinou selénu v plazme, selenoenzýmov a závažnosťou septického stavu [5]. Etiológia Stillovej choroby je dosiaľ neznáma. Najpravdepodobnejšou hypotézou je prehnaná reakcia organizmu na infekčné agens alebo určitý toxický substrát komplexnou interakciou. Popisovaný prípad približuje úspešnosť zvolenej terapie pri Stillovej chorobe komplikovanej systémovým prejavom zápalu spojeným zo zlyhávaním pečene.

Metodika

Vykonané terapeutické postupy sú na našej klinike rutinne používané. Pacienti, ktorým boli stanovené aktivity antioxidačných enzýmov, boli zaradení do štúdie Se-AOX schválenej Etickou komisiou Univerzitnej nemocnice L. Pasteura.

Kazuistika

Pacientka vo veku 31 rokov s dlhodobou imunosupresívnou liečbou kortikoidmi a s dokumentovanou Stillovou chorobou bola hospitalizovaná pre vertebroalgický syndróm v cervikálnej a lumbosakrálnej oblasti na neurologickom oddelení okresnej nemocnice. Priebeh hospitalizácie bol komplikovaný febrilitami a rozvojom šokového stavu, syndrómom systémovej zápalovej odpovede (SIRS) nejasnej etiológie a multiorgánovým zlyhávaním. U pacientky došlo k rozvoju diseminovanej intravaskulárnej koagulopatie (DIC). Pre zhoršujúci sa stav bola pacientka preložená na I. kliniku anestéziológie a intenzívnej medicíny (I. KAIM) Univerzitnej nemocnice L. Pasteura v Košiciach za účelom ďalšej intenzívnej terapie. Ihneď po prijatí na KAIM sa realizovala adjuvantná antioxidačná terapia kontinuálnou suplementáciou selénu v dávke 750 μg/24 hodín počas nasledujúcich šiestich dní. Výživa bola zabezpečená podávaním nutrientov enterálnym i parenterálnym prístupom. Nutričné prípravky obsahovali vyšší podiel rozvetvených esenciálnych aminokyselín (valín, leucín, izoleucín) a prekurzorov glutatiónu (cysteín, glutamín). Pravidelne boli merané aktivity antioxidačných enzýmov, ako aj štandardné zápalové parametre (tab. 1). Bola začatá oxygenoterapia a empirická kombinovaná širokospektrálna antimikrobiálna liečba. Pre cirkulačnú instabilitu si stav vyžiadal vazopresorickú podporu noradrenalínom v dávke 0,06 μg . kg^{-1} . min^{-1}. V laboratórnom skríningu pri prijatí dominovali elevované aminotransferázy, hyperbilirubinémia, zvýšená hladina urey v sére, anémia, leukocytóza, hypokoagulačný stav s trombocytopéniou v rámci DIC a zvýšené zápalové markery C-reaktívny proteín (CRP) a prokalcitonín (PCT) – viď tabuľka 1. Po konzultácii s hematológom bola realizovaná substitučná hemoterapia. Vzhľadom na rozvíjajúcu sa oligúriu, po konzultácii s nefrológom, bola indikovaná mimotelová eliminačná liečba (MEL) formou kontinuálnej veno-venóznej hemodialýzy (CVVHD, Ci-Ca modul). Do terapie boli zaradené hepatoprotektíva a vitamínová liečba. Po konzultácii s internistom stav hodnotený ako exacerbácia Stillovej choroby a následne aplikovaná pulzná liečba metylprednisolonom (iniciálne 500 mg i. v. počas 4 dní). Na 3. deň hospitalizácie bola pacientka pre progresiu res-

Tabulka 1. Laboratórne hodnoty počas hospitalizácii na I. klinike anestéziológie a intenzívnej medicíny

	Pri prijatí	2. deň terapie	6. deň terapie
Leukocyty (10^9/l)	13,05	12,77	8,54
Neutrofily/Lymfocyty (10^9/l)	10	11,5	13
Trombocyty (10^9/l)	41	45	68
Albumín (g/l)	30,7	29,2	30,9
Celkové bielkoviny (g/l)	51,6	48,4	50,8
Fibrinogén (g/l)	0,15	0,87	1,07
ALT (μkat/l)	69.1	22,4	6,44
AST (μkat/l)	173,1	22,4	1,65
Bilirubín celkový (mmol/l)	31	49,3	37,6
Kreatinín (μmol/l)	261,5	245,9	194,4
Urea (mmol/l)	11,34	8,56	10,52
Laktát (mmol/l)	7,16	3,15	2,31
CRP (mg/l)	78,6	39,08	4,77
Prokalcitonín (μg/l)	25,66	2,3	0,866
Protrombínový čas (%)	19	41	95
APTT (s)	54,3	41,9	30,2
GPx (μkat/l)	0,1166	0,1912	0,27
GR (μkat/l)	0,2666	0,4122	0,6833
SOD (U/ml)	3,975	4,112	4,450

piračnej insuficiencie intubovaná a prechodne napojená na umelú ventiláciu pľúc (UVP). Boli realizované pomocné zobrazovacie vyšetrenia v rámci detekcie zdroja ťažkej sepsy. CT vyšetrenie brucha nevylúčilo kolitídu v cékoascendentnej oblasti a v oblasti pravého colon transversum. Zároveň bolo prítomné edematózne presiaknutie oboch obličiek. CT vyšetrenie pľúc potvrdilo obojstranný fluidotorax a infiltratívne zmeny v dorzobazálnych častiach oboch pľúcnych krídel. Pre meningeálny syndróm a kvadruspasticitu bola realizovaná lumbálna punkcia, vyšetrenie likvoru však bolo negatívne. Po aplikovanej liečbe došlo k parciálnemu zlepšeniu laboratórnych parametrov. Naďalej pretrvávala anúria, boli nutné denné nefrologické konzília pre vyžadujúcu MEL, postupne z CVVHD zmenenej na intermitentnú hemodialýzu. Po týždni hospitalizácie na I. KAIM bol pacientkin celkový zdravotný stav zlepšený, pacientka bola afebrilná, so spontánnym dýchaním, parametre acidobázickej rovnováhy boli v norme, preto bola extubovaná, hemodynamicky stabilná bez nutnosti pokračovania v katecholamínovej liečbe. ATB liečba bola ukončená, antimykotikum ponechané len v profylaktickej dávke. Nutrícia bola naďalej zabezpečená kombináciou enterálnej a parenterálnej výživy.

Tabuľka 2. Laboratórne hodnoty počas hospitalizácie I. klinike internej medicíny

	Pri prijatí	Pri prepustení
Leukocyty (10^9/l)	norma	norma
ALT (μkat/l)	4,54	1,67
AST (μkat/l)	1,35	0,67
GMT (μkat/l)	9,68	6,9
ALP (μkat/l)	1,78	1,48
Kreatinín (μmol/l)	374,4	norma
Urea (mmol/l)	21,12	norma
CRP (mg/l)	Norma	norma

Po šiestich dňoch bolo parenterálne podávanie selénu ukončené. Pacientku sme v stabilizovanom stave preložili na jednotku intenzívnej starostlivosti I. internej kliniky UNLP.

Pri príjme na internú kliniku bola pacientka pri vedomí, afebrilná, bez prejavov respiračnej insuficiencie, hemodynamicky stabilná, naďalej pretrvávala anúria. Výsledky vstupných laboratórnych vyšetrení sú uvedené v tabuľke 2. Realizované kompletné mikrobiologické vyšetrenia vrátane hemokultúr boli negatívne. Pre akútne obličkové zlyhanie sme naďalej pokračovali v mimotelovej eliminačnej liečbe, s postupnou úpravou kreatinínu na 135,9 μmol . l^{-1}. Taktiež sme pokračovali v pulznej kortikoterapii (v dávke 250 mg metylprednisolonu na deň) s postupnou redukciou dávky až prechodom na perorálnu liečbu prednisonom v dávke 40 mg na deň. Pacientke bol odstránený centrálny venózny katéter, nutrícia bola zabezpečená už len enterálnou formou. Anemický syndróm sme korigovali krvnými prevodmi. Vzhľadom na postihnutie pečene bola pacientke počas hospitalizácie podávaná hepatoprotektívna liečba zahájená už na KAIM, pri ktorej došlo k parciálnemu poklesu aminotransferáz (tab. 2). Stav pacientky sa po celkovo 28 dňoch hospitalizácie výrazne zlepšil a pacientku sme prepustili do ambulantnej starostlivosti.

Diskusia

Exacerbácia Stillovej choroby u pacientky bola vyvolaná neznámou príčinou. Infekčná etiológia nebola potvrdená, po diagnostikovaní kritérií SIRS a odobratí hemokultúr boli preventívne podávané širokospektrálne antibiotiká. Laboratórne boli po príjme potvrdené známky rozvíjajúceho sa hepatálneho zlyhávania, enormne zvýšené aktivity hepatálnych enzýmov, pokles koagulačných parametrov a opakovaný sklon k hypoglykémiam. Stav si vyžiadal podávanie pulznej kortikoterapie, hepatoprotektív, umelú pľúcnu ventiláciu, mimotelovú eliminačnú liečbu a špecifickú nutričnú podporu, formou enterálnej a parenterálnej výživy rešpektujúcej hepatálne poškodenie.

Dôležitou úlohou terapie je ovplyvnenie SIRS, kedy dochádza k masívnej produkcii voľných kyslíkových radikálov (ROS) v organizme. ROS zohrávajú významnú amplifikačnú úlohu v úvodnej fáze SIRS. Ak produkcia reaktívnych foriem kyslíka ďaleko prevyšuje ich vychytávanie, dochádza k spúšťaniu kaskád imunitných a zápalových dejov, súborne označovaných ako oxidačný stres [6]. Ovplyvniť oxidačný stres podporou metabolizmu závisí aj od optimálne zvolenej parenterálnej a enterálnej výživy, ktorá okrem iného aktivuje antioxidačnú obranu organizmu a doplní chýbajúce

stopové prvky a spotrebované zásoby vitamínov. U kriticky chorých sa zdá byť obzvlášť prospešná parenterálna suplementácia selénu ako aj prekuzorov glutatiónu. Štúdie potvrdili zlepšenie klinických výsledkov pri podávaní selénu, ktorý napomáhal zlepšovaniu zdravotného stavu pacientov pri infekciách a orgánovom zlyhaní. Pri podávaní glutamínu v rozsiahlych štúdiách sa potvrdilo zníženie infekčných komplikácií u kriticky chorých pacientov [7].

Pečeň je ústredným metabolickým orgánom s exkrečnými, metabolickými, detoxikačnými, hematologickými, hemostatickými funkciami a radou ďalších dôležitých funkcií. V začiatočnej fáze hepatálneho zlyhania dochádza k vyššiemu metabolizmu proteínov s prevahou katabolických dejov. Ústrednú úlohu pri týchto dejoch zohrávajú katabolické hormóny. Prvotným inzultom zodpovedným za nasledujúce deje sú noxy rôznej etiológie, ako napríklad infekcie, sepsa a vplyv rôznych toxínov. Dochádza k akcelerácii zápalovej odpovede, do obehu sa uvoľňujú mediátory zápalu a zvyšuje sa tvorba proteínov akútnej fázy. Táto fáza je do určitej miery reverzibilná, za podmienky dostatočných kompenzačných mechanizmov, zahrňajúcich imunitné, metabolické a terapeutické faktory.

Umelá výživa u kriticky chorých je limitovaná funkčnosťou gastrointestinálneho traktu z hľadiska voľby výživy enterálnej, doplnkovej alebo parenterálnej. Druhou limitáciou je utilizácia živín v pečeni, ktorá pri hepatálnom zlyhávaní môže byť nedostatočná a je nevyhnutné podávať synteticky pripravené základné stavebné makromolekuly živín. Orgánovo špecifická výživa pri hepatálnom zlyhávaní je založená na využití anaplerotických (AS) a špecificky nutričných substrátov (ŠS) [8]. Narušením jednej čiastkovej reakcie dochádza k zastaveniu celej metabolickej cesty. Suplementáciou AS sa doplní substrát za prerušenou reakciou, čím môže sled biochemických reakcií ďalej pokračovať. Suplementácia ŠS za daných podmienok je výhodná z hľadiska lepšej utilizácie a zlepšenia energetickej rovnováhy [8]. Výhodnou alternatívou sa zdá byť výživa doplnková. Dôležitým nutrientom, ktorý by mala obsahovať enterálna výživa, sú mastné kyseliny. Predstavujú významný zdroj pre regeneráciu hepatocytov. Vhodné je aj podávanie prebiotík (laktulózy), respektive probiotík v rámci enterálnej výživy, z dôvodu zvrátenia hnilobných procesov v čreve na kvasné. Pri parenterálnej výžive je vhodné pacientom podávať 40–45 % rozvetvených aminokyselín, ako aj vyššie množstvá esenciálnych aminokyselín s výnimkou aromatických, ktorých príjem je potrebné redukovať [9].

Za priaznivých podmienok dochádza k obnoveniu fyziologických funkcií pečeňového tkaniva a dochádza k reparácii narušených pečeňových lalôčikov ad integrum. Pri nadmernom pôsobení noxy, môže táto fáza prekročiť kompenzačné limity a stať sa ireverzibilnou a vyústiť do fulminantného hepatálneho zlyhania [8]. Dôležitú úlohu v tomto procese zohrávajú aktivované Kupfferove bunky. Sú to mononukleárne makrofágy, ktoré pod vplyvom noxy uvoľňujú enormné množstvo voľných radikálov (ROS) do svojho okolia. Tvorba ROS za fyziologických podmienok prebieha kontinuálne v rámci aeróbneho metabolizmu. ROS slúžia podobne ako aj v iných tkanivách na elimináciu baktérií a sú spúšťačom uvoľňovania signálnych molekúl. Tkanivá sú voči ROS chránené antioxidačným systémom. ROS v pečeni účinkujú na všetky dôležité biomolekuly, proteíny, lipidy a nukleové kyseliny. Poškodenie bunkových membrán hepatocytov je spôsobené ich lipoperoxidáciou ako aj sekundárnym poškodením vznikajúcimi produktami lipoperoxidácie. ROS sú zodpovedné za zvýšenie aktivity fosfolipázy-A2, čím dochádza k štepeniu fosfolipidov bunkových membrán a poruchám intracelulárnej homeostázy Ca^{2+} [9].

Peroxidované lipidy sú následne rozložené na aldehydy, ketóny, alkoholy a laktóny, ktoré samotné majú toxické účinky. Dôležité je spomenúť rozklad hemoglobínu v pečeni, ktorý môže proces lipoperoxidácie iniciovať. Uvoľnená skupina hému, ktorá obsahuje ióny železa má katalytický účinok na tvorbu peroxidov. Podľa laboratórnych výsledkov dochádzalo k zvýšenej dekompozícii hemoglobínu, čím sa zvýšil aj obsah hemoproteínov, respektive iónov kovov, ktoré proces peroxidácie organických substrátov urýchľovali. Zvýšenie aminotransferáz, bilirubínu, zníženie albumínov potvrdzuje poškodenie pečene. Okrem klinických príznakov poškodenia obličiek to potvrdzujú aj zvýšenia kreatinínu a močoviny v krvi. Pritom nie je možné vylúčiť ani postihnutie svalov, nakoľko aminotransferázy sú súčasťou každej bunky včítane svalov a na zvýšení hodnôt sa mohli podieľať aj svalové aminotransferázy. Podobne kreatinín vzniká vo svaloch odbúravaním kreatínfosfátu, ale na zvýšení kreatinínu v sére môže pôsobiť znížené vylučovanie obličkami. Spomínané látky sa ale dialýzou odstraňujú.

Za fyziologických okolností je glykogén uskladnený v pečeni ľahko mobilizovateľný, a je preto dôležitým zdrojom glukózy pre ostatné orgánové systémy. Narušením funkcie glykogenfosforylázy dochádza k poklesu jeho premeny na D-glukózu. Pacienti s hepatálnym zlyhávaním sú preto náchylní na hypoglykémiu. Dochádza k poklesu proteosyntézy, čo sa prejavuje znížením hladín celkových bielkovín a dochádza najmä k poklesu plazmatického albumínu, prealbumínu, transferínu, transkortínu a retinol-viažúceho proteínu [9]. U pacientky z kazuistiky boli zaznamenané znížené hladiny celkových bielkovín a najmä albumínu v krvi počas hospitalizácie na I. KAIM. Ďalej dochádza pri hepatálnom zlyhaní k výraznému zvýšeniu plazmatickej koncentrácie aromatických kyselín fenylalanínu, tyrozínu a tryptofánu, ktoré sú za fyziologických podmienok metabolizované prevažne v pečeni. Narušenie metabolických ciest vedie k ich akumulácii v krvi. Narušenie ureosyntézy spôsobuje vzostup amoniaku v krvi, ktorý sa v pokročilých štádiách ochorenia podieľa na rozvoji hepatálnej encefalopatie [8].

Esenciálne aminokyseliny s rozvetveným uhľovodíkovým reťazcom valín, leucín a izoleucín sú len nepatrne metabolizované v pečeni. Sú významným zdrojom energie pre svaly, ktoré ich aktívne vychytávajú

z krvi a môžu nahrádzať energiu pochádzajúcu z glukózy. Ich aminoskupiny tlmia acidózu tým, že sa premenia na NH^{4+} a sú vylučované obličkami. Podávanie spomínaných aminokyselín do výživy preto mohlo pozitívne vplývať aj na regeneráciu svalov a kompenzovanie acidózy, ktorá sa prejavila v danom prípade aj zvýšením laktátu v krvi. Podávanie glutamínu, ktorého syntéza v organizme vyžaduje ATP tiež mohlo významne pozitívne vplývať na regeneračné procesy, pretože glutamín je východiskovou látkou pri syntéze pyrimidínových a purínových báz, ktoré sú základnými bázami nukleových kyselín, ale aj energeticky bohatých trifosfátov ako ATP, GTP, UDP atď. Tie majú významnú úlohu okrem syntetických dejov i v regulácii metabolizmu prenosom signálov a usmerňovaním metabolizmu, napr. druhý poslovia (cAMP, cGMP). Prebytočný glutamín nevyužitý k syntéze spomenutých báz môže poskytnúť amoniak, ktorý pribratím H^+ kompenzuje acidózu, podobne ako amoniak z rozvetvených aminokyselín.

Selén sa uplatňuje ako kofaktor enzýmu glutatión peroxidázy, ktorý patrí do skupiny enzýmov vykazujúcich peroxidázovú aktivitu [10]. Jej hlavnou úlohou je ochrana bunkových štruktúr pred oxidačným poškodením. Biochemická funkcia GPx je redukovať lipidové peroxidy na im korešpondujúce alkoholy a redukovať voľný peroxid vodíka na vodu. Substrátom pre túto reakciu je glutatión (GSH) [11, 12]. Suplementáciou glutamínu alebo jeho prekurzorov sa zvyšuje efektivita účinku GPx. Regenerácia oxidovanej formy glutatiónu (GSSG) na GSH je katalyzovaná glutatión reduktázou (GR). Priame meranie dynamiky antioxidačných enzýmov u pacientky z kazuistiky poukazuje na ich aktiváciu suplementovanými mikronutrientmi. U pacientky z kazuistiky dochádza aj k zvýšeniu aktivity GR, čo je možné vysvetliť súčasným podávaním prekurzorov syntézy GSH. Zvýšená hodnota GPx v šiesty deň od aplikácie prvej dávky selénu a glutamínu svedčí o zvýšenej aktivite antioxidačného systému. Zabudovanie selénu do selenoproteínu, akým je GPx, a zvýšenie jej aktivity má význam v zabránení monoredukcie peroxidov na .OH, čo nie je enzymatická reakcia, ale môže byť značne urýchlená prítomnosťou kovov, napr. z porušených molekúl, kde sa ióny kovov často nachádzajú v ich aktívnom centre. Metaloproteínom je aj superoxid dizmutáza (SOD), ktorá je ďalším dôležitým antioxidačným enzýmom. Jeho funkciou je rýchla dizmutácia $O_{2.}^-$ na kyslík a peroxid vodíka. V prostetickej skupine tohto enzýmu sa nachádza meď, zinok alebo horčík. Pred zahájením liečby bola aktivita tohto enzýmu znížená. Počas liečby došlo k zvýšeniu aktivity aj u tohto enzýmu. Biochemické parametre iných metaloproteínov sledované neboli. Tento jav bol sprevádzaný poklesom plazmatickej hladiny CRP, čo je známkou efektívnej terapie systémovej zápalovej odpovede. Pomer neutrofilov a lymfocytov má priaznivú klesajúcu tendenciu aj napriek stúpajúcej hodnote leukocytov. Pomer neutrofilov a lymfocytov jednoducho a spoľahlivo vystihuje mieru závažnosti oxidačného stresu a systémového zápalu. Predošlé štúdie popisujú súvislosť medzi závažnosťou klinického stavu a mierou neutrofílie a lymfocytopénie [13]. Výsledky tejto kazuistiky potvrdzujú priaznivý vplyv suplementácie selénu na SIRS a obnovu hepatálnej funkcie. Zvýšenie aktivít antioxidačných enzýmov u pacientky z kazuistiky poukazuje na ich aktiváciu suplementovanými mikronutrientmi.

Záver

Stillova choroba je ochorenie neznámej etiológie, vyžadujúce dlhodobú imunosupresívnu liečbu. Akútna exacerbácia ochorenia môže viesť k rôznym orgánovým dysfunkciám a z nich vyplývajúcim klinickým prejavom. Jedným z najzávažnejších je akútna hepatálna insuficiencia, ktorá pri neadekvátnej terapii môže viesť k fulminantnému zlyhaniu pečene. Liečba je vysoko komplexná, vyžadujúca multidisciplinárny prístup. Pochopenie patofyziologických pochodov pri stavoch spojených so zlyhávaním pečene má kľúčovú úlohu pri výbere orgánovo špecifickej nutrície, ktorá adekvátne reaguje na metabolické potreby organizmu. Využitie podpornej antioxidačnej terapie formou selénových preparátov a prekurzorov glutatiónu sa zdá byť výhodné pri ovplyvnení systémovej zápalovej odpovede a zlepšení klinického stavu.

Použité skratky:

AS	– anaplerotický nutričný substrát
ATB	– antibiotiká
ATP	– adenozíntrifosfát
Ci-Ca	– modul citrátový – kalciový modul
cAMP	– cyklický adenozínmonofosfát
cGMP	– cyklický guanozínmonofosát
CVVHD	– Continuous venovenous hemodiafiltration
CRP	– C-reaktívny proteín
DIC	– disseminovaná intravaskulárna koagulopatia
GCS	– Glasgow coma scale
GPx	– glutatión peroxidáza
GR	– Glutatión reduktáza
GSH	– redukovaná forma glutatiónu
GSSG	– oxidovaná forma glutatiónu
GTP	– guanozíntrifosfát
MEL	– mimotelová eliminačná liečba
$O_{2.}^-$	– superoxid
.OH	– hydroxylový radikál
PCT	– prokalcitonín
ROS	– reaktívne formy kyslíka
SIRS	– Systemic Inflammatory Response Syndrome
SOD	– superoxid dismutáza
ŠS	– špecifický nutričný substrát
UDP	– uridíntrifosfát
UVP	– umelá ventilácia pľúc

Literatura

1. **Bywaters, E. G. L.** Still´s disease in the adult. *Ann. Rheum. Dis.*, 1971, 30, p. 121–132.
2. **Keher, J. P.** Free radicals as mediators of tissue injury and disease. *Critical rev. Toxicol.*, 1993, 23, p. 21–48.
3. **Geoghegan, M., Mcauley, D., Eaton, S., Powel-Tuck, J.** Selenium in critical illness. *Critical Care*, 2006, 12, p. 136–141.
4. **Kočan, L., Firment, J., Šimonová, J., Vašková, J., Guzy, J.** Suplementácia selénu u pacientov s ťažkou akútnou pankreatitídou. *Rozhl. v Chirurgii*, 2010, 89, 1, p. 518–521.
5. **Gartner, R., Albrich, W., Angustwurm, M. W.** The effect of selenium supplementation on the outcome of patients with severe systemic inflammation, burn and trauma. *Biofactors*, 2001, 14, p. 199–204.
6. **Imlay, J.** Pathways of oxidative damage. *Annual Review of Microbiology*, 2003, 57, p. 395–418.
7. **Andrews, P. J.** Selenium and glutamine supplements: where are we heading? A critical care perspective. *Current Opinion in Clinical Nutrition and Metabolic Care*, 2010, 13, p. 192–197.
8. **Zadák, Z.** *Umělá výživa při poškození jater.* In Zadák, Z. Výživa v intenzivní péči. Grada Publishing a.s. : Praha, 2008, p. 359–365, ISBN: 978-80-247-2844-5.
9. **Turecký, L.** *Poškodenie pečene voľnými radikálmi.* In Ďuračková, Z. Voľné radikály a antioxidanty v medicíne II. Slovac Academic Press s.r.o. 1999, p. 210-218,ISBN: 80-88908-46-9.
10. **Berger, M., Chiolero, R.** Antioxidant supplementation in sepsis and systematic inflammatory response syndrome. *Critical Care Medicine*, 2007, 35 p. 584–590.
11. **Forceville, X.** Seleno-enzymes and seleno-compounds: the two faces of selenium. *Critical Care,* 2006, 10, p. 180.
12. **Muller, F. L., Lustgarten, M. S., Jang, Y., Richardson, A., Van Remmen, H.** Trends in oxidative aging theories. *Free Radic. Biol. Med.*, 2007, 43, p. 477–503.
13. **Záhorec, R.** Pomer neutrofilov a lymfocytov – rýchly a jednoduchý ukazovateľ systémového zápalu a stresu u pacientov v kritických stavoch. *Bratislavské Lekárske Listy*, 2001, 102, p. 5–14.

Došlo dne 11. 8. 2011.
Přijato dne 11. 11. 2011.

Adresa pro koresponddenci:
MUDr. Ladislav Kočan
I. klinika anestéziológie a intenzívnej medicíny
Univerzitná nemocnica L. Pasteura
Trieda SNP 1
041 90 Košice
Slovenská republika
e-mail: kocanladislav@yahoo.com

2.5.3 Závery štúdie SE-AOX

Deficit selénu u kriticky chorých pacientov sme potvrdili aj našimi meraniami prostredníctvom atómovej absorbčnej spektrofotometrie. Výsledky týchto meraní boli prezentované na postgraduálnom kurze Sepsy a MODS v Ostrave 2010.

- *Doterajšie skúsenosti liečby Selénom u kriticky chorých / J. Firment,* ***L. Kočan,*** *J. Kubálková. [Up to now experiences in selenium treatment in criticaly ill]. In: 12. postgraduální kurz Sepse a MODS : 26.-29. leden 2010. - Ostrava, 2010. - ISBN 9788025480397. - S. [1-3].*

Vyhodnotením výsledkov štúdie Se-AOX neboli zistené rozdiely v mortalite, výskyte renálneho zlyhania a respiračných funkcií medzi jednotlivými skupinami a podskupinami ($p>0,05$). V obidvoch skupinách, selén versus placebo, bolo preukázané, že pacienti s vyšším skóre APACHE II a SOFA mali preukázateľne vyššiu úmrtnosť ($p<0,05$).
Medzi skupinami a podskupinami neboli zistené významné rozdiely dynamických zmien sledovaných biochemických parametrov (CRP, laktát, celkové bielkoviny, albumín, urea) ($p>0,05$), hematologických a koagulačných parametrov (počet leukocytov a ich populácii, trombocytov, fibrinogénu) ($p>0,05$) počas terapie. Zmeny aktivít antioxidačných enzýmov v sledovaných podskupinách potvrdili nárast aktívít GPx u pacientov, ktorým bol suplementovaný Se ($p<0,05$). Pacientom v podskupine OI < 200, ktorým sa suplementoval selén, sme zaznamenali signifikantné zlepšenie oxygenačného indexu na konci terapie selénom ($p<0,05$), ale bez ďalšieho klinicky významnej korelácie. Výsledky štúdie nepreukázali výrazný klinický benefit u pacientov, ktorým sa suplementoval selén oproti placebo-skupine, tento rozdiel nebol zaznamenaný ani medzi jednotlivými podskupinami.
Zároveň výsledky štúdie Se-AOX korelovali s výsledkami ďalších, v tom čase aktuálnych prospektívnych randomizovaných klinických štúdií, akými bola štúdia SIGNET Andrews a kol., 2011 (4), ako aj štúdie Valenta a kol., 2011 (3). Na základe v tej dobe zistených výsledkov boli doplnené odporúčania pre liečbu sepsy mikroprvkami zhrnuté v Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Medicine, v ktorých sa podávanie selénu pri liečbe sepsy a septického šoku ďalej neodporúča (silné odporúčanie, stredná kvalita dôkazov) (21).

Závery štúdie Se-AOX boli publikované vo: Wiener Klinische Wochenschrift - ISSN 0043-5325, IF 1,3, Q2 a Clinical Biochemistry - ISSN 0009-9120, IF 2,3, Q2.

original article

Wien Klin Wochenschr (2013) 125:316–325
DOI 10.1007/s00508-013-0371-x

Wiener klinische Wochenschrift
The Central European Journal of Medicine

Restoration of antioxidant enzymes in the therapeutic use of selenium in septic patients

Vašková Janka, Kočan Ladislav, Firment Jozef, Vaško Ladislav

Received: 3 December 2012 / Accepted: 10 April 2013 / Published online: 4 May 2013

Summary A prospective observational study of parenteral selenium supplementation started in January 2008 which included 72 septic patients with APACHE II scores ranging from 19 to 40 after admission.

Patients were divided into two major groups: one with a continual infusion of sodium selenite at 750 μg/24 h for 6 days and a placebo group followed by subgroups according to the presence or absence of surgical procedure. Routine biochemical and hematological parameters were determined continuously. Sequential Organ Failure Assessment (SOFA) scores were calculated in two-day intervals.

Patients who died had a higher Acute Physiology and Chronic Health Evaluation (APACHE) II score, lower albumin on the 3rd days of therapy and higher C-reactive protein (CRP) on the 6th days of therapy. Statistically, there was no significant difference in the comparison of CRP, fibrinogen, albumin, plasma proteins, or neutrophil to lymphocyte counts during the 6 days in all subgroups. There was a significant difference in the comparison of leukocytes on the 6th day of therapy. Glutathione peroxidase and glutathione reductase activity was increased in selenium subgroups with negative correlation in placebo subgroups during the therapy. A downward trend in SOD activity, more appreciable in selenium groups, seemed to be a reflection of lower superoxide radical production. This is biased more as a result of GPx activity restoration, preventing further peroxidation of organic substrates and cyclic formation of other radicals, than actual attenuation of their production.

Selenium substitution increased selenium dependent antioxidant enzyme activity and, in comparing mortality in groups, we found a 16.7 % decrease in mortality in favor of supplementation with selenium.

Keywords: Oxidative stress, Sepsis, Selenium, Artificial nutrition

L. Kočan, PhD (✉) · Assoc. Prof. J. Firment, PhD
1st Clinic of Anaesthesiology and Intensive Care Medicine,
Louis Pasteur University Hospital in Košice, Tr. SNP 1,
04066 Košice, Slovak Republic
e-mail: kocanladislav@yahoo.com

J. Vašková, PhD · Assoc. Prof. L. Vaško, PhD
Institute of Medical and Clinical Biochemistry,
Faculty of Medicine, Pavol Jozef Šafárik University in Košice,
Košice, Slovak Republic

Wiederherstellung der Aktivität von antioxidativen Enzymen durch Selen bei septischen Patienten

Zusammenfassung Eine prospektive Beobachtungsstudie der Wirkung einer parenteralen Gabe von Selen wurde im Jänner 2008 begonnen. Es wurden 72 Patienten mit Sepsis und einem APACHE II Score zwischen 19 und 40 bei Aufnahme in die Studie aufgenommen.

Die Patienten wurden in 2 große Gruppen eingeteilt: einer Gruppe wurde eine kontinuierliche Infusion von 750 μg Na-Selenit/24 h 6 Tage lang verabreicht – der anderen Placebo. Es wurden dann Subgruppen gebildet, je nachdem, ob operative Maßnahmen gesetzt wurden. Routine biochemische und hämatologische Parameter wurden kontinuierlich erhoben. Die SOFA Scores wurden alle 2 Tagen errechnet.

Die verstorbenen Patienten hatten einen höheren APACHE II Score, sowie ein niedrigeres Albumin am 3. Tag und ein höheres CRP am 6. Tag. Statistisch bestand kein signifikanter Unterschied im Vergleich des CRP, des Fibrinogens, des Albumins, der Plasmaproteine bzw. der Neutrophilen und Lymphozyten Zahl aller Subgrup-pen während der 6 Beobachtungstage. Die Leukozyten-zahl war am 6. Tag statistisch signifikant unterschied-lich. Die Aktivität der Glutathionperoxidase (GPx) und der Glutathionreduktase war in den Selen-Subgruppen

während der Therapie erhöht mit einer negativen Korrelation in den Placebo-Untergruppen.

Ein Abwärtstrend der SOD Aktivität, der in den Selen-Untergruppen deutlicher war, schien eine Folge einer geringeren Produktion von Superoxyd Radikalen zu sein. Wahrscheinlich ist das aber eher auf eine Wiederherstellung der GPx Aktivität (die vor einer weiteren Peroxydation der organischen Substrate und einer zyklischen Bildung von anderen Radikalen schützt) als auf eine tatsächliche Verminderung ihrer Produktion zurückzuführen.

Substitution mit Selen erhöhte die Aktivität der Selenabhängigen antioxidativen Enzyme. Ein Vergleich der Mortalität beider Gruppen ergab eine um 16,7 % niedrigere Sterberate der mit Selen behandelten Patienten.

Schlüsselwörter: Oxidativer Stress, Sepsis, Selen, Künstliche Ernährung

Introduction

Many serious medical problems originate from conditions of oxidative stress, that are amplified in the immune response. Its activation role in the pathogenesis of syndromes such as systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, septic shock, and in various disease states such as acute pancreatitis is well known, and also in other diseases encountered in the intensive care unit (ICU) [16]. Treatment of such diseases is very complex, involving causal treatment, support, and assistance. An individual approach is used from one patient to another. Oxidative stress can be influenced by choosing the optimal enteral and parenteral nutrition, which can, among other things, activate the body's antioxidant defense and add missing trace elements and vitamins which have been consumed. The current multicenter study confirmed the importance of supplementation of these nutrients to support antioxidant capacity of the organism. Decreased plasma levels of antioxidants are caused by increased antioxidant consumption in plasma and tissues, necessary for the destruction of free radicals. The decrease is considerable in uric acid, protein and thiol groups, unconjugated bilirubin, ascorbic acid, alpha-tocopherol, beta-carotene, lycopene, antioxidant enzymes, glutathione, and trace elements such as selenium and zinc [7].

Absorption of dietary selenium in the inorganic form is limited to about 50 %, while the organic form is readily taken up, with up to 100 % of organic dietary selenium being absorbed. After resorption in the small intestine, it is transported in the blood bond to selenoprotein P and its plasma level is sensitive to psychological and physical stress. Also selenoprotein P represents a stock potential in which over 50 % of plasma selenium is stored. The level of this protein is a determinant of the total selenium status [28], which is, however, insufficient in acute conditions. The safe oral intake for healthy people with normal renal function is 50–200 μg/day [15]. Selenium balance in the body is regulated by the kidneys. Adults excrete 50–60 % of selenium in the urine. Selenium in the body forms part of some proteins, most of which play an important role in protecting cell structures from oxidative damage. The most important macromolecule of this group is an enzyme, glutathione peroxidase [28], although selenium is also found in the active sites of two other important enzymes in the metabolism of thyroid hormones: jodotyronine-5-deiodinase, and that of nucleotides, thioredoxin reductase.

Growing clinical studies in patients with SIRS, sepsis, burns, trauma, Acute respiratory distress syndrome (ARDS), and pancreatitis suggest that selenium supplementation may be advantageous. Administration of selenium-containing supplements appears to be beneficial in terms of reducing mortality [13]. Ingestion of 4 mg of sodium selenite pentahydrate is considered nontoxic in healthy humans [12]. Critically ill patients have lower levels of selenium in their blood compared with the healthy population, so are much more able to tolerate the increased supply of selenium without showing signs of toxicity [2]. An intake of 1,000 μg/day of sodium selenite pentahydrate administered intravenously was tolerated well by patients [13]. Several clinical studies have been devoted to the issue of selenium antioxidant therapy in selected pathologies [2, 12, 15, 18]. Individual studies addressing this issue are varied in respect to the dose and duration of selenium administration, the form of administration (bolus vs. continuous administration) and controversial results. The reality clarified for us the relationship between the dose and the clinical response. On this basis, we developed a methodology of the study named Se-AOX in early 2008. From previous studies and meta-analyses, it has been concluded that a safe interval of administration of sodium selenite pentahydrate falls in the range of 600–800 μg per day. However, other studies were also published later in which high doses of sodium selenite pentahydrate were administered on the first day at 1,000 μg/day and from days 2 to 14 at 1,500 μg/day in an attempt to exploit the prooxidant properties of selenium in the initial stage of oxidative stress on neu-tralization of microbial pathogens and the next phase of its antioxidant properties after installation into proteins. The results of the study demonstrated no statistically sig-nificant reduction in mortality in any groups [1, 29].

In our prospective observational study, Se-AOX, we studied the dynamic changes of antioxidant enzymes, the development of biochemical and hematological parameters of sepsis, and 28-day mortality in critically ill patients with APACHE II (median 24, min 19, max 40) and SOFA (median 9, min 4, max 14), treated with sodium selenite pentahydrate at a dose of 750 μg/day as a continuous infusion for 6 days.

Patients, materials and methods

The Ist Clinic of Anaesthesiology and Intensive Care Medicine, University Hospital of Louis Pasteur in Košice, Slovak Republic started a prospective observational study, Se-AOX, in 2008. The study looked at the effects of

parenteral selenium supplementation in septic patients and subsequent monitoring of dynamic changes in the activities of selected antioxidant enzymes and the development of clinical status. A protocol has been developed, on the basis of which the University ethics committee approved the study 109/2011.

As the study was observational, therapeutic procedures were performed in our clinic routinely and most of the patients had altered consciousness, the patients' special informed consent was not requested. The Se-AOX study enrolled 72 patients with an APACHE II score between 19 and 24 points at the ages from 23 to 79 years, who developed sepsis, severe sepsis or septic shock during hospitalization as defined by the International Sepsis Definitions Conference [19]. A SOFA score was calculated in the time periods of 1–2 days (T1), 3–4 days (T2) and 5–6 days (T3) according to an internet calculator (http://www.sfar.org/scores2/sofa2.php).

The Se-group consisted of 35 patients who received selenium supplementation during hospitalization in the form of sodium selenite pentahydrate at 750 μg/day for 6 days immediately after admission to our department (1,000 μg of sodium selenite pentahydrate =333 μg of selenium) (Selenase, Vivax; selenium hereinafter). The placebo group consisted of 37 patients who received con-tinuous saline NaCl 50 ml/day for 6 days as a continuous infusion (excluding additional infusion therapy). Patients were further divided into four subgroups according to whether they received surgery. The A1 subgroup con-sisted of 23 patients who received selenium supplements postoperatively. Subgroup A2 consisted of 21 patients treated with placebo during the postoperative period. The B1 subgroup consisted of 12 patients who received selenium supplements without undergoing surgery. Finally, the B2 group consisted of 16 patients treated with placebo without undergoing surgery (Fig. 1).

The nutritional strategy (enteral vs. parenteral, quali-tative and quantitative characteristics of the diet) was dependent on the patient's diagnosis and nutritional sta-tus, as well as the standard therapeutic approaches to the disease.

Food administration was cyclical, to stimulate the diurnal rhythm. The daily dosage of glucose sugar was from 1.5 g/kg up to 5.5 g/kg. The total daily supply of amino acids ranged from 0.8 to 2.5 g/kg (0.5–0.75 g/kg for renal and liver injury, 0.7–1.0 g/kg in stable patients, 1.0–1.5 g/kg in a poor state of nutrition and catabolism, and 1.5–2.0 g/kg for severe malnutrition). Substitution of fat was performed after the acute stage of the dis-ease subsided and was administered at 0.5 g/kg/day. All groups were also administered antioxidants parenterally: 1,000 mg/24 h vitamin C, 30 mg/24 h vitamin E, 100 mg/24 h thiamine, 100 mg/24 h pyridoxine, and 1,000 μg/24 h cobalamin. The determination of biochemical and hematological parameters of blood samples formed part of the routine diagnostic methods. Data collection for the Se-AOX study was performed at two-day inter-vals: T1 (1st–2nd day), T2 (3rd–4th day) and T3 (5–6th day). Kinetic methods for estimating the activities of glutathione peroxidase (GPx, E.C. 1.11.1.9) and glutathione reductase (GR, E.C.1.6.4.2) were performed using a kit (Sigma-Aldrich, Germany) and that of superoxide dismutase (SOD, E.C. 1.15.1.1) by means of the SOD-Assay Kit-WST (Fluka, Japan) following the user manual provided. A control group of 30 healthy volunteers was used for comparison with the development activity of antioxidant enzymes in the critically ill. Statistical significance was determined by T-test (calculated from mean, SD) baseline vs. 6–7th day (T3) and Pearson's Chi-square selenium vs. non-selenium group from baseline to 6–7th day. Differences were considered significant at $p<0.05$. Possible benefit from the treatment was calculated by number needed to treat (NNT) test.

Results

There was a decrease in the median SOFA scores of surgical patients, in both selenium and placebo groups (A1 and A2), and nonsurgical patients (B1) at time periods T1, T2, and T3 (Table 1). This suggested a reduction in the number of organ failures and an improvement in health. There was a decrease in median CRP levels in all groups followed (Table 2). There were no significant differences ($p>0.05$) between the observed biochemical parameters shown in Table 2. In group A1—surgical patients receiving selenium supplements—median platelet values were increased (Table 3), suggesting both the incorporation of selenium and the mitigation of the systemic inflammatory response. Through the course of treatment, there was a decreasing tendency for the median values of white blood cells in both groups of nonsurgical patients (B1 and B2) which translates to a significant change in values between groups A and B at T3. Patients in both the A subgroups had a decreased 28-day mortality of 16.7 % in comparison to group B (Table 4). The reduction was not statistically significant ($p>0.05$), although the figure is not at all negligible. Using NNT, we can state with 95 % certainty that the absolute reduction in 28-day mortality was 16.6 % and that every 7th patient benefits from the treatment.

High SOD activity values were observed during this period in surgical patients (Table 5). The median of the measured values has a slightly decreasing trend in groups A1 and A2; however, unlike in groups without selenium administration, these values returned toward the median of healthy volunteers. The activity of GPx was increased in the interval T1–T3 in patients supplemented with selenium from groups A1 and B1, but was pronounced in the group in surgical patients, A1. It is in these patients that the median values were closest to the median values of healthy volunteers, albeit without reaching them. Similarly, an upward trend could be observed in group B1. A much weaker upward trend could be observed in the group with surgery without selenium supplementation (A2), and in group B2 (significance at $P<0.05$ in comparison to B1). Median values of GR in A1 are very close to the median values of healthy volunteers, although there is a slight downward trend when compared with baseline

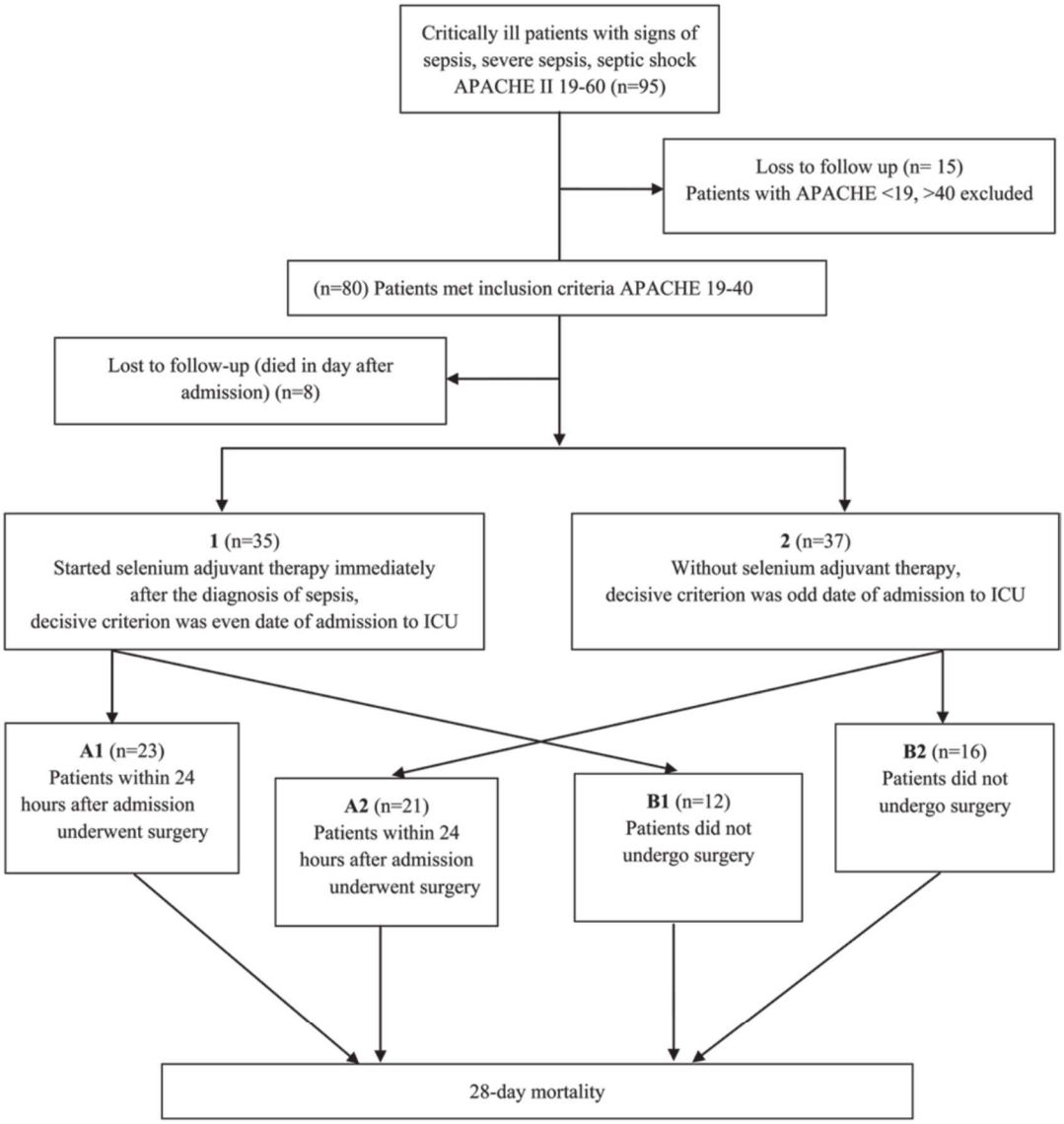


Fig. 1 Flow diagram of prospective observational study Se-AOX in septic patients in groups with selenium adjuvant therapy *1* and placebo *2*, with surgical (A) and without surgical performance (B)

values during T2-T3 (only for median values). The case with A2 is similar. The differences in the changes in GR activity are generally available for monitoring when comparing groups A and B. The median B values are lower, although the differences between the groups are not statistically significant. This may be a result not only of fewer patients, as they were divided into four groups, but also of the range of values measured and thus higher deviations from the mean.

Discussion

Normally, there is homeostasis between the production of reactive oxygen radicals and their removal by endogenous antioxidant scavengers, nonenzymatic antioxidant supplementation, and diet. Oxidative stress results from an impaired balance of excessive reactive oxygen species (ROS) production, including superoxide, hydrogen peroxide and hydroxyl radicals and/or inadequate antioxidant defense. Severe infections and inflammatory

Table 1. Demographic characteristics of the patients in groups with selenium adjuvant therapy A1-with surgical and B1-without surgical performance and placebo groups A2-with surgical and B2-without surgical performance

Characteristics	Median (min-max)		Median (min-max)	
	A1	A2	B1	B2
N	23	21	12	16
Age	(23–78) 53	(30–77) 60	(28–68) 47	(40–79) 53
Gender (M/F)	17/6	14/7	6/6	12/4
APACHE score	(19–39) 24	(19–38) 23	(20–40) 24	(19–37) 30
SOFA score T1	(4–14) 10	(6–13) 9	(3–14) 8	(4–14) 9
SOFA score T2	(1–14) 9	(4–17) 8	(1–14) 8	(2–12) 9
SOFA score T3	(1–15) 8	(2–19) 6	(2–14) 6	(3–14) 9
Sepsis	4	5	3	5
Severe sepsis	5	8	5	4
Septic shock	14	8	4	7
Disseminated intravascular coagulation	4	1	1	2
Bronchopneumonia	8	7	6	8
ALI	8	11	2	3
ARDS	11	1	5	6
Nosocomial infections	11	6	4	9
Dialysis	13	6	5	5
Admission diagnosis				
Respiratory failure	21	12	7	14
Coma (status after CPR)	–	2	2	3
Cardiac disease	1	–	1	3
Hemato-oncological disease	–	–	2	4
Peritonitis	8	7	–	–
Pancreatitis	10	2	–	–
State after neurosurgical procedure	2	–	–	–
State after cardiosurgical procedure	1	1	–	–
State after urological procedure	1	3	–	–
Trauma	2	1	–	–
Hemorrhagic shock	3	2	–	–

Table 2. Comparison of the levels of selected parameters in plasma over T1–T3 day interval

Parameter (reference values)	Time interval	Median (min-max)		*P*	Median (min-max)		*P*	*P* (A vs. B)
		A1	A2		B1	B2		
Total plasma protein (64.0–83.0 g/l)	T1	49.0 (36.0–62.2)	49.0 (39.8–63.4)	0.09	50.5 (36.0–77.9)	46.8 (27.4–78.5)	0.90	0.22
	T2	50.6 (34.0–62.5)	51.2 (42.2–70.0)	0.96	50.0 (31.5–68.0)	50.1 (31.5–73.4)	0.40	0.56
	T3	53.6 (36.3–60.3)	50.0 (34.1–65.0)	0.23	49.9 (28.1–73.8)	49.8 (34.6–70.4)	0.99	0.37
Albumin (35.0–52.0 g/l)	T1	22.8 (13.4–39.0)	25.3 (20.2–37.9)	0.23	25.3 (14.5–50.0)	25.6 (34.6–43.7)	0.77	0.19
	T2	19.7 (12.3–32.3)	27.0 (21.6–32.1)	0.09	22.9 (14.9–39.4)	20.7 (14.3–37.2)	0.17	0.45
	T3	21.8 (12.9–39.2)	26.4 (17.8–35.3)	0.56	24.5 (12.5–39.2)	23.1 (13.4–35.6)	0.48	0.89
CRP (0.1–5.0 mg/l)	T1	180.4 (38.2–482.0)	148.8 (23.6–367.2)	0.12	103.4 (4.5–308.8)	156.3 (15.6–445.3)	0.60	0.22
	T2	190.2 (27.4–356.6)	97.0 (12.2–428.5)	0.20	93.5 (12.2–444.5)	108.5 (51.6–386.5)	0.61	0.47
	T3	168.7 (20.7–482.1)	98.3 (2.7–242.8)	0.07	115.5 (10.2–318.0)	95.0 (36.1–249.0)	0.31	0.21
Fibrinogen (1.8–3.5 g/l)	T1	5.4 (2.8–8.7)	4.4 (0.8–8.4)	0.25	4.6 (1.9–8.4)	4.2 (2.0–8.9)	0.79	0.51
	T2	5.7 (1.8–8.9)	4.9 (1.2–7.0)	0.22	4.4 (1.1–9.0)	5.1 (3.0–14.0)	0.25	0.92
	T3	5.3 (1.7–10.9)	5.0 (2.5–8.5)	0.16	5.0 (1.3–7.8)	5.0 (2.7–12.0)	0.42	0.58

Table 3. Comparison of the number of blood components and ratios of neutrophils/lymphocytes in subgroups

Parameter (reference values)	Time interval	Median (min-max)		*P*	Median (min-max)		*P*	*P* (A vs. B)
		A1	A2		B1	B2		
Platelets (150–350 × 10^9/l)	T1	152.5 (21.7–499.3)	233.0 (41.7–410.0)	0.64	204.0 (6.6–550.0)	121.3 (30.7–326.5)	0.10	0.53
	T2	192.2 (30.7–432.3)	198.0 (27.7–322.0)	0.99	132.0 (49.0–445.0)	104.0 (12.5–414.0)	0.70	0.76
	T3	221.5 (48.0–483.0)	153.0 (13.0–337.0)	0.32	123.0 (57.5–477.0)	84.5 (16.5–526.0)	0.92	0.41
WBC (4.0–10.0 × 10^9/l)	T1	14.8 (5.5–32.2)	13.4 (3.8–21.4)	0.56	15.7 (3.1–30.7)	10.8 (1.9–62.2)	0.16	0.96
	T2	12.6 (3.1–31.3)	10.3 (5.5–30.9)	0.38	12.3 (4.1–29.3)	12.8 (2.1–34.9)	0.05	0.96
	T3	13.4 (1.5–25.0)	14.2 (6.5–27.4)	0.48	9.9 (3.5–18.6)	8.5 (1.0–29.3)	0.09	0.003*
Neutrophils (33–80 %)	T1	15.33 (2.95–32.47)	12.1 (3.2–18.4)	0.32	11.0 (6.0–28.1)	9.4 (0.9–24.8)	0.32	0.11
	T2	11.25 (2.59–29.60)	8.0 (5.0–16.4)	0.86	11.7 (5.6–24.9)	9.0 (0.9–19.4)	0.84	0.93
	T3	13.40 (1.30–17.80)	6.8 (1.7–3.7)	0.78	11.2 (1.1–20.1)	8.1 (4.6–24.7)	0.47	0.74
Lymphocytes (15–35 %)	T1	1.02 (0.33–8.24)	1.1 (0.5–2.5)	0.28	1.1 (0.7–1.8)	0.9 (0.1–2.6)	0.49	0.16
	T2	1.11 (0.31–8.19)	1.1 (0.2–2.2)	0.73	1.2 (0.6–2.3)	0.8 (0.1–2.3)	0.66	0.59
	T3	0.75 (0.14–9.10)	1.8 (0.2–2.4)	0.92	1.1 (0.1–2.0)	1.1 (0.3–2.3)	0.54	0.78
Neu/Ly ratio (0.94–5.33)	T1	5.38 (2.80–8.71)	4.4 (0.8–8.4)	0.43	4. 6 (1. 9–8.4)	4.2 (2.0–8.9)	0.50	0.18
	T2	5.73 (1.79–8.93)	4.9 (1.2–7.0)	0.25	4.4 (1.2–9.0)	5.1 (3.0–14.0)	0.90	0.12
	T3	5.32 (1.73–10.85)	5.0 (2.5–8.5)	0.95	5.0 (1.3–7.8)	5.0 (2.7–12.0)	0.42	0.23

*$p < 0.01$

Table 4. Mortality of patients in the groups dosed with selenium and without selenium administration ($\chi^2 = 1.985$, $p = 0.159$)

Group of patients	Se-adjuvant therapy	Mortality		Total
		Survived	Died	
1	+	20	15	35
2	–	15	22	37
Total		35	37	72

Table 5. Dynamic changes in the activities of SOD, GPx, and GR in subgroups A and B at time intervals T1, T2, and T3

Parameter (healthy volunteers-median (min-max)	Time interval	Median (min-max)		*P*	Median (min-max)		*P*	*P* (A vs. B)
		A1	A2		B1	B2		
SOD 3.11 (2.44–5.21) (U/ml)	T1	4.83 (3.65–8.89)	4.89 (3.80–8.81)	0.89	3.96 (3.44–6.95)	4.01 (3.44–7.69)	0.99	0.35
	T2	4.36 (3.38–6.03)	3.89 (3.28–5.74)	0.54	4.05 (3.26–5.55)	4.11 (2.98–6.01)	0.42	0.30
	T3	4.25 (3.15–6.19)	3.69 (3.05–5.13)	0.19	3.95 (3.25–6.05)	3.88 (3.56–5.23)	0.99	0.07
GPx 1.12 (0.858–6.066) (µkat/l)	T1	0.177 (0.017–1.002)	0.182 (0.017–0.995)	0.97	0.215 (0.050–0.80)	0.123 (0.064–0.760)	0.96	0.73
	T2	0.333 (0.011–1.480)	0.155 (0.048–0.367)	0.50	0.325 (0.117–1.317)	0.200 (0.012–1.766)	0.14	0.85
	T3	0.833 (0.105–1.650)	0.194 (0.093–0.267)	0.16	0.692 (0.067–1.233)	0.300 (0.019–1.017)	0.04*	0.28
GR 1.15 (0.567–3.917) (µkat/l)	T1	1.084 (0.033–1.278)	1.076 (0.034–1.219)	0.98	0.283 (0.033–1.770)	0.283 (0.033–1.770)	0.94	0.06
	T2	0.801 (0.117–4.947)	0.486 (0.133–3.383)	0.44	0.367 (0.017–1.932)	0.660 (0.050–1.113)	0.17	0.63
	T3	0.852 (0.050–4.600)	0.580 (0.033–2.217)	0.41	0.458 (0.017–3.083)	0.712 (0.083–1.602)	0.11	0.80

*$p < 0.05$

conditions in sepsis, septic shock, and SIRS cause periods of strong oxidative stress, which is characterized by a dramatic decrease in the levels of antioxidants and their cofactors, leading to the development of severe complications such as ARDS and multiple organ failure (MOF).

Septic patients have a significantly reduced level of plasma selenium with antioxidant, anti-inflammatory, and immunological functions [14, 26], which is directly related to their high mortality rates. Although low levels of selenium in the plasma do not necessarily indicate a lack of selenium, it is reflected in selenoprotein activity, particularly in reduced GPx activity, thereby weakening the antioxidant enzyme system, or systems, for which the activity is necessary. This suggests that selenium deficiency in the critically ill is one of the causes of failure to cope with conditions of oxidative stress [2, 25].

Following the selenium status is difficult because of the redistribution of selenoproteins like albumin in septic patients due to increased permeability of blood vessels into the tissues, where it is preferentially incor-

porated into various selenoproteins [29]. The primary effect of sodium selenite is its prooxidant character, and its function as an antioxidant bound into selenoenzymes. Therefore, we have primarily chosen to follow changes in the activity of the antioxidant enzyme GPx. Since GPx belongs to a group of eight enzymes exhibiting substrate-specific peroxidase activity, which may be dependent or independent of selenium, the properly chosen methodology made it possible to measure the serum selenoenzyme activities which eliminate peroxides and selenones faster than thiols. During these reactions, two electrons are transferred from reduced glutathione to peroxide which then decomposes to water or the corresponding alcohol.

Patients who have been administered with selenium supplementation had a gradual increase in the GPx enzyme activity measured at time intervals T1, T2, and T3 (Table 5). Group A1 surgical patients had lower median values of GPx activity compared with patients in non-surgical group B1 (Table 5), which may reflect the higher stress load to that group. GPx enzyme activity values in the A1 subgroup at the time interval T3 (5–6th day) are higher than those in subgroup B1. This may indicate higher antioxidant defense activity in surgical patients. Comparing surgical and nonsurgical subgroups, patients had comparable median values of GPx activity at time T1. During therapy, a significant increase in GPx activity occurred in the A1 subgroup not only compared to subgroup A2, but also compared to nonsurgical patients. A significant increase in activation occurred in patients receiving selenium supplements in A1 vs. B2 ($p<0.05$), although the last day revealed that levels still remained below average compared with healthy subjects. The results, in agreement with previous studies as discussed by Mishra et al. [25] suggest a restoration of selenoenzyme activities due to trace element supplementation. However, it is known that plasma GPx is an easily renewable selenoprotein, while other components of blood containing GPx like erythrocytes and platelets are still required to restore long-term supplementation [10]. According to Manzanares et al. [21] and Valenta et al. [29], GPx activity increases from the first day of Se supplementation and peaks at day 7, before falling on the 10th day regardless of continued supplementation. For this reason, we refrained from monitoring enzymatic activities further. A possible explanation for this phenomenon is considered to be enzyme inhibition or saturation due to lack of precursor synthesis, such as selenium hydride or selenocysteine, or limited synthesis of glutathione due to glutamine or cysteine deficiency [22]. Patients included in the study were given an enteral or parenteral form of alanylglutamine and other glutathione precursors to enhance protein synthesis.

SOD activity has been identified as a suitable parameter for assessing oxidative stress in septic patients. Increased SOD activity was detected in the plasma and in various organs, e.g., heart, liver, and kidneys with increased formation of O_2^-. In addition, plasma SOD activity has been identified as predictive for patient survival for approximately 3 h after the development of sepsis [27]. Higher SOD activities were observed in patients with septic shock [17]. The patients who have been reported as having low levels of SOD had a higher mortality [3]. However, little is known as yet about the activity of this enzyme in relation to selenium supplementation. There were significant differences in the treatment groups between the median values of input SOD activities of surgical and nonsurgical patients (Table 5). This phenomenon may be related to higher production of O_2^- in surgical patients, which requires more powerful elimination. During therapy, there was only a slight decrease in SOD activity in all four subgroups. Some authors believe, and we identify with this statement, as we will explain later, that the increase in SOD activity is associated with activation of neutrophils and macrophages, which leads to the production of O_2^- in defending the body against microorganisms, as these are dismutated by the catalytic effectiveness of SOD. The increase in SOD activity may also occur due to the increased production of free radicals by lipid peroxidation. Increasing SOD activity, however, is an effective antioxidant defense [8]. Under normal circumstances, O_2^- is formed by monovalent reduction of molecular oxygen in every living organism, and especially within the mitochondrial electron transport. Dismutation of O_2^- to hydrogen peroxide through SOD activity without concomitant rapid degradation of resulting peroxide by catalase and GPx may lead to the formation of a reactive hydroxyl radical. SOD activity values at T1 are higher than in healthy subjects, indicating that there is SOD activity without selenoenzyme support, but prooxidant in terms of the reduced effective defenses against emerging peroxide. Antioxidant defense support by selenium and glutathione precursor supplementation act in the sequence of reactions beyond those catalyzed by SOD. We can assume, therefore, that an increase in peroxide formation occurs as a result of increased SOD activity, whose excess generally causes inhibition of peroxidases and can count as another reason for the low GPx activity. The downward trend of SOD activity, more appreciable in A groups, seems to be a reflection of lower O_2^- production in time intervals T2 and T3 in all four subgroups (A vs. B not quite statistically significant at T3). We cannot draw a conclusion on the reduction of endogenously formed GPx, preventing further peroxidation of organic substrates and cyclic formation of other radicals, due to slight changes in the environment, such as changes in the number of neutrophils (Table 3).

Another important enzyme involved in oxidation-reduction reactions in the elimination of free radicals is GR, which regenerates oxidized glutathione. The group of surgical patients in the initial measurement had higher GR activities than the nonsurgical group. Increased GR activity suggests an increased need for reduction of oxidized glutathione and indirectly points to intense oxidative stress in surgical patients. Subset A had an irregular downward trend following dynamic development of GR activity during T2–T3, as in a subgroup B1. Measuring the

GR activity in non-selenium subsets B2 confirmed only a small gradual rise in the median values (Table 5).

On the one hand, we think that the generally low GR activity in critically ill patients compared to healthy subjects may be due to GSH level depletion. As already mentioned, there is limited synthesis of glutathione owing to glutamine or cysteine deficiency [22]. Supplementation of glutathione synthesis precursors was used in an attempt to counteract this deficiency. Given that its precursors were equally covered in all patients, we may take into consideration synthetic liver function. The liver is the main source of GSH exported into blood. The export of GSH and its conjugates from liver cells occurs via transporters, referred to as organic anion-transporting polypeptides. These are generally believed to carry out electroneutral exchange, in which the cellular uptake of organic anions is coupled to the efflux of anions such as HCO_3^-, GSH, GSSH, and/or glutathione S-conjugates [6, 20]. The production in the liver and export from it are related to GSH functions, and at least two principles may be implicated. The primary GSH function here is directed toward detoxification of injurious external agents to prevent damage to the organism. The second principle is related to high-intensity oxygen-based metabolism and detoxification of certain compounds by internal organs. We did not measure GSH levels, but its synthesis can be sufficient to indirectly assess the other substances that are exclusively synthesized in the liver. When comparing the values of albumin and fibrinogen (Table 2) no significant differences were found between the groups particularly in the monitored period, even with regard to the administration of selenium. Also, severe liver failure would lead to a decrease in CRP [23], although there was no notion of a significant decrease at any time intervals T1, T2, and T3.

On the other hand, a significant fact for persistent low GR activities must then be the availability of NADPH, as the hydrogen required for this reduction of glutathione by GR comes from NADPH arising from the pentose phosphate pathway (PFP). Since NADPH is needed to drive redox reactions as a strong reducing agent, the NADP+/NADPH ratio is kept very low [30]. Here, we must consider the glucose metabolism in sepsis, where hyperglycemia is a common feature. The reduction of glutathione reductase activity may be directly caused by glucose or glucose-6-phosphate glycation [5]. The speed of glycation is dependent upon the amount of open-chain sugar, in which the carbonyl group is available for Schiff base formation. We assume that this toxic effect of glucose is at least partly prevented by strict glycemic control and, as such, other possible toxic effects and inhibition of PFP alone will not be mentioned. It remains, therefore, only a possible use of NADPH. Basically, we come around in a seemingly vicious circle, as the oxidative PFP produces NADPH, feeding either glutathione reductase to maintain glutathione in its reduced state (antioxidant) or NADPH oxidase and NO synthase for producing radicals and NO, respectively (prooxidant) [32]. This prooxidative phase is especially developed in septic patients, as the internal conditions indicate the preferential use of NADPH for NADPH oxidases in phagocytic and nonphagocytic cells as well as the use of NO synthase to form radicals in cooperation with myeloperoxidase (see characteristic parameters in Table 3 also e.g., CRP and fibrinogen in Table 2). It is true that the diagnosis of severe sepsis in critically ill patients is often very difficult and changes in body temperature, heart rate, white blood cells count, and respiratory rate show low diagnostic specificity. Positive blood cultures are often caught later as the situation requires. The biochemical parameters for the aforementioned purpose are set by CRP and procalcitonin [23]. For low and irregular measurements of procalcitonin, we did not use it as a reference marker in patients enrolled in the study. To complete our account, we will argue that the rise of plasma CRP occurs after 4–6 h after the evocative stimulus, doubling every 8 h before peaking between 36 and 50 h. The average values of CRP in SIRS are around 100 mg/l, in sepsis 150 mg/l, and as high as 170 mg/l for severe sepsis. After the disappearance of the stimulus, CRP gradually decreases, with a half-life of 19 h. If the noxa persists, CRP can be raised for a long time. The values reported in Table 2 would only confirm persisting prooxidant conditions.

Generally, slower growth in GR activities when compared to GPx toward the values measured in healthy subjects also refers to the activation of another very important redox system. The thioredoxin system participates in both antioxidant defense and regulation of cellular signal transmission, transcription, cell growth, and apoptosis and its function is therefore critical for cell survival. The reaction substrate is thioredoxin, which is oxidized. The regeneration is catalyzed by selenoenzyme thioredoxin reductase, which is important to consider in terms of selenium supplementation. Thioredoxin reductase is the only known enzyme that reduces oxidized thioredoxin but the reduction requires NADPH-like GR. In view of the increased demands for protein synthesis in septic patients, it is likely that there is a preferred restorative functionality of the system not only because of its importance, but also due to the fact that it is also a selenoenzyme. As yet, there are no data about any developmental changes in GR activity, so none have mentioned selenoenzyme thioredoxin reductase in septic patients after selenium administration.

For our consideration of the assessment of the clinical status of development in all four subgroups, we used a comparison of the number of blood components and ratios of neutrophils/lymphocytes in all subgroups (Table 3). Sepsis is an obvious risk factor for thrombocytopenia in critically ill patients, highlighting that thrombocytopenia is dependent on the severity of sepsis [9]. Platelets themselves are capable of producing the activation of free radicals and thus participate in the elimination of bacteria [4]. A decrease in thrombocytes in septic patients is typical during the first 4 days in the ICU. The subgroups did not reveal any statistically significant differences (Table 3), which would indicate the same degree of severity of the clinical condition of the sub-

groups. Compared with the reference levels, there is an increase in the total number of leukocytes in the blood to >12,000 mm^3 (leukocytosis), which is typical for a bacterial infection or a decrease <4,000 mm^3 (leukopenia) in long-term viral or bacterial infections [11], which leads to the depletion of reserves and incentive mechanisms. Statistical analysis confirmed no differences between groups in the time intervals T1 and T2 ($p>0.05$) in the leukocyte count, which can testify the comparable status of infection development intensity in both groups during treatment with selenium but no longer at T3. Difference between A and B is considered to be very statistically significant. Also, the number of days to reduce the number of lymphocytes was dependent on the extent of surgery or aggravated during SIRS. The average time of lymphocytopenia is 2–7 days [31]. The rapid development of changes in the population of white blood cells reflects the immune system's response to postoperative stress, SIRS, and sepsis [24]. A correlation was found between the severity of clinical status and the degree of neutrophilia and lymphocytopenia. The ratio of neutrophils and lymphocytes simply and reliably portrays the severity of inflammation [31]. In our Se-AOX study, there were no statistically significant differences ($p>0.05$) (Table 3) between the placebo group and subgroups A1, A2, B1, and B2 at intervals T1, T2, and T3 when comparing neutrophils/lymphocytes ratios.

In conclusion, the comparison of routine biochemical, hematological, and coagulation parameters between the groups and subgroups did not show any statistically significant differences, which is comparable with the results of several international multicenter randomized studies. An increase in GPx activity occurred after initiation of selenium supplementation. We consider the major finding of our study that, even after treatment with selenium, the antioxidant enzyme activities in patients do not reach the level of those of healthy people. An increase in GPx was observed in all groups and subgroups, but pronounced in subgroups containing surgical patients.

This raises the question of the use of higher therapeutic daily doses of selenium, which could be up to twice the dose used in this study. The benefits of using a bolus of sodium selenite pentahydrate are arguable at the beginning of therapy, as is the use of its prooxidant effect in the initial phase of sepsis, though rather for the nonsurgical group of patients. Surgical patients, on the other hand, are assumed to be able to manage an acute increase in oxidative stress thanks to the intervention, which the body usually responds to immediately. That being said, recent studies dealing with the issue of administration of high doses of selenium have not yet produced conclusive results. SOD activity has been shown to be a good and relatively quick indicator of a decrease in superoxide radical formation when monitoring the effectiveness of the selected treatment. Monitoring GR activity appears to be meaningful and informative for considering patient success. We therefore consider GR when considering elective treatment in relation to its physiological and biochemical importance and possibility of inactivation.

Based on the results obtained by us in the Se-AOX study we can assume that even if supplementation of sodium selenite pentahydrate does not belong to the "live-saving therapy" it could help to balance oxidative stress during systemic inflammation reaction, emphasizing on the efficiency of the dose used in surgical patients. It is still necessary to collect and analyze the results for a better indication of drug administration in various disease states which require further clinical studies.

Acknowledgements
Work was supported from Slovak grant agency for Science VEGA no. 1/1236/12 and 1/0624/08. Ethics committee of Pavol Jozef Šafárik University in Košice approved the study 109/2011.

Conflict of interest
None of the authors has any financial/commercial conflicts of interest with the published data.

References

1. Andrews PJ, Avenell A, Noble DW, Campbell MK, Croal BL, Simpson WG, Vale LD, Battison CG, Jenkinson DJ, Cook JA, Scottish Intensive care Glutamine or seleNium Evaluative Trial Trials Group. Randomized trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. BMJ. 2011; 342:d1542, doi:10.1136/bmj.d1542.
2. Angstwurm MW, Engelmann L, Zimmermann T, Lehmann C, Spes CH, Abel P, Strauss R, meier-Hellmann A, Insel R, Radke J, Schüttler J, Gärtner R. Selenium in Intensive Care (SIC): results of a prospective, randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. Crit Care Med. 2007;35(1):118–26.
3. Bela P, Bahl R, Sane AS, Sawant PH, Shah VR, Mishra VV, Trivedi HL. Oxidative stress status: possible guideline for clinical management of critically ill patients. Panminerva Med. 2001;43(1):27–31.
4. Bhat MA, Bhat JI, Kawoosa MS, Ahmad SM, Ali SW. Organism-specific platelet response and factors affecting survival in thrombocytopenic very low birth weight babies with sepsis. J Perinatol. 2009;29(10):702–8.
5. Blakytny R, Harding JJ. Glycation (non-enzymic glycosylation) inactivates glutathione reductase. Biochem J. 1992;288(Pt 1):303–7.
6. Briz O, Romero MR, Martinez-Becerra P, Macias RI, Perez MJ, Jimenez F, San Martin FG, Marin JJ. OATP8/1B3-mediated cotransport of bile acids and glutathione: an export pathway for organic anions from hepatocytes? J Biol Chem. 2006;281(41):30326–35.
7. Chan WS, Dedon CP. The biological and metabolic fates of endogenous DNA damage products. J Nucleic Acids. 2010;13, Article ID 929047, doi:10.4061/2010/929047.
8. de Menezes CC, Dorneles AG, Sperotto RL, Duarte MM, Schetinger MR, Loro VL. Oxidative stress in cerebrospinal fluid of patients with aseptic and bacterial meningitis. Neurochem Res. 2009;34(7):1255–60.
9. Faviere W, Boechat T. Sepsis: thrombocytopenia is bad, not recovering thrombocytopenia is too bad. Crit Care. 2011;15(Supp 1):P440.

10. Finley JW, Duffield A, Ha P, Vanderpool RA, Thomson CD. Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium. Br J Nutr. 1999;82(5):357–60.
11. Firment J, Hudak V, Grendel T. Difficulties in implementation of the recommendations for the diagnosis and treatment of severe sepsis and sepsis shock. Anesteziologie a Intenzivní Medicína 2008;19(5):252–9.
12. Forceville X, Laviolle B, Annane D, Vitoux D, Bleichner G, Korach JM, Cantais E, Georges H, Soubirou JL, Combes A, Bellissant E. Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study. Crit Care. 2007;11(4):R73.
13. Geoghegan M, McAuley D, Eaton S, Powell-Tuck J. Selenium in critical illness. Curr Opin Crit Care. 2006;12(2):136–41.
14. Hardy G, Hardy I. Selenium: the Se-XY nutraceutical. Nutrition. 2004;20(6):590–3.
15. Heyland D. Selenium supplementation in critically ill patients: can too much of a good thing be a bad thing? Crit Care. 2007;11(4):153.
16. Ince C. The microcirculation is the motor of sepsis. Crit Care. 2005;9(Supp 4):13–9.
17. Kharb S, Singh V, Ghalaut PS, Sharma A, Singh GP. Role of oxygen free radicals in shock. J Assoc Physicians India. 2000;48(10):956–7.
18. Kuklinski B, Zimmermann T, Schweder R. Decreasing mortality in acute pancreatitis with sodium selenite. Clinical results of 4 years antioxidant therapy. Med Klin (Munich). 1995;90 (Supp 1):36–41.
19. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, Cohen J, Opal SM, Vincent JL, Ramsay G. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med. 2003;29:530–8.
20. Mahagita C, Grassl SM, Piyachaturawat P, and Ballatori N. Human organic anion transporter 1B1 and 1B3 function as bidirectional carriers and do not mediate GSH-bile acid cotransport. Am J Physiol Gastrointest Liver Physiol 2007;293(1):G271–8.
21. Manzanares W, Biestro A, Torre MH, Galusso F, Facchin G, Hardy G. High-dose selenium reduces ventilator-associated pneumonia and illness severity in critically ill patients with systemic inflammation. Intensive Care Med. 2011;37(7):1120–7.
22. Manzanares W, Hardy G. The role of prebiotics and synbiotics in critically ill patients. Curr Opin Clin Nutr Metab Care. 2008;11(6):782–9.
23. Meisner M. Biomarkers of sepsis: clinically useful? Curr Opin Crit Care. 2005;11(5):473–80.
24. Menges T, Engel J, Weters I, Wagner RM, Little S, Ruwoldt R, Wollbrueck M, Hempelmann G. Changes in blood lymphocyte populations after multiple trauma: association with posttraumatic complications. Crit Care Med. 1999;27(4):733–40.
25. Mishra V, Baines M, Perry SE, McLaughlin PJ, Carson J, Wenstone R, Shenkin A. Effect of selenium supplementation on biochemical markers and outcome in critically ill patients. Clin Nutr. 2007;26(1):41–50.
26. Rayman MP. The importance of selenium to human health. Lancet, 2000;356(9225):233–41.
27. Ritter C, Andrades M, Frota Júnior ML, Bonatto F, Pinho RA, Polydoro M, Klamt F, Pinheiro CT, Menna-Barreto SS, Moreira JC, Dal-Pizzol F. Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation. Intensive Care Med. 2003;29(10):1782–9
28. Salama A, Sakr Y, Reinhart K. The role of selenium in critical illness: Basic science and clinical implications. Indian J Crit Care Med. 2007;11(3):127–38.
29. Valenta J, Brodska H, Drabek T, Hendl J, Kazda A. High-dose selenium substitution in sepsis: a prospective randomized clinical trial. Intensive Care Med. 2011;37(5):808–15.
30. Wollin MS, Ahmad M, Gao Q, Gupte SA. Cytosolic NAD(P)H regulation of redox signaling and vascular oxygen sensing. Antioxid Redox Signal. 2007;9(6):671–8.
31. Záhorec R. Ratio of neutrophil to lymphocyte counts-rapid and simple parameter of systemic inflammation and stress in critically ill. Bratisl Lek Listy. 2001;102(1):5–14.
32. Zuurbier CJ, Eerbeek O, Goedhart PT, Struys EA, Verhoeven NM, Jakobs C, Ince C. Inhibition of the pentose phosphate pathway decreases ischemia-reperfusion-induced creatine kinase release in the heart. Cardiovascular Res. 2004;62:145–53.

Clinical Biochemistry 47 (2014) 44–50

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Selenium adjuvant therapy in septic patients selected according to Carrico index

Ladislav Kočan [a], Janka Vašková [b,*], Ladislav Vaško [b], Jana Šimonová [a], Róbert Šimon [c], Jozef Firment [a]

[a] 1st Clinic of Anaesthesiology and Intensive Care Medicine, Louis Pasteur University Hospital in Košice, Slovak Republic
[b] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Slovak Republic
[c] 2nd Clinic of Surgery, Louis Pasteur University Hospital in Košice, Slovak Republic

ARTICLE INFO

Article history:
Received 24 March 2014
Received in revised form 3 July 2014
Accepted 4 July 2014
Available online 12 July 2014

Keywords:
Oxidative stress
Oxygenation index
Renal functions
Selenium
Sepsis

ABSTRACT

Objectives: The objective of this paper is to highlight the selected group of patients in which adjuvant therapy seems to have a more pronounced positive effect.

Design and methods: 65 septic patients from the prospective observational study Se-AOX (2008–2012) (ClinicalTrials.gov Identifier: NCT02026856) were divided into a Se group, receiving sodium selenite in a continual infusion of 750 μg/24 h for 6 days, and a placebo group. They were subsequently divided into subgroups according to the initial Carrico index (CI) on the day of admission: CI > 200 and CI < 200. Dynamical changes in glutathione peroxidase (GPx), glutathione reductase (GR) and superoxide dismutase activities were recorded at two day intervals. Clinical parameters and mortality were compared.

Results: The CI increased in subgroup Se-CI < 200 with negative correlation against subgroup Placebo-CI < 200 during the last measuring period ($p < 0.02$). GPx activity increased in selenium subgroups with negative correlation against placebo subgroups ($p < 0.01$). SOD activity was elevated in all subgroups in comparison with values of healthy subjects.

Conclusions: Adjuvant selenium therapy seems to be beneficial for a selected group of patients with acute lung injury. However, as is clear from the results discussed, this is not the case with persistent renal failure, as this leads to an inability to maintain synthetic renal function and ensure GPx synthesis.

Introduction

Sepsis is associated with systemic mediator-induced alterations to oxygen utilization, including increased oxygen demand, altered oxygen extraction, and decreased myocardial contractility [1]. When developing global respiratory insufficiency and hypoxia, where the Carrico index—ratio of partial pressure arterial oxygen and fraction of inspired oxygen (PaO_2/FiO_2) is less than 280, it is necessary to intubate the patient and start artificial ventilatory support. Acute lung injury (ALI) is a multifactorial problem. The basis for establishment may be interstitial pulmonary edema, dystelectases, fluidothorax or increased pulmonary shunts. At the inception of acute respiratory distress syndrome (ARDS) with a CI less than 200, special protection is required in the form of mechanical ventilation [2]. A significant increase in the fraction of inspired oxygen is required in cases of worse tissue oxygenation. These factors are the cause of worsening CI and further damage to the lung tissue [3].

Hawker et al. [4] presented that selenium (Se) deficiency can augment pulmonary injury from high concentrations of inspired oxygen. Septic patients have a significantly reduced level of plasma Se with antioxidant, anti-inflammatory and immunological functions [5–7], and are exposed to mechanical ventilation with high concentrations of inspired oxygen at the same time. The discovery of glutathione peroxidase (GPx) in the cytosol lent support to the protective role of Se. Se is an essential component of GPx which acts synergistically with tocopherol in the regulation of lipid peroxidation. In tandem with catalase, it degrades hydrogen peroxide to water (or corresponding alcohols) via glutathione reductase and flavin adenine dehydrogenase (FAD) in the pentose phosphate shunt [8].

Several clinical studies have been devoted to the issue of selenium antioxidant therapy in selected pathologies [5,9–11]. Individual studies addressing this issue are varied in respect to the dose and duration of selenium administration, and the form of administration (bolus vs. continuous administration) and show conflicting results. However, other studies were also published later which demonstrated no statistically significant reduction in mortality in any groups [12,13]. Despite findings from previous studies that plasma GPx is an easily renewable selenoprotein, and that other components of blood containing GPx, like erythrocytes and platelets, are still required to restore long-term

* Corresponding author at: Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Tr. SNP 1, 040 66 Košice, Slovak Republic.
E-mail address: janka.vaskova@upjs.sk (J. Vašková).

http://dx.doi.org/10.1016/j.clinbiochem.2014.07.004
0009-9120/

supplementation [14], selenium supplementation is still not advised "as standard" to the critically ill.

Considering the above-mentioned pathophysiological importance of Se metabolism, and especially its antioxidant role in lung injury, we (within Se-AOX study from 2008 to 2012) purposefully selected a group of patients to test the hypothesis that the selected dose of selenium would decrease the severity of the disease, and improve Carrico index.

Materials and methods

From January 2008 to December 2012 at Clinic I of Anaesthesiology and Intensive Care Medicine, University Hospital of Louis Pasteur in Košice, Slovak Republic a prospective observational study, Se-AOX, was conducted to look at selenium supplementation. This study looked at parenteral administration of selenium to septic patients and subsequently monitored dynamic changes of selected activities of antioxidant enzymes and the development of clinical status. The university ethics committee approved the study 109/2011, ClinicalTrials.gov Identifier: NCT02026856. 72 patients were enrolled in the whole Se-AOX with an APACHE II score between 19 and 24 points at ages between 23 and 79 years old who developed sepsis, severe sepsis or septic shock during hospitalization as defined by the International Sepsis Definitions Conference [15]. A SOFA score was calculated at the time periods 1–2 days (T1), 3–4 days (T2) and 5–6 days (T3) according to an internet calculator (http://www.sfar.org/scores2/sofa2.php), and more detailed information about criteria for the diagnosis of sepsis is dealt with by Vašková et al. [16].

65 patients were included in the evaluation (Table 1). The study was conducted only in the Louis Pasteur University Hospital. Due to the severity of the condition, the selection criteria could not include more patients for that period. Regarding the ventilation period, we excluded patients who had received mechanical ventilation for less than 24 h. We had supposed shorter exposure of oxygen radicals in the air mix with artificial ventilation. Neuromuscular disease and terminal illness were also excluding criteria; see flow-diagram (Fig. 1). The CI as PaO_2/FiO_2 (partial pressure of oxygen in arterial blood/fraction of inspired oxygen) ratio in patients was determined daily. Patients were divided into two groups. Se-group consisted of 31 patients, who received selenium supplementation during hospitalization in the form of sodium selenite pentahydrate at 750 mg/day for 6 days as a continual infusion immediately after admission to our department (1000 mg of sodium selenite pentahydrate = 333 μg of selenium) (Selenase, Vivax; selenium hereinafter). At the same time, alanyl glutamine solution was administered via a central venous catheter at a daily dose 100 ml (2 g) for 6 days. The placebo group consisted of 34 patients who received continuous saline NaCl 50 ml/day for 6 days as a continuous infusion (excluding additional infusion therapy). Patients were further divided into subgroups according to CI on the day of admission to ICU. 200 was chosen as the threshold CI value.

The nutritional strategy (enteral vs. parenteral, qualitative and quantitative characteristics of the diet) was dependent on the patient's diagnosis and nutritional status, as well as the standard therapeutic approaches to the disease. Some of the patients were without any enteral or parenteral nutrition longer than 48 h (8 patients in selenium and 10 patients in non-selenium group). The reason of this strategy was the presence of the acute phase of septic shock. Counts of this patients in both groups were similar and without statistical significant differences in mortality when compared patients with enteral and parenteral nutrition. Food administration was cyclical, to stimulate the diurnal rhythm. The daily dosage of glucose ranged from 1.5 g/kg up to 5.5 g/kg. The total daily supply of amino acids ranged from 0.8 to 2.5 g/kg (0.5 to 0.75 g/kg for renal and liver injury, from 0.7 to 1.0 g/kg in stable patients, 1.0 to 1.5 g/kg in a poor state of nutrition and catabolism, and 1.5 to 2.0 g/kg for severe malnutrition). Enteral nutrient intake (Nutrison-Multifibre) was provided via naso-gastric tube and parenteral nutrition solutions (SmofKabiven and Aminomix II) were administered intravenously. Fat substitution (Smoflipid and Omegaven) was performed after the acute stage of the disease subsided and was administered at 0.5 g/kg/day. All groups were also administered antioxidants parenterally: ascorbic acid 1000 mg/24 h, tocopherol 30 mg/24 h, 100 mg/24 h thiamine, 100 mg/24 h pyridoxine and 1000 μg/24 h cobalamin.

The determination of biochemical and hematological parameters of blood samples formed part of the routine diagnostic methods. Data collection for the Se-AOX study was performed at two-day intervals: T1 (1st–2nd day), T2 (3rd–4th day) and T3 (5th–6th day). Kinetic methods for estimating the activities of glutathione peroxidase (GPx, E.C. 1.11.1.9) and glutathione reductase (GR, E.C.1.6.4.2) were performed using a kit (Sigma-Aldrich, Germany) and that of superoxide dismutase (SOD, E.C. 1.15.1.1) by means of the SOD-Assay Kit-WST (Fluka, Japan) following the user manual provided.

Descriptive statistics methods were used to evaluate the results. Differences between continuous variables were analyzed by a non-parametric Mann–Whitney test. Statistical significance was determined by Pearson's Chi-square of selenium vs. non-selenium groups from the baseline to the 6–7th day. Possible benefits from the treatment were calculated by NNT test.

Results

There were no statistically significant differences at the time intervals T1, T2 and T3 ($p > 0.05$) when comparing the plasma levels of total protein, albumin, CRP and fibrinogen, lactate, creatinine and urea between subgroups: Se-CI > 200 vs Placebo-CI > 200 and

Table 1
Demographic characteristics of the patients in groups with selenium adjuvant therapy divided according to Carrico index (CI) under or above 200 and placebo groups with oxygenation index under or above 200.

Characteristics	Se-CI > 200 (min–max) med	Placebo-CI > 200 (min–max) med	Se-CI < 200 (min–max) med	Placebo-CI <200 (min–max) med
N	14	19	17	15
Age	(23–78) 53	(30–77) 60	(28–68) 47	(40–79) 53
Gender (M/F)	7/7	12/7	12/5	12/4
APACHE score	(19–40) 23	(19–38) 23	(19–39) 25	(20–36) 28
SOFA score in T1	(6–14) 11	(6–14) 9	(5–14) 10	(6–13) 9
Severe sepsis	10	12	7	9
Septic shock	4	7	8	6
Disseminated intravascular coagulation	2	2	1	2
Bronchopneumonia	9	7	12	9
Surgical performance	9	11	12	9
Nosocomial infections[a]	11	14	12	10
Dialysis	8	5	9	6
-CVVH/CRRT[b] 5 and more days	5	2	3	1

[a] Cultivated pathogens: *Staphylococcus aureus*, *Enterococcus* sp., *Candida* sp., *Pseudomonas aeruginosa* (bloodstream), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Acinetobacter* sp., *Klebsiella pneumonia* (respiratory tract), *E. coli*, *Candida* sp., *Pseudomonas aeruginosa* (urinary tract).
[b] CVVH/CRRT—Continuous Veno-Venous Hemofiltration/Continuous Renal Replacement Therapy.

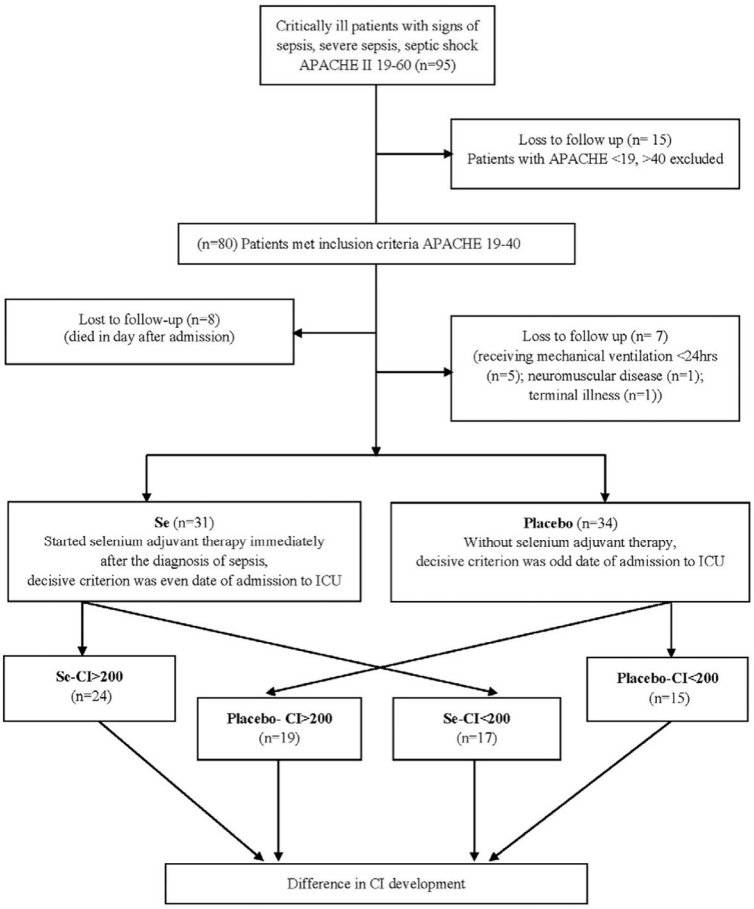


Fig. 1. Flow diagram. Patients were reselected according to Carrico index and selenium administration. Worse Carrico index indicates reduced pulmonary function and dysfunction of alveolar–capillary membrane. The Carrico index was measured every day during the whole period of study observation. Patients receiving mechanical ventilation for <24 h were excluded. Other excluding criteria were neuromuscular disease and terminal illness.

sub-Se-CI < 200 vs Placebo-CI < 200 (Table 2). The plasma levels of measured parameters showed statistical similarity. Median values of creatinine were also comparable, but with very high maximum values ensured in groups with CI > 200. Compliance to these conditions is important for the antioxidant response followed, including sufficient amounts of amino acids supplemented for the formation of glutamine and protein components of antioxidant enzymes.

Comparing the number of white blood cells, neutrophils, lymphocytes, and platelets (Table 2), a statistically significant decrease was found in total white blood cell count at time T3 between subgroups Se-CI > 200 vs Placebo-CI > 200 favoring the placebo subgroups ($p < 0.05$). There were no statistically significant differences at the time intervals T1, T2 and T3 when comparing other blood elements ($p > 0.05$). This fact testifies to the similarity of groups in the study intervals and the same degree of severity of the clinical status between subgroups.

Comparing the CI (Fig. 2) in individual subgroups, a statistically significant increase in CI was found at time T3 between subgroups Se-CI < 200 and Placebo-CI < 200 in favor of selenium subgroups ($p < 0.02$). A statistically significant increase in GPx activity at time T3 was found in all subgroups with selenium supplementation ($p < 0.01$) (Table 3). No statistically significant differences were found between subgroups at the time intervals T1, T2 and T3 ($p > 0.05$) in the activities of antioxidant enzymes GR and SOD.

Discussion

In a prospective observational study with AOX we followed the dynamic development of biochemical and hematological parameters of sepsis and dynamic changes of antioxidant enzymes in critically ill patients ($n = 65$) with APACHE II (median 24, min. 19, max. 40) and SOFA (median value 9, min. 4 max. 14). There was no evidence of differences between subgroups when comparing the changes in organ dysfunction by SOFA score intervals T1, T2, and T3 See Fig. 3. Similarly, no differences were found in mortality between Se and placebo subgroups (Table 4).

The liver is the central body of metabolic excretion, metabolic, detoxifying, hematological, and hemostatic functions, and a number of

Table 2
Comparison of the levels of selected parameters in plasma over the T1–T3 day interval.

Parameters (reference values)	Time interval	(min–max) med		P	(min-max) med		P
		Se-CI > 200	Placebo-CI > 200		Se-CI < 200	Placebo-CI < 200	
Total plasma protein	T1	(38.5–61.7) 50.95	(36.0–57.5) 48.38	0.2	(36.0–77.9) 50.95	(38.5–61.7) 46.8	0.60
(64.0–83.0 g l^{-1})	T2	(34.0–62.5) 50.6	(42.2–70.0) 51.2	0.85	(34.0–70.0) 50.0	(34–68.051.0	0.76
	T3	(36.3–64.7) 54.4	(28.1–77.9) 49.8	0.43	(34.0–65.0) 52.4	(28–78) 49.8	0.82
Albumin	T1	(13.0–39.0) 25.95	(11.2–50.0) 22.5	0.72	(15.5–38.0) 23.2	(15.0–46.0) 25.6	0.07
(35.0–52.0 g l^{-1})	T2	(15.1–31.9) 22.03	(14.9–39.4) 21.05	0.70	(14.9–39.4) 22.9	(14.3–37.2) 20.7	0.23
	T3	(14.2–39.2) 24.95	(14.9–39.4) 21.05	0.80	(12.9–35.5) 22.3	(12.5–39.2) 24.7	0.70
CRP	T1	(38.2–482.0) 180.4	(23.6–367.2) 148.8	0.43	(6.6–370.4) 192.1	(4.5–445.3) 103.4	0.33
(0.1–5.0 mg l^{-1})	T2	(12.4–428.5) 181.2	(8.4–220.4) 94.1	0.19	(38.1–280.0) 162.0	(3.1–444.5) 122.5	0.88
	T3	(2.7–323.3) 129.2	(21.7–180.0) 92.4	0.24	(16.0–482) 126.5	(10–318.0) 170.0	0.85
Fibrinogen	T1	(0.8–8.7) 4.1	(2.0–8.4) 4.9	0.38	(1.3–9.5) 5.4	(1.9–25.0) 4.1	0.31
(1.8–3.5 g l^{-1})	T2	(1.2–8.9) 4.7	(3.0–7.2) 4.2	0.72	(2.3–7.8) 5.95	(1.1–14.0) 6.1	0.77
	T3	(1.0–9.0) 4.75	(2.0–8.4) 4.9	0.60	(2.5–10.8) 5.2	(1.3–12.0) 5.1	0.97
Lactate	T1	(1.3–6.2) 3.0	(1.0–6.3) 3.2	0.98	(1.4–6.2) 2.0	(1.1–12.9) 2.9	0.08
(0.6–2.1 mmol l^{-1})	T2	(0.9–4.3) 1.85	(1.1–7.2) 2.1	0.87	(1.1–5.9) 2.2	(0.9–7.2) 2.2	0.85
	T3	(1.0–3.8) 1.7	(1.1–5.8) 1.6	0.24	(1.0–6.9) 2.1	(1.2–4.7) 2.3	0.30
Creatinine	T1	(41.2–689.6) 145	(62.1–601.1) 149	0.9	(71.9–370.5) 214.1	(42.8–315.3) 110	0.33
(53–115 μmol l^{-1})	T2	(44.9–375.8) 126.5	(53.4–298.7) 109.4	0.73	(57.0–318.7) 142.4	(56.1–446) 185.3	0.91
	T3	(36–649) 123.5	(57–213) 104.1	0.65	(87–312) 130	(75.4–391.5) 128.6	0,29
Blood urea nitrogen	T1	(2–44) 12	(2–46) 11	0.36	(5–29) 13	(3–37) 6	0.15
(2.6–8.1 mmol l^{-1})	T2	(6–35) 10	(3–32) 13.4	0.45	(2–41) 15	(3–32) 11	0.85
	T3	(3–44) 12.5	(5–25) 14.5	0.78	(1–30) 11	(5–28) 11	0.89
WBC	T1	(5.6–32.2) 13.1	(5.1–62.2) 11.1	0.92	(3.8–25.2) 17	(0.9–25)11.9	0.18
(4.0–10.0 × 10^9 l^{-1})	T2	(3–31.3) 11.45	(8–34.9) 13	0.46	(5.5–21.6) 12.4	(0.1–29.3) 9.4	0.20
	T3	(1.5–27.4) 13.1	(1.7–18.6) 8.9	***0.05***	(6.3–23) 13.4	(3.5–21.2) 11.6	0.24
Neutrophils	T1	(3.0–32.5) 12.75	(4.1–28.1) 10.1	0.57	(3.2–21.4) 15.3	(0.8–27.2) 9.4	0.33
(33–80%)	T2	(2.6–29.6) 12.8	(5.8–24.3) 9.7	0.60	(3.7–18.3) 10	(0.9–24.9) 9.6	0.74
	T3	(1.3–21.9) 10.9	(3.3–16.3) 8.9	0.36	(3.7–17.8) 12	(1.1–20.1) 9.2	0.43
Lymphocytes	T1	(0.5–8.2) 1.1	(0.1–2.6) 1.0	0.76	(0.3–2.3) 1.0	(0.0–2.3) 0.9	0.43
(15–35%)	T2	(0.3–8.2) 0.9	(0.4–1.9)1.2	0.26	(0.2–2.2) 1.4	(0.1–2.3) 0.9	0.33
	T3	(0.1–9.1) 0.7	(0.6–2.0) 1.1	0.31	(0.2–3.6) 0.9	(0.1–1.8) 1.1	0.89
Platelets	T1	(22–499) 178	(31–390) 128	0.82	(47–410) 199	(6.6–550) 163	0.38
(150–350 × 10^9 l^{-1})	T2	(31–430) 137	(35–429) 120	0.79	(28–432) 221	(12.5–445) 113	0.20
	T3	(48–337) 145	(17–477) 123	0.36	(13–483) 231	(49–426.5) 136	0.45

other important functions. In the early stage of hepatic failure there is higher degradation of proteins by predominantly catabolic pathways. There is an acceleration in the inflammatory response, an increase in inflammatory mediators and increase in the production of acute phase proteins being released into the circulation. Through examination of non-specific inflammatory parameters fibrinogen and C-reactive protein at time intervals T1, T2 and T3, we found a statistical similarity between groups ($p > 0.05$) with generally elevated levels. Under favorable conditions, the physiological function of liver tissue is restored and the damaged liver lobules are repaired *ad integrum*. The excessive action of *noxa* may exceed the compensation limits and become irreversible, resulting in hepatic failure [5]. This leads to a decrease in protein synthesis, which results in decreased levels of total protein and manifests mainly as a decrease in plasma albumin, prealbumin, transferrin, transcortin and retinol-binding protein [17]. The same statistical similarity was recorded between groups when comparing low plasma levels of albumin, and total protein ($p > 0.05$). Although, hypoalbuminemia is quite common in critically ill patients, especially in severe sepsis, on the one hand it is interpreted as a normal compensatory mechanism when inflammatory mediators increase vascular permeability to promote escape of circulatory albumin into extravascular space which may provide protection from oxidative stress [18]. On the other hand, however, there is also the possibility of reduced production by the liver, which may be due to the persistent lack of capacity in the plasma compartment that influences the development of illness and outcome of patients. Hepatic failure confirms, in addition to clinical signs of kidney damage, serum creatinine and blood urea nitrogen levels (Table 2). Creatinine, however, is also formed by cleavage of phosphocreatine in the muscles, but the increase in serum creatinine can be further increased by reduced renal excretion. But both are removed by dialysis, and a number of patients from each group underwent dialysis (at least 5 patients in the placebo-CI > 200 and not more than 9 in Group Se-CI < 200) (Table 1).

Monitoring non-specific inflammatory parameters and proteosynthetic processes is a very complex process, and especially difficult to evaluate in the specific pathophysiology of organ dysfunction. The interpretation of results is also hampered by the diversity of applications and types of artificial nutrition. Although, Montejo et al. [19] pointed out that diet enriched with pharmaconutrients in patients requiring enteral nutrition could have beneficial effects. A diet enriched with omega-3/omega-6 fatty acids and elevated antioxidants led to improvements in clinical outcomes of patients with ALI/ARDS [20].

An assessment of the function these organs have on the metabolism of therapeutic agents like selenium is of particular importance. Septic patients have a significantly reduced level of plasma selenium with antioxidant, anti-inflammatory and immunological functions [6,7]. This is directly related to their high mortality rates. It was observed that Se (in the form of sodium selenite or selenium methionine) travels in one of three directions, into plasma without being taken up by liver; via the hepatopancreatic subsystem, or alternatively, via the lymphatic system into the plasma; or taken up by the liver. From there, some is returned to the intestine via pancreatic secretion of bile, some enters plasma, and the rest enters a pool considered as tissues. From this pool, selenium moves through delay compartments into the plasma or into other tissue pools, including red blood cells. From the plasma, Se returns to the liver, tissues, or urine [21]. Following absorption, the liver appeared to extract 50% of the Se, with the remainder staying in the plasma or entering the lymph [21].

According to the determined parameters, liver function is impaired and Se cannot be expected to be incorporated into forms of transport (selenoproteins P) or bound to albumin bound with sufficient tissue redistribution. GPx is also a selenoenzyme, which is also an important part of the antioxidant mechanism of glutathione. From the data measured in T1 (Table 3) it is evident that its activity in all groups is very low when compared to the values measured in healthy volunteers [16].

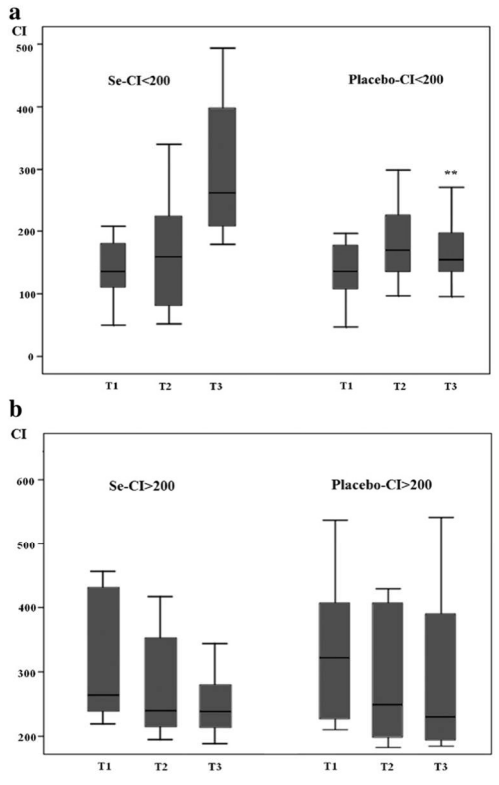


Fig. 2. Comparison of CI groups with a placebo. A statistically significant increase in CI was found when comparing oxygenation indexes at time T3 between subgroups Se-CI < 200 vs Placebo-CI < 200 in favor of selenium subgroups (**$p < 0.02$).

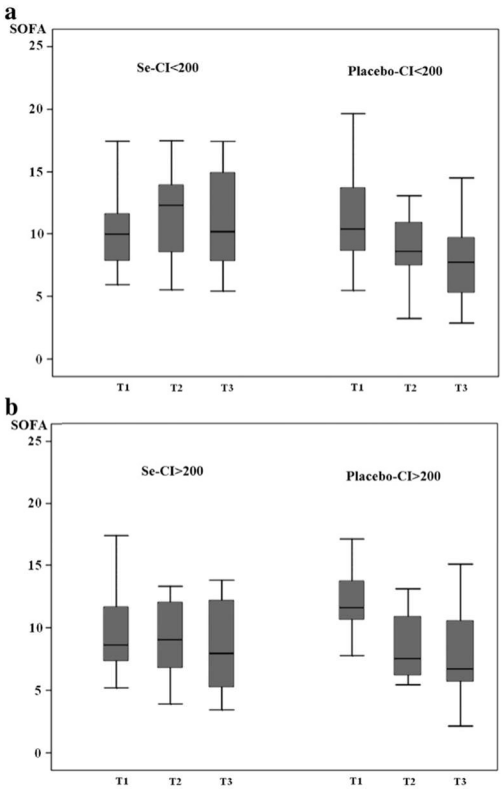


Fig. 3. The dynamic development of SOFA- score in patients divided into subgroups according to CI on the day of admission to ICU.

After six days of Se supplementation at T3, there were significant differences in GPx activities in both Se groups in favor of Se groups when compared to the placebo. This would clearly refute impaired liver function, as the activity of GPx isoforms are regulated by the Se status. This essentially eliminated the possibility of comparable conditions for biochemical parameters in all other groups. Kidney proximal tubular cells are the main source of GPx activity in the plasma [22]. El-far et al. [23] found a highly significant negative correlation between GPx activity in plasma and serum creatinine, and also blood urea nitrogen in patients with renal impairment or chronic renal failure on hemodialytic maintenance. Each group included a number (usually less than half) of patients who were on dialysis (Table 1) and, of those, some required Continuous Veno-Venous Hemofiltration/Continuous Renal Replacement Therapy (CVVH/CRRT) for 5 days or more, indicating renal impairment or chronic renal failure throughout the period of selenium administration. Due to the reasons mentioned above, the last subgroup can be considered confusing, as the Se adjuvant therapy is ineffective in this case. GPx uses glutathione as the reducing agent to convert substrate peroxides to water and oxidized glutathione is recycled back to glutathione by

Table 3
Comparison of plasma levels of antioxidant enzymes in selenium and placebo groups.

Parameters	Time interval	(min–max) med		P	(min–max) med		P
		Se-CI > 200	Placebo-CI > 200		Se-CI < 200	Placebo-CI < 200	
GPx	T1	(0.02–3.28) 0.19	(0.11–1.89) 0.21	0.96	(0.02–1.66) 0.16	(0.08–1.32) 0.12	0.71
(μkat l^{-1})	T2	(0.01–2.98) 0.30	(0.01–1.22) 0.21	0.71	(0.12–2.67) 0.40	(0.12–1.77) 0.21	0.66
	T3	(0.10–1.23) 0.52	(0.01–0.59) 0.14	***0.01***	(0.18–2.97) 0.53	(0.05–2.02) 0.09	***0.01***
GR	T1	(0.03–1.77) 0.33	(1.01–3.11) 1.31	0.76	(0.04–1.78) 0.61	(0.18–1.99) 0.48	0.87
(μkat l^{-1})	T2	(0.02–4.95) 0.61	(0.08–1.42) 0.42	0.61	(0.03–2.95) 0.87	(0.05–2.22) 0.57	0.18
	T3	(0.02–2.82) 0.50	(0.08–2.27) 0.59	0.81	(0.02–4.60) 1.18	(0.03–1.27) 0.61	0.37
SOD	T1	(3.43–6.45) 4.51	(3.21–6.15) 4.48	0.83	3.50–8.89) 4.41	.42–9.01) 4.38	0.39
(μkat l^{-1})	T2	(3.34–6.05) 4.01	(2.98–5.74) 3.98	0.67	(3.26–6.12) 4.34	(3.36–6.01) 3.84	0.09
	T3	(3.15–6.08) 4.5	(3.11–5.34) 4.21	0.43	(3.25–6.19) 4.17	(3.05–5.23) 3.76	0.40

Table 4
Mortality of patients divided into groups according to CI > 200 and CI < 200 on the day of admission (T1).

Group of patients	Mortality		Total	Statistics
	Survived	Died		
Se-CI > 200	7	7	14	$\chi^2 = 0.022$
Placebo-CI > 200	9	10	19	$p = 0.88$
Total	16	17	33	NNT = 39
Se-CI < 200	10	7	17	$\chi^2 = 2.079$
Placebo-CI < 200	5	10	15	$p = 0.15$
Total	15	17	32	NNT = 4

GR. There were no statistically significant differences between groups; however, there is an obvious upward trend in the activity of GR in Se groups. In Se-CI < 200, the values are comparable with a group of healthy volunteers [16].

SOD activity was identified a survival predictor for patients for about 3 h after the development of sepsis [24]. Higher SOD activity was observed in patients with septic shock. Kharb et al. [25] and Bela et al. [26] showed that patients with a low level of SOD had a higher mortality. In our study groups, however, the mean input SOD activity values were found to be elevated when compared to the activity in healthy volunteers in all subgroups [16]. An activation of extracellular SOD has a strict dependence on reduced glutathione or a low redox potential of the cell [27]. This phenomenon may be related to the higher production of superoxide radicals resulting from the greater oxidative stress in septic patients, which requires an increase in elimination, but also leads to higher peroxide formation, whose elimination is dependent on the activity of peroxidases. As antioxidant enzymes commonly use the oxido-reduction properties of metal cofactors for catalytic activity, there is also the possibility for supplementation redox active trace elements in critical illness [28]. In the course of therapy in the Se-AOX study there was only a slight decrease in SOD activity in all four subgroups.

Comparison of the number of leukocytes revealed a statistically significant decrease in total white blood cell count at time T3 between subgroups Se-CI > 200 vs Placebo-CI > 200 in favor of the placebo ($p < 0.05$). Severe sepsis is characterized by a rise in the total number of leukocytes in the blood of >12 000 mm^3. This is typical of bacterial infection, while a decrease <4000 mm^3 is typical of viral infections or prolonged bacterial infections [29], leading to the depletion of reserves and incentive mechanisms of the body.

In our study, Se-AOX we found a remarkable and statistically significant improvement in respiratory function, quantified on the basis of CI index. A statistically significant increase in CI was found between the subgroups when comparing the CI at time T3 between subgroups Se-CI < 200 and Placebo-CI < 200 in favor of subgroups supplemented with selenium ($p < 0.05$). Hawker et al. [4] showed that manifestation of pulmonary oxygen toxicity was more severe in Se deficient animals. Furthermore, the findings of higher lung Se concentration after exposure to 95% O_2 for 36 h than after air exposure in Se supplemented animals suggest that Se is redistributed into the lungs in response to oxidative stress. This will take a more positive meaning for significantly higher values of GPx, which are found in groups supplemented with Se.

Conclusions

It can be assumed that the lung tissue in bronchopneumonia ARDS is exposed to higher oxidative stress, and thus the effect of reactive oxygen species generated endogenously and exogenously is higher. This is especially true when administered to high fractions of inspired oxygen FiO_2 during mechanical ventilation. This seems to account for the most serious damage to the lung parenchyma, as the most intense stress reaction takes place there. It also offers the greatest potential target for therapeutic antioxidant molecules. The subgroup with lower CI had improved pulmonary functions after selenium administration.

Selenium administration appeared to be beneficial in these cases. We expect that the percentage would have been even higher if it had not been for the proportion of patients that had undergone continual renal replacement therapy for more than 5 days. This therapy prevented the synthetic function of GPx creation from selenium administration.

Conflict of interest

None of the authors has any financial/commercial conflicts of interest with the published data.

Acknowledgments

The study was supported by grants of Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic nos. 1/0799/09 and 1/1236/12.

References

[1] Vincent JL. The international sepsis forum's frontiers in sepsis: high cardiac output should be maintained in severe sepsis. Crit Care 2003;7:276–8.
[2] Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschker R, et al. Surviving sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. Crit Care Med 2008;36:296–327.
[3] Varga M. Bronchial hyperreactivity, 1. I-med; 2011. p. 1–31 [http://www.i-med.sk/moodle/login/index.php.].
[4] Hawker FH, Ward HE, Steward PM, Wynne LA, Snitch PJ. Selenium deficiency augments pulmonary toxic effects of oxygen exposure in the rat. Eur Respir J 1993;6:1317–23.
[5] Angstwurm MW, Engelmann L, Zimmermann T, Lehmann C, Spes CH, Abel P, et al. Selenium in Intensive Care (SIC): results of a prospective, randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. Crit Care Med 2007;35:118–26.
[6] Hardy G, Hardy I. Selenium: the Se-XY nutraceutical. Nutrition 2004;20:590–3.
[7] Rayman MP. The importance of selenium to human health. Lancet 2000;356:233–41.
[8] Chu FF, Doroshow JS, Esworthy RS. Expression, characterization and tissue distribution of a new cellular Se-dependent glutathione peroxidase, GSHPX-GI. J Biol Chem 1993;268:2571–6.
[9] Forceville X, Laviolle B, Annane D, Vitoux D, Bleichner G, Korach JM, et al. Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study. Crit Care 2007;11:R73.
[10] Heyland D. Selenium supplementation in critically ill patients: can too much of a good thing be a bad thing? Crit Care 2007;11:153.
[11] Kuklinski B, Zimmermann T, Schweder R. Decreasing mortality in acute pancreatitis with sodium selenite. Clinical results of 4 years antioxidant therapy. Med Klin (Munich) 1995;90:36–41.
[12] Andrews PJ, Avenell A, Noble DW, Campbell MK, Croal BL, Sipmson WG, et al. Scottish Intensive care Glutamine or seleNium Evaluative Trial Trials Group. Randomised trial of glutamine, selenium, or both, to supplement parenteral nutrition for critically ill patients. BMJ 2011;342:d1542. http://dx.doi.org/10.1136/bmj.d1542.
[13] Valenta J, Brodska H, Drabek T, Hendl J, Kazda A. High-dose selenium substitution in sepsis: a prospective randomized clinical trial. Intensive Care Med 2011;37:808–15.
[14] Finley JW, Duffield A, Ha P, Vanderpool RA, Thomson CD. Selenium supplementation affects the retention of stable isotopes of selenium in human subjects consuming diets low in selenium. Br J Nutr 1999;82:357–60.
[15] Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. Intensive Care Med 2003;29:530–8.
[16] Vašková J, Kočan L, Vaško L, Firment J. Restoration of antioxidant enzymes in therapeutic use of selenium in septic patients. Wien Klin Wochenschr 2013;125:316–25.
[17] Briz O, Romero MR, Martinez-Becerra P, Macias RI, Perez MJ, Jimenez F, et al. OATP8/1B3-mediated cotransport of bile acids and glutathione: an export pathway for organic anions from hepatocytes? J Biol Chem 2006;281:30326–35.
[18] Hwang TL. Potential use of albumin administration in severe sepsis. J Chin Med Assoc 2009;5:225–6.
[19] Montejo JC, Zarazaga A, López-Martínez J, Urrútia G, Roqué M, Blesa AL, et al. Immunonutrition in the intensive care unit. A systematic review and concensus statement. Clin Nutr 2003;22:221–3.
[20] Pontes-Arruda A, Demichele S, Seth A, Singer P. The use of an inflammation-modulating diet in patients with acute lung injury or acute respiratory distress syndrome: a meta-analysis of outcome data. J Parenter Enter Nutr 2008;32:596–605.
[21] Wastney ME, Combs Jr GF, Canfield WK, Taylor PR, Patterson KY, Hill AD, et al. A human model of selenium that integrates metabolism from selenite and selenomethionine. J Nutr 2011;141:708–17.
[22] Avissar N, Omt D, Yagil Y, Horowitz S, Watkins RH, Kerl EA, et al. Human kidney proximal tubules are the main source of plasma glutathione peroxidase. Am J Physiol 1994;266:C367–75.

[23] El-far MA, Bakr MA, Farahat SE, Abd El-Fattah EA. Glutathione peroxidase activity in patients with renal disorders. Clin Exp Nephrol 2005;9:127–31.
[24] Ritter C, Andrades M, Frota Júnior ML, Bonatto F, Pinho RA, Polydoro M, et al. Oxidative parameters and mortality in sepsis induced by cecal ligation and perforation. Intensive Care Med 2003;29:1782–9.
[25] Kharb S, Singh V, Ghalaut PS, Sharma A, Singh GP. Role of oxygen free radicals in shock. J Assoc Physicians India 2000;48:956–7.
[26] Bela P, Bahl R, Sane AS, et al. Oxidative stress status: possible guideline for clinical management of critically ill patients. Panminerva Med 2001;43:27–31.
[27] Carroll MC, Giroaurd JB, Ulloa JL, Subramaniam JR, Wong PC, Valentine JS, et al. Mechanisms for activating Cu- and Zn-containing superoxide dismutase in the absence of CCS Cu chaperone. Proc Natl Acad Sci U S A 2004;101:5964–9.
[28] Rech M, To L, Tovbin A, Smoot T, Mlynarek M. Heavy metal in the intensive care unit: a review of current literature on trace element supplementation in critically ill patients. Nutr Clin Pract 2014;29:78–89.
[29] Záhorec R, Firment J, Straková J, Mikula J, Malik P, Novák I, et al. Epidemiology of severe sepsis in intensive care units in the Slovak Republic. Infection 2005;33:122–8.

Publikácia: **Selenium adjuvant therapy in septic patients selected according to Carrico index**. bola ocenená Lekárskou Fakultou Univerzity Pavla Jozefa Šafárika v Košiciach pre Oblasť výskumu 18. Lekárske, farmaceutické a nelekárske zdravotnícke vedy ako:

„**Najvýznamnejšie vedecké práce zamestnancov Univerzity Pavla Jozefa Šafárika v Košiciach publikované v roku 2014 podľa fakúlt a akreditovaných oblastí výskumu**“ (Príloha č.1).

2.5.4 Citácie štúdie SE-AOX v Metaanalýzach a Systémových prehľadoch

Výsledky štúdie Se-AOX boli doteraz citované v nasledujúcich systematických prehľadoch a metaanalýzach:

1. Manzanares W, Lemieux M, Elke G, Langlois PL, Bloos F, Heyland DK. High-dose intravenous selenium does not improve clinical outcomes in the critically ill: a systematic review and meta-analysis. Crit Care. 2016 Oct 28;20(1):356. doi: 10.1186/s13054-016-1529-5. PMID: 27788688; PMCID: PMC5084353.

2. Zhao Y, Yang M, Mao Z, Yuan R, Wang L, Hu X, Zhou F, Kang H. The clinical outcomes of selenium supplementation on critically ill patients: A meta-analysis of randomized controlled trials. Medicine (Baltimore). 2019 May;98(20):e15473. doi: 10.1097/MD.0000000000015473. PMID: 31096444; PMCID: PMC6531101.

3. Mousavi MA, Saghaleini SH, Mahmoodpoor A, Ghojazadeh M, Mousavi SN. Daily parenteral selenium therapy in critically ill patients: An updated systematic review and meta-analysis of randomized controlled trials. Clin Nutr ESPEN. 2021 Feb;41:49-58. doi: 10.1016/j.clnesp.2020.11.026. Epub 2021 Jan 19. PMID: 33487307.

4. Jaff S, Zeraattalab-Motlagh S, Amiri Khosroshahi R, Gubari M, Mohammadi H, Djafarian K. The effect of selenium therapy in critically ill patients: an umbrella review of systematic reviews and meta-analysis of randomized controlled trials. Eur J Med Res. 2023 Feb 28;28(1):104. doi: 10.1186/s40001-023-01075-w. PMID: 36849891; PMCID: PMC9972714.

3 RESPIRAČNÉ ZLYHANIE NA PODKLADE VÍRUSOVEJ INFEKCIE A JEHO LIEČBA

V priebehu posledných pár rokov sa naplno preukázal deštrukčný potenciál vírusových pneumónii, pôsobiaci na pľúcne tkanivo. Okrem pochopenia zápalových mechanizmov vedúcich k poškodeniu alveolárnych jednotiek sa výrazne zdokonaľovali možnosti umelej pľúcnej ventilácie, ktorých cieľom bolo zlepšenie prežívania pacientov v akútnej fáze ochorenia, ako aj redukcia postinfekčných komplikácií.

3.1 Viachladinová ventilácia pľúc

Umelá pľúcna ventilácia je základným postupom orgánovej podpory pacientov, u ktorých došlo k vzniku závažnej poruchy ventilačnej alebo oxygenačnej funkcie respiračného systému alebo sú takouto poruchou aktuálne ohrození. Medzi tieto stavy patrí syndróm akútnej respiračnej tiesne (ARDS), kardiogénny a nekardiogénny pľúcny edém, kontúzie pľúc, závažné pneumónie a ďalšie kritické stavy. Terapia závažnej respiračnej insuficiencie vyžaduje zabezpečenie dýchacích ciest a realizáciu umelej pľúcnej ventilácie s voľbou adekvátneho ventilačného režimu s individuálnym nastavením ventilačných parametrov vzhľadom na vlastnosti respiračného systému daného pacienta. Vulnerabilné pľúcne tkanivo, ktoré je poškodené už primárnym inzultom, je v priebehu liečby ďalej ohrozené samotnou umelou pľúcnou ventiláciou. Nepriaznivo na pľúcne tkanivo pôsobia vysoké inspiračné tlaky, ktorých následkom môže byť barotrauma, ako aj kaskádová aktivácia autoimunitných procesov, ktorých výsledkom je biotrauma pľúc. Pod pojmom biotrauma pľúc rozumieme preukázaný vplyv agresívnej ventilácie (vysoké špičkové inspiračné tlaky, extrémny vplyv strižných síl pri otváraní alveol a podobne), ktoré participujú na rozvoji lokálnej zápalovej odpovede v pľúcach, následkom čoho dochádza ku excesívnej tvorbe reaktívnych molekúl a tvorbe proinflamatórnych cytokínov. Tieto závery vychádzajú z viacerých klinických štúdií, v ktorých bola porovnávaná vysokotlaková ventilácia s „protektívnym“ spôsobom ventilácie.

Ranieri M a spol. zistili, že „tradičná UVP“ (V_T 11 ml*kg^{-1}, PEEP 6.7 cm H_2O) u pacientov s ARDS je spojená s významným nárastom pľúcnej a systémovej koncentrácie proinflamatórnych cytokínov (TNF-a, IL-1b, IL-6, IL-8) v porovnaní s „protektívnym“ spôsobom ventilácie (V_T 7.6 ml*kg^{-1}, PEEP 14.8 cm H_2O). Plötz F. a spol.

zistili, že ventilačný režim (V_T 10 ml*kg^{-1}, PEEP 4 cm H_2O) už po dvoch hodinách aplikácie vedie k nárastu TNF-a a IL-6 v bronchoalveolárnej laváži u detí bez predchádzajúceho pľúcneho poškodenia. Tieto nálezy potvrdili existenciu VILI (ventilator-induced lung injury) v humánnej medicíne. Patogenetický mechanizmus biotraumy predpokladá mechanické pôsobenie umelej ventilácie na pľúca (vysoké špičkové tlaky, over-distenzia), energie dodávanej pri UVP ventilátorom, ktorá je pohltená pľúcami a aplikácie vysokých koncentrácii inspirovaného kyslíka. Tieto vplyvy vedú k funkčným zmenám buniek v pľúcnom tkanive (up-regulation) a následnému vzniku zápalovej reakcie. Zápalová odpoveď sa cytologicky prejavuje infiltráciou tkanivových štruktúr pľúc a bronchoalveolárnej tekutiny neutrofilmi (23 - 26).

Zápalová reakcia indukovaná mechanickými inzultmi v pľúcach môže zhoršovať už existujúci infekčný zápalový proces v samotných pľúcach. Cestou produkcie zápalových mediátorov môže ďalej podporiť rozširovanie zápalovej reakcie, ako aj apoptotických biochemických signálov na sekundárne orgány.

Pri ARDS a ťažkých pneumóniách je prítomné závažné poškodenie pľúcneho parenchýmu s redukciou alveolárnej plochy, čoho následkom dochádza k narušeniu výmeny plynov medzi vonkajším a vnútorným prostredím. Jedným z vážnych a ťažko riešiteľných problémov je nehomogénne poškodenie pľúcneho tkaniva, ktoré zapríčiňuje nehomogénnu distribúciu plynov. Rozdiely v časových konštantách (τ) jednotlivých kompartmentov v pľúcach sú natoľko evidentné, že pri optimálnom nastavení parametrov UVP pre jeden či dva kompartmenty, je nastavenie parametrov (frekvencia, pomer dôb Ti : Te, prietoky plynov) pre ďalšie pľúcne kompartmenty nevyhovujúce, alebo výrazne suboptimálne. Praktickým riešením je zavedenie viachladinovej (multilevel) ventilácie. Doterajšie experimentálne a klinické poznatky poukazujú na terapeutickú efektivitu viachladinovej ventilácie, ktorou je dosiahnutá redistribúcia alebo zlepšená distribúcia plynov z kompartmentov s krátkou časovou konštantou do oblastí s dlhšou časovou konštantou (24, 26 - 27).

V rámci niekoľkých pilotných projektov bol náš vedecko-lekársky tím na Klinike anestéziológie a intenzívnej medicíny vo Východoslovenskom ústave srdcových a cievnych chorôb v Košiciach, zapojený do klinických štúdií, ktorých úlohou bola aplikácia matematických ventilačných modelov do klinickej praxe pri troj-štvor hladinovej ventilácii u pacientov s nehomogénnym postihnutím pľúc. Predmetom výskumu bola ventilácia pľúc pri nehomogénnom pľúcnom poškodení a jeho prevencia a neinvazívna diagnostika. Pri týchto viachladinových ventilačných režimoch základnú ventilačnú hladinu predstavoval PS alebo PC ventilačný režim s nastavenými parametrami a to: frekvenciou dýchania, inspiračných

tlakov, pomeru Ti : Te, PEEP. Nad základnou hladinou boli na pozadí nastavené nadstavbové ventilačné režimy, a to pri trojhladinovej (3-level) ventilácii PEEPh1 a frekvencii PEEPh1. Pri štvorhladinovej (4-level) ventilácii boli nastavenia ventilátora doplnené o ďalšie parametre a to PEEPh2 a frekvencia PEEPh2. Frekvencie nadstavbových hladín ako aj aplikovaný tlak boli nižšie ako parametre základnej hladiny.

3.2 COVID - 19

V roku 2019 moderný svet začala ohrozovať globálna pandémia ochorenia COVID-19, ktorá bola spôsobená prenosom akútneho infekčného ochorenia vyvolaného koronavírusom SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2).
Aj keď COVID-19 v tom čase vykazoval nižšiu mortalitu (0,25 až 3 %) v porovnaní so SARS (10 %), MERS (34 %) alebo H1N1 (17 %). Intenzita jeho šírenia v konečnom dôsledku viedla k vysokému počtu úmrtí. V niektorých európskych krajinách dosiahla mortalita na SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) 7-10 % z celkového počtu nakazených, čo predstavovalo 10 násobne vyššiu úmrtnosť v porovnaní s infekciou chrípky. Mortalita ochorenia COVID-19 vzhľadom na ľahšie šírenie ochorenia a postihnuté vysoké percento populácie bola v absolútnych číslach vysoká (28). Ochorenie COVID-19 vedie k rozvoju celého spektra respiračných následkov s extrémne vysokou incidenciou pneumónie a syndrómu akútnej respiračnej tiesne (29).

Na Slovensku sa prvý prípad objavil 6. marca 2020. V rámci svetovej iniciatívy hľadania efektívnych preventívno-liečebných postupov bol náš vedecko-lekársky tím zapojený do grantových projektov podporovaných EU zameraných na zlepšenie programov ventilácie nehomogénnych pľúc.

Grantové projekty:

1. Operačný program Integrovaná infraštruktúra pre projekt „Návrh a implementácia pokročilých metód ventilačnej liečby a diagnostiky vírusových pneumónií vrátane COVID-19 s možnosťou ich rýchleho osvojenia“, kód 313011ASX1, akronym IPMVDCov, spolufinancovaný zo zdrojov Európskeho fondu regionálneho rozvoja v spolupráci s Ministerstvom školstva, výskumu, vývoja a mládeže Slovenskej republiky.

2. Operačný program Integrovaná infraštruktúra pre projekt „Výskum a vývoj systému zefektívnenia ventilácie pacientov s COVID-19 alebo iným nehomogénnym poškodením pľúc" kód 313011ATG9 spolufinancovaný zo zdrojov Európskeho fondu regionálneho rozvoja v spolupráci s Ministerstvom školstva, výskumu, vývoja a mládeže Slovenskej republiky.

3.3 Práce súvisiace s grantovými projektami

Práce súvisiace s grantovými projektmi boli publikované v časopise Anestéziológia a intenzívna medicína ISSN 1339-4177.

- *Rybár D, Depta F, **Kočan L**, Grendel T, Nosáľ N, Imrecze Š, Török P. Hélium a jeho možné využitie v intenzívnej medicíne. Anestéziológia a intenzívna medicína. 2023;12(2):50-54.*

- *Török P, Rybár D, Depta F, Nosáľ M, **Kočan L**, Donič V, Grendel T, Firment P, Imrecze Š, Firment J. F 0.1 (Flow in 1d.00 msec.) ako parameter ventilačného úsilia počas ventilačnej podpory. Anestéziológia a intenzívna medicína. 2023;12(2):55-57.*

- *Török P, Rybár D, Depta F, Nosáľ M, **Kočan L**, Donič V Grendel T, PhD., MUDr. Firment P, Imrecze Š, Firment J. Nehomogénne pľúcne poškodenie – teória vplyvu časového faktora na výmenu plynov v pľúcach. Anestéziológia a intenzívna medicína. 2023;12(1):11-15.*

- *Török P, Depta F, Nosáľ M, **Kočan L**, Donič V, Grendel T, Rybár D, Imrecze Š, Firment J. Základné fyzikálne, matematické a biologické princípy očisťovania dýchacích ciest pri umelej ventilácii pľúc. Anestéziológia a intenzívna medicína. 2022;11(2):51-55.*

- *Nosáľ M, **Kočan L,** Török P, Donič V, Grendel T, Rybár D, Depta F, Firment P, Imrecze Š. Fyziológia a patofyziológia problémov spojených s dlhodobou mechanickou ventiláciou pľúc. Anestéziológia a intenzívna medicína. 2022;11(1):11-14.*

- ***Kočan L**, Vašková J, Török P, Donič V, Grendel T, Nosáľ M, Rybár D, Depta F, Firment P, Imrecze Š. Terapeutické možnosti vodíka pri vybraných patologických stavoch u kriticky chorých pacientov. Anestéziológia a intenzívna medicína. 2022;11(1):15-19.*

Za doterajšie výsledky súvisiace s menovanými grantovými projektmi bola udelená nášmu lekársko-vedeckému tímu **CENA ZA TRANSFER TECHNOLÓGIÍ NA SLOVENSKU 2023** v kategórii **INOVÁCIA.** Národným portálom pre transfer technológií
Názov projektu: Zariadenie na umelú ventiláciu pľúc s identifikáciou nehomogenity distribúcie plynov a spôsob riadenia zariadenia pri umelej ventilácii pľúc. Majiteľ technológie: Univerzita Pavla Jozefa Šafárika v Košiciach (Príloha č.2).

Pôvodcovia: doc. MUDr. Pavol Török, CSc., prof. MUDr. Viliam Donič, PhD. , MUDr. Tomáš Grendel, PhD., **MUDr. Ladislav Kočan PhD.,** MUDr. Martin Nosáľ, MUDr. Dušan Rybár, PhD., MUDr. Filip Depta, MUDr. Štefan Imrecze PhD., doc. MUDr. Jozef Firment, PhD., MUDr. JUDr. Peter Firment

3.4 Oxidačný stres pri vírusovej pneumónii

Experimentálne štúdie na zvieracích modeloch zameraných na výskum priebehu akútneho respiračného syndrómu (ARDS) pri vírusových infekciách potvrdili prítomnosť excesívnej zápalovej reakcie v pľúcnom tkanive.
V priebehu týchto zložitých interakcií zohráva dôležitú úlohu vrodená imunita a aktivácia dráh súvisiacich s progresiou oxidačného stresu. Úloha polymorfonukleárov v tvorbe ROS je dlhodobo známa. Zložité interakcie vedú k aktivácii NF-kB v imunukompetentných bunkách, a to indukciou zápalu a aktiváciou signálnych dráh hostiteľskej bunky sprostredkovaných proteinkinázami p38 MAPK a zapojením neštruktúrnych 3a proteínov, kódovaných vírusovým genómom SARS-CoV a ďalších procesov, ktoré vedú k zvýšenej indukcii bunkovej apoptózy.
Ďalšie publikované práce Lin a kol. poukazujú na súvislosť medzi vírusovou infekciou a aktiváciou excesívnej tvorby ROS vo vývojovej rade bielych krviniek, promonocytov HL-CZ bunkách. Ich aktivácia je indikovaná vírusovou proteázou 3CLPro (3-chymotrypsin like protease). Tieto zložité interakcie môžu byť základom progresie vážneho poškodenia pľúc u

infikovaných jedincov, poháňaného aktiváciou dráh oxidačného stresu zložito spojených s vrodenou imunitou. Okrem zápalovej reakcie spustenej vírusovou infekciou, zvýšený prooxidačný stav v pľúcnom tkanive dopĺňa možná biotrauma pľúc spôsobená energiou prenesenou z ventilátora na tkanivá pľúc, ďalej inspirácia vysokých frakcií kyslíka ako aj celkový septický stav pacienta (30, 31).

3.5 Postpneumonická fibróza pľúc

Pľúcna fibróza je chronickou komplikáciou závažnej vírusovej pneumónie, častokrát sprevádzanej ARDS. Vo všeobecnosti sú pľúca veľmi fragilné voči rôznym noxám. Zraniteľnosť vychádza zo samotnej štruktúry pľúcneho tkaniva, ktorou je nízka hustotu buniek v pomere k objemu pľúc (32). Intersticiálne pľúcne abnormality vznikajúce v nadväznosti na ťažké postihnutie pľúc rôznej etiológie sú známou komplikáciou už z éry pred pandémiou COVID-19. Nálezy fibrotických zmien na pľúcach boli popísané u pacientov s potvrdenou infekciou SARS-CoV Severe-Acute-Respiratory-Syndrome coronavirus) alebo MERS-CoV (Middle-East-Respiratory-Syndrome coronavirus). Vo väčšine štúdií zaoberajúcich sa problematikou rozvoja post-vírusovej pľúcnej fibrózy je cytokínová búrka považovaná za primárny patogenetický mechanizmus. Celý tento stav výrazne urýchľuje prítomný oxidačný stres, pri ktorom dochádza ku amplifikácii kaskádových dejov a k ďalšiemu masívnemu uvoľňovaniu prozápalových cytokínov v rámci aberantnej zápalovej reakcie. Rozvoju pľúcnej fibrózy ďalej napomáha dysregulácia reparačných dejov (32, 33). Vírusové infekcie, medzi ktoré patrí COVID-19, vyvolávajú zmeny v bunkovom a molekulárnom prostredí pľúcneho tkaniva, čím spúšťajú nadmernú expresiu zápalových cytokínov, najmä transformujúceho rastového faktora-β1 (TGF-β), tumor nekrotizujúceho faktora-α (TNF-α) interleukín-1 (IL-1) a interleukín-6 (IL-6). Tieto mediátory stimulujú proliferáciu alveolárnych buniek typu 2 a nábor fibroblastov, čo vyvrcholí zvýšenou produkciou a ukladaním extracelulárnej matrix (ECM). Tento proces ohrozuje architektúru alveolárno-kapilárnej membrány, čo vedie k zhoršenej výmene plynov a hypoxémii. Alveolárna regenerácia po akútnom poškodení pľúc bola pozorovaná v rámci zvieracích štúdií, v ktorých sa preukázala kľúčová funkcia pneumocytov II typu (AT2). AT2 bunky ako základné štrukturálne zložky alveolárneho epitelu sú schopné sa ďalej proliferovať a diferencovať sa na pneumocyty I typu (AT1). AT1 bunky vytvárajú funkčnú štruktúru alveolov (32, 34, 35).

Vírus SARS-CoV-2 môže priamo infikovať AT2 bunky a spôsobiť ich masívnu apopotózu. Dôležitú úlohu v tomto procese zohrávajú zápalové mechanizmy. Nie je známe, či alveolárna regenerácia prebieha rovnakým spôsobom u ľudí po poškodení pľúc vyvolanom infekciou SARS-CoV-2 ako na zvieracích modeloch. Rovnako otázny je časový interval regenerácie. Pri dlhotrvajúcom a deštruktívnom poškodení pľúc dochádza v miestach poškodenia k tvorbe a hromadeniu väzivových vlákien. Jedná sa o reparačný mechanizmus, pri ktorom sa vytvára väzivové tkanivo. Charakteristická je kumulácia väziva v mieste poškodenia epitelu a endotelu s významnou redukciou funkcie pľúc a zvýšenou morbiditou. Pľúcna fibróza je bežným výsledkom väčšiny chronických zápalových pľúcnych porúch a môže mať vplyv na funkcie pľúc, čo v konečnom dôsledku vedie k respiračnému zlyhaniu a smrti.. Proces tvorby väziva závisí aj od závažnosti a trvania poškodenia, keďže dlhotrvajúce poškodenia majú tendenciu rozvinúť sa na fibrózu na rozdiel od poškodení malého rozsahu. Zistilo sa, že interakcie medzi rôznymi typmi buniek sú veľmi dôležité pre nástup fibrózy (36). Za kľúčové v tomto procese sa považujú mezenchymálne bunky a fibroblasty. Ďalšími dôležitými procesmi sú aktivácia glykolýzy vo fibroblastoch po poškodení pľúc a kaskáda enzymatických aktivácií, ktoré zvyšujú proliferáciu buniek, syntézu kolagénu a produkciu sekundárnych metabolitov, čím podporujú fibrózu. Pri aktivácii fibroblastov hrá dôležitú úlohu zvýšená glutaminolýza a oxidácia mastných kyselín. Zároveň rezidentné monocyty a makrofágy majú regulačnú úlohu v procesoch tkanivovej fibrózy. Pomáhajú iniciovať, udržiavať a upravovať poškodenie tkaniva (35, 36). Úroveň zápalovej reakcie je rozhodujúcim faktorom či dôjde k regenerácii alebo fibróze pľúcneho tkaniva. Oba procesy náhrady poškodeného tkaniva sú často sekvenčné a vzájomne prepojené. V prípade obmedzeného poškodenia sa regenerácia zvyčajne vyskytuje prioritne, aby sa obnovila integrita a funkcia tkaniva. Ak tento proces zlyhá v dôsledku vážneho poškodenia iniciuje sa tvorba väziva, ktorá môže viesť k chronickému ochoreniu pľúc alebo kolapsu. To naznačuje, že regenerácia je plnohodnotný proces, zatiaľ čo tvorba väziva je dobrá len vtedy, keď je mierna. V prípade vírusovej infekcie je zápalové poškodenie výrazné a fibróza je dominantným procesom v porovnaní s regeneráciou (34, 35).

Otázkou je reverzibilita započatej fibrogenézy jej možná prevencia, ako aj následná rekonvalescencia pacientov a kvalita života vrátane trvalých positnfekčných následkov. Táto téma je aktuálna vzhľadom na veľké percento pacientov po prekonanej COVID infekcii a ďalšej predikcie ich prognózy. Prvé skúsenosti so závažnou vírusovou pneumóniou a problematickou ventiláciou ťažko postihnutých pľúc sme získali na našom pracovisku počas epidémie prasacej chrípky H1N1. Do sledovanej skupiny pacientov v septickom šoku v rámci

štúdie Se-AOX bol v roku 2012 zaradený 30 ročný pacient so závažným priebehom vírusovej pneumónie H1N1. U pacienta bola prvýkrát použitá viachladinová ventilácia vrátane intravenóznej suplementácie selénu a glutamínu. Pacient po 60-tich dňoch opúšťa nemocnicu v zlepšenom stave avšak s ťažko postihnutými pľúcami do domácej opatery. Pacient bol ďalej sledovaný počas nasledujúcich 10 rokov, s realizovanými opakovanými RTG vyšetreniami pľúc, ktoré poukazovali na postupné zlepšenie stavu pľúcneho parenchýmu. V roku 2022 pacient tragicky zahynul a telo bolo odoslané na anatomicko-patologicku pitvu. Závery z histologických nálezov pľúcneho tkaniva poukazujú na úplnú reparáciu poškodeného tkaniva. Táto kazuistika bola publikovaná v karentovom medicínskom časopise MEDICINE Baltimore ISSN: 1536-5964 Q3, Impact Factor: 1.6.

Clinical Case Report

Medicine

OPEN

Full recovery of lung tissue after severe viral pneumonia H1N1

A case report with 10 years follow-up

Ladislav Kočan, MD, PhD[a], Jozef Firment, MD, PhD[b], Ingrid Pirníková, MD[b], Silvia Farkašová Iannaccone, MD, PhD[c], Dušan Rybár, MD, PhD[a], Juliána Gnoriková, MD[d], Ján Korček, MD[b], Hana Kočanová, MD[e], Pavol Török, MD, PhD[a], Simona Rapčanová, MD[f], Janka Vašková, Dr, PhD[g,*]

Abstract

Rationale: World healthcare frequently faced severe viral pneumonia cases in the last decades, due to pandemic situations such as H1N1, MERS-CoV, and SARS-COVID-19.

Patient concerns: The impact of viral infection on lung structure, lung function, and overall mortality was significant. The quality of life and assumed life expectancy was decreased with the supposed development of lung fibrosis in involved survived patients.

Diagnoses: We described the course and treatment of severe pneumonia H1N1 in a 30-year-old patient.

Interventions: Patient was included in a study regarding the therapeutic efficacy of selenium ClinicalTrials.gov ID: NCT02026856 with 10 years follow-up with concurrently documented X-ray lung examinations and final histology of lung tissue after sudden death.

Outcomes: All sequential examinations and histological findings show a healing trend with the final full recovery of lung tissue.

Abbreviations: AT1 = alveolar type I cell, AT2 = alveolar type II cell, CT = computed tomography, H1N1 = type of influenza A virus, swine flu, PEEP = positive end-expiratory pressure, SARS-Cov-2 = severe acure respiratory syndrome-related Coronavirus 2.

Keywords: covid-19, H1N1, lung fibrosis, pneumonia, recovery, viral infection

1. Introduction

Pneumonia causes high morbidity and mortality with high hospitalization rate worldwide. Viral pneumonias are characterized by epidemic and pandemic spread in certain time cycles. Examples are the Spanish flu (1918–1920) with an estimated 500 million infected, 50 to 100 million victims, the Asian flu (1957–1958) responsible for 1 to 1.5 million deaths, or the Hong Kong flu, which broke out in in 1969, with an estimated 750 million infected patients and up to 1 million victims.[1] In the interpandemic period, viral pneumonias are considered less serious compared to bacterial pneumonias. However, mortality from viral lung infections has increased dramatically in the last decade with the emergence of new strains of viruses such as the pandemic A type of influenza A virus, swine flu (H1N1) 2009 virus, which is a combination of swine, avian and human influenza viruses, and as well as the emergence of a new strain of the Middle East respiratory syndrome Coronavirus in the Middle East.[2] Since 2019, the human society has faced a new pandemic threat caused by the severe acure respiratory syndrome-related Coronavirus 2 (SARS-CoV-2) virus, in various evolving variants. The manifestation of the infection is the disease COVID-19 with the dominance of respiratory symptoms, including viral pneumonia.[3]

The treatment strategy of mild respiratory forms of COVID-19 to severe clinical conditions associated with respiratory

This publication is the result of the project implementation: Design and implementation of advanced methods of lungs ventilation treatment and diagnosis of viral pneumonia, including Covid-19 with the possibility of their rapid adoption", ITMS2014+: 313011ASX1 supported by the Operational Programme Integrated Infrastructure, funded by the ERDF.

Informed consent about study enrollment and follow up was obtained from participant included in the study during the first hospital admission in 2011.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

[a] Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Košice, Slovak Republic, [b] Ist Clinic of Anestesiology and Intensive Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice and Louis Pasteur University Hospital, Košice, Slovak Republic, [c] Department of Forensic Medicine, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic, [d] Department of Anaesthesiology and Intensive Care Medicine, Štefan Kukura Hospital with Policlinic, Michalovce, Slovak Republic, [e] Clinic of Anaesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Košice, Slovak Republic, [f] Europainclinics, Bardejov, Slovak Republic, [g] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic.

** Correspondence: Janka Vašková, Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Tr. SNP 1, Košice 04066, Slovak Republic (e-mail: janka.vaskova@upjs.sk).*

How to cite this article: Kočan L, Firment J, Pirníková I, Farkašová Iannaccone S, Rybár D, Gnoriková J, Korček J, Kočanová H, Török P, Rapčanová S, Vašková J. Full recovery of lung tissue after severe viral pneumonia H1N1: A case report with 10 years follow-up. Medicine 2023;102:8(e33052).

Received: 24 January 2023 / Accepted: 1 February 2023

http://dx.doi.org/10.1097/MD.0000000000033052

failure with the development of the acute respiratory distress syndrome and associated complications has brought a spectrum of new therapeutic procedures. At the same time, new data on the health status of patients after overcoming more severe forms of COVID-19 point to a new problem, which is a kind of "hidden pandemic" of post-COVID consequences. The conclusions of observational clinical studies show that up to 90% of patients hospitalized with COVID-19 have symptoms of dyspnea, reduced diffusion capacity of the lungs, related to lung tissue damage, and subsequently a decrease in physical activity after infection. In more than 50% of patients symptoms disappear within 3 months, while it is assumed that these patients are going to have a complete regeneration of lung tissue within 9 months after the end of hospitalization. A significant part of the population has some pre-disease lung tissue damage already, and almost 10% of all patients show fibrotic lung damage even before the infection of COVID-19. Predisposed patients are exposed to a greater risk of developing post-COVID fibrotic remodeling of the lung parenchyma. The 2% to 6% incidence of post-COVID pulmonary fibrosis can be estimated after moderate respiratory involvement. The rate of lung remodeling increases with the severity of the disease course.[4] However, not all clinically serious pneumonias caused by viruses lead immediately to fibrosis.

The case report shows a retrospective view of the regenerative processes of the patient's lung parenchyma after an H1N1 infection with acute respiratory distress syndrome in the acute phase of the disease, his physical lung computed tomography (CT) and X-ray findings during the next 10 years of follow-up, and histological findings of lung tissue from the patient's autopsy.

2. Case report

A 30-year-old patient hospitalized in January 2011 at the Intensive Care Unit of University Hospital due to bilateral pneumonia presented with respiratory failure with an oxygenation index < 200. At admission, intubation and initiation of controlled ventilation was started. Pressure controlled ventilation with fraction of inspired oxygen of 0.8, positive end-expiratory pressure (PEEP) 9 cm H_2O, pressure controlled ventilation 22 cm H_2O and minute ventilation 12 l/minute was applied along with sedation, continuous administration of muscle relaxants, and prone positioning. Vasopressor support with a norepinephrine dose of up to 0.15 µg/kg/minute was necessary to stabilize circulation. Empirical antibiotic treatment and anticoagulation therapy was started (Fig. 1).

Viral infection of influenza A type H1N1 was diagnosed by the PCR method, thus a virostatic agent Oseltamivir was added. Restrictive fluid administration was maintained daily. Antifungal agent Voriconazol was later added for the positive galactomannan test. The patient's condition deteriorated as he developed severe sepsis. *Pseudomonas aeruginosa* was confirmed from the bronchoalveolar lavage leading to the adjustment of antibiotic treatment (Fig. 1) and adding immunomodulation therapy with intravenous immunoglobulin and Polyoxidonium. On the 5th day the so called programmed multilevel ventilation mode performed by the Chirana AURA V (Slovakia) ventilator was applied for the first time with basic PEEP level 7 to 9 cm H_2O, pressure control 12 to 10 cm H_2O, frequency of 22 to 23/minute, upper PEEP level of 5 to 6 cm H_2O was applied 4 to 5 times per minute, resulting in minute ventilation of 12 to 8 l/minute. Cortisol therapy started from the 5th day of hospitalization in a daily dose of 200 mg, later reduced to 100 mg, followed by reduction to 50 mg per day during the overall 15-day corticosteroid therapy. On 5th day, selenium adjuvant therapy started too. Patient was enrolled in the study Se-AOX approved by the ethical committee of Pavol Jozef Šafárik University in Košice under number 109/2011; ClinicalTrials.gov Identifier: NCT02026856. Selenium in the form of sodium selenite pentahydrate at 750 mg/day for 6 days as a continual infusion was supplemented (1000 mg of sodium selenite pentahydrate = 333 µg of selenium) (Selenase, Vivax). During the severe sepsis, renal insufficiency developed with oliguria and an increase in blood levels of the urea and creatinine which necessitated the initiation of continuous veno-venous hemodialysis. Diarrhea developed 11 days from admission.

In the next course, the patient´s lung compliance and resistance started to improve. A gradual ventilation weaning was started by switching the 3-level ventilation to pressure control and later to pressure support ventilation mode with Pps 10 to 6 cm H_2O, PEEP 7 to 5 cm H_2O and minute ventilation 10-7 l/minute. On the 14th day a CT scan of the lungs showed bilaterally present diffuse opacities of the milk glass type with thickening of the interlobular septation (Fig. 2). Peribronchovascular irregular consolidations were present in the upper and posterobasal lung segments of the lower lung lobes with a negative bronchogram. In superposition with the described changes, there was a suspicion of micro- and macrocystic remodeling of the lung parenchyma (so called honeycombing) on the periphery of the left lingular lung segments and the right medial segment of the middle lung lobe. The pleural spaces were free from fluid effusions. On the 15th day of hospitalization, percutaneous dilatation tracheostomy was performed. 17 days after admission, clipping was attempted for gastrointestinal bleeding from a duodenal ulcer- unsuccessfully, therefore, and a laparotomy with successful duodenotomy was performed in the D2 to 3 region. In the postoperative period, paralytic ileus developed and was treated conservatively. Subsequently, enteral nutrition was started once again. In the next course of hospitalization, repeated attempts to wean the patient from controlled ventilation were made. Concurrently, withdrawal symptoms appeared after long-term application of opioids, therefore, and neuroleptics were started. The patient gradually improved and was able to breathe spontaneously for several hours a day through a T-tube with intermittent pressure support ventilation. Continuous renal replacement therapy was switched to intermittent hemodialysis.

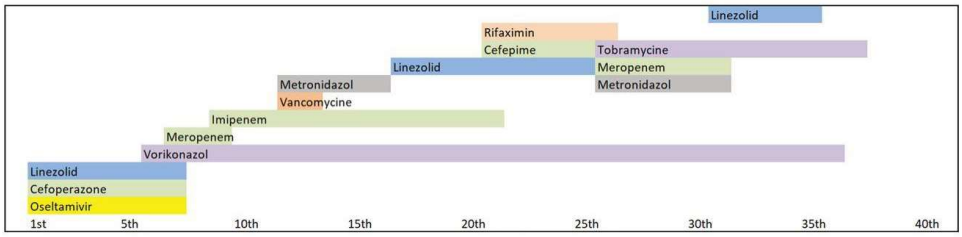


Figure 1. Time sequence of dynamic changes in antimicrobial treatment according to empirical experiences of the Intensive Care Unit, prospectively medication followed according to microbial culture and its sensitivity, and individual patient toleration to drugs.

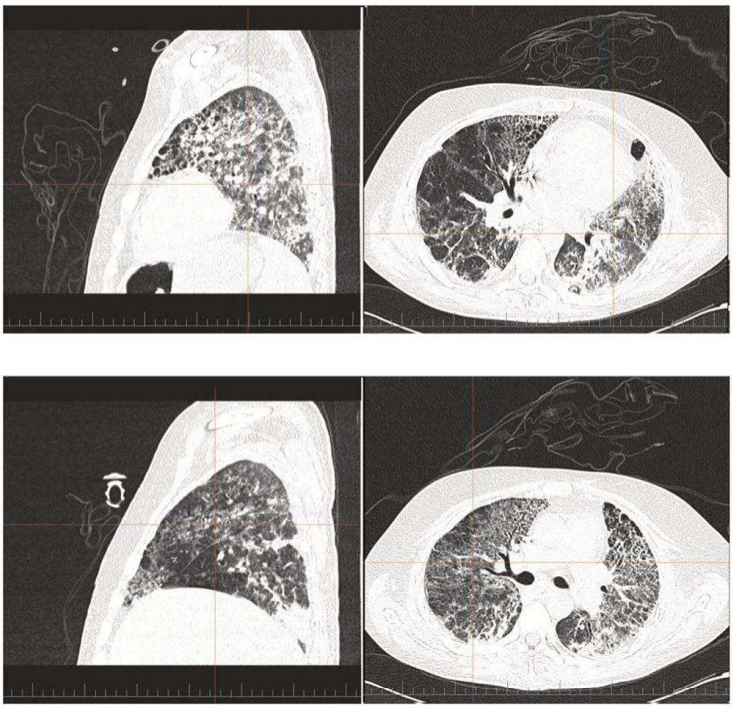


Figure 2. CT sagittal and a coronal view of lungs. In the upper row, the red cross marker is focused on the left lung. In lower row, the red cross marker is focused on the right lung. CT = computed tomography.

After repeated negative cultures from biological samples, antibiotic treatment was terminated. At that time, the patient was on an oral full-fledged diet and adhered to a complex rehabilitation process. In the following days, the patient developed a polyuric phase of acute kidney injury followed by a gradual recovery of renal functions. On the 47th day, the patient was transferred to the intensive care unit of the regional hospital. The patient further improved and was discharged to outpatient care.

In 2012 and 2014 the patient was treated by the emergency medical service for difficult breathing leading to admission to the local hospital's internal medicine department.

In April 2018, the patient was hospitalized in the local hospital's intensive care unit due to acute pancreatitis as a result of excessive alcohol consumption, confirmed by laboratory and CT examination. The condition was complicated by the development of disorientation, agitation, and delirium, which necessitated the use of sedatives and antipsychotics. Despite the treatment, the condition did not improve, furthermore, and respiratory insufficiency developed. The patient was intubated and controlled ventilation was started and vasopressors were temporarily used. The following day, the symptoms improved, sedation was gradually discontinued, ventilation support was reduced and finally the patient was extubated. He was transferred to the surgical department. The control X-ray of the chest from this period describes findings without serious pathology, presenting indistinct irregularities of the course bronchovascular pattern in the lower lung fields (Fig. 3). The patient was discharged home after 7 days. Control X-ray examination in following 2-years period shows Figure 4.

In November 2021, the patient was transported by the emergency medical service to the local hospital's emergency department after falling on the street. Coincidentally, a diagnosis of COVID-19 by antigen testing was made. The patient was examined by a traumatologist, a neurologist, and an internist. A small subarachnoid hemorrhage was found on the left temporal side on the head CT scan. Laboratory results showed a significant elevation of hepatic enzymes, leukopenia, thrombocytopenia, moderate elevation of C-reactive protein, D-dimers, and fibrinogen. Abdominal ultrasound revealed cirrhosis of the liver. The patient had repeated grand mal seizures and developed delirium. In the evening, a cardiac arrest suddenly occurred. Immediately, advanced cardiopulmonary resuscitation was started but was unsuccessful and the patient died. An autopsy was indicated.

Autopsy was performed at the Medico-Legal and Pathological-Anatomical Department of Health Care Surveillance Authority in Košice within 24 hours after death. Death was attributed to severe cerebral edema following traumatic brain injury (subarachnoid hemorrhage, focal contusions of the brain). The right and left lungs weighted 535 g and 515 g respectively. The pleural surfaces were smooth with a mild accumulation of anthracotic pigment deposited along lymphatic routes in the pleura (anthracosis). The upper lobes demonstrated dilatation of air spaces; the lower lobes were dry, firm, airless and consolidated. The parenchyma of the lower lobes was pink-red to red-purple in color with a meaty appearance and showed no expression of fluid from the cut surfaces upon compression (Fig. 5). The bronchi were white-gray with no sign of fluid collection. Histological examination of hematoxylin-eosin stained sections taken from both lungs

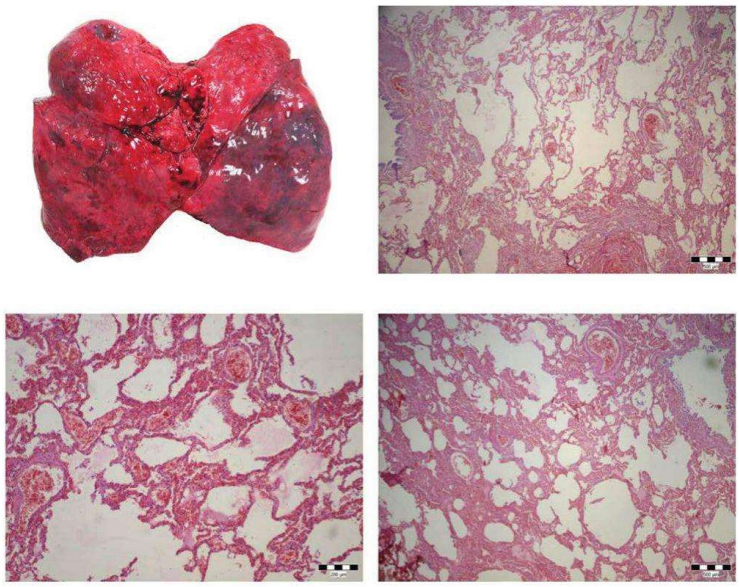


Figure 5. A: Lungs: the upper lobes with dilatation of air spaces; the lower lobes were dry, firm, airless and consolidated. B: Lungs: acute congestion, focal hemorrhage, focal emphysema, mild alveolar edema, scant interstitial lymphoplasmacytic infiltrate, some interstitial fibrosis (hematoxylin and eosin, x40). C: Lungs: acute congestion, hemorrhage, mild alveolar edema, focal emphysema (hematoxylin and eosin, x100). D: Lungs: acute congestion, focal hemorrhage, focal emphysema, mild alveolar edema, scant interstitial lymphoplasmacytic infiltrate, some interstitial fibrosis (hematoxylin and eosin, x40).

patients indicate that signs of pulmonary fibrosis may subside over time.[4] The mentioned case shows the high regenerative capacity of the lungs after overcoming severe viral pneumonia, while the initial prognostic assumption was the fibrotic remodeling of the lung tissue. This topic is relevant due to the large percentage of patients who have overcome the COVID infection and the further prediction of their prognosis.

In general, lungs are very fragile against various noxae. Vulnerability stems from the very structure of the lung tissue, which is the low density of cells in relation to the lung volume. Lung function depends on the arrangement of cells forming alveolar septa.[5] The entry gate of the SARS Cov-2 virus into the cells of the respiratory system is the membrane receptor for angiotensin-converting enzyme 2 or the membrane protein transmembrane serine protease 2. The virus is able to infect alveolar type I cells (AT1) and alveolar type II cells (AT2). It is believed that only a small population of AT2 cells express the angiotensin-converting enzyme 2 receptor on their surface.[6] The most vulnerable cells are located on the surface of the alveoli.[7] Damage to AT1 stimulates the rapid proliferation and differentiation of AT2 cells, which also regenerates the tissue barrier function.[5] Alveolar regeneration after acute lung injury has been observed in animal studies demonstrating the key function of AT2 cells. AT2 cells, as basic structural components of the alveolar epithelium, are able to further proliferate and differentiate into AT1 cells. AT1 cells form the functional structure of the alveoli. However, evidence of lung tissue regeneration in humans is absent, probably due to the lack of lung samples obtained. The SARS-CoV-2 virus can directly infect AT2 pneumocytes and cause their massive apoptosis. Inflammatory mechanisms play an important role in this process. It is not known whether alveolar regeneration occurs in the same way in humans after lung injury induced by SARS-CoV-2 infection as in animal models. The time interval of regeneration is also questionable. In rodent animal models, lung regeneration and recovery of function takes several weeks.[8]

Studies dealing with the recovery of respiratory functions in humans point to processes that last several years, which implies a longer onset of differentiation of AT2 cells, in contrast to animal studies on rodents, in which this process lasts only a few weeks.[8] In case of long-lasting and destructive damage to the lungs, the formation and accumulation of fibrous connective tissue (fibrosis) occurs in the places of damage. It is a reparative mechanism in which fibrous tissue is formed. Accumulation of fibrous tissue at the site of damage to the epithelium and endothelium with a significant reduction in lung function and increased morbidity is characteristic. Pulmonary fibrosis is a common outcome of most chronic inflammatory lung disorders and can affect lung function, ultimately leading to respiratory failure and death.[9] The process of fibrous tissue formation also depends on the severity and duration of the injury, as long-lasting injuries tend to develop into fibrosis in contrast to small-scale injuries. Interactions between different cell types have been found to be very important for the onset of fibrosis.[8] Mesenchymal cells and fibroblasts are considered to be key in this process. Other important processes are the activation of glycolysis in fibroblasts after lung injury and a cascade of enzymatic activations that increase cell proliferation, collagen synthesis, and production of secondary metabolites, thereby promoting fibrosis. Increased glutaminolysis and fatty acid oxidation play an important role in the activation of fibroblasts. At the same time, resident monocytes and macrophages have a regulatory role in tissue fibrosis processes. They help initiate, maintain, and repair tissue damage.[8,10]

The level of the inflammatory response is a decisive factor whether regeneration or fibrosis occurs. Both processes of damaged tissue replacement are often sequential and interconnected. In the case of limited damage, regeneration usually occurs preferentially to restore tissue integrity and function. If this process fails due to severe damage, fibrous tissue formation is initiated, and which can lead to chronic lung disease or collapse. This

suggests that regeneration is a full-fledged process, while fibrous tissue formation is only good when it is moderate. In the case of viral infection, and inflammatory damage is prominent and fibrosis is the dominant process before regeneration.[10,11] Factors such as the amount and types of cells that are damaged, the disruption of barrier function, the intensity and duration of the local immune response can be a predictor of whether the damaged lung regenerates ad integrum or the formation of fibrous tissue leads to chronic lung disease.[5] Study Se-AOX, in which this patient was also included, pointed out, for example, to a significant improvement of respiratory functions quantified on the basis of Carrico index in early selenium adjuvant therapy.[12]

4. Conclusion

The case report of a patient affected by severe viral pneumonia points to an example of possible complete regeneration of lung tissue, despite severe lung damage. Pathological changes in cellular organization within reparations include the formation of fibrous connective tissue, which inevitably changes the critical structure of lung properties and leads to poorer lung function. However, clinical experience and several studies have shown that the respiratory system has an extensive capacity to respond to noxae by regenerating damaged cells or proliferating and differentiating progenitor cells or changing the function of already existing differentiated cells. Pathological studies show that in damaged lung tissue, AT2 cells are the most extensive proliferating population in severely damaged lungs. Alveolar regeneration can be initiated especially in patients with less severe viral pneumonia.

Author contributions

Conceptualization: Ladislav Kočan, Jozef Firment, Pavol Török, Janka Vašková.
Data curation: Ladislav Kočan, Jozef Firment, Ingrid Pirniková, Silvia Farkašová Iannaccone, Dušan Rybár, Juliána Gnoriková, Ján Korček, Pavol Török.
Formal analysis: Ladislav Kočan, Ingrid Pirniková, Silvia Farkašová Iannaccone, Dušan Rybár, Juliána Gnoriková, Ján Korček, Hana Kočanová, Pavol Török, Simona Rapcanova, Janka Vašková.
Investigation: Ladislav Kočan, Jozef Firment, Janka Vašková.
Methodology: Ladislav Kočan, Juliána Gnoriková, Ján Korček.
Supervision: Jozef Firment, Silvia Farkašová Iannaccone.
Validation: Ladislav Kočan, Jozef Firment, Ingrid Pirniková, Simona Rapcanova.
Writing – original draft: Ladislav Kočan, Silvia Farkašová Iannaccone, Dušan Rybár, Hana Kočanová, Pavol Török, Janka Vašková.
Writing – review & editing: Ladislav Kočan, Jozef Firment, Silvia Farkašová Iannaccone, Hana Kočanová, Simona Rapcanova, Janka Vašková.

References

[1] Akin L, Gözel MG. Understanding dynamics of pandemics. Turk J Med Sci. 2020;50:515–9.
[2] Al-Baadani AM, Elzein FE, Alhemyadi SA, et al. Characteristics and outcome of viral pneumonia caused by influenza and Middle East respiratory syndrome-coronavirus infections: a 4-year experience from a tertiary care center. Ann Thorac Med. 2019;14:179–85.
[3] McDonald LT. Healing after COVID-19: are survivors at risk for pulmonary fibrosis? Am J Physiol Lung Cell Mol Physiol. 2021;320:L257–65.
[4] Kotton DN, Morrisey EE. Lung regeneration: mechanisms, applications and emerging stem cell populations. Nat Med. 2014;20:822–32.
[5] Lucas A, Yasa J, Lucas M. Regeneration and repair in the healing lung. Clin Transl Immunology. 2020;9:e1152.
[6] Zou X, Chen K, Zou J, et al. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med. 2020;14:185–92.
[7] Zhao F, Ma Q, Yue Q, et al. SARS-CoV-2 infection and lung regeneration. Clin Microbiol Rev. 2022;35:e0018821.
[8] Chen J, Wu H, Yu Y, et al. Pulmonary alveolar regeneration in adult COVID-19 patients. Cell Res. 2020;3:708–10.
[9] Kobayashi Y, Tata A, Konkimalla A, et al. Persistence of a regeneration-associated, transitional alveolar epithelial cell state in pulmonary fibrosis. Nat Cell Biol. 2020;22:934–46.
[10] Gupta SK, Srivastava M, Minocha R, et al. Alveolar regeneration in COVID-19 patients: a network perspective. Int J Mol Sci. 2021;22:11279.
[11] Yu M, Liu Y, Xu D, et al. Prediction of the development of pulmonary fibrosis using serial thin-section CT and clinical features in patients discharged after treatment for COVID-19 pneumonia. Korean J Radiol. 2020;21:746–55.
[12] Kočan L, Vašková J, Vaško L, et al. Selenium adjuvant therapy in septic patients selected according to carrico index. Clin Biochem. 2014;47:44–50.

4 ÚLOHA OXIDAČNÉHO STRESU V CHRONICKEJ BOLESTI

Oxidačný stres sa podieľa na rozvoji mnohých patologických stavov vrátane syndrómov spojených s chronickou neuropatickou bolesťou. Neuropatická bolesť vzniká z komplexnej súhry viacerých mechanizmov vrátane periférnej a centrálnej senzitizácie, ektopickej aktivity v primárnych aferentných vláknach, zmien v regulácii iónových kanálov, ako aj nerovnováhy medzi excitačnou a inhibičnou intercelulárnou a extracelulárnou signalizáciou. Pribúdajúce dôkazy odhalili, že „zápal nervového systému (angl. neuroinflammation – ďalej len neurozápal)" a oxidačná dysfunkcia zohrávajú rozhodujúcu úlohu pri indukcii a udržiavaní neuropatickej bolesti. Závery experimentálnych prác poukazujú na prítomnosť vysokých koncentrácií oxidačne aktívnych molekúl, ktoré sú medziproduktami oxidačno-redukčných reakcií v poškodených tkanivách v súvislosti s prítomnou neuropatickou bolesťou (37, 38).

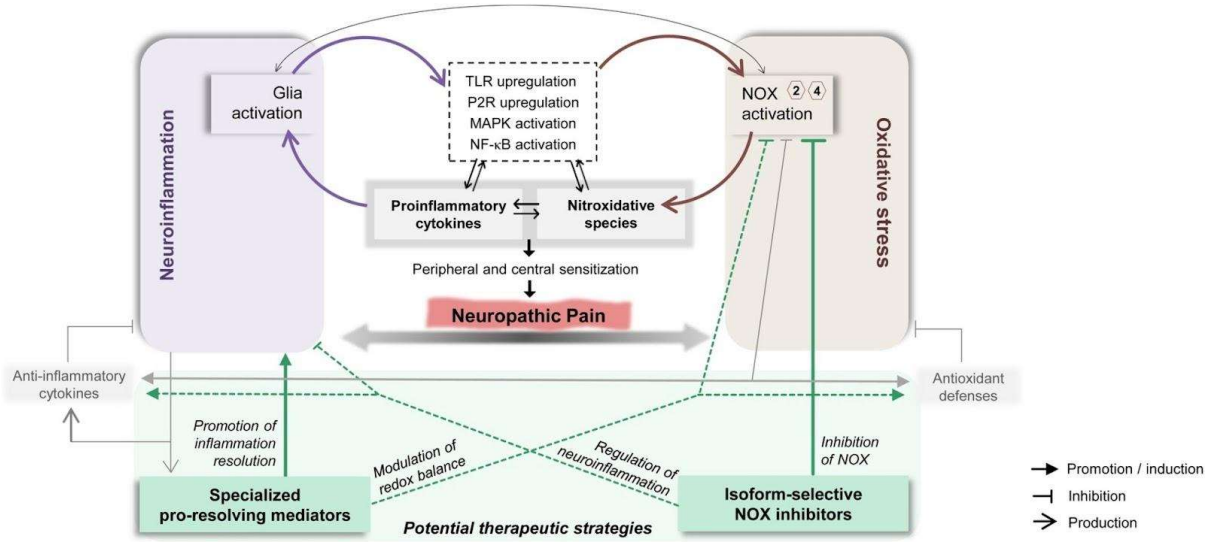
Oxidačný stres môže vyvolať negatívne zmeny najmä v centrálnom nervovom systéme vzhľadom na vysokú zraniteľnosť neurónov a gliových buniek voči oxidačným vplyvom a metabolickým zmenám, ktoré prebiehajú v rámci neurozápalu. Z experimentálnych výskumov vyplýva, že oxidačný stres hrá dôležitú úlohu v rámci viacerých mechanizmov, ktoré sú zapojené do nociceptívnej modulácie, centrálnej senzitizácie a priebehu neurozápalovej odpovede.

Vo všeobecnosti zápal predstavuje základnú biologickú reakciu, ktorá je prísne regulovaná súhrou rôznych mechanizmov, pričom jej cieľom je obnovenie integrity tkaniva. Neurozápal je forma zápalovej reakcie, ktorá sa týka periférnej a centrálnej nervovej sústavy. Je charakterizovaný zvýšenou vaskulárnou permeabilitou, infiltráciou tkaniva imunitnými bunkami, aktiváciou gliových buniek, šírením genetickej proinflamačnej informácie makrofágmi do iných častí nervového systému a jeho okolia a zvýšenou produkciou zápalových mediátorov v nervovom tkanive. Dôležitým prooxidačným enzýmom v neurozápalových reakciách je NADPH oxidáza (NOX), ktorá katalyzuje premenu kyslíka na superoxidový anión radikál podľa rovnice:

$$NADPH + 2O_2 \leftrightarrow NADP^+ + 2O_2^- + H^+$$

Ďalším významným enzýmom je enzým NO-syntáza (NOS) katalyzujúca reakciu, ktorej výsledkom je reaktívna forma dusíka (RNS), NO. Tieto molekuly, podobne ako reaktívne

formy kyslíka, majú okrem významných prooxidačných vlastností aj dôležitú signalizačnú funkciu. V rámci patofyziologických ciest vzniku neuropatickej bolesti NOS zvyšujú excitabilitu nervových vlákien, a to prevažne nepriamo, cestou mitochondriálneho poškodenia (38, 39).



Obrázok č.3: Prehľad interakcií a vzájomných vzťahov medzi neurozápalovými mechanizmami a oxidačným stresom pri rozvoji chronickej bolesti. TLR - Toll-like receptor, P2R - Purinérgny 2 receptor, MAPK - mitogénom aktivovaná proteinová kináza, NF-κB - nukleárny faktor kappa B (Zdroj: Teixeira-Santos 2020).

Zápalové reakcie sú riadené komplexnou sieťou regulačných mechanizmov, ktoré kontrolujú priebeh, intenzitu a supresiu škodlivých dôsledkov zápalu. Za fyziologických okolností je zápalová kaskáda po dosiahnutí obranných alebo reparačných cieľov potlačená biochemickými a imunitnými mechanizmami. Ak však tieto mechanizmy regulácie zlyhajú, môže vzniknutá situácia viesť k dlhodobej a nekontrolovateľnej zápalovej reakcii, čo má za následok rozvoj chronických stavov, medzi ktoré patrí aj rozvoj chronickej neuropatickej bolesti.

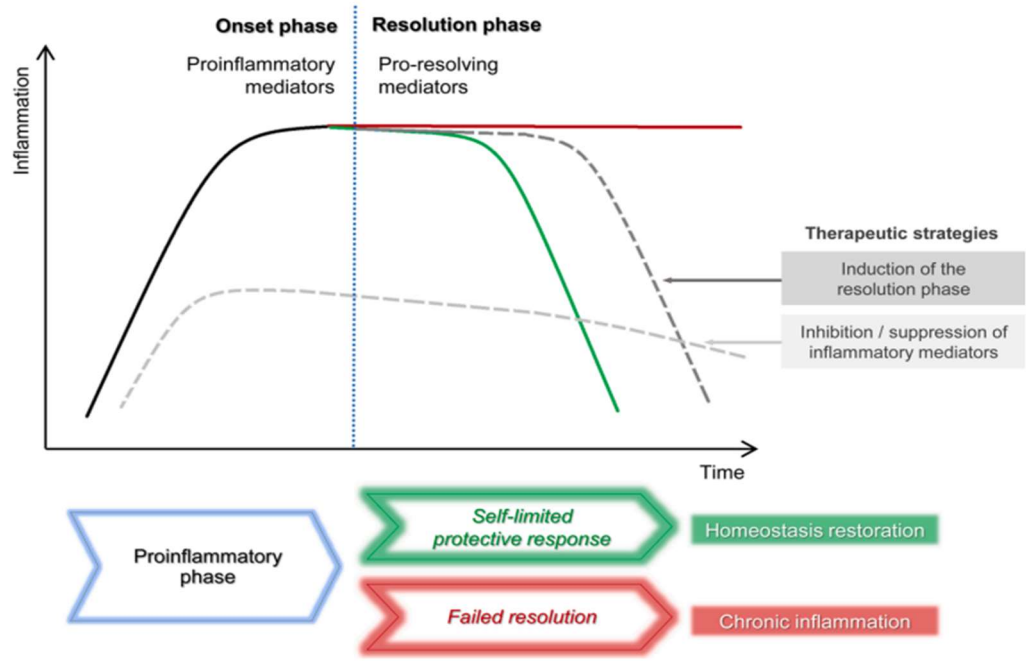
Pribúdajúce dôkazy poukazujú na to, že v procesoch regulácie zohrávajú úlohu viaceré významné molekuly akými sú špecializované „pro-resolving" mediátory, medzi ktoré patria deriváty odvodené od kyseliny arachidonovej (lipoxíny) a mediátory odvodené od polynenasýtených mastných kyselín: kyselina eikozapentaénová, kyselina dokozapentaénová a kyselina dokozahexaénová (resolvíny, maresíny a protektíny). Tieto molekuly sú významné inhibítory NOX a ich deficit sa spája s vyššou intenzitou neurozápalovej odpovede (38, 39).

Liečba chronickej bolesti je komplexná. Opiera sa o komplexnú farmakologickú, intervenčnú a nefarmakologickú liečbu, a však naďalej sa hľadajú ďalšie efektívne možnosti terapie.

Nové experimentálne štúdie poukazujú na možnosti ovplyvnenia neurozápalu aj ďalšími cestami v rôznych fázach zápalovej reakcie. Tieto terapeutické prístupy je možné rozdeliť do dvoch hlavných línií, z ktorých každá sa zameriava na odlišné fázy zápalového procesu.

Intervencie konvenčnej protizápalovej liečby sa primárne zameriavajú na inhibíciu prozápalových mediátorov za účelom zmiernenia symptómov a obmedzenia zápalovej reakcie. Jedná sa o aplikáciu potentných antioxidantov v priebehu celej periódy zápalovej reakcie.

Stratégie „pro-resolution": Na rozdiel od nešpecifickej všeobecnej liečby, stratégie „pro-resolution" sú zamerané na protizápalové procesy, uľahčenie včasnej obnovy tkanivovej homeostázy a minimalizáciu rizika vzniku chronického zápalu. Do tejto línie liečby sa radí skupina tzv. pro-resolving mediátorov (PRM), ktoré pôsobia ako aktívne inhibítory syntázy oxidu dusnatého (NOX). PRM majú schopnosť supresiou modulovať zápalovú odpoveď a tým brániť možnej chronifikácii bolesti.



Obrázok č.4: Zobrazenie jednotlivých fáz neurozápalovej odpovede v patogenéze neuropatickej bolesti (Zdroj: Teixeira-Santos, 2020).

Ďalšou významnou antioxidačnou molekulou, ktorá potláča nežiadúce oxidačno-redukčné deje v rámci neurozápalu a pozitívne ovplyvňuje priebeh chronifikácie bolesti je glutatión. Glutatión hrá kľúčovú úlohu pri ochrane nervových buniek pred poškodením a degeneráciou. Pomáha udržiavať štrukturálnu integritu a funkciu neurónov, čím predchádza alebo zmierňuje poškodenie nervov spôsobené rôznymi noxami, akými sú napríklad oxidačný stres, zápalové reakcie a neurotoxické látky. Zachovaním fyziologickej integrity nervových štruktúr môže glutatión zmierniť symptómy neuropatickej bolesti a podporiť regeneráciu nervov
Ďalším mechanizmom, ktorým glutatión nepriamo ovplyvňuje moduláciu a percepciu bolesti je zapojenie sa do regulačných procesov glutamátergného prenosu. Glutamát je kľúčový neurotransmiter zapojený do signalizácie bolesti a dysregulácia glutamátergného prenosu prispieva k rozvoju neuropatickej bolesti. Ukázalo sa, že glutatión moduluje hladiny a aktivitu glutamátu, čím ovplyvňuje vnímanie a prenos bolesti. Reguláciou glutamátovej neurotransmisie môže glutatión zmierniť hypersenzitivitu neuropatickej bolesti. Glutatión sa ďalej podieľa na regulácii endogénnych systémov modulácie bolesti, ako je napríklad endogénny opioidný systém. Zvýšením aktivity týchto systémov môže glutatión prispieť k zmierneniu symptómov neuropatickej bolesti (38, 39).

Prehľadový článok o charakteristike glutatiónu a jeho potenciálnych terapeutických účinkoch publikoval náš lekársko-vedecký tím v časopise Molecules Q2, IF 4.6.

- *Vašková J, Kočan L, Vaško L, Perjési P. Glutathione-Related Enzymes and Proteins: A Review. Molecules. 2023 Feb 2;28(3):1447. doi: 10.3390/molecules28031447. PMID: 36771108; PMCID: PMC9919958.*

Vplyv oxidačného stresu a možnosti jeho supresie pri rozvoji neurozápalu, neuropatickej bolesti a pooperačnej kognitívnej dysfunkcie u pacientov po kardiochirurgických a nekardiochirurgických výkonoch skúmame v aktuálne prebiehajúcej prospektívnej observačnej klinickej štúdii NeuOX-postSurg Trial NCT06391866.

molecules

MDPI

Review

Glutathione-Related Enzymes and Proteins: A Review

Janka Vašková [1,*], Ladislav Kočan [2], Ladislav Vaško [1] and Pál Perjési [3,*]

[1] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, 040 11 Košice, Slovakia
[2] Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, 040 11 Košice, Slovakia
[3] Institute of Pharmaceutical Chemistry, University of Pécs, 7600 Pécs, Hungary
* Correspondence: janka.vaskova@upjs.sk (J.V.); pal.perjesi@gytk.pte.hu (P.P.); Tel.: +42-155-234-3232 (J.V.)

Abstract: The tripeptide glutathione is found in all eukaryotic cells, and due to the compartmentalization of biochemical processes, its synthesis takes place exclusively in the cytosol. At the same time, its functions depend on its transport to/from organelles and interorgan transport, in which the liver plays a central role. Glutathione is determined as a marker of the redox state in many diseases, aging processes, and cell death resulting from its properties and reactivity. It also uses other enzymes and proteins, which enables it to engage and regulate various cell functions. This paper approximates the role of these systems in redox and detoxification reactions such as conjugation reactions of glutathione-S-transferases, glyoxylases, reduction of peroxides through thiol peroxidases (glutathione peroxidases, peroxiredoxins) and thiol–disulfide exchange reactions catalyzed by glutaredoxins.

Keywords: cell; redox homeostasis; glutathione; glutathionylation; glutathione system; glutathione enzyme

Citation: Vašková, J.; Kočan, L.; Vaško, L.; Perjési, P. Glutathione-Related Enzymes and Proteins: A Review. *Molecules* **2023**, *28*, 1447. https://doi.org/10.3390/molecules28031447

Academic Editor: Hyun-Ock Pae

Received: 15 January 2023
Revised: 30 January 2023
Accepted: 31 January 2023
Published: 2 February 2023

1. Introduction

Glutathione (GSH) was first isolated in 1888 by De-Rey-Pailhade. He named the substance *phylothion*, the Greek expression for sulfur loving [1]. Its structure was controversial for several years. Initially, it was described as a sulfur-containing dipeptide [2]. Later the structure was refined, demonstrating that the substance is a tripeptide, γ-Glu-Cys-Gly [3–5]. Other related compounds, such as γ-Glu-Cys-Gly-spermidine and $(\gamma\text{-Glu-Cys})_n$-Gly in *E. coli* and plants, were also described [6]. The thiol group of the cysteine residue enables GSH to function as both a reducing agent and a nucleophilic center [7]. Glutathione occurs in two free forms: the reduced (GSH) thiol and the oxidized (GSSG) disulfide forms (Figure 1). In addition, it can be bound to proteins and other thiols, affecting their activity. In its reduced and oxidized forms (GSH, GSSG), glutathione is ubiquitous in mammalian cells ranging in 1–10 mM concentrations [8]. Under physiological conditions, more than 98% of total GSH occurs in the reduced form [9,10]. It is an essential antioxidant against reactive oxygen and nitrogen species [11]. The compound plays a critical role in maintaining the redox homeostasis of the cells and in cell cycle regulation, apoptosis, immunological defense, and pathological abnormalities [8]. Furthermore, it is one of the endogenous substances involved in the metabolism of endogenous (e.g., estrogens, leukotrienes, prostaglandins) and exogenous compounds (e.g., drugs, non-energy-producing xenobiotics) [12]. These latter transformations could be the molecular basis for eliminating foreign substances from the body. In this review, the role of glutathione and glutathione-dependent enzymes in the maintenance of redox homeostasis is summarized.

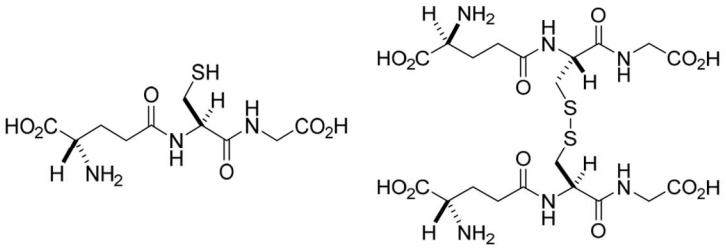


Figure 1. Structure of reduced (GSH) and oxidized (GSSG) forms of glutathione.

2. Glutathione

Together with glutaredoxins (Grx), GSH acts to reduce disulfide bonds and is, in turn, oxidized to glutathione disulfide (GSSG), which is reduced by NADPH-dependent glutathione reductase. The GSH/GSSG, NADPH/$NADP^+$, Grx-SH/Grx-SS, and Trx-SH/Trx-SS are the most important redox couples in maintaining cellular redox homeostasis [13]. The standard apparent redox potential (E'^o) of GSH is −288 mV (pH 7, 298.15 K, 0.25 M ionic strength), which is well between the most negative H^+/H_2 (−423 mV) and the most positive, O_2/H_2O (+849 mV) redox couples [14]. Accordingly, the GSH/GSSG redox couple can readily interact with most physiologically relevant redox couples, undergoing reversible oxidation or reduction [7].

Given the availability of glutathione in the cells, the reactions of protein thiols are mediated by multiple enzymes and enzyme systems, thus allowing it to participate in the abovementioned functions and regulatory pathways. Among them are glutaredoxins, which are central in the response against oxidative stress as the biological activity of many proteins are modified by the formation of GSH-mixed disulfides. Furthermore, other redox-maintaining enzymes such as glutathione peroxidases, and detoxification enzymes, glyoxylases, are closely related to carbohydrate metabolism [15,16]. Thus, the involvement of glutathione and its activity in the cell represents a wide range of biological and biochemical processes. The consequence of its deficiency results in increased stress conditions, which is the basis of the pathophysiology of many organ or tissue-specific diseases such as inflammation, virus infections (HIV), sickle cell anemia, cancer, diabetes, heart attack, stroke, liver disease, cystic fibrosis, Alzheimer's, and Parkinson's disease [17,18].

2.1. The Role of the Liver in Glutathione Synthesis and Distribution

Synthesis of GSH occurs in the cytoplasm in all cells in two subsequent ATP-dependent reactions catalyzed by glutamate-cysteine ligase and GSH synthetase, from where it is transported to other organelles and extracellular space [8,19]. Glycine, glutamate, and cysteine as nonessential amino acids can be obtained from dietary sources or synthesis.

The liver removes a significant amount of resorbed cysteine from the portal vein [20]. However, cysteine can be synthesized by methionine transsulfuration in the liver [21]. The liver is responsible for the metabolism of up to half of the daily methionine intake, predisposing the liver to almost exclusive transsulfuration activity and being the most important in interorgan GSH homeostasis [22]. Thus, a considerable amount of GSH is produced by the liver and released into plasma and bile [22]. Rat liver cytosolic GSH has a half-life of 2–3 h [8], and the daily turnover for GSH is estimated to be higher than cysteine turnover in the body protein pool, around 40 mmol per day [21]. Transsulfuration is not present in the fetus, newborn infants, or patients with cirrhosis [23]. Cirrhosis causes a decrease in methionine adenosyltransferase activity following a reduction in S-adenosylmethionine production and lower effectivity of the transsulfuration pathway [24]. Glutathione concentration within extracellular fluids and blood plasma reaches only several μM; however, in some extracellular fluids, such as lung lining fluid, 100–400 μM levels have been detected [25,26].

2.2. Cell Uptake and Metabolism of Glutathione

To date, two mechanisms of glutathione uptake into mammalian cells are known [19]. The most common one is primarily associated with the activity of γ-glutamyl transpeptidase (GGT) (Figure 2). GGT is localized to the cell surface and cleaves only extracellular substrates, GSH, and oxidized GSH (GSSG), its most abundant ones. The amide bond between the glutamine γ-carboxyl and the cysteine amino units does not allow cleavage of GSH by cellular and circulating serum peptidases [27]. It is hydrolyzed by the γ-glutamyltranspeptidase (GGT) to glutamate and Cys-Gly. Cys-Gly can be cleaved by membrane-bound dipeptidases (MDBs) or intracellular Cys-Gly peptidases. Cellular uptake of Cys-Gly or the individual Cys, Gly, and glutamate units serve as precursors for intracellular GSH synthesis. GGT is expressed on the luminal surface of excretive and absorptive cells that line glands and ducts throughout the body, with the highest level of GGT activity in the kidney and pancreas ducts [28]. It is nearly absent, however, from the hepatocytes and cardiac myocytes [7]. The absence of GGT activity on the apical surface of the kidney's proximal tubules by genetic disorder results in glutathionuria [29,30].

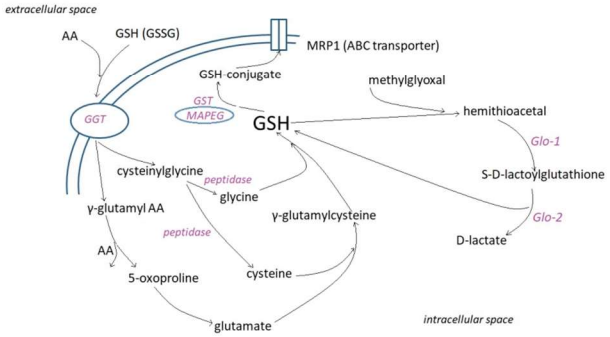


Figure 2. Involvement of γ-glutamyl transpeptidase (GGT), glutathione-S-transferases (GST), their subfamily of Membrane Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG), and glyoxylases (Glo) in the intracellular metabolism of GSH. MRP1 (multidrug resistance-associated protein 1) transporter facilitates the unidirectional transport of conjugates.

GGT has multiple functions, including catalytic transfer of γ-glutamyl groups to amino acids and short peptides, hydrolysis of GSH to glutamyl moiety and cysteinyl glycine, and catabolism of GSH conjugates [31]. GGT allows hydrolysis of a broad range of γ-glutamyl amides and transpeptidation of amino acids or dipeptides [32]. So GSH, its *S*-conjugates, GSSG, γ-glutamyl di- or tripeptides, glutamine, l-α-methyl derivatives of γ-glutamyl amides, various lipid-derived mediators (e.g., leukotriene C4), geranylgeranyl, poly-γ-glutamyl derivatives serve as substrates of GGT [33–36]. Many tumor cells express GGT on their entire cell surface and can therefore cleave GSH not only in the ductal but also in interstitial fluid and blood [37]. GGT expression provides tumor cells with an additional source of cysteine and cystine from the breakdown of extracellular GSH and GSSG [38].

Besides the GGT pathway, there is evidence of Na^+-dependent and Na^+-independent glutathione transport systems for glutathione cell uptake expressed in the renal basolateral membrane [38,39], the small intestine [40], and the brain [41]. In the renal basolateral membrane, two Na^+-independent Organic Anion Transport systems (OAT1 and OAT3) [42] and the Na^+-dependent dicarboxylate carriers are the most important organizations [43,44]. On the other hand, the plasma membrane glutathione efflux can be facilitated by specifically or ubiquitously expressed membrane proteins and anion channels such as multidrug resistance-associated proteins (MRP1-5), Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), Arginine/Ornithine Transport ATP-binding Proteins (OATP 1,2), and ATP-Binding Cassette superfamily G member 2 (ABCG2) [19].

2.3. Intracellular Distribution and Functions of GSH

Within the cell, there are three main glutathione pools. The cytosol (80–85%), the mitochondria (10–15%), and the endoplasmic reticulum [45–47]. Studies by Birk et al. and Montero et al. [48,49] pointed out that the total glutathione content in the lumen of the endoplasmic reticulum even exceeds the entire cellular glutathione content. GSH and GSSG concentrations depend on the subcellular compartment, the cell type, and the organism. Accordingly, the redox potential of the GSSG/2GSH system varies from tissue to tissue, from organism to organism. This relies on the proportion of GSH and GSSG and the total concentration of glutathione, which is quite challenging to estimate their actual concentration and ratio in vivo [50,51]. For example, taking the local pH and GSSG/2GSH ratios into consideration, cytosolic $E_{pH7.0}$ = −289 mV (or even lower), mitochondrial matrix $E_{pH7.0}$ = −296 mV (or even lower), and human plasma $E_{pH7.4}$ = −118 mV half-cell reduction potentials (E_{hc}) have been estimated [52]. Furthermore, a correlation has been found between the cell cycle, the condition of the cell (stressed, apoptotic, etc.), and the GSSG/2GSH ratio. For instance, in cell proliferation (E_{hc} = ~−240 mV), in cell differentiation (E_{hc} = ~−200 mV), and in apoptosis (E_{hc} = ~−170 mV), which can be applicable for a better understanding of oxidative stress [13,53]. Van't Erve et al. [54] found that GSSG/2GSH levels and reduction potential in erythrocytes reflect genetic differences between individuals.

Cytoplasmic glutathione levels impact glutathione diffusion through nuclear pore complexes [55], playing a role in oxidative signaling during proliferation, epigenetic control of histone activity, and the cell cycle control, mainly in the S + G_2/M phase [56,57]. ATP-dependent transporters have also been reported to import glutathione into the nucleus [58].

Glutathione synthesis occurs only in the cytosol; thus, the mitochondrial pool is supplied by GSH transport and maintained by reducing its oxidized form via the activity of glutathione reductase. Glutathione passes the mitochondrial outer membrane through the mitochondrial porin, a voltage-dependent anion channel (VDAC). As a negatively charged molecule, glutathione cannot diffuse through the mitochondrial inner membrane. Its transport into the mitochondrial matrix is either active or provided in exchange for another anion [7]. Six of the eight anion carriers have the potential for GSH import through the inner membrane into mitochondria. Monocarboxylate, dicarboxylate (DIC), 2-oxoglutarate (OGC), tricarboxylate (or citrate), glutamate-hydroxide, glutamate-aspartate transporters involved in the transport of GSH also provide intermediates of the Krebs cycle and the gluconeogenesis pathway [59]. DIC and OGC were identified as major GSH transporters, although at the expense of Krebs cycle intermediates [60]. Around 70–80% of GSH transport could be associated with DIC and OGC activity in the kidney, but only about 45–50% of liver mitochondria [61]. DIC imparts malate (malonate or succinate) in exchange for phosphate, sulfate, and thiosulfate. Malate conversion into oxalacetate, followed by the formation of phosphoenolpyruvate, is limited for gluconeogenesis in the cytosol. Reduction in DIC expression leads to decreased glutathione levels and impaired complex I activity [62]. OGC transfers 2-oxoglutarate substituting dicarboxylate [63], thus regulating respiration and glycolysis. While succinate from the matrix side increases the affinity of OGC to malate, substrates such as phenyl succinate, pyridoxal phosphate, retinoic acid, and ethanol cause inhibition of OCG. Reduced activity of OCG leads to lower energy production, increased oxidative stress, and it could be the basis of liver or nervous tissue diseases [64–66]. GSSG is not transported out from mitochondria [67].

The endoplasmic reticulum offers a unique setting concerning GSH homeostasis. It contains the thiol oxidase Ero1, which catalyzes the formation of disulfides transmitted to folding substrates via protein disulfide isomerase (Pdi1). Both reduced and oxidized forms of glutathione are transported into the endoplasmic reticulum at different rates, with a preference for the reduced form [68]. Since GSH is oxidized but not reduced in the ER, GSH must be imported into the ER, while GSSG is exported to the cytosol [69]. A study by Ponsero et al. [70] brought up the finding of facilitated diffusion of GSH through the Sec61 protein translocation complex. In the sarcoplasmic reticulum, ryanodine receptor

calcium channel type 1 (RyR1) was suggested to play an important role [71]. However, Bachhawat et al. [19] pointed out that this might result from the S-glutathionylation of several cysteine residues within the RyR1 molecule. To maintain GSH homeostasis, part of GSSG is transported to the cytosol through vesicular transport [72]. Most GSSG reacts with proteins or protein disulfide isomerase involved in oxidative protein folding [64]. A lower GSH:GSSG ratio results in more oxidizing conditions (−240 mV) [70] in the endoplasmic reticulum allowing protein disulfide formation.

GSH plays an essential role not only in the peripheral tissues but in the central nervous system (CNS) as well. Brain tissues are rich in unsaturated fatty acids. Due to their relatively low levels of antioxidants or antioxidant enzymes, they are rather sensitive to oxidative damage. The most important small molecular CNS antioxidants are GSH, ascorbic acid (vitamin C), and α-tocopherol (vitamin E) [73]. Among these antioxidants, GSH seems to be the determining agent because it is selectively decreased in the brains of patients with these neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease, and Amyotrophic lateral sclerosis) [74]. Therefore, regulating the redox state by intracellular GSH is crucial for maintaining cellular functions under physiological and pathological conditions.

In the central nervous system, besides the functional neurons, there are several other types of cells for the nervous system to function properly. This is where a set of glial cells intervene, which make up 25–50% of the nerve mass [75]. The most common type of glial cells in the CNS are the astrocytes and the microglia. Synthesis of GSH occurs both in the neurons and the glial cells. In an early work by Rice and Russo-Menna (1998) [76], GSH levels of glutathione in neurons and glia were reported to be 2.5 nM and 3.8 mM, respectively. The authors found that ascorbate predominates in neurons (10 mM), whereas GSH is slightly predominant in glia. According to the above, GSH supplementation seems promising for treating patients with neurodegenerative diseases.

2.4. Acid–Base Properties

The acid–base properties of glutathione (GSH) have long been the focus of scientific interest. It has three acidic (thiol, glycinyl carboxyl, glutamyl carboxyl) and one basic (amino) functional group. Accordingly, in an aqueous solution, glutathione can exist in four different macroscopic protonation states:

$$L^{3-} \overset{K_1}{\leftrightarrow} HL^{2-} \overset{K_2}{\leftrightarrow} H_2L^{-} \overset{K_3}{\leftrightarrow} H_3L \overset{K_4}{\leftrightarrow} H_4L^{+}$$

where L^{3-} is the fully deprotonated, H_4L^{+} is the fully protonated GSH molecule.

Since the HL^{2-} and the H_3L forms have four protonation isomers (microspecies) each, and the H_2L^{-} form has six microspecies, the molecule has sixteen different protonation states (microspecies) altogether [77].

The micro and sub-micro protonation constants characterize the acid–base properties at the submolecular level [78]. These constants allow quantification of the proton binding capacity of submolecular basic units when the protonation states of all other sites are defined in the molecule [79]. Group constants are special micro constants when the rest of the groups in the molecule are far enough apart, and their protonation does not affect the basicity of the group [80]. The rotational state of the flexible parts of the molecules is defined by the sub-micro constants when protonation occurs [81]. The correct characterization of the basicity of the sites of protonation of multidentate ligands can be conducted using the micro and sub-micro constants. In addition, this group of constants is used to measure the concentration of different protonation forms, of which the principal form is not always the reactive form in chemical and biological processes. [82–86]. The macroscopic protonation constants (K1-K4) determined by ^{1}H NMR-pH titrations were as follows: logK1 9.65; logK2 8.78; logK3 3.52; and logK4 2.22 [77].

The obtained values were found to be very similar to those determined in earlier works of Pirie and Pinhey [87] (9.62, 8.66, 3.53, 2.12), Li et al. [88] (9.65, 8.75, 3.59), and

Martin and Edsall [89] (9.62, 8.74). The results demonstrated that the first and the second protonation constants were predominated by the overlapping protonation of the amino and the thiolate site, the amino being typically more favored. The carboxylate groups also protonated in an overlapping fashion, the glycinyl carboxylate being more basic. It is worth mentioning that the protonated amino group makes the inherently more basic glutamyl carboxylate more acidic [77].

It is important to note that the physico-chemical properties (e.g., complex formation, nucleophilic reactivity, redox properties) and biological functions of glutathione could be significantly different at different protonation states (i.e., in solutions with different pH values) [90–93] and its redox behavior [94,95]. Furthermore, ionic strength and the nature of ionic media also affect the acid–base characteristics of glutathione [96].

2.5. Antioxidant Properties

The pKa value of GSH (ranging from 8.6 to 8.8 [87–89] results in low thiol reactivity in the cellular environment [97]. Still, high GSH concentrations enable some reducing activities against oxidizing agents in the cell [98]. GSH, for example, can reduce H_2O_2, resulting in GSSG and water [99]. The rate of reaction depends on the cellular GSH level and the ratio of GSH to H_2O_2 concentrations [100]. Recently, Zinatullina et al. [101] confirmed that the oxidation of GSH is accompanied by radical formation. GSH reacts with the majority of free radicals generating thiyl radicals. Consecutive reactions of the radicals with a thiolate anion and molecular oxygen lead to disulfide and superoxide radicals formation [102]. Furthermore, γ-glutamylcysteine, a GSH precursor, was found to decompose H_2O_2 similarly to glutathione peroxidase-1 [103].

Glutathione exists in 100 μM concentrations as glutathione persulfide (GSSH) [104], the latter exhibiting higher activities due to its higher nucleophilic power than GSH [105]. Under specific conditions, GSSH reacts with H_2O_2, while GSH does not [106]. Furthermore, its reactions with one-electron oxidants are faster than similar reactions of thiols [107]. GSSH are intermediates in the synthesis of iron-sulfur clusters and mitochondrial H_2S oxidation [108–110]. GSH can react with HS^- catalyzed by sulfide quinone oxidoreductase or thiosulfate sulfurtransferase, forming GSSH, which can reduce oxidized thioredoxin. Single-domain sulfurtransferase (TSTD1, known as rhodanese) and mercapto pyruvate sulfurtransferase can also directly transfer sulfides to GSH and the thioredoxin antioxidant systems [111]. Mutations in persulfite dioxygenase, oxidizing GSSH to sulfite and GSH, are bases for autosomal-recessive inherited ethylmalonic encephalopathy [112].

2.6. Redox Signaling Properties

Signaling is the process that makes cells capable of reacting to the change in their environment (intercellular signaling) or their homeostasis (intracellular). The initial step of the process is the interaction of the signaling particles (ligands) with the target molecule (receptor). The well-known signaling mechanisms involve protein–protein interactions, allosteric changes induced by the binding of ligands, proteolytic processing, and chemical modifications such as acylation, acetylation, alkylation, and phosphorylation of proteins. On the contrary, redox signaling is the transduction of signals based on the transfer of electrons. Redox signaling involves a broad spectrum of pathways involving free radicals, redox-active metals (e.g., iron, copper), or reductive equivalents [74]. Here only those pathways are mentioned that are based on a modification of signaling proteins through the modification of one amino acid, cysteine.

The physiological level of hydrogen peroxide) (H_2O_2) and nitric oxide ($^{\cdot}NO$) can selectively react with the thiol function of the cysteinyl residues at the active site of the proteins (receptors, enzymes, transporters, etc.). Accordingly, the receptor-mediated stimulation of the H_2O_2 and $^{\cdot}NO$ production are part of normal physiology; this is especially true for the longer-lived H_2O_2. [113]. However, overproduction of these and related species (ROS and RNS) lead to irreversible oxidation of the thiol residues and impairs cellular protein functions [114,115]. The GSSG/2GSH redox system is fundamental in the cells and, together

with other redox-active couples (including NADPH/NADP+, Trx-SH/Trx-SS), regulates and maintains the appropriate cellular redox status. For example, the GSSG/2GSH half-cell reduction potential differed in cell proliferation, differentiation, and apoptosis [13,53]. Thus, changes in the GSSG/2GSH ratio are fundamental in controlling signal transduction that supports cell cycle regulation and other cellular processes [55].

The functions and activities of GSH as the main regulator of cellular redox status and redox signal transduction have been reviewed [17,116–119]. GSH acts protectively against oxidative stress by reacting directly with $^{\cdot}NO$, superoxide anion radical ($O_2^{\cdot -}$), H_2O_2, hydroxyl radical ($^{\cdot}OH$), peroxinitrite anion ($ONOO^-$), and the lipid peroxidation product 4-hydroxy-2-nonenal (4-HNE) [116,117]. Such reactions directly modify the cellular GSSG/2GSH half-cell potential, a physiological signaling event. Furthermore, changing the GSH level results in a selective change in the activity of the thioredoxin/glutathione systems [118], the glutaredoxin/glutathione system [119], and the activity of some GST isoforms. The latter protein family is involved not only in the metabolism of xenobiotics but also of endogenous compounds which play critical roles in regulating signaling pathways [120–122].

2.7. Reactions with Electrophilic Xenobiotics

Glutathione-S-transferases (GST) lower the pKa of GSH thiol under 6, enhancing rates of nucleophilic addition and substitution reactions with electrophylic xenobiotics (Figure 2). These reactions are examples of Phase II bioconjugation reactions, most of which result in reduced toxic effects of the parent compounds or their metabolites [98,123]. Other enzymes/enzyme systems, e.g., selenium-containing glutathione peroxidases (GPx) or peroxiredoxins (Prdx), use GSH to reduce various peroxides and hydroperoxides. Glyoxalase (Glo) performs conjugation of GSH with the glycolysis byproduct methylglyoxal to form (S)-lactoylglutathione (Figure 2). Moreover, glyoxalase II (Glo-2) catalyzes S-glutathionylation using (S)-lactoylglutathione [124].

3. The Glutathione Peroxidase System

The glutathione or glutathione peroxidase system consists of glutathione peroxidase (GPx) and glutathione reductase (GR). In the decomposition reaction of H_2O_2 or other organic peroxides (HOOR), two molecules of GSH reduce the substrate to H_2O or the corresponding alcohol (HOR) and restore the enzyme forming GSSG with concomitant formation of GSSG and H_2O.

$$2\,GSH + HOOR \rightarrow GSSG + HOR + H_2O \quad (1)$$

GSSG can be excreted from the cell or recycled by GR using the reducing power of NADPH (Figure 3). NADPH arises in two reactions of the pentose phosphate pathway, which is the most potent source of it. However, NADPH can also be formed directly in the mitochondria by $NAD(P)^+$ transhydrogenase, mitochondrial/cytosolic NADP-dependent isocitrate dehydrogenase, or cytosolic malate dehydrogenase [125].

GR is a homodimeric flavoprotein consisting of 52 kD monomers. Except for synthesis, the activity of GR represents a second source of GSH in the cytosol and some organelles, such as mitochondria. Although inhibition of GR has been reported to cause a depletion of GSH and accumulation of GSSG [126], a comprehensive study of the GR and the cellular thiol redox system is missing [127]. Inhibition of the enzyme has also been related to the toxicity of various chemicals and metals [128,129].

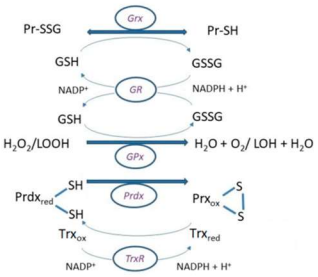


Figure 3. Basic reaction mechanisms of glutathione peroxidase (GPx), and glutaredoxin in (de)glutathionylation using (GSH)GSSG, respectively, and reduction of GSSG by the activity of glutathione reductase (GR) with reducing the power of NADPH + H^+. Reduction of peroxiredoxin (Prdx) after disposal of peroxides is ensured by thioredoxin (Trx), which is reduced by consumption of NADPH + H^+ in catalytic efficiency of thioredoxin reductase (TrxR).

The term glutathione peroxidase (GPx) describes only a small subgroup of the peroxidases [130], which belong to a group of phylogenetically related enzymes. GPx 1–4 are selenoproteins with selenocysteine (SeCys) in the catalytic center. GPx6 is a human selenoprotein [131]. Their important antioxidant function was shown in various places and cell structures: GPx1 is ubiquitous in the cytosol and mitochondria, GPx2 in the intestinal epithelium, and GPx3 in the plasma; all three work in the aqueous phase reducing H_2O_2 and free fatty acid peroxides [131]. GPx4 protects mainly membranes by reducing phospholipid and cholesterol peroxides [131,132]. Gpx5, which contains cysteine instead of Se in the active center, is a secretory enzyme of the epididymis. GPx6 is a human selenoprotein and is formed by the olfactory epithelium. GPx7 and GPx8 are also CysGPx with low peroxidase activity. GPx1, 2, 3, 4, 5, and 6 are homotetramers, which could determine their specificity for hydrogen peroxide. GPx4, 7, and 8 are monomers. This structure probably enables the reaction with more complex lipid hydroperoxides, but this has been proven only for GPx4 [132]. The catalytic center of GPx was first characterized as a triad consisting of SeCys or Cys, Gln, and Trp. It was later found to be a tetrad with Asn. A conservative feature for these GPx is the presence of a second or even a third cysteine residue.

The reaction mechanism differs between individual GPx isoforms, whose activity requires GSH. In general, they do not form a ternary complex between the enzyme, hydroperoxide, and GSH, but the reaction has a concomitant oxidation and a reduction part. In the oxidation part, deprotonation takes place in the same way. The side chains of the Glu, Try, and Asp residues form a highly nucleophilic region in the enzyme's active center, where oxidation of the active site selenocysteine (RSeH) or cysteine (RSH) occurs after binding the peroxide. This reaction results in the formation of a selenenic acid (RSeOH) derivative. The selenenic acid is then converted back to the selenol (RseH) by a two-step process that begins with a reaction with GSH to form the GS-SeR and water. A second GSH molecule reduces the GS-SeR intermediate back to the selenol, releasing GS-SG as the byproduct [52,132]. A simplified representation (with H_2O_2 as a substrate) is shown below:

$$RSeH + H_2O_2 \rightarrow RSeOH + H_2O \quad (2)$$

$$RSeOH + GSH \rightarrow GS\text{-}SeR + H_2O \quad (3)$$

$$GS\text{-}SeR + GSH \rightarrow GS\text{-}SG + RSeH \quad (4)$$

Glutathione reductase then reduces the oxidized glutathione to complete the cycle:

$$GS\text{-}SG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+ \quad (5)$$

Selenium deficiency results in increased GSH synthesis in the liver with accompanying release to the plasma [133]. Increased plasma GSH led to cysteine depletion, impaired protein synthesis, decreased GPx, and increased GST activities [134]. Usually, GPx requires GSH in millimolar concentrations in the intracellular space, and plasma GSH reaches micromolar concentrations, which questions GPx's antioxidant function [135]. However, within the cell, in the cytosol and mitochondria, the GPx system appears to be very efficient in the elimination of H_2O_2 due to the low (100–200 μM) K_m value of the enzyme [136] and the range of substrates [137]. Mimicking GSH, γ-glutamylcysteine can be used by GPx1 as a cofactor [103].

4. Glutaredoxins (Grx)

The thiol oxidoreductase glutaredoxins (Grx) are small proteins reducing various protein disulfides (PrSSPr) and GSH-protein mixed disulfides (PrSSG), where the electron donor is glutathione [138]. Grxs catalyze glutathionylation, post-transcriptional modifications, and the disulfide exchange between GSSG and protein thiols (PrSH) [139] (Figure 3). Grx-catalyzed (de)glutathionylation is an important event in signal transductions and serves as the primary protective mechanism against the irreversible oxidation of cysteine residues [115]. As mentioned above, the standard cell potential changes depending on the environment and the cell itself. Cell proliferation occurs at approximately −240 mV, differentiation at about −200 mV, and apoptosis at around −170 mV [55]. Changes in the GSH/GSSG redox potential can be sensed by Grxs, which operate as GSH-dependent reductases at about −240 mV and GSSG-dependent oxidases at about −170 mV [140].

Grxs are characterized by their active site motif. Dithiol-type Grx (class I) enzymes have a Cys-Pro-Tyr-Cys active site, while monothiol Grx (class II) enzymes do not contain a thiol at the C-terminus of the active site (Cys-Gly-Phe-Ser). Dithiol Grxs and monothiol Grxs with one Grx domain are found in all living organisms. Multi-domain monothiol Grxs (PICOTs, PKC-interacting cousin of thioredoxin) are present in eukaryotic cells. These contain an N-terminal Trx-like domain and three C-terminal monothiol Grxs domains [141]. Two other regions were recognized near the active site, the Grx characteristic motif GG and the TVP, which are involved in binding GSH [142].

4.1. Glutathionylation

Glutathionylation involves the reversible attachment of glutathione to cysteine residues in target proteins. Conditions of elevated oxidative stress increase the levels of protein glutathionylation. The glutathionylation/deglutathionylation cycle is viewed as a process that acts primarily against ROS/RNS via reducing aberrant cysteine modifications and thereby preventing the formation of damaging irreversible cysteine modifications.

There are three pathways of glutathionylation. (a) The thiol-disulfide exchange between GSSG and PrSH is accomplished at a low GSH:GSSH ratio. The reactivity of PrSH depends on the thiol pKa [143]. (b) The oxidation of the PrSH yields a thiyl radical ($RS^{\cdot}$), which reacts with the deprotonated form of glutathione (GS^-), forming a mixed disulfide radical ($RSSG^{\cdot -}$). After the loss of an electron, a mixed disulfide (RSSG) and a superoxide anion radical ($O_2^{\cdot -}$) are formed [144]. (c) Mixed disulfides can also be formed with low molecular weight thiols with indistinct biological relevance. As Lushchak [60] discussed, inhibition of glutathione reductase, phosphofructokinase, fatty acid synthase, or activation of fructose-1,6-bisphosphatase by CoASSG was shown.

Cysteine residues of proteins with a low pKa are targets for redox modulation under oxidative or nitrosative stress conditions. The primary products of these oxidative transformations are the respective thiyl radicals ($PrS^{\cdot}$). These reactive intermediates can react with glutathione (GSH) to form stable glutathionylated protein disulfides (PrSSG) to prevent their further oxidation with molecular oxygen. The protected protein thiol can be regenerated by the deglutathionylation process (e.g., through a reaction with another GSH molecule). Under oxidative stress, the thiyl radical can be further oxidized to form sulfenic (RSOH), sulfinic (RSO_2H), or sulfonic acid (RSO_3H) derivatives of the

proteins. Both sulfenic and sulfinic acids of proteins can be reduced by Trx and sulfiredoxin, respectively [145–147]. In contrast, sulfonic acid cannot be reduced. Both sulfenic and sulfinic acids of proteins can be conjugated to GSH to form S-glutathionylated proteins via glutathione S-transferases (GSTs), Grx, or nonenzymatically. Glutathionylation was referenced to cytoskeletal proteins, metabolic, redox enzymes, cyclophilin, stress proteins, nucleophosmin, transgelin, galectin, and fatty acid binding protein [148], affecting their activity either in activation or decrease.

4.2. Deglutathionylation

Deglutathionylation undergoes cleavage of the disulfide linkage of the glutathionylated protein with another GSH molecule (Figure 3). The reaction can proceed (a) either in a mixed disulfide intermediate with an N-terminal thiol active site; (b) in a mixed disulfide intermediate by the attack of a second GSH molecule; or (c) by non-covalent binding of the thiol function of both an *N*-terminal thiol active site and GSH-coordinating metal cofactor in the [Fe-S] binding Grx subgroup [142]. The motif in the active site and the type of disulfide bond in the target protein are decisive for the reaction mechanism [149]. In the reaction mechanism of monothiol Grxs, the reduction of glutathionylated proteins (PrSSG) begins with a nucleophilic attack of the *N*-terminal cysteine. As a result, glutathionylated Grx and reduced substrate protein are released. The Grx-SG intermediate is cleaved by a GSH molecule, resulting in reduced Grx and GSSG, which is subsequently reduced by GR [150] (Figure 3). In the mechanism of dithiol Grxs, the reduction of PSSG and mixed disulfides begins with a nucleophilic attack of the *N*-terminal cysteine, but GSH is released. The Grx-protein intermediate is reduced by the second C-terminal active cysteine of Grx, forming oxidized Grx and reduced protein [151,152]. Dithiol Grx can also use monothiol mechanisms. However, both mechanisms are critically dependent on the availability of reduced GSH [153].

Apart from oxidoreductase activity, both classes of Grx proteins can bind [Fe-S] clusters. Class II enzymes are essential in the processes of regulation of Fe metabolism. Their function depends on the [Fe-S] binding capacity and not on the reductase activity [154]. In addition, Grxs have dehydroascorbate reductase and transhydrogenase activity, catalyzing denitrosylation and partial cystine conversion [155].

Monothiol Grxs (Grx3 and Grx5) form an iron–sulfur complex. Both isoforms can transfer iron to specific proteins. However, monothiol Grxs cannot deglutathionylate target proteins [156]. Grx3, localized in the cytosol, has a unique domain structure consisting of an *N*-terminal Trx-homology domain [141,157]. The first discovered function of Grx3 was related to that of protein kinase C theta, and in T-cells, Grx3 colocalizes with it, hence the name PICOT [157]. Since Grx3 is expressed in a wide variety of organs and tissues, it has been proposed as a redox sensor in signal transduction in response to reactive oxygen and nitrogen species [158]. Nuclear Grx3 has a role in the epigenetic regulation of chromatin by regulating the methylation of myelin transcription factor 1 and cell proliferation [159,160]. Grx5 participates in the biogenesis of [4Fe–4S] clusters by interacting with ISCA1 of the mitochondrial homolog of the iron–sulfur cluster assembly and ISCA2 of the cytosolic iron cluster [161,162]. Grx5 forms a cluster in the cytosol with a family of BolA-like proteins (regulatory DNA-binding proteins) for the maturation of iron–sulfur proteins [163].

Grx1 and Grx2 are dithiol Grxs. Most human Grx1 is found in the cytosol, less in the nucleus [164] and the mitochondrial intermembrane space [165]. Grx1, unlike Trx, is not an essential protein [98]. Grx1 activity depends on the redox state of the cells, especially the GSH/GSSG ratio [166]. In addition to deglutathionylation activity, Grx1 has also been able to denitrosylate protein Cys-NOs and prevent the pro-apoptotic effect of nitric oxide in tumor cell lines and cardiomyocytes [167,168]. Grx2 is about 20 times less abundant than Grx1 [169]. Depending on gene splicing, it is localized in mitochondria, cytosol, or nucleus [170]. Like Grx1, it catalyzes the reduction of disulfides mixed with GSH with a higher affinity but with a lower turnover rate [171]. However, these two proteins behave differently in response to an oxidative environment. While Grx1 is inhibited

when other structural cysteine residues are oxidatively modified [154], Grx2 is activated. The different response to oxidative conditions is due to the ability of Grx2 to form [Fe–S] clusters [172]. The [Fe–S] clusters act as sensors for Grx2 activity under oxidative conditions [154]. Outside the active site, two cysteines form a [2Fe–2S]-bridged dimer that is enzymatically inactive. Oxidative stress increases GSSG concentration and reduces the availability of GSH for coordination of the [Fe–S] complex, leading to cluster degradation and formation of enzymatically active Grx2 monomers [154]. Grx2 can cycle and accept electrons from thioredoxin reductase1 (TrxR1) [171]. In mitochondria, Grx2 has been shown to efficiently catalyze (de)glutathionylation of complex I and SOD1 [173,174].

5. Peroxiredoxins (Prdx)

Peroxiredoxins (Prdxs) are cysteine-dependent peroxidase enzymes [132,175], whose low Km for H_2O_2 (10 μM) and their ubiquity, comprising up to 0.8% of total protein in some animal cells predispose them for reduction H_2O_2 [176]. However, they can also reduce peroxynitrite, peroxynitrous acid, and lipid peroxides [177,178]. Their peroxidatic functions overlap with GPx and catalase, and their catalytic efficiency is lower (~10^5 M^{-1} s^{-1}) compared to GPx (~10^8 M^{-1} s^{-1}) and catalase (~10^6 M^{-1} s^{-1}) [179]. Furthermore, comparing Prdx Km for H_2O_2 with that of GPx and catalase exceeding even the millimolar range [180] suggests that the role of Prdx is rather as a sensor of H_2O_2 [178] than oxidative stress condition reversal.

Prdxs are divided into the subgroups Prdx1/AhpC, Prdx5, Prdx6, Tpx (thiol peroxidase), PrdxQ/BCP, and AhpE. Human Prdxs can be posttranscriptionally modified by glutathionylation, acetylation, ubiquitination, oxidation (RSOH, RSSR, RSO_2, RSO_3), S-nitrosylation, phosphorylation [181] or tyrosine nitration [182]. Prdxs proceed the same catalytic cycle, where the active site cysteine (peroxidatic cysteine, Cys_P) reduces peroxides and forms Cys_P-sulfenic acid (RSOH), releasing water or the corresponding alcohol. Some Prdxs contain a second, so-called resolving cysteine (Cys_R), which reacts with RSOH forming disulfide (Cys_P-S-S-Cys_R) and water [183]. Cys_R can originate from the adjacent monomer, the same monomer, glutathione, or a redox-relay binding partner [184]. Accordingly, six human Prdxs isoforms are diversified into three subgroups.

In general, the Prdx1 subfamily enzymes are the most highly expressed, making up 0.1–1% of the soluble protein in the cell. The "typical 2-Cys" Prdxs are homodimers with two active sites (having both a Cys_P and Cys_R). The disulfide bond is formed between the two subunits in the reaction of RSOH and Cys_R of the other subunit. Reduction of disulfide bond is catalyzed by Trx (Figure 3), tryparedoxin, or alkyl hydroperoxide reductase [179,185]. In the reduced state, PrdxI, II, and IV form decamers or dodecamers such as PrdxIII [186]. Reduced decamers show efficient peroxidase activity and, depending on other posttranslational modifications, form high molecular weight oligomers associated with cell cycle checkpoints, chaperones, and various intracellular processes [187–189]. The "atypical 2-Cys" Prdxs (Prdx5) are monomers forming intramolecular disulfide since both Cys_R and Cys_P are within the same molecule; their reduction is achieved by Trx. The "atypical 2-Cys" Prdxs can form dimers independently of the redox state [179]. The "1-Cys" Prdxs (Prdx6) contains only Cys_P in the *N*-terminus [190]. The resolving electron donor thiol can be glutathione, allowing the formation of a mixed disulfide, while the second donor thiol enables the reduction of the formed disulfide bonding. Ascorbate, lipoic acid, and cyclophilin, but most commonly GSH, can serve as electron donors for disulfide reduction [179,185,191]. Prdx6 reduces phospholipid hydroperoxides using GSH, and also the GST P1-1 class showed the ability to act as phospholipase A_2 [192]. Hyperoxidation, formation of RSO_2H or RSO_3H, and phosphorylation regulate the activity of Prdxs [181]. The "1-Cys" Prdxs are resistant to hyperoxidation. Hyperoxidation can be repaired by sulfiredoxin, but not in human Prdx6 [193].

6. Glutathione-S-Transferases (GST)

GSTs belong to the Phase II biotransformation enzymes catalyzing the GSH-mediated peroxide reduction [194] and conjugation of GSH with a variety of reactive electrophiles, most commonly generated by cytochrome P450 metabolism [195]. GSTs expressed ubiquitously, but tissue-specific distribution is probably an adaptive response against endo- and exogenous metabolites [196]. GSTs comprise two distinct superfamilies, membrane-bound microsomal and soluble cytosolic. In humans, cytosolic GSTs are encoded by 16 genes, while the microsomal, at least by six genes, in addition to significant genetic polymorphisms [197]. According to the degree of sequence identity and localization, the cytosolic GSTs (cGSTs) are divided into alpha, mu, pi, omega, theta, delta, sigma, and zeta (A, M, P, O, T, D, S, Z) classes. Mitochondrial GSTs (mGSTs) are divided into A, M, P, and kappa (K) classes. A novel superfamily designated MAPEG (Membrane Associated Proteins in Eicosanoid and Glutathione metabolism) includes members of widespread origin with diversified biological functions. Members of this family are leukotriene C-4 synthase, 5-lipoxygenase activating protein, prostaglandin E synthase, and microsomal glutathione S-transferases (MGST) 1, 2 and 3 [198,199].

Due to polymorphisms, gene duplication, and genetic recombination, GSTs have multiple isoenzymes with overlapping substrate specificity and diversity [200]. In humans, the highest cytosolic GST activity level is present in the liver, whereas the kidney, lung, and intestine show lower activity levels than that of the liver at 22, 66, and 63%, respectively [201]. Intracellularly, some specific GST activities also were detected in the plasma membrane, outer mitochondrial membrane, and nucleus [198].

In mammals, GSTs exist as homodimers with analogous tertiary structures [202]. All GSTs have a basic protein fold comprising two subunits with C-terminal and N-terminal domains. The N-terminal domain includes a thioredoxin-like fold, β-α-β-α-β-β-α, where β-β-α motif, known as G-site, serves as the binding site for GSH through the γ-glutamyl unit. The C-terminal domain is diverging [202–204]. The conserved proline residue at the N-terminal β3 strand ensures catalytic function and stability of thioredoxin-like proteins [205]. The G-site sequence similarity divides GST into two subgroups. Tyrosine-type GSTs contain Tyr residue (T- or P-class), which activates GSH [206]. Replacement of Tyr by Phe reduces the catalytic activity [207]. The Ser/Cys-type GSTs (O-class) used Ser or Cys to form mixed disulfides with GSH. These GSTs are more involved in redox reactions [208]. Selectivity for the substrates is determined by high variations in hydrophobic amino acid residues in the cleft between domains, called the H-site [208].

GSTs transfer GSH to several various electrophilic compounds [209]. The reactions with some compounds, such as benzyl and phenethyl isothiocyanates and alkyl dihalides, can be reversible, increasing their toxicity [210]. Some classes conjugate GSH with epoxides and catalyze isomerization or reduction of harmful peroxides [52]. It was shown that the physiological function of Z-class GSTs is the cis-trans isomerization of 4-maleylacetoacetate to 4-fumarylacetoacetate [211]. The A-class GSTs display selenium-independent GPx activity, thereby reducing phospholipid peroxides and cholesterol hydroperoxides within the membrane without phospholipase A_2-mediated release [212]. Anionic A-class GSTs also efficiently conjugate 4-hydroxynonenal, balancing lipid production and peroxidation [213]. Furthermore, isomerization of the double bond in selected 3-oxo-Δ^5-steroids releasing 3-oxo-Δ^4-steroids has been detected in some A-class GSTs [214]. S-class GSTs enable anti-, proinflammatory, and immunomodulatory functions [215]. From this class, prostaglandin-D_2 synthase and prostaglandin-E_2 synthase catalyze the cleavage of prostaglandin H_2, forming prostaglandin-D_2 or E_2 [216]. The enzyme leukotriene-C_4 synthase (MAPEG) catalyzes the conjugation of GSH with epoxide leukotriene A_4 [217]. Unique blood-barrier functions were described for M-class GSTs in the testis and brain [218]. O-class GSTs were able to modulate ryanodine receptor calcium release channels in cardiac muscle due to structural similarities to Chloride Intracellular Channel Proteins (CLIC) [219]. Approximately 15% sequence identity was found between O-class GSTs and CLIC1 [219]. CLIC proteins contain Grx-like active site motiv, Cys-Pro-(Phe/Ser)-(Ser/Cys), present also in

O-class GSTs [208,220,221]. CLIC, however, bind GSH covalently creating a mixed disulfide, unlike classical GSTs, which bind GSH in the active site non-covalently but with high affinity [220]. Finally, as indicated by the structural similarity, (de)glutathionylation activity by Menon and Board [222] but also dehydroascorbate reductase, S-(phenylacyl)glutathione reductase [223,224] activities of GSTO1-1 were confirmed. In P-class GSTs, chaperone functions and the influence of the MAPK pathway through JNK and TRAF2 modulation in response to oxidative/nitrosative stress were also detected [225]. One of the unwanted consequences and the subject of intensive ongoing research is resistance to drugs owing to increased GSTs activities [226].

7. Glyoxylases (Glo)

The glyoxalase system is a ubiquitous enzymatic network present in the cytoplasm, and some of them are also in the nucleus. It consists of glyoxalase 1 (Glo-1), glyoxalase 2 (Glo-2), and reduced glutathione (GSH) (Figure 2), which perform an essential metabolic function in cells by detoxifying methylglyoxal (MG) and other endogenous harmful metabolites into non-toxic D-lactate [227,228]. As discussed in Rabbani et al. [229], in mammals, methylglyoxal arises in 0.05–0.1% as a minor product from (a) glyceraldehyde-3-phosphate and dihydroxyacetone phosphate degradation in glycolysis, (b) oxidation of acetone by cytochrome P450, (c) oxidation of aminoacetone by semicarbazide amine oxidase, and (d) degradation of glycated proteins and monosaccharides. Methylglyoxal, whose formation can reach 3 mg/kg body weight/day [230], is a glycating agent, forming mainly arginine-derived hydroimidazolone adducts, DNA adducts, and isomeric imidazopurinones [231]. In the glyoxalase system, the rate-limiting enzyme is glyoxalase 1 (Glo-1, lactoylglutathione lyase). Methylglyoxal undergoes spontaneous thiolation with GSH, followed by the Glo-1 catalyzed conversion of methylglyoxal thioacetal to (S)-lactoylglutathione [232,233]. Studies have revealed that Glo-1 is a dimeric metal ion-dependent isomerase converting various glutathione-hemithioacetals to glutathione thioesters [234]. The activity of Glo-1 can be modified by phosphorylation or nitrosylation. While acetylation and oxidation have no effect, acylation of GSH inhibits Glo-1 activity [235]. Glo-2 is a thioesterase catalyzing the hydrolysis of (S)-lactoylglutathione to D-lactate and GSH. Glo-2 predominantly interacts with glutathione moieties allowing hydrolysis of a variety of glutathione substrates [234,236,237]. Glo-3, found in bacteria, catalyzes the conversion of methylglyoxal to D-lactate without the participation of GSH. DJ-1 and its homologs may display this function in humans [232].

Dicarbonyl stress causes protein modification and misfolding, affecting their structure and function, increasing the importance of Glo-1 in detoxification and its implication in the pathophysiology of diseases [238]. Moreover, there is evidence that the Glo-1 gene is a hotspot for copy-number variation associated with multidrug resistance in tumor chemotherapy [239].

8. Conclusions

Glutathione reaches the highest concentration in cells, with the predominant component being the reduced form. An electrochemical potential of a redox couple GSH/GSSG at different pH within cell compartments allows reversibility of oxidation or reduction reactions, thereby mediating a cell redox signaling mechanism. Several enzymes use glutathione in reaction mechanisms and fulfill a variety of protective, defensive, synthetic, or signaling roles in cellular metabolism. Either it can be through redox reaction in reduction of peroxides by thiol peroxidases or most common reversible modification, S-glutathionylation by thiol transferases or in conjugation reactions of toxic metabolites through glyoxalase or a variety of other compounds by glutathione-S-transferases. It also raises the question of the suggested genetic basis for differences in glutathione levels. Glutathione is undoubtedly part of a vast complex of cellular machinery processes. Therefore, monitoring it as a marker of specific conditions and dynamic changes in its concentration but also in some systems of which it is a part has a significant value.

Author Contributions: Conceptualization, J.V. and L.V.; writing—original draft preparation, J.V., L.V., L.K. and P.P.; writing—review and editing, J.V. and P.P.; visualization, L.K. and P.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Lillig, C.H.; Berndt, C. Preface. Cellular functions of glutathione. *Biochim. Biophys. Acta* **2013**, *1830*, 3137–3138. [CrossRef] [PubMed]
2. Hopkins, F.G. On an autoxidizable constituent of the cell. *Biochem. J.* **1921**, *15*, 286–305. [CrossRef] [PubMed]
3. Hopkins, F.G. On glutathione, a reinvestigation. *J. Biol. Chem.* **1929**, *84*, 269–320. [CrossRef]
4. Hunter, G.; Eagles, B.A. Glutathione. A critical study. *J. Biol. Chem.* **1927**, *72*, 147–166. [CrossRef]
5. Simoni, R.D.; Hill, R.L.; Vaughan, M. The discovery of glutathione by F. Gowland Hopkins and the beginning of biochemistry at Cambridge University. *J. Biol. Chem.* **2002**, *277*, 27–28. [CrossRef]
6. Alanazi, A.M.; Mostafa, G.A.E.; Al-Badr, A.A. Glutathione. *Profiles Drug Subst. Excip. Relat. Methodol.* **2015**, *40*, 43–158.
7. Lash, L.H. Mitochondrial glutathione transport: Physiological, pathological and toxicological implications. *Chem. Biol. Interact.* **2006**, *163*, 54–67. [CrossRef]
8. Lu, S.C. Glutathione synthesis. *Biochim. Biophys. Acta* **2013**, *1830*, 3143–3153. [CrossRef]
9. Ballatori, N.; Krance, S.M.; Notenboom, S.; Shi, S.; Tieu, K.; Hammond, C.L. Glutathione dysregulation and the etiology and progression of human diseases. *Biol. Chem.* **2009**, *390*, 191–214. [CrossRef]
10. Forman, H.J.; Zhang, H.; Rinna, A. Glutathione: Overview of its protective roles, measurement, and biosynthesis. *Mol. Asp. Med.* **2009**, *30*, 1–12. [CrossRef]
11. Jozefczak, M.; Remans, T.; Vangronsveld, J.; Cuypers, A. Glutathione is a key player in metal-induced oxidative stress defenses. *Int. J. Mol. Sci.* **2012**, *13*, 3145–3175.
12. Dickinson, D.A.; Forman, H.J. Cellular glutathione and thiols metabolism. *Biochem. Pharmacol.* **2002**, *64*, 1019–1026. [CrossRef] [PubMed]
13. Jones, D.P. Redox potential of GSH/GSSG couple: Assay and biological significance. *Methods Enzymol.* **2002**, *348*, 93–112. [PubMed]
14. Alberty, R.A. Standard apparent reduction potentials of biochemical half reactions and thermodynamic data on the species involved. *Biophys. Chem.* **2004**, *111*, 115–122. [CrossRef] [PubMed]
15. López-Lázaro, M. A new view of carcinogenesis and an alternative approach to cancer therapy. *Mol. Med.* **2010**, *16*, 144–153.
16. Xue, M.; Weickert, M.O.; Qureshi, S.; Kandala, N.B.; Anwar, A.; Waldron, M.; Shafie, A.; Messenger, D.; Fowler, M.; Jenkins, G.; et al. Improved Glycemic Control and Vascular Function in Overweight and Obese Subjects by Glyoxalase 1 Inducer Formulation. *Diabetes* **2016**, *65*, 2282–2294. [CrossRef]
17. Aquilano, K.; Baldelli, S.; Ciriolo, M.R. Glutathione: New roles in redox signaling for an old antioxidant. *Front. Pharmacol.* **2014**, *5*, 196.
18. Townsend, D.M.; Tew, K.D.; Tapiero, H. The importance of glutathione in human disease. *Biomed. Pharmacother.* **2003**, *57*, 145–155. [CrossRef]
19. Bachhawat, A.K.; Thakur, A.; Kaur, J.; Zulkifli, M. Glutathione transporters. *Biochim. Biophys. Acta* **2013**, *1830*, 3154–3164. [CrossRef]
20. Garcia, R.A.; Stipanuk, M.H. The splanchnic organs, liver and kidney have unique roles in the metabolism of sulfur amino acids and their metabolites in rats. *J. Nutr.* **1992**, *122*, 1693–1701. [CrossRef]
21. Stipanuk, M.H.; Dominy, J.E., Jr.; Lee, J.I.; Coloso, R.M. Mammalian cysteine metabolism: New insights into regulation of cysteine metabolism. *J. Nutr.* **2006**, *136*, 1652S–1659S. [CrossRef] [PubMed]
22. Ookhtens, M.; Kaplowitz, N. Role of the liver in interorgan homeostasis of glutathione and cyst(e)ine. *Semin. Liver Dis.* **1998**, *18*, 313–329. [CrossRef]
23. Lu, S.C. Regulation of glutathione synthesis. *Mol. Asp. Med.* **2009**, *30*, 42–59. [CrossRef] [PubMed]
24. Lu, S.C.; Mato, J.M. S-adenosylmethionine in liver health, injury, and cancer. *Physiol. Rev.* **2012**, *92*, 1515–1542. [CrossRef] [PubMed]
25. Cantin, A.M.; North, S.L.; Hubbard, R.C.; Crystal, R.G. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J. Appl. Physiol.* **1987**, *63*, 152–157. [CrossRef] [PubMed]

26. van der Vliet, A.; O'Neill, C.A.; Cross, C.E.; Koostra, J.M.; Volz, W.G.; Halliwell, B.; Louie, S. Determination of low-molecular-mass antioxidant concentrations in human respiratory tract lining fluids. *Am. J. Physiol.* **1999**, *276*, L289–L296. [CrossRef]
27. Lieberman, M.W.; Wiseman, A.L.; Shi, Z.Z.; Carter, B.Z.; Barrios, R.; Ou, C.N.; Che'vez-Barrios, P.; Wang, Y.; Habib, G.M.; Goodman, J.C.; et al. Growth retardation and cysteine deficiency in gammaglutamyl transpeptidase-deficient mice. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 7923–7926. [CrossRef]
28. Kiba, N. Enzymes in physiological samples. In *Encyclopedia of Analytical Science*, 2nd ed.; Worsfold, P., Poole, C.F., Eds.; Elsevier: Amsterdam, The Netherlands, 2005; pp. 536–544.
29. Griffith, O.W.; Meister, A. Translocation of intracellular glutathione to membrane-bound γ-glutamyl transpeptidase as a discrete step in the γ-glutamyl cycle: Glutathionuria after inhibition of transpeptidase. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 268–272. [CrossRef] [PubMed]
30. Njalsson, R.; Norgren, S. Physiological and pathological aspects of GSH metabolism. *Acta Paediatr.* **2005**, *94*, 132–137. [CrossRef]
31. Csanaky, I.; Gregus, Z. Role of glutathione in reduction of arsenate and of gamma-glutamyltranspeptidase in disposition of arsenite in rats. *Toxicology* **2005**, *207*, 91–104. [CrossRef]
32. Calvio, C.; Romagnuolo, F.; Vulcano, F.; Speranza, G.; Morelli, C.F. Evidences on the role of the lid loop of γ-glutamyltransferases (GGT) in substrate selection. *Enzym. Microb. Technol.* **2018**, *114*, 55–62. [CrossRef] [PubMed]
33. Allison, D. γ-Glutamyl transpeptidase: Kinetics and mechanism. *Methods Enzymol.* **1985**, *113*, 419–437. [PubMed]
34. Lam, B.K.; Austen, K.F. Leukotriene C4 synthase: A pivotal enzyme in cellular biosynthesis of the cysteinyl leukotrienes. *Prostag. Other Lipid Mediat.* **2002**, *68–69*, 511–520. [CrossRef]
35. Lu, E.; Wolfreys, F.D.; Muppidi, J.R.; Xu, Y.; Cyster, J.G. S-Geranylgeranyl-L-glutathione is a ligand for human B cell-confinement receptor P2RY8. *Nature* **2019**, *567*, 244–248. [CrossRef] [PubMed]
36. Tate, S.S.; Meister, A. Interaction of γ-glutamyl transpeptidase with amino acids, dipeptides and derivatives and analogs of glutathione. *J. Biol. Chem.* **1974**, *249*, 7593–7602. [CrossRef]
37. Hanigan, M.H.; Frierson, H.F., Jr.; Swanson, P.E.; De Young, B.R. Altered expression of gamma-glutamyl transpeptidase in human tumors. *Hum. Pathol.* **1999**, *30*, 300–305. [CrossRef]
38. Hanigan, M.H. Gamma-glutamyl transpeptidase: Redox regulation and drug resistance. *Adv. Cancer Res.* **2014**, *122*, 103–141.
39. Lash, L.H.; Jones, D.P. Transport of glutathione by renal basal-lateral membrane vesicles. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 55–60. [CrossRef]
40. Lash, L.H. Renal glutathione transport: Identification of carriers, physiological functions, and controversies. *Biofactors* **2009**, *35*, 500–508. [CrossRef]
41. Iantomasi, T.; Favilli, F.; Marraccini, P.; Magaldi, T.; Bruni, P.; Vincenzini, M.T. Glutathione transport system in human small intestine epithelial cells. *Biochim. Biophys. Acta* **1997**, *1330*, 274–283. [CrossRef]
42. Kannan, R.; Mittur, A.; Bao, Y.; Tsuruo, T.; Kaplowitz, N. GSH transport in immortalized mouse brain endothelial cells: Evidence for apical localization of a sodium-dependent GSH transporter. *J. Neurochem.* **1999**, *73*, 390–399. [CrossRef] [PubMed]
43. Lash, L.H.; Putt, D.A. Renal cellular transport of exogenous glutathione: Heterogeneity at physiological and pharmacological concentrations. *Biochem. Pharmacol.* **1999**, *58*, 897–907. [CrossRef] [PubMed]
44. Lash, L.H. Role of glutathione transport processes in kidney function. *Toxicol. Appl. Pharmacol.* **2005**, *204*, 329–342. [CrossRef] [PubMed]
45. Giustarini, D.; Galvagni, F.; Tesei, A.; Farolfi, A.; Zanoni, M.; Pignatta, S.; Milzani, A.; Marone, I.M.; Dalle-Donne, I.; Nassini, R.; et al. Glutathione, glutathione disulfide, and S-glutathionylated proteins in cell cultures. *Free Radic. Biol. Med.* **2015**, *89*, 972–981. [CrossRef] [PubMed]
46. Hwang, C.; Sinsky, A.J.; Lodish, H.F. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* **1992**, *257*, 1496–1502. [CrossRef]
47. Yuan, L.; Kaplowitz, N. Glutathione in liver diseases and hepatotoxicity. *Mol. Asp. Med.* **2009**, *30*, 29–41. [CrossRef]
48. Birk, J.; Meyer, M.; Aller, I.; Hansen, H.G.; Odermatt, A.; Dick, T.P.; Meyer, A.J.; Appenzeller-Herzog, C. Endoplasmic reticulum: Reduced and oxidized glutathione revisited. *J. Cell Sci.* **2013**, *126*, 1604–1617. [CrossRef]
49. Montero, D.; Tachibana, C.; Rahr Winther, J.; Appenzeller-Herzog, C. Intracellular glutathione pools are heterogeneously concentrated. *Redox Biol.* **2013**, *1*, 508–513. [CrossRef]
50. Kojer, K.; Bien, M.; Gangel, H.; Morgan, B.; Dick, T.P.; Riemer, J. Glutathione redox potential in the mitochondrial intermembrane space is linked to the cytosol and impacts the Mia40 redox state. *EMBO J.* **2012**, *31*, 3169–3182. [CrossRef]
51. López-Mirabal, H.R.; Winther, J.R. Redox characteristics of the eukaryotic cytosol. *Biochim. Biophys. Acta* **2008**, *1783*, 629–640.
52. Deponte, M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochim. Biophys. Acta* **2013**, *1830*, 3217–3266. [PubMed]
53. Aw, T.Y. Cellular redox: A modulator of intestinal epithelial cell proliferation. *News Physiol. Sci.* **2003**, *18*, 201–204. [CrossRef] [PubMed]
54. Van 't Erve, T.J.; Wagner, B.A.; Ryckman, K.K.; Raife, T.J.; Buettner, G.R. The concentration of glutathione in human erythrocytes is a heritable trait. *Free Radic. Biol. Med.* **2013**, *65*, 742–749. [CrossRef] [PubMed]
55. Schafer, F.Q.; Buettner, G.R. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. *Free Radic. Biol. Med.* **2001**, *30*, 1191–1212.

56. Bellomo, G.; Palladini, G.; Vairetti, M. Intranuclear distribution, function and fate of glutathione and glutathione-S-conjugate in living rat hepatocytes studied by fluorescence microscopy. *Microsc. Res. Tech.* **1997**, *36*, 243–252. [CrossRef]
57. Markovic, J.; Borrás, C.; Ortega, A.; Sastre, J.; Viña, J.; Pallardó, F.V. Glutathione is recruited into the nucleus in early phases of cell proliferation. *J. Biol. Chem.* **2007**, *282*, 20416–20424. [CrossRef]
58. Pallardó, F.V.; Markovic, J.; García, J.L.; Viña, J. Role of nuclear glutathione as a key regulator of cell proliferation. *Mol. Asp. Med.* **2009**, *30*, 77–85. [CrossRef]
59. Palmieri, F. The mitochondrial transporter family (SLC25): Physiological and pathological implications. *Pflügers Arch.* **2004**, *447*, 689–709. [CrossRef]
60. Lushchak, V.I. Glutathione homeostasis and functions: Potential targets for medical intervention. *J. Amino Acids* **2012**, *2012*, 736837. [CrossRef]
61. Zhong, Q.; Putt, D.A.; Xu, F.; Lash, L.H. Hepatic mitochondrial transport of glutathione: Studies in isolated rat liver mitochondria and H4IIE rat hepatoma cells. *Arch. Biochem. Biophys.* **2008**, *474*, 119–127. [CrossRef]
62. Kamga, C.K.; Zhang, S.X.; Wang, Y. Dicarboxylate carrier-mediated glutathione transport is essential for reactive oxygen species homeostasis and normal respiration in rat brain mitochondria. *Am. J. Physiol. Cell Physiol.* **2010**, *299*, C497–C505. [CrossRef]
63. Booty, L.M.; King, M.S.; Thangaratnarajah, C.; Majd, H.; James, A.M.; Kunji, E.R.S.; Murphy, M.P. The mitochondrial dicarboxylate and 2-oxoglutarate carriers do not transport glutathione. *FEBS Lett.* **2015**, *589*, 621–628. [CrossRef] [PubMed]
64. Cione, E.; Pingitore, A.; Perri, M.; Genchi, G. Influence of all-trans-retinoic acid on oxoglutarate carrier via retinoylation reaction. *Biochim. Biophys. Acta* **2009**, *1791*, 3–7. [CrossRef] [PubMed]
65. Coll, O.; Colell, A.; García-Ruiz, C.; Kaplowitz, N.; Fernández-Checa, J.C. Sensitivity of the 2-oxoglutarate carrier to alcohol intake contributes to mitochondrial glutathione depletion. *Hepatology* **2003**, *38*, 692–702. [CrossRef] [PubMed]
66. Ribas, V.; García-Ruiz, C.; Fernández-Checa, J.C. Glutathione and mitochondria. *Front. Pharmacol.* **2014**, *5*, 151. [PubMed]
67. Yin, F.; Sancheti, H.; Cadenas, E. Mitochondrial thiols in the regulation of cell death pathways. *Antioxid. Redox Signal.* **2012**, *17*, 1714–1727. [CrossRef]
68. Bánhegyi, G.; Lusini, L.; Puskás, F.; Rossi, R.; Fulceri, R.; Braun, L.; Mile, V.; di Simplicio, P.; Mandl, J.; Benedetti, A. Preferential transport of glutathione versus glutathione disulfide in rat liver microsomal vesicles. *J. Biol. Chem.* **1999**, *274*, 12213–12216. [CrossRef]
69. Bulleid, N.J.; Ellgaard, L. Multiple ways to make disulfides. *Trends Biochem. Sci.* **2011**, *36*, 485–492. [CrossRef]
70. Ponsero, A.J.; Igbaria, A.; Darch, M.A.; Miled, S.; Outten, C.E.; Winther, J.R.; Palais, G.; D'autreaux, B.; Delaunay-Moisan, A.; Toledano, M.B. Endoplasmic Reticulum Transport of Glutathione by Sec61 Is Regulated by Ero1 and Bip. *Mol. Cell* **2017**, *67*, 962–973. [CrossRef]
71. Csala, M.; Fulceri, R.; Mandl, J.; Benedetti, A.; Banhegyi, G. Ryanodine receptor channel dependent glutathione transport in the sarcoplasmic reticulum of skeletal muscle. *Biochem. Biophys. Res. Commun.* **2001**, *287*, 696–700. [CrossRef] [PubMed]
72. Appenzeller-Herzog, C.; Riemer, J.; Zito, E.; Chin, K.-T.; Ron, D.; Spiess, M.; Ellgaard, L. Disulphide production by Ero1 alpha-PDI relay is rapid and effectively regulated. *EMBO J.* **2010**, *29*, 3318–3329. [CrossRef]
73. Aoyama, K. Glutathione in the Brain. *Int. J. Mol. Sci.* **2021**, *22*, 5010. [CrossRef]
74. Aoyama, K.; Nakaki, T. Impaired glutathione synthesis in neurodegeneration. *Int. J. Mol. Sci.* **2013**, *14*, 21021–21044. [PubMed]
75. von Bartheld, C.S.; Bahney, J.; Herculano-Houzel, S. The search for true numbers of neurons and glial cells in the human brain: A review of 150 years of cell counting. *J. Comp. Neurol.* **2016**, *524*, 3865–3895.
76. Rice, M.E.; Russo-Menna, I. Differential compartmentalization of brain ascorbate and glutathione between neurons and glia. *Neuroscience* **1998**, *82*, 1213–1223. [CrossRef] [PubMed]
77. Mirzahosseini, A.; Somlyay, M.; Noszál, B. The comprehensive acid–base characterization of glutathione. *Chem. Phys. Lett.* **2015**, *622*, 50–56. [CrossRef]
78. Mazák, K.; Noszál, B. Advances in microspeciation of drugs and biomolecules: Species-specific concentrations, acid-base properties and related parameters. *J. Pharm. Biomed. Anal.* **2016**, *130*, 390–403. [CrossRef]
79. Bjerrum, N. Dissociation constants of polybasic acids and their application to the calculation of molecular dimensions. *Z. Phys. Chem.* **1923**, *106*, 219–242. [CrossRef]
80. Noszál, B. Group constant: A measure of submolecular basicity. *J. Phys. Chem.* **1986**, *90*, 4104–4110. [CrossRef]
81. Fujiwara, S.; Ishizuka, H.; Fudano, S. NMR study of amino acids and their derivatives. *Chem. Lett.* **1974**, *3*, 1281–1284. [CrossRef]
82. Noszál, B.; Scheller-Krattiger, V.; Martin, R.B. A unified view of carbon bound hydrogen exchange of H(2) in imidazoles and H(8) in purine nucleosides and their metal ion complexes. *J. Am. Chem. Soc.* **1982**, *104*, 1078–1081. [CrossRef]
83. Noszál, B.; Rabenstein, D.L. Nitrogen-protonation microequilibria and C(2)-deprotonation microkinetics of histidine, histamine, and related compounds. *J. Phys. Chem.* **1991**, *95*, 4761–4765. [CrossRef]
84. Szakács, Z.; Noszál, B. Determination of dissociation constants of folic acid, methotrexate, and other photolabile pteridines by pressure-assisted capillary electrophoresis. *Electrophoresis* **2006**, *27*, 3399–3409. [CrossRef] [PubMed]
85. Orgován, G.; Tihanyi, K.; Noszál, B. NMR analysis, protonation equilibria and decomposition kinetics of tolperisone. *J. Pharm. Biomed. Anal.* **2009**, *50*, 718–723. [CrossRef] [PubMed]
86. Tóth, G.; Baska, F.; Schretner, A.; Rácz, Á.; Noszál, B. Site-specific basicities regulate molecular recognition in receptor binding: In silico docking of thyroid hormones. *Eur. Biophys. J.* **2013**, *42*, 721–730. [CrossRef]
87. Pirie, N.W.; Pinhey, K.G. The titration curve of glutathione. *J. Biol. Chem.* **1929**, *84*, 321–333. [CrossRef]

88. Li, N.C.; Gawron, O.; Bascuas, G. Stability of zinc complexes with glutathione and oxidized glutathione. *J. Am. Chem. Soc.* **1954**, *76*, 225–229. [CrossRef]
89. Martin, R.B.; Edsall, J.T. Glutathione: Ionization in basic solutions and molecular rearrangement in strongly acid solution. *Bull. Soc. Chim. Biol.* **1958**, *40*, 1763–1771.
90. Dorcák, V.; Krężel, A. Correlation of acid–base chemistry of phytochelatin PC2 with its coordination properties towards the toxic metal ion Cd(II). *Dalton Trans.* **2003**, *11*, 2253–2259. [CrossRef]
91. Mah, V.; Jalilehvand, F. Mercury(II) complex formation with glutathione in alkaline aqueous solution. *J. Biol. Inorg. Chem.* **2008**, *13*, 541–553. [CrossRef]
92. Noszál, B.; Szakács, Z. Microscopic protonation equilibria of oxidized glutathione. *J. Phys. Chem. B* **2003**, *107*, 5074–5080. [CrossRef]
93. Wang, X.; Li, K.; Yang, X.D.; Wang, L.L.; Shen, R.F. Complexation of Al(III) with reduced glutathione in acidic aqueous solutions. *J. Inorg. Biochem.* **2009**, *103*, 657–666. [CrossRef]
94. Gough, J.D.; Lees, W.J. Effects of redox buffer properties on the folding of a disulfide-containing protein: Dependence upon pH, thiol pK_a, and thiol concentration. *J. Biotechnol.* **2005**, *115*, 279–290. [CrossRef]
95. Madej, E.; Wardman, P. The oxidizing power of the glutathione thiyl radical as measured by its electrode potential at physiological pH. *Arch. Biochem. Biophys.* **2007**, *462*, 94–102. [CrossRef]
96. Cigala, R.M.; Crea, F.; De Stefano, C.; Lando, G.; Milea, D.; Sammartano, S. Modeling the acid–base properties of glutathione in different ionic media, with particular reference to natural waters and biological fluids. *Amino Acids.* **2012**, *43*, 629–648. [CrossRef]
97. Harris, T.K.; Turner, G.J. Structural basis of perturbed pKa values of catalytic groups in enzyme active sites. *IUBMB Life* **2002**, *53*, 85–98. [CrossRef]
98. Matsui, R.; Ferran, B.; Oh, A.; Croteau, D.; Shao, D.; Han, J.; Pimentel, D.R.; Bachschmid, M.M. Redox Regulation via Glutaredoxin-1 and Protein *S*-Glutathionylation. *Antioxid. Redox Signal.* **2020**, *32*, 677–700. [CrossRef]
99. Chatgilialoglu, C.; Bowry, V.W. Why Not Trans? Inhibited Radical Isomerization Cycles and Coupling Chains of Lipids and Alkenes with Alkane-thiols. *J. Org. Chem.* **2018**, *83*, 9178–9189. [CrossRef]
100. Abedinzadeh, Z.; Gardes-Albert, M.; Ferradini, C. Kinetic study of the oxidation mechanism of glutathione by hydrogen peroxide in neutral aqueous medium. *Can. J. Chem.* **1989**, *67*, 1247–1255. [CrossRef]
101. Zinatullina, K.M.; Kasaikina, O.T.; Kuz'min, V.A.; Khrameeva, N.P. Interaction of Glutathione with Hydrogen Peroxide: A Kinetic Model. *Kinet. Catal.* **2019**, *60*, 266–272. [CrossRef]
102. Winterbourn, C.C. Radical Scavenging by Thiols and the Fate of Thiyl Radicals. In *Oxidative Stress and Redox Regulation*; Jakob, U., Reichmann, D., Eds.; Springer: Dordrecht, The Netherlands, 2013; pp. 43–58.
103. Quintana-Cabrera, R.; Bolaños, J.P. Glutathione and γ-glutamylcysteine in hydrogen peroxide detoxification. *Methods Enzymol.* **2013**, *527*, 129–144.
104. Kasamatsu, S.; Nishimura, A.; Morita, M.; Matsunaga, T.; Abdul Hamid, H.; Akaike, T. Redox signaling regulated by cysteine persulfide and protein polysulfidation. *Molecules* **2016**, *21*, 1712. [CrossRef]
105. Sawa, T.; Ono, K.; Tsutsuki, H.; Zhang, T.; Ida, T.; Nishida, M.; Akaike, T. Reactive cysteine persulphides: Occurrence, biosynthesis, antioxidant activity, methodologies, and bacterial persulphide signalling. *Adv. Microb. Physiol.* **2018**, *72*, 1–28.
106. Ida, T.; Sawa, T.; Ihara, H.; Tsuchiya, Y.; Watanabe, Y.; Kumagai, Y.; Suematsu, M.; Motohashi, H.; Fujii, S.; Matsunaga, T.; et al. Reactive cysteine persulfides and S-polythiolation regulate oxidative stress and redox signaling. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 7606–7611. [CrossRef]
107. Chauvin, J.-P.R.; Griesser, M.; Pratt, D.A. Hydropersulfides: H-atom transfer agents par excellence. *J. Am. Chem. Soc.* **2017**, *139*, 6484–6493. [CrossRef]
108. Libiad, M.; Motl, N.; Akey, D.L.; Sakamoto, N.; Fearon, E.R.; Smith, J.L.; Banerjee, R. Thiosulfate sulfurtransferase-like domain-containing 1 protein interacts with thioredoxin. *J. Biol. Chem.* **2018**, *293*, 2675–2686. [CrossRef]
109. Filipovic, M.R.; Zivanovic, J.; Alvarez, B.; Banerjee, V. Chemical biology of H_2S signaling through persulfidation. *Chem. Rev.* **2018**, *118*, 1253–1337.
110. Mueller, E.G. Trafficking in persulfides: Delivering sulfur in biosynthetic pathways. *Nat. Chem. Biol.* **2006**, *2*, 185–194. [CrossRef]
111. Kruithof, P.D.; Lunev, S.; Aguilar Lozano, S.P.; de Assis Batista, F.; Al-Dahmani, Z.M.; Joles, J.A.; Dolga, A.M.; Groves, M.R.; van Goor, H. Unraveling the role of thiosulfate sulfurtransferase in metabolic diseases. *Biochim. Biophys. Acta Mol. Basis Dis.* **2020**, *1866*, 165716. [CrossRef] [PubMed]
112. Kabil, O.; Motl, N.; Strack, M.; Seravalli, J.; Metzler-Nolte, N.; Banerjee, R. Mechanism-based inhibition of human persulfide dioxygenase by γ-glutamyl-homocysteinyl-glycine. *J. Biol. Chem.* **2018**, *293*, 12429–12439. [CrossRef]
113. Sies, H.; Jones, D.P. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nat. Rev. Mol. Cell Biol.* **2020**, *21*, 363–383.
114. Giustarini, D.; Rossi, R.; Milzani, A.; Colombo, R.; Dalle-Donne, I. S-glutathionylation: From redox regulation of protein functions to human diseases. *J. Cell. Mol. Med.* **2004**, *8*, 201–212. [CrossRef]
115. Mieyal, J.J.; Chock, P.B. Posttranslational modification of cysteine in redox signaling and oxidative stress: Focus on s-glutathionylation. *Antioxid. Redox Signal.* **2012**, *16*, 471–475. [CrossRef]
116. Forman, H.J.; Fukuto, J.M.; Miller, T.; Zhang, H.; Rinna, A.; Levy, S. The chemistry of cell signaling by reactive oxygen and nitrogen species and 4-hydroxynonenal. *Arch. Biochem. Biophys.* **2008**, *477*, 183–195. [CrossRef]

117. Forman, H.J.; Ursini, F.; Maiorino, M. An overview of mechanisms of redox signaling. *J. Mol. Cell. Cardiol.* **2014**, *73*, 2–9. [CrossRef]
118. Ren, X.; Zou, L.; Zhang, X.; Branco, V.; Wang, J.; Carvalho, C.; Holmgren, A.; Lu, J. Redox Signaling Mediated by Thioredoxin and Glutathione Systems in the Central Nervous System. *Antioxid. Redox Signal.* **2017**, *27*, 989–1010. [CrossRef]
119. Lou, M.F. Glutathione and Glutaredoxin in Redox Regulation and Cell Signaling of the Lens. *Antioxidants* **2022**, *11*, 1973. [CrossRef]
120. Pajaud, J.; Kumar, S.; Rauch, C.; Morel, F.; Aninat, C. Regulation of signal transduction by glutathione transferases. *Int. J. Hepatol.* **2012**, *2012*, 137676. [CrossRef]
121. Laborde, E. Glutathione transferases as mediators of signaling pathways involved in cell proliferation and cell death. *Cell Death Differ.* **2010**, *17*, 1373–1380. [CrossRef]
122. Singh, R.R.; Reindl, K.M. Glutathione S-transferases in cancer. *Antioxidants* **2021**, *10*, 701. [CrossRef]
123. Wang, J.Q.; Yang, Y.; Cai, C.Y.; Teng, Q.X.; Cui, Q.; Lin, J.; Assaraf, Y.G.; Chen, Z.S. Multidrug resistance proteins (MRPs): Structure, function and the overcoming of cancer multidrug resistance. *Drug Resist. Updat.* **2021**, *54*, 100743. [PubMed]
124. Ercolani, L.; Scirè, A.; Galeazzi, R.; Massaccesi, L.; Cianfruglia, L.; Amici, A.; Piva, F.; Urbanelli, L.; Emiliani, C.; Principato, G.; et al. A possible S-glutathionylation of specific proteins by glyoxalase II: An in vitro and in silico study. *Cell Biochem. Funct.* **2016**, *34*, 620–627. [CrossRef] [PubMed]
125. Rydström, J. Mitochondrial NADPH, transhydrogenase and disease. *Biochim. Biophys. Acta.* **2006**, *1757*, 721–726. [CrossRef] [PubMed]
126. Cereser, C.; Boget, S.; Parvaz, P.; Revol, A. Thiram-induced cytotoxicity is accompanied by a rapid and drastic oxidation of reduced glutathione with consecutive lipid peroxidation and cell death. *Toxicology* **2001**, *163*, 153–162. [CrossRef]
127. Zhao, Y.; Seefeldt, T.; Chen, W.; Wang, X.; Matthees, D.; Hu, Y.; Guan, X. Effects of glutathione reductase inhibition on cellular thiol redox state and related systems. *Arch. Biochem. Biophys.* **2009**, *485*, 56–62. [CrossRef]
128. Arning, J.; Dringen, R.; Schmidt, M.; Thiessen, A.; Stolte, S.; Matzke, M.; Bottin-Weber, U.; Caesar-Geertz, B.; Jastorff, B.; Ranke, J. Structure-activity relationships for the impact of selected isothiazol-3-one biocides on glutathione metabolism and glutathione reductase of the human liver cell line Hep G2. *Toxicology* **2008**, *246*, 203–212. [CrossRef]
129. Franco, J.L.; Posser, T.; Mattos, J.J.; Sánchez-Chardi, A.; Trevisan, R.; Oliveira, C.S.; Carvalho, P.S.; Leal, R.B.; Marques, M.R.; Bainy, A.C.; et al. Biochemical alterations in juvenile carp (*Cyprinus carpio*) exposed to zinc: Glutathione reductase as a target. *Environ. Res.* **2008**, *66*, 88–89. [CrossRef]
130. Maiorino, M.; Ursini, F.; Bosello, V.; Toppo, S.; Tosatto, S.C.; Mauri, P.; Becker, K.; Roveri, A.; Bulato, C.; Benazzi, L.; et al. The thioredoxin specificity of Drosophila GPx: A paradigm for a peroxiredoxin-like mechanism of many glutathione peroxidases. *J. Mol. Biol.* **2007**, *365*, 1033–1046. [CrossRef]
131. Toppo, S.; Flohé, L.; Ursini, F.; Vanin, S.; Maiorino, M. Catalytic mechanisms and specificities of glutathione peroxidases: Variations of a basic scheme. *Biochim. Biophys. Acta* **2009**, *1790*, 1486–1500. [CrossRef]
132. Flohé, L.; Toppo, S.; Cozza, G.; Ursini, F. A comparison of thiol peroxidase mechanisms. *Antioxid Redox Signal.* **2011**, *15*, 763–780. [CrossRef]
133. Hill, K.E.; Burk, R.F.; Lane, J.M. Effect of selenium depletion and repletion on plasma glutathione and glutathione-dependent enzymes in the rat. *J. Nutr.* **1987**, *117*, 99–104. [CrossRef] [PubMed]
134. Burk, R.F.; Hill, K.E.; Awad, J.A.; Morrow, J.D.; Lyons, P.R. Liver and kidney necrosis in selenium-deficient rats depleted of glutathione. *Lab. Investig.* **1995**, *72*, 723–730. [PubMed]
135. Hatfield, D.L.; Gladyshev, V.N. How selenium has altered our understanding of the genetic code. *Mol. Cell. Biol.* **2002**, *22*, 3565–3576. [CrossRef]
136. Woo, H.A.; Yim, S.H.; Shin, D.H.; Kang, D.; Yu, D.Y.; Rhee, S.G. Inactivation of peroxiredoxin I by phosphorylation allows localized H(2)O(2) accumulation for cell signaling. *Cell* **2010**, *140*, 517–528. [CrossRef]
137. Seiler, A.; Schneider, M.; Förster, H.; Roth, S.; Wirth, E.K.; Culmsee, C.; Plesnila, N.; Kremmer, E.; Rådmark, O.; Wurst, W.; et al. Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. *Cell Metab.* **2008**, *8*, 237–248. [CrossRef]
138. Gallogly, M.M.; Starke, D.W.; Mieyal, J.J. Mechanistic and kinetic details of catalysis of thiol-disulfide exchange by glutaredoxins and potential mechanisms of regulation. *Antioxid. Redox Signal.* **2009**, *11*, 1059–1081. [CrossRef] [PubMed]
139. Hurd, T.R.; Filipovska, A.; Costa, N.J.; Dahm, C.C.; Murphy, M.P. Disulphide formation on mitochondrial protein thiols. *Biochem. Soc. Trans.* **2005**, *33*, 1390–1393. [CrossRef]
140. Aslund, F.; Berndt, K.D.; Holmgren, A. Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria. *J. Biol. Chem.* **1997**, *272*, 30780–30786. [CrossRef]
141. Isakov, N.; Witte, S.; Altman, A. PICOT-HD: A highly conserved protein domain that is often associated with thioredoxin and glutaredoxin modules. *Trends Biochem. Sci.* **2000**, *25*, 537–539. [CrossRef]
142. Lillig, C.H.; Berndt, C.; Holmgren, A. Glutaredoxin systems. *Biochim. Biophys. Acta* **2008**, *1780*, 1304–1317.
143. Di Simplicio, P.; Cacace, M.G.; Lusini, L.; Giannerini, F.; Giustarini, D.; Rossi, R. Role of protein -SH groups in redox homeostasis–the erythrocyte as a model system. *Arch. Biochem. Biophys.* **1998**, *355*, 145–152. [CrossRef] [PubMed]
144. Jung, C.H.; Thomas, J.A. S-glutathiolated hepatocyte proteins and insulin disulfides as substrates for reduction by glutaredoxin, thioredoxin, protein disulfide isomerase, and glutathione. *Arch. Biochem. Biophys.* **1996**, *335*, 61–72. [CrossRef] [PubMed]

145. Cooper, J.L.; Pinto, J.T.; Callery, P.S. Reversible and irreversible protein glutathionylation: Biological and clinical aspects. *Expert Opin. Drug Metab. Toxicol.* **2011**, *7*, 891–910. [CrossRef] [PubMed]
146. Findlay, V.J.; Townsend, D.M.; Morris, T.E.; Fraser, J.P.; He, L.; Tew, K.D. A novel role for human sulfiredoxin in the reversal of glutathionylation. *Cancer Res.* **2006**, *66*, 6800–6806. [CrossRef] [PubMed]
147. Holmgren, A.; Lu, J. Thioredoxin and thioredoxin reductase: Current research with special reference to human disease. *Biochem. Biophys. Res. Commun.* **2010**, *396*, 120–124. [CrossRef]
148. Fratelli, M.; Demol, H.; Puype, M.; Casagrande, S.; Eberini, I.; Salmona, M.; Bonetto, V.; Mengozzi, M.; Duffieux, F.; Miclet, E.; et al. Identification by redox proteomics of glutathionylated proteins in oxidatively stressed human T lymphocytes. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 3505–3510. [CrossRef]
149. Rouhier, N.; Lemaire, S.D.; Jacquot, J.P. The role of glutathione in photosynthetic organisms: Emerging functions for glutaredoxins and glutathionylation. *Annu. Rev. Plant Biol.* **2008**, *59*, 143–166. [CrossRef]
150. Nordstrand, K.; Sandström, A.; Aslund, F.; Holmgren, A.; Otting, G.; Berndt, K.D. NMR structure of oxidized glutaredoxin 3 from Escherichia coli. *J. Mol. Biol.* **2000**, *303*, 423–432. [CrossRef]
151. Begas, P.; Liedgens, L.; Moseler, A.; Meyer, A.J.; Deponte, M. Glutaredoxin catalysis requires two distinct glutathione interaction sites. *Nat. Commun.* **2017**, *8*, 14835. [CrossRef]
152. Ukuwela, A.A.; Bush, A.I.; Wedd, A.G.; Xiao, Z. Reduction potentials of protein disulfides and catalysis of glutathionylation and deglutathionylation by glutaredoxin enzymes. *Biochem. J.* **2017**, *474*, 3799–3815. [CrossRef]
153. Ogata, F.T.; Branco, V.; Vale, F.F.; Coppo, L. Glutaredoxin: Discovery, redox defense and much more. *Redox Biol.* **2021**, *43*, 101975. [PubMed]
154. Lillig, C.H.; Berndt, C.; Vergnolle, O.; Lönn, M.E.; Hudemann, C.; Bill, E.; Holmgren, A. Characterization of human glutaredoxin 2 as iron-sulfur protein: A possible role as red ox sensor. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 8168–8173. [CrossRef]
155. Prigge, J.R.; Coppo, L.; Martin, S.S.; Ogata, F.; Miller, C.G.; Bruschwein, M.D.; Orlicky, D.J.; Shearn, C.T.; Kundert, J.A.; Lytchier, J.; et al. Hepatocyte Hyperproliferation upon Liver-Specific Co-disruption of Thioredoxin-1, Thioredoxin Reductase-1, and Glutathione Reductase. *Cell Rep.* **2017**, *19*, 2771–2781. [CrossRef] [PubMed]
156. Tamarit, J.; Bellí, G.; Cabiscol, E.; Herrero, E.; Ros, J. Biochemical characterization of yeast mitochondrial Grx5 monothiol glutaredoxin. *J. Biol. Chem.* **2003**, *278*, 25745–25751. [CrossRef] [PubMed]
157. Witte, S.; Villalba, M.; Bi, K.; Liu, Y.; Isakov, N.; Altman, A. Inhibition of the c-Jun N-terminal kinase/AP-1 and NF-κB pathways by PICOT, a novel protein kinase C-interacting protein with a thioredoxin homology domain. *J. Biol. Chem.* **2000**, *275*, 1902–1909. [CrossRef] [PubMed]
158. Haunhorst, P.; Hanschmann, E.M.; Bräutigam, L.; Stehling, O.; Hoffmann, B.; Mühlenhoff, U.; Lill, R.; Berndt, C.; Lillig, C.H. Crucial function of vertebrate glutaredoxin 3 (PICOT) in iron homeostasis and hemoglobin maturation. *Mol. Biol. Cell* **2013**, *24*, 1895–1903. [CrossRef] [PubMed]
159. Pandya, P.; Pasvolsky, R.; Babichev, Y.; Braiman, A.; Witte, S.; Altman, A.; Isakov, N. PICOT binding to the polycomb group protein, EED, alters H3K27 methylation at the MYT1 PRC2 target gene. *Biochem. Biophys. Res. Commun.* **2019**, *509*, 469–475. [CrossRef]
160. Pandya, P.; Isakov, N. PICOT promotes T lymphocyte proliferation by down-regulating cyclin D2 expression. *World J. Immunol.* **2020**, *10*, 1–12. [CrossRef]
161. Brancaccio, D.; Gallo, A.; Mikolajczyk, M.; Zovo, K.; Palumaa, P.; Novellino, E.; Piccioli, M.; Ciofi-Baffoni, S.; Banci, L. Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery. *J. Am. Chem. Soc.* **2014**, *136*, 16240–16250. [CrossRef]
162. Banci, L.; Brancaccio, D.; Ciofi-Baffoni, S.; Del Conte, R.; Gadepalli, R.; Mikolajczyk, M.; Neri, S.; Piccioli, M.; Winkelmann, J. [2Fe-2S] cluster transfer in iron-sulfur protein biogenesis. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 6203–6208. [CrossRef]
163. Nasta, V.; Giachetti, A.; Ciofi-Baffoni, S.; Banci, L. Structural insights into the molecular function of human [2Fe-2S] BOLA1-GRX5 and [2Fe-2S] BOLA3-GRX5 complexes. *Biochim. Biophys. Acta Gen. Subj.* **2017**, *1861*, 2119–2131. [CrossRef] [PubMed]
164. Rozell, B.; Barcena, J.A.; Martinez-Galisteo, E.; Padilla, C.A.; Holmgren, A. Immunochemical characterization and tissue distribution of glutaredoxin (thioltransferase) from calf. *Eur. J. Cell Biol.* **1993**, *62*, 314–323.
165. Pai, H.V.; Starke, D.W.; Lesnefsky, E.J.; Hoppel, C.L.; Mieyal, J.J. What is the functional significance of the unique location of glutaredoxin 1 (GRx1) in the intermembrane space of mitochondria? *Antioxid. Redox Signal.* **2007**, *9*, 2027–2033. [CrossRef] [PubMed]
166. Ukuwela, A.A.; Bush, A.I.; Wedd, A.G.; Xiao, Z. Glutaredoxins employ parallel monothiol-dithiol mechanisms to catalyze thiol-disulfide exchanges with protein disulfides. *Chem. Sci.* **2017**, *9*, 1173–1183. [CrossRef] [PubMed]
167. González, R.; López-Grueso, M.J.; Muntané, J.; Bárcena, J.A.; Padilla, C.A. Redox regulation of metabolic and signaling pathways by thioredoxin and glutaredoxin in NOS-3 overexpressing hepatoblastoma cells. *Redox Biol.* **2015**, *6*, 122–134. [CrossRef]
168. Inadomi, C.; Murata, H.; Ihara, Y.; Goto, S.; Urata, Y.; Yodoi, J.; Kondo, T.; Sumikawa, K. Overexpression of glutaredoxin protects cardiomyocytes against nitric oxide-induced apoptosis with suppressing the S-nitrosylation of proteins and nuclear translocation of GAPDH. *Biochem. Biophys. Res. Commun.* **2012**, *425*, 656–661. [CrossRef]
169. Lundberg, M.; Fernandes, A.P.; Kumar, S.; Holmgren, A. Cellular and plasma levels of human glutaredoxin 1 and 2 detected by sensitive ELISA systems. *Biochem. Biophys. Res. Commun.* **2004**, *319*, 801–809. [CrossRef]

170. Lundberg, M.; Johansson, C.; Chandra, J.; Enoksson, M.; Jacobsson, G.; Ljung, J.; Johansson, M.; Holmgren, A. Cloning and expression of a novel human glutaredoxin (Grx2) with mitochondrial and nuclear isoforms. *J. Biol. Chem.* **2001**, *276*, 26269–26275. [CrossRef]
171. Johansson, C.; Lillig, C.H.; Holmgren, A. Human mitochondrial glutaredoxin reduces S-glutathionylated proteins with high affinity accepting electrons from either glutathione or thioredoxin reductase. *J. Biol. Chem.* **2004**, *279*, 7537–7543. [CrossRef]
172. Hashemy, S.I.; Johansson, C.; Berndt, C.; Lillig, C.H.; Holmgren, A. Oxidation and S-nitrosylation of cysteines in human cytosolic and mitochondrial glutaredoxins: Effects on structure and activity. *J. Biol. Chem.* **2007**, *282*, 14428–14436. [CrossRef]
173. Wu, H.; Xing, K.; Lou, M.F. Glutaredoxin 2 prevents H2O2-induced cell apoptosis by protecting complex I activity in the mitochondria. *Biochim. Biophys. Acta Bioenerg.* **2010**, *1797*, 1705–1715. [CrossRef] [PubMed]
174. Ferri, A.; Fiorenzo, P.; Nencini, M.; Cozzolino, M.; Pesaresi, M.G.; Valle, C.; Sepe, S.; Moreno, S.; Carrì, M.T. Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. *Hum. Mol. Genet.* **2010**, *19*, 4529–4542. [CrossRef]
175. Rhee, S.G.; Woo, H.A. Multiple functions of peroxiredoxins: Peroxidases, sensors and regulators of the intracellular messenger H_2O_2, and protein chaperones. *Antioxid. Redox Signal.* **2011**, *15*, 781–794. [CrossRef] [PubMed]
176. Georgiou, G.; Masip, L. Biochemistry. An overoxidation journey with a return ticket. *Science* **2003**, *300*, 592–594. [CrossRef] [PubMed]
177. Bryk, R.; Griffin, P.; Nathan, C. Peroxynitrite reductase activity of bacterial peroxiredoxins. *Nature* **2000**, *407*, 211–215. [CrossRef] [PubMed]
178. Rhee, S.G.; Woo, H.A.; Kil, I.S.; Bae, S.H. Peroxiredoxin functions as a peroxidase and a regulator and sensor of local peroxides. *J. Biol. Chem.* **2012**, *287*, 4403–4410. [CrossRef]
179. Wood, Z.A.; Schröder, E.; Robin Harris, J.; Poole, L.B. Structure, mechanism and regulation of peroxiredoxins. *Trends Biochem. Sci.* **2003**, *28*, 32–40. [CrossRef]
180. Nadeem, M.S.; Khan, J.A.; Murtaza, B.N.; Muhammad, K.; Rauf, A. Purification and properties of liver catalase from water buffalo (*Bubalus bubalis*). *South Asian J. Life Sci.* **2015**, *3*, 51–55. [CrossRef]
181. Rhee, S.G.; Woo, H.A. Multiple functions of 2-Cys peroxiredoxins, I and II, and their regulations via posttranslational modifications. *Free Radic. Biol. Med.* **2020**, *152*, 107–115. [CrossRef]
182. Randall, L.; Manta, B.; Nelson, K.J.; Santos, J.; Poole, L.B.; Denicola, A. Structural changes upon peroxynitrite-mediated nitration of peroxiredoxin 2; nitrated Prx2 resembles its disulfide-oxidized form. *Arch. Biochem. Biophys.* **2016**, *590*, 101–108. [CrossRef]
183. Nelson, K.J.; Perkins, A.; Van Swearingen, A.E.D.; Hartman, S.; Brereton, A.E.; Parsonage, D.; Salsbury, F.R., Jr.; Karplus, P.A.; Poole, L.B. Experimentally dissecting the origins of peroxiredoxin catalysis. *Antioxid. Redox Signal.* **2018**, *28*, 521–536. [CrossRef] [PubMed]
184. Bolduc, J.; Koruza, K.; Luo, T.; Malo Pueyo, J.; Nghia Vo, T.; Ezeriņa, D.; Messens, J. Peroxiredoxins wear many hats: Factors that fashion their peroxide sensing personalities. *Redox Biol.* **2021**, *42*, 101959.
185. Monteiro, G.; Horta, B.B.; Pimenta, D.C.; Augusto, O.; Netto, L.E. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 4886–4891. [CrossRef] [PubMed]
186. Phillips, A.J.; Littlejohn, J.; Yewdall, N.A.; Zhu, T.; Valéry, C.; Pearce, F.G.; Mitra, A.K.; Radjainia, M.; Gerrard, J.A. Peroxiredoxin is a Versatile Self-Assembling Tecton for Protein Nanotechnology. *Biomacromolecules* **2014**, *15*, 1871–1881. [CrossRef] [PubMed]
187. Kim, Y.; Jang, H.H. Role of Cytosolic 2-Cys Prx1 and Prx2 in Redox Signaling. *Antioxidants* **2019**, *8*, 169. [CrossRef]
188. Phalen, T.J.; Weirather, K.; Deming, P.B.; Anathy, V.; Howe, A.K.; van der Vliet, A.; Jonsson, T.J.; Poole, L.B.; Heintz, N.H. Oxidation state governs structural transitions in peroxiredoxin II that correlate with cell cycle arrest and recovery. *J. Cell Biol.* **2006**, *175*, 779–789. [CrossRef]
189. Saccoccia, F.; Di Micco, P.; Boumis, G.; Brunori, M.; Koutris, I.; Miele, A.E.; Morea, V.; Sriratana, P.; Williams, D.L.; Bellelli, A.; et al. Moonlighting by different stressors: Crystal structure of the chaperone species of a 2-Cys peroxiredoxin. *Structure* **2012**, *20*, 429–439. [CrossRef]
190. Rhee, S.G.; Chae, H.Z.; Kim, K. Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radic. Biol. Med.* **2005**, *38*, 1543–1552. [CrossRef]
191. Manevich, Y.; Feinstein, S.I.; Fisher, A.B. Activation of the antioxidant enzyme 1-CYS peroxiredoxin requires glutathionylation mediated by heterodimerization with pi GST. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 3780–3785. [CrossRef]
192. Schremmer, B.; Manevich, Y.; Feinstein, S.I.; Fisher, A.B. Peroxiredoxins in the lung with emphasis on peroxiredoxin VI. *Subcell. Biochem.* **2007**, *44*, 317–344.
193. Poole, L.B.; Nelson, K.J. Distribution and features of the six classes of peroxiredoxins. *Mol. Cells* **2016**, *39*, 53–59. [PubMed]
194. Aniya, Y.; Imaizumi, N. Mitochondrial glutathione transferases involving a new function for membrane permeability transition pore regulation. *Drug Metab. Rev.* **2011**, *43*, 292–299. [CrossRef] [PubMed]
195. Kural, C.; Kocdogan, A.K.; Şimşek, G.G.; Oguztuzun, S.; Kaygın, P.; Yılmaz, I.; Bayram, T.; Izci, Y. Glutathione S-transferases and cytochrome P450 enzyme expression in patients with intracranial tumors: Preliminary report of 55 patients. *Med. Princ. Pract.* **2018**, *28*, 56–62. [CrossRef] [PubMed]
196. Raza, H. Dual localization of glutathione S-transferase in the cytosol and mitochondria: Implications in oxidative stress, toxicity and disease. *FEBS J.* **2011**, *278*, 4243–4251. [CrossRef]

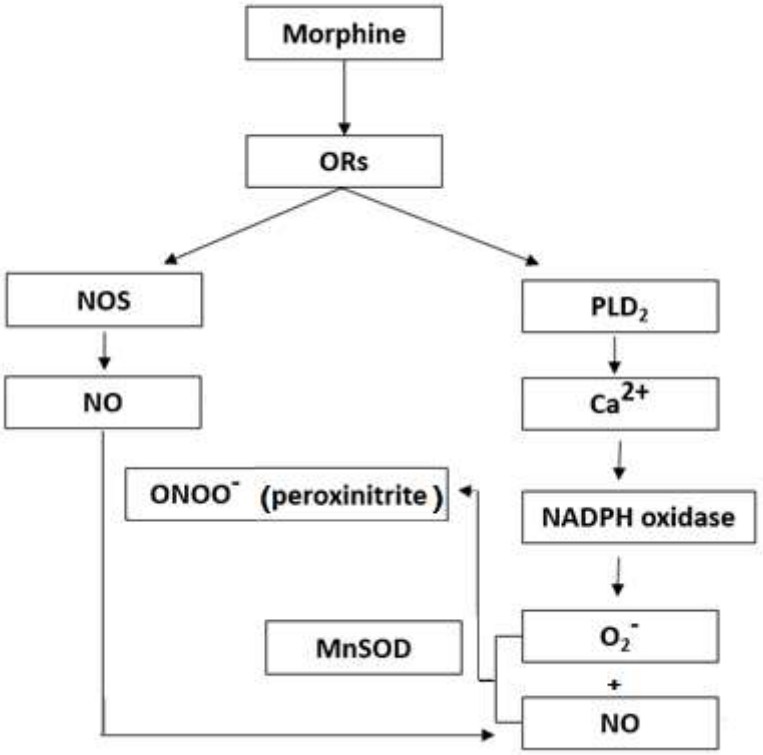
197. Hayes, J.D.; Strange, R.C. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology* **2000**, *61*, 154–166. [CrossRef]
198. Hayes, J.D.; Flanagan, J.U.; Jowsey, I.R. Glutathione transferases. *Annu. Rev. Pharmacol. Toxicol.* **2005**, *45*, 51–88. [CrossRef]
199. Chatterjee, A.; Gupta, S. The multifaceted role of glutathione S-transferases in cancer. *Cancer Lett.* **2018**, *433*, 33–42. [CrossRef]
200. Townsend, D.; Tew, K. The role of glutathione-*S*-transferase in anti-cancer drug resistance. *Oncogene* **2003**, *22*, 7369–7375. [CrossRef]
201. Pacifici, G.; Franchi, M.; Bencini, C.; Repetti, F.; Di Lascio, N.; Muraro, G. Tissue distribution of drug-metabolizing enzymes in humans. *Xenobiotica* **1988**, *18*, 849–856. [CrossRef]
202. Oakley, A. Glutathione transferases: A structural perspective. *Drug Metab. Rev.* **2011**, *43*, 138–151. [CrossRef]
203. Ladner, J.E.; Parsons, J.F.; Rife, C.L.; Gilliland, G.L.; Armstrong, R.N. Parallel evolutionary pathways for glutathione transferases: Structure and mechanism of the mitochon-drial class kappa enzyme rGSTK1-1. *Biochemistry* **2004**, *43*, 352–361. [CrossRef] [PubMed]
204. Li, J.; Xia, Z.; Ding, J. Thioredoxin-like domain of human kappa class glutathione transferase reveals sequence homology and structure similarity to the theta class enzyme. *Protein Sci.* **2005**, *14*, 2361–2369. [CrossRef] [PubMed]
205. Nathaniel, C.; Wallace, L.A.; Burke, J.; Dirr, H.W. The role of an evolutionarily conserved cis-proline in the thioredoxin-like domain of human class Alpha glutathione transferase A1-1. *Biochem. J.* **2003**, *372*, 241–246. [CrossRef] [PubMed]
206. Atkinson, H.J.; Babbitt, P.C. Glutathione transferases are structural and functional outliers in the thioredoxin fold. *Biochemistry* **2009**, *48*, 11108–11116. [CrossRef]
207. Stenberg, G.; Board, P.G.; Mannervik, B. Mutation of an evolutionarily conserved tyrosine residue in the active-site of a human class alpha-glutathione transferase. *FEBS Lett.* **1991**, *293*, 153–155. [CrossRef]
208. Board, P.G.; Coggan, M.; Chelvanayagam, G.; Easteal, S.; Jermiin, L.S.; Schulte, G.K.; Danley, D.E.; Hoth, L.R.; Griffor, M.C.; Kamath, A.V.; et al. Identification, characterization, and crystal structure of the omega class glutathione transferases. *J. Biol. Chem.* **2000**, *275*, 24798–24806. [CrossRef]
209. Mannervik, B.; Board, P.G.; Hayes, J.D.; Listowsky, I.; Pearson, W.R. Nomenclature for mammalian soluble glutathione transferases. *Methods Enzymol.* **2005**, *401*, 1–8.
210. Hayes, J.D.; Pulford, D.J. The glutathione *S*-transferase gene family: Regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 445–600.
211. Fernández-Cañón, J.M.; Peñalva, M.A. Characterization of a fungal maleylacetoacetate isomerase gene and identification of its human homologue. *J. Biol. Chem.* **1998**, *273*, 329–337. [CrossRef]
212. Yang, Y.; Sharma, R.; Zimniak, P.; Awasthi, Y.C. Role of α class glutathione S-transferases as antioxidant enzymes in rodent tissues. *Toxicol. Appl. Pharmacol.* **2002**, *182*, 105–115. [CrossRef]
213. Yang, J.Y.; Sharma, R.; Sharma, A.; Awasthi, S.; Awasthi, Y.C. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochim. Pol.* **2003**, *50*, 319–336. [CrossRef]
214. Johansson, A.S.; Mannervik, B. Human glutathione transferase A3-3, a highly efficient catalyst of double-bond isomerization in the biosynthetic pathway of steroid hormones. *J. Biol. Chem.* **2001**, *276*, 33061–33065. [CrossRef]
215. Flanagan, J.U.; Smythe, M.L. Sigma-class glutathione transferases. *Drug Metab. Rev.* **2011**, *43*, 194–214. [CrossRef]
216. Kanaoka, Y.; Ago, H.; Inagaki, E.; Nanayama, T.; Miyano, M.; Kikuno, R.; Fujii, Y.; Eguchi, N.; Toh, H.; Urade, Y.; et al. Cloning and crystal structure of hematopoietic prostaglandin D synthase. *Cell* **1997**, *90*, 1085–1095. [CrossRef]
217. Welsch, D.J.; Creely, D.P.; Hauser, S.D.; Mathis, K.J.; Krivi, G.G.; Isakson, P.C. Molecular cloning and expression of human leukotriene-C4 synthase. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 9745–9749. [CrossRef]
218. Campbell, E.; Takahashi, Y.; Abramovitz, M.; Peretz, M.; Listowsky, I. A distinct human testis and brain mu-class glutathione S-transferase. Molecular cloning and characterization of a form present even in individuals lacking hepatic type mu isoenzymes. *J Biol Chem.* **1990**, *265*, 9188–9193. [CrossRef] [PubMed]
219. Dulhunty, A.; Gage, P.; Curtis, S.; Chelvanayagam, G.; Board, P. The glutathione structural family includes a nuclear chloride channel and a ryanodine receptor calcium release channel modulator. *J. Biol. Chem.* **2001**, *276*, 3319–3323. [CrossRef]
220. Harrop, S.J.; DeMaere, M.Z.; Fairlie, W.D.; Reztsova, T.; Valenzuela, S.M.; Mazzanti, M.; Tonini, R.; Qiu, M.R.; Jankova, L.; Warton, K.; et al. Crystal structure of a soluble form of the intracellular chloride ion channel CLIC1 (NCC27) at 1.4-A resolution. *J. Biol Chem.* **2001**, *276*, 44993–45000. [CrossRef] [PubMed]
221. Wilce, M.C.; Parker, M.W. Structure and function of glutathione S-transferases. *Biochim. Biophys. Acta* **1994**, *1205*, 1–18. [CrossRef] [PubMed]
222. Menon, D.; Board, P.G. A role for glutathione transferase Omega 1 (GSTO1-1) in the glutathionylation cycle. *J. Biol. Chem.* **2013**, *288*, 25769–25779. [CrossRef]
223. Board, P.G.; Anders, M.W. Glutathione transferase omega 1 catalyzes the reduction of *S*-(phenacyl)glutathiones to acetophenones. *Chem. Res. Toxicol.* **2007**, *20*, 149–154. [CrossRef]
224. Schmuck, E.M.; Bard, P.G.; Whibread, A.K.; Tetlow, N.; Cavanaugh, J.A.; Blackburn, A.C.; Masoumi, A. Characterization of the monomethylarsonate reductase and dehydroascorbate reductase activities of omega class glutathione transferase variants. Implications for arsenic metabolism and the age-at-onset of Alzheimer's and Parkinson's diseases. *Pharmacogenet. Genom.* **2005**, *15*, 493–501. [CrossRef]

225. Dong, S.; Sha, H.; Xu, X.; Hu, T.; Lou, R.; Li, H.; Wu, J.; Dan, C.; Feng, J. Glutathione S-transferase π: A potential role in antitumor therapy. *Drug Des. Dev. Ther.* **2018**, *12*, 3535–3547. [CrossRef]
226. Townsend, D.M.; Findlay, V.L.; Tew, K.D. Glutathione S-transferases as regulators of kinase pathways and anticancer drug targets. *Methods Enzymol.* **2005**, *401*, 287–307.
227. Antognelli, C.; Ferri, I.; Bellezza, G.; Siccu, P.; Love, H.D.; Talesa, V.N.; Sidoni, A. Glyoxalase 2 drives tumorigenesis in human prostate cells in a mechanism involving androgen receptor and p53-p21 axis. *Mol. Carcinog.* **2017**, *56*, 2112–2126. [CrossRef]
228. Sousa Silva, M.; Gomes, R.A.; Ferreira, A.E.; Ponces Freire, A.; Cordeiro, C. The glyoxalase pathway: The first hundred years . . . and beyond. *Biochem. J.* **2013**, *453*, 1–15. [CrossRef]
229. Rabbani, N.; Xue, M.; Thornalley, P.J. Dicarbonyls and glyoxalase in disease mechanisms and clinical therapeutics. *Glycoconj. J.* **2016**, *33*, 513–525. [CrossRef]
230. Ellis, K.J. Human body composition: In vivo methods. *Physiol. Rev.* **2000**, *80*, 649–680.
231. Thornalley, P.J.; Waris, S.; Fleming, T.; Santarius, T.; Larkin, S.J.; Winklhofer-Roob, B.M.; Stratton, M.R.; Rabbani, N. Imidazopurinones are markers of physiological genomic damage linked to DNA instability and glyoxalase 1-associated tumour multidrug resistance. *Nucleic Acids Res.* **2010**, *38*, 5432–5442. [CrossRef]
232. He, Y.; Zhou, C.; Huang, M.; Tang, C.; Liu, X.; Yue, Y.; Diao, Q.; Zheng, Z.; Liu, D. Glyoxalase system: A systematic review of its biological activity, related-diseases, screening methods and small molecule regulators. *Biomed. Pharmacother.* **2020**, *131*, 110663. [CrossRef]
233. Sarker, M.K.; Lee, J.H.; Lee, D.H.; Chun, K.H.; Jun, H.S. Attenuation of diabetic kidney injury in DPP4-deficient rats; role of GLP-1 on the suppression of AGE formation by inducing glyoxalase 1. *Aging* **2020**, *12*, 593–610. [PubMed]
234. Thornalley, P.J. The glyoxalase system in health and disease. *Mol. Asp. Med.* **1993**, *14*, 287–371.
235. Birkenmeier, G.; Stegemann, C.; Hoffmann, R.; Gunther, R.; Huse, K.; Birkemeyer, C. Posttranslational modification of human glyoxalase 1 indicates redox-dependent regulation. *PLoS ONE* **2010**, *5*, e10399.
236. Limphong, P.; Nimako, G.; Thomas, P.W.; Fast, W.; Makaroff, C.A.; Crowde, M.W. *Arabidopsis thaliana* mitochondrial glyoxalase 2–1 exhibits beta-lactamase activity. *Biochemistry* **2009**, *48*, 8491–8493. [PubMed]
237. Wendler, A.; Irsch, T.; Rabbani, N.; Thornalley, P.J.; Krauth-Siegel, R.L. Glyoxalase II does not support methylglyoxal detoxification but serves as a general trypanothione thioesterase in African trypanosomes. *Mol. Biochem. Parasitol.* **2009**, *163*, 19–27. [PubMed]
238. Hara, T.; Toyoshima, M.; Hisano, Y.; Balan, S.; Iwayama, Y.; Aono, H.; Fatamura, Y.; Osada, H.; Owada, Y.; Yoshikawa, T. Glyoxalase I disruption and external carbonyl stress impair mitochondrial function in human induced pluripotent stem cells and derived neurons. *Transl. Psychiatry* **2021**, *11*, 275.
239. Xue, M.; Shafie, A.; Qaiser, T.; Rajpoot, N.M.; Kaltsas, G.; James, S.; Gopalakrishnan, K.; Fisk, A.; Dimitriadis, G.K.; Grammatopoulos, D.K.; et al. Glyoxalase 1 copy number variation in patients with well differentiated gastro-entero-pancreatic neuroendocrine tumours (GEP-NET). *Oncotarget* **2017**, *8*, 76961–76973. [PubMed]

5 OXIDAČNÝ STRES A OPIOIDY

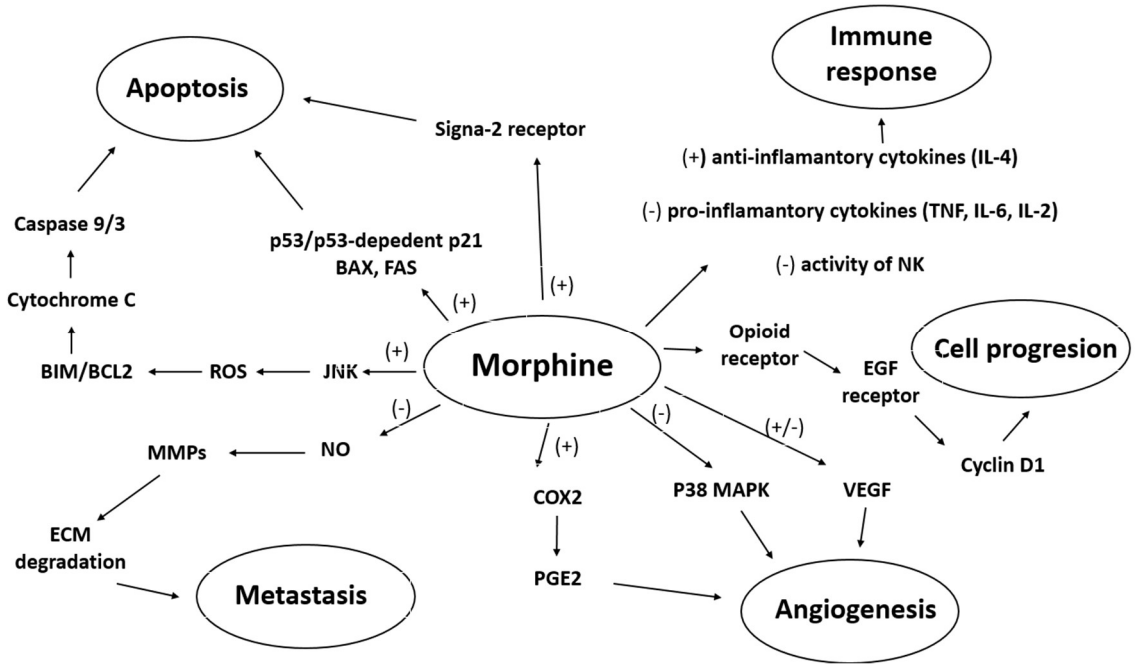
Viaceré štúdie poukazujú na koreláciu medzi vznikom oxidačného stresu a jeho následnými komplikáciami v súvislosti s liečbou opioidmi. Najviac podozrivým liečivom s týmto vedľajším účinkom sa javí byť morfín. Tento efekt je pravdepodobný aj pri semisyntetických molekulách odvodených od morfínu, ktoré sa pri odbúravaní v organizme menia na morfín alebo jeho metabolity. Štúdie iných opioidov zamerané sa nadmernú produkciu voľných radikálov doposiaľ neboli uskutočnené v dostatočnom rozsahu.

V zásade genéza tvorby reaktívnych foriem kyslíka a dusíka v spojitosti s dlhodobým užívaním morfínu môže prebiehať dvoma možnými biochemickými cestami. Sú to reakcie, ktoré nasledujú po aktivácii enzýmu NO-syntázy alebo po aktivácii enzýmu fosfolypázy D2. Tieto cesty môžu v organizme prebiehať súčasne (40).



Obrázok č.5: Vzťah medzi morfínom a tvorbou voľných radikálov. Diagram schematicky znázorňuje predpokladané dráhy, ktorými môžu opioidné receptory aktivované morfínom indukovať oxidačný stres v cieľových bunkách. Skratky: ORs - Opioidné receptory, ONOO- - peroxynitrit (peroxynitritový anión), PLD2 - foslfolipáza D2, MnSOD - Superoxiddizmutáza (Mn-kofaktor) (Zdroj: spracované podľa Skrabalova, 2013).

Štúdie, ktoré skúmali problematiku krátkodobého a dlhodobého pôsobenia morfínu poukazujú na signifikantný pokles hladín glutatiónu v mozgovom tkanive a v pečeni hlodavcov, ako aj v mozgovom tkanive ľudí. Zároveň bolo zistené, že chronická liečba morfínom viedla k zníženiu aktivít antioxidačných enzýmov superoxiddizmutázy, katalázy, glutatión peroxidázy a ďalších enzýmov zahrnutých do antioxidačnej obrany (40, 41).
Na oslabení antioxidačnej aktivity u jedincov zaťažených opioidmi sa podieľa veľa ďalších vnútorných, ako aj vonkajších faktorov. Tento efekt závisí od doby trvania liečby, množstva podávaného morfínu, od možných interakcií s inými liečivami, ako aj od zmien v metabolizme pri rôznych ochoreniach. Vo všeobecnosti sa nadmerná tvorba reaktívnych foriem kyslíka a dusíka a súčasne ich znížená eliminácia antioxidačnými molekulami, spája so vznikom oxidačného stresu (42). Tento efekt bol zaznamenaný v rôznych predklinických a klinických štúdiách, a to pri krátkodobom ako aj dlhodobom podávaní morfínu.



Obrázok č.6: Vplyv morfínu na rozličné endogénne procesy. ORs - Opioidné receptory, ONOO- peroxynitrit (peroxynitritový anión), PLD2 - fosfolipáza D2, MnSOD - Superoxiddizmutáza (Mn-kofaktor), VEGF - Vascular endothelial growth factor (rastový faktor cievneho endotelu), EGF - Epidermal growth factor (rastový faktor epidermy), NK - c-Jun N-terminal kinase, MMPs - matrixové metaloproteinázy, BAX - Bcl-2-like protein 4, BIM - Bcl-2-like protein 11, BCL2 - B-cell lymphoma 2, FAS - Fas receptor (Zdroj: Gach, 2011).

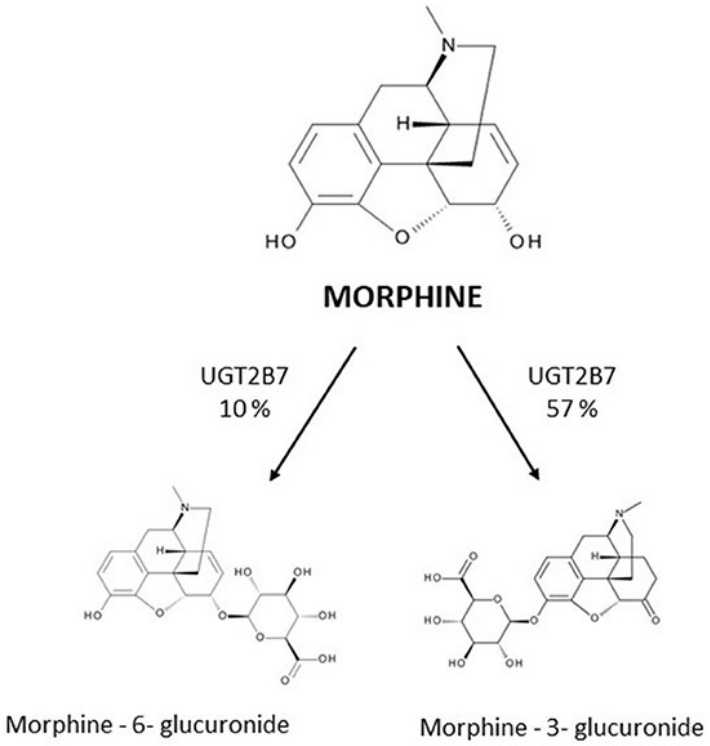
Štúdia sledujúca oxidačné poškodenie ciev preukázala tvorbu reaktívnych foriem kyslíka po dlhodobom podávaní morfínu v endotelových bunkách laboratórnych zvierat. Tento efekt bol neskôr preukázaný aj na endotelových bunkách ľudí. Vysvetlením tohto javu môže byť znížená koncentrácia oxidu dusnatého, zhoršená vazodilatácia, ako aj následný rozvoj aterosklerózy (43). Vplyvom pôsobenia morfínu bolo zaznamenané zvýšenie koncentrácie peroxidu vodíka v prefrontálnom kortexe a v striatum potkanov. Zároveň boli v hipokampe a v pečeni namerané zvýšené koncentrácie produktov lipidovej peroxidácie (44).

Zvýšená tvorba voľných kyslíkových radikálov, a to najmä superoxidu, bola preukázaná aj v tkanivových makrofágoch. Naproti tomu výsledky experimentálnych štúdií sledujúcich bunkové línie ľudského neuroblastómu SH-SY5Y po podávaní morfínu nepreukázali jeho prooxidatívny efekt a ani urýchlenie apoptózy týchto buniek. Tento dôkaz môže svedčiť pre zvýšenú rezistenciu nádorového tkaniva voči tvorbe radikálov a následnému poškodeniu (45).

Dôležitou reaktívnou molekulou zapojenou do oxidačných procesov iniciovaných morfínom je peroxynitrit, ktorý sa tvorí v reakcii superoxidového aniónového radikálu s oxidom dusnatým. Tieto molekuly slúžia ako signálne molekuly nielen pri rozvoji oxidačného stresu, ale sú zároveň zahrnuté do patogenézy vzniku hyperalgézie ako aj opiátovej tolerancie. Tieto zistenia boli potvrdené pri inhibícii syntézy NO a superoxidu (46).

Medzi hlavné enzýmy, ktorých aktivitou sa tvoria NO a superoxid sú syntáza oxidu dusnatého a enzýmy rodiny NOX (NADPH-oxidázy). Zníženie tvorby radikálov vedie k zníženiu pravdepodobnosti vytvorenia morfínom indukovanej antinociceptívnej tolerancie. Tento efekt bol dokázaný na geneticky modifikovaných laboratórnych myšiach s deficienciou NOS, u ktorých po dlhodobom podávaní morfínu nedošlo k rozvoju morfínom indukovanej antinociceptívnej tolerancie. Morfínom indukovaná aktivácia NADPH oxidázy, ktorá následne katalyzuje tvorbu superoxidu a aktivuje makrofágy, je jedným z viacerých príkladov prepojenia účinku morfínu a jeho vplyvu na imunitný systém. Rovnako dôležité sú neuroimunitné procesy aktivované zvýšenou produkciou prozápalových cytokínov (TNF-α, IL-1β, IL6). Táto signálna interakcia je sprostredkovaná cez μ-opioidný receptor, čím následne dochádza ku aktivácii fosfolipázy D a k následnej kaskáde biochemických dejov, ktoré vedú k zvýšeniu intracelulárneho Ca^{2+}. Pri zvýšenom podávaní morfínu bola zistená zvýšená expresia nikotínamidadeníndinukleotidfosfát (NMDA) receptorov. Tento jav v klinickej praxi môže prispievať k vytvoreniu morfínovej závislosti a tolerancie. K zníženiu glukuronidácie morfínu enzýmom UDP-glukuronozyltransferázou (UGT), môže prispieť

obmedzený metabolizmus glukózy vplyvom morfínu ako aj samotné ochorenie. Táto metabolizácia prebieha v pečeni. Je však nutné podotknúť, že UGT má viaceré genetické polymorfizmy, ktoré vykazujú rôznu aktivitu (47).

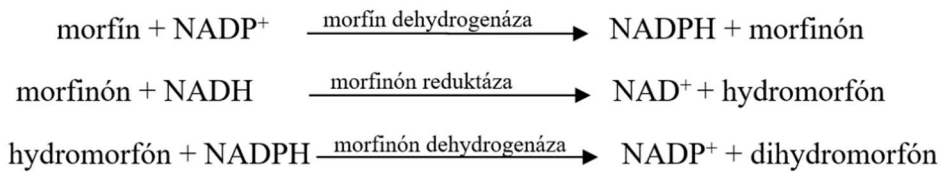


Obrázok č.7: Metabolická degradácia morfínu (Zdroj: De Gregori 2012).

Niektoré štúdie potvrdili, že opiáty ovplyvňujú metabolizmus glukózy a hladiny hormónov regulujúcich glukózu (48). Napríklad pri nádorových ochoreniach sa kyselina glukurónová tvorí v pečeni viac z glykogénu alebo aj z glykogénnych aminokyselín než z glukózy. Preto látky, ktoré majú byť metabolizované UGT spôsobujú pokles glykogénu. V štúdii Favaro a kol. dokázali, že pri nádorových ochoreniach nedochádza k mobilizácii glykogénu (49).
Bezprostredne z uvedeného môže vyplývať niekoľko skutočností, ktoré významne zasahujú a menia charakter redoxných procesov:
1. Obmedzená aktivita UGT kvôli nedostatku kyseliny glukurónovej a s tým aj metabolizmus podávaných liečiv.
2. Ovplyvnenie redoxného stavu obmedzením najväčšieho zdroja tvorby NADPH v bunke – pentozafosfátového cyklu a to: glukóza-6-fosfátdehydrogenázy (G6PDH). Je známe, že podávanie morfínu vedie k deaktivácii G6PDH. Dochádza k tomu vytváraním konjugátov morfín-G6PDH (50). Práve preto sa tento druhý bod zdá byť závažnejší než nedostatok kys.

glukurónovej, či predtým spomínaná alterácia aktivity polymorfných foriem UGT, pretože morfín môže byť metabolizovaný alternatívne a to dvojako:

A)



NADPH je pritom vyžadované pre aktivitu:

1. NADPH-oxidáz, avšak nakoľko je ich aktivita výrazná najmä u buniek imunitného systému a tie využívajú k jeho tvorbe glutamín, nepredpokladáme preto obmedzenie produkcie superoxidu.
2. syntázy oxidu dusnatého (NOS). Dostatok kofaktorov a substrátov značne ovplyvňuje aktivitu všetkých troch izoforiem NOS. V prípade nedostatku NADPH, tetrahydrobiopterínu, či aj substrátu arginínu (alebo dokonca aj jeho chýbaniu) dochádza k tzv. odpojeniu aktivity NOS, pričom sa tvorí súčasne NO aj superoxid, ktoré reagujú za vzniku peroxynitritu (51). Pterín pôsobí ako účinný vychytávač O^{2-}•, čím predchádza rýchlej reakcii O^{2-}• s NO a tvorbe ONO^{2-} a uvoľňuje ho vo forme H_2O_2. Jedine v prípade nedostatku hemu NOS nie sú schopné viazať tetrahydrobiopterín a nekatalyzujú tvorbu NO (52).
3. Glutatiónreduktázy, ktorej účinkom dochádza prostredníctvom NADPH k spätnej redukcii oxidovaného glutatiónu (GSSG) na redukovaný (GSH).

B) cytochrómami:

Morfín, oxymorfín aj hydromorfón môžu byť metabolizované N-demetyláciou enzýmami cytochrómu P450 (CYP3A4 a CYP2C8). Tento reakčný mechanizmus je taktiež závislý na NADPH. Z neho preberá elektróny na vytvorenie redukovaného hemu. Redukovaný hem umožní naviazanie O2 a oxidáciu substrátu vložením jedného kyslíka do substrátu a druhého do molekuly vody. Reakciu je možné znázorniť takto:

Perferylový komplex $[Fe^{5+} = O]^{3+}$, ktorý sa tvorí na heme prijatím elektrónu je schopný namiesto OH skupiny naviazať iba H čím sa tvoria aj alkylové radikály. Radikály poškodzujú bunkové štruktúry reakciami s proteínmi za vzniku stabilných adduktov. Okrem toho sa ich aktivitou ako vedľajší produkt môže tvoriť singletový kyslík, vrámci reakcií sú však schopné redukovať molekulárny kyslík na superoxid a/alebo H_2O_2 (5). Výrazný vplyv tu zohráva aj unikátna vlastnosť enzýmu syntázy oxidu dusnatého (NOS). Enzým je podobný cytochrómu P450 a katalyzuje iné oxidačné metabolické reakcie, v ktorých sa taktiež tvoria radikálové intermediáty. Mechanizmus oxidácie látok je možné vysvetliť mechanizmom cytochrómu P450. Cytochróm P450$[Fe^{5+} = O]^{3+}$ odtrhnutím vodíka zo substrátu vytvorí cytochróm P450$[Fe^{4+}$ - $OH]^{3+}$ a zodpovedajúci radikál (53). Skutočnosť, že NOS metabolizuje a ovplyvňuje účinnosť a toleranciu k morfínu naznačujú aj niektoré štúdie, napríklad už dostatkom arginínu (54). Ďalšie štúdie poukázali na antinociceptívny účinok morfínu, ktorý bol zaznamenaný pri aplikácii L-arginínu. Naproti tomu podávanie D-arginínu tento efekt nemalo. Čo jednoznačne naznačuje výrazný vplyv NOS na metabolizmus a účinnosť morfínu, ako aj tvorbu reaktívnych častíc, nakoľko NOS má obmedzenú substrátovú špecificitu a k syntéze NO nedokáže D-arginín využiť (53, 55).

5.1 Štúdia Opioid-Redox Study

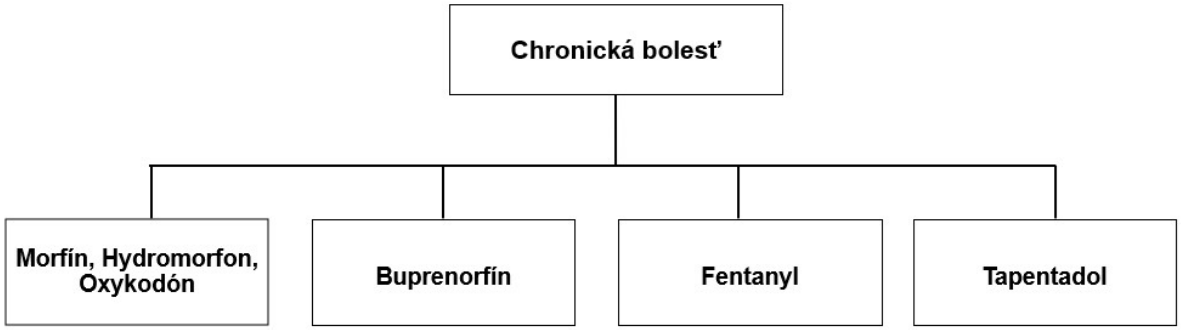
Viaceré dôkazy z experimentálnych štúdií, ako aj výsledky niektorých klinických štúdií poukazujú na úlohu opioidov pri vzniku oxidačného stresu. Tento jav sa rovnako predpokladá u pacientov s chronickou bolesťou, odkázaných na dlhodobé užívanie silných opioidov, ktoré sú pilierom liečby neuropatickej bolesti. Štúdie v tejto skupine pacientov absentujú a to najmä pre vysoko komplexnú problematiku spojenú s polymorbiditou daných pacientov s rôznymi pridruženými ochoreniami, odlišnou dĺžkou liečby analgetikami, individuálnym dávkovaním analgetík, ako aj liekovými interakciami, farmakokinetikou a farmakodynamikou celého spektra liečiv, ktoré pacienti užívajú.

Z dôvodu nejasných dôkazov o intenzite oxidačného stresu u pacientov s chronickou bolesťou liečených opioidmi pre silnú bolesť náš lekársko-vedecký tím realizuje prospektívnu observačnú multicentrickú klinickú štúdiu pod názvom Opioid-Redox Study. Tento projekt je realizovaný v spolupráci s Algeziologickou klinikou SZU Fakultnej nemocnice s poliklinikou F. D. Roosevelta v Banskej Bystrici, Ambulanciou bolesti Algmed v Košiciach,

Ústavom lekárskej biochémie Univerzity Pavla Jozefa Šafárika v Košiciach a Východoslovenským ústavom srdcových a cievnych chorôb a.s. v Košiciach.

Štúdia bola zaregistrovaná v medzinárodnej databáze klinických štúdií „clinicaltrials.gov“ pod registračným číslom NCT03105232. Cieľom štúdie je monitorovať klinický stav pacientov, zmeny aktivít antioxidačných enzýmov a redoxných kapacít po začatí liečby opioidmi pre silnú bolesť, ako aj v priebehu ich užívania.

Tento projekt aktuálne prebieha vo fáze náboru pacientov do klinickej štúdie. Po splnení inklúznych kritérií a zaradení do štúdie sú pacienti rozdelení do 4 skupín:



Obrázok č.8: Flow diagram klinickej štúdie Opioid-Redox Study.

Hodnotia sa nepriame markery oxidačného stresu, medzi ktoré patria: aktivita glutatión peroxidázy, glutatión reduktázy, katalázy, superoxiddizmutázy a plazmatických hladín glutatiónu. Monitorované sú klinické parametre ako doterajšia spotreba analgetík, vyšetrenie zamerané na typ bolesti (nociceptívna vs. neuropatická) hodnotením senzitívnych dotazníkov PainDetect, DN4, a LANSS Pain škály. Jednotlivé merania sa realizujú v intervaloch: pred začatím užívania opiátov, po 6 mesiacoch a po 12 mesiacoch danej opiátovej liečby.

Niektoré štúdie zaoberajúce sa problematikou dlhodobého užívania opiátových analgetík a následnými zmenami oxidačno-redukčných dejov, poukázali na nižšiu úroveň nežiadúcich oxidačných účinkov morfínu v porovnaní s ekvivalentnými dávkami iných opioidov (47, 56).

Pilotné výsledky štúdie Opioid-Redox Study poukazujú na rovnakú intenzitu prebiehajúcich antioxidačných reakcií u všetkých skupín pacientov, ktorí sú na dlhodobej opiátovej liečbe. Bol zaznamenaný signifikantný vzostup plazmatickej aktivity enzýmov - glutatión peroxidázy, glutatión reduktázy a signifikantné zvýšenie plazmatickej koncentrácie glutatiónu. Zároveň došlo u týchto sledovaných skupín k signifikantnému poklesu aktivít superoxid dizmutázy, v porovnaní s kontrolnou skupinou zdravých jedincov, čo poukazuje na

zvýšenú úroveň prebiehajúcich antioxidačných reakcií u pacientov s chronickou bolesťou po 6 mesiacoch na opiátovej liečbe. Tieto údaje naznačujú možnú prítomnosť oxidačného stresu v opiátovej skupine.

Doterajšie závery tejto práce boli prezentované na medzinárodnej konferencii:

- *Šimonová J, Vašková J, Ogurčáková D,* ***Kočan L****. Martuliak I, Rapčan R, Šimon R. Antioxidant capacities of patients with chronic pain after strong opioid treatment. Pain in Europe XI Valencia, European Pain Federation EFIC 2019.*

Práce súvisiace s problematikou vplyvu opiátov na rozvoj oxidačného stresu boli publikované v recenzovaných medicínskych časopisoch a časopisoch WOS:

- *Ogurčáková D,* ***Kočan L****, Šimonová J, Martuliak I, Sabol F, Vašková Janka. Oxidative stress in patients receiving long-term opioid therapy. Laboratórna diagnostika: recenzovaný časopis pre pracovníkov diagnostických laboratórií. ISSN 1335-2644. - Roč. 24, č. 1 (2019), s. 57-61.*

- ***Kočan L****, Martuliak I, Ogurčáková D, Vašková J, Kočanová H. Oxidačný stres u pacientov dlhodobo liečených opiátmi. Paliatívna medicína a liečba bolesti. - ISSN 1337-9917. - Roč. 8, supl. 2 (2015), s. 23.*

- *Ogurčáková D,* ***Kočan L****, Šimonová J, Martuliak I, Sabol F, Vašková Janka. Plasma antioxidant status in patients undergoing long-term opioid treatment. Medical Science. ISSN 2321-7367. - Roč. 26, č. 124 (2022). s 1-7.* DOI: 10.54905/disssi/v26i124/ms217e2319.

Medical Science

pISSN 2321–7359; eISSN 2321–7367

Plasma antioxidant status in patients undergoing long-term opioid treatment

Ogurčáková Daniela[1,2], Kočan Ladislav[3], Šimonová Jana[4], Martuliak Igor[5], Sabol František[6], Vašková Janka[2*]

To Cite:
Ogurčáková D, Kočan L, Šimonová J, Martuliak I, Sabol F, Vašková J. Plasma antioxidant status in patients undergoing long-term opioid treatment. Medical Science, 2022, 26, ms217e2319.
doi:

Author affiliations
[1]Pain management clinic Algmed, Rastislavova 45, 04001 Košice, Slovak Republic
[2]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, 040 11 Košice, Slovak Republic
[3]Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic
[4]1st Clinic of Anaesthesiology and Intensive Care Medicine, Louis Pasteur University Hospital, SNP 1, 040 11 Košice, Slovak Republic
[5]Department of Algeziology, F.D. Roosevelt Teaching Hospital with Policlinic Banská Bystrica and Slovak Medical University in Banská Bystrica, nám. Ludvika Svobodu 8, 97401 Banská Bystrica, Slovak Republic
[6]Department of Cardiac Surgery, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic

***Corresponding author**
Janka Vašková, assoc. prof. Dr. PhD.
Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice,
Tr. SNP 1, 04066 Košice, Slovak Republic
Email: janka.vaskova@upjs.sk

Peer-Review History
Received: 26 May 2022
Reviewed & Revised: 27/May/2022 to 09/June/2022
Accepted: 09 June 2022
Published: 11 June 2022

Peer-review Method
External peer-review was done through double-blind method.

URL: https://www.discoveryjournals.org/medicalscience

ABSTRACT

Background: Opioid treatment is now an integral part of pharmacotherapy for severe chronic malignant and non-malignant pain. Currently, there is a sufficient selection of opioids to allow individualized pain treatment. Several experimental studies have confirmed the effect of opioids on oxidative stress. The aim of this work is to determine the presence of redox changes occurring as a result of long-term opioid use in patients with chronic pain. *Results:* Six months of opioid use for severe pain was evaluated in 37 patients. Patients formed three groups depending opioid treatment (oxycodone, fentanyl and tapentadol) and were compared with 42 healthy probands. Compared to control, activities of superoxide dismutase were decreased, while those of glutathione peroxidase and glutathione reductase were significantly increased in all groups. Together with lowered levels of reduced glutathione, this indicated conditions of oxidative stress. There were no differences between treatment groups. *Conclusion:* It is necessary to know the risks of side effects and provide patients with possible solutions. At this stage and with this number of subjects, we can conclude that neither the form of administration nor the type of opioid has any effect on reducing oxidative stress from opioid metabolism in the treatment of severe pain.

Keywords: antioxidant enzymes, pain, glutathione, opioids, oxidative stress

1. INTRODUCTION

Chronic pain and non-malignant pain is a widespread serious public health problem (Breivik et al., 2006; Ripamonti, 2012). There are no clear epidemiological studies available in the European Union; however, approximately 50% of adults suffer from one or more chronic pain with an incidence of moderate to severe pain in the European population at 20%. 70% of patients are in active age, 11% of patients are untreated, and 50% do not have enough pain treatment. The prevalence is higher in women and the number of patient's increases with age, reaching 80% in geriatric patients. The economic costs, namely indirect costs due to incapacity for work, are not negligible. The most common chronic pains such as back pain, osteoarthritis, headache, and neuralgia are often considered to be a normal part of life. The treatment of chronic pain is the elimination of pain and restoration of all

functions (physical, mental, social); in the most optimal case, this includes the possibility of returning to work (Breivik et al., 2006; 2013).

The choice and management of analgesic treatment is based on patient data regarding the intensity and nature of the pain and on the specific clinical condition. It does not determine the origin of the pain (malignant, non-malignant), but in chronic pain: the procedure follows a "bottom-up" approach (step up). In intense acute pain, parenteral administration of an analgesic, possibly also an opioid (in cases of angina pectoris, heart attack, renal and gallbladder colic) is appropriate; otherwise non-invasive administration of analgesics (*per os*, transdermal, *per rectum*) is preferred. Regarding time, analgesics with a rapid onset of action are the most advantageous in acute pain; in chronic pain, analgesics are administered "on an hourly basis" and thus the development of pain is prevented.

Opioid analgesics are essential for pain management (Gilson et al., 2011) due to efficiency and safety treatment under competent physicians. The biopsychosocial status in non-terminal patients with chronic pain should be considered by physician to set up a treatment plan with patient motivation to reach functional goals (Von Korff et al., 2011; Kalso et al., 2004). Morphine is considered to be the gold standard of opioid treatment, with the properties of other opioids derived from it. Many side effects of opioid treatment are under thorough investigation. Recent findings suggest that long-term opioid treatment may contribute to oxidative stress, which is a serious pathological problem due to its key role in the pathogenesis of many diseases (Cacciapuoti, 2016).

Recent studies have also revealed new roles for oxidative stress or reduced antioxidant activities relevant to mitochondria functions behind the development of a migrains (Ferroni et al., 2018). Clinical trials focusing on the redox state of patients taking opioids for chronic pain are still lacking. Due to unclear evidence of oxidative stress intensity in chronic pain patients dosed with opioids for severe pain we have started a prospective multicentre observational study. The aim of the study was to monitor the clinical condition of patients, as well as changes in the activities of antioxidant enzymes after starting opioid treatment for severe pain and during their use.

2. MATERIALS AND METHODS

The study was carried out in co-operation of three pain treatment centres, Pain management clinic Algmed in Košice, Department of Algesiology, F.D. Roosevelt Hospital in Banská Bystrica, and the East Slovak Institute of Cardiovascular Diseases in Košice. The Ethic Committee of Faculty of Medicine Pavol Jozef Šafárik University in Košice no. 1N/2017, Ethic Committee of Slovak Medical University in Banská Bystrica 28/11/2016, and Ethic Committee of East Slovak Institute of Cardiovascular Diseases in Košice no. 1/2019/VUSCH/EK approved the study. The study was registered in the International Database U.S. National Institutes of Health ClinicalTrials.gov under the number NCT03105232. Patients recruitment started august 2020 and was completed December 2021.

The course of the research was explained to potential study participants and, after signing informed consent, followed up with a pain specialist. Screening of parameters was carried out, namely: consumption of analgesics, pain type examination (nociceptive vs. neuropathic - filling out the questionnaires for Pain Detect, DN4, and numeric pain scale), and demographic data (weight, height, age). The inclusion criteria of patients were: non-malignant pain, no previous opioid treatment for visceral, neuropathic, or nociceptive, pain and age over 18 years. Patients with oncological conditions were excluded. Enrolled participants were divided into 4 groups: group 1 - control group (C), healthy individuals (42); group 2 (O) - patients with chronic pain using morphine, hydromorphone, oxycodone, or buprenorphine (14); group 3 (F) - patients with chronic pain receiving transdermal fentanyl patch (12); group 4 (T) - patients with chronic pain using tapentadol (11). The second examination followed six months from the start of opioid use for severe pain. During the examination, blood for biochemical analysis was collected, patients completed a Pain Detect questionnaires, DN4, and Lanss Pain Scale, and clinical parameters were measured.

Bicinchoninic acid was used for blood plasma protein determination. Glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities were determined according to the procedures given by the kit manufacturer (Sigma-Aldrich, Germany). Superoxide dismutase activities were set according to the SOD-Assay KIT-WST (Fluka, Japan). The concentration of reduced glutathione (GSH) was determined by the method originally described by Floreani et al. (1997).

Descriptive statistics were used to characterise groups of patients. A T-test was used to compare values within groups against the corresponding control. Intragroup differences at two sampling times were determined by one-way analysis of variance followed by Tukey-post hoc test. Intergroup differences within parameters were detected by a Mann-Whitney test. Differences were considered significant at $p < 0.05$, $p < 0.01$, $p < 0.001$.

3. RESULTS AND DISCUSSION

After screening the patients who met the conditions and completed both samplings (also after 6 months), 37 patients were included in the study. The measurements of antioxidant parameters in plasma were compared with a group of 42 healthy individuals. Results (Figure 1 and 2) show that SOD activities were decreased in each treatment group when compared to control before the start of therapy and after 6 months regardless of the form of drug administered and the type of opioid. Activities of GPx and GR were increased in comparison to control at both time points. The activities of both GPx and GR enzymes have an even greater tendency to increase 6 months after opioid treatment in all groups. In group 3, there was a statistically significant increase in GPx ($p=0.0044$) over 6 months (Table 1). Morphine treatment affects antioxidant enzyme activities, as morphine-dependent rhesus macaques have been observed with GPx, SOD increased after 140 days of morphine treatment (Pérez-Casanova et al., 2008). Other studies have shown that these enzyme activities were reduced by morphine (Payabvash et al., 2006; Sumathi et al., 2011; Zhou et al., 2011; Roziski et al., 2013). It becomes obvious that this can be affected by many different factors including dosage, exposure time, and species exposed.

The catalytic activity of SOD is the dismutation of the superoxide radical ($O_2^{\cdot-}$) to hydrogen peroxide. Hydrogen peroxide as well as other hydro peroxides is converted to water or the corresponding alcohols by GPx. Glutathione is co-oxidized in the reaction, and reduced back by GR, thus providing an effective antioxidant response. The increased activities of these two glutathione-related enzymes are confirmation of oxidative stress conditions in patients suffering from pain, and very likely suppression or inactivation of SOD. Moreover, $O_2^{\cdot-}$ as well as peroxynitrite anion (ONO_2^-), are known as pro-nociceptive species (Salvemini and Newmann, 2009; 2010). Under conditions of oxidative stress, inflammation contributes even more significantly to raising the concentration of $O_2^{\cdot-}$ through several sources. In the respiratory chain within mitochondria, these include lipoxygenase, cyclooxygenase, NOX enzyme induction, and concomitantly the formation of the ONO_2^- as a result of thiol oxidation in xanthine dehydrogenase, and uncoupling of NOS activity (Vašková et al., 2016).

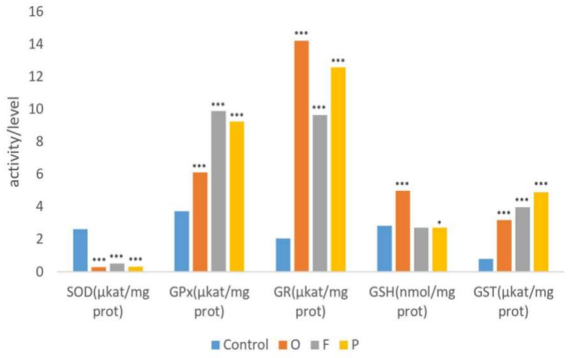


Figure 1 Antioxidant enzyme activities and reduced glutathione concentrations in patients treated with opioids for severe pain compared to healthy subjects at the beginning of treatment period. Statistical significance at *$p < 0.05$ a ***$p < 0.001$

Reactive oxygen species (ROS) formation has been observed even at low doses of morphine in vascular endothelial cells (mouse and human) (Hsiao et al., 2009). Macrophages have also been found to be morphine-induced generating $O_2^{\cdot-}$ (Bhat et al., 2004). Other morphine-induced sources are activated nitric oxide synthase (NOS) and NADPH oxidase. Activation of NOS leads to increase NO production and subsequent SOD nitration. Inactivation of SOD leads to the formation of ONO_2^- in a reaction between $O_2^{\cdot-}$ and NO. Although $O_2^{\cdot-}$ is an initiator of the formation of other ROS, ONO_2^- together with NO depletion have deleterious effects on tissues and their functions (Vašková et al., 2016). All these ROS are involved in the pain sensitisation, opiate-induced hyperalgesia and antinociceptive tolerance (Salvemini et al., 2009).

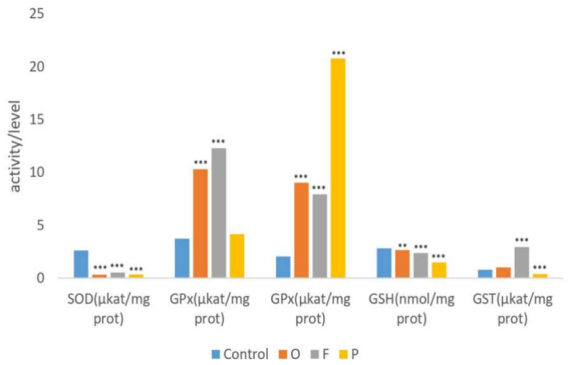


Figure 2 Antioxidant enzyme activities and reduced glutathione concentrations in patients treated with opioids for severe pain compared to healthy subjects 6 months after starting therapy. Statistical significance at **p < 0.01 and ***p < 0.001.

SOD is inactivated *in vivo* through nitration with NO-derived oxidants (ONO_2^-) and hydroxylation (hydroxyl radical) (Janssen-Heininger et al., 2005). Salvemini et al., (2011) reported MnSOD inactivation as an essential element for increased production of O_2^- and ONO_2^- in nociceptive signalling from the results of several studies of their research group. Inactivation of NOS or inhibition of nitration and inactivation of SOD made it possible to prevent the formation of morphine-induced antinociceptive tolerance (Muscoli et al., 2007).

It was found that hydrogen peroxide is capable of SOD inactivation, yet too slow to be the cause of inactivation under physiological conditions (Escobar et al., 1996). However, relatively higher concentrations of hydrogen peroxides (and hydroperoxides) are capable of substrate peroxidase inactivation (Olorunniji et al., 2009), which was not confirmed by the results of our study. Increased activities of GPx, synergistically acting GR together with lowered concentrations of GSH (especially 6 months after starting therapy) only support the response to oxidative stress conditions (Figure 1 and 2). Levels of GSH were significantly higher when compared with control (p < 0,001) in group treated with oxycodone, hydromorphone at the beginning, and unchanged in group with fentanyl, they markedly decreased 6 months after starting therapy (p < 0,001).

Table 1 Further description of the results of the antioxidant parameters in patients treated with opioids for severe pain compared to healthy subjects before at the beginning (0) and 6 months of treatment (6). Significance at b <0.01, c<0.05

Group	t	SOD (μkat/mg prot) med (min-max)	GPx (μkat/mg prot) med (min-max)	GR (μkat/mg prot) med (min-max)	GSH (nmol/mg prot) med (min-max)	GST (μkat/mg prot) med (min-max)
O	0	0.24 (0.16-0.99)	6.61 (0.99-11.6)	14.06 (1.37-27.29)	9.76 (0.78-12.23)	3.35 (0.97-4.53)
	6	0.24 (0.22-0.61)	5.60 (0.94-29.11)	9.18 (4.65-13.12)	1.86 (1.70-5.21)	0.55 (0.20-2.74)
F	0	0.22 (0.19-1.21)	9.98 (1.09-26.94)[b]	9.23 (3.32-19.07)	2.51 (1.01-4.38)	3.08 (0.15--10.36)
	6	0.27 (0.19-1.39)	7.00 (1.12-29.11)[b]	9.75 (1.83-10.39)	2.51 (0.56-3.96)	1.55 (0.60-9.01)
T	0	0.23 (0.17-1.21)	6.72 (2.23-21.04)	12.52 (2.53-22.58)	2.39 (0.76-4.83)	4.19 (0.18-12.23)[c]
	6	0.24 (0.21-0.63)	5.45 (0.69-6.35)	22.32 (11.7-28.34)	1.33 (1.19-1.97)	0.44 (0.19-0.56)[c]

Unlike other opioids, morphine is more associated with the induction of oxidative stress either by the formation of ROS or reduction the activity of antioxidants (Skrabalová et al., 2013; Zahmatkesh et al. 2017). Several studies demonstrated that both acute and chronic morphine exposure can lead to significant reductions in GSH levels in rodent and human brains, serum and liver (Abdel-Zaher et al., 2010; Cemek et al., 2011; Guzmán et al., 2006; Mannelli et al., 2009; Ozmen et al., 2007; Payabvash et al., 2006; Skoulis et al., 1989; Sumathi et al., 2011; Todaka et al., 2005). Methadone and buprenorphine treatment showed similar results (Leventelis, et al., 2019).

In addition to the examined function of glutathione within this study, this molecule is much more widely involved in redox reactions, e.g. reacting directly with $O_2^{\cdot-}$, H_2O_2 and NO, participating in disulphide interchange, amino acid transport into the cells in the γ-glutamyl cycle and conjugation with electrophilic compounds catalysed by GST. A significant increase in GST activities was found in every group at the beginning of treatment (Figure 1). After 6 months, there was a decrease in GST activity in group 2 with no difference from the control (Figure 2). Yet, in the group treated with tapentadol, activities decreased in comparison with control, and there was the significant difference between times of sampling ($p=0.0019$) in this group (Table 1). Myers et al., (2010) found a six-fold increase in protein level and expression of liver GST isoenzyme after administration of oxycodone to rats for 8 days. However, in the case of this study, the first measurements were not made after the short-term effect of the opioids, but before their action. Observed increases in activities of GST may be due to the response to the oxidative stress conditions in patients. In 6 month of opioid administration, there was decrease in GST activity, in all three groups, however most in group 4. Comparable effect in long-term morphine use was showed on rat model study (Samarghandian et al., 2014).

4. CONCLUSION

Although some studies pointed to lower levels of adverse effects in comparison to equivalent doses of opioids such as morphine, the results of our study did not show significant changes between the monitored antioxidant parameters in the three various opioid treatment groups over a period of half a year. In all three groups, a very similar pattern of action was found on antioxidant enzymes, conjugation with glutathione and the effect of reduced glutathione levels.

Acknowledgement

We would like to thank the Department of Medical and Clinical Biochemistry for the support.

Author contributions

KL, MI and JV conceived and designed the study. Data collection and measurements were performed by DO, ŠJ and MI. The manuscript was written by DO, JV and SF, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

Study was supported by grant VEGA 1/0782/15.

Ethical approval

The study was approved by the Ethic Committee of Slovak Medical University in Banská Bystrica 28/11/2016, the Ethic Committee of East Slovak Institute of Cardiovascular Diseases in Košice no. 1/2019/VUSCH/EK and the Ethic Committee of Faculty of Medicine Pavol Jozef Šafárik University in Košice no. 1N/2017 approved the study.

Conflicts of interest

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES AND NOTES

1. Abdel-Zaher AO, Abdel-Rahman MS, ELwasei FM. Blockade of nitric oxide overproduction and oxidative stress by Nigella sativa oil attenuates morphine-induced tolerance and dependence in mice. Neurochem Res 2010; 35: 1557-1565. doi: 10.1007/s11064-010-0215-2.

2. Bhat RS, Bhaskaran M, Mongia A, Hitosugi N, Singhal PC. Morphine-induced macrophage apoptosis oxidative stress and strategies for modulation. J Leukoc Biol 2004; 75: 1131–1138. doi: 10.1189/jlb.1203639.
3. Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain 2006; 10: 287-333. doi: 10.1016/j.ejpain.2005.06.009.
4. Breivik H, Eisenberg E, O'Brien T. OPEN Minds. The individual and societal burden of chronic pain in Europe: the case for strategic prioritisation and action to improve knowledge and availability of appropriate care. BMC Public Health 2013; 13: 1229. doi: 10.1186/1471-2458-13-1229.
5. Cacciapuoti F. Oxidative Stress as "Mother" of Many Human Diseases at Strong Clinical Impact. J Cardiovasc Med Cardiol 2006; 3: 001-006. doi: 10.17352/2455-2976.000020.
6. Cemek M, Büyükokuroğlu ME, Hazman Ö, Bulut S, Konuk M, Birdane Y. Antioxidant enzyme and element status in heroin addiction or heroin withdrawal in rats: effect of melatonin and vitamin E plus Se. Biol Trace Elem Res 2011; 139: 41-54. doi: 10.1007/s12011-010-8634-0.
7. Escobar JA, Rubio, MA, Lissi EA. SOD and catalase inactivation by singlet oxygen and peroxyl radicals. Free Rad Biol Med 1996; 20: 285-290. doi: 10.1016/0891-5849(95)02037-3.
8. Ferroni P, Barbanti P, Della-Morte D, Palmirotta R, Jirillo E, Guadagni F. Redox Mechanisms in Migraine: Novel Therapeutics and Dietary Interventions. Antioxid Redox Signal 2018; 28: 1144-1183. doi: 10.1089/ars.2017.7260.
9. Floreani M, Petrone M, Debetto P, Palatini P. A comparison between different methods for the determination of reduced and oxidized glutathione in mammalian tissues. Free Radic Res 1997; 26: 449-455. doi: 10.3109/10715769709084481.
10. Gilson AM, Maurer MA, Ryan KM, Skemp-Brown M, Husain A, Cleary JF. Ensuring patient access to essential medicines while minimizing harmful use: a revised World Health Organization tool to improve national drug control policy. J Pain Palliat Care Pharmacother 2011; 25: 246-251. doi: 10.3109/15360288.2011.599485.
11. Guzmán DC, Vázquez IE, Brizuela NO, Alvarez RG, Mejía GB, García EH, Santamaría D, de Apreza Ml, Olguín HJ. Assessment of oxidative damage induced by acute doses of morphine sulfate in postnatal and adult rat brain. Neurochem Res 2006; 31: 549-554. doi: 10.1007/s11064-006-9053-7.
12. Hsiao PN, Chang MC, Cheng WF, Chen CA, Lin HW, Hsieh CY, Sun WZ. Morphine induces apoptosis of human endothelial cells through nitric oxide and reactive oxygen species pathways. Toxicol 2009; 256: 83-91. doi: 10.1016/j.tox.2008.11.015.
13. Janssen-Heininger Y, Ckless K, Reynaert N, van der Vliet A. SOD inactivation in asthma: bad or no news?. Am J Pathol 2005; 166(3):649-652. doi:10.1016/s0002-9440(10)62286-9.
14. Kalso E, Edwards JE, Moore AR, McQuay HJ. Opioids in chronic non-cancer pain: systematic review of efficacy and safety. Pain 2004; 112: 372-380. doi: 10.1016/j.pain.2004.09.019.
15. Leventelis C, Goutzourelas N, Kortsinidou A, Spanidis Y, Toulia G, Kampitsi A, Tsitsimpikou C, Stagos D, Veskoukis AS, Kouretas D. Buprenorphine and methadone as opioid maintenance treatments for heroin-addicted patients induce oxidative stress in blood. Oxid Med Cell Longev 2019; 2019: 9417048. doi: 10.1155/2019/9417048.
16. Mannelli P, Patkar A, Rozen S, Matson W, Krishnan R, Kaddurah-Daouk R. Opioid use affects antioxidant activity and purine metabolism: preliminary results. Hum Psychopharmacol 2009; 24: 666-675. doi: 10.1002/hup.1068.
17. Muscoli C, Cuzzocrea S, Ndengele MM, Mollace V, Porreca F, Fabrizi F, Esposito E, Masini E, Matuschak GM, Salvemini D. Therapeutic manipulation of peroxynitrite attenuates the development of opiate-induced antinociceptive tolerance in mice. J Clin Invest 2007; 117: 3530-3539. doi: 10.1172/JCI32420.
18. Myers AL, Hassan HE, Lee IJ, Eddington ND. Repeated administration of oxycodone modifies the gene expression of several drug metabolising enzymes in the hepatic tissue of male Sprague-Dawley rats, including glutathione S-transferase A-5 (rGSTA5) and CYP3A2. J Pharm Pharmacol 2010; 62: 189-196. doi: 10.1211/jpp.62.02.0006.
19. Olorunniji F, Iniaghe MO, Adebayo JO, Malomo SO, Adediran SA.Mechanism-based inhibition of myeloperoxidase by hydrogen peroxide:enhancement of inactivation rate by organic donor substrates. Open Enzym Inhib J 2009; 2: 28–35.
20. Ozmen I, Naziroğlu M, Alici HA, Sahin F, Cengiz M, Eren I. Spinal morphine administration reduces the fatty acid contents in spinal cord and brain by increasing oxidative stress. Neurochem Res 2007; 32: 19-25. doi: 10.1007/s11064-006-9217-5.
21. Payabvash S, Beheshtian A, Salmasi AH, Kiumehr S, Ghahremani MH, Tavangar SM, Sabzevari O, Dehpour AR. Chronic morphine treatment induces oxidant and apoptotic damage in the mice liver. Life Sci 2006; 79: 972-980. doi: 10.1016/j.lfs.2006.05.008.
22. Pérez-Casanova A, Husain K, Noel RJ Jr, Rivera-Amill V, Kumar A. Interaction of SIV/SHIV infection and morphine on plasma oxidant/antioxidant balance in macaque. Mol Cell Biochem 2008; 308: 169-175. doi: 10.1007/s11010-007-9625-0.
23. Rozisky JR, Laste G, de Macedo IC, Santos VS, Krolow R, Noschang C, Vanzella C, Bertoldi K, Lovatel GA, de Souza IC, Siqueira IR, Dalmaz C, Caumo W, Torres IL. Neonatal morphine administration leads to changes in hippocampal BDNF levels and antioxidant enzyme activity in the adult life of rats. Neurochem Res 2013; 38: 494-503. doi: 10.1007/s11064-012-0941-8.
24. Salvemini D, Little JW, Doyle T, Neumann WL. Roles of reactive oxygen and nitrogen species in pain. Free Radic Biol

Med 2011; 51: 951-966. doi: 10.1016/j.freeradbiomed.2011.01.026.
25. Salvemini D, Neumann W. Targeting peroxynitrite driven nitroxidative stress with synzymes: A novel therapeutic approach in chronic pain management. Life Sci 2010; 86(15-16):604-614. doi: 10.1016/j.lfs.2009.06.011.
26. Salvemini D, Neumann WL. Peroxynitrite: a strategic linchpin of opioid analgesic tolerance. Trends Pharmacol Sci 2009; 30: 194-202. doi: 10.1016/j.tips.2008.12.005.
27. Samarghandian S, Afshari R, Farkhondeh T. Effect of long-term treatment of morphine on enzymes, oxidative stress indices and antioxidant status in male rat liver. Int J Clin Exp Med 2014; 7: 1449-1453. PMID: 24995110.
28. Skoulis NP, James RC, Harbison RD, Roberts SM. Depression of hepatic glutathione by opioid analgesic drugs in mice. Toxicol Appl Pharmacol 1989; 99: 139-147. doi: 10.1016/0041-008x(89)90119-1.
29. Skrabalova J, Drastichova Z, Novotny J. Morphine as a Potential Oxidative Stress-Causing Agent. Mini Rev Org Chem 2013; 10: 367-372. doi: 10.2174/1570193X113106660031.
30. Sumathi T, Nathiya VC, Sakthikumar M. Protective Effect of Bacoside-A against Morphine-Induced Oxidative Stress in Rats. Indian J Pharm Sci 2011; 73: 409-415. doi: 10.4103/0250-474X.95624.
31. Todaka T, Ishida T, Kita H, Narimatsu S, Yamano S. Bioactivation of morphine in human liver: isolation and identification of morphinone, a toxic metabolite. Biol Pharm Bull 2005; 28: 1275-1280. doi: 10.1248/bpb.28.1275.
32. Vašková J, Kočan L, Vaško L. Oxidative Stress and Opioids. Glob J Anesthesiol 2016; 3: 020-029. doi: 10.17352/2455-3476.000027.
33. Von Korff M, Kolodny A, Deyo RA, Chou R. Long-term opioid therapy reconsidered. Ann Intern Med 2011; 155: 325-328. doi: 10.7326/0003-4819-155-5-201109060-00011.
34. Zahmatkesh M, Kadkhodaee M, Salarian A, Seifi B, Adeli S. Impact of opioids on oxidative status and related signalling pathways: An integrated view. J Opioid Manag 2017; 13: 241-251. doi: 10.5055/jom.2017.0392.
35. Zhou J, Li Y, Yan G, Bu Q, Lv L, Yang Y, Zhao J, Shao X, Deng Y, Zhu R, Zhao Y, Cen X. Protective role of taurine against morphine-induced neurotoxicity in C6 cells via inhibition of oxidative stress. Neurotox Res 2011; 20: 334-342. doi: 10.1007/s12640-011-9247-x.

6 ĎALŠIE PRÁCE SPOJENÉ S TEMATIKOU AKÚTNEJ A CHRONICKEJ BOLESTI

Rozsiahlou činnosťou, ktorá sa stala predmetom mojej ďalšej medicínskej práce a klinického výskumu je liečba a štúdium akútnej a chronickej bolesti, keďže bolesť predstavuje bežný, avšak rozsiahly medicínsky problém týkajúci sa hospitalizovaných ako aj ambulantných pacientov.

6.1 Problematika bolesti

Bolesť je komplexný fenomén, ktorý postihuje milióny ľudí na celom svete a jej zvládanie zostáva hlavnou výzvou pre systémy zdravotnej starostlivosti. Nová revidovaná definícia Medzinárodnej spoločnosti pre štúdium bolesti (IASP) charakterizuje bolesť ako nepríjemný senzorický a emocionálny zážitok, ktorý je spojený so skutočným alebo potenciálnym poškodením tkaniva. Definícia ďalej poukazuje na individuálny aspekt každého jedinca a zdôrazňuje rozdiel medzi nocicepciou a bolesťou, keďže bolesť sa nedá odvodiť len zo samotnej aktivity senzorických neurónov. Tvrdí, že v priebehu života jedinca sa vytvárajú behaviorálne vzorce súvisiace s prežívaním bolesti. Zdôrazňuje ďalej to, že ak pacient vyjadruje svoju bolesť, tak táto informácia má byť rešpektovaná. Verbálny popis bolesti je len jedným z viacerých prejavov (expresie) bolestí a neschopnosť pacienta komunikovať neznamená, že pacient neprežíva pocit bolesti (57). Bolesť je možné definovať podľa rôznych kritérií. Na základe časového faktora trvania bolesti rozlišujeme bolesť akútnu a bolesť chronickú. Časovým medzníkom je trvanie bolesti viac alebo menej ako 3 mesiace.

Akútna bolesť je v určitom zmysle chápaná ako eudynia, takzvaná „dobrá bolesť“, ktorá je signálom akútneho poškodenia tkaniva a teda má ochranný význam. Avšak táto predstava stráca na význame za okolností, ktoré majú závažný dopad na klinický stav pacienta. Táto situácia nastáva pri jej silnej intenzite, kedy svojimi metabolickými a imunologickými zmenami môže poškodiť zdravie jedinca. Vhodným príkladom je pooperačná bolesť u hospitalizovaných pacientov. Jej mnohostranné negatívne dopady na perspektívu zdravia pacienta sú vyjadrené v tabuľke (58, 59).

ASPEKT	MORBIDITA
klinický	oddialenie hojenia vyvolané zvýšenou sympatikotóniou
	↑ incidencia insuficiencie anastomóz
	↑ incidencia pľúcnych komplikácií, vrátane pneumónií, pri šetrení dýchania vyvolanom bolesťou
	↑ riziko trombotických komplikácií
	↑ mortalita
	pretrvávajúca hyperadrenergná odpoveď s hypertenziou
pacienta	zbytočné utrpenie
	poruchy spánku
	retencia moču
	obmedzená mobilita, dýchanie, ↓ autonómia
	strach a obavy
	zbytočná čiastočná alebo úplná neschopnosť so stratou produktivity práce
	pomalšia rekonvalescencia normálnych funkcií a životného štýlu
	↓ kvalita života počas rekonvalescencie
	perspektívny
	predĺžený pobyt na JIS alebo v nemocnici
perspektívny	predĺžený pobyt na JIS alebo v nemocnici
	↑ riziko komplikácií a nákladov na starostlivosť
	↑ riziko chronickej bolesti a následných nákladov na starostlivosť
	dôsledkom nedostatočnej kontroly akútnej bolesti je zlá úroveň zdravotníckej starostlivosti

Tabuľka č. 1: Negatívne dopady pooperačnej bolesti (Zdroj Kulichová 2011).

Chronická bolesť je patologickou bolesťou s predpokladaným pretrvávaním viac ako 3 až 6 mesiacov, teda doby potrebnej na hojenie tkaniva. Často nemá jednoznačne definovanú príčinu a chápeme ju ako „škodlivú bolesť“ – maldýniu. Nekontrolovaná sa stáva zmyslom pacientovho života. Odlíšenie chronickej od akútnej bolesti je založené nielen na časovom faktore jej trvania a symptomatológii (charakter, kvalita bolesti, psychické zmeny a podobne), ale predovšetkým vo vyvíjajúcich sa patofyziologických zmenách nervového systému. Tieto zmeny sú následne sprevádzané typickým “algickým” správaním jedinca. (61, 60).

Bez ohľadu na to, či je príčina známa alebo ide o bolesť bez zrejmej organickej podstaty, chronická bolesť nie je symptómom ochorenia, ale stáva sa samostatným ochorením s komplikovanou etiológiou, patofyziológiou a symptomatikou (59, 61).

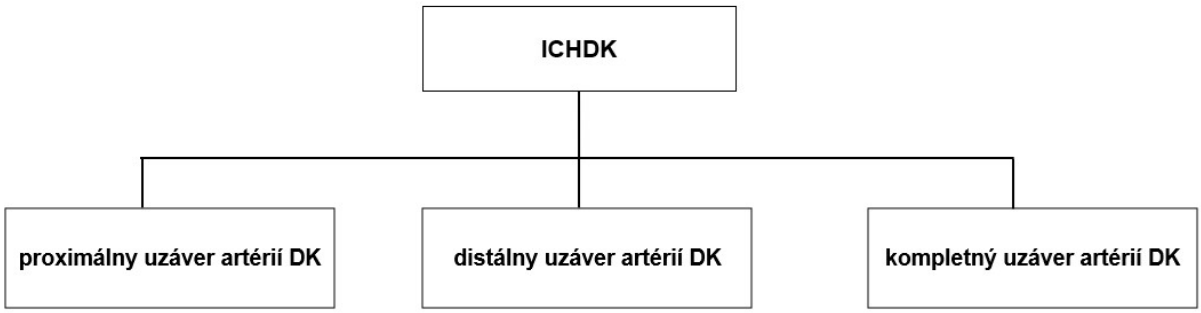
6.2 Projekty manažmentu terapie bolesti v nemocničnom zariadení VÚSCH a.s.

Riešením akútnej bolesti v zdravotníckom zariadení Východoslovenskom ústave srdcových a cievnych chorôb a.s. v Košiciach (VÚSCH a.s.), bolo vytvorenie podmienok pre vznik Služby akútnej bolesti – (angl. - Acute Pain Servis - APS), vytvorenie tímu APS a rozdelenie úloh pre členov tímu APS na jednotlivých oddeleniach, ako aj etablácie vypracovaných štandardných postupov liečby pooperačnej bolesti na jednotlivých oddeleniach. V rámci tohto projektu boli zavedené do praxe nové techniky pre liečbu prelomovej bolesti, akými boli periférne nervové blokády, fasciálne blokády a kryoablačné techniky po kardiochirurgických výkonoch, sternotómiach a torakotómiach. Výsledky týchto metód boli sledované v klinickej štúdii VUSCH/POPT1study NCT03915301 a ďalej prezentované na kongresoch Donovaly 2023, Brno 2023 a publikované v časopise Medical science, indexovanom vo WOS.

- ***Kocan L**., Rapčan R., Rapcanova S., Varhol J., Vašková J. Cryoablation of the intercostal nerve after mini-thoracotomy procedures: Pilot prospective interventional clinical study. Medical Science. ISSN 2321-7367. Volume 27, Issue 142, December 2023. DOI: https://doi.org/10.54905/disssi.v27i142.e384ms3258*

Problematika chronickej bolesti v zdravotníckom zariadení VÚSCH a.s. bola riešená založením Ambulancie chronickej bolesti v roku 2018, ktorá je určená prevažne pre pacientov s vysokým kardiovaskulárnym rizikom. Okrem štandardnej komplexnej farmakoterapie akútnej a chronickej bolesti sa zaoberáme intervenčnými výkonmi realizovanými pod ultrazvukovou a skiaskopickou navigáciou. V rámci sledovania klinického stavu pacientov s chronickou ischemickou chorobou dolných končatín (ICHDK) po intervenčných výkonoch zameraných na terapeutické ovplyvnenie lumbálneho sympatikového nervového systému sa realizuje v spolupráci s Technickou Univerzitou v Košiciach prospektívna observačná klinická štúdia: Tevi-LuSy-Study.

Štúdia je registrovaná v medzinárodnej databáze „clinicaltrials.gov“ pod registračným číslom NCT06111599. Klinická štúdia je finančne podporená grantom: Slovenskej spoločnosti pre štúdium a liečbu bolesti 24-3055/069.



Obrázok č.9: Flow diagram klinickej štúdie Tevi-LuSy-Study.

Prvotné výsledky štúdie demonštrujú zvýšenie tkanivovej perfúzie dolných končatín, ktoré boli pôvodne bez priameho prietoku v dôsledku chronickej oklúzie tepien. Zvýšenie perfúzie bolo nepriamo zaznamenané termografickým meraním teplotných zmien v angiozómoch po lumbálnej sympatikovej blokáde. Tieto zistenia naznačujú, že lumbálna sympatiková blokáda môže byť prospešná pre pacientov s ICHDK.

Pilotný článok: Thermovision controlled lumbar sympathetic blockade in chronic limb-threatening ischemia treatment - pilot trial, bol publikovaný v karentovom časopise: Vasa - European Journal of Vascular Medicine, Q4, IF 1,8.

- ***Kočan L**, Rajťúková V, Rašiová M, Kočanová H, Rapčanová S, Rapčan R, Martuliak I, Hudák R, Rybár D, Vašková J, Hudák M. Thermovision controlled lumbar sympathetic blockade in chronic limb-threatening ischemia treatment - pilot trial. Vasa. 2023 Mar;52(2):133-135. doi: 10.1024/0301-1526/a001053. PMID: 36872886.*

Letter to the editor

Thermovision controlled lumbar sympathetic blockade in chronic limb-threatening ischemia treatment – pilot trial

Ladislav Kočan[1,2], Viktória Rajťúková[4], Mária Rašiová[3], Hana Kočanová[5], Simona Rapčanová[6], Róbert Rapčan[6], Igor Martuliak[7], Radovan Hudák[4], Dušan Rybár[1], Janka Vašková[8], and Marek Hudák[3]

[1] Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Košice, Slovak Republic
[2] Poliklinika Terasa, EuroPainClinics, Košice, Slovak Republic
[3] Department of Angiology, East Slovak Institute of Cardiovascular Disease, Košice, Slovak Republic
[4] Department of Biomedical Engineering and Measurement, Faculty of Mechanical Engineering, Technical University of Košice, Slovak Republic
[5] Clinic of Anaesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Košice, Slovak Republic
[6] EuroPainClinics, Košice, Slovak Republic
[7] Pain Clinic, Faculty Hospital A. D. Roosevelt Banská Bystrica, Slovak Republic
[8] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University, Košice, Slovak Republic

Chronic limb-threatening ischemia (CLTI) is the end stage of lower extremity artery disease with rising prevalence [1]. The prognosis of CLTI patients is alarming and even more devastating when revascularization is not possible [2].

One of the supportive treatment possibilities for CLTI patients is lumbar sympathectomy (LS) causing irreversible damage to a part of the lumbar sympathetic trunk, thus decreasing peripheral resistance of lower limb arteries controlled by sympathetic activity [3]. The LS is standardly performed on the desired side according to vascular pathology at the L3 spinal level. However, it causes anatomically nonspecific blockade of the lumbar sympathicus, thus it can often block both sides.

The angiosome is defined as a three-dimensional network of vessels in all tissue layers between the skin and bone. Two types of anastomotic arteries between adjacent angiosomes were identified: similar-caliber ("true") anastomotic arteries and reduced-caliber ("choke") that ordinarily exist in a state of reduced caliber during routine circulation and are controlled by sympathetic tone [4].

In the most common angiosome model, the foot consists of six angiosomes originating from the 3 main source arteries: posterior tibial artery (PTA), anterior tibial artery (ATA) and peroneal artery (PA).

Dermatomes represent the surface area of angiosomes. Therefore it makes sense to evaluate perfusion in angiosomes by temperature changes in dermatomes caused by vasodilatation enhanced due to sympathectomy. Anatomical relationship between dermatomes and angiosomes of the foot shows Figure 1.

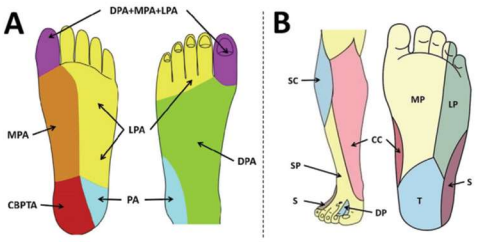


Figure 1. Anatomical relationship between angiosomes and dermatomes of the foot. (A) Angiosomes. MPA: angiosome of the medial plantar artery; LPA: angiosome of the lateral plantar artery (MPA and LPA are perfused by the posterior tibial artery); CBPTA: calcaneal branch of posterior tibial artery (PTA) angiosome (MPA, LPA and CBPTA create together one angiosome perfused by PTA); DPA: angiosome of the dorsalis pedis artery (angiosome perfused by the anterior tibial artery); PA: peroneal artery angiosome. (B) Dermatomes. SC: lateral sural cuta-neous nerve (L5-S2); CC: saphenous medial crural cutaneous nerve (L3-4); SP: superficial peroneal nerve (L4-S1); DP: deep peroneal nerve (L4-5); LP: dermatome of the lateral plantar nerve (S1-2); MP: dermatome of the medial plantar nerve (L4-5); T: dermatome of the tibial nerve (S1-2); S: dermatome of sural nerve (S1-2). SP and DP create together the surface area of DPA/ATA angiosome. LP, MP and T create together the surface area of the PTA angiosome. S is the surface area of the PA angiosome.

We have hypothesized, that alteration in sympathetic tone could cause dilation and reorientation of flow in choke vessels and this way sympathectomy may increase the blood flow from adjacent angiosomes with patent arteries to an angiosome perfused by an occluded arteries which should be associated with an increase of temperature [5].

Vasa (2023), *52* (2), 133–135
https://doi.org/10.1024/0301-1526/a001053

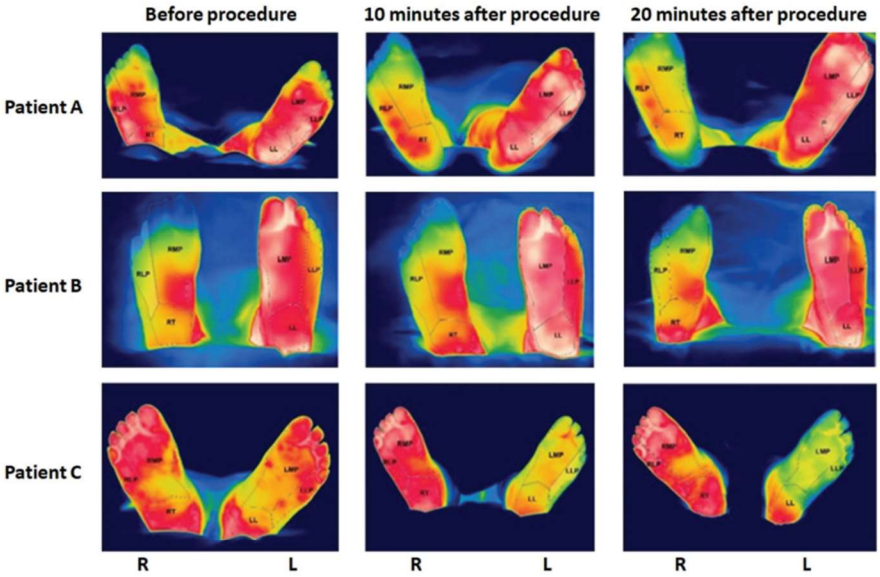


Figure 2. Images of thermal changes of the foot in Patient A, B and C taken by FLIR SC660 thermal image camera before, 10 minutes and 20 minutes after the procedure with detailed area segmentation. R: right side of the patient; L: left side of the patient.

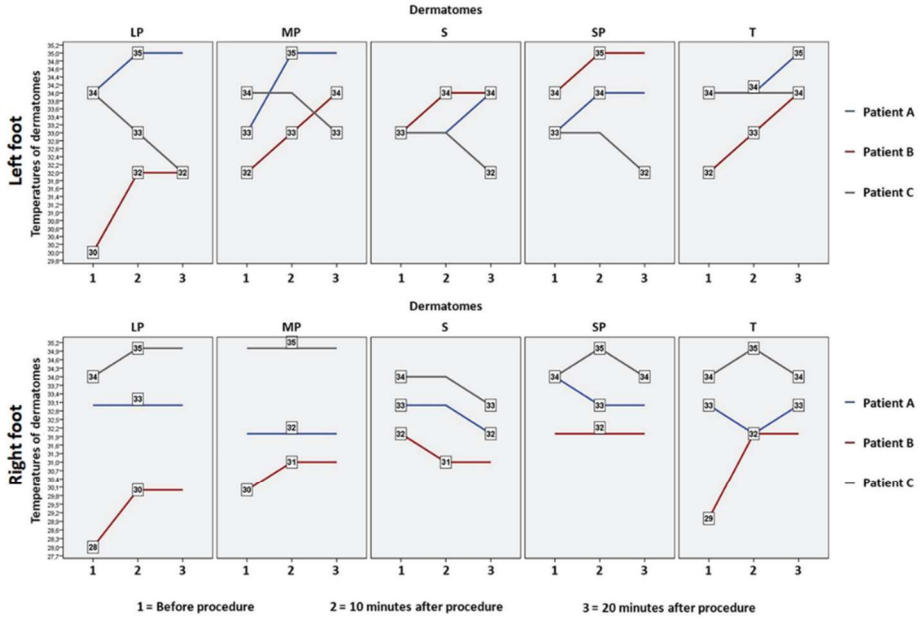


Figure 3. Line graphs showing changes of temperatures in each foot dermatome of all patients before, 10 minutes and 20 minutes after the procedure (LS).

In our pilot study, we measured changes in foot temperature by Infrared thermography before LS, than 10 and 20 minutes after LS in three CLTI patients (A, B, C) with chronic lower limb wound and ischemia at least grade 1 according to WIfI classification.

Clinically relevant change in temperature was arbitrarily considered an increase of more than 1 °C after the procedure. The study protocol was approved by an ethical committee.

All patients underwent angiography before LS. Patient A had three vessels runoff on the right limb and 2 vessels runoff – ATA and PA, PTA was occluded on the left limb. Angiographic findings of patient B were similar on both limbs, PTA was bilaterally occluded. Patient C had dominantly venous wounds due to post thrombotic syndrome. The severity lesions of below-the-knee arteries were up to 50% bilaterally in this patient.

Comparing the left and right foot, a relevant increase in temperature was observed 20 minutes after the procedure in patient A on the left foot in lateral and medial plantar dermatomes ranging from 1.7 °C to 2.1 °C (Figures 2 and 3) that corresponds to PTA angiosome. In patient B, there was an apparent change in temperature from 1.5 °C to 2.1 °C (left foot) and from 1.2 °C to 3.0 °C (right foot) in lateral, medial plantar, and tibial areas bilaterally (Figures 2 and 3) that also corresponds to PTA angiosome. Patient C had no relevant increase in temperature (Figures 2 and 3). Improvement in wound healing and partial reduction of wound size was observed in patients A and B only.

We have documented a relevant increase of temperature after LS only in dermatomes which create surface areas of angiosomes perfused by occluded arteries, probably due to the opening of choke vessels after the LS procedure. However, our findings are too preliminary and need to be verified by another study with a higher sample size. Despite this, we have performed LS in several CLTI patients with no option for revascularization (but already without thermal imaging).

References

1. de Graaff JC, Ubbink DT, Legemate DA, de Haan RJ, Jacobs MJ. Interobserver and intraobserver reproducibility of peripheral blood and oxygen pressure measurements in the assessment of lower extremity arterial disease. J Vasc Surg. 2001;33(5): 1033–40.
2. Wang Z, Hasan R, Firwana B, Elraiyah T, Tsapas A, Prokop L, et al. A systematic review and meta-analysis of tests to predict wound healing in diabetic foot. J Vasc Surg. 2016;63(2 Suppl): 29S–36S.e1–2.
3. Cronenwett JL, Lindenauer SM. Direct measurement of arteriovenous anastomotic blood flow aMer sympathectomy. Surgery. 1977;82(1):82–9.
4. Taylor GI, Palmer JH. The vascular territories (angiosomes) of the body: experimental study and clinical applications. Br J Plast Surg. 1987;40(2):113–41.
5. Taylor GI, Palmer JH. Angiosome theory. Br J Plast Surg. 1992;45:327–8.

History
Submitted: 07.11.2022
Accepted after revision: 10.01.2023
Published online: 06.03.2023

Conflict of Interest
There are no conflicts of interest existing.

ORCID
Viktória Rajťúková
https://orcid.org/0000-0002-7180-2874
Radovan Hudák
https://orcid.org/0000-0003-1060-0539
Dušan Rybár
https://orcid.org/0000-0003-0803-3213
Janka Vašková
https://orcid.org/0000-0003-0465-7950
Marek Hudák
https://orcid.org/0000-0003-0820-1328

Correspondence address
Marek Hudák, MD, PhD
East Slovak Institute of Cardiovascular Diseases:
Vychodoslovensky Ustav Srdcovych a Cievnych Chorob AS
Ondavská 8
04011 Košice
Slovakia

mhudak@vusch.sk

Ďalšie práce spojené s ambulantnou algeziologickou prácou boli publikované v recenzovaných medicínskych časopisoch Radiology Case Reports Q3, Neurologie pro praxi, Polish Journal of Sports Medicine Q4 a časopise Bolest:

- ***Kočan L**, Rapčan R, Griger M, Rapčanová S, Kantárová D, Török P, Vašková J. Deciphering the enigmatic symptoms of Pancoast tumors: Navigating the complex landscape of pain management-A case report. Radiol Case Rep. 2024 Feb 20;19(5):1810-1814. doi: 10.1016/j.radcr.2024.01.091.*

- *Dvorák M, Želinský Ľ, Horný V, Rapčan R, Neuwirth J, Griger M, **Kočan L**. Multidisciplinárny prístup v diagnostike vertebrogénnych ochorení z pohľadu neurológa a fyziatricko-rehabilitačného lekára. Neurol. praxi. 2019;20(6):417-420. ISSN 1213-1814.*

- *Neuwirth J, Griger M, Rapčan R, Dvorák M, Želinský Ľ, Horný V, **Kočan L**. Multidisciplinárny prístup v diagnostike vertebrogénnych ochorení z pohľadu rádiológa a intervenčného algeziológa. Neurol. praxi. 2019;20(6):423-427. ISSN 1213-1814.*

- *Mláka J, Matias M, Rapčan R, Rapčanová S, **Kočan L**. Perkutánne endoskopické techniky v diagnostike a liečbe vertebrogénnych ochorení: epiduroskopia a endoskopická diskektómia. Neurol. praxi. 2019;20(6):428-432. ISSN 1213-1814.*

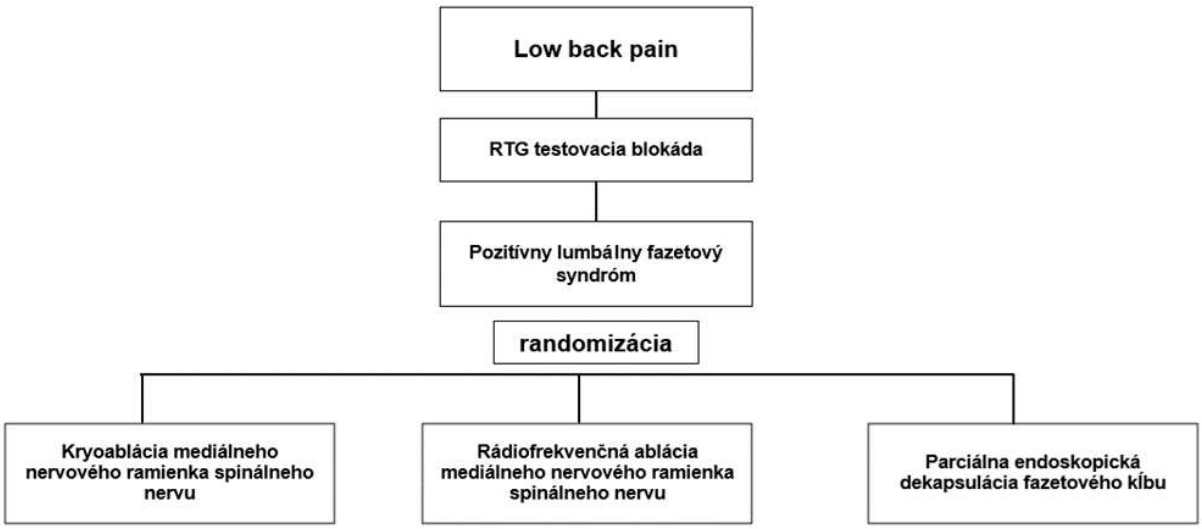
- *Rapčan R, Martuliak I, Lejčko J, Illéš R, Krajčovič M, **Kočan L**. Neuromodulačné techniky v riešení vertebrogénnych bolestivých syndrómov. Neurol. praxi. 2019;20(6):433-438.*

- *Rapčan R, Poliak Ľ, Rapčanová S, Lenčéš P, Burianek M. Vašková J. **Kočan L**. The influence of various sport activities on the degeneration of intervertebral discs. Polish Journal of Sports Medicine. 2023. 3. 115-121. ISSN 1232-406X.*

- *Tirpák R, Rapčan R, Griger M, Mláka J, Burianek M, Poliak Ľ, Lenčeš P, Matias M, **Kočan L.** Terapeutické možnosti a technika periradikulární terapie při kořenových syndromech. Bolest. 22. č 1. 2019. 21-28. ISSN 1212-0634.*

- *Poliak Ľ, Rapčan R, Griger M, Mláka J, Burianek M, Tirpák R, Lenčeš P, Matias M,* ***Kočan L.*** *Komplexná liečba radikulárneho syndrómu (prehľad terapeutických možností). Bolest. 22 č.2. 2019. 1-8. ISSN 1212-0634.*

Výsledky intervenčných techník zameraných na liečbu chronickej bolesti chrbta u pacientov s pozitívnym fazetovým syndrómom sme sledovali v multicentrickej randomizovanej klinickej štúdii EPCS XIr NCT04684303. V štúdii sme skúmali efektivitu intervenčných výkonov u pacientov s bolesťou dolnej časti chrbta po pozitívnom testovaní zameranom na mediálne nervové ramienka dorzálnej vetvy spinálneho nervu, ktoré inervujú fazetové kĺby v lumbosakrálnej oblasti. Po diagnostikovaní lumbálneho fazetového syndrómu, splnení inklúznych kritérií pre klinickú štúdiu boli pacienti randomizáciou rozdelení do 3 skupín podľa nasledujúceho flow diagramu.



Obrázok č.10: Flow diagram klinickej štúdie EPCS XIr.

Výsledky prezentované na kongrese World Institute of Pain - WIP XI WORLD CONGRESS BUDAPEST 2022, poukázali na signifikantné zlepšenie parametrov ($p < 0.05$) bolesti dolnej časti chrbta, ako aj bolestí vyžarujúcich do dolných končatín a signifikantného zlepšenia hodnotených dotazníkov kvality života Oswestry a EuroQuol v časových obdobiach po 3, 6,

a 12 mesiacoch od realizovaného výkonu. Zároveň medzi skupinové porovnanie medzi skupinami kryoablácia (n =70), rádiofrekvenčná ablácia (n=40) a endoskopická parciálna dekapsulácia (n=50) nepotvrdili signifikantné rozdiely vo všetkých sledovaných časových periódach $p > 0.05$.

Porovnanie metodických postupov rádiofrekvenčnej ablácie a kryoablácií kolmým a paralelným uložením kryosondy bolo publikované v medicínskom časopise Radiology Case Reports IF 1,0, Q4.

- ***Kočan L**, Rapčan R, Sudzina R, Rapčanová S, Rybár D, Mláka J, Kočanová H, Buriánek M, Vašková J. Radiofrequency denervation and cryoablation of the lumbar zygapophysial joints in the treatment of positive lumbar facet joint syndrome - a report of three cases. Radiol Case Rep. 2022 Sep 26;17(12):4515-4520. doi: 10.1016/j.radcr.2022.09.010.*

Radiology Case Reports 17 (2022) 4515–4520

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/radcr

Case Report

Radiofrequency denervation and cryoablation of the lumbar zygapophysial joints in the treatment of positive lumbar facet joint syndrome – a report of three cases ☆,☆☆

Ladislav Kočan, MD, PhD[a,b], *Róbert Rapčan, MD, PhD*[c], *Rudolf Sudzina, MD, PhD*[d], *Simona Rapčanová, MD*[c], *Dušan Rybár, MD*[a], *Juraj Mláka, MD*[b], *Hana Kočanová, MD*[e], *Miroslav Buriánek, MD*[f], *Janka Vašková, PhD*[g,*]

[a] *Clinic of Anesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic*
[b] *EuroPainClinics, Poliklinika Terasa, Toryská 1, 040 11 Košice, Slovak Republic*
[c] *EuroPainClinics, Na hradbách 94/3, 085 01 Bardejov, Slovak Republic*
[d] *Neurological Clinic, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovak Republic*
[e] *Clinic of Anesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Košice, Slovak Republic*
[f] *EuroPainClinics, Starochodovská 1750/91, 149 00 Prague 4, Czech Republic*
[g] *Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Tr. SNP 1, 040 11, Košice, Slovak Republic*

ARTICLE INFO

Article history:
Received 27 July 2022
Revised 30 August 2022
Accepted 4 September 2022
Available online 26 September 2022

Keywords:
Cryoablation
Facet joint
Radiofrequency ablation
Zygapophysial joint

ABSTRACT

Radiofrequency denervation of the zygapophysial (facet) joints is a frequently performed procedure for chronic low back pain. However, cryoablation represents a novel therapeutic approach for this condition. We observed and analyzed 3 cases with confirmed positive lumbar facet joint syndrome. Our results show a significant improvement in the clinical state of the patients in the first and third months after the procedure. The 6-month follow-up examination demonstrates a recurrence of pain and a gradual deterioration in the quality of life with a lasting partial pain-relief effect. Thermal radiofrequency denervation and cryoablation of the lumbar zygapophysial joints represent an effective, albeit temporary treatment option for lumbar facet joint syndrome patients, resulting from the pathophysiology of sensory nerve regeneration after destructive procedures. This type of treatment can be used repeatedly in the case of a positive response.

☆ Competing Interests: None of the authors has financial/commercial conflicts of interest with the published data.
☆☆ Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
* Corresponding author.
E-mail address: janka.vaskova@upjs.sk (J. Vašková).
https://doi.org/10.1016/j.radcr.2022.09.010

Introduction

Back pain is the most common type of pain in humans. More than 80% of the global population will experience low back pain at least once in their life and it is considered chronic if it lasts 3 months or longer [1–3]. The main cause is degenerative changes of spine structures including the facet joints, which can be a potentially important source of symptoms because of the high level of mobility and load forces, especially in the lumbar area [4,5]. Treatment of facet joint syndrome is multidisciplinary, and when conservative methods are not sufficient, radiofrequency thermal ablation is performed. More recently, cryoablation has emerged as an alternative interventional treatment option of facet joint syndrome [6,7].

In the following 3 case studies, we describe the treatment of facet joint syndrome using radiofrequency thermal ablation and cryoablation techniques placing the electrodes perpendicularly and in parallel with the nerve course. Cryoablation was performed with a CRYO-S PAINLESS device with carbon dioxide (−78°C) as a working medium for 2 minutes in 2 cycles for one nerve. This device also contains an integrated neurostimulator. When proper cryoprobe position was confirmed in multiple views, the impedance was checked and followed by sensory stimulation with a current of 50 Hz and motor stimulation with a current of 2 Hz. We followed these patients before the procedure, 4 weeks, 12 weeks, and 6 months after the procedure. Measured parameters included lower back pain intensity and intensity of pain of the lower extremities based on the Numeric Rating Scale (NRS) from 0 to 10. We used quality of life questionnaires, Oswestry Disability Index (ODI), which is focused on performing everyday activities affected by lower back pain, where the score 0 means the best possible status and 100 the worst possible status. We also used the licensed EQ-5D-5L questionnaire, evaluating the quality of life through 5 dimensions: mobility, self-care, everyday activities, pain/discomfort, and anxiety/depression. Each dimension is rated by the patient from 1 to 5, where a score of 1 means the best condition and a score of 5 means the worst possible condition. The EQ-VAS (EQ visual analogue scale) describes the current health condition, where 0 means the worst possible state, and 100 means the best possible state. This clinical trial was approved by the ethical committee of the Medical Faculty of the Pavol Jozef Safarik University in Košice, with the registration number 75/EK/15, as well as being registered in the international database clinicaltrials.gov under the registration number NCT03039296. All patients signed an informed consent about the participation in the study and publishing the clinical results.

Case series

Patient 1

Seventy-one-year-old patient, pensioner, with chronic lower back pain in the lumbo-sacral area after 6 spinal surgeries in the past, currently on combined pharmacological therapy: buprenorphine transdermal patch 35 ug/h and pregabalin 75 mg twice daily. In the past, the patient had repeated caudal pressure blocks (5 in total) with minimal therapeutic effect. The first MRI (magnetic resonance imaging) examination describes a multilevel herniation of the intervertebral discs L3/L4, L4/L5, listhesis of the L5 vertebra, and multilevel bilateral facet joint arthritis. Currently, the patient is experiencing a dull pain in the lumbo-sacral area with an NRS intensity of 9/10 radiating to the lower extremities. The patient underwent bilateral test blockade of the medial branches of the dorsal ramus of the spinal nerve (DRSN) at the interventional pain management clinic, after which they described 100% pain relief lasting for 48 hours. Subsequently, the patient underwent bilateral radiofrequency denervation of the medial branches of the DRSN innervating the facet joints L3/L4, L4/L5, and L5/S1. The radiofrequency probe was placed in parallel with the anticipated anatomical course of the sensory nerve (Fig. 1).

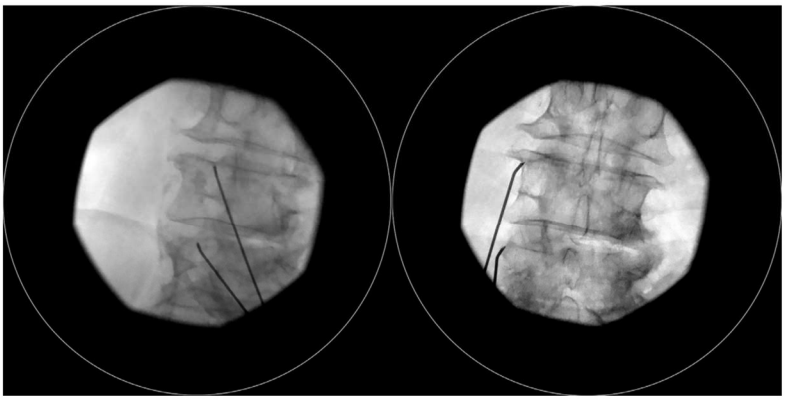


Fig. 1 – Final placement of the radiofrequency electrodes at the L3/L4 and L4/L5 facet joint level left side, anterior and oblique projections.

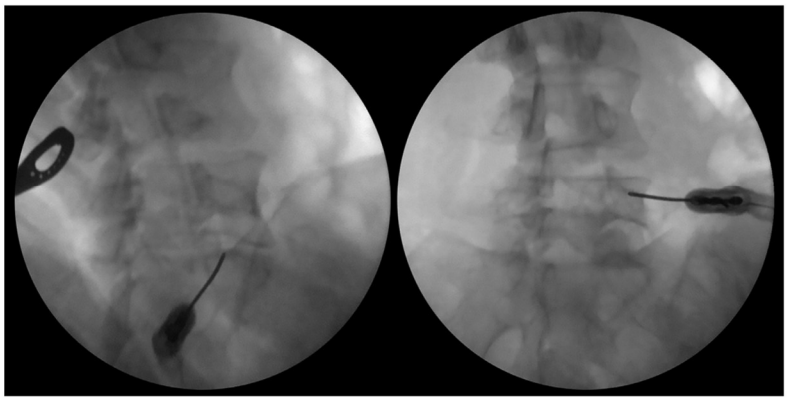


Fig. 2 – Final perpendicular placement of the cryoprobe to the L5/S1 and L4/L5 facet joints on the right, shown in the oblique projection.

Patient 2

Forty-five-year-old patient working as a teacher and artistic carpenter was repeatedly hospitalized at the neurological ward for attacks of back pain in the lumbosacral area. The current MRI of the low back spine shows multilevel discopathy, moderate ventrolisthesis of L5, minimal dorsolisthesis of L3 to L4. Further, it shows an L5/S1 intervertebral disc herniation affecting the posterior lower border of the L5 vertebra with the size 5.8 mm and a multilevel herniation. The patient has intense pain of their lumbar spine area radiating to the right lower limb, paresthesias on the lateral border of their thigh, they have problems being seated for a longer period of time. The patient has been using NSAIDs (nonsteroidal anti-inflammatory drugs) in combination with Tramadol/Paracetamol tablets during intense bouts of pain. After a bilateral test blockade, the patient felt 70% relief of pain for 12 hours. They then underwent cryoablation of the medial branches of the facet joints L3/L4, L4/L5, L5/S1. The cryoprobe was placed perpendicularly to the expected anatomical course of the sensory nerve (Fig. 2).

Patient 3

Sixty-four-year-old patient with a desk job, complains of intense pain of their lumbar spine radiating to both lower limbs, more on the right, lasting approximately 4 years. The pain occurs mainly at night. The current MRI shows no compression of any structures, numerous Schmorl's nodes in vertebral bodies, L3/L4 and L4/L5 segments shows MODIC1 and MODIC2 (modic type endplate changes, status 1 and 2) changes. Clinical examination revealed pseudoradicular syndrome, provocation tests for the SI joins were negative without a sensory or motor deficit in the lower limbs. Analgesic therapy consisted of sporadic use of NSAIDs and metamizol. The patient underwent a bilateral test facet joint blockade, with significant pain relief about 90% directly after the procedure lasting up to the night hours. Here, we performed a bilateral thermal ablation of the medical branches (DRSN) L3, L4, and L5 with cryoanalgesia. The cryoprobe was placed in parallel with the expected anatomical course of the sensory nerve (Fig. 3).

In all cases, we noted a significant improvement of pain intensity in the 1st and 3rd months after the procedure with a lasting pain relief effect or slightly recurring pain at the 6 months follow-up (Table 1). Similar results were noted in patients monitored by ODI, EQ-VAS, and the EQ-5D-5 dimensions with a lasting positive treatment effect as well as a slight worsening at the 6 months follow-up (Tables 1 and 2).

Discussion

Facet joint syndrome is linked with the dysfunction in the joint of the same name, which becomes the source of pain. The most common causes of this are trauma, more commonly repeated microtrauma in the facet joint area caused by repeated extension of the lumbar spine or with overhead activities, as well as sports involving tiresome extension exercises of the lumbar spine. One of the most common causes is the damage of the intervertebral disc, which causes a disruption in the biomechanics of the facet joints. This can cause their subluxation, microtrauma, swelling of the synovium surrounding the facet joints, leading to synovitis. A hypertonic contraction of surrounding structures occurs as a defence mechanism, which leads to the worsening of pain. An important step in the diagnosis of lumbar facet joint syndrome is a test blockade of the medial branch DRSN. Precise diagnostics is important, similarly as it was performed in patients from the case series where, after injecting a small amount of local anesthetic (0.5 mL 0.5 % bupivacaine) under X-ray guidance to the sensory medial branch DRSN, significant pain relief was achieved for numerous hours. This test was performed twice in each patient. After a positive test result, cryoablation or radiofrequency denervation was performed, which provided

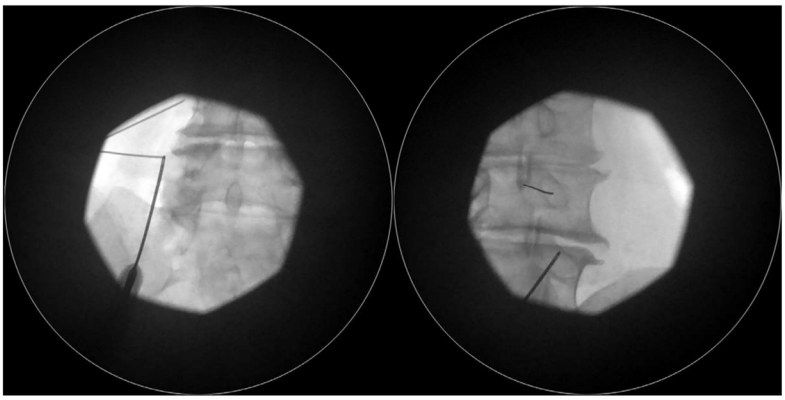


Fig. 3 – The final parallel placement of the cryoprobe to the L4/L5 facet joint on the left in the AP projection and parallel placement of the cryoprobe to the L4/L5 facet joint on the right, shown in the oblique projection.

Table 1 – Trend of change in the clinical status in the Oswestry Disability Index (ODI) and EQ-VAS (EQ visual analogue scale) parameters and pain intensity assessed by the Numerical Rating Scale (NRS) at each time interval in the lumbo-sacral back pain and pain radiating to the lower extremities followed in patients 1, 2, and 3.

Patient (number)/technical aspects	Parameters	Follow-up			
		Before procedure	1 month	3 month	6 month
Radiofrequency ablation	ODI	45	5	9	22
	EQ-VAS	50	90	80	60
	NRS back pain	9	2	1	5
	NRS leg pain	3	1	1	2
Cryoablation perpendicularly	ODI	63	31	31	45
	EQ-VAS	75	85	85	55
	NRS back pain	8	4	4	6
	NRS leg pain	8	5	5	8
Cryoablation in parallel	ODI	56	4	9	29
	EQ-VAS	55	70	85	65
	NRS back pain	7	4	2	7
	NRS leg pain	7	4	4	6

the patient with pain relief for a couple of months. Both of these procedures cause targeted physical damage of the nerve in a limited area, either by the action of an electromagnetic field, increased temperatures in the desired area, or cold temperatures. These physical effects cause reversible damage to the nerve, a so-called axonotmesis, where axonal damage occurs with the preservation of the epineurium. During this damage, a defect in the transmission of nerve signals occurs, which clinically manifests as the disappearance of pain. Distally from the nerve damage, Wallerian degeneration occurs, as well as changes of the myelin sheath, but the periphery of the nerve maintains its electrical irritability for 5-10 days. After a couple of days, the body of the neuron is activated – sprouting; each nerve fiber from the damaged axon regenerates at the speed of 1 mm per day [7,8]. The return of pain after neural ablation indicates nerve regeneration after peripheral nerve damage after a couple of weeks or months. This trend of pain recurrence was demonstrated in all patients.

In clinical practice, we are also met with unsuccessful thermal ablation procedures. This can have numerous causes. The most common cause is multifactorial etiology of lower back pain. Facet joint arthropathy is often connected with discopathy, radiculopathy, spinal canal stenosis, and myofascial syndrome. The co-existence of these co-morbidities means that treating only one of them can lead to unsuccessful pain relief. Another cause can be a false positive diagnostic blockade, by injecting too much local anesthesia and its spread to the surrounding structures, which could also be the sources of pain [9–11]. A high percentage of positive reactions after a placebo dose are the cause of false positive results [12]. Aberrant nerve sprouting could also be the cause of unsuccessful treatment. Okuyama et al. discovered, that after RF ablation of the myocardium, aberrant nerve sprouting occurs already after 2 hours, and it is probable that the same process occurs after RF ablation of the medial branches of the DRSN [13,14]. The choice of facet joint thermal ablation technique (radiofre-

Table 2 – Trend of changes in the patients' assessment of everyday activities at each time interval, where a score of 5 indicates the worst possible status and the score 1 means the best possible status.

Patient/technical aspect	Mobility/ follow-up			
	Before procedure	1 month	3 month	6 month
Radiofrequency ablation	4	2	2	3
Cryoablation perpendicularly	4	2	2	3
Cryoablation in parallel	3	1	2	2
	Self-care/ follow-up			
Radiofrequency ablation	3	1	1	3
Cryoablation perpendicularly	3	2	2	3
Cryoablation in parallel	4	1	1	2
	Usual activities/follow-up			
Radiofrequency ablation	4	2	2	3
Cryoablation perpendicularly	4	2	1	3
Cryoablation in parallel	4	2	2	2
	Pain and discomfort/follow-up			
Radiofrequency ablation	4	1	2	3
Cryoablation perpendicularly	3	1	2	3
Cryoablation in parallel	4	1	2	2
	Anxiety and depression/follow-up			
Radiofrequency ablation	3	1	1	2
Cryoablation perpendicularly	3	1	2	3
Cryoablation in parallel	4	1	2	3

quency or cryoablation) as well as probe placement can also have an influence on the final treatment effect, seeing as they have different nerve damage mechanisms. The choice of interventional thermal ablation method is not strictly defined, seeing as there have been no published EBM results on the superiority of one technique over the other. In terms of the result, the shape and extent of the lesion which forms around the tip of the electrode are important, and they depend on its physical properties and treatment procedure. Placement of the RF electrode during radiofrequency denervation is defined by SIS guidelines based on EBM, which consists of placing the probe in parallel with the anatomic course of the nerve [1]. The reason is to damage the nerve the most efficiently. Placement of the cryoablation electrode is not yet clearly determined, which is why in our patients, we placed them in the 2 most common used locations in practice – perpendicular to and in parallel with the nerve course.

Conclusion

Due to the diverse etiology of lower back pain, it is imperative to determine the cause of the pain to plan the treatment procedure. To correctly determine the source of lower back pain, we have to perform a complex evaluation. In a precisely diagnosed lumbar facet joint syndrome, thermal ablation techniques represent a relevant therapeutic tool.

Patient consent

Written informed consent for the publication of this case report was obtained from each patient.

REFERENCES

[1] Manchikanti L, Pampati V, Fellows B, Bakhit CE. Prevalence of lumbar facet joint pain in chronic low back pain. Pain Phys 1999;2(3):59–64.

[2] Bogduk N. Practice guidelines for spinal diagnostic and treatment procedures. San Francisco: International Spine Intervention Society, United States; 2013. p. 684. (in Eng.).

[3] Geraci MC, Baker RM, Sasso RC. Lumbar zygapophysial joint evaluation and treatment. Curr Concepts 2005:10–17.

[4] Lau P, Mercer S, Govind J, Bogduk N. The surgical anatomy of lumbar medial branch neurotomy (facet denervation). Pain Med 2004;5(3):289–98. doi:10.1111/j.1526-4637.2004.04042.x.

[5] Manchikanti L, Boswell MV, Singh V, Derby R, Fellows B, Falco FJ, et al. Comprehensive review of neurophysiologic basis and diagnostic interventions in managing chronic spinal pain. Pain Phys 2009;12(4):E71–120. doi:10.36076/ppj.2009/12/E121.

[6] Venglarčík M, Mláka J, Griger M, Tirpák R, Vinklerová V, Kočan L, et al. Radiofrequency denervation of facet joints in low back pain therapy at lumbar facet syndrome. Neurol praxi 2017;18(1):38–42 (in Slovak). doi:10.36290/neu.2017.061.

[7] Perolat R, Kastler A, Nicot B, Pellat JM, Tahon F, Attye A, et al. Facet joint syndrome: from diagnosis to interventional management. Insights Imaging 2018;9(5):773–89. doi:10.1007/s13244-018-0638-x.

[8] Menorca RM, Fussell TS, Elfar JC. Nerve physiology: mechanisms of injury and recovery. Hand Clin 2013;29(3):317–30. doi:10.1016/j.hcl.2013.04.002.

[9] Dreyfuss P, Halbrook B, Pauza K, Joshi A, McLarty J, Bogduk N. Efficacy and validity of radiofrequency neurotomy for chronic lumbar zygapophysial joint pain. Spine (Phila Pa 1976) 2000;25(10):1270–7. doi:10.1097/00007632-200005150-00012.

[10] Schwarzer AC, Wang SC, Bogduk N, McNaught PJ, Laurent R. Prevalence and clinical features of lumbar zygapophysial joint pain: a study in an Australian population with chronic

low back pain. Ann Rheum Dis 1995;54(2):100–6. doi:10.1136/ard.54.2.100.
[11] Cohen SP, Strassels SA, Kurihara C, Forsythe A, Buckenmaier CC 3rd, McLean B, et al. Randomized study assessing the accuracy of cervical facet joint nerve (medial branch) blocks using different injectate volumes. Anesthesiology 2010;112(1):144–52. doi:10.1097/ALN.0b013e3181c38a82.
[12] Lord SM, Barnsley L, Bogduk N. The utility of comparative local anesthetic blocks versus placebo-controlled blocks for the diagnosis of cervical zygapophysial joint pain. Clin J Pain 1995;11(3):208–13. doi:10.1097/00002508-199509000-00008.
[13] Okuyama Y, Pak HN, Miyauchi Y, Liu YB, Chou CC, Hayashi H, et al. Nerve sprouting induced by radiofrequency catheter ablation in dogs. Heart Rhythm 2004;1(6):712–17. doi:10.1016/j.hrthm.2004.09.012.
[14] Kim MH, Kim SW, Ju CI, Chae KH, Kim DM. Effectiveness of repeated radiofrequency neurotomy for facet joint syndrome after microscopic discectomy. Korean J Spine 2014;11(4):232–4. doi:10.14245/kjs.2014.11.4.232.

6.3 Multicentrické projekty spojené s liečbou a výskumom akútnej a chronickej bolesti

Ďalšou dôležitou činnosťou zameranou na liečbu bolesti je aktívna spolupráca s viacerými algeziologickými ambulanciami, klinikami neurológie a neurochirurgie v rámci Slovenska a Českej republiky. Významná spolupráca prebieha s Ústavom merania Slovenskej Akadémie Vied (ÚM SAV). Ako dôležitý výskumný počin považujeme vytvorenie a vedenie multicentrických klinických štúdií zameraných na hodnotenie kvality života u pacientov s akútnou a chronickou bolesťou po intervenčných algeziologických výkonoch, a to prostredníctvom dotazníkov Oswestry Disability Index a EuroQuol EQ-5D-5L. V súčinnosti s ÚM SAV sme stanovili koeficienty štatistických váh pre štatistickú analýzu dotazníkov EQ 5D-5L na modeloch skupín pacientov s akútnou a chronickou bolesťou pre regióny Česka a Slovenska. Túto metodiku je možné ďalej využívať pre hodnotenie kvality života rôznych diagnóz v rozličných medicínskych odboroch. Medzi naše hlavné klinické štúdie, v ktorých bola hodnotená kvalita života pacientov patria: Prospektívna randomizovaná multicentrická klinická štúdia EPCS II (zameraná na chronickú bolesť u pacientov s postlaminektomickým syndrómom – „failed back surgery syndrome“ - FBSS) a Prospektívna observačná klinická štúdia EPCS V (zameraná na liečbu akútnej bolesti u pacientov s akútnym radikulárnym syndrómom, spôsobeným útlakom koreňa herniovaným diskom alebo sequestrom).

Výsledky štúdie EPCS II boli publikované v medicínskych časopisoch: Pain Medicine IF 3,1, Q1, Wiener klinische Wochenschrift IF 2,6 Q2 a Bratislava Medical Journal, IF 1,4, Q4. Výsledky poukazujú na klinický benefit u pacientov s FBSS po epiduroskopickom výkone, a to v zlepšení intenzity bolesti v dolnej časti chrbta a výsledkov dotazníkov Oswestry a EuroQuol po 6 mesiacoch ($p < 0.05$). V skupine, v ktorej bola vykonaná epiduroskopia a simultánna aplikácia liečiv do epidurálneho priestoru sme zaznamenali signifikantne predĺžený pozitívny efekt v parametri intenzita bolesti vyžarujúcej do dolných končatín po 12 mesiacoch ($p < 0.05$).

Najdôležitejšie publikácie štúdie EPCS II:

- *Griger M, **Kocan L**, Rapcan R, Matias M, Burianek M, Kocanova H, Rapcanova S, Mlaka J, Zahorec R, Vaskova J. Epiduroscopic intervention in patients with a failed back surgery syndrome. Bratisl Lek Listy. 2020;121(10):727-732. doi: 10.4149/BLL_2020_119. IF 1,7, Q3*

- *Rapčan R, **Kočan L**, Witkovsky V, Mláka J, Griger M, Burianek M, Rapčanová S, Hammond A, Poliak Ľ, Tirpák R, Šimonová J, Sabol F, Vašková J. EQ-5D-5L questionnaire as suitable assessment of quality of life after epiduroscopy : Multicenter randomized double-blind pilot study. Wien Klin Wochenschr. 2020 Sep;132(17-18):526-534. doi: 10.1007/s00508-019-01590-z. IF 1,6, Q3*

- *Rapčan R, **Kočan L**, Mláka J, Burianek M, Kočanová H, Rapčanová S, Hess M, Hammond A, Griger M, Venglarčík M, Gajdoš M, Vašková J. A Randomized, Multicenter, Double-Blind, Parallel Pilot Study Assessing the Effect of Mechanical Adhesiolysis vs Adhesiolysis with Corticosteroid and Hyaluronidase Administration into the Epidural Space During Epiduroscopy. Pain Med. 2018Jul 1;19(7):1436-1444. doi: 10.1093/pm/pnx328. IF 2,8 Q1*

Výsledky štúdie EPCS II boli citované v Metaanalýzach, Systémových prehľadoch, a medzinárodných odporúčaniach:

- Geudeke MW, Krediet AC, Bilecen S, Huygen FJPM, Rijsdijk M. Effectiveness of Epiduroscopy for Patients with Failed Back Surgery Syndrome: A Systematic Review and Meta-analysis. Pain Pract. 2021 Apr;21(4):468-481. doi: 10.1111/papr.12974.

- Papalia GF, Russo F, et al. V. Non-Invasive Treatments for Failed Back Surgery Syndrome: A Systematic Review. Global Spine J. 2023 May;13(4):1153-1162. doi: 10.1177/21925682221141385.

- Manchikanti L, Racz GB, , et all. Epidural Interventions in the Management of Chronic Spinal Pain: American Society of Interventional Pain Physicians (ASIPP) Comprehensive Evidence-Based Guidelines. Pain Physician. 2021 Jan;24(S1):S27-S208. PMID: 33492918.

Pain Medicine 2018; 19: 1436–1444
doi: 10.1093/pm/pnx328

OXFORD

NEUROMODULATION & INTERVENTION SECTION

Original Research Article

A Randomized, Multicenter, Double-Blind, Parallel Pilot Study Assessing the Effect of Mechanical Adhesiolysis vs Adhesiolysis with Corticosteroid and Hyaluronidase Administration into the Epidural Space During Epiduroscopy

Róbert Rapčan, MD,[*,†,‡] Ladislav Kočan, MD, PhD,[§] Juraj Mláka, MD,[¶] Miroslav Burianek, MD, MBA,[*] Hana Kočanová, MD,[‖] Simona Rapčanová, MD,[*] Michael Hess, MD,[‖‖] Anthony Hammond, MD,[**] Martin Griger, MD,[†] Michal Venglarčík, MD, PhD,[‡] Miroslav Gajdoš, MD, PhD,[††] and Janka Vašková, PhD[‡‡]

[*]Europainclinics, Prague, Czech Republic; [†]Europainclinics, Nové Mesto, Slovak Republic; [‡]Europainclinics, Bardejov, Slovak Republic; [§]Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Košice, Slovak Republic; [¶]Europainclinics, Poliklinika Terasa, Košice, Slovak Republic; [‖]Clinic of Anaesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Košice, Košice, Slovak Republic; [‖‖]Wirbelsäuleninstitut, Munich, Munich, Germany; [**]Kent Institute of Medicine and Surgery, Kent, UK; [††]Department of Neurosurgery, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, and Louis Pasteur University Hospital, Košice, Slovak Republic; [‡‡]Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

Correspondence to: Janka Vašková, PhD, Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, 040 66 Košice, Slovak Republic. Tel: 421552343232; Fax: 421552343401; E-mail: janka.vaskova@upjs.sk.

Conflicts of interest: None of the authors has any financial/commercial conflicts of interest with the published data.

Abstract

Objective. Epiduroscopy is a proven method of diagnosis and treatment for chronic radicular pain after spinal surgery, which is known as failed back surgery syndrome (FBSS). The aim of the study was to compare the efficacy of drugs (the enzyme hyaluronidase and corticosteroid DEPO-Medrol) administrated into the epidural space during epiduroscopy, performed within the ventral and ventro-lateral epidural space with a focus on releasing foraminal adhesions.

Methods. Forty-eight patients with diagnosed FBBS were randomized into two groups before epiduroscopy. Group A received the standard treatment—mechanical lysis of fibrotic tissue in the epidural space. Group B received hyaluronidase and corticosteroid methylprednisolone acetate during the procedure. Subjects were followed for six and 12 months via scheduled double-blinded examinations by pain physicians. Leg and back pain intensity was assessed by an 11-point numerical rating scale, and patients' functional disability was assessed by the Oswestry Disability Index (ODI).

Results. Study subjects showed a significant decrease in ODI score in both groups ($P < 0.05$). Significantly lower pain scores for leg pain ($P < 0.05$) and back pain ($P < 0.05$) were also recorded after the six-month follow-up. However, the one-year follow-up showed a return to the baseline ODI values of most monitored pain scores in both groups ($P > 0.05$). Improvement was only noted on the NRS for back pain at one-year follow-up ($P < 0.05$).

Conclusions. A significant improvement of leg and back pain was found in both groups after six

months. ODI was significantly improved only in group B in both the six- and 12-month intervals. Back pain at one-year follow-up was only improved in group B.

Key Words. Adhesiolysis; Back Pain; Epiduroscopy; Failed Back Surgery Syndrome; Hyaluronidase

Introduction

Medical experts and researchers have investigated various types of optical visualization of human cavities for decades, with varying degrees of success [1]. Epiduroscopy is a relatively new technique used in the evaluation and treatment of low back pain via advancements in optical fiber technology. As a minimally invasive, endoscopic technique, it allows for direct endoscopic imaging of the epidural space and helps with pain management for patients suffering from post–lumbar surgery syndrome (PLSS) and other causes of low back pain and radiculopathy [2]. Epiduroscopy, the direct visualization of the epidural space with a flexible endoscope, has been performed in some places for years, but its significance is still questionable. For example, it has been shown to be more sensitive than magnetic resonance imaging (MRI) in detecting epidural fibrosis. According to literature, in patients with failed back surgery syndrome, MRI showed epidural fibrosis in 16.1% of patients whereas epiduroscopy showed epidural fibrosis in 91% of patients [3]. A systematic review of the literature regarding the effectiveness of spinal endoscopic adhesiolysis in managing chronic intractable pain from PLSS indicated an evidence level of II-1 or II-2 based on the US Preventive Services Task Force (USPSTF) criteria, and one randomized trial gave it a recommendation of 1 C/strong [4]. The authors of this study have been using epiduroscopy for a number of years, and they see a potential for this method to enable physicians to perform a detailed examination of the spinal canal with optic visualization of the particular structures. This type of examination will provide detailed information about the presence of postoperative fibrosis, adhesions, inflammatory changes, or any other pathological change within the epidural space. The current technique also allows the utilization of optical visualization for targeted therapeutical interventions in the epidural space, such as the removal of adhesions and fibrotic changes, or targeted administration of medication. According to the literature, in patients with failed back surgery syndrome (FBSS), epidurally applied corticosteroids reach the intended level in only 26% of cases [3]. Anterior epiduroscopy and epiduroscopic laser neural decompression (ELND) have recently been introduced in the treatment of herniated disc decompressions and chronic low back and radicular pain, respectively [2]. Recent publications describe the use of Fogarty catheters and resablation to remove adhesions attached to the dura [3].

There is some controversy regarding the theory behind the role of postoperative fibrosis and epidural adhesions in the etiology of FBSS. This is a syndrome in which patients do not experience an improvement in their clinical status after successful back surgery or, after a minor improvement, their status deteriorates without any correlations with MRI. The formation of scar tissue near the nerve root is a common occurrence after back surgery and is called epidural fibrosis: Scar tissue might be a major cause of postoperative pain, commonly called FBSS. This epidural scarring can cause pain for many reasons; for example, the nerves may be trapped by scars, while veins in the epidural space press down upon the nerves and become enlarged, putting pressure on them [5]. The decreased nourishment of nerve tissue and traction of epidural adhesions on the dural sac can also contribute to the etiopathogenesis of the complex pain syndrome in FBSS. Stenosis of the spinal canal by fibrosis is also a justifiable factor in the worsening of the clinical prognosis in a patient after back surgery. If the aforementioned statements do have a clinical foundation, the removal of fibrosis and adhesions should lead to an improvement in the clinical state of the patient even without the administration of anti-inflammatory medications, depomedrol, and hyaluronidase.

On the other hand, it has been scientifically proven that hyaluronidase inhibits cellular recruitment, edema formation, and pro-inflammatory mediator production, resulting in decreased adherence of leukocytes to blood vessels and tissue infiltration [6].

The goals of our study are to evaluate the changes in the clinical state of patients with FBSS after the endoscopic removal of fibrosis and adhesions and to compare between an exclusively mechanical intervention and a mechanical intervention with targeted administration of depot corticosteroids and hyaluronidase.

Methods

A randomized controlled trial with a parallel group study design was used. The present study was approved by the Ethics Committee of Louis Pasteur University Hospital in Košice (approval number 75/EK/15) and registered at clinicaltrials.gov with registration ID NCT02459392. Written informed consent was obtained from all participants. All patients with failed back surgery syndrome who were indicated to undergo an epiduroscopy procedure were recruited from one of the three pain clinics in Bratislava, Bardejov, and Košice in the Slovak republic. Inclusion criteria were age 18 years or older, written informed consent, symptoms of FBSS, permanent low back pain with dominant (more than 60%) radiation to lower extremities despite previous epidural corticosteroid injections, current magnetic resonance imaging (no older than three months) without serious spinal stenosis and serious radicular compression. Exclusion criteria were the presence of annihilating phenomena (loss of sensitivity of the skin, loss of coordination of the lower extremities, problems with urination

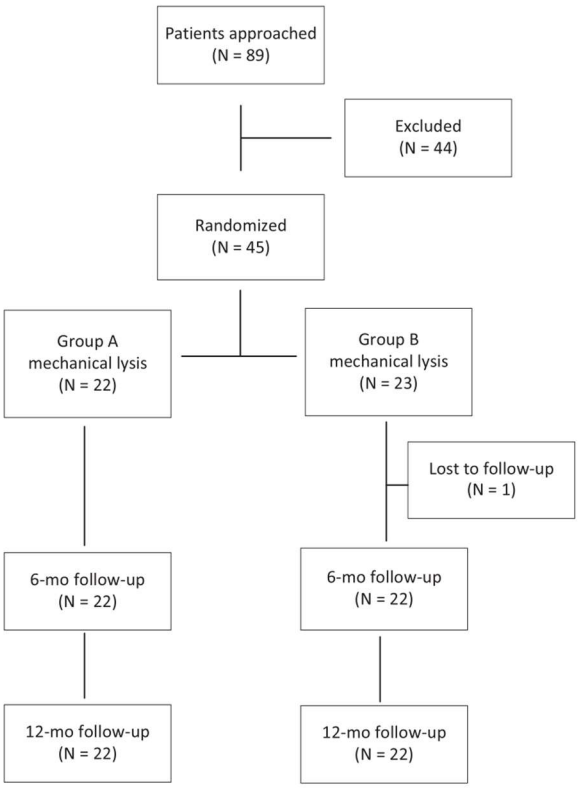


Figure 1 Flow chart of patient selection, enrollment, and follow-up in the study.

or defecation), presence of infection, neoplasms, and lack of patient approval.

All eligible patients were approached. After written informed consent was obtained, participants were allocated into study groups according to computer randomization software. Each patient had obtained a unique clinical trial ID number, which was generated by computer software before epiduroscopy. Blinding at the time of randomization was maintained with a sealed envelope given to the anesthesiologist managing the patient. The anesthesiologist was not involved in data collection. Study outcome measurements were obtained by an independent research team from the Medical Faculty, Pavol Jozef Šafárik University in Košice. Study continuance was maintained by an independent clinical study coordinator from the East Slovak Institute of Cardiovascular Disease in Košice, and the report was prepared in accordance with the Consolidated Standards of Reporting Trials (the "CONSORT statement"). All data were assembled in a protected and encrypted database accessible only to the study coordinator including statisticians from an independent statistical institution and local study site coordinators.

Patients diagnosed with failed back surgery syndrome (FBSS) were enrolled in the study. Patients underwent at least one previous back surgery with ongoing pain radiating to the lower extremities with or without back pain, which was still present after periradicular therapy or caudal blockade (less than 50% visual analog scale [VAS] relief of pain 30 days after intervention) performed by a pain management specialist. A patient who had met the inclusion criteria was informed by the examining doctor about the study and given the opportunity to participate. They were informed about the intervention procedure, epiduroscopy performed by a flexible fiberoptic endoscope with a video-guided catheter (Myelotec, Inc., Roswell, GA, USA), strictly within the ventral and ventro-lateral epidural space with focus on

releasing foraminal adhesions. Consequently, in the case of agreement, the patient signed the informed consent documents about the interventional procedure, epiduroscopy, and their informed consent to participate in the study.

The mechanical adhesiolysis itself was performed by three different tools: laser, radiofrequency probe, or a balloon catheter. The choice of the instrument was made by the surgeon according to his clinical preference. The total volume of our standard pharmacological mixture was 30 mL per foraminal level (20 mL of the mixture bupivacaine 0.5%, 5 mL methylprednisolone, 80 mg saline, and 150 I.U. of hyaluronidase, Hylase "Dessau", in 10 mL saline). The maximum volume injected was never more than 60 mL. Patients were randomly split into two groups (Figure 1). The first group (Group A) underwent epiduroscopy (5 mL of 0.5% bupivacaine was injected; the total volume injected was supplemented up to 20 mL with saline), during which only a mechanical lysis of the epidural fibrotic attachments was performed by either laser (four patients), radiofrequency (15 patients), or the balloon technique (three patients). The second group (Group B) underwent epiduroscopy, during which mechanical lysis of the epidural fibrotic attachments was performed by laser (five patients), radiofrequency (16 patients), or the balloon technique (four patients), as in group A (5 mL of 0.5% bupivacaine). At the same time, a solution of hyaluronidase (Hylase "Dessau" 150 I.U. in 10 mL of saline) and injectable corticosteroid methylprednisolone acetate (DEPO-Medrol) 80 mg were administered to the patient into the place of conflict (the depression in the spinal root by fibrosis). After completing the first protocol of the preoperative examination, this protocol was sent to the coordinator of the study as well as the researcher in charge of processing study data. The coordinator of the study planned the first postoperative examination six months after the procedure and the second postoperative examination after 12 months following the procedure. The first and second postoperative examinations of the patient were performed by a different physician (not the one performing the actual procedure), or at a different pain management clinic. They performed a pain assessment of the patient while blinded to which procedure they had undergone (endoscopy only including mechanical lysis or with the administration of the drugs) and completed the pain management protocol of the study. Primary outcomes were pain intensity spreading in the back and legs and also evaluation of the Oswestry Disability Index (ODI). Patient Status Score (PSS) is a grading scale from 0–4, where 0 means the patient is without pain, has a normal life, normal job, is able to exercise; and 4 means the patient needs help to take care of themselves and is bed-ridden. The Patient Self-Content Score (PSCS) is evaluated on a scale from 0 to 10, where the patient describes his satisfaction with the procedure at six- and 12-month follow-up (0 being the worst and 10 being the best). There were no important changes to the methods after the study commenced.

Table 1 Characteristics of patients in groups divided according to the performed procedure

	Group A (Min–Max) Med	Group B (Min–Max) Med
Participants, No.		
Before procedure	22	23
6-mo follow-up	22	22
12-mo follow-up	22	22
Age, y	(35–70) 54	(33–69) 46.5
Sex (F/M)	10/12	12/11
ASA	(1–3) 2	(1–3) 2
BMI	22	20
Pain in dermatomes according to examination before procedure		
L2	0	1
L3-L4	1	0
L4-L5	4	5
L5	2	6
L5-S1	5	3
S1	4	4
Mechanical therapeutic intervention		
Balloon	3	4
Laser	4	5
Radiofrequency	15	16

Descriptive statistical methods were used to evaluate the results (mean, median, maximum, minimum, and SD). Examination of the distributional form for score and time data was determined by box plots. Each box plot indicated minimal value, lower quartile (lowest 25% of data), median, upper quartile (highest 25% of data), and maximal value. Normality of data distribution was assessed by the Shapiro-Wilk test. Homogeneity of variances was estimated using the Levene test. Differences between continuous variables were analyzed by a nonparametric Kruskal-Wallis one-way test. A paired Student *t* test was used to assess the statistical significance of changes within each treatment group. *P* values of less than 0.05 were considered significant. Statistical analysis was performed with the SPSS version 11.0 statistical software package.

Results

Of the 86 admitted patients with FBSS, 45 fulfilled the selection criteria and were randomized into two groups (Group A—mechanical lysis, Group B—mechanical lysis and drugs) and then underwent epiduroscopy. One patient from group B was lost during follow-up. There were no unexpected side effects. The baseline demographic and characteristic values are summarized in Table 1 and were similar in both groups.

A significant improvement was recorded in ODI in both groups after six months ($P < 0.05$), which indicated

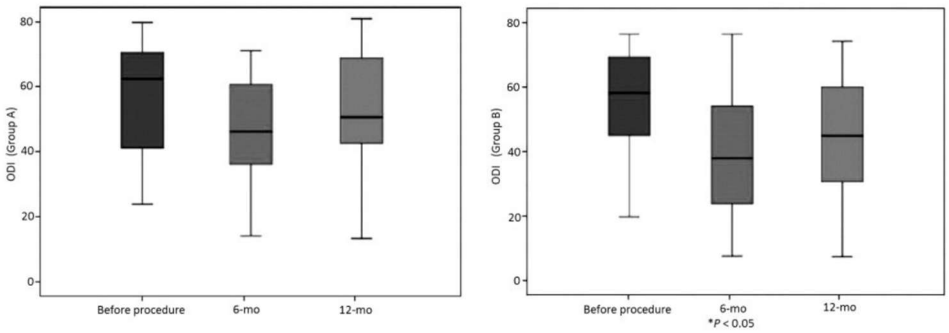


Figure 2 Evaluation of patient functional disability by the Oswestry Disability Index indicating significant improvement in comparison with baseline after six months, with return to baseline values after 12 months in both groups.

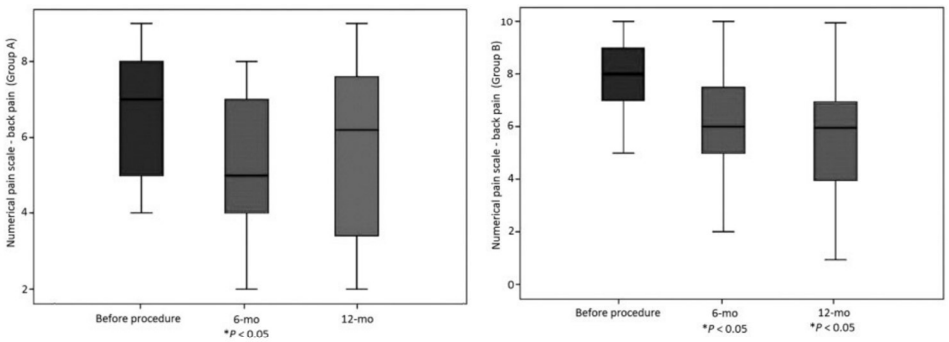


Figure 3 Evaluation of back pain intensity in patients indicating significant reduction in low back pain in both groups after six months, with persisting significant improvement after 12 months in group B.

that subjects had greater clinical improvement. After 12 months, the ODI score was the same as before the procedure, which showed a return to the previous state (Figure 2). A reduction in low back pain was recorded after six months in both groups ($P > 0.05$), but this did not persist until 12 months in Group A ($P > 0.05$) (Figure 3). A similar reduction in pain after six months was found on examination of leg pain ($P < 0.05$), but the level of pain in the legs had reverted to baseline after 12 months in both groups ($P > 0.05$) (Figure 4).

The changes between both groups are summarized in Table 2. No difference was found between Group A and Group B in ODI scoring values nor in the numerical pain scale for leg pain and back pain before the procedure (T0) or at six months (T1) and 12 months (T2) after procedure ($P > 0.05$) in all observed parameters. There was no difference in PSS between Group A and Group B. We found significant worsening of PSCS in Group A at $P < 0.05$, but not in Group B.

Discussion

Everything we do regarding success or failure in pain medicine is ultimately expressed at the cellular level and represents changes in electrical patterns, neurotransmitters, and metabolism. Epidural fibrosis has been described as a common phenomenon with a place among the major causes of continued pain after surgical intervention [7]. As Baber and Erdek [8] pointed out, scar formation is part of the healing process after spinal surgery, like any other surgical procedure, resulting in fibrosis within the epidural space. Epidural fibrosis can be

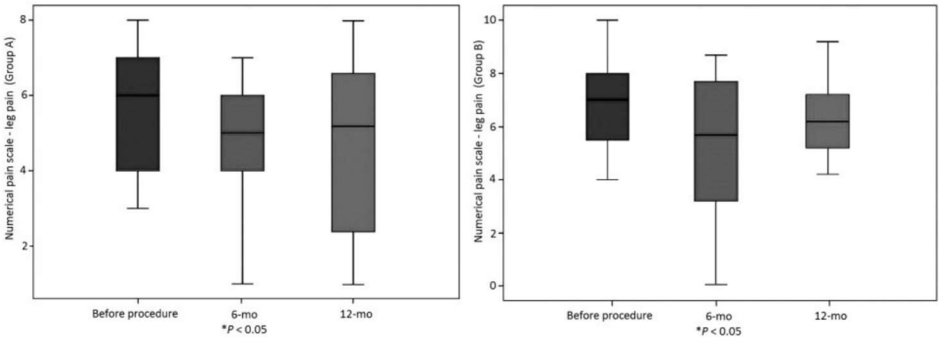


Figure 4 Evaluation of leg pain intensity in patients indicating significant reduction in leg pain in both groups after six months.

Table 2 Comparison of the mean values of recorded parameters between groups in the 6 and 12 months following the procedure

Parameters	Time Interval	Group A (Min–Max) Med	SD	Group B (Min–Max) Med	SD	Mean Difference	95% CI	*P* Value
Oswestry Disability Index	Before procedure	(31–80) 65	15.57	(20–76) 58	18.46	−5.192	−15.485 to 5.101	0.313
	6-mo follow-up	(12–76) 47	18.63	(8–76) 38	21.77	−8.921	−21.090 to 3.248	0.148
	12-mo follow-up	(18–82) 54	17.78	(12–74) 48	19.14	−8.591	−19.833 to 2.651	0.131
Numerical pain scale– back pain	Before procedure	(4–9) 7	1.64	(0–10) 8	2.19	1.022	−0.150 to 2.194	0.086
	6-mo follow-up	(2–8) 5	1.97	(0–10) 6	2.85	0.561	−0.921 to 2.043	0.449
	12-mo follow-up	(4–9) 7	1.63	(1–10) 6	2.33	−0.727	−1.951 to 0.496	0.237
Numerical pain scale–leg pain	Before procedure	(3–8) 6	1.72	(4–10) 7	1.54	0.868	−0.071 to 1.806	0.069
	6-mo follow-up	(1–7) 5	1.56	(0–9) 6	2.78	0.887	−0.476 to 2.251	0.193
	12-mo follow-up	(3–8) 6	1.54	(1–9) 6	2.11	−0.045	−1.254 to 1.163	0.940
Patient status score	Before procedure	(1–4) 3	0.728	(1–4) 3	0.700	0.197	−0.239 to 0.632	0.367
	6-mo follow-up	(0–4) 2	0.976	(0–3) 2	0.839	−0.47	−0.604 to 0.632	0.864
	12-mo follow-up	(1–4) 2	0.728	(1–4) 3	1.071	−0.054	−0.620 to 0.512	0.848
Patient self-content Score	6-mo follow-up	(0–10) 7	3.171	(0–10) 7	2.864	0.329	−1.583 to 2.240	0.73
	12-mo follow-up	(0–10) 5	3.092	(0–10) 6	4.025	1.560	−0.722 to 3.841	0.171

CI = confidence interval.

caused by chronic inflammatory changes also from chronic spinal cord injury. The pathophysiological background of epidural fibrosis is the inflammatory reaction of the arachnoid mater [9]. The pain is a characteristic manifestation of inflammation there [10]; however, fibrotic adhesions themselves cause back and leg pain by compressing nerve roots, decreasing the range of motion in the back and introducing pain with movement [11]. Despite multiple studies, the relationship between fibrosis and pain is still not entirely resolved [12]. In earlier studies, active signs of root inflammation were seen in only six patients from the 20 studied [13], or none [7]. Therefore, we did not focus on following the signs or markers of inflammation in fibrosis during epiduroscopy.

This study concerns an initial evaluation of an initial group of patients over a one-year period after a spinal endoscopic procedure. In our study, an attempt was made to show that the targeted destruction of post-operative fibrosis and adhesions has the potential to improve the patient's clinical condition in cases of

Table 3 Comparison of patients' pain intensity, self-content score, patient status score, and functional disability inside the groups in the 6- and 12-month postoperative examination periods

Parameters	Groups	Before Procedure Mean	6-mo Follow-up Mean	Mean Difference	95% CI	*P* Value	12-mo Follow-up Mean	Mean Difference	95% CI	*P* Value
Oswestry Disability Index	A	59.41	49.18	10.227	−0.117 to 20.676	0.055	53.68	4.331	−5.857 to 14.520	0.400
	B	54.22	40.26	13.957	1.960 to 25.953	0.024*	45.09	9.126	−2.191 to 20.444	0.111
Numerical pain scale–back pain	A	6.50	5.09	1.409	0.078 to 2.285	0.037*	6.45	−0.182	−1.175 to 0.811	0.714
	B	7.52	5.65	1.870	0.357 to 3.382	0.017*	5.73	1.794	0.431 to 3.158	0.011*
Numerical pain scale–leg pain	A	6.05	4.59	1.318	0.320 to 2.317	0.011*	5.77	0.136	−0.856 to 1.129	0.783
	B	6.91	5.48	1.435	0.101 to 2.768	0.036*	5.73	1.095	−0.22 to 2.211	0.061
Patient status score	A	2.57	1.96	−0.609	−1.120 to −0.097	0.021*	2.43	−0.130	−0.563 to 0.302	0.546
	B	2.76	2.32	−0.853	−1.340 to −0.366	0.067	2.38	−0.111	−0.463 to 0.102	0.181
Patient satisfaction score	A		6.57				4.19	2.381	0.427 to 4.334	0.018*
	B		6.90				5.75	1.150	−1.086 to 3.386	0.305

CI = confidence interval.
*statistical significance at: $P < 0.05$

FBSS diagnosis. Clinical effectiveness of spinal endoscopy with adhesiolysis from prospective trials [13,14], retrospective trials [15–18], and case reports showed evidence for moderate short-term pain relief and limited evidence for long-term pain relief. Moreover, Manchikanti et al. [7] have shown that the targeted injection of local anaesthetic and steroid can be significantly effective for patients as short-term pain relief. The study by Kim et al. [19] has aided in the increase of knowledge in this area, the conclusions of which show the long-term benefits of the application of hyaluronidase along with a steroid. This study was therefore aimed to compare the effectiveness of the mechanical removal of fibrosis with mechanical intervention and targeted administration of corticosteroids and hyaluronidase in pain relief.

Our results show improvement in pain relief in group B at 12 months, with group A demonstrating an improvement only up to the six-month follow-up. Because of the interval of improvement and a consecutive return to the original state, we assume that either new fibrotic changes might develop or a repeated attempt is required to achieve better clinical improvement. In some patients, there was a significant improvement with a long-term effect, and this improvement lasted even after the 12-month follow-up. At this stage, we can hypothetically suggest an improved selection of patients suitable for an epiduroscopy procedure with a higher chance of significant clinical improvement. However, based on empirical relations between the clinical outcome of epiduroscopies and the number of open spinal surgical interventions, it seems logical that a higher number of open surgical interventions in the spinal canal will lead to increased formation of fibrosis and worsening of the prognosis for an epiduroscopy procedure. Mild-to-moderate fibrosis, in conjunction with local pain reproduction, was an indicator of a more favorable outcome than severe fibrosis [20]. The amount of nerve root damage, where the pain is distributed in the corresponding dermatomes, should also be considered an important parameter. Severe damage to the nerve roots gives only a small chance of a good clinical prognosis in an epiduroscopy procedure, and it might be more effective to implant a spinal cord stimulator. This theory has not yet been completely proven in an adequate amount of studies. In conclusion, information obtained through lumbosacral epiduroscopy has significant diagnostic and prognostic value and may be helpful in the management of patients with low back pain and/or leg pain in general [21]. Even more detailed knowledge of the anatomy, histology, and pathology of both the intact and pathological epidural space will improve our ability to understand the pathophysiology of back and leg pain. The question is whether this potential should also be implemented in routine diagnostics and treatment of all patients with back pain, as it is in the diagnosis and treatment of joint diseases and diseases of the abdominal cavity.

Conclusion

Forty-four patients with six- and 12-month follow-up intervals were evaluated in the initial phase of our study. It concerned patients after a minimum of one back surgery without a satisfying clinical result regarding pain relief in the back and the lower limbs. In the first group of patients, the main goal was to remove, under endoscopic guidance, the postoperative fibrosis or adhesions in the vicinity of nerve roots, where pain is distributed in the corresponding dermatomes. A radiofrequency probe, laser, or balloon was used to work in the anterior epidural space. Subsequently, the posterior epidural space was always examined endoscopically, and in the case of visible adhesions fixated on the dural sac, an attempt was made to remove them. The same procedure was performed in the second group of patients, with the additional administration of hyaluronidase and a mix of Depo-Medrol with a local anaesthetic after mechanical intervention. An improvement was observed at six-month follow-up in both groups, regarding back and lower limb pain. At 12-month follow-up, the effect of pain relief in the lower limbs was insignificant in both groups. Pain relief in the low back was only seen after 12 months in the group of participants who had received medication. ODI was significantly improved only in group B at six-month follow-up. A limitation of the study was the relatively small sample size. The continuation will be the enrollment of a higher number of patients to collect and evaluate more data. Based on results of our study, epiduroscopy has great potential to become an effective and well-accepted diagnostic and therapeutic tool in the treatment of FBSS symptoms.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1 Di Donato A, Fontana C, Alemanno D, Di Giacomo A. Epiduroscopy in treatment of degenerative chronic low back pain: A prospective analysis and follow-up at 60 months. *Clin Res Regul Aff* 2010;27(3):69–74.

2 Sayhan H, Beyaz SG. Pain management. In: Prostran M, ed. *Epiduroscopy (Epidural Endoscopy)*. Croatia: InTech; 2016:115–41.

3 Kallewaard JW, Vanelderen P, Richardson J, et al. Epiduroscopy for patients with lumbosacral radicular pain. *Pain Pract* 2014;14(4):365–77.

4 Helm S, Racz GB, Gerdesmeyer L, et al. Percutaneous and endoscopic adhesiolysis in managing low back and lower extremity pain: A systematic review and meta-analysis. *Pain Physician* 2016;19(2):E245–81.

5 Bellini M, Barbieri M. A comparison of non-endoscopic and endoscopic adhesiolysis of epidural fibrosis. *Anaesthesiol Intensive Ther* 2016;48(4): 266–71.

6 Fronza M, Muhr C, da Silveira DS, et al. Hyaluronidase decreases neutrophils infiltration to the inflammatory site. *Inflamm Res* 2016;65(7): 533–42.

7 Manchikanti L, Rivera JJ, Pampati V, et al. Spinal endoscopic adhesiolysis in the management of chronic low back pain: A preliminary report of a randomized, double-blind trial. *Pain Physician* 2003;6:259–67.

8 Baber Z, Erdek MA. Failed back surgery syndrome: Current perspectives. *J Pain Res* 2016;9:979–87.

9 Han X, Tian X, Baklaushev VP, et al. Epidural fibrosis post laminectomy is the unappreciated cause of spinal cord compression post chronic spinal cord injury. *Biomed Res* 2017;28: 4756–61.

10 Masopust V, Häckel M, Netuka D, et al. Postoperative epidural fibrosis. *Clin J Pain* 2009;25(7):600–6.

11 Chan CW, Peng P. Failed back surgery syndrome. *Pain Med* 2011;12(4):577–606.

12 Lee F, Jamison DE, Hurley RW, Cohen SP. Epidural lysis of adhesions. *Korean J Pain* 2014;27(1):3–15.

13 Geurts JW, Kallewaard JW, Richardson J, Groen GJ. Targeted methylprednisolone acetate/hyaluronidase/clonidine injection after diagnostic epiduroscopy for chronic sciatica: A prospective, 1-year follow-up study. *Reg Anesth Pain Med* 2002;27:343–52.

14 Richardson J, McGurgan P, Cheema S, Prasad R, Gupta S. Spinal endoscopy in chronic low back pain with radiculopathy. A prospective case series. *Anaesthesia* 2001;56(5):454–60.

15 Manchikanti L, Pampati V, Bakhit CE, Pakanati RR. Non-endoscopic and endoscopic adhesiolysis in post-lumbar laminectomy syndrome: A one-year outcome study and cost effectiveness analysis. *Pain Physician* 1999;2:52–8.

16 Manchikanti L, Pakanati RR, Pampati V. The value and safety of epidural endoscopic adhesiolysis. *Am J Anesthesiol* 2000;27:275–9.

17 Saberski LR. A retrospective analysis of spinal canal endoscopy and laminectomy outcomes data. *Pain Physician* 2000;3(2):193–6.

18 Krasuski P, Poniecka AW, Gal E, et al. Epiduroscopy: Review of technique and results. *Pain Clinic* 2001;13(1):71–6.

19 Kim SB, Lee KW, Lee JH, Kim MA, An BW. The effect of hyaluronidase in interlaminar lumbar epidural injection for failed back surgery syndrome. *Ann Rehabil Med* 2012;36(4):466–73.

20 Bosscher HA, Heavner JE. Lumbosacral epiduroscopy findings predict treatment outcome. *Pain Pract* 2014;14(6):506–14.

21 Bosscher HA, Heavner JE. Diagnosis of the vertebral level from which low back or leg pain originates. A comparison of clinical evaluation, MRI and epiduroscopy. *Pain Pract* 2012;12(7): 506–12.

Wien Klin Wochenschr
https://doi.org/10.1007/s00508-019-01590-z

Wiener klinische Wochenschrift
The Central European Journal of Medicine

EQ-5D-5L questionnaire as suitable assessment of quality of life after epiduroscopy

Multicenter randomized double-blind pilot study

Róbert Rapčan · Ladislav Kočan · Viktor Witkovsky · Juraj Mláka · Martin Griger · Miroslav Burianek · Simona Rapčanová · Anthony Hammond · Ľubomír Poliak · Róbert Tirpák · Jana Šimonová · František Sabol · Janka Vašková

Received: 26 July 2019 / Accepted: 3 December 2019

Summary
Background Epiduroscopy is a well-established diagnostic and to certain level therapeutic tool in complex situations, where conventional methods such as magnetic resonance imaging (MRI) lack power or resolution to detect pathological changes. Such a situation is primarily failed back surgery syndrome (FBSS) but also radicular pain without surgery. The aim of this study was to determine the effectiveness of epiduroscopic treatment in patients with FBSS.
Methods A total of 79 patients with FBSS were randomized into 2 groups. The first group underwent epiduroscopy and received mechanical lysis of adhesions only, the second group received also medication into the epidural space (methylprednisolone and hyaluronidase). Patients were subsequently followed for 12 months, with evaluation also after 6 months post-epiduroscopy. Patients were checked in terms of mobility, self-care, usual activities, pain/discomfort and anxiety/depression as defined in the 5-dimensional EQ-5D-5L questionnaire and to asses suitability of this questionnaire in chronic pain states. Data were collected using EQ-5D-5L questionnaire and also quality of life (QoL) questionnaire.
Results In the terms of ability to walk (dimension mobility) and also ability to do housework, study or leisure activities (dimension usual activity) patients improved in both groups after 6 and 12 months after epiduroscopy. In pain dimension there was improvement mainly after 6 months which correlated also with self-care dimension and quality of life self-assessment. Results in anxiety/depression dimension were mixed.
Conclusion Epiduroscopy appears to be a beneficial procedure for both patient groups, especially after 6 months, with some benefit remaining after

R. Rapčan · M. Griger · Ľ. Poliak
Europainclinics, Kominárska 21/5, 831 04 Bratislava, Slovakia

L. Kočan
Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovakia

V. Witkovsky
Institute of Measurement Science, Slovak Academy of Sciences, Dúbravská cesta 9, 841 04 Bratislava, Slovakia

J. Mláka
Poliklinika Terasa, Europainclinics, Toryská 1, 040 11 Košice, Slovakia

R. Tirpák · F. Sabol
Department of Cardiac Surgery, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Košice, Slovakia

R. Rapčan · M. Burianek · S. Rapčanová
Europainclinics, Starochodovská 1750, 149 00 Prague 4, Czech Republic

A. Hammond
Kent Institute of Medicine and Surgery, Newnham Court Way, Bearsted, ME14 5FT, Kent, UK

J. Šimonová
1st Clinic of Anaesthesiology and Intensive Care Medicine, Louis Pasteur University Hospital, SNP 1, 040 11 Košice, Slovakia

assoc. prof. Dr. J. Vašková, Ph.D. (✉)
Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University, Trieda SNP 1, 040 66 Košice, Slovakia
janka.vaskova@upjs.sk

R. Rapčan
Europainclinics, Štefánikova 1599/82, 085 01 Bardejov, Slovakia

Published online: 07 January 2020

12 months. The EQ-5D-5L questionnaire seems to be a suitable and comprehensive way to assess patient health in chronic pain states.

Keywords Back pain · Epiduroscopy · Failed back surgery syndrome · Hyaluronidase · EQ-5D-5L questionnaire

Introduction

Failed back surgery syndrome (FBSS) is one of the difficult to treat conditions that occur after spinal surgery and it is also believed to be one of frequent causes of neuropathic pain. Patients with FBSS often suffer from epidural, intraneural, or perineural fibrosis and scar tissue [1]. The potential pain source can be any anatomical structure that contains nociceptive fibres or their combination. Treatment of FBSS is very complex and often unsuccessful. Patients are suffering and their life is limited by a strong continual pain, disturbance of physical health, psychological state, level of independence, and social relationships. Quality of life for patients living with chronic disease is a priority. One of the ways how to measure and specify quality of life is to use the EQ-5D-5L questionnaire.

Currently, epiduroscopy is well established as a diagnostic tool for different back pain syndromes which cannot be explained by other diagnostic methods, such as magnetic resonance (MR) imaging [2, 3, 18]. It can also be used as a treatment modality in certain cases of radicular pain or failed back surgery syndrome [4, 5]. There are several mechanisms by which epiduroscopy can be a therapeutic, not only diagnostic, tool. For example, because epidural fibrosis or scar formation around nerves can be one of the possible causes of low back pain and/or radicular pain in FBSS [2] mechanical lysis of these adhesions reduces nerve oppression, improve nutrient supply, and thus contribute to the decrease of patient's pain. Epiduroscopy also allows for a more targeted delivery of drugs as compared to conventional routes [6, 7]. Moreover, infusion of normal saline used to open the epidural space may have a dilutional effect on inflammatory mediators released from disc or facet joints [3].

The aim of this study was to determine the effectiveness of epiduroscopic treatment in patients with failed back surgery syndrome in terms of mobility, self-care, usual activities, pain/discomfort and anxiety/depression as defined in 5-dimensional EQ-5D-5L questionnaire. As an extension of another ongoing study [8] the differences of quality of life between study groups were also analyzed.

Patients, material and methods

This study was a randomized, multicenter double blinded clinical trial with two parallel groups. Ethical approval for this study was provided by the University Hospital ethics committee in Košice (approval number: 75/EK/15). Registration was accomplished at clinicaltrials.gov with registration ID NCT PRS: NCT02459392. Permission for using the questionnaire for a study issue was given in February 2017 by EuroQol group. Participants also completed a quality of life (QoL) questionnaire. Overall health status was evaluated by the visual analogue scale (EQ-VAS) measured on the numerical scale 0–100 (100 means the best health and 0 means the worst health).

The study participants with chronic pain caused by a previous spine surgery were recruited from pain clinics located in Košice, Bardejov and Bratislava in the Slovak Republic. Epiduroscopy was performed by EuroPainClinics® health company in Košice. Coordinator of the study was recruited from the medical faculty of Pavol Jozef Šafárik in Košice and data were collected in the encrypted cloud database. All medical visits were coordinated by independent coordinators from the East Slovak Institute for Cardiovascular Diseases. The inclusion criteria for patient enrolment were: patients with FBSS, up-to-date magnetic resonance imaging, lesions without serious spinal stenosis, lesions without serious radicular compression, lesions without serious intervertebral disc herniation, age of 18 years or older and signed written informed consent. Exclusion criteria were as follows: patients not capable of consenting, pregnant women or women of child-bearing age, cauda equine syndrome. After meeting the inclusion criteria, the patients were allocated according to computer randomization software into two groups, group A and group B. Each patient obtained a unique clinical trial ID number, which was generated by a computer software before epiduroscopy. The protocol and quality of life questionnaires were filled out by each involved participant before the operation. Blinding at the time of randomization was maintained with a sealed envelope given to the anesthesiologist managing the patient. Group A underwent epiduroscopy where only mechanical lysis of the epidural fibrotic attachments was performed by either laser, radiofrequency or balloon technique with 20 mL saline at the end of the procedure. Group B underwent epiduroscopy with the same types of mechanical lysis as in the previous group, but additionally, a solution of hyaluronidase (RIEMSER Pharma GmbH, Greifswald-Insel Riems, Germany, 150 I.U. in 10 mL of saline) and injectable corticosteroid methylprednisolone acetate 80 mg were administered to the epidural space precisely into the place of conflict (the depression in the spinal root by fibrosis). The coordinator of the study planned the first postoperative examination 6 months after the procedure and the second postoperative examination after 12 months following the procedure. The first and second postoperative examinations of the patient were performed by a different physician (not the one performing the actual procedure), or at a different pain management clinic. They performed a pain

Table 1 Characteristics of patients divided into groups according simple randomization

	Group A (min–max) med	Group B (min–max) med
Participants (n)		
Before procedure	40	39
6-month follow-up	40	38
12-month follow-up	40	38
Age (years)	(35–70) 54	(33–69) 46.5
Sex (F/M)	22/18	19/20
ASA	(1–3) 2	(1–3) 2
BMI	22	20
Pain in dermatomes according to examination before procedure		
L2	0	2
L3–L4	1	2
L4–L5	15	11
L5	7	8
L5–S1	9	7
S1	8	8
Mechanical therapeutic intervention		
Balloon	3	4
Laser	4	5
Radiofrequency	15	16
Complications		
Bladder paralysis	1	9
Neurological deficiency in dermatomes	1	0
Dura mater puncture	5	3
Infection, bleeding	0	0

(min-max) med (minimum-maximum) median, *ASA* American Society of Anesthesiologists physical status classification system, *BMI* body mass index

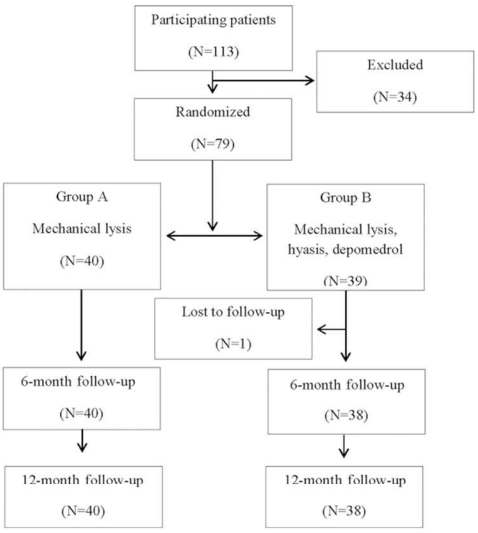


Fig. 1 Scheme of patient selection, enrolment, and follow-up in the study

assessment of the patient while blinded to which procedure they had undergone (endoscopy only including mechanical lysis or with the administration of the drugs) and completed the pain management protocol of the study. The study followed the details of patient selection as well as instrumental equipment, execution of the procedure according to Rapčan et al. [8].

Participants who voluntarily signed the informed consent and were eligible for this study were randomly assigned to one of two groups in January 2016 before their operation. Computer-generated random numbers were evoluted as a single sequence of random assignments based on simple randomization (Table 1). Patients in group A underwent only mechanical lysis of adhesions and fibrosis in epidural space. Patients in group B underwent mechanical lysis joined with drugs administration into the epidural space: corticoid and enzyme hyaluronidase, as stated in Rapčan et al. [8]. The interventional pain management specialist was informed about the next advance after the beginning of the operation which was based on previous randomization. Unique ID was given to each participant. The first follow-up examination took place at the pain clinic after six months and the second follow-up after twelve months (Fig. 1). Physicians and patients were blinded in both cases. Patients at each of the three meetings with the physician completed EQ-5D-5L questionnaire. Each dimension of the questionnaire (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) is scored on a scale of 5 levels (I, II, III, IV, and V), with level I indicating the least severe (optimal health) state and level V indicating most severe (critical health) state.

Nonparametric statistical methods were used for statistical processing of the EQ-5D-5L questionnaire data. In particular, the analyses are based on the Wilcoxon rank-sum test and the nonparametric estimator of the reliability parameter R. Wilcoxon rank-sum test is a nonparametric test of the null hypothesis that it is equally likely that a randomly selected value (the questionnaire response) from one group will be less than or greater than a randomly selected value from a second group. The reliability parameter R is a parameter well-known in stress-strength modelling and is frequently used also in medicine for treatment comparisons. R is defined as a probability $Prob(X < Y)$, with values from the interval (0, 1), where X and Y are random variables associated with the considered probability distributions. Under null hypothesis $Prob(X < Y) = Prob(X > Y)$ the estimated confidence interval covers the expected value 0.5 with stated probability. Otherwise, if the estimated confidence interval does not cover the value 0.5, it was concluded that the alternative is to be preferred. In a nonparametric set-up, the estimator of the reliability

Table 2 Patients' answers distribution in percentage

Level/time (months)		Group A					Group B				
		I	II	III	IV	V	I	II	III	IV	V
Mobility	0	0.0	47.5	42.5	10.0	0.0	0.0	43.60	51.30	5.10	0.0
	6	5.0	52.5	32.5	10.0	0.0	10.50	39.50	26.30	23.70	0.0
	12	2.5	42.5	45.0	10.0	0.0	7.80	21.0	41.0	30.20	0.0
Self care	0	5.0	22.5	62.5	10.0	0.0	8.7	20.5	67.8	3.0	0.0
	6	10.0	35.0	47.5	5.0	2.5	10.5	36.8	44.7	5.4	2.6
	12	7.5	22.5	55.0	12.5	2.5	15.8	18.4	55.3	7.9	2.6
Usual activities	0	0.0	17.5	75.0	7.5	0.0	0.0	17.9	71.8	10.3	0.0
	6	5.0	35.0	47.5	12.5	0.0	7.9	34.2	50.0	7.9	0.0
	12	2.5	5.0	77.5	12.5	2.5	5.0	34.2	52.9	7.9	0.0
Pain/ discomfort	0	0.0	12.5	67.5	17.5	2.5	0.0	11.9	77.9	5.1	5.1
	6	5.0	17.5	50.0	20.0	7.5	5.2	23.7	63.2	7.9	0.0
	12	2.5	7.5	75.0	10.0	5.0	7.7	23.7	62.2	3.2	3.2
Anxiety/ depression	0	17.5	75.0	5.0	2.5	0.0	7.7	69.0	23.3	0.0	0.0
	6	15.0	77.5	7.5	0.0	0.0	8.9	87.5	3.6	0.0	0.0
	12	25.0	50.0	20.0	5.0	0.0	29.0	63.5	7.5	0.0	0.0

parameter R is functionally related to the Wilcoxon rank-sum test statistic. For more details see Kotz et al. [9] and Zhou [10]. Analyses using MATLAB (R2018b) with Statistics and Machine Learning Toolbox Version 11.4 (The MathWorks, Inc., Natick, MA, USA) were performed. A p-value less than 0.05 was considered statistically significant. For analysis of the Quality of Life (QoL) questionnaire data, Shapiro-Wilk, Levene, and paired t-test, using SPSS Version 11.0 statistic software package, were performed. P values of less than 0.05 were considered significant.

Results

The following notation was used for the specific groups of patients: A0, A6, A12, B0, B6, B12. Two basic groups of patients (A patients with mechanical lysis only and B patients with mechanical lysis joined with drug administration) were considered. Each group of patients was observed at three different time periods (0 at the beginning of the study, 6 after 6 months from the beginning of the study, and 12 after 12 months from the beginning of the study). The responses of the patients of the two groups to the 5-dimensional EQ-5D-5L questionnaire at all three times are summarized in Table 2.

The effectiveness of epiduroscopic treatment in patients with failed back syndrome was assessed by testing statistical hypotheses on equality of probability distributions of the questionnaire responses in the considered (sub) groups of patients against the prespecified one-sided alternative hypotheses. The hypotheses assume that the distribution of response levels is stochastically smaller, with better health status for one group that the other, in terms of mobility, self-care, usual activities, pain/discomfort and anxiety/depression as defined in 5 dimensional EQ-5D-5L questionnaire. Moreover, as a summary compound measure, we have also considered the comparisons of the combined (weighted) questionnaire values, with carefully prespecified weighing for each questionnaire dimension, which combines the information from all 5 dimensions of the EQ-5D-5L questionnaire. For each subject the combined value Q was calculated by the following formula: $Q = 1 - w_0 - w_1 - w_2 - w_3 - w_4 - w_5$, with the weights specified in the Table 3. For more details on the different weighing strategies see e.g. Szende et al. [11].

For specific sub-groups of patients, denoted by A0, A6, A12, B0, B6, and B12, we tested the hypotheses on pairwise comparisons. For comparison of any two groups of patients based on the observed questionnaire responses we computed the p-value from the Wilcoxon rank-sum test for testing the null hypothesis of equality of distributions of the questionnaire responses against the (specified) one-sided alternative hypothesis. Moreover, we also estimated the reliability parameter R together with the associated two-sided 95% confidence interval, calculated from the bootstrap distribution of the responses in group 1 and in group 2 (in this study we have used the bootstrap samples of size $N = 1000$). Table 4 presents the observed p-values of the Wilcoxon rank-sum test and the estimated reliability parameter R with the 95% confidence interval for each considered hypothesis on equality of probability distributions of the questionnaire responses in the considered groups of patients. For example, the notation B6 < A6 indicates testing the null hypothesis about equality of the distributions for groups B6 and A6, i.e. H0: B6 ~ A6, against the alternative hypothesis HA: B6 < A6 (i.e. better health status in the considered dimension for the patients in group B6 than in the group A6).

As is shown in Table 4, there was improvement in mobility after 6 months with statistical significance at $p = 0.0637$ and after 12 months ($p < 0.05$) in

Table 3 The weights used for computing the weighted questionnaire value Q, which combines the information from all 5 dimensions of the EQ-5D-5L questionnaire

Weight	Value	EQ-5D-5L dimension	Condition
w_0	0.1279	All	Any level in questionnaire is II or III
w_0	0.2288	All	Any level in questionnaire is IV or V
w_0	0.0000	All	Otherwise
w_1	0.0659	Mobility	Mobility level is II or III
w_1	0.1829	Mobility	Mobility level is IV or V
w_1	0.0000	Mobility	Otherwise
w_2	0.1173	Self-care	Self-care level is II or III
w_2	0.1559	Self-care	Self-care level is IV or V
w_2	0.0000	Self-care	Otherwise
w_3	0.0264	Usual activities	Usual activities level is II or III
w_3	0.0860	Usual activities	Usual activities level is IV or V
w_3	0.0000	Usual activities	Otherwise
w_4	0.0930	Pain/discomfort	Pain/discomfort level is II or III
w_4	0.1639	Pain/discomfort	Pain/discomfort level is IV or V
w_4	0.0000	Pain/discomfort	Otherwise
w_5	0.0891	Anxiety/depression	Anxiety/depression level is II or III
w_5	0.1290	Anxiety/depression	Anxiety/depression level is IV or V
w_5	0.0000	Anxiety/depression	Otherwise
For each subject the value Q was calculated by the formula $Q = 1 - w_0 - w_1 - w_2 - w_3 - w_4 - w_5$, with the specified weights			

group A. In group B, we recorded a statistically significant improvement in mobility after 6 and 12 months ($p < 0.05$). There was a significant improvement in the performance of normal daily activities in patients, in all groups A and B after 6 and 12 months ($p < 0.05$). Following the pain parameter, group B included patients with significantly smaller pain A0 vs. B0 ($p < 0.05$). Subsequently, the reduction in pain was recorded only in A6 and A12 groups ($p < 0.05$) during further measurements. In groups B6 and B12 this trend was not recorded; however, the differences in the pain parameter between A and B after 6 and 12 months were not recorded. Improvement in self-care was observed only in group A after 6 months ($p < 0.05$). After 12 months no improvement in self-care was observed in either group compared to pre-operative baseline. An improvement in the sense of anxiety and depression in a given disease varied in both groups. Significant improvements were seen in group A and B after 6 and 12 months ($p < 0.05$). The statistical weighting of values, based on index demographic conversion, showed improvement in group B after 6 and 12 months and group A after 12 months ($p < 0.05$).

Normal distribution of EQ-VAS on the numerical scale (0–100) was evaluated with the Shapiro–Wilk test. Homogeneity of variances was estimated using the Levene test. When conditions of homogeneity and normality of variances were met between both groups, for comparing the means we used paired t-test (Table 5, Fig. 2). The comparison shows that significant changes were found in both groups at the 6-month period measurement following the procedure.

Interpretation of previous medications in all included patients was extremely difficult and not suitable for statistical evaluation due to the irregular drugs intake, different dosing in all drugs, and high variability of dosing per day even in a single patient. Some of the patients, despite strong pain, did not use any opioids because of opioid intolerance occurrence, which was noticed in around 60% (Fig. 3 and 4).

Discussion

Chronic pain especially related to FBSS is very difficult to assess properly, as it affects the life of individuals on various levels. Typically used is the measure of pain in the form of visual analogue pain score (VAS), Oswestry disability index and patient's status score as appropriate parameters of dynamic changes in the patient health; however, it could have some limitations. Visual analogue pain score seems to be prone to bias and therefore not very suitable for solitary assessment of these conditions [12]. Neuropathic pain is a complex problem relating to pain centralization in the central nervous system, changes in pain perception, long-term suffering and subsequent behavioral changes [13]. Use of different outcome domains or combination of VAS and another multilevel tool is recommended [14] to assess this condition. Another suitable complementary description method of global patient health evolution after epiduroscopic intervention is EQ-5D questionnaire. It is not specific to one disease, available for self-completion, easy to complete and covers all important aspects of chronic pain states. The longer 5-level version was used, as it pro-

Table 4 Mobility (left) vs. self-care (right) *p*-values, usual activities (left) vs. pain/discomfort (right) *p*-values, anxiety/depression (left) vs. weighted questionnaire (right) *p*-values of the Wilcoxon ranksum test for testing the null hypothesis of equality of distributions of the questionnaire responses against the specified one-sided alternative, and the estimated reliability

Hypothesis	*p*-value	R	Lower	Upper	Hypothesis	*p*-value	R	Lower	Upper
Mobility					*Self-care*				
A6 < A0	0.0637	0.5954	0.4691	0.7118	A6 < A0	0.0497*	0.5984	0.4878	0.7053
A12 < A0	0.0301*	0.6164	0.5053	0.7355	A12 < A0	0.2474	0.5401	0.4257	0.6638
A12 < A6	0.4052	0.5156	0.3944	0.6389	A12 < A6	0.7830	0.4522	0.3470	0.5703
B6 < B0	0.0474*	0.6049	0.4895	0.7298	B6 < B0	0.1828	0.5540	0.4318	0.6646
B12 < B0	0.0199*	0.6272	0.5145	0.7271	B12 < B0	0.5325	0.4956	0.3826	0.6093
B12 < B6	0.4052	0.5156	0.3885	0.6371	B12 < B6	0.7830	0.4522	0.3359	0.5741
B0 < A0	0.5653	0.4904	0.3811	0.6093	B0 < A0	0.1947	0.5481	0.4349	0.6545
B6 < A6	0.5022	0.5000	0.3719	0.6233	B6 < A6	0.5022	0.5000	0.3726	0.6222
B12 < A12	0.5022	0.5000	0.3688	0.6240	B12 < A12	0.5023	0.5000	0.3830	0.6212
Usual activities					*Pain/Discomfort*				
A6 < A0	0.0129*	0.6273	0.5141	0.7283	A6 < A0	0.0019*	0.6694	0.5589	0.7674
A12 < A0	0.0228*	0.6132	0.5092	0.7220	A12 < A0	0.0033*	0.6546	0.5457	0.7546
A12 < A6	0.6104	0.4834	0.3705	0.6028	A12 < A6	0.6584	0.4778	0.3743	0.5859
B6 < B0	0.0139*	0.6282	0.5277	0.7291	B6 < B0	0.0520	0.5870	0.4818	0.6835
B12 < B0	0.0236*	0.6147	0.5155	0.7257	B12 < B0	0.0856	0.5698	0.4706	0.6680
B12 < B6	0.6104	0.4834	0.3580	0.6011	B12 < B6	0.6584	0.4778	0.3705	0.5838
B0 < A0	0.5561	0.4929	0.3942	0.5939	B0 < A0	0.0378*	0.5981	0.4910	0.6990
B6 < A6	0.5023	0.5000	0.3802	0.6184	B6 < A6	0.5024	0.5000	0.3850	0.6056
B12 < A12	0.5023	0.5000	0.3802	0.6163	B12 < A12	0.5026	0.5000	0.3920	0.5994
Anxiety/Depression					*Weighted questionnaire*				
A6 < A0	0.7800	0.4655	0.3786	0.5493	A6 < A0	0.1597	0.5625	0.4484	0.6878
A12 < A0	0.1647	0.5523	0.4378	0.6595	A12 < A0	0.0368*	0.6112	0.4869	0.7297
A12 < A6	0.0417*	0.5876	0.4896	0.6863	A12 < A6	0.1726	0.5581	0.4322	0.6731
B6 < B0	0.0423*	0.5830	0.4882	0.6687	B6 < B0	0.4459	0.5081	0.4001	0.6201
B12 < B0	0.0031*	0.6518	0.5439	0.7395	B12 < B0	0.0102*	0.6407	0.5331	0.7470
B12 < B6	0.0417*	0.5876	0.4875	0.6863	B12 < B6	0.0159*	0.6316	0.5114	0.7521
B0 < A0	0.9812	0.3933	0.2984	0.4936	B0 < A0	0.1827	0.5554	0.4436	0.6910
B6 < A6	0.5035	0.5000	0.4259	0.5755	B6 < A6	0.4545	0.5072	0.3816	0.6230
B12 < A12	0.5024	0.5000	0.3878	0.6153	B12 < A12	0.1167	0.5740	0.4516	0.6980

*Statistically significant at $p < 0.05$

Table 5 Comparison of differences in patients' status within and between groups according to QoL questionnaire

Parameters	Time interval	A			B				
Comparison between the groups	–	*Mean*	*SD*	–	*Mean*	*SD*	–	*CI 95%*	*p*
	Before procedure	43.67	13.94	–	43.56	14.657	–	–2.931–3.136	0.946
	6-month follow-up	50.97	17.977	–	50.37	16.643	–	–7.572–8.783	0.882
	12-month follow-up	47.32	16.639	–	42.66	18.745	–	–1.982–11.298	0.164
Comparison inside the groups	–	*SD*	*CI 95%*	*p*	*SD*	*CI 95%*	*p*	–	–
	Before procedure vs. 6-month follow-up	18.891	–13.79 to –1.71	0.013*	15.435	–11.495 to –1.348	0.015*	–	–
	Before procedure vs. 12-month follow-up	17.763	–8.33 to 3.03	0.351	13.924	–0.761 to 8.393	0.100	–	–

*Statisitically significant at $p < 0.05$
SD standard deviation, *CI* confidence interval

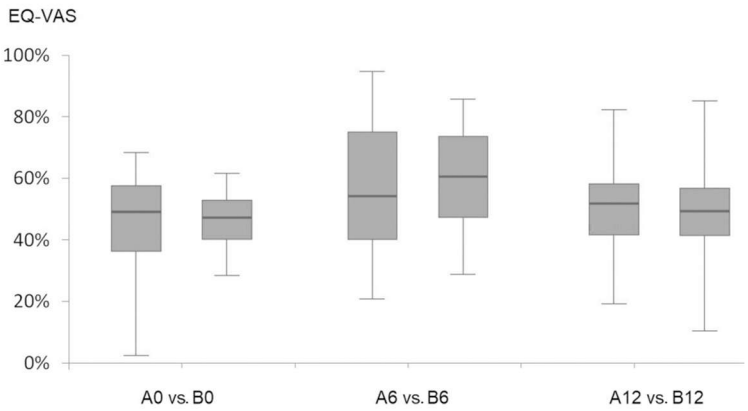


Fig. 2 Evaluation of patients' Quality of life between both groups before treatment, 6 and 12 months after treatment

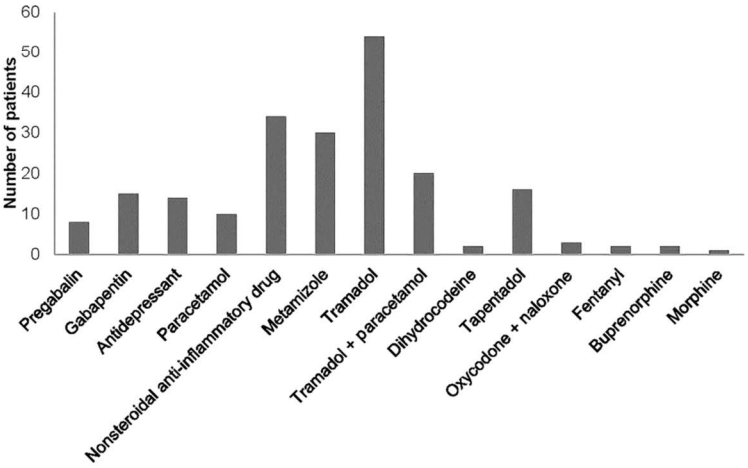


Fig. 3 Analysis of analgesic medication treatment in all included patients before epiduroscopy

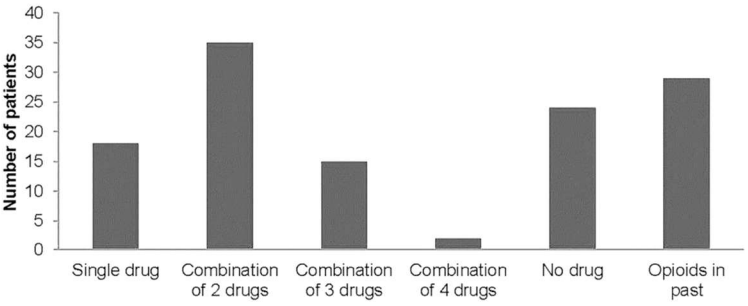


Fig. 4 Strategy of previous analgesic medication treatment in all included patients before epiduroscopy

vides better sensitivity in detecting subtle changes as compared with its original 3-level version [15].

Aim of this multicenter pilot double blinded trial was to investigate the health state in the five dimensions of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression of subjects with FBSS after epiduroscopy. In this paper, we have suggested weigths derived from EQ-5D-3L European weights constructed using data from 6 countries: Finland, Germany, The Netherlands, Spain, Sweden, and the UK. Although not all of the studies included were representative of the country in which they were carried out and the data from other European countries were not available, there are sufficient data from different European regions to make European dataset moderately representative for Europe. Statistical extrapolation from EQ-5D-3L to EQ-5D-5L was determined as a next step. This may lead to slightly different average quality of life values in individual Central European countries. Previous partial results from our investigation showed improvement in Oswestry disability index and numerating rating scale (NRS) for leg and back pain after epiduroscopy in group A (mechanical lysis) and group B (mechanical lysis and administrated drugs, Hyasis and Depomedrol) after 6 months. Improvement was only noted on the NRS for back pain at the 1-year follow-up ($p < 0.05$). These partial results were processed on the relatively small size of patients: 22 in A group and 22 in B group. [8].

We present an enlarged examination of 79 patients randomly divided into the 2 groups focused on health-related quality of life measured by EQ-5D-5L questionnaire and quality of life by the NRS. In the mobility dimension focused mainly on the persons' walking ability, we recorded improvement in all groups 6 and 12 months after epiduroscopy. These findings demonstrate the positive effect of the procedure, which could be caused not only by releasing the fibrotic compression in the epidural space, but also with the possible suppression of the cytokine cascade by saline flush. Spinal stenosis and fibrotic processes in the epidural space have in general a negative major impact on the patient quality of life. Moreover, the cytokine reduction may have a short-term effect by reducing cytokine pain mediators, such as prostaglandin E2, but it could also slow down the fibrotic formation by reducing the focal aseptic inflammatory reaction as a long-term effect.

Usual activities measures performance in work, study, housework, family or leisure activities and they are certainly closely connected to the mobility, which brings the same improvement in the observed result for all groups after 6 and 12 months. By comparing the entry data (in the time period 0) for the dimension of pain/discomfort between group A and group B, a statistically significant difference was found. We did not find statistically significant differences by comparing the entry data (in the time period 0) of other dimensions (mobility, self-care, usual activities, and anxiety/depression). This event was caused by a fact that majority of the participants in group A marked the level of their pain as number IV, participants in group B marked the level of their pain as number III. This phenomenon could be visible only by using EQ-5D-5L questionnaire. On the other hand, this situation would probably be undetected by EQ-5D-3L questionnaire form. Randomization bias was recorded only in the pain dimension. In all other parameters, randomization was done appropriately. As a result of this event, improvement of the pain parameter was noticed only in all A groups; however, the pain intensity between groups A6 and B6 and also between groups A12 and B12 was not significant, which is indicated on the same intensity of pain in groups after 6 and 12 months. In the future, this bias will probably be eradicated by enrolling larger number of participants.

Self-care dimension is related to the ability to wash or dress oneself. Results describe only improvement in group A after 6 months which can be related to pain improvement. Insignificant improvement in group B after 6 months followed pain relief in the observed group and may be related to the fact that the pain intensity B0 vs. B6 was not significantly altered by the procedure, which was transferred to the self-care dimension. This also correlates with the results of the quality of life measured by 100-point numerical rating scale ($p < 0.05$). Mixed results for anxiety and depression show how difficult and complex the problem of treating neuropathic pain is in adults, which can subsequently indicate the need to use co-analgetic drugs such as andipressant drugs (tricyclic antidepressants, selective serotonin reuptake inhibitors or serotonin-norepinephrine reuptake inhibitors) or anticonvulsants to effectively help to improve the outcome in indicated cases [16]. The results of our study, well described and summarized in weighted statistical analysis, have been considered as a positive outcome. Improvement after endoscopic treatment in patients with chronic low back pain is not so distinct in comparison with the treatment of acute low back pain, but our results follow conclusions of clinical trials focused on spinal cord stimulation treatment of failed back surgery syndrome [17]. Standard treatment in patients with failed back surgery syndrome is chosen based on the current magnetic resonance imaging examination. According to this result the need for a neurosurgical intervention is either confirmed or rejected. After the exclusion of a surgical operation, all patients undergo a clinical examination and precise interventional diagnostics to exclude other sources of pain, such as Z-joint syndrome, sacroiliac joint or intervertebral disc pain source. If nothing is found, epiduroscopy is indicated. Alongside interventional and other pain diagnostic methods, all patients are considered to get full suitable pharmacological and physio-rehabilitation treatment [18, 19].

Conclusion

Epiduroscopy appears to be a beneficial procedure for both patient groups, especially after 6 months, with some benefits remaining after 12 months. A disadvantage at this stage may be the relatively low number of patients, which limits the possibility to distribute and evaluate patients according to decades (20–30 years, 31–40 years, 41–50 years, 51–60 years, 61–70 years, 80 years and over); however, the results of the study clearly show that the quality of health was improved after 6 and 12 months in almost all parameters. This is not consistent with the VAS where a previous study showed improvement after 12 months in group B alone, and only isolated in back pain (paradoxically, the expectation was greater for lower limb pain). This indicates that the VAS is a subjective parameter prone to bias and is clearly not sufficient for a comprehensive assessment of a chronic pain states. The EQ-5D-5L appears to be a convenient and comprehensive way to evaluate a patient with chronic pain.

Conflict of interest R. Rapčan, L. Kočan, V. Witkovsky, J. Mláka, M. Griger, M. Burianek, S. Rapčanová, A. Hammond, Ľ. Poliak, R. Tirpák, J. Šimonová, F. Sabol, and J. Vašková declare that they have no competing interests.

References

1. Kamieniak P, Bielewicz J, Grochowski C, Litak J, Bojarska-Junak A, Daniluk B, et al. The elevated serum level of IFN-γ in patients with failed back surgery syndrome remains unchanged after spinal cord stimulation. Dis Markers. 2019;2019:2606808.
2. Bossher HA, Heavner JE. Incidence and severity of epidural fibrosis after back surgery: an endoscopic study. Pain Pract. 2010;10(1):18–24.
3. Kallewaard JW, Vanelderen P, Richardson J, Van Zundert J, Heavner J, Groen GJ. Epiduroscopy for patients with lumbosacral radicular pain. Pain Pract. 2014;14(4):365–77.
4. Bellini M, Barbieri M. A Comparison of non-endoscopic and endoscopic adhesiolysis of epidural fibrosis. Anaesthesiol Intensive Ther. 2016;48(4):266–71.
5. Di Donato A, Fontana C, Alemanno D, Di Giacomo A. Epiduroscopy in treatment of degenerative chronic low back pain: a prospective analysis and follow-up at 60 months. Clin Res Regul Aff. 2010;27(3):69–74.
6. Fredman B, Nun MB, Zohar E, Iraqi G, Shapiro M, Gepstein R, et al. Epidural steroids for treating "failed back surgery syndrome": is fluoroscopy really necessary? Anesth Analg. 1999;88(2):367–72.
7. Geurts JW, Kallewaard JW, Richardson J, Groen GJ. Targeted methylprednisolone acetate/hyaluronidase/clonidine injection after diagnostic epiduroscopy for chronic sciatica: a prospective, 1-year follow-up study. Reg Anesth Pain Med. 2002;27(4):343–52.
8. Rapčan R, Kočan L, Mláka J, Burianek M, Kočanová H, Rapčanová S, et al. A randomized, multicenter, double-blind, parallel pilot study assessing the effect of mechanical adhesiolysis vs adhesiolysis with corticosteroid and hyaluronidase administration into the epidural space during epiduroscopy. Pain Med. 2018;19(7):1436–44.
9. Kotz S, Lumelskii Y, Pensky M, editors. The stress-strength model and its generalizations: theory and applications. New York: World Scientific; 2003.
10. Zhou W. Statistical inference for P(X<Y). Stat Med. 2008;27(2):257–79.
11. Szende A, Oppe M, de Charro F. EQ-5D value sets: inventory, comparative review and user guide. Berlin: Springer; 2007.
12. Carlsson M. Assessment of chronic pain. I. Aspects of the reliability and validity of the visual analogue scale. Pain. 1983;16(1):87–101.
13. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. Lancet Neurol. 2010;9(8):807–19.
14. Dworkin RH, Turk DC, Farrar TJ, Haythornthwaite JA, Jensen MP, Katz NP, et al. Core outcome measures for chronic pain clinical trials: IMMPACT recommendations. Pain. 2005;113(1–2):9–19.
15. Herdman M, Gudex C, Lloyd A, Janssen MF, Kind P, Parkin D, et al. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). Qual Life Res. 2011;20(10):1727–36.
16. Gustavsson A, Bjorkman J, Ljungcrantz C, Rhodin A, Rivano-Fischer M, Sjolund KF, et al. Pharmacological treatment patterns in neuropathic pain—lessons from Swedish administrative registries. Pain Med. 2013;14(7):1072–80.
17. Palmer N, Guan Z, Chai NC. Spinal cord stimulation for failed back surgery syndrome, patient selection considerations. Transl Perioper Pain Med. 2019;6(3):81–90.
18. Vanelderen P, Van Boxem K, Van Zundert J. Epiduroscopy: the missing link connecting diagnosis and treatment? Pain Pract. 2012;12(7):499–501.
19. Aasa B, Berglund L, Michaelson P, Aasa U. Individualized low-load motor control exercises and education versus a high-load lifting exercise and education to improve activity, pain intensity, and physical performance in patients with low back pain: a randomized controlled trial. J Orthop Sports Phys Ther. 2015;45(2):77–85. B1–4.

Výsledky štúdie EPCS V poukazujú na vysokú mieru efektivity endoskopickej diskektómie u pacientov s akútnym radikulárnym syndrómom spôsobeným herniáciou alebo sekvestráciou medzistavcového disku. Signifikantné zlepšenie sme zaznamenali v sledovaných parametroch a to: redukcia intenzity bolesti ($p < 0.05$) a hodnotených dotazníkoch EuroQuol a Oswestry ($p < 0.05$) po 6 a 12 mesiacoch po výkone.

Tieto výsledky boli publikované v časopisoch Bratislava Medical Journal, IF 1,2, Q3 a Medicine IF 1,6, Q3.

- *Mlaka J, Rapcan R, Burianek M, Rapcanova S, Gajdos M, Kocanova M, Griger M, Kovalicova L, Vaskova J,* ***Kocan L****. Endoscopic discectomy as an effective treatment of a herniated intervertebral disc. Bratisl Lek Listy. 2020;121(3):199-205. doi: 10.4149/BLL_2020_030. PMID: 32115977.*

- *Rapčan R,* ***Kočan L****, Witkovsky V, Rapčanová S, Mláka J, Tirpák R, Burianek M, Kočanová H, Vašková J, Gajdoš M. Endoscopic discectomy of the herniated intervertebral disc and changes in quality-of-life EQ-5D-5L analysis. Medicine (Baltimore). 2023 Jun 30;102(26):e34188. doi: 10.1097/MD.0000000000034188. PMID: 37390280; PMCID: PMC10313260.*

DOI: 10.4149/BLL_2020_030

Bratisl Med J 2020; 121 (3)
199–205

CLINICAL STUDY

Endoscopic discectomy as an effective treatment of a herniated intervertebral disc

Mlaka J[1], Rapcan R[2], Burianek M[3], Rapcanova S[2], Gajdos M[4], Kocanova H[5], Griger M[6], Kovalicova L[2], Vaskova J[7], Kocan L[8]

EuroPainClinics, Kosice, Slovakia. **kocanladislav@gmail.com**

ABSTRACT

OBJECTIVE: Prospective observational multicentre two-arm parallel study describing clinical outcome after endoscopic discectomy provided via transforaminal and interlaminar approach.

BACKGROUND: Endoscopic lumbar discectomy (ELD) is a percutaneous minimally invasive procedure for the treatment of herniated lumbar discs. Herniations at lumbar intervertebral disc levels of L1/2, L2/3, L3/4 and L4/5 are mostly accessed by the transforaminal (TF) approach. However, due to the anatomic position of the iliac crest, the L5/S1 level might not be reachable by the transforaminal approach, while the interlaminar (IL) percutaneous approach should be a suitable alternative.

METHODS: In a prospective observational multicentre clinical trial NCT0274311, we compared the clinical outcomes of two groups of patients who underwent ELD via IL (83) and TF (103) approach. The subjects were followed for 12 months via planned examinations by pain physicians. The levels of leg pain and back pain intensity were assessed by an 11-point numerical ratings scale (NRS). Patient's functional disability was assessed by the Oswestry Disability Index (ODI).

RESULTS: Study subjects showed a significant decrease in ODI scores in both groups ($p<0.001$) The values of mean preoperative ODI in TF and IL groups were 39.1±15.7 and 43.4±16, respectively. Postoperative values in the latter groups were 14.8±14.9 and 17.5±14.3, respectively. Significantly lower pain scores for leg pain ($p<0.001$) and back pain ($p<0.001$) were also recorded at 12-month follow-ups.

CONCLUSION: Because both procedures are strictly percutaneous; they are now more commonly performed by interventional pain physicians as a safe and effective alternative to open surgical spine procedures *(Tab. 3, Fig. 7, Ref. 19)*. Text in PDF *www.elis.sk*.

KEY WORDS: endoscopic discectomy, disc herniation, transforaminal, interlaminar, pain, quality of life, radicular syndrome.

Introduction

Lumbosacral radicular syndrome is the most common cause of neuropathic pain in our population (1). The most common reason for the development of acute lumbosacral radicular syndrome is the herniation of an intervertebral disc. The peripheral nerve damage leads to significant cellular and molecular changes not only at the level of axon, but also at that of DRG. The inflammatory cascade is set off directly by the nerve damage, as well as due to the presence of highly inflammatory material from the nucleus of the disc. Inflammation induces the production of neurotrophins and changes the neuronal transmission. In the place of the damaged axons and mainly in the corresponding DRG, ectopic discharges are fired, which explains the formation of radicular pain (2).

The mechanisms of central sensitisation often propagate pain to the neighbouring spinal segments. That is why, if there is a pathology seen in numerous segments on an MRI, it is necessary to perfectly correlate the MRI results with the clinical signs. When in doubt which root is causing the symptoms, it is possible to use a diagnostic root block in conjunction with physical examination and MRI (3).

There is no clear evidence that an early surgical decompression of a herniated disc has a better long-term outcome than conservative therapy. Moreover, there are a large number of opinions about how soon one should perform the surgical procedure (4).

In general, an early intervention is indicated in patients with the extinction phenomenon – sensory or motor. In patients without extinction phenomena and only with radicular pain, an early surgical decompression enables a significantly quicker return to a normal pain-free life. In the past couple of years, minimally in-

[1]EuroPainClinics, Kosice, Slovakia, [2]EuroPainClinics, Bardejov, Slovakia, [3]EuroPainClinics, Prague, Czech Republic, [4]Louis Pasteur University Hospital, Neurosurgery Clinic, Kosice, Slovakia, [5]Clinic of Anaesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Kosice, Slovakia, [6]EuroPainClinics, Bratislava, Slovakia, [7]Department of Medical and Clinical Biochemistry, Faculty of Medicine, PJ Safarikiensis University, Kosice, Slovakia, and [8]Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Kosice, Slovakia

Address for correspondence: L. Kocan, MD, PhD, Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, 040 11 Kosice, Slovakia
Phone: +421 557 891 100

vasive procedures have been coming forward in the field of surgical interventions. Endoscopic discectomy is among the most sparing procedures, which is performed by a spinal surgeon or an interventional pain physician.

In contrast to an open surgery, an endoscopic discectomy causes less trauma to the surrounding tissues, there is minimal blood loss and a decreased risk of post-operative fibrosis. Long-term results are, however, equivalent to an open surgery approach (5). The procedure only requires hospitalisation of the patient for one day, after which the patient leaves for home.

Materials and methods

Ethics

Ethical approval for this study (Ethical Committee No EK:9N-2015) was provided by the Ethical Committee of Medical faculty, University of PJ Safarikiensis in Kosice. The study was registered in ClinicalTrials.gov (NCT02742311). All patients gave their consent after receiving extensive information about the study.

Study design

The study is a prospective observational multicentre two-arm parallel study of endoscopic discectomy provided in acute sciatica caused by a herniated intervertebral disc. We independently investigated the efficacy of two surgical approaches: transforaminal and interlaminar approach. Included pain clinics with recruitment and postoperative follow-up were situated in Bratislava, Kosice and Bardejov. The investigation of clinical parameters was based on a numerating rating scale of pain (NRS), Oswestry disability index (ODI), patient status, patient satisfaction score and EQ-VAS. We collected all data before the procedure and 12 months after the procedure. All patients had their unique ID code generated after being enrolled in the study. To secure the safety of data, they were encrypted in an established online database. An independent study coordinator from the East Institute of Cardiovascular Diseases was appointed to superintend during the study process.

Patients

Patients classified by the American Society of Anesthesiologists physical status (ASA PS) classes I, II, or III aged between 18–75 with acute sciatica, scheduled for endoscopic discectomy after obtaining their informed consent were enrolled into the study.

Indication criteria included unilateral lumbosacral radicular pain lasting less than one year, with or without signs of radiculopathy, correlated with isolated single level disc herniation on MRI, responding neither to conservative therapy nor to transforaminal steroids 2 weeks after injection.

Exclusion criteria included multisegmental or bilateral pain, herniations in multiple adjacent segments, severe disc degeneration, radiological or clinical signs of segmental instability, pathology not suitable for endoscopic treatment e.g. bulging disc, severe MODIC changes in adjacent endplates or very large sequesters.

The study was aimed at performing a parallel statistical comparison of two endoscopic approaches, namely transforaminal (103 patients) and interlaminar (83 patients) ways of treatment of lesions in the epidural space at the level of lumbar and sacral spine segments examined before the procedure and after 12 months (Fig. 1).

Surgical procedure

Transforaminal endoscopic discectomy (TFED)

The procedure is done under monitored anaesthesia care with the patient in a prone position. After aseptic preparation of the operation field, a local infiltration of skin and muscle with trimecaine was performed. At a site located 10 to 14 cm from the midline, a skin incision was made, and under fluoroscopic control, a needle and a guide-wire were inserted to the level of the upper third of the superior articular process (SAP) of the affected spine segment. After puncture channel dilation, a Tom Shidi needle was delicately hammered through the SAP not exceeding the medial pedicular line. A part of SAP was removed with a series of bone drills. After inserting the endoscope, the remaining part of the *ligamentum flavum* was removed. The nerve root and disc material were identified

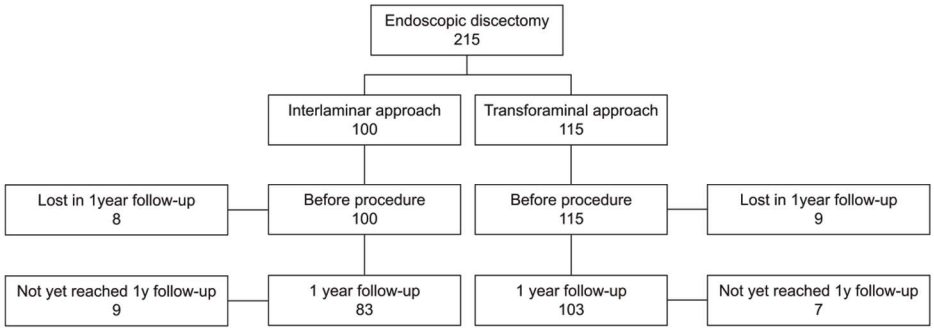


Fig. 1. Flow chart of patient selection, enrolment, and follow-up in the study.

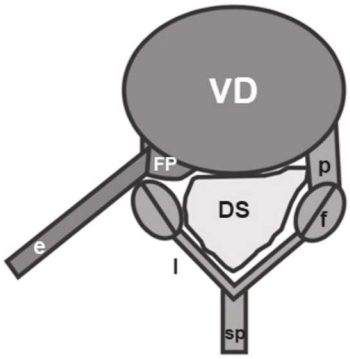


Fig. 2. Endoscopic transforaminal approach – axial view: VD – vertebral disc; FP – foraminal protrusion; l – lamina; p – pedicle; sp – spinous process; f – facet joint; DS – dural sac; e – endoscope.

and the nerve was mobilised with a flexible probe. The protrusion was removed with a grasper. The posterior annulus radiofrequency shrinkage and coagulation of epidural veins was performed with the Elliquence TriggerFlex probe. After the free pulsation of the traversing nerve root had been observed, the endoscope was removed and skin sutures applied.

Foraminal and paramedial hernias were surgically treated through a foraminal approach.

This technique uses a natural entrance into the spinal canal (neuroforamen) (Fig. 2).

The working channel for the endoscope is inserted lateromedially through the paravertebral musculature. During TFED, it is not necessary to disrupt the dorsal border of the spinal canal (skeletal lamina of the vertebra and *ligamentum flavum*). The risk of post-operative fibrosis formation is, therefore, minimal.

Interlaminar endoscopic discectomy (ILED)

The procedure is done under general anaesthesia care with the patient in a prone position. After aseptic preparation of the operation field, local infiltration of skin and muscle with trimecaine was performed (6). The puncture point for the guiding tubus at the affected disc level was established as a site located laterally from the spinous process, medio-caudally from the upper lamina, and over the epidural window. Subsequently, in an AP view, the guiding tubus was inserted towards the epidural window on the affected side. The insertion of the tubus was gradually checked in an AP lateral view until contact with the vertebral lamina. A working channel for the endoscope was established. Under endoscopic control, the ligamentum flavum was dissected to enter the epidural space. The nerve root, dura and disc protrusion were identified. The protrusion was removed with a grasper. Posterior annulus radiofrequency shrinkage and coagulation of epidural veins was performed with an Elliquence TriggerFlex probe. After the free pulsation of the traversing nerve root had been observed, the endoscope was removed and skin sutures applied.

When it was technically more difficult to reach central protrusions or in case of more voluminous sequesters especially at the L5/S1 level, the interlaminar approach was used (Fig. 3). A working channel with an endoscope was introduced in the midline, very similar to microdiscectomy. To enter the spinal canal, it is only needed to disrupt a part of the *ligamentum flavum*. The dural sac is then moved medially with a special manoeuvre to enable work in the central part of the channel.

Anaesthesia

Anaesthesia was provided by an anaesthesiology team. During the procedure, the patient was analgosedated. 10ug Sufentanil and 50–400 mg Propofol was used. During the tunnelling of the working channel, the patient was under total intravenous anaesthesia with protective lung ventilation. During the procedure, basic vital functions were monitored, namely the non-invasive blood pressure monitoring, heart rate, oxygen saturation, and respiratory rate.

Scoring systems

The monitored parameters included interval scales and index scoring systems as follows: numerical pain scale of legs, numerical pain scale of the lower back, Oswestry disability index, patient status, and patient satisfaction score.

Numerical pain scale

The numerical pain scale is an internationally accepted tool to determine the intensity of pain. The patient uses an 11-point scale (0–10) to describe their intensity of pain, where 0 means no pain and 10 means the strongest possible pain the patient can imagine.

Oswestry disability index

Oswestry disability index is derived from the Oswestry low back pain questionnaire which quantifies disability caused by pain

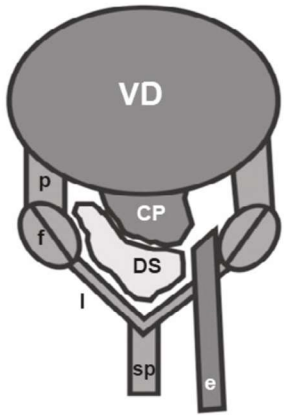


Fig. 3. Endoscopic interlaminar approach axial view: VD – vertebral disc; CP – central protrusion; l – lamina; p – pedicle; sp – spinous process; f – facet joint; DS – dural sac; e – endoscope.

of the lower back. ODI is presently the gold standard method of measuring the level of disability, as well as quality of life in people suffering from lower back pain. The questionnaire includes 10 questions about the intensity of pain, lifting weights, level of self-care, ability to walk, sit and lead a sexual life, ability to stand, have a social life, quality of sleep and ability to travel. For each question the patient can choose from 6 different answers describing various alternatives. The answers are in an order from most to least favourable. The patient chooses the answer which most closely relates to their condition. The answers are evaluated from 0 to 5 points. The most favourable answer is evaluated with 0 points (the lowest level of disability) and the last answer with 5 points (the highest level of disability). The number of points obtained from answers to each of 10 questions is summed together and multiplied by 2. The resulting number is the ODI score.

Patient status

This is a questionnaire, which directly describes the self-sufficiency and mobility of the patient. It is graded on a scale from 0 to 5, where each number represents the patient's state as follows: 0 – pain-free, i.e. the patient leads a normal life, works, does sports, etc; 1 – the patient is able to perform basic daily activities (does not participate in sports), is employed part-time; 2 – the patient is not able to work, but can take care of himself/herself; 3 – the patient is not independent, needs help with care; 4 – the patient needs home care and is bed-ridden.

Patient satisfaction score

Patient satisfaction score (PSS) – on a scale from 0 to 10, the patient can grade their satisfaction with the performed medical procedure and its end-result, where the score 0 means the worst satisfaction and 10 highest satisfaction.

EQ VAS

The EQ VAS is a part of EuroQol licensed questionnaire and records the respondent's self-rated health on a 20 cm vertical, visual analogue scale (0–100) with endpoints labelled as 'the best health you can imagine' (100) and 'the worst health you can imagine'(0). This information can be used as a quantitative measure of health and quality of life as judged by the individual respondents.

Statistical analysis

The statistical test (parametric/non-parametric) was chosen based on normality and number of data.

Tab. 1. Characteristics of patients in groups divided according to the surgical approach.

Characteristic	Transforaminal	Interlaminar
Gender	M 51pt/F 52pt	M 44pt/F 39pt
Age	(min 18–max 78) med 47	(min 23–max 76) med 43
ASA	(min 1–max 3) med 2	(min 1–max 3) med 2
L1–L2	0	0
L2–L3	0	0
L3–L4	14	1
L4–L5	52	5
L5–S1	41	77

M – male; F – female; ASA – The American Society of Anesthesiologist's physical status classification system.

Results

Table 1 shows the demographic characteristic for both groups. The results in Table 2 demonstrate that using either of surgical approaches led to a significant improvement in the evaluated parameters after 12 months with $p < 0.001$. The intensity of lower back pain was considered less intense than that of the leg pain before the procedure. There was a significant improvement in the intensity of back and leg pain after 12 months, where average values of the intensity of the pain were more-or-less the same (Fig. 4 and 5). The 95% probability of the replicability of the estimate for the given values is lower in the IL group, however, the values for both groups overlap. During the evaluation of the grade of disability according to ODI, it is also clear that after performing either of types of surgical procedure, there is a substantial improvement in the quality of life of the patient at the 12-month follow up. There are

Tab. 2. Comparison of patient pain intensity, self-content score, patient status score, Quality of life (VAS) and functional disability in both groups aftera 12-month postoperative examination period.

Parameters	Groups	Before procedure			12 months follow-up			CI 95 %	p
		Mean	St. error mean	SD	mean	St. error mean	SD		
Oswestry disability index	IL group	43.36	1.94	15.98	14.75	1.24	14.91	16.288 to 26.856	<0.001
	TF group	39.09	1.71	14.01	17.52	1.18	14.31	16.315 to 26.83	<0.001
Numerical pain scale Back pain	IL group	4.31	0.36	3.27	2.18	0.23	2.11	1.370 to 2.895	<0.001
	TF group	5.35	0.29	2.97	2.41	0.19	1.94	2.345 to 3.530	<0.001
Numerical pain scale leg pain	IL group	7.19	0.22	2.01	2.46	0.23	2.11	4.161 to 5.309	<0.001
	TF group	7.34	0.21	2.07	2.23	0.19	1.93	4.590 to 5.623	<0.001
Patient status score	IL group	2.27	0.10	0.85	1.10	0.11	0.94	0.939 to 1.407	<0.001
	TF group	2.44	0.08	0.84	1.12	0.10	0.09	1.113 to 1.527	<0.001
Patient satisfaction score	IL group	x	x	X	7.6	0.27	2.48	7.05 to 8.14	X
	TF group	x	x	X	7.96	0.24	2.42	7.49 to 8.43	X
Quality of life	IL group	49.13	1.73	15.74	72.60	1.91	17.36	–28.54 to –18.39	<0.001
	TF group	49.40	1.55	15.69	70.10	1.80	18.21	–25.39 to –16.00	<0.001

IL – interlaminar; TL – transforaminal; St. error mean – standard error of the mean; SD – standard deviation; CI – confidence interval

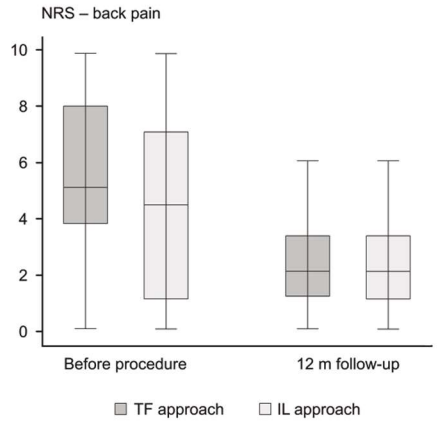


Fig. 4. Evaluation of back pain intensity in patients indicating significant reduction in low back pain in both groups after 12 months. NRS – numerating rating scale; TF – transforaminal; IL – interlaminar.

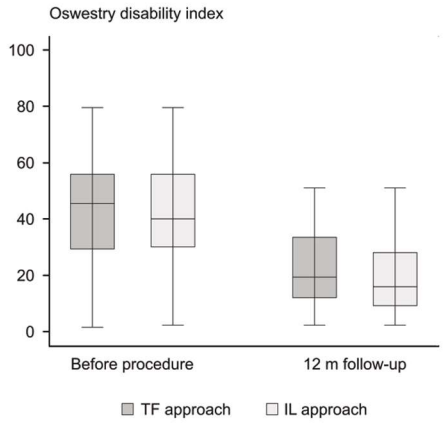


Fig. 6. Evaluation of patient functional disability by the Oswestry Disability Index indicating significant improvement in comparison with baseline after 12 months in both groups. TF – transforaminal; IL – interlaminar.

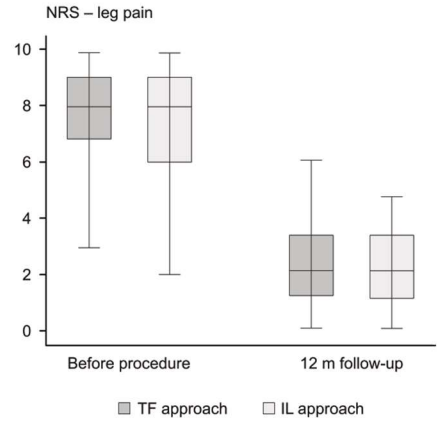


Fig. 5. Evaluation of back pain intensity in patients indicating significant reduction in leg pain in both groups after 12 months. NRS – numerating rating scale; TF – transforaminal; IL – interlaminar.

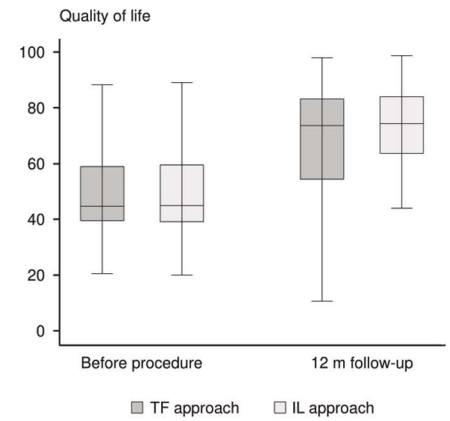


Fig. 7. Evaluation of patient quality of life measured on visual analogue scale indicating significant improvement in comparison with baseline after 12 months in both groups. TF – transforaminal; IL – interlaminar.

only minor differences in the CI values for both of the groups. The improvement was comparable in both groups of patients (Fig. 6).

In parallel, the evaluation of self-sufficiency and mobility of patients in the PS questionnaire demonstrated a comparable and significantly improved patient status after 12 months in both groups (Tab. 2). Their condition improved in 12 months from an average of "inability to work" to an average of "ability to take part in basic daily activities". Although, as in NRS, the CI is lower in the IL group, the ranges of both groups overlap. Considering the general patient status, the patients in both groups expressed a rather high satisfaction with the medical procedure in the PSS questionnaire, being mostly graded as 7 or 8. Self-assessment of the quality of life of the patients (EQ-VAS) demonstrated a significant improvement after the procedure in both groups (Tab. 2, Fig. 7). The rate of complications is summarized in Table 3.

Discussion

The dominant position among the surgical approaches to a lumbar intervertebral disc herniation and the decompression of a spinal nerve used to belong to open surgical procedures, most commonly to hemi-laminectomy (7, 8). The strategy of surgical

Tab. 3. The rate of complications.

Complications	TF 103pt	%	IL 83pt	%
Nerve root injury	0	0	0	0
New level herniation	3	2.91	4	4.82
New level operation	2	1.94	2	2.41
Surgical errors	0	0	0	0
Dural puncture	1	0.97	1	1.2
Hematoma	0	0	0	0
Wound complications	0	0	0	0
Re-herniations	5	4.85	4	4.82
Reoperations	5	4.85	4	4.82

TF – transforaminal; IL – interlaminar

approaches has since gradually started focusing on the minimally-invasive surgical options, where micro-discectomies have become the dominant choice. The trend of minimizing the surgical field in the treatment of symptomatic intervertebral disc herniations lead to the development of the endoscopic discectomy technique, (9) which has two approaches; one is interlaminar, and the other is the transforaminal approach, depending on the type of herniation and its height (10). Moreover, this surgical approach has been added to the portfolio of not only spinal surgeons, but also interventional pain management specialists. The comparison of our results between the transforaminal and interlaminar approach after a 12-month follow-up demonstrated a high effectivity of treatment in minimising back pain $p < 0.001$, as well as a significant decrease in the radiation of pain to the leg $p < 0.001$.

The inability to carry out basic daily activities due to lower back pain is expressed by the Oswestry disability index calculated using a questionnaire based on the intensity of pain, carrying out basic daily activities, lifting bigger loads, walking, sitting, standing, sleeping, social life, sex life and traveling (11).

The character of acute pain with lumbosacral radicular syndrome is apparent by adjusting and regaining positive values in the monitored ODI score. The ODI scores and results from the patient quality of life questionnaire EuroQuol that we obtained in this study demonstrate a significant improvement after 12 months to the procedure ($p < 0.001$). The effectiveness of endoscopic discectomy procedures is comparable to an open surgery as well as to a microdiscectomy surgical approach (12, 13). All of these surgical approaches are of high effectiveness with excellent outcomes (5, 14). During this study, we noted a small number of complications linked to the surgical procedure. Some of the complications include nerve root injury, new level herniation, new level operation, surgical errors, dural puncture, epidural hematoma, wound complications, re-herniations and reoperations. From a meta-analysis comparing the prevalence of complications after lumbar open microdiscectomy, microendoscopic discectomy (MED) and percutaneous microdiscectomy, a smaller number of post-operative complications was found with endoscopic procedures, as well as a decrease in post-operative pain (15). This is due to minimal manipulation of the surrounding tissue. Another notable discovery in the peri-operative period was the reduction in anaesthesia-related complications. The procedure is done with the patient in a prone position, which in general causes a number of disadvantages in total anaesthesia. This is logistically the most difficult position due to the challenges associated with providing adequate oxygenation, ensuring adequate ventilation, maintaining haemodynamics, and securing intravenous lines and tracheal tube. The access to the patient's airway is also poor. Pressure sores, vascular compression or brachial plexus injuries can occur (6). However, endoscopic discectomies are performed with analgosedation combined with local anaesthesia. During the almost whole procedure, the patient communicates with the surgeon and anaesthesiologist and can notify them if they feel any unpleasant sensation or new pain associated with the medical instrument touching a spinal nerve (16, 17).

As compared to the open surgical approach, the advantage of a minimally invasive endoscopic discectomy lies in its radical influence on the cause of pain without further chronification, and with minimal damage it causes to the paravertebral musculature. This is confirmed upon the follow-up MRI tests which demonstrate significant atrophy of the paravertebral musculature after a microdiscectomy approach, while after an endoscopic procedure, such changes are not visualized. This phenomenon is most probably linked to the incisional denervation of muscles. Patients after endoscopic discectomy maintain their muscular corset, which enhances early rehabilitation as well as post-operation functionality (4, 18).

Beside the removal of the extruded fragment, the endoscopic discectomy techniques also preserve the *ligamentum flavum*, decrease bleeding in the epidural space and effectively resolve sciatica symptoms with a lower risk of recurrence and formation of epidural fibrosis.

In general, an early intervention is indicated in patients with the extinction phenomenon – sensory or motor. In patients without extinction phenomena and with radicular pain only, an early surgical decompression enables a significantly quicker return to their normal pain-free life (3, 19).

Conclusion

Endoscopic discectomy belongs to the keyhole techniques. It has become a domain not only for spinal surgeons, but also for interventional pain physicians. It presents a valuable surgical approach due to its cautiousness around surrounding tissues; it demonstrates a significant reduction in post-operative complications linked with the formation of epidural fibrosis, not to forget the benefit of early convalescence, additional decreased costs of hospitalisation, as well as decreased risk of pain becoming chronic (10). After the procedure, the patient is able to walk out of the operation theatre on his own, and, while adhering to a strict regime, can recover at home.

References

1. Van Boxem K, Cheng J, Patijn J, Van Kleef M et al. Lumbosacral Radicular Pain. Evidence-Based Interventional Pain Medicine: According to Clinical Diagnoses 2011; 10 (4): 71–86.

2. Van Boxem K, Huntoon M, Van Zundert J, Patijn J, Van Kleef M, Joosten EA. Pulsed radiofrequency: a review of the basic science as applied to the pathophysiology of radicular pain: a call for clinical translation. Regional Anesthesia and Pain Medicine. 2014; 39 (2): 149–159.

3. Manchikanti L, Buenaventura RM, Manchikanti KN et al. Effectiveness of therapeutic lumbar transforaminal epidural steroid injections in managing lumbar spinal pain. Pain Physician 2012; 15 (3): 199–245.

4. Weinstein JN, Lurie JD, Tosteson TR et al. Surgical vs nonoperative treatment for lumbar disk herniation: the Spine Patient Outcomes Research Trial (SPORT) observational cohort. JAMA 2006; 296 (20): 2451–2459.

5. Cong L, Zhu Y, Tu G. A meta-analysis of endoscopic discectomy versus open discectomy for symptomatic lumbar disk herniation. Eur Spine J 2016; 25 (1): 134–143.

6. Rozet I, Vavilala MS. Risks and benefits of patient positioning during neurosurgical care. Anesthesiol Clin 2007; 25 (3): 631–53.

7. Munting E, Röder C, Sobottke R, Dietrich D, Aghayev E. Patient outcomes after laminotomy, hemilaminectomy, laminectomy and laminectomy with instrumented fusion for spinal canal stenosis: a propensity score-based study from the Spine. Tango registry Eur Spine J 2015; 24 (2): 358–368.

8. Oktem IS, Akdemir H, Kurtsoy A, Koc RK, Menku A, Tucer B. Hemilaminectomy for the removal of the spinal lesions. Spinal Cord 2000; 38 (2): 92–96.

9. Seiger A, Gadjradj PS, Harhangi BS et al. PTED study: design of a non-inferiority, randomised controlled trial to compare the effectiveness and cost-effectiveness of percutaneous transforaminal endoscopic discectomy (PTED) versus open microdiscectomy for patients with a symptomatic lumbar disc herniation. BMJ Open 2017; 21; 7 (12): e018230.

10. Li ZZ, Hou SX, Shang WL, Song KR, Zhao HL. The strategy and early clinical outcome of full-endoscopic L5/S1 discectomy through interlaminar approach. Clin Neurol Neurosurg 2015; 133: 40–45.

11. Khan I, Bydon M, Archer KR et al. Impact of Occupational Characteristics on Return to Work for Employed Patients after Elective Lumbar Spine Surgery. Spine J 2019; 9: S1529–9430.

12. Choi KC, Shim HK, Kim JS et al. Cost-effectiveness of microdiscectomy versus endoscopic discectomy for lumbar disc herniation. Spine J 2019; 19 (7): 1162–1169.

13. Liu X, Yuan S, Tian Y, Wang L, Gong L, Zheng Y, Li J. Comparison of percutaneous endoscopic transforaminal discectomy, microendoscopic discectomy, and microdiscectomy for symptomatic lumbar disc herniation: minimum 2-year follow-up results. J Neurosurg Spine 2018; 28 (3): 317–325.

14. Lee SH, Chung SE, Ahn Y, Kim TH, Park JY, Shin SW. Comparative radiologic evaluation of percutaneous endoscopic lumbar discectomy and open microdiscectomy: a matched cohort analysis. Mount Sinai J Med 2006; 73 (5): 795–801.

15. Shriver MF, Xie JJ, Tye EY, Rosenbaum BP, Kshettry VR, Benzel EC, Mroz TE. Lumbar microdiscectomy complication rates: a systematic review and meta-analysis. Neurosurg Focus 2015; 39 (4).

16. Henmi T, Terai T, Hibino N et al. Percutaneous endoscopic lumbar discectomy utilizing ventral epiduroscopic observation technique and foraminoplasty for transligamentous extruded nucleus pulposus: technical note. J Neurosurg Spine 2016; 24 (2): 275–280.

17. Zhu YJ, Ying GY, Chen AQ et al. Minimally invasive removal of lumbar intradural extramedullary lesions using the interlaminar approach. Neurosurg Focus. 2015; 39 (2).

18. Lequin MB, Verbaan D, Jacobs WC et al. Surgery versus prolonged conservative treatment for sciatica: 5-year results of a randomised controlled trial. BMJ Open 2013; 28: 3 (5).

19. Vialle LR, Vialle EN, Henao Suárez JE, Giraldo G. Lumbar disc herniation. Revista Brasileira de Ortopedia 2010; 45 (1): 17–22.

Received October 24, 2019.
Accepted December 5, 2019.

Observational Study

Medicine

OPEN

Endoscopic discectomy of the herniated intervertebral disc and changes in quality-of-life EQ-5D-5L analysis

Róbert Rapčan, MD, PhD, FIPP[a,b,c], Ladislav Kočan, MD, PhD, FIPP[d,*], Viktor Witkovsky, CSc[e], Simona Rapčanová, MD[f], Juraj Mláka, MD, FIPP, EDRA[b], Róbert Tirpák, MD, FIPP[b], Miroslav Burianek, MD[f], Hana Kočanová, MD[g], Janka Vašková, PhD[h], Miroslav Gajdoš, CSc[i]

Abstract

Herniated lumbar discs are a common cause of low back pain, which can negatively impact the quality of life of working-age individuals. This study aimed to evaluate changes in the quality of life in patients with sciatica who underwent endoscopic discectomy, a minimally invasive surgical procedure. The study (ClinicalTrials.gov NCT02742311) included 470 patients who underwent transforaminal, interlaminar, or translaminar endoscopic discectomy. Quality of life and pain perception were evaluated by comparing statistically weighted values of EQ-5D-5L, EQ-VAS, Oswestry disability index, and numerical pain scales for lower limb and back pain before and 12 months after the endoscopic procedure. After the procedure, there was a significant improvement in the reduction of back and lower limb pain, as well as in all monitored questionnaires ($P < .001$), which persisted 12 months after the endoscopy. All evaluated dimensions of the EQ-5D-5L questionnaire indicated a significant improvement in the assessed quality of life ($P < .001$). The study showed that percutaneous endoscopic lumbar discectomy is an effective pain-treating intervention that can improve the quality of life. There was no observed difference in the percentage of complications or re-herniations when comparing the transforaminal and interlaminar, approaches.

Abbreviations: CI = confidence interval, HRQoL = health-related quality of life, ILED = interlaminar endoscopic discectomy, LDH = lumbar disc herniation, NRS = numerical rating scale, ODI = Oswestry disability index, PELD = percutaneous endoscopic lumbar discectomy, TFED = transforaminal endoscopic discectomy.

Keywords: back pain, discectomy, EQ-5D-5L questionnaire, leg pain, quality of life

1. Introduction

Low back pain with sciatica caused by lumbar disc herniation (LDH) affects nearly 60% of patients and is increasingly common as the global population ages. This condition is particularly prevalent in the working-age population, with 70% of disability caused by LDH occurring in people aged 20 to 65 years. The diagnosis of LHD is confirmed by magnetic resonance imaging, and the major symptoms include low back pain and sciatica. However, there is no clear evidence regarding the best treatment approach.[1]

Recently, percutaneous endoscopic lumbar discectomy (PELD) has been used as an alternative to open discectomy. PELD is a minimally invasive technique that reduces the use of general anesthesia and results in less damage to surrounding soft tissues and paravertebral musculature, faster wound healing time, reduced spine instability, and shorter hospitalization.[2–4] The health-conditioned quality of life in patients with LHD is a result of both subjective perception of the disease and objective conditions.[5–7] Therefore, various questionnaires are used to evaluate the health-related quality of life (HRQoL) of patients undergoing treatment.[8,9]

The authors have no funding to disclose.

Written informed consent has been obtained from the patients to publish this paper.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Faculty of Medicine, Pavol Jozef Šafárik University in Košice No EK:9N-2015. The study was registered in ClinicalTrials.gov (NCT02742311, April 19, 2016).

[a] Europainclinics, Bratislava, Slovak Republic, [b] Europainclinics, Poliklinika Terasa, Košice, Slovak Republic, [c] Europainclinics, Bardejov, Slovak Republic, Slovak Republic, [d] Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Košice, Slovak Republic, [e] Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovak Republic, [f] Europainclinics, Prague, Czech Republic, [g] Clinic of Anaesthesiology and Intensive Care Medicine, Railway Hospital and Clinic Košice, Košice, Slovak Republic, [h] Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Trieda SNP 1, Košice, Slovak Republic, [i] Department of Neurosurgery, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, and Louis Pasteur University Hospital, Košice, Slovak Republic.

** Correspondence: Ladislav Kočan, Clinic of Anaesthesiology and Intensive Care Medicine, East Slovak Institute of Cardiovascular Disease, Ondavská 8, Košice 04011, Slovak Republic (e-mail: kocanladislav@yahoo.com).*

How to cite this article: Rapčan R, Kočan L, Witkovsky V, Rapčanová S, Mláka J, Tirpák R, Burianek M, Kočanová H, Vašková J, Gajdoš M. Endoscopic discectomy of the herniated intervertebral disc and changes in quality-of-life EQ-5D-5L analysis. Medicine 2023;102:26(e34188).

Received: 8 May 2023 / Received in final form: 12 June 2023 / Accepted: 13 June 2023

http://dx.doi.org/10.1097/MD.0000000000034188

This study aimed to assess changes in the quality of life in patients with sciatica who underwent endoscopic discectomy using the EuroQol 5-dimension questionnaire. The study also evaluated the Oswestry disability index (ODI), the numerical scale of quality of life, and numerical scales of leg pain and back pain. The statistical weight values for the studied countries were determined. The results showed significant improvements in all monitored parameters, including a reduction in back and lower limb pain, as well as improvements in HRQoL that persisted 12 months after the endoscopic procedure. PELD was found to be an effective intervention for treating pain, and no significant differences were observed in complications or re-herniations when comparing the different approaches.

2. Materials and methods

2.1. Patients

Between January 2016 and June 2021, we enrolled 470 patients who underwent ELD. These patients were between the ages of 18 to 80 years old and were classified according to their physical status using the American Society of Anesthesiology grading system (classes I, II, and III) (Fig. 1). The study was conducted in pain clinics located in Bratislava, Bardejov, Košice, Prague, and Brno in the Slovak and Czech Republic, and approved by a clinical investigation and recruitment process.

Inclusion criteria for the study were: being 18 years old or older, having signed informed consent, presenting with magnetic resonance imaging evidence of intervertebral disc herniation or sequestration, and experiencing permanent pain radiating to the lower limbs despite prior epidural steroid injection or similar interventions in conjunction with conservative treatment (such as rehabilitation and pharmacological treatment).

Exclusion criteria were: bleeding diathesis, urination or defecation problems, presence of infection or neoplasm, possible pregnancy, and patient disapproval. The procedure was performed by either a spine surgeon or an interventional pain management specialist, and each patient received a unique clinical trial ID number.

The primary outcomes of the study were the intensity of pain radiating to the back and legs, measured using the numerical rating scale (NRS) (ranging from 0 to 10), and the evaluation of the ODI, which assesses the extent of low back pain-related disability on a scale of 0 to 100%. Scores between 0 and 20% indicate minimal disability, while scores between 81 and 100% indicate bedridden patients or those with exaggerated symptoms. We also assessed the EuroQol survey using the EQ-5D-5L questionnaire. The descriptive system consists of five dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with scores ranging from 1 to 5 (representing the best to worst state). The EuroQol survey also includes the EQ-VAS vertical visual analog scale, which utilizes a grading scale from 0 to 100 to evaluate the quality of life. A score of 0 indicates "The worst health you can imagine," while a score of 100 indicates "The best health you can imagine."

No significant changes were made to the methods after the study commenced. Prior to the operation, the NRS for back and leg pain, EQ-VAS, and ODI were assessed in one of the pain clinics participating in the study. The second assessment was conducted 12 months after the procedure, either during an appointment in one of the included centers or via a phone call follow-up, to assess pain severity by NRS and neurological state.

2.2. Surgery

2.2.1. Transforaminal endoscopic discectomy (TFED). This technique uses a natural entrance into the spinal canal (neuroforamen). The working channel for the endoscope is

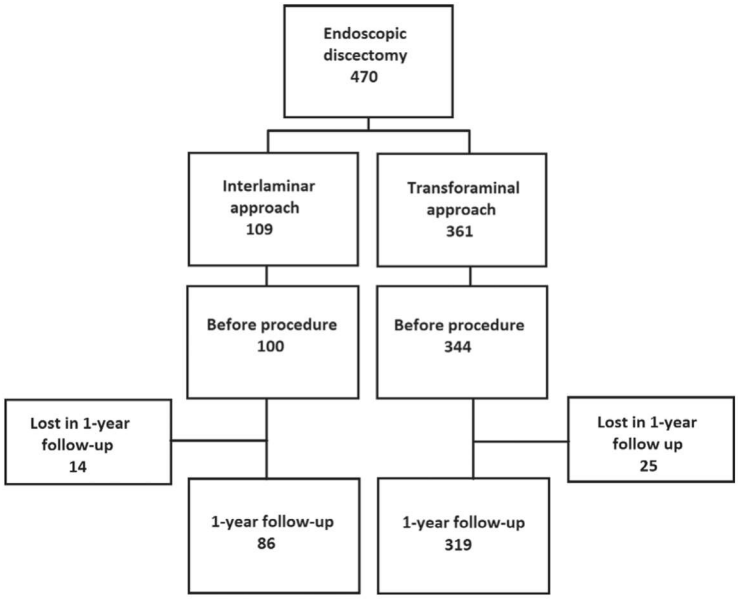


Figure 1. Flow chart of patient selection, enrollment, and follow-up in the study.

inserted through the paravertebral musculature latero-medially. During TFED, it is not necessary to disrupt the dorsal border of the spinal canal (the skeletal lamina of the vertebra and the ligamentum flavum). The risk of postoperative fibrosis formation is, therefore, minimal.

2.2.2. Interlaminar endoscopic discectomy (ILED). With more central protrusions that are harder to reach or more voluminous sequesters, especially at the L5/S1 level, the interlaminar approach was used. The working channel with the endoscope was introduced in the midline, very similar to a microdiscectomy. To enter the spinal canal, only a small part of the ligamentum flavum needs to be removed. The dural sac is then moved medially with a special maneuver to enable working in the central part of the channel.

2.2.3. Translaminar endoscopic discectomy. In cases, where entry into the spinal canal slightly above or below the interlaminar windows was desirable, translaminar endoscopic discectomy was used. The working channel for the affected disc level was inserted at the lateral inferior part of the lamina. Part of the lamina and ligamentum flavum are removed. This approach is very similar to ILED.

2.3. Anesthesia

The perioperative management of patients in our study involved several components. Prior to the operation, patients were premedicated with midazolam in a peroral dose ranging from 4 to 7.5 mg. During the procedure, standard monitoring was conducted, including electrocardiography, monitoring of oxygen saturation, and noninvasive blood pressure measurements.

During the procedures, the patients were positioned in the prone position. We employed the propofol-remifentanil target-controlled infusion sedation technique. Throughout the sedation process, the patients maintained consciousness and were responsive to our inquiries. They breathed spontaneously with the assistance of oxygen masks. The target-controlled infusion sedation technique was maintained by administering a continuous infusion of remifentanil at a rate ranging from 0.05 to 2 μg/kg/min. Additionally, propofol was administered with dosages varying from 0.5 to 4.0 μg/ml, depending on the specific needs of the surgical procedure.

During the operation, the surgical operator infiltrated the wound and operating canal with a mixture of local anesthetics. This combination consisted of 0.5% levobupivacaine and 1% trimecaine, with a total volume of up to 20 ml.

After the operation, patients were transferred to the recovery room where they were observed for up to 3 hours. During this time, standard monitoring was performed based on the patient's clinical situation. This included continuous monitoring of vital signs such as heart rate, blood pressure, respiratory rate, and oxygen saturation. Additionally, any specific postoperative complications or concerns were addressed and managed accordingly.

To manage postoperative pain, patients received intravenous paracetamol at a dose of 1 g. For at-home medication, patients were prescribed tramadol at a dosage of 100 mg every 12 hours, diclofenac at a dosage of 75 mg per day, and paracetamol at a dosage of 500 mg every 8 hours.

These medications and interventions were part of our standardized protocol for perioperative management in our study. They were designed to ensure adequate pain control and patient comfort during the postoperative period.

2.4. Statistical methods

Statistical analysis of the EQ-5D-5L questionnaire scores (in each of the 5 dimensions considered) was conducted using Wilcoxon rank-sum test and, alternatively, the estimated reliability parameter (together with the associated confidence intervals [CIs]). The Wilcoxon rank-sum test is a standard statistical, nonparametric test that checks whether 2 independent samples come from continuous probability distributions (populations) with the same medians. Although the original version of the test was designed to compare continuous distributions, a simple generalization is possible for applications with discrete distributions (which is the case when we try to compare the distribution of questionnaire scores reflecting the degree of the symptom tested on a scale from 1 to 5). Secondly, as an alternative, we have used the estimated reliability parameter $R = Prob(X < Y)$. For more details see Kotz et al,[10] Zhou,[11] and Rapčan et al.[12]

Descriptive statistical methods were also used to evaluate the results (mean, median, maximum, minimum, and SD). Examination of the distributional form for score and time data was determined by box plots. Each box plot indicated minimal value, lower quartile (lowest 25% of data), median, upper quartile (highest 25% of data), and maximal value. Normality of data distribution was assessed by the Shapiro–Wilk test. Homogeneity of variances was estimated using the Levene test. Differences between continuous variables were analyzed by a nonparametric Kruskal–Wallis 1-way test. A paired Student *t* test was used to assess the statistical significance of changes within each treatment group. *P* values of <0.05 were considered significant. Analyses were performed using MATLAB (R2021a) with Statistics and Machine Learning Toolbox version 12.1 (The MathWorks, Inc., MA) and the statistical software package SPSS version 11.0 (Chicago, IL) .

3. Results

Four hundred seventy patients were included in the statistical processing. Three hundred nineteen patients underwent TFED and 86 patients ILED with a 1-year follow-up (Fig. 1). Several parameters were evaluated (Table 1). Prior to discectomy, the patient's quality of life was assessed using an ODI questionnaire, a subsequent calculation of the ODI index, and a 5-dimensional EQ-5D-5L questionnaire was also assessed. The third questionnaire evaluating the quality of life from 0 to 100 was determined on the EQ-VAS scale. We also compared the intensity of back pain and leg pain measured on the numeric rating scale (0–10).

Comparing the parameters: ODI index, NRS, and EQ-VAS before the operation and 12 months after the operation showed a significant improvement in the reduction of back pain, painful lower limbs, as well as significant changes in the evaluated questionnaires $P < .001$ (Table 2). Before surgery, the median values of NRS for lower limb pain were 9, and NRS for back pain was 8.

Table 1

Descriptive characteristics of the group of examined patients.

Surgical approach	Transforaminal	Interlaminar
	319	86
Gender M/F	140/179	660/26
Age	Min/Max/Med	Min/Max/Med
	21/79/45	18/74/51
Level of herniation		
L1/L2	0	0
L2/L3	3	0
L3/L4	18	0
L4/L5	169	0
L5/S1	122	86
L3/L4 + L4/L5	6	0
L4/L5 + L5/S1	1	0
Reoperations %	7%	6%

Table 2

Evaluation of and Oswestry disability index, EQ-VAS, and pain intensity in the lower back and lower limbs according to the NRS in patients before surgery and 12 months after endoscopic discectomy.

Parameters	Time interval	Mean	SD	SEM	95% CI	*P* value
Oswestry disability index	Before procedure	54.11	20.71	1.186	36.71–41.38	<.001
	12 months follow-up	15.06				
EQ-VAS	Before procedure	39.13	30.32	1.506	−43.20 to −37.28	<.001
	12 months follow-up	79.38				
Numerical pain scale	Before procedure	7.83	3.32	0.919	4.69–5.44	<.001
Back pain	12 months follow-up	2.77				
Numerical pain scale	Before procedure	7.96	3.50	0.201	5.16–5.95	<.001
Leg pain	12 months follow-up	2.4				

CI = confidence interval, NRS = numerical rating scale, SD = standard deviation.

After 12 months, the median values for both groups in terms of back and leg pain dropped to 2, as shown in the box plots in Figure 2. The box plots represent the median, the box the 25th to 75th percentile, and the branches the range of the data, with a significant difference before and after the procedure. Furthermore, there was a statistically significant improvement in the quality of life, as indicated by the ODI and EQ-VAS questionnaires (Fig. 3), with $P < .001$. The responses of patients before the procedure and at the 1-year follow-up, according to the 5-dimensional EQ-5D-5L questionnaire, are summarized in Table 3 and Figure 3.

We formally evaluated the efficacy of endoscopic discectomy treatment in patients with sciatica. To do this, we tested statistical hypotheses about the equality of medians in the probability distributions of questionnaire responses in the patient groups against prespecified 1-sided alternative hypotheses. The alternative hypotheses stated that the distribution of response scores in the posttreatment group is stochastically smaller and has better quality-of-life measures than the distribution of response scores in the pretreatment group in terms of mobility, self-care, everyday activities, pain/discomfort, and anxiety/depression as defined in the 5-dimensional questionnaire EQ-5D-5L. Alternatively, we estimated the corresponding values of the reliability parameters along with the associated 2-sided 95% CIs. Alternatively, we estimated the corresponding values of the reliability parameters along with the associated 2-sided 95% CIs. As a summary measure, we also considered the combined (weighted) questionnaire responses using a carefully determined weighting. In particular, for each subject the combined value Q was calculated by the following formula: $Q = 1 - w_0 - w_1 - w_2 - w_3 - w_4 - w_5$, with the weights specified in Table 3. For more details on the different weighing strategies and for discussion on selecting the specific weights see, for example, Rapčan et al,[12] and Szende.[13]

Table 4 shows the calculated P values of the Wilcoxon rank-sum test and the estimated values of the reliability parameter R with the associated 2-sided 95% CI. These results are presented for all hypotheses about the equality of the probability distributions of the questionnaire responses in the patient groups considered for each of the 5 dimensions of the EQ-5D-5L questionnaire, that is mobility, self-care, everyday activities, pain/discomfort, anxiety/depression, and the combined weighted questionnaire score. The Wilcoxon rank-sum test convincingly rejected all considered null hypotheses of equality of the medians of the distributions of the questionnaire responses before and after treatment against the stated 1-sided alternative, which states that the respective dimension of the EQ-5D-5L questionnaire response is better after treatment than before treatment (After < Before). Furthermore, the estimated values of the reliability parameters and the corresponding 95% CIs show that the calculated values for all considered EQ-5D-5L dimensions are higher than 0.5, which clearly confirms the results of the statistical tests.

4. Discussion

This study aimed to evaluate clinical parameters, including pain intensity, ODI, and EQ-5D-5L scores, in patients with acute sciatica pain before and after endoscopic discectomy in the Slovak and Czech Republic. The study aimed to test the comprehensibility and face validity of EQ-5D-5L scores compared to other health measurement tools such as the ODI and NRSs for pain.

For all patients with LHD in the clinical trial, conservative therapy was initiated after the onset of clinical symptoms for 6 to 8 weeks. This included a combination of steroids, nonsteroidal anti-inflammatory drugs, physical therapy, epidural steroid injections, and rest. If the clinical state did not improve or worsened over time, the treatment strategy was changed to an endoscopic surgical approach. Although there are no proven differences in outcome between conservative and surgical treatment, timely neurosurgical treatment can lead to good long-term results. Nerve decompression performed at the right time is a good prognostic factor for neuronal recovery and can help prevent further irreversible neuronal damage and pain chronification.[14,15]

Among the monitored patients, the majority had a single-level herniated intervertebral disc in the intervertebral spaces L5/S1 (108 patients) and L4/L5 (169 patients). We observed a persistent improvement in the perception of back pain and pain radiating to the lower limbs, with a significant improvement in quality of life 1 year after the follow-up as measured by the EQ-VAS visual scale and the Oswestry disability questionnaire. These findings are consistent with the results of other clinical trials using various surgical approaches for lumbar herniated discs. A systematic review and meta-analysis of 99 articles focused on various spinal conditions found that spine surgery was associated with improved HRQoL for all groups in which postoperative scores were measured. The analysis included 22,312 cases for EQ-5D utilities, 2312 cases for SF-6D utilities, and 11,927 cases for SF-36 PCS scores with a median follow-up time of 12 months. Clinical trials monitoring the treatment of lumbar herniated discs with open surgical approaches, such as hemilaminectomy and microdiscectomy, have also shown the benefit of the procedures and improved patient quality of life after surgery. However, open surgery is associated with disadvantages such as extensive retraction and dissection of paraspinal muscles, longer operating time, larger wounds, and bone resection.[10,16] Minimally invasive surgical approaches offer several advantages over open surgery, including the preservation of paraspinal structures and a reduction in postoperative pain, which often leads to earlier discharge. Additionally, these procedures can be performed under local anesthesia.[16]

A meta-analysis was conducted to compare the effectiveness of open lumbar surgery with lumbar endoscopic discectomy. The analysis included 9 randomized controlled trials with 1092 patients. The results showed that endoscopic surgery had slightly better clinical outcomes than open surgery based on

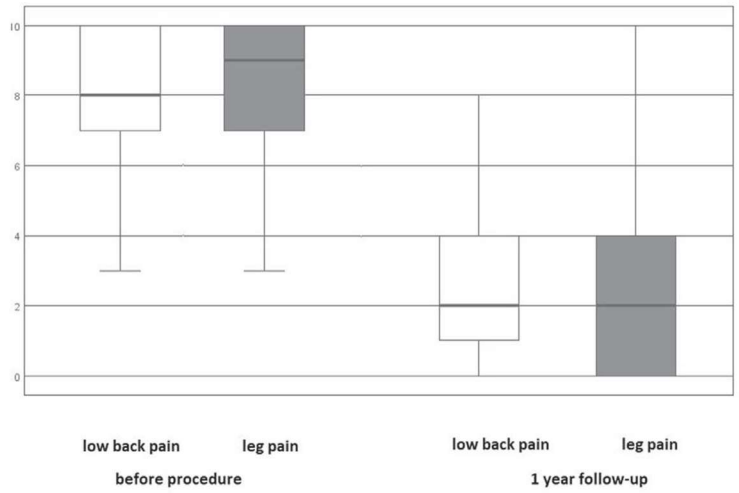


Figure 2. Evaluation of patients' scores on the Numerical Pain Scale (NRS) for lower limb and back pain, before treatment, and 12 months after treatment.

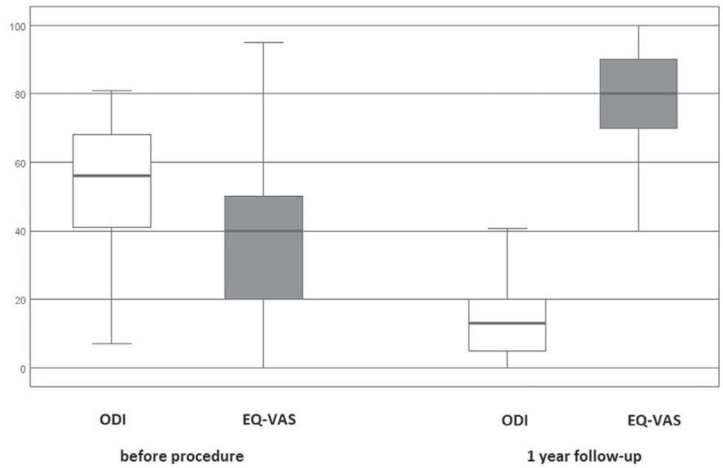


Figure 3. Evaluation of patients' scores of Oswestry Disability Index (ODI) and overall health score (EQ-VAS) before treatment, and 12 months after treatment. Numerical scale (0–100) of ODI and EQ-VAS before the procedure and at the 1-year follow-up.

the Macnab criteria, but this difference was not clinically significant. However, patients who underwent endoscopic surgery reported significantly higher satisfaction rates, lower intraoperative blood loss volume, and shorter hospital stays.[6]

Similarly, a meta-analysis compared the efficacy of PELD with other surgeries for LDH and demonstrated better outcomes of PELD in 14 clinical trials involving a total of 2528 patients. PELD had a shorter duration of surgery, lower blood loss, and similar complications compared to other surgeries for LDH. However, PELD operations resulted in a higher recurrence rate (relative risk = 1.65, 95% CI: 1.08–2.52; $P = .021$). There were no significant differences in quality of life and ODI before and after the procedure between the interventions. The study's limitation was the relatively low number of clinical trials included.[5] Nevertheless, published results concerning open-operative techniques are also associated with a good prognosis.

To evaluate the quality of life, we chose the EQ-5D-5L questionnaire as it offers a comprehensive assessment of a patient's health status. This questionnaire measures 5 dimensions of perceived problems, each with 5 levels of severity, which improves sensitivity and reduces the ceiling effect. The EQ-VAS scale, which is easier to complete and score, was also used. The "Usual activities" dimension evaluates a patient's performance in work, studying, housework, family, or leisure activities, as well as other dimensions like mobility, self-care, everyday activities, and anxiety/depression. Our analysis shows

Table 3

The weights used for computing the weighted questionnaire value Q, which combines the information from all 5 dimensions of the EQ-5D-5L questionnaire.

Weight	Value	EQ-5D-5L dimension	Condition
w0	0.1279	All	Any level in questionnaire is 2 or 3
w0	0.2288	All	Any level in questionnaire is 4 or 5
w0	0.0000	All	Otherwise
w1	0.0659	Mobility	Mobility level is 2 or 3
w1	0.1829	Mobility	Mobility level is 4 or 5
w1	0.0000	Mobility	Otherwise
w2	0.1173	Self-care	Self-care level is 2 or 3
w2	0.1559	Self-care	Self-care level is 4 or 5
w2	0.0000	Self-care	Otherwise
w3	0.0264	Usual activities	Usual activities level is 2 or 3
w3	0.0860	Usual activities	Usual activities is 4 or 5
w3	0.0000	Usual activities	Otherwise
w4	0.0930	Pain/discomfort	Pain/discomfort level is 2 or 3
w4	0.1639	Pain/discomfort	Pain/discomfort level is 4 or 5
w4	0.0000	Pain/discomfort	Otherwise
w5	0.0891	Anxiety/depression	Anxiety/depression level is 2 or 3
w5	0.1290	Anxiety/depression	Anxiety/depression level is 4 or 5
w5	0.0000	Anxiety/depression	Otherwise

For each subject, the value Q was calculated by the formula
$Q = 1 - w_0 - w_1 - w_2 - w_3 - w_4 - w_5$, with the specified weights.

Table 4

Calculated *P* values of the Wilcoxon rank-sum test and the estimated values of the reliability parameter *R*.

EQ-5D-5L dimension	Alternative hypothesis	Wilcoxon test *P* value	Reliability parameter *R* *Prob (After < Before)*	Lower	Upper
Mobility	After<Before	<.001	0.9112	0.8911	0.9298
Self-care	After<Before	<.001	0.9224	0.9031	0.9402
Usual activities	After<Before	<.001	0.9093	0.8881	0.9291
Pain/discomfort	After<Before	<.001	0.9320	0.9140	0.9486
Anxiety/depression	After<Before	<.001	0.6353	0.6051	0.6650
Weighted questionnaire	After<Before	<.001	0.9426	0.9258	0.9583

Calculated *P* values of the Wilcoxon rank-sum test and the estimated values of the reliability parameter *R* (shown together with the associated 2-sided 95% confidence interval) for 5 dimensions of the EQ-5D-5L questionnaire: mobility, self-care, usual activities, pain/discomfort, anxiety/depression, and also the combined weighted questionnaire result. These are used for testing the null hypothesis of equality of medians of the distributions of the questionnaire responses before and after treatment against the specified 1-sided alternative which specifies that the particular dimension of the EQ-5D-5L questionnaire response is better after the treatment than before the treatment (After < Before). The null hypothesis is rejected if the result of the Wilcoxon test is considered statistically significant (here at $P < 0.05$) or alternatively, if the reliability parameter *R* and all values included in the associated 95% confidence interval are above 0.5.

a significant improvement in the quality of life lasting >1 year after endoscopic surgery ($P < .001$). We also calculated the statistically weighted questionnaire values for each dimension of the EQ-5D-5L questionnaire in the Slovak and Czech region, which also indicate a significant improvement in social quality of life ($P < .001$).

Despite our study involving patients with a mean age of 46.3 years, the clinical improvement, reduction in lower limb pain, and improved quality of life after surgery are consistent with a 2-year follow-up study by Peng et al[6] in younger patients with a mean age of 35.6 years.

An interesting finding from a prospective cohort study by Kapetanakis et al[4] is that neither gender, muscle mass, nor body mass index has a significant effect on the final outcome of endoscopic discectomy.

A systematic analysis of clinical trials comparing the effectiveness of transforaminal and interlaminar endoscopic approaches in treating LHD, which reviewed 26 clinical trials involving 3294 patients, demonstrated a significant therapeutic benefit with both interventional approaches. However, the transforaminal approach was associated with higher efficacy, shorter operation time, and lower blood loss.[7]

In our clinical study, we selected the safest and most effective possible approach for each patient. For patients with higher levels of L3/4 and L4/5 herniations, we preferred the transforaminal approach. For herniations in the lower spinal segments L5/S1 due to anatomical conditions of the pelvis, we chose the interlaminar approach more often than the transforaminal approach. Comparing both approaches, we did not observe a higher percentage of complications or re-herniation.

We consider analgosedation with monitoring as a safe and beneficial method for endoscopic discectomy. It allows for effective communication with the patient, ensuring safety and minimizing the risk of nerve damage. Additionally, it promotes early recovery and a faster return to consciousness, while reducing the occurrence of postoperative nausea and vomiting. Despite potential challenges in airway access, we did not encounter any cases of acute respiratory failure requiring intubation.

4.1. Limitations

Our clinical trial on acute sciatica treatment had limitations that should be addressed. Firstly, using a prospective observational design instead of a randomized controlled trial introduced the potential for selection bias. The absence of a control group made it challenging to compare treatment effectiveness accurately. Including a control group would have enabled a more robust evaluation of endoscopic discectomy, microdiscectomy, and conservative treatments. The study involved 470 patients, but a broader age range (18–79 years) may have influenced the results. Additionally, a loss to follow-up of 39 patients resulted in missing data for statistical analysis, potentially impacting the robustness and generalizability of our findings. Future studies should minimize loss to follow-up and ensure comprehensive data collection. Recognizing these limitations is crucial for interpreting our findings accurately and planning future research in acute sciatica treatment.

5. Conclusions

Endoscopic discectomy is a highly effective minimally invasive surgical method for treating LHD, with a significant impact on the patient's clinical state. Clinical assessments of the quality of life using different types of measurement instruments are useful in demonstrating the efficacy of pain-treating interventions.

Author contributions

Conceptualization: Ladislav Kočan, Juraj Mláka, Róbert Tirpák.
Data curation: Róbert Rapčan, Ladislav Kočan, Viktor Witkovsky.
Formal analysis: Róbert Rapčan, Ladislav Kočan, Viktor Witkovsky, Miroslav Gajdoš.
Funding acquisition: Ladislav Kočan, Simona Rapčanová, Juraj Mláka.
Investigation: Ladislav Kočan, Viktor Witkovsky, Simona Rapčanová, Juraj Mláka, Róbert Tirpák, Hana Kočanová.
Methodology: Ladislav Kočan, Hana Kočanová.
Project administration: Ladislav Kočan, Janka Vašková.
Resources: Ladislav Kočan, Miroslav Burianek, Janka Vašková.
Software: Róbert Rapčan.
Supervision: Róbert Rapčan, Ladislav Kočan, Juraj Mláka.
Validation: Róbert Rapčan, Ladislav Kočan, Miroslav Burianek.
Visualization: Miroslav Burianek.
Writing – original draft: Ladislav Kočan, Simona Rapčanová, Róbert Tirpák, Hana Kočanová.
Writing – review & editing: Ladislav Kočan, Simona Rapčanová, Hana Kočanová.

References

[1] Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396:1204–22.

[2] Zhou T, Ma T, Gu Y, et al. Percutaneous Transforaminal Endoscopic Surgery (PTES) for treatment of lumbar degenerative disease in patients with underlying diseases: a retrospective cohort study of 196 Cases. J Pain Res. 2023;16:1137–47.

[3] van der Windt DA, Simons E, Riphagen II, et al. Physical examination for lumbar radiculopathy due to disc herniation in patients with low-back pain. Cochrane Database Syst Rev. 2010;2:CD007431.

[4] Gadjradj P, Biswadjiet H, Amelink J, et al. Percutaneous transforaminal endoscopic discectomy versus open microdiscectomy for lumbar disc herniation: a systematic review and meta-analysis. Spine. 2021;46:538–49.

[5] Mlaka J, Rapcan R, Burianek M, et al. Endoscopic discectomy as an effective treatment of a herniated intervertebral disc. Brat Lek Listy. 2020;121:199–205.

[6] Cong L, Zhu Y, Tu G. A meta-analysis of endoscopic discectomy versus open discectomy for symptomatic lumbar disk herniation. Eur Spine J. 2016;25:134–43.

[7] Pak DJ, Yong RJ, Kaye AD, et al. Chronification of pain: mechanisms, current understanding, and clinical implications. Curr Pain Headache Rep. 2018;22:9.

[8] Gempt J, Jonek M, Ringel F, et al. Long-term follow-up of standard microdiscectomy versus minimal access surgery for lumbar disc herniations. Acta Neurochir. 2013;15:2333–8.

[9] Bosković K, Cigić T, Grajić M, et al. The quality of life of patients after a lumbar microdiscectomy: a four-year monitoring study. Clin Neurol Neurosurg. 2010;112:557–62.

[10] Kotz, S., Lumelskii, Y., Pensky, M. The stress-strength model and its generalizations: theory and applications. WorldScientific: NewYork, 2003; pp. 272.

[11] Zhou W. Statistical inference for P(X<Y). Stat Med. 2008;27:257–79.

[12] Rapčan R, Kočan L, Witkovsky V, et al. EQ-5D-5L questionnaire as suitable assessment of quality of life after epiduroscopy: multicenter randomized double-blind pilot study. Wien Klin Wochenschr. 2020;132:526–34.

[13] Szende, A., Oppe, M., Devlin, N. (2007). EQ-5D value sets: inventory, comparative review and user guide. Springer: London, 2007; pp. 91.

[14] Foster NE, Konstantinou K, Lewis M, et al. The clinical and cost-effectiveness of stratified care for patients with sciatica: the SCOPiC randomised controlled trial protocol (ISRCTN75449581). BMC Musculoskelet Disord. 2017;18:172.

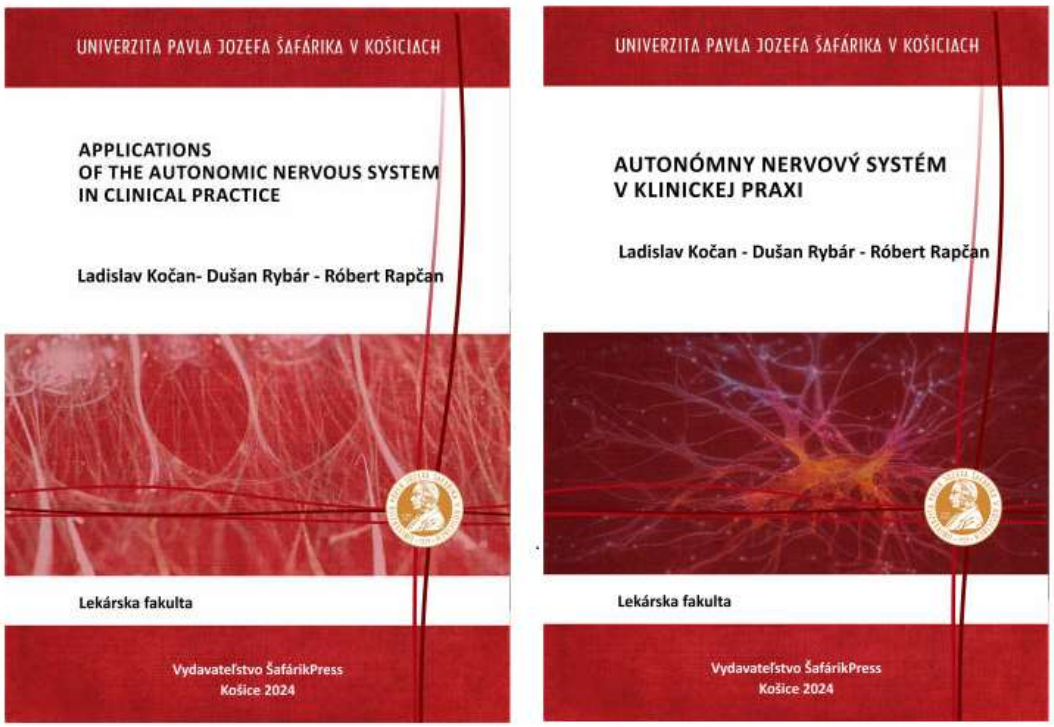
[15] Stochkendahl M, Kjaer P, Hartvigsen J, et al. National clinical guidelines for non-surgical treatment of patients with recent onset low back pain or lumbar radiculopathy. Eur Spine J. 2018;27:60–75.

[16] Kapetanakis S, Gkasdaris G, Thomaidis T, et al. Comparison of quality of life between men and women who underwent transforaminal percutaneous endoscopic discectomy for lumbar disc herniation. Int J Spine Surg. 2018;12:475–82.

7 UČEBNICOVÉ VYSOKOŠKOLSKÉ TEXTY

Ďalším našim významným počinom bolo vydanie dvoch publikácií učebnicových vysokoškolských textov zameraných na špecifiká vegetatívneho nervového systému v slovenskom a anglickom jazyku.

Cieľom týchto publikácii je poskytnúť komplexný a objektívny pohľad na anatomické a fyziologické špecifiká vegetatívneho nervového systému a implementáciu týchto znalostí do klinickej praxe. V publikácii popisujeme v rámci jednotlivých anatomických úsekov vegetatívneho nervového systému možnosti terapeutických intervencií pre vybrané diagnózy. Efektivita jednotlivých intervenčných výkonov pre dané ochorenie je vyjadrená v texte úrovňou dôkazov podľa aktuálnych odporúčaní medicíny založenej na dôkazoch. Publikácie sú určené predovšetkým pre študentov piateho a šiesteho ročníka všeobecného lekárstva a pre lekárov pracujúcich v odbore anestéziológia, intenzívna medicína a algeziológia.



- ***Kočan L**, Rybár D, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. https://doi.org/10.33542/ANS-0304-3.*
- ***Kočan L**, Rybár D, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. https://doi.org/10.33542/ANS-0312-8.*

Kapitoly v slovenskom jazyku

- ***Kočan L***. 1 MEDICÍNA ZALOŽENÁ NA DÔKAZOCH. In *Kočan **L**, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 36 – 41.*

- ***Kočan L***. 2 VEGETATÍVNY SYSTÉM CERVIKO-TORAKÁLNEJ OBLASTI V KLINICKEJ PRAX. In *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 42-52.*

- ***Kočan L***. 3 AUTONÓMNA KONTROLA KARDIOVASKULÁRNEHO SYSTÉMU PROSTREDNÍCTVOM BARORECEPTOROV In: *Kočan L*. In *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 53-56.*

- ***Kočan L***. 4 AUTONÓMNA REGULÁCIA SRDCA PRI SRDCOVOM ZLYHANÍ. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 57-64.*

- ***Kočan L***. 5 CHEMORECEPTORY. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 67 – 74.*

- ***Kočan L***. 6 VEGETATÍVNA INERVÁCIA PĽÚC A JEJ ÚČASŤ V PATOGENÉZE VYBRANÝCH PĽÚCNYCH OCHORENÍ. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 67 -74.*

- ***Kočan L***. 7 HRUDNÝ SYMPATIKUS. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 75 – 84.*

- ***Kočan L***. 8 ONKOLOGICKÁ ABDOMINÁLNA BOLESŤ. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 83 - 94.*

- ***Kočan L***. 9 LUMBÁLNY SYMPATIKUS. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 95 -103.*

- ***Kočan L***. 10 DYSFUNKCIE KRANIÁLNEHO PARASYMPATIKOVÉHO SYSTÉMU. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 104 – 117.*

- ***Kočan L***. 11 KLINICKÝ VÝZNAM PÔSOBENIA BLÚDIVÉHO NERVU. In: *Kočan **L**, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 118 – 126.*

- ***Kočan L***. 12 MOŽNOSTI OVPLYVNENIA SAKRÁLNEJ PARASYMPATIKOVEJ INERVÁCIE. In: *Kočan L, Rybár R, Rapčan R. Autonómny nervový systém v klinickej praxi. ŠafárikPress Publishing. 2024 ISBN 978-80-574-0304-3. s 127 – 128.*

Kapitoly v anglickom jazyku

- ***Kočan L***. 1 EVIDENCE-BASED MEDICINE. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 35 – 39.*

- ***Kočan L***. 2 THE AUTONOMIC SYSTEM OF CERVICO-THORACIC REGION IN CLINICAL PRACTICE. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 40 – 50.*

- ***Kočan L***. 3 AUTONOMOUS CONTROL OF THE CARDIOVASCULAR SYSTEM VIA BARORECEPTORS. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 51 – 53.*

- ***Kočan L***. 4 AUTONOMIC REGULATION OF THE HEART IN HEART FAILURE. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. 54 – 60.*

- ***Kočan L***. 5 CHEMORECEPTORS. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. 61 – 62.*

- ***Kočan L***. 6 AUTONOMIC INNERVATION OF THE LUNGS AND ITS INVOLVEMENT IN THE PATHOGENESIS OF SELECTED DISEASES. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 63 – 70.*

- ***Kočan L***. 7 THORACIC SYMPATHETIC INNERVATION. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 71 – 80.*

- ***Kočan L***. 8 ONCOLOGICAL ABDOMINAL PAIN. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 81 – 90.*

- ***Kočan L***. 9 LUMBAR SYMPATHETIC CHAIN. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 91 -90.*

- ***Kočan L***. 10 DYSFUNCTION OF THE CRANIAL PARASYMPATHETIC SYSTEM. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 100 -112.*

- ***Kočan L***. 11 CLINICAL SIGNIFICANCE OF THE VAGUS NERVE. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 113 – 121.*

- ***Kočan L***. 12 THERAPEUTIC APPROACHES TARGETING SACRAL PARASYMPATHETIC INNERVATION. In: *Kočan L, Rybár R, Rapčan R. Applications of the Autonomic Nervous System in Clinical Practice. ŠafárikPress Publishing 2024. ISBN 978-80-574-0312-8. s 122 – 123.*

8 VZDELÁVACIE AKTIVITY TEMATICKY SA VZŤAHUJÚCE K HABILITAČNEJ PRÁCI A GRANTOVÉ PROJEKTY

8.1 Prednášky na medzinárodných podujatiach

- ***Kočan L*** *,Vašková J, Vaško L. Kočanová H. Oxidative stress and opioids. XVII. česko-slovenské dialógy o bolesti a XXIII. slovenské dialógy o bolesti. Bratislava 2015*

- ***Kočan L****, Rapčan R, Burianek M, Kočanová H. Medicína založená na dôkazoch v intervenčnej algeziológii.IM, XVII. česko-slovenské dialógy o bolesti a XXIII. slovenské dialógy o bolesti. Bratislava 2015*

- *Rapčan R,* ***Kočan L****, Burianek M, Mláka J, Rapčanová S, Griger M, Gajdoš M. Effect of drugs administration in to the epidural space during epiduroscopy. The symposium on clinical pain trials in Europe. London 2017*

- ***Kočan L****, Rapčan R, Wikovský V, Kočanová H. EQ-5D-5L questionnaire as a suitable assessment of quality of life after percutaneous endoscopic techniques in treatment of low*

back pain XXI. česko-slovenské dialógy o bolesti a XXVII. slovenské dialógy o bolesti. Slovenská lekárska spoločnosť, Stará Lesná, Vysoké Tatry 2019

- *Šimonová J, Vašková J, Ogurčáková D, **Kočan L**. Martuliak I, Rapčan R, Šimon R. Antioxidant capacities of patients with chronic pain after strong opioid treatment. Pain in Europe XI Valencia, European Pain Federation EFIC 2019.*

- *Rapčan R, **Kočan L**, Hess M, Mláka J, Griger M, Poliak Ľ, Matias M, Burianek M. Comparison of clinical outcomes after endoscopic discectomy between interventional pain physician and spine surgeon. The 24th Annual Gabor Racz Advanced Interventional Pain Conference and Workshop. World Institute of Pain 2019.*

- ***Kočan L**, Rapčan R, Depta F, Rybár D, Kolesár A, Varhol J. Cryoablation as a suitable pain relieving method after thoracotomy. XXIII.CESKO-SLOVENSKE DIALOGY O BOLESTI, Zvolen 2022*

- ***Kočan L**. Lumbar Sympatholysis. STEP BY STEP APPROACH TO FIPP EXAM. EPC. Essen 2022.*

- *Rapčan R, **Kočan L**, Mláka J, Matias M, Łokas K, Tirpák R. Percutaneous lumbar endoscopic discectomy. ESRA Congress. Thessaloniki 2022*

8.2 Popularizačné aktivity tematicky sa vzťahujúce k habilitačnej práci

- USG v liečbe bolesti- Hands on Workshop, Zvolen, 2022 - inštruktor

- 1st PANEUROPE INTERVENTIONAL PAINMANAGEMENT SERIES
  USG navigation, Praha 2023 - inštruktor

- ADVANCES IN PAIN THERAPY - RTG navigation, Frankfurt 2024 - inštruktor

8.3 Grantové projekty

1. Európsky fond regionálneho rozvoja. Operačný program Integrovaná infraštruktúra pre projekt „Návrh a implementácia pokročilých metód ventilačnej liečby a diagnostiky vírusových pneumónií vrátane COVID-19 s možnosťou ich rýchleho osvojenia“, kód 313011ASX1, akronym IPMVDCov, spolufinancovaný zo zdrojov Európskeho fondu regionálneho rozvoja v spolupráci s Ministerstvom školstva, výskumu, vývoja a mládeže Slovenskej republiky. Trvanie grantu: 02/2020 - 06/2023 - **Spoluriešiteľ**

2. Európsky fond regionálneho rozvoja. Operačný program Integrovaná infraštruktúra pre projekt „Výskum a vývoj systému zefektívnenia ventilácie pacientov s COVID-19 alebo iným nehomogénnym poškodením pľúc" kód 313011ATG9 spolufinancovaný zo zdrojov Európskeho fondu regionálneho rozvoja v spolupráci s Ministerstvom školstva, výskumu, vývoja a mládeže Slovenskej republiky. Trvanie grantu: 04/2021 - 05/2023 - **Spoluriešiteľ**

3. Grant Slovenskej spoločnosti pre štúdium a liečbu bolesti. „Termovíziou kontrolované lumbálne sympatikové blokády pri ischemickej chorobe dolných končatín“. Číslo grantu 24-3055/069. Trvanie grantu: 1/2024-31/2025 - **Hlavný riešiteľ**

ZOZNAM POUŽITÝCH SKRATIEK A SYMBOLOV

APACHE	Acute Physiology and Chronic Health Evaluation
AR	adrenoreceptory
ASIPP	Americká spoločnosť intervenčných lekárov bolesti
ARDS	syndróm akútnej respiračnej tiesne
AT2	pneumocyty II typu
AT1	pneumocyty I typu
BAX	Bcl-2-like protein 4
BCL2	B-cell lymphoma 2
BIM	Bcl-2-like protein 11
ECM	extracelulárna matrix
EBM	medicíny založenej na dôkazoch
EGF	Epidermal growth factor (rastový faktor epidermy)
FAS	Fas receptor
GPx	glutatiónperoxidáza
GR	glutatiónreduktáza
GSSG	oxidovaný glutatión
GSH	redukovaný glutatión
H_2O_2	peroxid vodíka
$HO^{\bullet}$	hydroxylový radikál
ICHDK	ischemická choroba dolných končatín
IL	interleukín
MAPK	mitogénom aktivovaná proteínová kináza,
MERS-CoV	Middle-East-Respiratory-Syndrome coronavirus
MMPs	matrixové metaloproteinázy
MnSOD	Superoxiddizmutáza (Mn-kofaktor)
NF-κB	nukleárny faktor kappa B
NK c-Jun	N-terminal kinase
NO	oxid dusnatý
NOS	syntáza oxidu dusnatého
NOX	NADPH oxidáza
$O_2^{\bullet-}$	superoxidový radikál
P2R	Purinérgny 2 receptor
PLD2	fosfolipáza D2
RNS	reaktívna forma dusíka
ROS	Reaktívne formy odvodené od kyslíka
SARS-CoV	Severe-Acute-Respiratory-Syndrome coronavirus
SOD	superoxiddizmutáza
τ	časová konštanta
TGF-β	transformujúci rastový faktora-β1
TLR	Toll-like receptor
TNF-α	tumor nekrotizujúci faktor-α
ORs	Opioidné receptory
$ONOO^-$	peroxynitrit (peroxynitritový anión)
UGT	UDP-glukuronozyltransferáza
VEGF	Vascular endothelial growth factor (rastový faktor cievneho endotelu)

ZOZNAM OBRÁZKOV

ZOZNAM TABULIEK

ZOZNAM PRÍLOH

Literatúra

1. Firment J, Hudák V, Capková J, ,Šimonová J, Grochová M. Aktualizácia medzinárodných odporúčaní pre manažment sepsy a septického šoku. Anestéziológia a intenzívna medicína. (2021). 2021;10(2)
2. Evans L, Rhodes A, Alhazzani W, Antonelli M, Coopersmith CM, French C, Machado FR, Mcintyre L, Ostermann M, Prescott HC, Schorr C, Simpson S, Wiersinga WJ, Alshamsi F, Angus DC, Arabi Y, Azevedo L, Beale R, Beilman G, Belley-Cote E, Burry L, Cecconi M, Centofanti J, Coz Yataco A, De Waele J, Dellinger RP, Doi K, Du B, Estenssoro E, Ferrer R, Gomersall C, Hodgson C, Møller MH, Iwashyna T, Jacob S, Kleinpell R, Klompas M, Koh Y, Kumar A, Kwizera A, Lobo S, Masur H, McGloughlin S, Mehta S, Mehta Y, Mer M, Nunnally M, Oczkowski S, Osborn T, Papathanassoglou E, Perner A, Puskarich M, Roberts J, Schweickert W, Seckel M, Sevransky J, Sprung CL, Welte T, Zimmerman J, Levy M. Surviving sepsis campaign: international guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021 Nov;47(11):1181-1247. doi: 10.1007/s00134-021-06506-y.
3. Valenta J, Brodska H, Drabek T, Hendl J, Kazda A. High-dose selenium substitution in sepsis: a prospective randomized clinical trial. Intensive Care Med. 2011 May;37(5):808-15. doi: 10.1007/s00134-011-2153-0.
4. Andrews PJ, Avenell A, Noble DW, Campbell MK, Battison CG, Croal BL, Simpson WG, Norrie J, Vale LD, Cook J, de Verteuil R, Milne AC; Trials Management Group. Randomised trial of glutamine and selenium supplemented parenteral nutrition for critically ill patients. Protocol Version 9, 19 February 2007 known as SIGNET (Scottish Intensive care Glutamine or seleNium Evaluative Trial). Trials. 2007 Sep 20;8:25. doi: 10.1186/1745-6215-8-25.
5. Vašková, J., Vaško, L., Kron, I.: Oxidative processes and antioxidative metaloenzymes. In: Antioxidant enzyme. - Rijeka : Intech, 2012. - ISBN 9789535107897. - S. 19-58 .
6. Zhang YY, Ning BT. Signaling pathways and intervention therapies in sepsis. Signal Transduct Target Ther. 2021 Nov 25;6(1):407. doi: 10.1038/s41392-021-00816-9.
7. Geoghegan M, McAuley D, Eaton S, Powell-Tuck J. Selenium in critical illness. Curr Opin Crit Care. 2006 Apr;12(2):136-41. doi: 10.1097/01.ccx.0000216581.80051.d6.
8. Hatwalne MS. Free radical scavengers in anaesthesiology and critical care. Indian J Anaesth. 2012 May;56(3):227-33. doi: 10.4103/0019-5049.98760.

9. Vetrani C, Costabile G, Di Marino L, Rivellese AA. Nutrition and oxidative stress: a systematic review of human studies. Int J Food Sci Nutr. 2013 May;64(3):312-26. doi: 10.3109/09637486.2012.738651.
10. Tsermpini EE, Glamočlija U, Ulucan-Karnak F, Redenšek Trampuž S, Dolžan V. Molecular Mechanisms Related to Responses to Oxidative Stress and Antioxidative Therapies in COVID-19: A Systematic Review. Antioxidants (Basel). 2022 Aug 19;11(8):1609. doi: 10.3390/antiox11081609.
11. Firment J. Studená A. a kol. Anesteziológia a intenzívna medicína. 2014. ŠafárikPress. st.285-309. ISBN 978-80-8152-084-6.
12. Singer P, Blaser AR, Berger MM, Calder PC, Casaer M, Hiesmayr M, Mayer K, Montejo-Gonzalez JC, Pichard C, Preiser JC, Szczeklik W, van Zanten ARH, Bischoff SC. ESPEN practical and partially revised guideline: Clinical nutrition in the intensive care unit. Clin Nutr. 2023 Sep;42(9):1671-1689. doi: 10.1016/j.clnu.2023.07.011.
13. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, McCarthy MS, Davanos E, Rice TW, Cresci GA, Gervasio JM, Sacks GS, Roberts PR, Compher C; Society of Critical Care Medicine; American Society for Parenteral and Enteral Nutrition. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr. 2016 Feb;40(2):159-211. doi: 10.1177/0148607115621863.
14. Vetrani C, Costabile G, Di Marino L, Rivellese AA. Nutrition and oxidative stress: a systematic review of human studies. Int J Food Sci Nutr. 2013 May;64(3):312-26. doi: 10.3109/09637486.2012.738651.
15. Tsermpini EE, Glamočlija U, Ulucan-Karnak F, Redenšek Trampuž S, Dolžan V. Molecular Mechanisms Related to Responses to Oxidative Stress and Antioxidative Therapies in COVID-19: A Systematic Review. Antioxidants (Basel). 2022 Aug 19;11(8):1609. doi: 10.3390/antiox11081609.
16. Firment J, Hulin I. Zahorec index or Neutrophil-to-lymphocyte ratio, valid biomarker of inflammation and immune response to infection, cancer and surgery. Bratisl Lek Listy. 2024;125(2):75-83. doi: 10.4149/BLL_2024_012. PMID: 38219059.
17. Manzanares W, Dhaliwal R, Jiang X, Murch L, Heyland DK. Antioxidant micronutrients in the critically ill: a systematic review and meta-analysis. Crit Care. 2012 Dec 12;16(2):R66. doi: 10.1186/cc11316.

18. Kuklinski B, Zimmermann T, Schweder R. Letalitätssenkung der akuten Pankreatitis mit Natriumselenit. Klinische Resultate einer vierjährigen Antioxidanzientherapie [Decreasing mortality in acute pancreatitis with sodium selenite. Clinical results of 4 years antioxidant therapy]. Med Klin (Munich). 1995 Jan 15;90 Suppl 1:36-41. German. PMID: 7715583.
19. Angstwurm MW, Engelmann L, Zimmermann T, Lehmann C, Spes CH, Abel P, Strauss R, Meier-Hellmann A, Insel R, Radke J, Schüttler J, Gärtner R. Selenium in Intensive Care (SIC): results of a prospective randomized, placebo-controlled, multiple-center study in patients with severe systemic inflammatory response syndrome, sepsis, and septic shock. Crit Care Med. 2007 Jan;35(1):118-26. doi: 10.1097/01.CCM.0000251124.83436.0E.
20. Sakr Y, Reinhart K, Bloos F, Marx G, Russwurm S, Bauer M, Brunkhorst F. Time course and relationship between plasma selenium concentrations, systemic inflammatory response, sepsis, and multiorgan failure. Br J Anaesth. 2007 Jun;98(6):775-84. doi: 10.1093/bja/aem091.
21. Forceville X, Laviolle B, Annane D, Vitoux D, Bleichner G, Korach JM, Cantais E, Georges H, Soubirou JL, Combes A, Bellissant E. Effects of high doses of selenium, as sodium selenite, in septic shock: a placebo-controlled, randomized, double-blind, phase II study. Crit Care. 2007;11(4):R73. doi: 10.1186/cc5960.
22. Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, Kumar A, Sevransky JE, Sprung CL, Nunnally ME, Rochwerg B, Rubenfeld GD, Angus DC, Annane D, Beale RJ, Bellinghan GJ, Bernard GR, Chiche JD, Coopersmith C, De Backer DP, French CJ, Fujishima S, Gerlach H, Hidalgo JL, Hollenberg SM, Jones AE, Karnad DR, Kleinpell RM, Koh Y, Lisboa TC, Machado FR, Marini JJ, Marshall JC, Mazuski JE, McIntyre LA, McLean AS, Mehta S, Moreno RP, Myburgh J, Navalesi P, Nishida O, Osborn TM, Perner A, Plunkett CM, Ranieri M, Schorr CA, Seckel MA, Seymour CW, Shieh L, Shukri KA, Simpson SQ, Singer M, Thompson BT, Townsend SR, Van der Poll T, Vincent JL, Wiersinga WJ, Zimmerman JL, Dellinger RP. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017 Mar;43(3):304-377. doi: 10.1007/s00134-017-4683-6.
23. Ranieri VM, Giuliani R, Dambrosio M, Carravetta G, Cinnella G, Brienza N, Pesce C, Marella G. Effetto della PEEP sui volumi polmonari in pazienti affetti da broncopatia cronica ostruttiva (BPCO) durante ventilazione meccanica [Effect of PEEP on the

pulmonary volume in patients with chronic obstructive bronchopathy during mechanical ventilation]. Minerva Anestesiol. 1991 Oct;57(10):848. Italian. PMID: 1961513.

24. Török P, Šalantay J, Čandík P, Drbjáková E, Saladiak S, Göryová J, Popaďák J, Lakatoš I. Trojhladinová ventilácia pľúc (3LV® – 3 level ventilation) – prvé klinické skúsenosti. Anest. intenziv. Med., 19, 2008, č. 2, s. 105–109.
25. Török P. NOVINKY V ANESTÉZIOLÓGII, ALGEZIOLÓGII A INTENZÍVNEJ MEDICÍNE 2009. CEEA. Základné princípy umelej ventilácie pľúc a základné ventilačné režimy. 2009. s. 117-132. ISBN 978-80-89334-03-2.
26. Čandík P, Rybár D, Depta F, Sabol F, Kolesár A, Galková K, Török P, Doničová V, Imrecze Š, Nosáľ M, Donič V. Relationship between dynamic expiratory time constant tau(edyn) and parameters of breathing cycle in pressure support ventilation mode. Physiol Res. 2018 Dec 18;67(6):875-879. doi: 10.33549/physiolres.933750. Epub 2018 Sep 11. PMID: 30204464.
27. Depta F, Török P, G Miller A, Firment P, Leškanič J, Porubän A, Halaš P, Mandinec S, Filka V, Zajac H, Gentile MA, Zdravkovic M. Programmed multi-level ventilation in COVID-19-related acute respiratory distress syndrome: a multi-center retrospective observational study. J Int Med Res. 2022 May;50(5):3000605221101970. doi: 10.1177/03000605221101970. PMID: 35634917; PMCID: PMC9158417.
28. Firment P., Palenčíková E., Turčan A., Mavrodiev B. Pacienti s ochorením COVID-19 na OAIM, odporúčania a skúsenosti z druhej vlnyAnestéziolgia a intenzívna medicína, 2021; 10(1):6-16
29. Laššán Š, Laššánová M. Post-COVID-19 pľúcna fibróza a možnosti jej liečby. Praktické lekárnictvo. 2023;13(4).
30. Xu W, Zhao T, Xiao H. The Implication of Oxidative Stress and AMPK-Nrf2 Antioxidative Signaling in Pneumonia Pathogenesis. Front Endocrinol (Lausanne). 2020 Jun 17;11:400. doi: 10.3389/fendo.2020.00400.
31. Du L, Xie Y, Zheng K, Wang N, Gao M, Yu T, Cao L, Shao Q, Zou Y, Xia W, Fang Q, Zhao B, Guo D, Peng X, Pan JA. Oxidative stress transforms 3CLpro into an insoluble and more active form to promote SARS-CoV-2 replication. Redox Biol. 2021 Nov 26;48:102199. doi: 10.1016/j.redox.2021.102199.
32. Kočan L, Firment J, Pirníková I, Farkašová Iannaccone S, Rybár D, Gnoriková J, Korček J, Kočanová H, Török P, Rapčanová S, Vašková J. Full recovery of lung tissue after severe viral pneumonia H1N1: A case report with 10 years follow-up. Medicine (Baltimore). 2023 Feb 22;102(8):e33052. doi: 10.1097/MD.0000000000033052.

33. Zhao F, Ma Q, Yue Q, Chen H. SARS-CoV-2 Infection and Lung Regeneration. Clin Microbiol Rev. 2022 Apr 20;35(2):e0018821. doi: 10.1128/cmr.00188-21.
34. Yu M, Liu Y, Xu D, Zhang R, Lan L, Xu H. Prediction of the Development of Pulmonary Fibrosis Using Serial Thin-Section CT and Clinical Features in Patients Discharged after Treatment for COVID-19 Pneumonia. Korean J Radiol. 2020 Jun;21(6):746-755. doi: 10.3348/kjr.2020.0215.
35. Gupta SK, Srivastava M, Minocha R, Akash A, Dangwal S, Dandekar T. Alveolar Regeneration in COVID-19 Patients: A Network Perspective. Int J Mol Sci. 2021 Oct 19;22(20):11279. doi: 10.3390/ijms222011279.
36. Chen J, Wu H, Yu Y, Tang N. Pulmonary alveolar regeneration in adult COVID-19 patients. Cell Res. 2020 Aug;30(8):708-710. doi: 10.1038/s41422-020-0369-7.
37. Hendrix J, Nijs J, Ickmans K, Godderis L, Ghosh M, Polli A. The Interplay between Oxidative Stress, Exercise, and Pain in Health and Disease: Potential Role of Autonomic Regulation and Epigenetic Mechanisms. Antioxidants (Basel). 2020 Nov 23;9(11):1166. doi: 10.3390/antiox9111166.
38. Teixeira-Santos L, Albino-Teixeira A, Pinho D. Neuroinflammation, oxidative stress and their interplay in neuropathic pain: Focus on specialized pro-resolving mediators and NADPH oxidase inhibitors as potential therapeutic strategies. Pharmacol Res. 2020 Dec;162:105280. doi: 10.1016/j.phrs.2020.105280.
39. Carrasco C, Naziroğlu M, Rodríguez AB, Pariente JA. Neuropathic Pain: Delving into the Oxidative Origin and the Possible Implication of Transient Receptor Potential Channels. Front Physiol. 2018 Feb 14;9:95. doi: 10.3389/fphys.2018.00095.
40. Skrabalova J, Drastichova Z, Novotny J. Morphine as a Potential Oxidative Stress-Causing Agent. Mini Rev Org Chem. 2013 Nov;10(4):367-372. doi: 10.2174/1570193X113106660031.
41. Zhang YT, Zheng QS, Pan J, Zheng RL. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin Pharmacol Toxicol. 2004 Aug;95(2):53-8. doi: 10.1111/j.1742-7843.2004.950202.x.
42. Gach K, Wyrębska A, Fichna J, Janecka A. The role of morphine in regulation of cancer cell growth. Naunyn Schmiedebergs Arch Pharmacol. 2011 Sep;384(3):221-30. doi: 10.1007/s00210-011-0672-4.
43. Hsiao PN, Chang MC, Cheng WF, Chen CA, Lin HW, Hsieh CY, Sun WZ. Morphine induces apoptosis of human endothelial cells through nitric oxide and reactive oxygen

species pathways. Toxicology. 2009 Feb 4;256(1-2):83-91. doi: 10.1016/j.tox.2008.11.015.

44. Rozisky JR, Laste G, de Macedo IC, Santos VS, Krolow R, Noschang C, Vanzella C, Bertoldi K, Lovatel GA, de Souza IC, Siqueira IR, Dalmaz C, Caumo W, Torres IL. Neonatal morphine administration leads to changes in hippocampal BDNF levels and antioxidant enzyme activity in the adult life of rats. Neurochem Res. 2013 Mar;38(3):494-503. doi: 10.1007/s11064-012-0941-8.
45. Lin X, Li Q, Wang YJ, Ju YW, Chi ZQ, Wang MW, Liu JG. Morphine inhibits doxorubicin-induced reactive oxygen species generation and nuclear factor kappaB transcriptional activation in neuroblastoma SH-SY5Y cells. Biochem J. 2007 Sep 1;406(2):215-21. doi: 10.1042/BJ20070186.
46. Salvemini D, Neumann WL. Peroxynitrite: a strategic linchpin of opioid analgesic tolerance. Trends Pharmacol Sci. 2009 Apr;30(4):194-202. doi: 10.1016/j.tips.2008.12.005.
47. Giorgi M, Meizler A, Mills PC. Pharmacokinetics of the novel atypical opioid tapentadol following oral and intravenous administration in dogs. Vet J. 2012 Dec;194(3):309-13. doi: 10.1016/j.tvjl.2012.05.019.
48. Azod L, Rashidi M, Afkhami-Ardekani M, Kiani G, Khoshkam F. Effect of opium addiction on diabetes. Am J Drug Alcohol Abuse. 2008;34(4):383-8. doi: 10.1080/00952990802122580.
49. Favaro E, Bensaad K, Chong MG, Tennant DA, Ferguson DJ, Snell C, Steers G, Turley H, Li JL, Günther UL, Buffa FM, McIntyre A, Harris AL. Glucose utilization via glycogen phosphorylase sustains proliferation and prevents premature senescence in cancer cells. Cell Metab. 2012 Dec 5;16(6):751-64. doi: 10.1016/j.cmet.2012.10.017.
50. Chiu ML, Ytterberg AJ, Ogorzalek Loo RR, Loo JA, Monbouquette HG. Characterization of morphine-glucose-6-phosphate dehydrogenase conjugates by mass spectrometry. Bioconjug Chem. 2011 Aug 17;22(8):1595-604. doi: 10.1021/bc2001352.
51. Presta A, Siddhanta U, Wu C, Sennequier N, Huang L, Abu-Soud HM, Erzurum S, Stuehr DJ. Comparative functioning of dihydro- and tetrahydropterins in supporting electron transfer, catalysis, and subunit dimerization in inducible nitric oxide synthase. Biochemistry. 1998 Jan 6;37(1):298-310. doi: 10.1021/bi971944c.
52. Klatt P, Pfeiffer S, List BM, Lehner D, Glatter O, Bächinger HP, Werner ER, Schmidt K, Mayer B. Characterization of heme-deficient neuronal nitric-oxide synthase reveals

a role for heme in subunit dimerization and binding of the amino acid substrate and tetrahydrobiopterin. J Biol Chem. 1996 Mar 29;271(13):7336-42. doi: 10.1074/jbc.271.13.7336.

53. Porasuphatana S, Tsai P, Rosen GM. The generation of free radicals by nitric oxide synthase. Comp Biochem Physiol C Toxicol Pharmacol. 2003 Mar;134(3):281-9. doi: 10.1016/s1532-0456(02)00271-5.
54. Karami R, Hosseini M, Khodabandehloo F, Khatami L, Taiarani Z. Different effects of L-arginine on morphine tolerance in sham and ovariectomized female mice. J Zhejiang Univ Sci B. 2011 Dec;12(12):1016-23. doi: 10.1631/jzus.B1100029.
55. Hsiao PN, Chang MC, Cheng WF, Chen CA, Lin HW, Hsieh CY, Sun WZ. Morphine induces apoptosis of human endothelial cells through nitric oxide and reactive oxygen species pathways. Toxicology. 2009 Feb 4;256(1-2):83-91. doi: 10.1016/j.tox.2008.11.015.
56. Nadeau SE, Wu JK, Lawhern RA. Opioids and Chronic Pain: An Analytic Review of the Clinical Evidence. Front Pain Res (Lausanne). 2021 Aug 17;2:721357. doi: 10.3389/fpain.2021.721357.
57. IASP Announces Revised Definition of Pain - International Association for the Study of Pain (IASP) (iasp-pain.org)
58. Stamer UM, Liguori GA, Rawal N. Thirty-five Years of Acute Pain Services: Where Do We Go From Here? Anesth Analg. 2020 Aug;131(2):650-656. doi: 10.1213/ANE.0000000000004655. PMID: 32011394.
59. Kulichová M. Galová M, Čiljaková Z, Cígerová D, Machlicová V, Bakošová E. Servis akútnej bolesti: pooperačná bolesť, potreba a možnosti liečby, organizácia. Paliat. med. liec. boles., 2011, 4(2): 61–63.
60. Martuliak I.:Mediátory bolesti. In: Martuliak, I., Patofyziológia bolesti, Martimed, s.r.o. 2014, s. 10 - 58, ISBN 978-80-971753-0-6.
61. Martuliak I. Farmakoterapia bolesti pre lekárov a farmaceutov. Martimed. 2019. 20-45 ISBN: 978-80-971753-1-3.

Komentár

Názov práce: *Úloha oxidačného stresu v patogenéze septických stavov, respiračného zlyhania na podklade vírusovej infekcie a bolestivých syndrómov*

Habilitačná práca je predkladaná ako komentovaný súbor vybraných publikácií.

Prvým tematickým okruhom habilitačnej práce je vplyv oxidačného stresu u septických pacientov. Klinický výskum bol zameraný na sledovanie klinického stavu u kriticky chorých pacientov s ťažkou sepsou a septickým šokom po parenterálnej suplementácii pentahydrátu sleničitanu sodného prostredníctvom prospektívnej observačnej klinickej štúdie Se-AOX.

V práci sú prezentované 3 kazuistiky úspešnej liečby septických stavov vrátane aplikácie selénu. Finálne výsledky štúdie Se-AOX boli publikované v dvoch karentovaných medicínskych článkoch v časopisoch Wiener Klinische Wochenschrift a Clinical Biochemistry. Závery týchto prác podporujú dôkazy o suplementácii selénu ako o inferiórnej podpornej liečbe. Práce boli citované v tematických metaanalýzach a systémových prehľadoch. Článok v Clinical Biochemistry bol ocenený Lekárskou fakultou Univerzity Pavla Jozefa Šafárika ako najlepšia publikácia za rok 2014.

V druhom tematickom celku sa diskutuje o problematike vírusových pneumónií vrátane pneumónií spojených s vírusovou infekciou COVID-19, vplyvu oxidačného stresu na rozvoj pľúcnej fibrózy, ako aj aplikáciou viac-hladinových ventilačných režimov do klinickej praxe. Klinický výskum v tejto oblasti bol realizovaný za pomoci dvoch grantov financovaných z Európskeho fondu regionálneho rozvoja - Operačný program Integrovaná infraštruktúra. Výsledky tejto práce boli ocenené CENOU ZA TRANSFER TECHNOLÓGIE NA SLOVENSKU 2023 v kategórii INOVÁCIA. V habilitačnej práci uvádzam z tohto celku súbor 6 publikovaných prác v časopise Anestéziológia a intenzívna medicína kategorizovaných ako pôvodné práce a prehľadové články, ako aj kazuistiku publikovanú v karentovom časopise Medicine.

Tretí okruh práce je zameraný na pôsobenie oxidačného stresu na rozvoj chronickej bolesti a neurozápalu. Venuje sa aktuálnym teoretickým poznatkom v tejto oblasti. Informácie o potenciálnom efekte antioxidačných molekúl popisuje prehľadový článok publikovaný v karentovom časopise Molecules.

Štvrtý okruh popisuje možný prooxidačný vplyv opiátov na amplifikáciu prooxidačných dejov a vysvetľuje koreláciu biochemických mechanizmov. Túto problematiku náš tím skúma v prospektívnej observačnej klinickej štúdii Opioid-Redox study, ktorá je zameraná na hodnotenie zmien redoxného stavu pre rôzne opiáty. Pilotné závery poukazujú na tieto skutočnosti a boli publikované v časopise Medical Science a ďalších recenzovaných časopisoch.

Piatym okruhom habilitačnej práce je opis lekársko-vedeckej činnosti zameranej na liečbu akútnej a chronickej bolesti, ktorú realizujeme v nemocničnom a mimo nemocničnom prostredí. Práce, ktoré súvisia so sledovaním akútnej pooperačnej bolesti, sú sledované v klinickej prospektívnej observačnej štúdii VUSCH/POPT1study. Efektivita terapeutických mini invazívnych intervencií zameraná na lumbálny sympatikový systém je prostredníctvom termálneho zobrazovania sledovaná v prospektívnej observačnej štúdii Tevi-Lusy Study. Tento projekt je finančne podporený grantom Slovenskej algeziologickej spoločnosti. Pilotný článok bol publikovaný v karentovom časopise VASA. Klinické štúdie zamerané na low-back pain s pozitívnym fazetovým syndrómom sú sledované v randomizovanej multicentrickej klinickej štúdii EPCS XIr. Parciálne výsledky boli publikované v časopise Radiology case reports a prezentované na medzinárodných konferenciách. Pacienti s postlaminektomickým syndrómom boli sledovaní v randomizovanej multicentrickej klinickej štúdii EPCS II a výsledky tejto štúdie boli publikované v karentovaných časopisoch Pain Medicine, Wiener Klinische Wochenschrift a Bratislava Medical Journal. V týchto prácach sme v spolupráci s Ústavom merania Slovenskej akadémie vied stanovili koeficienty štatistických váh pre dotazník kvality života EQ-5D-5L, pre región Českej a Slovenskej republiky. Tieto poznatky je možné využiť v širokom spektre medicínskych odborov pri hodnotení kvality života pacientov. Práce boli citované v tematickej metaanalýze a systémovom prehľade. Zmeny klinického stavu pacientov po endoskopickom výkone s akútnym radikulárnym syndrómom sme hodnotili v prospektívnej multicentrickej observačnej klinickej štúdii EPCS V. Závery tejto práce boli publikované v Bratislava Medical Journal a karentovom časopise Medicine.

Habilitation Thesis Abstract

Title: *The Role of Oxidative Stress in Septic Syndromes, Viral-Induced Respiratory Failure, and Associated Pain Syndromes*

This habilitation thesis is being presented as a synthesis of previously published scholarly works.

The first part of this habilitation thesis examines the influence of oxidative stress in septic patients. The clinical research focused on critically ill patients with severe sepsis and septic shock, investigating their clinical state after parenteral application of sodium selenite pentahydrate in a prospective observational clinical trial (Se-AOX). This thesis includes three case reports detailing successful treatments with selenium supplementation in patients with severe sepsis. The final outcomes of the Se-AOX trial were published in Klinische Wochenschrift and Clinical Biochemistry, with results indicating limited evidence for the efficacy of selenium supplementation. These papers were cited in thematic meta-analyses and systematic reviews. The article in Clinical Biochemistry was awarded Best Publication in 2014 by the Medical Faculty of Pavol Josef Safarik University.

The second part discusses viral pneumonias, including those related to COVID-19, and the potential influence of oxidative stress on the development of pulmonary fibrosis. It also addresses the application of multilevel ventilation modes in clinical practice. This research was funded by two grants from the European Fund for Regional Development – Operational Programme Integrated Infrastructure. The results were recognized with the Technological Transfer Award in the category of Innovation. This section includes six published works in Anaesthesiology and Intensive Care Journal (Slovak Republic), categorized as original research, summaries, and a case report published in the journal Medicine.

The third part focuses on the role of oxidative stress in the development of chronic pain and neuroinflammation. Current theoretical knowledge in this area is discussed, along with the potential effects of antioxidative molecules, as detailed in a publication in the journal Molecules.

The fourth part explores the pro-oxidative effects of opioids and their role in amplifying oxidative actions, explaining the biochemical mechanisms involved. This research is part of a prospective observational clinical trial, the Opioid-Redox Study, conducted by our medical

scientific team to evaluate redox changes associated with various opioids. Preliminary findings were published in the Medical Science journal and other review journals.

The fifth part of the thesis outlines our medical scientific work on acute and chronic pain management in both hospital and outpatient settings. Research on acute postoperative pain is conducted within the clinical prospective trial VUSCH/POPT1study. The efficacy of minimally invasive therapeutic interventions targeting the lumbar sympathetic system is assessed using thermal imaging in the Tevi-Lusy Study, a prospective observational clinical trial financially supported by the Slovak Pain Association. A pilot article from this study was published in VASA.

Clinical trials on low-back pain with positive facet syndrome are conducted in the multicenter randomized clinical trial EPCS XIr, with partial results published in Radiology Case Reports Journal and presented at international conferences. Patients with failed back surgery syndrome were studied in the multicenter randomized clinical trial EPCS II, with results published in Pain Medicine, Wiener Klinische Wochenschrift, and Bratislava Medical Journal. Collaboration with the Slovak Academy of Sciences, Institute of Measurements, led to the determination of statistical weights for the EQ-5D-5L quality of life questionnaire for the Czech and Slovak regions. This knowledge is applicable across various medical specializations to assess patient quality of life. These works were cited in thematic meta-analyses and systematic reviews. Changes in the clinical state of patients with acute radicular syndrome after endoscopic treatment were evaluated in the prospective multicenter observational clinical trial EPCS V, with conclusions published in Bratislava Medical Journal and Medicine.

PRÍLOHY

Príloha č. 1

UNIVERZITA PAVLA JOZEFA ŠAFÁRIKA V KOŠICIACH

**Najvýznamnejšie vedecké práce zamestnancov
Univerzity Pavla Jozefa Šafárika v Košiciach publikované v roku 2014
podľa fakúlt a akreditovaných oblastí výskumu**

Lekárska fakulta

Oblasť výskumu 18. Lekárske, farmaceutické a nelekárske zdravotnícke vedy

ADC – Vedecké práce v zahraničných karentovaných časopisoch
Mechírová, Eva – Danielisová, Viera, Domoráková, Iveta – Danková, Marianna – Stebnický, Milan – Mičková, Helena – Burda, Jozef:
Bradykinin preconditioning affects the number of degenerated neurons and the level of antioxidant enzymes in spinal cord ischemia in rabbits.
Acta histochemica. ISSN 0065-1281. - Vol. 116, no. 1 (2014), s. 252-257.

ADC – Vedecké práce v zahraničných karentovaných časopisoch
Kočan, Ladislav – Vašková, Janka – Vaško, Ladislav – Šimonová, Jana – Šimon, Róbert – Firment, Jozef:
Selenium adjuvant therapy in septic patients selected according to Carrico index.
Č. projektu: VEGA 1/0799/09.
Clinical Biochemistry. - ISSN 0009-9120. - Vol. 47, no. 15 (2014), s. 44-50.

ADC – Vedecké práce v zahraničných karentovaných časopisoch
Kaťuchová, Jana – Bober, Juraj – Harbuľák, Pavol – Hudák, Alexander – Gajdzík, Tomáš – Kalanin, Rastislav – Radoňak, Jozef:
Obesity as a risk factor for severe acute pancreatitis patients.
Wiener Klinische Wochenschrift. - ISSN 0043-5325. - Vol. 126, no. 7-8 (2014), s. 223-227.

Príloha č. 2

CENA

ZA TRANSFER TECHNOLÓGIÍ NA SLOVENSKU 2023

INOVÁCIA

Zariadenie na umelú ventiláciu pľúc s identifikáciou nehomogenity distribúcie plynov a spôsob riadenia zariadenia pri umelej ventilácii pľúc

doc. MUDr. Pavol Török, CSc.
prof. MUDr. Viliam Donič, PhD.
MUDr. Tomáš Grendel, PhD.
MUDr. Ladislav Kočan PhD.
MUDr. Martin Nosáľ

MUDr. Dušan Rybár, PhD.
MUDr. Filip Depta
MUDr. Štefan Imrecze PhD.
doc. MUDr. Jozef Firment, PhD.
MUDr. JUDr. Peter Firment

Univerzita Pavla Jozefa Šafárika v Košiciach

EURÓPSKA ÚNIA
Európsky fond regionálneho rozvoja
OP Integrovaná infraštruktúra 2014 – 2020

MINISTERSTVO ŠKOLSTVA, VEDY, VÝSKUMU A ŠPORTU SLOVENSKEJ REPUBLIKY

COINTT 2023

prof. PharmDr. Ján KYSELOVIČ, CSc.
generálny riaditeľ CVTI SR