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NONINVASIVE METHODS IN CARDIOLOGY 2009

on July 8, 2009

in the hall of Dean office on 2nd floor, Komenského nám. 2, Brno

organized by

Department of Functional Diagnostics and Rehabilitation
Department of Physiotherapy

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SPERM BANKING AND INFERTILITY TREATMENT IN MEN WITH TESTICULAR CANCER

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ABSTRACT

This study was designed to evaluate the use of sperm frozen before testicular cancer treatment and to analyse results of infertility treatment. A total of 166 male patients were referred for sperm freezing before testicular cancer treatment from 1995 to 2007. Cancer treatment, semen analysis, assisted reproduction techniques, and statistical evaluation were basic interventions. Sperm pathology analysis before and after cancer treatment, survival of patients, and pregnancy after infertility treatment were analysed. Testicular cancer was diagnosed and treated in 226 men from 1995 to 2007. A total of 166 men (73.5 %) decided to freeze their semen before cancer treatment. Seminoma was diagnosed in 87 men (52.4 %), nonseminomatous germ cell tumours (NSGCT) in 79 men (47.6 %). A total of 121 men (72.9 %) were of stage I. Azoospermia was diagnosed in 9 men (5.4 %), semen was cryopreserved in 157 patients. Median sperm concentration was 8.5 mil/ml in the seminoma group and 9.4 mil/ml in the NSGCT group – a non-significant difference. Until now, 5 patients (2.9 %) have died and 27 patients (17.2 %) have attended for infertility treatment. Median fresh sperm concentration was 4.4 mil/ml, median progressive sperm motility 3.7 %, azoospermia was found in 4 men (14.8 %). The confounding female infertility factor was found in 19 (70.4 %) female partners. Cryopreserved semen was used in 25 couples (92.6 %), intracytoplasmic sperm injection (ICSI) was the most effective procedure – 36 cycles resulted in 12 pregnancies and 9 deliveries. Testicular cancer survivors have a good chance of fathering a child by using cryopreserved sperm and ICSI procedure.

INTRODUCTION

Damage to reproductive function is a very frequent and well-documented side effect associated with the treatment

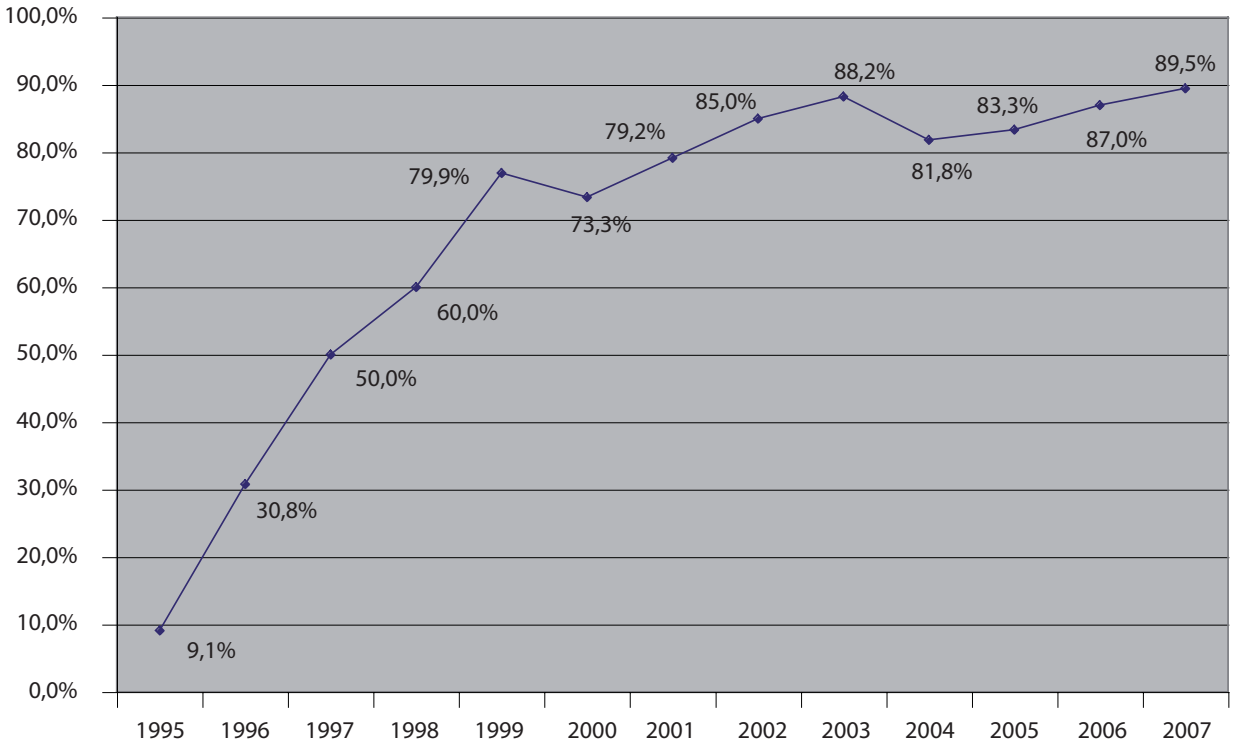

Figure 1
Sperm cryopreservation (%) in men with testicular cancer

of malignant testicular tumours. The first work describing chemotherapy-induced azoospermia was published in 1948 [1]. Variation in sperm quality in relation to the type of malignant tumour was also investigated [2]. The increasing success of testicular cancer treatment and determined efforts to improve the quality of life after successful treatment have turned attention to the preservation of reproductive function in young males [3]. The development of assisted reproduction techniques has brought about effective qualitative changes in this field [4, 5]. The collection, freezing, and long-term storage of sperm are currently considered to be the most effective method. The Assisted Reproduction Centre of the Department of Gynaecology and Obstetrics in collaboration with the Department of Urology launched their programme of sperm freezing and long-term storage before testicular cancer treatment in 1995.

PURPOSE

The objective of this study is to analyse the sperm counts of cancer patients and a possible correlation between sperm pathology and cancer diagnosis, and to make an overview of using frozen sperm during the 12 years of sperm banking.

MATERIAL AND METHODS

Between October 1995 and the end of December 2007 young men with testicular cancer were referred from the Department of Urology to the Assisted Reproduction Centre for sperm cryopreservation prior to treatment for testicular cancer. An obligatory examination of HIV 1, HIV 2, HBsAg, anti-HCV, and anti-HBC before storage of cryopreserved semen was introduced in 2003. Sperm counts were evaluated according to the respective WHO (World Health Organisation) laboratory manual [6] using the Neubauer counting chamber. Commercial media, Medi-Cult (Jyllinge, Denmark) or Vitrolife (Kungsbacka, Sweden) were used. The semen was mixed with a cryopreservation medium and placed in 2 ml Nunclon CryoTubes (Roskilde, Denmark) and frozen. Cryopreservation technology and the procedures used in the storage of frozen sperm samples were aimed at minimising the potential risks, including mistaken identity and transmission of infection. Sperm samples were frozen in a programmable Planer Kryo F10 (Sunbury-on-Thames, United Kingdom) instrument using a standard cooling curve. Samples from 1–3 collections before starting cancer treatment were frozen. The cryotubes were stored in liquid nitrogen at a temperature of –196 °C in

Table 1
Characteristics of patients referred for semen cryopreservation (n=166). NSGCT = nonseminomatous germ cell tumours

Age (years)	Mean 27.6	SD±4.4	Range 15 - 45	Median 27
Histological diagnosis	Seminoma: 87 men (52.4 %)	NSGCT: 79 men (47.6 %).		
Stage	I: 121 men (72.9 %),	II: 29 men (17.5 %)	III: 10 men (6.1%)	
	IV: 1 man (0.6 %)	Unknown: 5 men (2.9 %)		

Table 2
Semen analysis values before cryopreservation (n=166). (NSGCT = nonseminomatous germ cell tumours, NS = statistically non-significant)

	Seminoma (n=87)	NSGCT (n=79)	
Sperm concentration (10^6/ml)			
Mean	17,2	19,7	NS
Median	8,5	9,4	
Range	0 - 122	0 - 108	
S.D.	21,4	26,3	
Total sperm count ($10^{6)}$			
Mean	37	43	NS
Median	18,3	19,2	
Range	0 - 414	0 - 522	
S.D.	375	482	
Progressive sperm motility (%)*			
Mean	9,8	12,3	NS
Median	5,2	5,5	
Range	0 - 60	0 - 65	
S.D.	12,9	11,3	

* Nine patients with azoospermia were excluded from these analyses.

Table 3
Clinical results of infertility treatment with cryopreserved semen (n=25 couples).
(ICSI = intracytoplasmic sperm injection, IUI = intrauterine insemination, ET = embryotransfer, N.A. = not applicable)

	No. of couples	No. of cycles	No. of retrieved oocytes	Fertilisation rate	No. of transferred embryos/ET	No. of clinical pregnancies	Pregnancy rate (%)	Deliveries
ICSI	23	36	8.5±3.2	68,70%	1.9±0.3	12	33,3	9
IUI	2	5	N.A.	N.A.	N.A.	1	20,0	1

Table 4
Obligatory examinations before semen cryopreservation and storage according to Act No. 296/2008 (Czech Republic)

anti-HIV-1	Human immunodeficiency virus 1 antibody
anti-HIV-2	Human immunodeficiency virus 2 antibody
HBsAg	Hepatitis B surface antigen
anti-HBc	Hepatitis B core antibody
anti-HCV	Hepatitis C virus antibody
TPHA	Treponema pallidum haemagglutination

an LS 4800 container Tailor-Wharton HARSCO (Husum, Germany), with an indicator of the surface level and an alarm.
The assisted reproduction methods used complied with the respective standards of the centre [7]. The diagnoses and deceases were verified in the database of the National Oncological Register of the catchment area, in compliance with personal data protection. The Faculty Hospital Brno Ethics Committee's approval was obtained.
The study group was described using the basic descriptive statistics, where categorical variables were characterised using the percentage representations of individual categories, while continuous variables (age, sperm concentration and motility) were described using the mean, the median, the range of values (minimum and maximum), and standard deviations.
Statistical testing was used to confirm the hypothesis of whether or not the results of sperm counts correlated with the patient's diagnosis. The differences among groups of patients were tested using the Kruskal-Wallis test. When the influence of the diagnosis on the sperm count was significant, partial hypotheses were tested to see which particular diagnoses differed by their values (i.e. multiple comparisons of mean ranks). The critical limit for the level of significance was set to $p = 0.05$.

RESULTS

Testicular cancer was diagnosed and treated in 226 men (age 27.6±6.4) from 1995 to 2007. A total of 166 men (73.5 %) decided to freeze their semen before cancer treatment. The number of men asking for semen cryopreservation increased gradually from 9.1 % in 1995 to 89.5 % in 2007 (Figure 1). Seminoma was diagnosed in 87 men (52.4 %), nonseminomatous germ cell tumours (NSGCT) in 79 men (47.6 %). Patient characteristics including the stage of the tumour are given in Table 1. Azoospermia was diagnosed in 9 men (5.4 %), thus semen was cryopreserved in 157 patients. Sperm concentration was 17.2±21.4 (median 8.5) mil/ml in the seminoma group and 19.7±26.3 (median 9.4) mil/ml in the NSGCT group – a non-significant difference (Table 2). Statistical analysis did not find any significant difference of sperm count in relation to the stage of the cancer.
Until now, 5 patients (2.9 %) died and 27 testicular cancer survivors (17.2 %) with semen cryopreserved prior to cancer treatment have undergone infertility treatment. The interval between cryopreservation and infertility treatment ranged from 7 to 70 months (mean 22.2±14.7, median 18 months). In these men the mean fresh sperm concentration was 8.4±16.8 (median 4.4) mil/ml, progressive sperm motility 4.2±17.7

(median 3.7) %, azoospermia was found in 4 men (14.8 %). As these examinations were done after different time intervals following cancer treatment and after different chemotherapy protocols, the data were not sufficient for reliable statistical analysis.

A confounding female infertility factor was found in 19 (70.4 %) female partners. Cryopreserved samples were used in 25 couples (5 cycles of intrauterine insemination, 36 ICSI cycles). Clinical data and results of infertility treatment are given in Table 3. Fresh semen was used in 2 cases for 6 ICSI cycles and one clinical pregnancy resulted in spontaneous abortion. After failure of two ICSI cycles 3 couples (11.1 % of men coming for infertility treatment after sperm cryopreservation) decided to use intrauterine insemination with donor sperm, 5 cycles resulted in 2 pregnancies and deliveries.

DISCUSSION

The increasing frequency of testicular cancer in our study corresponds to the increasing incidence of this tumour in the Czech Republic. In 1977 the incidence of testicular cancer was 3.2/100 000 men, in 1995 it increased to 6.5, and in 2005 to 8.8/100 000 men. Approximately 68 % of them were in the range from 20 to 39 years. Advances in the diagnostics and management of this cancer and progress in its treatment contributed to a significant improvement in the survival rate of this very common malignancy of young men [8]. Stage I tumours were only 5 % in 1977 and improved gradually to 50 % in 1995 and 55 % in 2005. Similar findings are also reported by the Bourn Hall Clinic [9] and other clinics [10, 11].

Azoospermia was found in 5.4 % of the males referred for sperm cryopreservation, as compared to Lass et al. [9], who reported 17.3 %, and Kelleher et al. [12], who detected only 3.3 %. Severe abnormalities in sperm concentration and progressive motility were detected in both seminoma and NS-GCT groups, similar to other studies [13, 14].

The aetiology of impaired spermatogenesis in testicular cancer patients is not fully understood. Damage to the DNA of the sperm due to malignancy was confirmed [15]. However, the extent of damage to spermatogenesis does not correlate with the severity of the cancer [16]. The correlation between sperm pathology and testicular tumours is also known [17]. The impaired quality of sperm production is probably associated with disturbed differentiation of the testicle during the embryonic development of the gonad – the Testicular Dysgenesis Syndrome hypothesis [18]. This syndrome is manifested by the increased incidence of developmental defects of the genital (cryptorchism, hypospadias), spermatogenesis disorders, and testicular carcinomas. Testicular dysgenesis is caused by alteration in the development of the testicle by factors affecting endocrine regulation ("endocrine disruptors"). As spermatogenesis disorders correlate well with testicular carcinoma, close urological examination of men with severe sperm abnormalities is of importance [17]. When analysing impaired spermatogenesis in relation to the type of malignancy, we did not find any significant difference like Agarwal et al. [5].

After the completion of gonadotoxic therapy, the quality of sperm was significantly impaired. The resulting function of the gonad is affected by a number of factors, such as early diagnosis of the malignant disease, the chemotherapy regimens used, and the sperm count as determined prior to the start of therapy [19]. The recovery of spermatogenesis is usually very slow – for example, the mean period of time to achieve the best level of the sperm count is 51 months in patients who completed chemotherapy for leukaemia [20]. In the case of azoospermia, the methods of assisted reproduction based on the surgical collection of sperm provide worse results [21, 22]. Sperm cryopreservation performed prior to cancer therapy is therefore a prerequisite for the successful treatment of subsequent infertility. The main requirement of this programme is to establish a special cryobank to allow safe long-term storage of sperm samples. The operation of such a cryobank requires an exact database of patients and its own records. A proper examination complying with the law of the Czech Republic (Table 4) is a prerequisite of cryopreservation and storage of frozen semen. Another important aspect is that the patients should be given clear and relevant information on the possibilities and conditions of sperm cryopreservation and its use in future.

The fulfilment of this task requires close co-operation between the respective department of urology, the assisted reproduction centre capable of providing this kind of treatment, and the tissue bank. In the Brno Faculty Hospital, this interdisciplinary co-operation is facilitated [23, 24]. Thanks to a strong awareness among urology specialists of the possibilities and easy availability of sperm cryopreservation in our centre, the number of patients referred for this procedure has increased.

In our study 17.2 % of the males have come in for infertility treatment so far. This finding is higher than the data from the literature, for example 7.7 % published by Kelleher [25].

The reasons are not only in the area of patient health, but also in the social area, i.e. patients usually plan to start a family long after they have successfully completed therapy [26]. Another important aspect is that patients are afraid of the increased risk of congenital defects and malignant tumours in their offspring. Many detailed studies which have investigated this risk [3, 16, 27] have failed to prove its increase [28, 29]. Most males from our group who came in for infertility treatment had undergone successful treatment for testicular cancer and usually came 18 months after sperm cryopreservation.

Intrauterine insemination was performed in our clinic much less frequently (17.0 %) as compared to Schmidt et al. [29]; ICSI was used in 83.0 % of the treatment cycles.

The mortality rate of our group of patients was also analysed. According to the data obtained from the Oncological Register, only 2.9 % of males referred for sperm cryopreservation died, which corresponds to the total survival rate in patients with the early stage of testicular seminoma, which exceeds 95 % (30).

CONCLUSION

Sperm cryopreservation prior to gonadotoxic therapy is the basic method used to preserve reproductive potential for the survivors of cancer treatment. Cancer patient sperm banking programmes require close co-operation between the respective oncology department, the assisted reproduction centre, and the tissue bank. Sperm cryopreservation should be offered to every patient before therapy that could damage spermatogenesis.

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NEW TITANIUM β-ALLOYS FOR DENTAL IMPLANTOLOGY AND THEIR LABORATORY-BASED ASSAYS OF BIOCOMPATIBILITY

Bartáková S.[1], Prachár P.[1], Kudrman J.[2], Březina V.[3], Podhorná B.[2], Černochová P.[1], Vaněk J.[1], Strecha J.[4]

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ABSTRACT

In this study, we focused on biological aspects of the applicability of modern titanium alloys in dental medicine. Overall, 34 new titanium β-alloys with different amounts of niobium (Nb), molybdenum (Mo), tantalum (Ta), vanadium (Va), and iron (Fe) were tested. Their suitability for possible use in dental implants was evaluated with special regard to potential toxicity. To evaluate biocompatibility of β-alloys, we applied numerous biological tests focused on cell growth, cell adherence, cell dilatation, evaluation of toxicity, tests of chromosomal aberrance, and other parameters of biocompatibility. The results revealed that, due to elution of particles of intermetallic TiFe phase and consequent release of Fe ions, TiAlFe alloys were not suitable for dental implants. TiAlNb and TiTa alloys exhibited the required biocompatibility parameters. The TiAlV alloys were ranked as biotolerant. It might be concluded that TiMo alloys were acceptable for the production of dental implants but less suitable than TiAlNb and TiTa alloys.

INTRODUCTION

There is a relatively large time gap between the invention and the development of a new dental material and its application in clinical practice. Within this time, numerous material-related and laboratory-based tests, as well as preclinical and clinical trials, are done. The length of the period, however, might be shortened if effective multidisciplinary research is applied. Such research requires close collaboration among specialists from the fields of material engineering, dental implantation surgery, and stomatology. In our study aimed at the period of such tests in newly-developed alloys, we created a network of professional institutions comprising a stomatological clinic, a stomatological research centre, and several commercial companies in the field of biotests,

implantation surgery, and orthopaedics. In our study, we focused on the newly-developed titanium β-alloys, Ti38Nb and Ti35Nb6Ta, as well as on those with niobium (Nb), molybdenum (Mo), tantalum (Ta), vanadium (Va), and iron (Fe) tested for future use in dental implants. Due to the low value of Young modulus and relatively easy forming, even cold processing, the β-alloys have good characteristics for prospective application in dental implantology. Therefore, future dental implants would be prepared using routine foundry technologies. Undoubtedly, many new applications of β-alloys will appear in future dentistry [1]. It is, therefore, necessary to define requirements for mechanical characteristics in such materials and implants. First of all, mechanical and physical properties of the new cast material must be determined and compared to those of formed materials. Then, their possible application must reflect their performance in dental implants exposed to different load in a jaw. Especially the fatigue load might be a critical issue in some applications of dental implants. In this respect, it is highly desirable to compare the fatigue load in formed and cast alloys. Specifically for β-alloys, it is necessary to keep their properties constant, in spite of the fact that they may vary according to their complex microstructure, number and amount of alloy ingredients. The alloys may be deformed mainly by sliding or twinning and/or combination of the two. Additionally, the alloys may have unsuitable martensic structure. The fatigue properties of the alloys, especially the probability of microcrack initiation and their further development, depend strongly on alloy microstructure, composition, and temperature of processing. They may affect substantially the final properties of the alloy and the dental implant.

In our study, we focused on biological aspects of the applicability of modern titanium alloys in human medicine, dental implantology in particular. Within the last decades, numerous titanium alloys have been developed. These alloys possess suitable mechanical properties and a higher biocompatibility than traditional titanium-based materials [2]. In the Czech Republic, the majority of titanium dental alloys are of a high degree of purity abbreviated as Grade 2. For such purity, the maximum Fe content allowed is 0.25 % of weight. It is 0.20 %, and 0.08 % for carbon. The rigidness of such grade is within the range of 390–540 MPa. Formed materials obviously show rigidness at the upper limit. New materials could therefore show higher rigidness. They could also fit the high requirements for optimum biomechanical and biological interactions. The new materials should minimise fatigue and corrosion [3], which are the main reasons for implant rupture. Local load caused by chewing forces and the activity of oral cavity liquids may induce damage. It might be demonstrated as fretting damage, corrosion, and surface tension of implants. Generally, low pH and the resulting electrochemical changes in the oral cavity [4] may cause damage to dental implants, too. Normal values (pH about 7.0) may be decreased mainly in the close neighbourhood of haematomas. Interstitial liquids, especially chlorides, are the most aggressive agents to implants. They may induce pitting, corrosion along margins, crack corrosion, etc. Therefore, studies of the response of dental implant materials to a variety of mechanical, biochemical, and biological factors are highly desirable.

In our study, we focused on the behaviour of titanium alloys in biological environment and evaluation of corrosion and the toxic effect of eluted metal ions. Another aim was to analyse the likely reasons for biological rejection of titanium alloys. The aim of our study was to perform a series of tests of biocompatibility of β-alloys with special regard to those of Ti38Nb and Ti35Nb6Ta grade. A further aim was to point out several alloys with the best properties and thus most suitable for clinical dental applications. Our working hypothesis was that a change in the relative proportion of Ni, Mo, Ta, Va, and Fe in dental implants of β-alloys may cause substantial differences in their properties and suitability for application in human medicine. For this purpose, we selected several biological tests that are used in the evaluation of biocompatibility of materials. The biological tests were focused on the following fields: cell suspension growth in an elution from tested alloys, adherence, dilatation (spreading), and toleration tests. Last but not least, a test of atypical mitosis and a test of chromosomal aberrance were applied.

MATERIALS AND METHODS

Laboratory-based preparation of titanium alloys Titanium alloys were prepared in an electric arc furnace Leybold-Heraeus L2004 supplemented with a pump A2DS150 and an exhauster Roots RP1800. The alloys were smelted in water-cooled crystallisers in vacuum (10^{-2} to10^{-3} mbar) or under low pressure atmosphere of an inert gas (He or Ar, 300–400 mbar). The smelting was performed either by a melting electrode in vacuum or a non-melting tungsten electrode under He/Ar atmosphere. The power source of the whole system was capable of a maximum current of 2 500 A. For the typical melting process, 1200 A was typically used.

Several titanium alloys with different contents of admixtures were prepared. The relative shares of the admixtures were analysed by a certified laboratory (Chemopetrol, Litvínov, CZ) using standard chemical analyses for trace element determination. Table 1 summarises the alloys prepared and tested in our study.

Sample preparation for biological testing

From the laboratory-prepared alloys, small pieces were cut and subjected to a metallographic analysis and rigidness measurements. The samples were analysed in the initial

Table 1

Titanium alloys and their chemical composition. For trace elements, only the alloyed ones are listed. Other elements important for the alloy properties are listed as well. Key to abbreviations: Abbr. – abbreviation, Al – aluminium, V – vanadium, Ta – tantalum, Nb – niobium, Fe – iron, Mo – molybdenum, Zr – zirconium, O – oxygen, N – nitrogen

Alloy	Abbr.	Concentration [% weight]									
		Al	V	Ta	Nb	Fe	Mo	Zr	O	N	
Ti6Al2V	**V1**	6.56	1.75	-	-	-	-	-	0.119	0.028	
Ti6Al4V	**V2**	6.70	3.71	-	-	-	-	-	0.145	0.022	
Ti3Al2,5V	**V5**	3.26	2.21	-	-	-	-	-	0.091	0.009	
Ti6Al6V	**V3**	6.59	5.63	-	-	-	-	-	0.140	0.037	
Ti6Al8V	**V4**	6.85	7.85	-	-	-	-	-	0.133	0.036	
Ti6Al2Nb	**N1**	6.11	-	-	2.42	-	-	-	0.141	0.041	
Ti6Al4Nb	**N2**	5.91	-	-	4.84	-	-	-	0.095	0.012	
Ti6Al6Nb	**N3**	5.85	-	-	7.16	-	-	-	0.145	0.031	
Ti6Al7Nb	**N4**	5.93	-	-	8.36	-	-	-	0.149	0.033	
Ti6Al1Nb	**N5**	5.90	-	-	10.45	-	-	-	0.083	0.014	
Ti5Al1Fe	**F1**	5.15	-	-	-	1.10	-	-	0.150	0.037	
Ti5Al2,5Fe	**F2**	5.30	-	-	-	2.69	-	-	0.121	0.031	
Ti5Al4Fe	**F3**	5.38	-	-	-	4.22	-	-	0.131	0.036	
Ti5Al6Fe	**F4**	5.11	-	-	-	7.38	-	-	0.149	0.032	
Ti5Al8Fe	**F5**	5.00	-	-	-	10.18	-	-	0.137	0.030	
Ti5Ta	**M1**	-	-	5.39	-	-	-	-	0.178	0.043	
Ti10Ta	**M2**	-	-	9.83	-	-	-	-	0.185	0.035	
Ti15Ta	**M3**	-	-	14.80	-	-	-	-	0.192	0.031	
Ti20Ta	**M4**	-	-	19.70	-	-	-	-	0.154	0.024	
Ti25Ta	**M5**	-	-	24.39	-	-	-	-	0.198	0.020	
Ti15TaZr	**MZ3**	-	-	14.70	-	-	-	5.61	0.076	0.0058	
Ti30Ta	**M6**			28.95					0.089	0.0075	
Ti35Ta	**M7**			33.81					0.120	0.023	
Ti30Nb	**N6**				31.81				0.071	0.015	
Ti5Mo	**P1**						5.16		0.170	0.055	
Ti10Mo	**P2**						9.96		0.167	0.039	
Ti15Mo	**P3**						15.12		0.183	0.044	
Ti20Mo	**P4**						19.78		0.185	0.055	
Ti25Mo	**P5**						24.90		0.155	0.038	
Ti30Mo	**P6**						29.41		0.148	0.032	
Ti35Mo	**P7**						34.49		0.164	0.034	
Ti15Mo5Zr	**PZ3**						14.79	5.24	0.150	0.032	
Ti25Mo5Zr	**PZ2**						24.48	5.26	0.0077	0.0060	
Ti15Nb8Zr	**ZN1**				15.51			8.64	0.113	0.0098	

state after melting and at different states after annealing at 650 °C (15 min, air, abbreviated as Z in the following text), 850 °C (10 min, furnace, abbreviated as R), and 950 °C (10 min, furnace, abbreviated as P), respectively. For each sample and annealing type, microstructure of the alloy was evaluated. Microstructure was evaluated on a Lucia (NIKON) analysis system. The rigidness of the alloys was measured with a Vickers HV10 rigidometer. For selected samples, chemical analyses of phases were done using the microanalyser of a JOEL scanning electron microscope. For biological tests, cylinders of Ø 8x3 mm in diameter were prepared from the alloys.

Biological assays

To assess the interactions between the alloys and the biological material, several biological tests were applied. First, we used a test of cell growth in the elution of tested alloys for the evaluation of potential toxicity. Heteroploid cell lines were exposed to 4 treatments (concentrations), and positive and negative controls, respectively. The number of cells was counted in 1 ml of the elution every 24 h for 5 d. In the test of cell adherence, a cell suspension of the same lines was spread over the tested alloys and exposed for 24 h. Then, the cells were fixed in glutaraldehyde and prepared for analysis by a critical-point drying. The morphology of the adhered cells was evaluated

using a SEM (Vega Tescan microscope). The values obtained were compared to a standard surface (cover glass) and the relative number of adhered cells (an alloy/glass standard) was calculated. A dynamic dilatation (spreading) test of the cells was applied to evaluate the number of dilated cells adhered to a substrate with a membrane spread over the substrate after 6 h of exposition to the elution [5]. The other test was the tolerance test of a cell monolayer. A piece of the tested alloy was put into a cell culture monolayer in a cultivation flask. After 48 h exposition, the size of the death cell area was evaluated. A similar test was the test of cell tolerance in suspension to the tested material. The material of the tested alloy was put into a cultivation flask and then a cell suspension was added. The size of the area on the surface of the tested material on which adherence was inhibited was evaluated.

Induction of atypical mitosis was tested in a cell suspension inoculated to a cover glass and exposed for 72 h. Similarly to the cell growth test, 4 different concentrations of elution, and positive and negative controls were used. After the exposition, the number of metaphases, the number of cells possessing more than one nucleus, the number of multipolar divisions, and the mitotic index were evaluated. If there was more than 10 % of mitoses, the tested material was considered mutagenic. A clastogenic test, i.e. a test of induction of structural chromosomal aberrance, was applied to human leucocytes. After 72 h exposition, structural and numeric chromosome aberrances were counted and compared to positive and negative controls, respectively. To stop metaphase, colcemide was added to the cell monolayer 3 hours before harvesting. For details of the method see e.g. [6]. The last test that we applied was the test of chromosomal aberrance in human peripheral lymphocytes. In the test, fully heparinised blood is cultivated in the presence of PHA. Metaphased chromosomes were prepared. After 72 h exposition, chromosomal breaks were evaluated in 200 mitoses observed. In this case (e.g. the test on human leukocytes) more than 5 % of aberrant mitoses was assumed as a mutagenic effect.

RESULTS AND DISCUSSION

The screening test and two basic tests of cytocompatibility of selected pure metals showed that only four of the metals tested exhibited high biocompatibility (Table 2). Apart from Ti, also Ta, Nb, and Zr were highly biocompatible and thus potentially prospective for biocompatible alloys. Ti and Zr have similar properties. They are, therefore, substitutable. Ni and Ta also have similar properties. They can, therefore, be used in alloys with Zr and Ti, in which they enlarge the β phase. If their proportion was more than 30 %, then the resulting β alloy hardened by globular particles of the α phase. TiNb, TiTa alloys or their ternary combination are suitable for cold welding. This represents a great technological advantage in comparison to the traditionally used Ti6Al4V alloys. Moreover, TiNb and TiTa alloys do not form intermetallic phases, the properties of which differ from pure metals. On the other hand, there are some disadvantages. Tantalum has a high melting temperature, which is complicating for metallurgical processing. Therefore, alloys with Ni are preferred. In alloys with Nb, a proper share of Nb and processing is required to reach the β phase. When low Nb concentrations are used, then an undesirable martensite structure is reached (see Figure 1 left), characteristic of numerous intermartensite

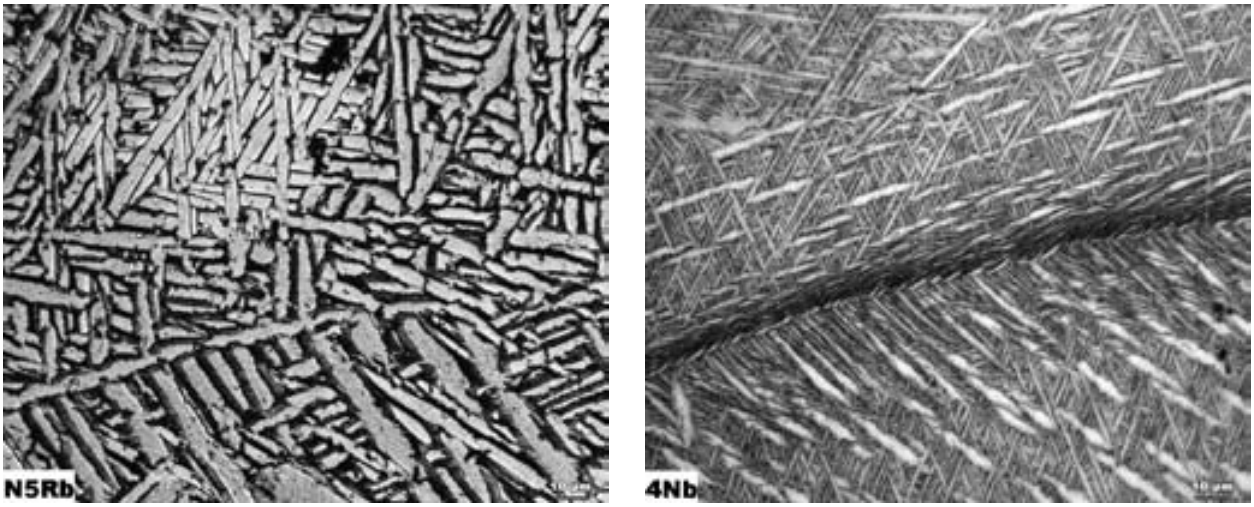

Figure 1
Microstructure of Ti6Al7Nb alloy after casting (left) and Ti6Al10Nb cooled 10 min at 850 °C/10 (right).

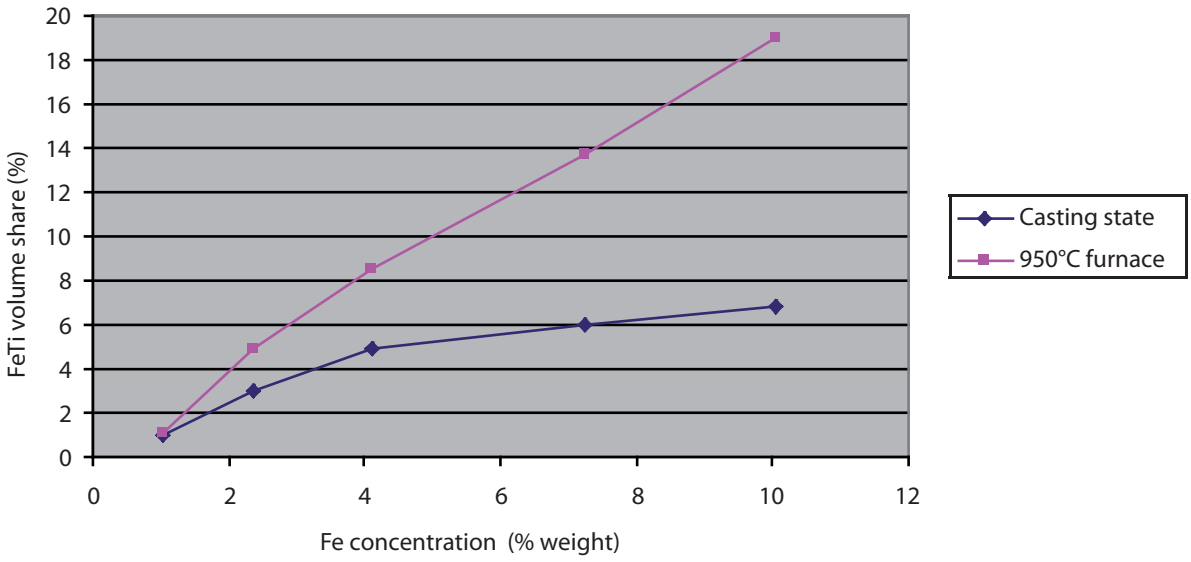

Figure 2
Relation of the volume share of intermetallic TiFe phase to Fe concentration and heat processing

spaces untransformed into the β phase. After slow cooling of the alloy, a typical combined α+β structure is reached (Figure 1 right). To achieve a proper β-phase alloy, higher Nb concentrations must be used (Ti35Nb).

A test of cell growth in the elution showed that the kinetics were of the same shape (data not shown) in TiAlFe, TiAlNb, and TiTa alloys. Similar growth curves were also found in an earlier study focused on titanium implants covered with a surface Cr-Co alloy [7]. For TiAlFe alloys, the number of cells found for particular lengths of exposition was the same, slightly lower if compared to a positive etalon. From this point of view, Fe alloys do not exhibit any toxic effect. A dilatation test showed a reduced number of dilated cells. The reduction was more pronounced in slowly cooled alloys. Moreover, a test of tolerance showed that around TiAlFe alloys a zone of toxic effect was formed. These results showed that TiAlFe alloys were not suitable for dental implants. The reason is the likely elution of particles of the intermetallic TiFe phase and release of Fe ions from the phase (see Figure 2).

Cell adherence to TiAlFe alloys was higher when the alloys were annealed at 950 °C and cooled in a furnace. In comparison to other metals in the alloy, TiAlFe exhibited lower values of cell adherence. A high proportion of the intermetallic phase reduced the number of atypical mitoses. We can, therefore, summarise that Fe caused negative effects in cells. A carcinogenic effect cannot be therefore excluded. TiAlFe alloys have poor biocompatibility and thus cannot be recommended for dental implants.

The results obtained in biological tests with TiAlNb alloys showed that they were very suitable for dental implant production. They exhibited the required biocompatibility parameters, even if the proportion of Nb was 35 %. It means that the binary system TiNb allows to prepare biocompatible β-alloys that would not be shaped under cool conditions. Generally, TiAlNb alloys might be recommended for the production of dental implants.

A biological test of TiAlV alloys resulted in slightly negative parameters. The TiAlV alloys are, however, still ranked as biotolerant. Moreover, some anomaly was observed, such as creation of fibrous cell structures and abnormalities of cell membranes. These abnormalities will be further analysed in detail in a separate paper. It can be stated that V-alloyed materials are acceptable and have recently been frequently used for dental implant production. They are, however, less suitable. There is the probable effect of V toxicity, in spite of the fact that V is well fixed in compact solution in TiAlV alloys.

TiTa alloys showed either very good biocompatibility or at least tolerance. The compatibility was high even when the Ta proportion was above 25 %. The results of all the tests indicated that Ta-alloyed materials were very suitable for dental implant production. It means that the binary system TiTa allows to prepare biocompatible β-alloys that would not be

Table 2
Summary of biocompatibility of metals presented in the alloys tested

Element	Dilatation test	Adherence test	Screening	Overall evaluation
Al	tolerant	tolerant	toxic	tolerant
Cu	toxic	toxic	toxic	toxic
In	toxic	toxic	tolerant	toxic
Mo	tolerant	tolerant	tolerant	tolerant
Nb	compatible	compatible	compatible	compatible
Pb	toxic	toxic	toxic	toxic
Ta	compatible	compatible	compatible	compatible
Ti	compatible	compatible	compatible	compatible
V	tolerant	toxic	tolerant	tolerant
Zn	toxic	toxic	toxic	toxic
Zr	compatible	compatible	compatible	compatible

shaped under cool conditions. TiTa alloys may therefore be recommended for the production of dental implants. Moreover, recent *in vitro* studies focused on the cytotoxicity of TiTa alloys [8, 9]support the idea of application of such materials in dental implantology.

A biological test of TiMo alloys showed somewhat lower biocompatibility than TiAlNb and TiTa alloys. However, TiMo alloys were evaluated as tolerant. It might be stated that TiMo alloys are acceptable for the production of dental implants but less suitable than TiAlNb and TiTa alloys.

Concluding remarks

Biological tests and controlled experiments performed *in vitro* undoubtedly provide valuable information on the biocompatibility of β titanium alloys. Such testing may point out important factors directly and indirectly involved in the process of dental implant functioning in patients. Recently, numerous pure titanium and α and β titanium alloys have been tested for the use in dental implants [10]. However, the rigidity of α and β type titanium alloys is still considerably greater than that of the cortical bone [11], although the rigidity of titanium alloys is less than that of Co-Cr type alloys and stainless steels used for biomedical applications. Therefore, the latest trend is to develop low-rigidity β-type titanium alloys. In such a material, a strongly limited or zero presence of toxic and allergic elements is a necessity. Such titanium alloys should have, apart from excellent mechanical properties and workability [12], also a high biocompatibility.

From the experimental results it can be seen that among the alloys tested, TiAlNb and TiTa alloys are potentially highly desirable for clinical use since they exhibit the best parameters in all the biologically-related features tested. Titanium alloys used in this study showed high cell adherence, similar to the results of the recent adsorption study [13, 14]. The biocompatibility of the beta titanium alloy might be excellent similarly as found in other β-alloys with Ni, Ta, and Zr [15]. In spite of the fact that there is only limited knowledge on long-term studies of titanium alloy effects in the oral cavity and thus on the incidence of sensitivity to titanium in the general population, we may recommend TiAlNb and TiTa alloys for clinical use. Their susceptibility to fretting corrosion and potential negative effects, such as small-scale debris production resulting in cellular reaction and osteolysis [16, 17, 18], will be the subject of our further study. It has been demonstrated in our earlier studies [19] that pure titanium dental implants did not increase the number of chromosomal aberrance. The results of our present study with β titanium alloys support this information. It may be concluded that β titanium alloys undoubtedly will enlarge the set of materials used at the Department of Stomatology, Masaryk University, Brno (for overview see e.g. [20]). However, careful preclinical testing will have to precede clinical application of dental implants made of β-alloys.

ACKNOWLEDGEMENTS

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LAPAROSCOPIC VERSUS LAPAROTOMIC APPENDECTOMY FOR GENERALISED PERITONITIS IN CHILDREN

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KEY WORDS

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ABSTRACT

The aim of our study was to compare the results obtained with traditional versus laparoscopic appendectomy in generalised peritonitis. All patients treated for generalised peritonitis at the Department of Paediatric Surgery, Orthopaedics and Traumatology (DPSOT) were monitored, with focus on differences occurring in the postoperative period following laparoscopic or open surgeries.

Our set of patients was established using medical records of patients treated at DPSOT between 2002 and 2008. The selection criteria included the basic diagnosis *(peritonitis diffusa)* and complex diagnostics, treatment, and follow-up care at DPSOT. In terms of the postoperative period, we observed the length of treatment at ICU, the total length of hospitalisation, leukocyte and CRP level patterns, and both early and subsequent complications. Group A consisted of 140 children who underwent open surgery. Group B consisted of 70 patients in whom laparoscopic surgery without conversion was performed.

The average length of intensive care after traditional open surgery for generalised peritonitis due to acute appendicitis was 6.3 days and the length of hospitalisation was 9.5 days. The average length of intensive care in patients who underwent laparoscopic surgery was 5.8 days and the length of hospitalisation was 8.9 days. The mean C-reactive protein levels on day 6 after appendectomy were 20.1 in group A and 22.0 in group B. The levels of circulating leukocytes on the same day were 5.3 in group A and 7.1 in group B.

Laparoscopic appendectomy for appendicitis in its late stage with advanced generalised peritonitis does not show increased occurrence of early or late complications compared to traditional laparotomic appendectomy. Traditional open appendectomy exhibits more late complications.

ABBREVIATIONS USED

DPSOT – Department of Paediatric Surgery, Orthopaedics and Traumatology, Faculty Hospital Brno
ICU – Intensive care unit

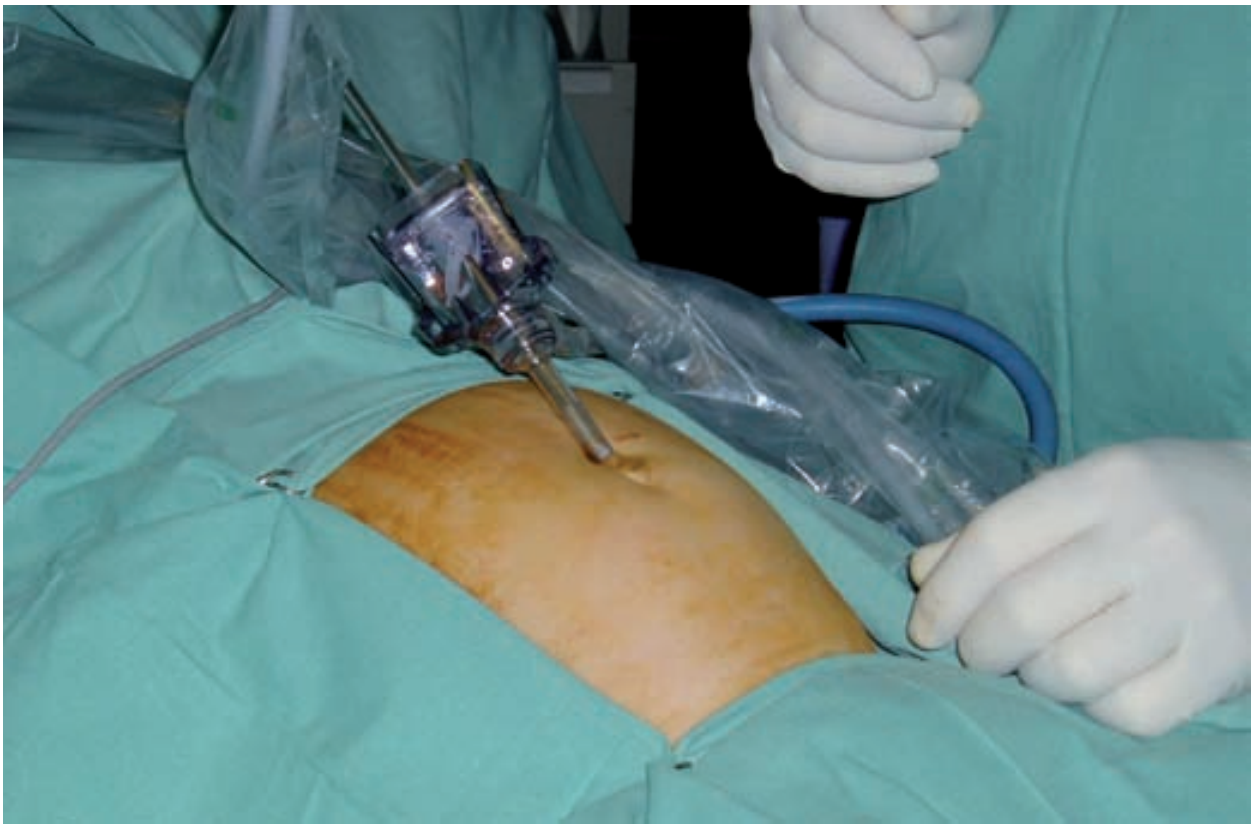
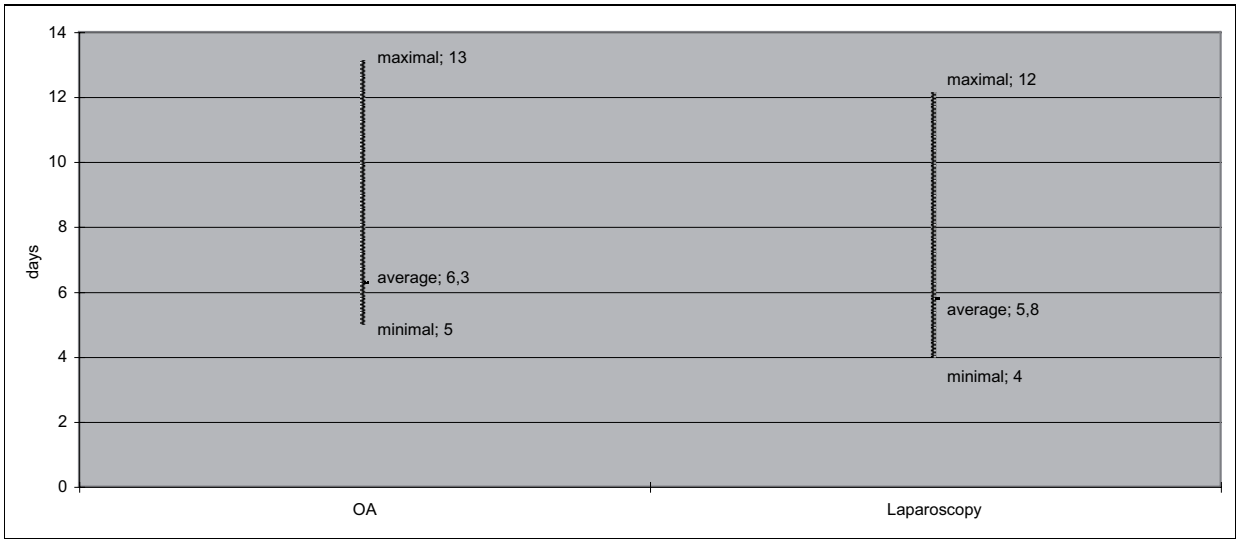


Figure 1
Transabdominal inserted laparoscope (archive of DPSOT)

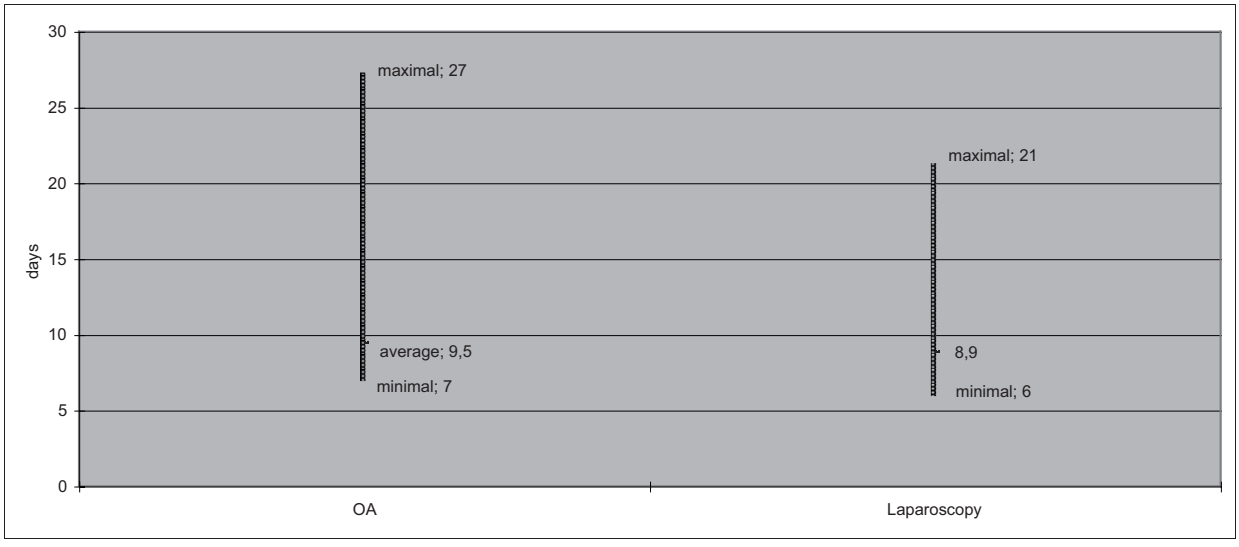
INTRODUCTION

Inflammation of the appendix of the caecum is one of the most common surgical emergencies of the abdomen treated at paediatric surgery departments. At the Department of Paediatric Surgery, Orthopaedics and Traumatology about 600 children (0–19 years) are admitted every year with suspected appendicitis, and in more than a half of the cases the diagnosis is confirmed by clinical examination and additional laboratory tests. The only causal treatment is early surgery performed by means of the classic, i.e. open, procedure or laparoscopically, a technique which has now been more commonly used. The choice of the surgical procedure is largely based on standard practice of the hospital. A large number of contradictory studies are available [1, 2], confirming or refuting the advantages of the mini-invasive laparoscopic procedure. As a result, the method is either widely accepted or classic laparotomy is preferred. Access to necessary equipment and experienced staff also play an important role [3]. A definite guide as to indication criteria for laparoscopic surgery cannot be determined and the two methods will therefore be still considered as the basic and adequate surgical procedures for acute appendicitis.

The aim of our study was to compare the results obtained with classic versus laparoscopic appendectomy in generalised peritonitis. Generalised peritonitis is in most cases associated with advanced inflammatory changes of the appendix in the form of gangrenous appendicitis, often perforated, and an appendix empyema or a periappendicular infiltrate frequently occur. Generalised peritonitis may also be accompanied by a smaller pelvic or bowel loop abscess. In all these cases, not only appendectomy, but also the abdominal cavity lavage is required. In terms of laparoscopy, the latter is more difficult to perform as extensively as with the open procedure. We observed all patients treated for generalised peritonitis at DPSOT and focused on differences occurring in the postoperative period following laparoscopic versus open surgeries. Initially, we formulated a null hypothesis, expecting no differences in the postoperative period after laparoscopic or laparotomic surgery.



Graph 1
Data distribution of hospitalisation lenght on ICU



Graph 2
Data distribution of hospitalisation lenght on surgery department

MATERIALS AND METHODS

Our set of patients was established using medical records of patients treated for generalised peritonitis at DPSOT between 2002 and 2008. Since 2002 we have been using a fully functional electronic hospital information system enabling us to search conveniently for all necessary information about these patients. The selection criteria included the basic diagnosis (K560 – *Peritonitis diffusa*) and complex diagnostics, treatment, and follow-up care at DPSOT. Within the analysed period, i.e. the last 7 years, 210 patients were selected based on these basic criteria. In the next phase of retrospective data collection we considered the choice of the surgical procedure and the postoperative

period. Concerning the latter, the following parameters were defined [4]:

- length of stay at ICU
- total length of hospitalisation
- patterns and stabilisation of leukocyte levels
- patterns and stabilisation of C-reactive protein levels
- early re-operation due to abscess
- occurrence of subsequent complications (adhesion – ileus)

The set of patients was divided into 2 groups. Group A consisted of 140 children who underwent open surgery. We did not distinguish between different laparotomy types (transverse incision, pararectal incision). In this group, we also included children who underwent laparotomic surgery after conversion from the originally selected procedure, i.e. laparoscopy. Group B consisted of 70 patients in whom laparoscopic surgery without necessary conversion was performed.

After compiling data tables, statistical evaluation was carried out for all defined parameters and the results of our retrospective study were determined.

RESULTS

The average length of intensive care after traditional open surgery for generalised peritonitis due to acute appendicitis was 6.3 days, whereas the average length of intensive care in patients who underwent laparoscopic surgery was 5.8 days. The total length of hospitalisation also shows no significant differences between the two methods: in group A it was 9.5 days and in group B 8.9 days. The mean C-reactive protein levels on day 6 after appendectomy were 20.1 in group A and 22.0 in group B. The levels of circulating leukocytes on the same day were 5.3 in group A and 7.1 in group B. No patients, whether treated with classic laparotomy or laparoscopy, exhibited early complications, such as an abdominal abscess or early bowel adhesions. In group A, we observed late complications in 8 cases, including clinical manifestations of bowel adhesions in the form of acute ileus in six of them (on day 78 after surgery on average). The remaining 2 cases exhibited late formation of an abscess focus (on day 40 after surgery on average). In children treated with laparoscopic surgery, ileus did not occur even during the minimum observation period of 1 year after surgery. Within the whole set of 70 patients, we recorded only one late complication and that was inflammatory penetration with subsequent cicatricial fibrosis and pain in *musculus psoas l. dx*.

In terms of statistical significance, our initially formulated null hypothesis was confirmed for all objective parameters defined for the evaluation of results achieved in acute appendicitis treatment. The total length of both intensive care and hospitalisation shows no differences between the two methods using a 5% significance level ($p1 = 0.0018$, $p2 = 0.0018$). The same situation occurred in case of C-reactive protein levels and leukocyte levels where both surgical procedures provided a statistically confirmed similarity of findings ($p3 = 0.0018$, $p4 = 0.0018$). Open laparotomy followed by appendectomy shows a higher incidence of subsequent complications and, in terms of statistical evaluation, it refutes our null hypothesis that had been initially formulated ($p5 = 0.145$). Within the whole set, we recorded zero mortality due to acute appendicitis and generalised peritonitis. In 4 patients included in group A, open surgery was required as a result of conversion from laparoscopy.

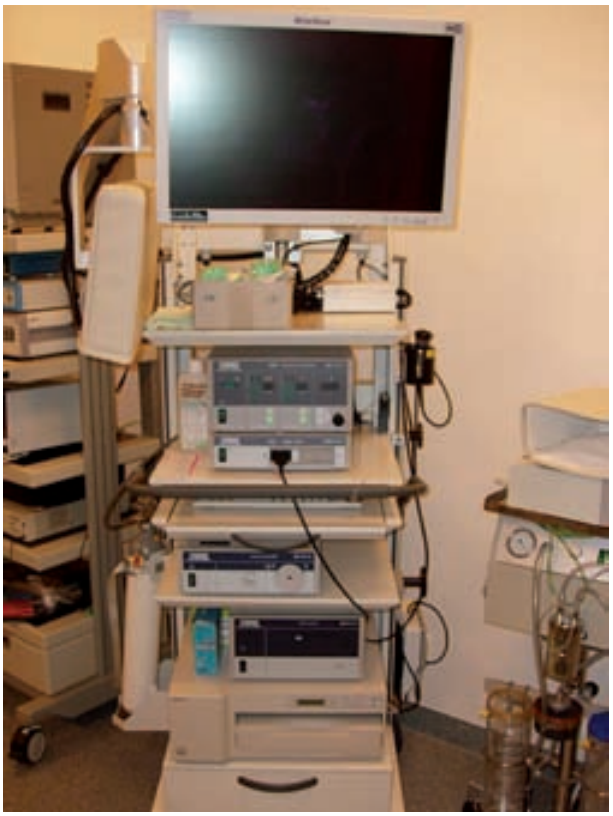
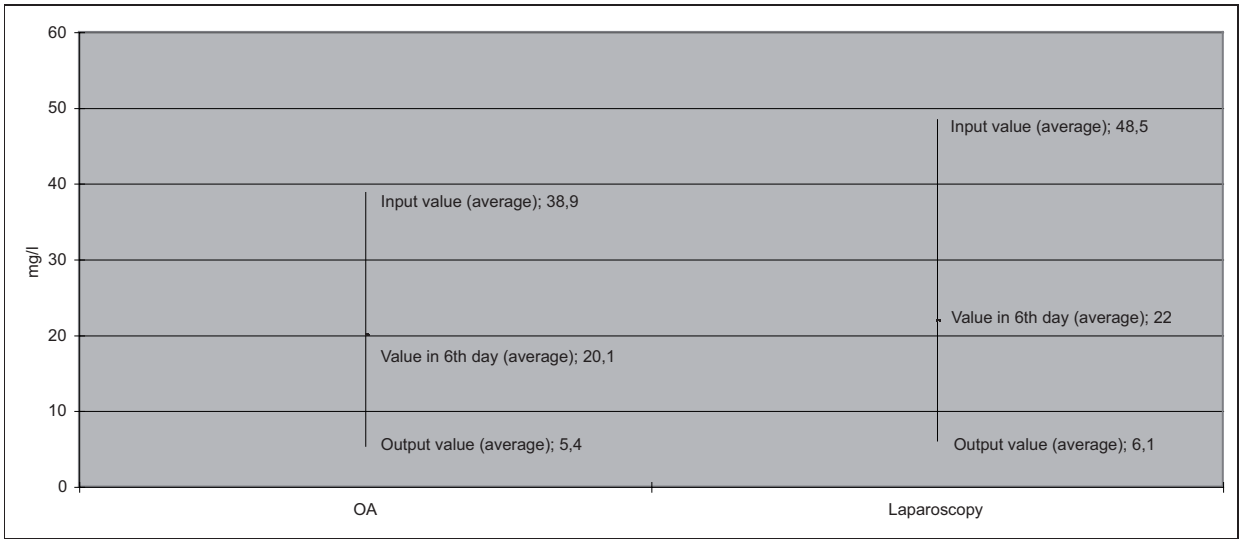


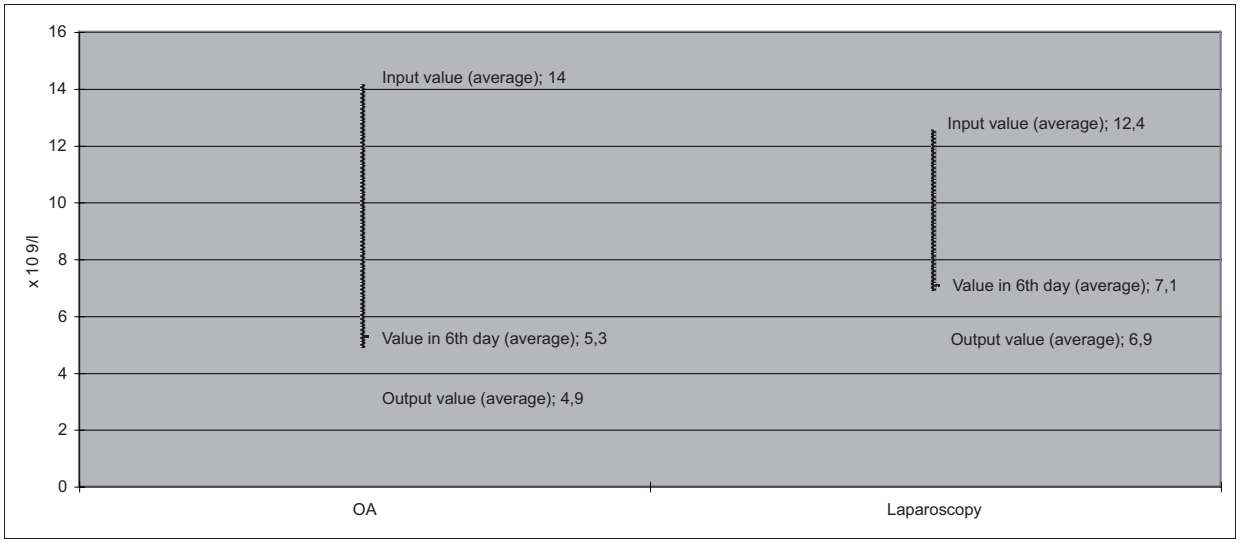
Figure 2
Laparoscopic set for paediatric endosurgery (archive of DPSOT)

DISCUSSION

The question of choosing the surgical procedure, i.e. whether to use laparoscopy or the traditional method, for inflammation of the vermiform appendage of the caecum has been under discussion ever since the introduction of the laparoscopic technique. Though the number of departments that



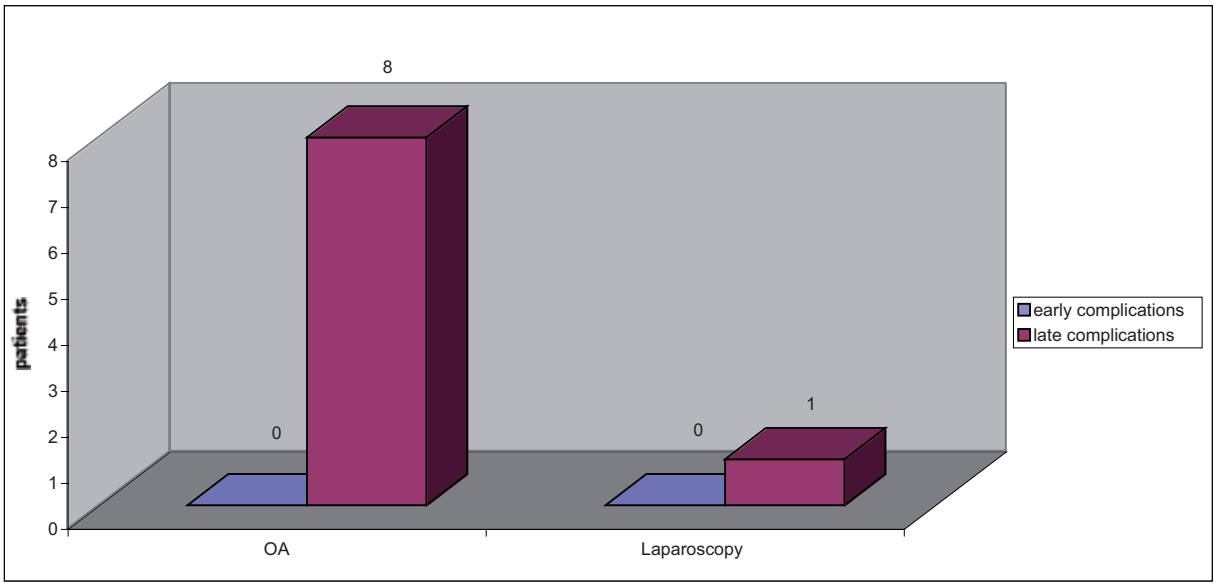
Graph 3
Data distribution of CRP level (6th day after operation)



Graph 4
Data distribution of WBC (6th day after operation)

prefer the mini-invasive laparoscopic procedure is growing, a final recommendation cannot be provided. In the end, it is always the responsibility of the surgeon. The day when one of the methods would be excluded from lege artis procedures is not likely to come.

As for laparoscopic surgeries, there is a large number of sceptics who regard the technical limitations of the performance during surgery as its disadvantage. However, gradual advances in surgical instruments and improving skills clearly show that human capability does not know any limits even in this area. One of the major practical limitations is time [4]: in laparoscopic appendectomy the operating time may be significantly longer than in laparotomic appendectomy. From this perspective, to prefer the latter seems essential and rational. By contrast, the atypical appendical position (retrocaecal, subhepatic) or a limited movement of the caecum



Graph 5
Frequency of early and late complications depending on surgical method

(subserous or sleeved) makes the open appendectomy quite difficult. With laparoscopy the operation procedure can be quicker and more comfortable for both the patient and the surgeon.

Another disadvantage associated with the laparoscopic method is a limited possibility of performing proper lavage of the inflamed peritoneal cavity [5]. Not only our study, but also several other studies provide valid information to disprove this assertion [4, 6]. Although the abdominal cavity is not widely opened and mechanically cleaned, compared to the classic procedure, we are able to perform revision of the peripheral areas of the abdominal cavity and individual organs more accurately. If we use adequate disinfection solutions, antibacterial measures can be regarded as sufficient. Moreover, in case of laparoscopy we can thoroughly suck out the remaining lavage liquid together with the remains of the fibrin clots, coagula, and tissue remains which are often found after surgery of generalised peritonitis.

The risks of laparoscopic surgeries, i.e. laparoscopic appendectomy, show a gradually decreasing tendency. This does not result only from the development of safer and more modern instruments, but it is also closely related to the adoption of safe surgical procedures. The insertion of the first port following Veress needle insertion has become less common and direct preparation of the abdominal cavity or monitored penetration by means of a transparent port with a camera are now preferred. The other ports are then inserted strictly under monitoring by a laparoscopic camera, and the incidence of injuries to organs in the abdominal cavity is comparable with the open procedure. In our set of patients, we did not record any iatrogenic injuries to organs in the abdominal cavity induced by the inserted port or a laparoscopic instrument. The percentage of this type of complications is usually about 1 ‰. The most commonly injured organ is the small intestine followed by the stomach, the inferior vena cava, and exceptionally some other organs (the aorta, the pancreas, etc.).

The question of safe preparation when using the electric field has also been widely discussed. Mostly Western countries and the USA intensively appeal for the safety of surgical procedures and the use of alternative means for early surface treatment. Within Western Europe the use of the harmonic scalpel has been promoted and in the USA the ENDO GIA stapler has been definitely recommended. Since they all represent stable economic systems of health care financing, it is only logical that their recommendations are not influenced by financial limitations. Retrospective studies provide numerous recommendations, suggesting an increased occurrence of complications after using monopolar electrocoagulation. Based on our experience, a large group of 210 patients treated for acute appendicitis did not exhibit any such complications. Even in the set of all patients treated laparoscopically at our department in the course of the last 7 years this problem could not be referred to as frequent. Patients who suffered an injury caused by monopolar electrocoagulation during laparoscopic surgery would

not exceed a few cases. The differences in effect between monopolar and bipolar coagulation are another subject for discussion. Our group of patients demonstrates the benefits of a bipolar machine, particularly in terms of reducing the number of perioperative complications with a tendency to bleed, which can lead to infection of haematoma remains at the appendectomy site. The risks of monopolar coagulation are indeed higher than in the case of bipolar forceps. However, we do not have statistical evidence for a higher incidence of injuries caused by electric current. The trend has obviously been set and all hospitals applying the laparoscopic procedure will probably aim at using disposable staplers, the harmonic scalpel, or new and more sophisticated methods.

CONCLUSION

Laparoscopic appendectomy for appendicitis in its late stage with advanced generalised peritonitis does not show increased occurrence of early or late postoperative complications compared to classic laparotomic appendectomy. A null hypothesis expecting no differences was successfully confirmed using a statistical significance level. On the contrary, traditional open appendectomy is accompanied by more late complications, such as bowel adhesions. Based on this study, we can recommend laparoscopy as adequate for appendectomy also in its late stage with inflammation extending into the peritoneum [7, 8].

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INTRAMEDULLARY NAIL USE FOR CORRECTIVE SURGERY OF DIAPHYSEAL FOREARM FRACTURES

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ABSTRACT

The aim of this study is to evaluate the results after reoperation of radial- and ulnar diaphyseal fractures using an interlocking intramedullary nail. A cohort of 30 patients, who were treated with a ForeSight (Smith&Nephew, USA) intramedullary nail between the years 2000 and 2005, were included in the study. In total, pseudoarthrosis formation was seen in 21 patients, and forearm refractures were seen in 9 patients. The average length of time between trauma and resurgery was 15 months (range of 4 to 32 months). Postoperatively, early and late complications were evaluated, as well as radiological and functional results. During resurgery, a total of 42 intramedullary nails were used in 30 cases of forearm fracture. Radiological proof of healing was observed in all patients: this was seen in 25 patients after 6 months, 3 patients after 12 months, and in 2 patients after 18 months. The average duration of healing was 18.45 weeks. Ranges of movement of the wrist, the forearm, and the elbow were classified according to the Anderson method. Excellent results were achieved in 9 patients, good results in 13 patients, and sufficient results in 8 patients. No patient exhibited insufficient results or complete rigidity of the wrist or the elbow. The results of our study corroborate the possibility of using stabilised intramedullary nailing for revision surgery of the forearm. The corrective surgery indicated must take into account the type of pseudoarthrosis present for a specific therapy, continually respecting the essentials of osteosynthesis stabilisation. A selective operative technique, meticulous follow-up of infections, and promotion of bone healing are paramount.

INTRODUCTION

Nowadays, the standard of primary treatment of radial- and ulnar diaphyseal fractures is operative treatment using plate fixation [2, 8]. Complications in healing after the use of this

technique occur quite infrequently, and most contemporary studies note the incidence of pseudoarthrosis formation at under 5 % [11,15,18]. Non-union of a diaphyseal forearm fracture is usually associated with either a complex injury, a complication such as an infection, or inadequate internal fixation [11].

The other motive for revision surgery of antebrachial diaphyseal fractures are refractures after removal of the osteosynthetic material.

The treatment of such non-union fractures, which are most frequently pseudoarthrosis formation, includes restoration of the correct length and rotation of the forearm so as to allow a physiological range of movement. A cohort of patients, for whom reosteosynthesis was indicated using intramedullary nailing because of an unhealed forearm fracture or refracture, has been evaluated in this study.

MATERIALS AND METHODS

Between the years 2000 and 2005 we performed corrective surgery for diaphyseal forearm fractures using the ForeSight intramedullary nail (Smith&Nephew, USA) in 30 patients. All patients were operated on in one clinic, with 6 surgeons alternating. The following patients were excluded from the study: those with pathological fractures, those with rheumatoid arthritis, those with a history of chronic steroid use, and those patients who did not show up for follow-up examination (4 patients).

Criteria for inclusion in the study involved:
Type of injury

- *Pseudoarthrosis formation,* which developed as a complication of plate osteosynthesis, external fixation, intramedullary pinning, or conservative therapy after primary reposition using plaster casts;
- *Refracture* (mostly after plate removal);
- *Re-osteosynthesis* performed by intramedullary nailing;
- A minimum of 18 months' *follow–up.*

For the purposes of this study, an atrophic pseudoarthrosis (delayed union) was defined as an unstable fracture with no signs of healing after a minimum of 4 months following injury. A hypertrophic pseudoarthrosis is an unstable fracture with overproduction of the callus, without signs of progressed healing up to 12 months after trauma with marked clinical symptomatology. Furthermore, a refracture was defined as a new fracture after minimal trauma in the original fracture line or in the area of the screw canal after plate extraction.

Primary injury

Our patient cohort included 18 men and 12 women of an average age of 36.2 years (range 19–74 years). The fracture was present on the left forearm in 21 patients and on the right forearm in 9 patients. The dominant extremity was affected in 13 cases (43.3 %). The cause of injury was a motor vehicle accident in 13 patients, falls in 11 patients, work-related injury in 5 patients, and a gunshot wound in one patient.

The primary injury in 21 patients was a diaphyseal fracture of both forearm bones; in 7 patients, an isolated fracture of the radial diaphysis; and in 2 patients, an isolated fracture of the ulna.

Eleven patients had a primarily opened fracture that was classified according to Gustillo-Anderson as stage I in 2 patients, stage II in 6 patients, and stage III in 3 patients.

Four patients had a concomitant injury of the ipsilateral upper extremity (two fractures + elbow subluxation, one diaphyseal fracture of the humerus, and one fracture of the distal humerus). Two patients had radial nerve paralysis: in one patient, this was due to an ipsilateral humeral diaphyseal fracture, and in the other patient, the paralysis occurred after primary osteosynthesis done at another clinic. In our study, no vascular injury was observed.

Primary therapy included plating in 17 cases, intramedullary stabilisation using Kirschner wires in 6 cases, external fixation in 2 cases, and plaster casting in 5 cases. Twenty-six patients were originally treated elsewhere (86.7%). A deep-seated infection occurred postoperatively in 4 patients, and this was treated by repeat debridement and parenteral antibiotic administration. Upon reoperation, no clinical or biochemical signs of active inflammation were observed.

Twice, a rupture of the extensor pollicis longus muscle tendon was noted after primary surgery. This complication was resolved during the corrective surgery by transposition of the tendon of the extensor indicis proprius muscle.

At the time of resurgery, 25 of 30 patients had had at least one previous surgery on the ipsilateral forearm. The following implants were selected during primary surgery:

- 3 plates unsuitable for the fracture type (1 – one-third tubular plate, 2–4.5mm-sized autocompressive plates);
- 3 plates of unsuitable length (only 2 screws inserted into one side of the fracture);
- 2 incorrectly placed screws (the screws crossed the fracture line).

The aforementioned patients, and those who had primarily stabilisation using intramedullary Kirschner wires (6 patients) or after primary reduction by plaster casting (5 patients), are considered by us to constitute the group of patients with suboptimal primary fixation (in total, 19 patients /63.3 %/).

Corrective procedure

Pseudoarthrosis or refracture was present at the time of reoperation in both forearm bones in 12 cases. These complications were isolated to the radius and the ulna in 13 and 5 cases,

Table 1
Patients with pseudoarthrosis – data

Patient (No.)	Age	Gender m/f	AO classification (primary fracture)	Surgical treatment	Type of pseudoarthrosis	Time of resurgery (months)
P1	27	f	C.2, closed	2x plate (3.5 mm)	atrophic PSA – both	12
P2	30	m	B.3., closed	2x plate (3.5 mm)	atrophic PSA – radius	15
P3	43	f	B.3., open G-A III	fixateur externe	atrophic PSA – ulna	4
P4	19	m	C.3., closed	2x plate (3.5 mm)	atrophic PSA – radius	8
P5	40	m	A.2., closed	1x plate (4.5 mm)	atrophic PSA – radius	10
P6	56	m	B.3., closed	2x plate (3.5 mm)	atrophic PSA – ulna	12
P7	48	f	A.2., open G-A II	1x 1/3 tub. plate (3.5 mm)	atrophic PSA – radius	21
P8	61	m	C.1., closed	2x plate (3.5 mm)	atrophic PSA – both	12
P9	74	f	B.2., closed	conservative, cast 14 w.	hypertrophic PSA – radius	15
P10	33	m	B.3., open G-A II	2x Kirschner wires	hypertrophic PSA – both	12
P11	44	m	C.3., closed	2x Kirschner wires	hypertrophic PSA – both	15
P12	27	m	A.2., G-A III stage	fixateur externe	hypertrophic PSA – radius	12
P13	29	f	C.2., closed	2x plate (3.5 mm)	hypertrophic PSA – both	15
P14	32	m	A.1., open G-A I stage	2x Kirschner wires	hypertrophic PSA – ulna	16
P15	27	m	C.1., closed	2x plate (3.5 mm)	hypertrophic PSA – both	18
P16	36	m	C.3., closed	conservative, cast 12 w.	hypertrophic PSA – both	13
P17	25	f	B.3., closed	2x plate (3.5 mm)	hypertrophic PSA – radius	15
P18	20	m	B.3., closed	conservative, cast 14 w.	hypertrophic PSA – both	12
P19	24	f	C.2., open G-A II	2x Kirschner wires	hypertrophic PSA – both	16
P20	22	f	A.2., closed	2x plate (3.5 mm)	hypertrophic PSA – radius	14
P21	40	m	C.3., open G-A II	2x Kirschner wires	hypertrophic PSA – radius	14

respectively. The length of time between trauma and reoperation was on average 15 months (range of 4–32 months).
In total, 21 patients had pseudoarthrosis formation (Table 1) and 9 patients had refractures (Table 2) of the forearm (4 fractures crossed the fracture line of the previous fracture and occurred after minimal trauma, while in 5 patients refracture occurred in the screw canal) (Figures 1a-f, 2a-e).

Interventions
The standard surgical approach involves an indirect closed reposition of the fracture. This technique was used primarily in refractures that had occurred after plate extraction, in pseudoarthrosis formation following conservative measures, or after intramedullary osteosynthesis using Kirschner wires. The procedure is performed on a radiolucent hand table, and the correct length and shape of the nail is confirmed using an X-ray intensifier and two malleable templates (radius – dorsoradial curvature, ulna – s-shaped curvature). The nailing of the ulna is accomplished through a port made in the proximal olecranon. The entry point for the nailing of the radius is made on the radial side of Lister's tubercle beneath the extensor carpi radialis brevis tendon. The ulna is nailed in the antegrade direction first, providing a more stable forearm for retrograde nailing of the radius.
Consequently, the medullary cavity of the fractured bone is gently manually reamed, and the medullary canal is widened to a length of between 0.5 and 1.0 mm greater than the selected nail diameter. After reaming, the nail is inserted.
In general, we perform bilateral interlocking as the standard for corrective surgery. Unilateral nailing is possible only in cases of "press-fit" nailing into the thin part of the medullary cavity (middle and distal third of the ulna; middle and proximal third of the radius). The fracture line should be simple, with optimal impaction, and the peripheral bone fragment must be long enough to secure a favourable rotational stability (minimum of 5 cm). Peroperative control of the rotational stability is essential. From the standpoint of stability, the high risk area is predominantly the proximal radius, where the deep branch of the radial nerve closely approximates to the neck of the radius. For this fact alone, unilateral stabilisation of the radius is recommended. The actual stabilisation is done using a free-hand technique.

Table 2
Patients with refractures – data

Patient (No)	Age	Gender m/f	AO classification (primary fracture)	Treatment	Time of removal (months)	Time delay of fracture after removal (months)	Adequate trauma	Fracture line
R1	54	f	A.3., open G-A II	2x Kirschner wires	6 months	8 months	fall from a tree	original fracture line
R2	19	m	B.3., open G-A III	conservative, cast 6 w.	0	14 months	minitrauma, walking	original fracture line
R3	25	f	A.2., closed	1x plate (3.5 mm)	18 months	4 months	minitrauma, bathroom	original fracture line
R4	20	m	B.3., open G-A I	conservative, cast 14 w.	0	12 months	regular fall	original fracture line
R5	41	m	A.2., closed	1x plate (3.5 mm)	12 months	20 months	bike accident	through the screw canal
R6	59	f	A.2., closed	1x plate (4.5 mm)	18 months	3 weeks	minitrauma, bathroom	through the screw canal
R7	40	m	C.3., open G-A II	2x plate (3.5 mm)	20 months	1 months	minitrauma, walking	through the screw canal
R8	22	f	B.3., closed	2x plate (3.5 mm)	12 months	3 months	minitrauma, home	through the screw canal
R9	45	m	B.1., closed	1x plate (3.5 mm)	15 months	5 months	regular fall	through the screw canal

To eliminate the risk of nerve damage, we adhere to the following approach:
After selecting the point of skin incision, blunt dissection with scissors and a forceps is used to prepare a canal for drilling. The drill bit is inserted with its protective sleeve into the soft tissue canal. Under radiological guidance, the drill bit is centred onto the bone and only then is pressure applied to the bone with the protective sleeve on the soft tissue. Damage to the deep branch of the radial nerve has not been observed using this technique.
An alternative surgical technique involved extraction of the failed implant, debridement of the sclerotic and devascularised bone, local removal of inflammatory and fibrous tissue, decortication of the principal fragments, and subsequent renewal of the length and rotation of the dislocated bone. Intramedullary nails were used for the stabilisation. In cases of atrophic pseudoarthrosis formation or with segmental bony defects, spongioplasty was performed. Autogenous spongious transplantation was used for the donor bone, and was taken from the crest of the iliac bone on the ipsilateral side. We did not use a bone-graft substitute in this study.

Clinical conclusions
Postoperatively, all early and late complications were studied, as well as the radiological and functional results.

Results
The average period of follow-up was 23 months (range of 18–42 months). An intramedullary nail was used in 30 forearm fractures, and a total of 42 nails were implanted (Table 3). Bilateral interlocking of the nail was done in 29 cases (69.0 %); unilateral interlocking, in 13 cases (31.0 %). Closed reposition was performed in 21 cases (50.0 %), mini-incision open reposition in 13 cases (30.9 %), and open reposition was done in 8 cases (19.1 %). The average length of surgery was 83 minutes (range of 30 to 180 minutes).

Adjuvant spongioplasty was performed in 8 patients. A supportive plaster cast was applied in 6 patients and was left in place for 4 weeks.

Healing
Radiological confirmation of healing (complete remodelling of the fracture with no gap between fragments) was seen in all patients (Table 4). This was observed in 25 patients within 6 months, in 3 patients within 12 months, and in 2 patients within 18 months (Table 5). The average length of time to healing was 18.45 weeks.

Complications
The following peroperative complications were noted:

Table 3
Type of corrective surgery and healing

Patient (No.)	Surgery	Spongiosaplasty	Locking screws	Cast (weeks)	Time of healing (radius + ulna/months)
P1	2x nail	*	bilat.	0	4 + 5
P2	1x nail	*	bilat.	0	6
P3	1x nail	0	bilat.	0	18
P4	1x nail	*	bilat.	0	4
P5	1x nail	*	bilat.	0	6
P6	1x nail	0	unilat.	0	3
P7	1x nail	*	unilat.	4	6
P8	2x nail	*	bilat.	0	5 +5
P9	1x nail	0	unilat.	4	3
P10	2x nail	0	bilat.	0	4 + 8
P11	2x nail	0	unilat.	0	4
P12	1x nail	0	bilat.	0	4
P13	2x nail	0	unilat.	4	5 + 6
P14	1x nail	0	bilat.	0	4
P15	2x nail	0	bilat.	0	14 + 4
P16	2x nail	0	bilat.	0	3 + 3
P17	1x nail	0	unilat.	0	4
P18	2x nail	0	bilat.	0	3 + 4
P19	2x nail	0	unilat.	4	2 + 2
P20	1x nail	0	unilat.	0	3
P21	1x nail	0	unilat.	0	4
R1	1x nail	0	bilat.	0	9
R2	2x nail	0	bilat.	0	3 + 3
R3	1x nail	0	unilat.	4	4
R4	2x nail	0	unilat.	0	3 + 3
R5	1x nail	*	bilat.	0	5
R6	1x nail	0	bilat.	0	5
R7	1x nail	0	unilat.	0	8
R8	2x nail	0	unilat.	4	4 + 3
R9	1x nail	*	bilat.	0	5

Table 4
Healing after resurgery according to individual types of fractures

	Number of patients	Average age	Average time to healing (weeks)
Refracture (of the original fracture line)	4	29.5	14.42
Refracture (of the screw canal)	5	41.5	21.30
Atrophic pseudoarthrosis formation	8	40.5	17.8
Hypertrophic pseudoarthrosis formation	13	33.3	19.56
Total number of patients	30	36.2	18.45

Table 5

Data of patients with impaired healing after corrective surgery. Three patients had delayed healing (8;8;9 months). A further 2 patients showed healing after 14 and 18 months. The time to complete healing is related to pseudoarthrosis formation. Healing occurred with no additional surgical intervention

Gender	Age	Reason for reoperation	Procedure	Bone	Time of healing (months)
M	40	Refracture in the screw canal after plate extraction	Nail, unilateral interlocking	Radius	8
F	54	Refracture in the previous fracture	Nail, bilateral interlocking	Ulna	9
F	43	Pseudoarthrosis formation	Nail, bilateral interlocking	Ulna	18
F	27	Pseudoarthrosis formation	Nail, bilateral interlocking	Radius + ulna	14 + 4
M	33	Pseudoarthrosis formation	Nail, bilateral interlocking	Radius + ulna	4 + 8

Table 6

Functional results according to range of movement

Anderson score	No. (%)
Complete range of movement*	9 (30.0)
Slight restriction in movement**	13 (43.3)
Severe restriction in movement ***	8 (26.7)
Complete rigidity	0 (0)

* less than 10% restriction of wrist-dorsipalmar flexion, less than 25% restriction of pronosupination
** less than 25% restriction of wrist-dorsipalmar flexion, less than 50% restriction of pronosupination
*** more than 25% restriction of wrist-dorsoplantar flexion, more than 50% restriction of pronosupination

Table 7

Results of evaluation of pain

Pain evaluation	No. (%)
No pain	20 (66.6)
Pain upon weight stress	8(26.7)
Pain at rest	2 (6.7)
Pain requiring chronic analgesic therapy	0 (0)

- 1 occurrence of nail impaction in the diaphyseal cavity and subsequent damage to the fixation thread during extraction. This was peroperatively corrected by exchanging the nail for a thinner one.
- 1 occurrence of a "fausse route" during reaming.
- 1 occurrence where it was necessary to remove a shot pellet (in a gunshot wound) from the medullary canal via open surgery, which impeded nailing.

In 3 cases, a late complication of partial migration of the locking screw in the nail was observed.

A discrepancy in the length of the distal radioulnar joint of more than 2 mm was observed in 2 patients after osteosynthesis (6.66 %).

Functional evaluation

The range of movement of the wrist, the forearm, and the elbow were evaluated by the Anderson method [3] (Table 6). Excellent results were achieved in 9 patients, good results were achieved in 13 patients, and satisfactory results were seen in 8 patients. No patients ended up with poor results of healing or complete rigidity of the wrist or the elbow.

Pain was evaluated with respect to difficulties at rest, upon weight stress, and severity requiring analgesic medication (Table 7).

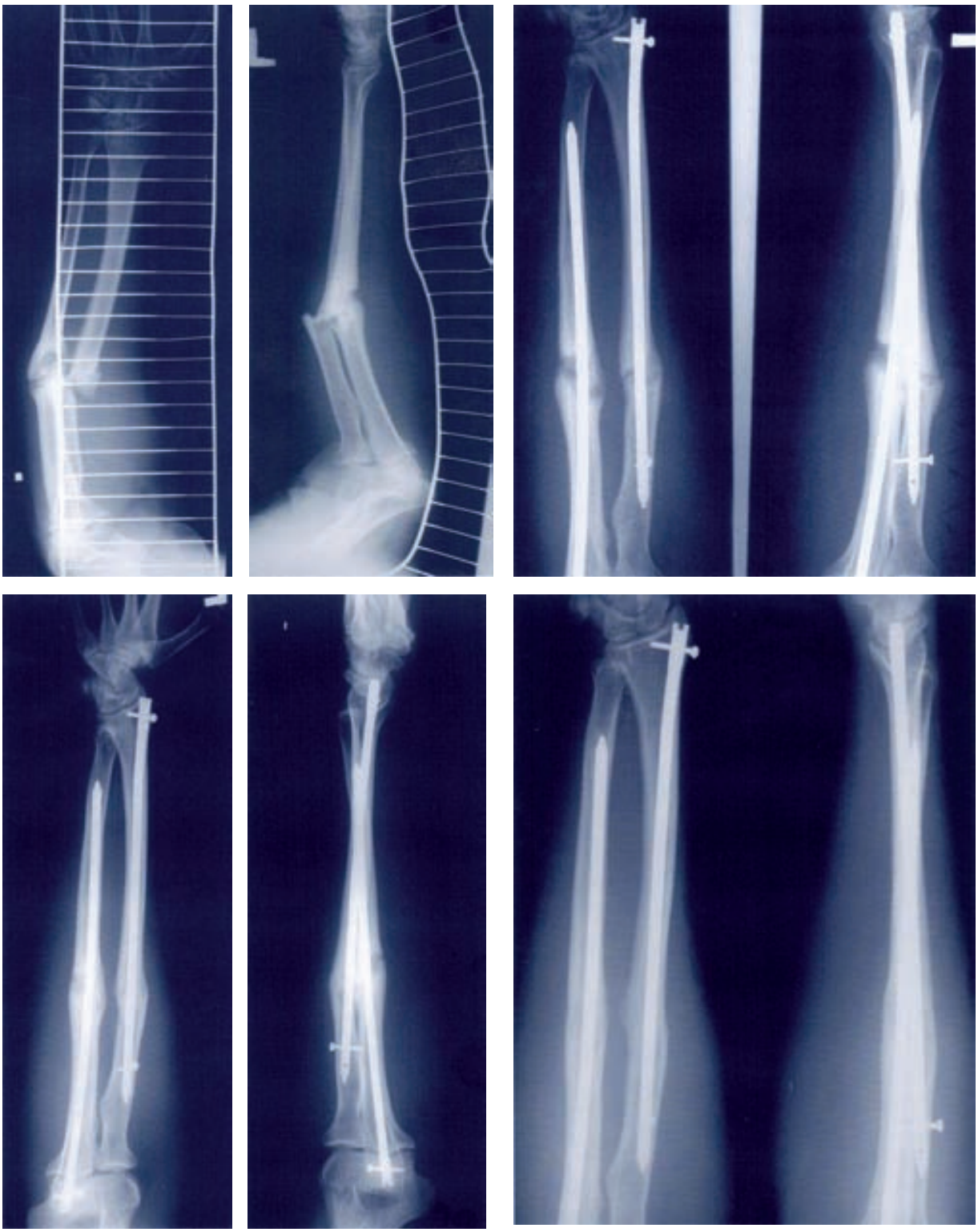

Figure 1
A 20-year-old patient after a fall from a tree treated conservatively by plaster casting for 14 weeks. Twelve months later, there was a refracture in the original fracture line after minimal trauma (a,b). Osteosynthesis of the forearm was done using a closed technique with intramedullary nailing (c). Follow-up X-rays after 8 weeks (d,e); complete healing after 12 weeks (f)

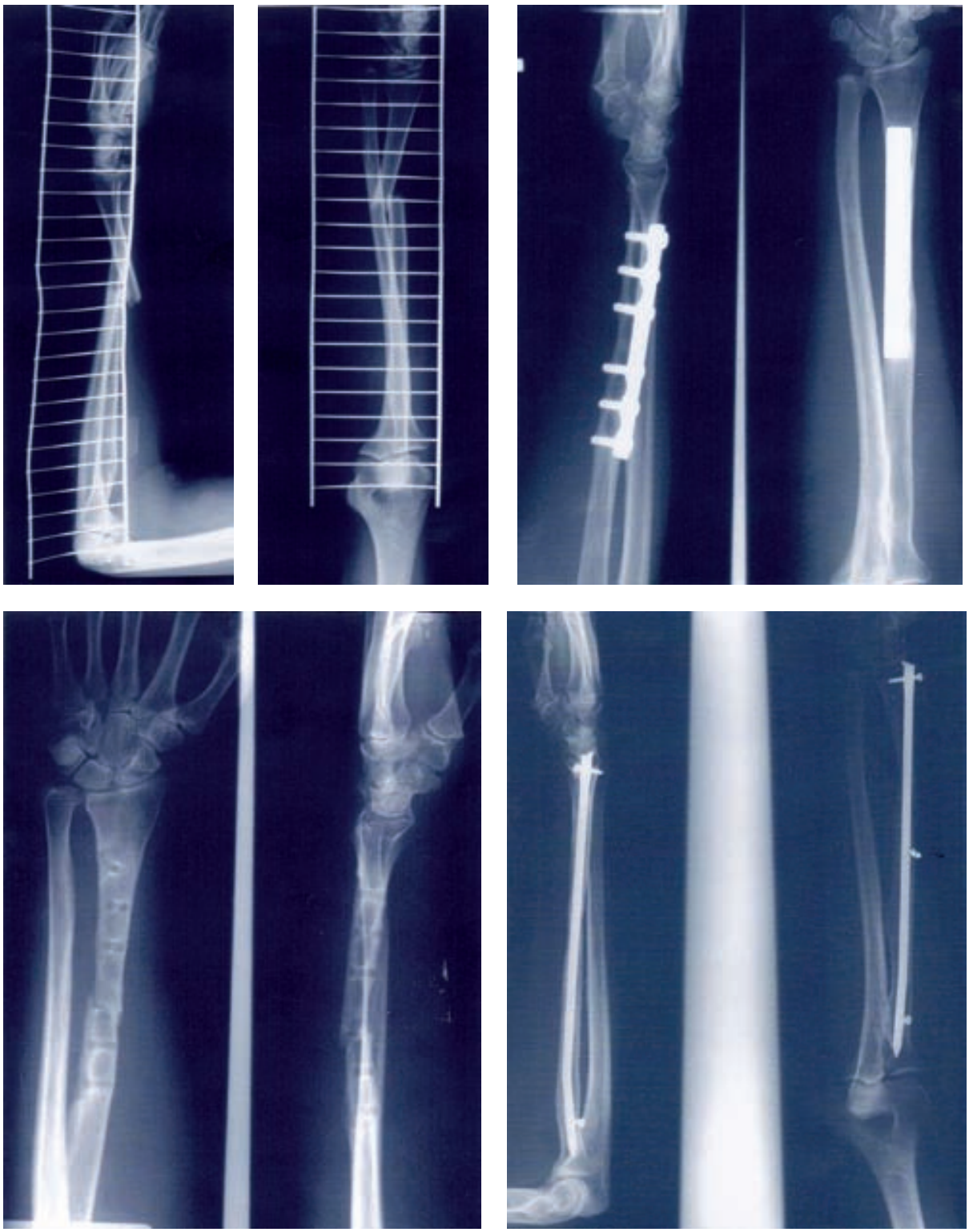

Figure 2
A 59-year-old patient after a motor vehicle accident with a Galleazzi fracture, type 22 – A2, according to AO classification (a,b). Treatment using plate osteosynthesis (c); extraction of the plate after 18 months; 3 weeks later, refracture in the screw canal after plating occurred after minimal trauma/fall in the bathroom, (d); Refracture was treated by nailing / X-ray healing after 5 months (e)

DISCUSSION

The notion of intramedullary osteosynthesis of the forearm was first published by Schon in 1913 [13]. The initial results were unsatisfactory, and even in 1957 Smith and Sage [14], in their analysis of 555 forearm fractures treated by a gamut of intramedullary implants (K wires, Rush-pins, …), noted a 20 % incidence of pseudoarthrosis formation [1]. This problem was justified on the grounds of insufficient angular and rotational stability of the implant materials used [12,14]. An improvement in the stability of the nail was forged by a change in the cross-section of the nail, and in later years various adjustments included the U-shaped Kuntscher nail, the triangular Save nail, the square-shaped Von Saal nail, and the quadratic Street nail. Further advancement of the rotational stability was achieved at the end of the 20th century, when the nail was supplemented with distal and proximal interlocking (i.e. ForeSight nail).

The incidence of disordered healing of diaphyseal forearm fractures is nowadays falling. The majority of contemporary studies cite a less than 5% incidence of pseudoarthrosis formation with the use of plates (11,15,18). Hence there are not many studies which evaluate corrective surgery of this area. One of the most comprehensive studies in English literature was published by Ring [11], who evaluated a cohort of 35 patients with diagnosed arthrotic ununited diaphyseal fractures of the forearm. He observed that the segmental bony defect was on average 2.2 cm in length (range between 1 and 6 cm). This was treated with the help of autologous spongious grafting and reoperation with plating. The period of healing was 6 months in all patients; the range of wrist movement was circa 2/3; the grip strength, in contradistinction to the opposite side, was on average 83 %. In evaluating the functional results according to Anderson, Rings achieved excellent results in 5 patients, good results in 18 patients, satisfactory results in 11 patients, and poor results (because of malunion) in 1 patient. Ring further noted that hypertrophic pseudoarthrosis formation does not typically occur in the forearm. An atrophic pseudoarthrosis formation with a segmental bony defect occurred more frequently. This opinion is however not shared by Babhulkar [2], who did not observe a majority of atrophic pseudoarthroses in his studies. In treating a total of 15 pseudoarthrosis formations of the radius and 21 cases of pseudoarthrosis formations of the ulna, he used plating (86.1 %) in favour over intramedullary nailing (13.9 %). The breakdown of individual types of pseudoarthrosis formations in our study more reflected those found by Babhulkar in that atrophic pseudoarthrosis more frequently arose in cases where the primary treatment involved an extensive approach and a gross injury to the periosteal vascular supply. In contrast, a higher incidence of hypertrophic pseudoarthrosis formation was observed after insufficient primary stability of the fracture area, which conferred a great degree of micromovement upon the local area (osteosynthesis using K wires, conservative measures, failure of plate fixation). A hypertrophic pseudoarthrosis formation is considered to be richly vascularised, and therefore quickly leads to bony healing after stable fixation and osteosynthesis. On the contrary, the atrophic pseudoarthrosis formation is poorly or inadequately vascularised, and is characterised by a low activity of osteoblasts, and bony healing is limited. This impediment requires resection of the dead tissue, as well as of the fibrous and inflammatory tissues, and the defect produced must be refilled with an autologous bony graft.

Refractures after plate extraction correspond primarily with injury to the periosteal circulation due to the implant, which leads to a pathophysiological process that ends in cortical atrophy and skeletal porosis. This problem is then directly related to the question of optimal timing of plate extraction. A precise time cannot be unequivocally recommended, but it should be no shorter than 12 months [5, 7, 9].

Studies that have evaluated the use of intramedullary nailing in corrective surgery of the forearm are indeed singular [4, 6, 16]. Hofmann [9] evaluated the use of a stabilisation nail in 7 reoperations after fracture of the ulna. The nail was successfully used even when the defect in the area of the pseudoarthrosis was extensive. We believe that a centrally-placed implant affords a better alignment biomechanically, and this stability is also conferred in the frontal and sagittal planes. It must be stressed that the plate is locally exposed to relatively great forces, which may lead to plate shattering or to screw loosening. This problem was partially resolved by the newer types of Locking Compression Plates or a PC-Fix type plate, which have more favourable biological conditions for healing.

In our study, the nail was used in 30 patients, who had 42 forearm fractures. The intramedullary nail will certainly not replace plating when used for corrective surgery, but in many cases it is advantageous to use it. When applying the nail, further weakening of the bone at the level of the stabilisation screws inserted into the plate does not occur. In addition, repeated deperiostation of the fragments does not occur. When performing reoperation with a plate, it is often consequently necessary to perform deperiostation to a great extent. The anatomical malleability (radius – dorsoradial curvature, ulna – s-shaped curvature) of the nail is a necessity and leads to a renewal of the physiological shape of the forearm bones. Reaming in the area of sclerosis after the preceding nailing is often tricky, and must be performed carefully and steadily, so that breakage of the reamer does not occur. In rigid pseudoarthroses, which are prone to incorrect positioning, closed reposition may be impossible, and mini-incision repositioning is therefore required. Following insertion of the

nail, the result of repositioning and freedom of wrist and elbow movement, as well as forearm rotation are checked. Rotational control is possible thanks to the static bilateral locking inherent to nailing. Stabilisation of the contralateral side is done by a free-hand technique.

Hypertrophic pseudoarthrosis formations arise mostly from insufficient stability. One must ensure an adequate period of rest to enable full healing of this pathology. In this type of pseudoarthrosis formation it is beneficial to use an intramedullary nail that is inserted by closed technique. During its application, the periosteal callus is not disturbed and, furthermore, gentle reaming of the medullary cavity stimulates healing – the so-called internal spongioplasty. Contraindications in using nails may be either a narrow medullary cavity (less than 3 mm) or an open epiphyseal plate in adolescents. A relative disadvantage is its high cost. In contrast, one advantage presented is the possibility of using the nail for a locally poor state of the soft tissues surrounding the pseudoarthrosis. It is also beneficial in treating segmental fractures but, most importantly, there is a very small percentage of infectious complications after intramedullary nailing.

For complete healing, an *atrophic pseudoarthrosis* requires supportive measures to stimulate bony healing. An essential component of the operative procedure is local debridement and decortication of the pseudoarthrosis formation. In this study, we have demonstrated the possibility of using nails even for this type of pseudoarthrosis. An important step in this surgery is open bone grafting.

It is necessary to confirm that the angle of reduced forearm bones is correct in both types of pseudoarthrosis formations. Healing in the incorrect position (a malunion) annuls the merit of the corrective surgery.

In conclusion, the results of our study confirm that intramedullary nailing can be used in corrective surgery of the forearm. The planned corrective procedure must take into consideration the type of pseudoarthrosis formation, be tailored to the individual involved, and, most of all, it must respect the tenets of stable osteosynthesis. Other important considerations are a differentiated operative procedure, the follow-up of infections, and supportive measures for bony healing.

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WOLF AND HELAL METATARSAL OSTEOTOMIES IN METATARSALGIA TREATMENT

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ABSTRACT

PURPOSE OF STUDY
A common reason for painful forefoot is plantar shift of metatarsal heads. Progression of this condition creates painful keratoses. The aim of our study was to evaluate the results after Wolf osteotomy of metatarsals and after Helal telescopic metatarsal osteotomy in comparable groups of patients.
MATERIAL
Wolf osteotomy was performed in 62 patients (75 feet) in the period of 2002 through 2005. The average follow-up was 31 months. There were 55 females (88.7 %) and 7 males (11.3 %) in the group. The mean age was 53.6 years (27–82 years). Forty-one patients (51 feet) appeared for follow-up examination.
Helal osteotomy was performed in 98 patients (112 feet) in the period of 2001 through 2003. The average follow-up was 34 months. There were 87 females (88.8 %) and 10 males (11.2 %). The mean age was 52.8 years (35–76 years). Seventy-one patients (76 feet) appeared for follow-up examination.
METHODS
We performed Wolf osteotomy with small Luer pliers in the region just proximal from the heads of the second, third, and fourth metatarsals. We removed a small part of the dorsal cortex and created a V-shaped notch based dorsally, leaving the plantar cortex intact. The plantar cortex was then fractured by closing the osteotomy with fingers and turning the metatarsal head upwards.
We performed Helal osteotomy using an oscillating saw on the border between the distal and the middle thirds of the metatarsal bone. The direction of the osteotomy was 45° obliquely distad and towards the planta. Then we gently shifted the distal fragment proximally and dorsally. We resected the overlapping part of the dorsal cortex of the distal fragment. We performed the same procedure on the second, third, and fourth metatarsals.
The Gainor scoring system was used for the evaluation of our results.

RESULTS
We achieved 76.5 % excellent, 13.7 % good, 5.9 % satisfactory and 3.9 % bad results in the group with Wolf osteotomy and 81.2 % excellent, 12 % good, 4.3 % satisfactory, and 2.5 % bad results in the group with Helal osteotomy.
DISCUSSION
The indication for Wolf or Helal osteotomies is a static deformity of the forefoot with plantar depression of the second, third, and fourth metatarsal heads and painful plantar callosities. The goal of both osteotomies is the elevation of metatarsal heads. The prerequisite of a good result is a careful surgical technique. Mistakes in the technique can influence the final result. None of the two methods requires internal fixation. Both procedures allow early stressing of the limb, even if with crutches. We have achieved similar results with both techniques in comparison with other publications.
CONCLUSIONS
Similar results can be achieved using both types of osteotomies. Both methods have an identical effect on pain relief in the forefoot caused by plantar shift of metatarsal heads. The advantage of both methods is early stressing of the operated foot.

INTRODUCTION

Wolf and Helal metatarsal osteotomies were used in our departments for the treatment of metatarsalgia. The aim of our study was to evaluate the results after both techniques, to discuss our experience, and to compare the results in both groups of patients. We also focused our attention to differences between both groups regarding pain relief and non-union.

MATERIAL AND METHODS

Wolf osteotomy was performed in 62 patients (75 feet) in the period of 2002 through 2005. The average follow-up was 31 months. There were 55 females (88.7 %) and 7 males (11.3 %) in the group. The mean age was 53.6 years (27–82 years). Forty-one patients (51 feet) appeared for follow-up examination.
Helal osteotomy was performed in 98 patients (112 feet) in the period of 2001 through 2003. The average follow-up was 34 months. There were 87 females (88.8 %) and 10 males (11.2 %). The mean age was 52.8 years (35–76 years). Seventy-one patients (76 feet) appeared for follow-up examination.
The indication for surgery in both groups was plantar metatarsalgia associated with plantar depression of the heads of the middle metatarsals. This deformity did not disappear by non-stressing, but the normal transverse arch of the forefoot could be achieved by pressure from the plantar side. This deformity was not rigid. Depression of the metatarsal heads caused their plantar prominence and painful plantar keratoses. Contraindications for surgery included infection, impaired vascularity, skin disturbances, and non-cooperation of the patient.
We performed radiographic analysis using an anteroposterior and a lateral radiograph of the foot in the standard and stress positions preoperatively. We also used an oblique dorsoplantar view in supine position and a plantodorsal view in prone position [3,7]. We assessed the divergence of the metatarsal bones in both standard view and the view in stressing position (Figure 1).
We used the Gainor scoring system [8] for the evaluation of clinical results (Tables 1, 2) involving walking, walking aids, needs for special shoes, cosmetic effect, pain relief, and satisfaction of the patient. Radiographically we evaluated the healing of the osteotomy and the occurrence of both delayed unions and non-unions.

Operative technique

Wolf osteotomy [17]: the operation is performed in general anaesthesia or in a foot block, usually with a tourniquet.

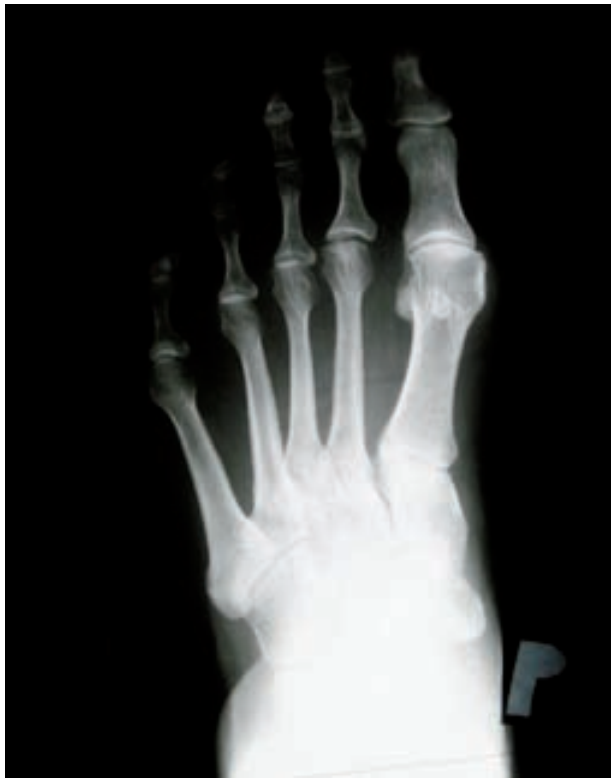

Figure 1
Radiograph in anteroposterior view – evidence of metatarsal divergence

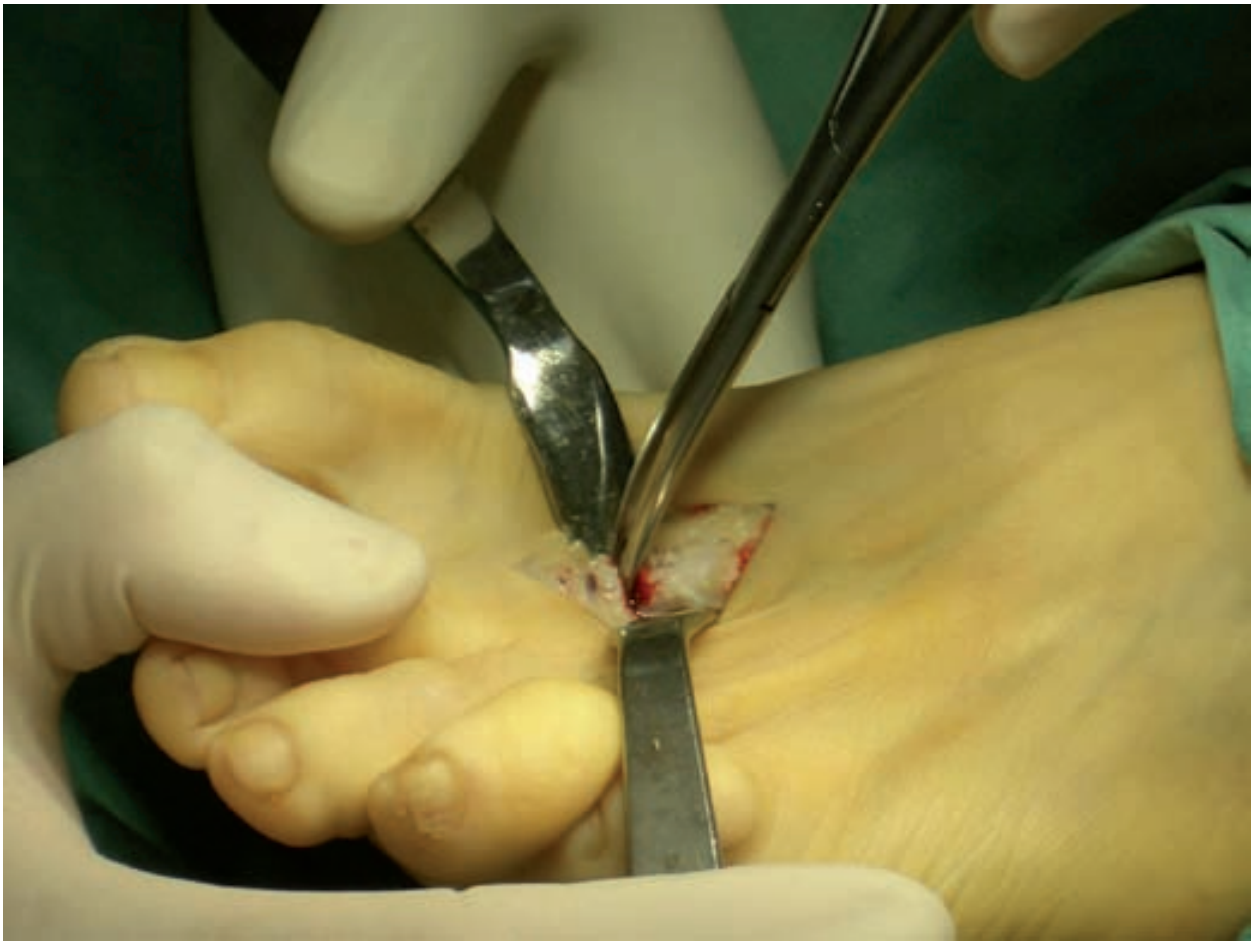

Figure 2
Wolf V-osteotomy with Luer pliers

We used two longitudinal incisions on the dorsum of the foot. We retracted the tendons of the extensor digitorum longus and brevis and the soft tissue to expose the metatarsal. At a point about 6 mm proximal to the metatarsal head, using a small rongeur (Luer, Liston), we created a V-shaped notch in the metatarsal down to but not including the plantar cortex. Then, by pressure on the plantar aspect of the metatarsal head, while stabilising the forefoot, we produced a greenstick fracture of the metatarsal shaft and closed the notch dorsally. We proceeded on the second, third, and fourth metatarsal bones. In that way we elevated the metatarsal heads dorsally and corrected the deformity. After releasing the tourniquet we stopped any bleeding and closed the wound in layers (Figure 3). We applied a soft dressing with a support underneath the metatarsal heads to maintain their position and model the transverse arch (Figure 4). Weight stressing was started with crutches the day after surgery. The patient was instructed to bear as much weight as possible on the forefoot, forcing the metatarsal heads superiorly to help keep the notch closed. The sutures were removed 12 to 14 days after surgery. Full weight stress without crutches was allowed after 4–6 weeks.

Helal osteotomy [10]: this is performed in general anaesthesia or in a foot block, usually with a tourniquet. We used two longitudinal incisions on the dorsum of the foot. Medial incision was placed over the second metatarsal and a lateral incision was placed between the third and fourth metatarsals. We retracted the extensor tendons and the soft tissue to expose the metatarsal. At the border between the middle and distal thirds of the metatarsal we performed an oblique osteotomy in plantar direction and distad at an angle of 45° with an oscillating saw (Figure 5). We performed this procedure on the second, third, and fourth metatarsal bones. Thereafter we shifted the distal fragment dorsally and proximally. We removed the overlapping dorsal cortex with Luer pliers. After releasing the

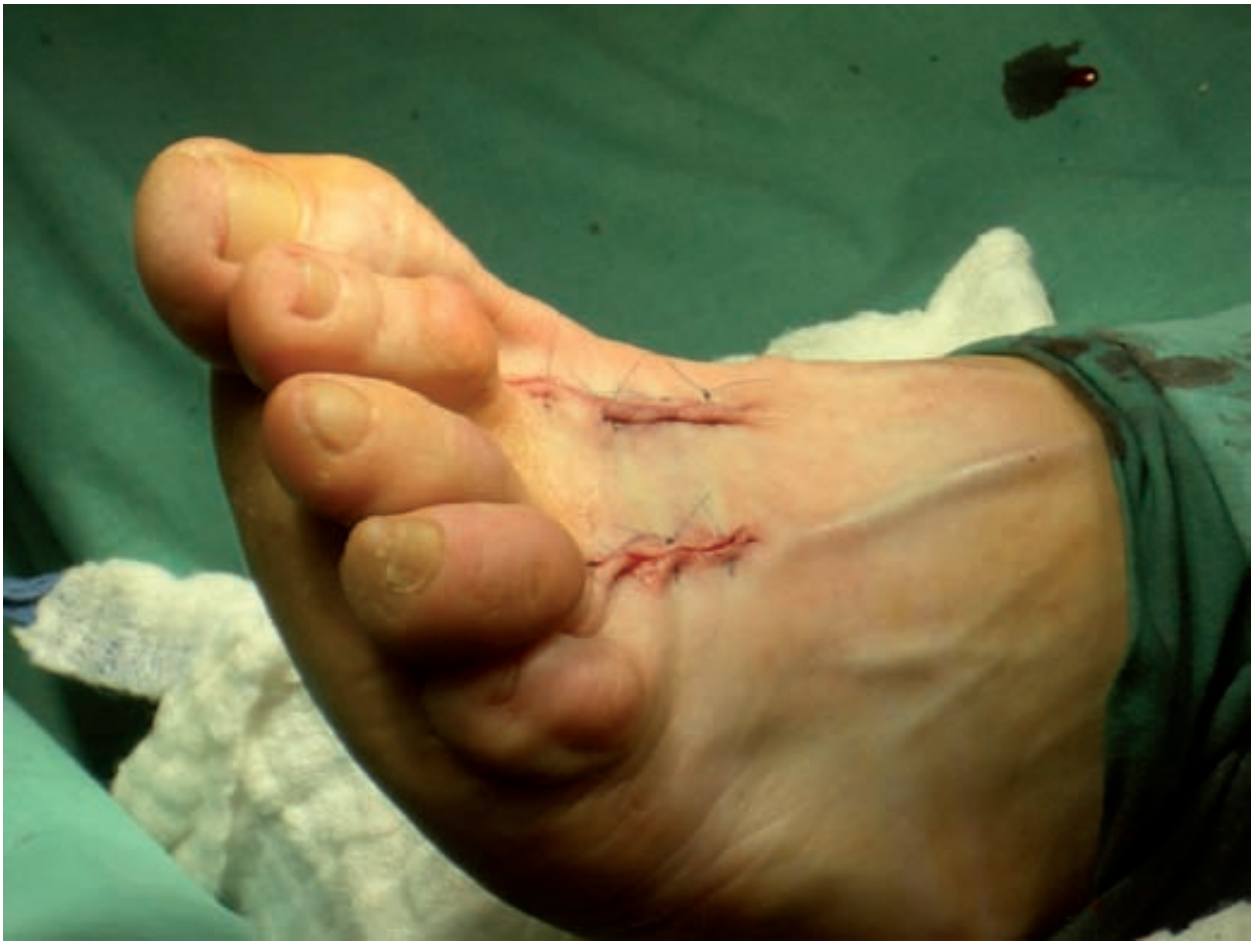

Figure 3
Closure of the wound, two dorsal incisions

tourniquet we stopped any bleeding and closed the wound in layers (Figure 3). We applied an elastic dressing with a support underneath the metatarsal heads (Figure 4). Postoperative management was the same as after Wolf osteotomy. Full weight stress without crutches was allowed after 6 weeks.

RESULTS

We evaluated both groups using the Gainor scoring system [8] (Tables 1, 2). In the group with Wolf osteotomy, we achieved 90.2 % excellent and good results; in the group with Helal osteotomy 91.5 % excellent and good results were achieved (Table 3). The final result was similar in both groups.
Our complications are listed in Table 4. We encountered superficial infection and marginal necrosis of the wound in 5 patients (12.2 %) after Wolf osteotomy and in 8 patients (11.3 %) after Helal osteotomy.

All cases healed with conservative methods without consequences. There was one case of deep infection (1.4 %) in the group with Helal osteotomy requiring a revision. It did not influence the final results in this case. Hypoesthesia of the operated foot was observed in one patient after Helal osteotomy (1.4 %) and in one patient after Wolf osteotomy (2.4 %). The hypoesthesia disappeared in both cases after 6 months.
There was one case of deep venous thrombosis after Wolf osteotomy (2.4 %) and three cases after Helal osteotomy (4.2 %). We did not encounter any painful or painless non-union in the groups with Wolf osteotomy, even if we disturbed the plantar cortex in two cases. The osteotomy was healed uneventfully. There were three cases (4.2 %) of painful non-union in the group with Helal osteotomy. In one case we performed a successful revision with subsequent healing of the bone. We observed a painless non-union in the group with Helal osteotomy in 17 patients (32.7 %).

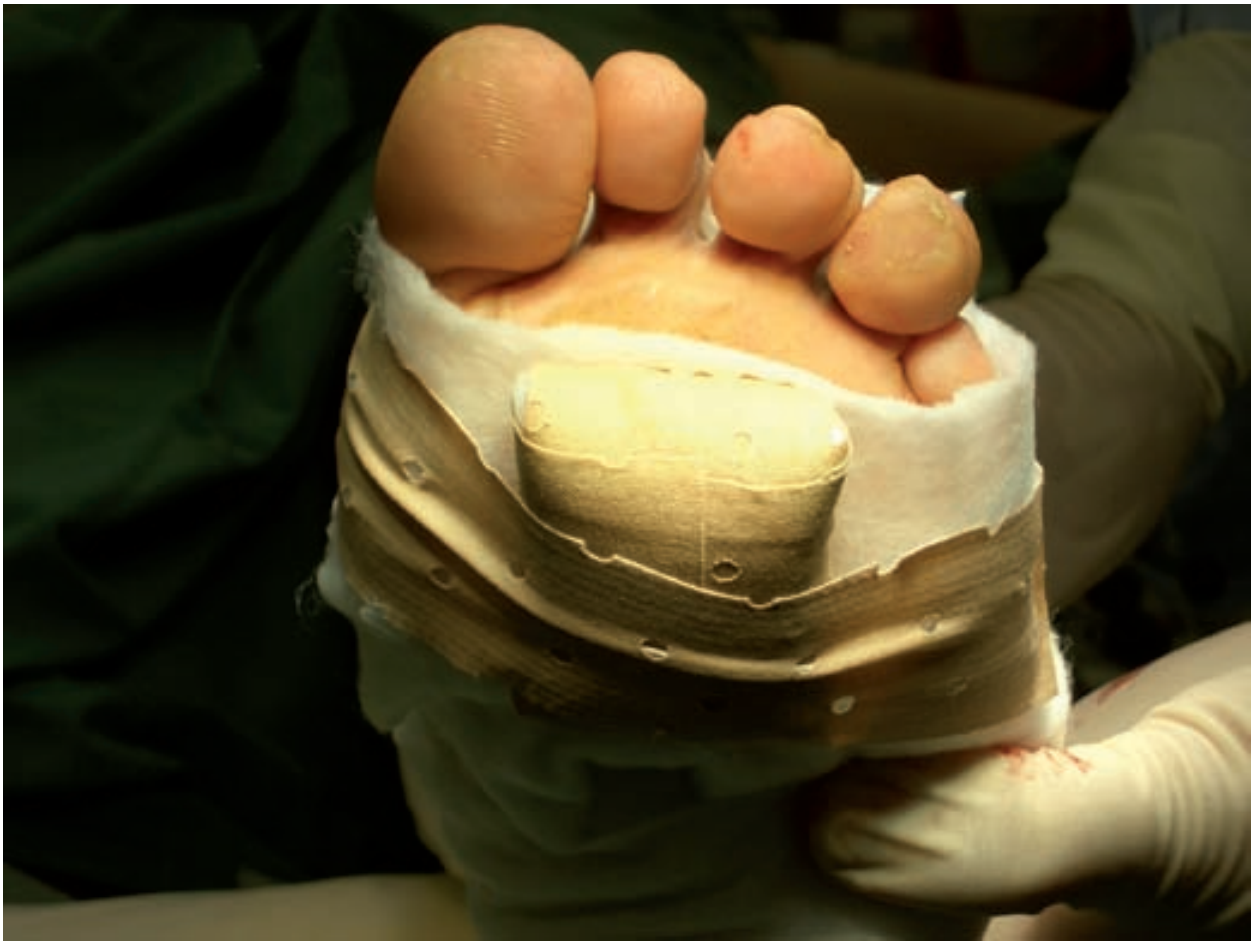

Figure 4
Inserted support underneath metatarsal heads

DISCUSSION

Metatarsalgia is a common complaint of many patients visiting outpatient orthopaedic departments. There are several causes of the pain distally from the Lisfranc's joint. One of them is overloading of the middle metatarsals. Plantar depression of the second, third, and fourth metatarsal heads is often associated with other static deformities of the forefoot (bunion, hammertoe, clawing toe) [11]. Overweight, improper footwear, and other factors cause weakness of short muscles and laxity of ligaments of the foot. The transverse arch of the foot with metatarsal heads is gradually depressed and the forefoot becomes wider. This deformity produces painful callosities underneath the metatarsal heads.

At the onset we started with conservative treatment. We recommend proper footwear, supports into the shoes, non-steroid anti-inflammatory drugs, weight reduction, physiotherapy, and change of activity. After exhausting all conservative measures we can choose from several shortening osteotomies (Giannestras, Hoffman, Thomas, Weil, Wolf, Helal) [2, 5, 9, 10, 13, 15, 16, 17]. The aim of the osteotomy is to create a balance of strengths influencing the metatarsal heads. This can be achieved by several means:

1. shortening osteotomy (Giannestras) [9],
2. change of the alignment of metatarsals (Wolf) [17],
3. dorsal and proximal shifts of the distal fragment of metatarsals (Helal) [10]. The same principle can be found in modified chevron osteotomy [12].

The operation technique described by Wolf [17] in 1973 consists in elevation of the depressed metatarsal heads and in achieving a horizontal alignment of the metatarsals with the axis of rotation in the region of the plantar cortex. Closed wedge osteotomy allows early postoperative partial weight stress [1, 4, 16]. Helal osteotomy produces shortening of the

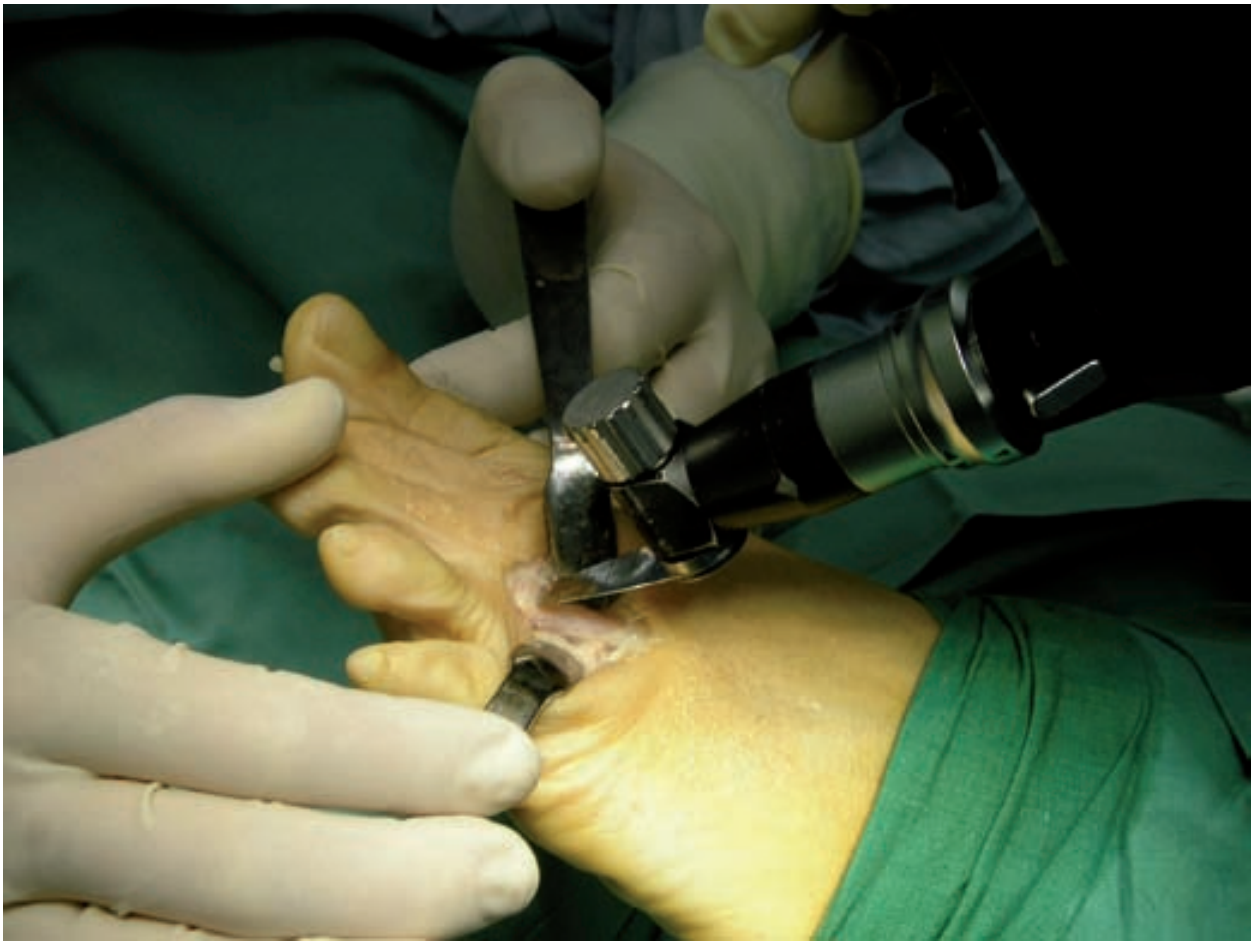

Figure 5
Helal telescoping osteotomy with oscillating saw

metatarsal by shifting the distal fragment dorsally and proximally. It does not change the longitudinal alignment of the metatarsals at the sagittal level. Early postoperative partial weight stress is also allowed [10, 16].

It is necessary to distinguish yet other causes of the painful forefoot (Morton's neuralgia, stress fractures, plantar fibromatosis, Freiberg's disease, vascular disorders, infections, tumours, etc.). We also have to exclude some other conditions such as rheumatoid arthritis, crystal arthropathy, gout, and psoriatic arthropathy [5].

An indication for Wolf and Helal osteotomy is a static deformity of the forefoot with plantar depression of the middle metatarsal heads. This deformity does not disappear by lifting the foot, but is correctable by pressure of fingers from below. This deformity is not rigid. Depression of metatarsal heads is associated with painful keratoses on the plantar side of the forefoot. The goal of the osteotomy made for pressure metatarsalgia is the elevation of metatarsal heads. There are several procedures influencing this deformity by different methods [9, 10, 12, 13, 16, 17].

Pilný [14] recommends to perform Wolf or Helal osteotomies in regional anaesthesia for the long-lasting analgesic effect and for a lower risk of complications than in general anaesthesia. We have to follow strictly all steps of the operation technique. Mistakes in the technique can adversely affect the final results. Wolf osteotomy should be performed in the subcapital region of the metatarsal. In the case of more proximal placement of the osteotomy we are unable to correct the alignment of the metatarsal. We also have to avoid producing longitudinal cracks of the metatarsal using Luer pliers and to avoid damage of the plantar cortex. In the case of disturbing the plantar cortex there is a risk of dislocation of the distal fragment and of formation of a non-union. After closing the osteotomy we have to maintain plantiflexion in

Table 1
Gainor score

1.	Walking distance	over 800 m up to 800 m at home	3 points 2 points 1 point
2.	Walking aids	none for other joints for operated foot	2 points 1 point 0 points
3.	Footwear	Ready-made special footwear	2 points 0 points
4.	Cosmetic effect	excellent good satisfactory bad	3 points 2 points 1 point 0 points
5.	Pain	painless small pain moderate pain severe pain	3 points 2 points 1 point 0 points

Table 2
Gainor scoring system

Points	**Result**
11–13	Excellent
8–10	Good
5–7	Satisfactory
0–4	Bad

Table 3
Results of Wolf and Helal osteotomies using Gainor scoring system

Results	Wolf osteotomy		Helal osteotomy	
Excellent	39	76.5 %	55	77.5 %
Good	7	13.7 %	10	14.1 %
Satisfactory	3	5.9 %	4	5.6 %
Bad	2	3.9 %	2	2.8 %

Table 4
Complications

	Wolf osteotomy (n) (%)		Helal osteotomy (n) (%)	
Superficial infection	5	12.2	8	11.3
Deep infection	0	0	1	1.4
Deep venous thrombosis	1	2.4	3	4.2
Hypoesthesia	1	2.4	1	1.4
Painful non-union	0	0	3	4.2
Painless non-union	0	0	17	32.7

the metatarsophalangeal joint at least in zero position. Otherwise, during the correction we could lever the distal fragment to dorsal angulation, which can lead to non-union.
It is necessary to perform Helal osteotomy at an angle of 45° for keeping the fragments in optimal contact. If we perform the osteotomy too much vertically, we do not achieve the telescoping of the fragments on each other; they could dislocate, which can lead to non-union. There is a good amount of trabecular bone at the level between the distal and middle thirds of the metatarsal for better healing.
There is no need for internal fixation in both osteotomies. Wolf osteotomy is performed with basic instruments and Luer pliers. In Helal osteotomy we need an oscillating saw. By comparison, we need special screws for internal fixation in Weil osteotomy [6, 15] and a wire in the Giannestras procedure [9].
We have achieved comparable clinical results in both groups. The only difference is a relatively high occurrence of painless pseudoarthroses after Helal osteotomy (32.7 %). They did not influence the final clinical results. Other complications are similar in both groups (Figure 4). The advantage of both methods is early weight stressing with the use of crutches [1, 4, 10, 17].
Helal [10] evaluated the results in a group of 38 patients (47 feet). He encountered one painless fibrous non-union in his study. He achieved 88.2 % of good results including pain relief and good recovery of the function. Wolf [17] published 85.3 % of excellent results in a group of 25 patients (31 feet) in his article in 1973. Ambler [1] achieved, with Wolf osteotomy in a group of 281 patients (340 feet), 96.5 % of good results regarding pain relief and remodelling of the transverse arch of the foot.

CONCLUSION

We have compared the clinical and radiological results after Wolf and Helal osteotomies performed for metatarsalgia with plantar depression of the middle metatarsal heads in similar groups of patients. We have achieved identical results in both groups. It is necessary to follow the correct operative technique and careful aftertreatment. The advantage of both procedures is low occurrence of complications and low demands on instruments for surgery. Both procedures can be performed in standard orthopaedic departments. Reasonable clinical and radiological results can be achieved in motivated patients.

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TOOTH-COLORED DENTAL RESTORATIVE MATERIALS IN PRIMARY DENTITION

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ABSTRACT

Restoration of decayed primary teeth is very important and significant not only for the maintenance of health and psychics of the child but also for the physiological development of permanent dentition. Esthetic materials were formulated for permanent teeth restoration, but they can also be used for the treatment of primary dentition, especially in minimally invasive techniques, atraumatic tooth restoration, and preventive interventions. Composite resins, glass ionomers, resin modified glass ionomers, and compomers are the materials of choice in the form of direct filling. Findings of individual authors on success of esthetic primary teeth restoration are, however, different and further well-founded clinical trials and experimental studies are necessary. Opinions on the decision-making process when choosing a restorative material are very similar and can be formulated in the following way: To receive reliable results with esthetic material restoration in primary dentition three conditions have to be present: isolation, cooperation, and time. Microscopic structure peculiarities of primary enamel and dentin and time until tooth exfoliation have to be taken into consideration too. One has to state, however, that the longevity of tooth-colored materials has not been found higher than that of amalgam. That is why dentists should consider the diagnosis, ease of material placement, oral hygiene, caries risk, esthetic demands, and financial aspects when choosing a restorative material for primary teeth.

ABBREVIATIONS USED

Bis-GMA – bisphenol-A – glycidyl methacrylate
HEMA – hydroxyl ethyl methacrylate
GIC – Glass ionomer cement
RMGIC – resin modified glass ionomer cement

Table 1
Classification of composites based on filler size (adapted according to 6)

Classification	Filler material (% weight content)	Average particle size (μm)
Macrofilled	Glass particles (78 %)	1–35 (conventional)
Microfilled	Amorphous silica (35 %–63 %)	0.01–0.1 (mean diameter 0.04)
Hybrid	Glass particles and fillers (>80 %)	5, conventional plus microfiller (two sizes)
Micro-Hybrid	Glass particles and microfillers(>80 %)	0.4–0.8
Nanofilled	Zirconium/silica or silica nanofillers and clusters (N/A)	5–20 nm Zr/Si or 75nm Si

INTRODUCTION

Restoration of decayed primary teeth is very important and significant not only for the healthy development and psychics of the child but also for the physiological development of permanent dentition. The choice of restorative materials for primary teeth is large at the present time. Whereas several years ago the only possibility was amalgam, today there are various alternatives at the dentist's disposal [1]. Increased attention to minimal intervention, combined with increased requests for more esthetical appearance, has created much broader use of newer restorative materials when treating dental caries in primary teeth. Amalgams have a known quality with long-proven history and research. They have been proven more economical for the dentist and the patient. Amalgams are easily completed and the margins are easily identifiable when carving. Radiographically, amalgams are easily distinguished from the tooth structure and enable the dentist to identify recurrent decay and margins. Amalgams have a long shelf life and easy storage of materials. Amalgam restoration has also been known for superior interproximal contacts and superior marginal integrity that becomes tighter with age. It also has a superior wear rate and can be extremely long-lasting [2]. The operative technique with amalgam is based on mechanistic principles. The box-shaped cavity preparation is not tooth-friendly to either healthy enamel or dentin, and the crown is weakened [3]. Marginal disintegration of amalgam fillings is material inherent and ultimately limits the longevity of restorations. A thickness of the amalgam filling less than 2 mm, particularly in Class II, commonly results in bulk fracture, dislodgment, or a combination of the two [4]. The esthetics of the amalgam filling is, however, unsatisfactory and can cause dark staining of the tooth and a tattoo of the gingival and buccal mucosa. Esthetically acceptable materials have been formulated in recent years and steadily improved especially as regards their physical properties. Esthetic materials were developed for permanent teeth restoration, but they can also be successfully used for the treatment of primary dentition, especially in minimally invasive techniques, atraumatic tooth restoration, and preventive interventions. Composite resins, glass ionomers, resin modified glass ionomers, and compomers are the materials of choice in the form of direct filling. Indirect filling (inlays), composite resins or ceramics are not used for primary teeth restoration.

Composite resin

In the early fifties, R. L. Bowen developed a polymer based on dimethacrylate chemistry. This polymer, generally known as bis-GMA or Bowen's resin, was made from a combination of bisphenol-A and glycidyl methacrylate. Since the advent of Bowen's resin, inorganic fillers were added to overcome the problem of shrinkage, thermal expansion, and low strength [5]. Current composite formulations contain high and low molecular weight monomers, inorganic fillers, silane coupling agents, polymerization inhibitors, initiators, and ultraviolet stabilizers. The high molecular weight monomers undergo polymerization via free radical addition to form rigid drops-linked polymers. Inorganic fillers serve multiple purposes in composites. Fillers occupy the spaces in between the resin matrix, which helps to reduce shrinkage upon polymerization. Fillers also impart greater strength, hardness, low water absorption, lower wear and greater esthetics upon polishing, and confer radiopacity as they contain barium, strontium or zinc [5].

Composites can be classified according to:

1. Type of fillers (quartz, fused silica, many types of glasses including aluminosilicates or borosilicates)
2. Size of fillers (macrofilled, microfilled) (Table 1)
3. The amount of filler used (percentage by weight or volume)
4. Method of curing (chemical activation and visible light activation)

Table 2
Evolution of dental bonding adhesives (adapted according to 48). Progression of dental adhesives

1960s and 1970s: First and Second Generations Did not recommend dental etching. Relied on adhesion to smear layer. Weak bond strength.	1980s: Third Generation Acid etching of dentin. Separate primer. Increased bond strength. Margin staining caused clinical failure over time.
Early 1990s: Fourth Generation Hybrid layer of dentin and collagen. Dental seal. Concept of wet bonding introduced. Technique sensitive.	Mid 1990s: Fifth Generation Combined primer and adhesive in one bottle. Maintained high bond strengths. Unit-dose packaging introduced.
Late 1990s, early 2000s: Sixth Generation Self-etching primers. Reduced incidence of post-treatment sensitivity. Bond strengths lower than fourth-and fifth-generation.	Late 2002: Seventh Generation All-in-One. Combines etching, priming and bonding. Single solution. Good bond strength and margin sealing.

Macrofilled composite resins were very hard and rough, which made them difficult to polish, and they tended to cause enamel abrasion. The surfaces often became rougher with wear and attracted plaque. Microfillers were then developed in 1970s. They were composed of colloidal silica 0.04 μm in size. These fillers rendered the composite more polishable and less shrinkage and higher wear resistant than the macrofillers. However, they have poor mechanical properties (use in low stress area). Hybrid composites combine glass particles with fillers of various sizes (aluminosilicates, quartz or barium aluminosilicate silica glasses). Hybrid composites are easier to polish and have better wear resistance than the microhybrid composite resins. Nanofilled composite resins can be excellently polished and have good mechanical properties [6].

Anchoring of a composite filling into the tooth structure was made possible by a revolutionary method – acid etching [7, 8]. The surface enamel is smooth and has little potential for bonding by micromechanical attachment. On treatment by certain acids, however, the structure of enamel may be modified considerably. Chemical treatment by acid etching enhances the topography of enamel, changing it from a low-reactive surface to a surface that is more susceptible to adhesion. The demineralization is selective because of the morphological disposition of the prisms [9]. Acid etching removes approximately 10 μm of enamel surface and creates a morphologically porous layer 5–50μm deep [10]. The surface free energy is doubled [11] and, as a result, the low viscosity fluid resin contacts the surface and is attracted to these microporosities through capillarity [12]. Therefore, resin tags are formed into microporosities of conditioned enamel and provide a resistant long-lasting bond by micromechanical interlocking with this tissue [13, 14, 15]. The type of resin applied to the etched enamel depends on the specific application used. For composite resins the mixed material may be applied directly to the etched enamel surface. The resin from the composite flows into the etched enamel and sets, forming rigid tags which retain the filling. More frequently, however, low viscosity resins, the so-called bonding resins enhancing the adhesive bond strength, are used. They consist of a resin similar to that used in composite material but contain no filler particles. It is fully fluid and readily flows into the etched enamel surface. Bonding readily occurs at the unfilled resin-to-composite interface. The resulting shear bond strength achieved between etched enamel and restorative resins is 16–20MPa.

Dentin adhesives

In most clinical situations, however, the restoration is bonded to the enamel and dentin. To interact with this intrinsically moist tissue, an adhesive system was needed that could diffuse under this condition. Formulation of dentin adhesives underwent a complicated development (Table 2). The most significant factor that limits bonding in the absence of any form of dentin pretreatment is the presence of the dentin smear layer. The smear layer is formed by the process of cavity preparation and extends over the whole prepared surface of the dentin and into the dentinal tubules (smear plug). A smear layer is present on the surface of freshly cut dentin irrespective of the method of mechanical tooth preparation. It is now

Table 3
Composition of glass ionomer cements

Powder/liquid materials	
Powder	Sodium aluminosilicate glass with about 20 % CaF or other minor additives
Liquid	Aqueous solutions of acrylic acid/itaconic acid copolymer or aqueous solutions of maleic acid polymer and tartaric acid in some products to control setting characteristics
Powder/water materials	
Powder	Sodium aluminosilicate glass with about 20 % CaF or other minor additives + vacuum-dried polyacid (acrylic, maleic or copolymers)
Liquid	Water (added by dropper) or dilute aqueous solution of tartaric acid

recognized that in order to form an effective bond and seal between a restorative resin and the dentin the smear layer must be removed, disturbed or modified in some way, which allows access to dentin. The liquids used for dentin pretreatment prior to bonding are called conditioners. They are generally acid solutions. Many acid solutions have been employed as conditioners. It is advantageous if the acid used for dentin conditioning can also be used for acid etching enamel [16]. The conditioning stage can be viewed as the first of three stages – the other two being priming and bonding. Having conditioned the dentin in order to remove or modify the smear layer the next stage is the priming stage. This stage is a key stage in the procedure as it is designated to change the chemical nature of the dentin surface and to overcome the normal repulsion between the hydrophilic dentin and the hydrophobic resin. Most adhesive systems that use the total-etch technique (etching with 37% phosphoric acid) have in their formulation low-viscosity hydrophilic monomers diluted in organic solvents with a high potential of volatization to displace the moisture of the conditioned dentin. The primer components (e.g. hydroxyl ethyl methacrylate – HEMA) are called bipolar because they have 2 functional groups (hydrophilic and hydrophobic). The hydrophilic end has the ability to interact even under moist conditions and the hydrophobic end has chemical affinity with the methacrylate group of the bonding resin matrix or with the composite resin [17]. Some adhesives contain inorganic filler particles to increase the film thickness and cohesive strength. After priming the nature of the dentin surface is significantly changed; it becomes more hydrophobic and ready to accept the resin-based bonding agent. The bonding agent is normally a fluid resin able to flow over and wet the primed surface to complete the formation of an effective bond. Efficient dentin conditioning removes the smear layer and smear plug and also causes a significant decalcification of the intertubular dentin to a depth of a few microns. The decalcification process leaves a three-dimensional collagenous network which can be infiltrated by the primer and resin to form a resin infiltrated/reinforced hybrid layer. The need to shorten the time-consuming application led to a simplification of the procedures for conditioners, primers and bonding agents, and to techniques combining these procedures into one. Dentin adhesives are currently classified into two major categories: etched-and-rinsed and self-etched adhesives based on the mechanisms by which they interact with the smear layer [18]. The etched-and-rinsed types remove the smear layer before application of the resin, while the self-etched adhesives incorporate the smear layer into a hybrid coating [19]. The original self-etched systems included two steps: application of an acidic self-etching primer followed by a separate bonding step. The self-etch adhesive system relies on the use of non-rinse acidic methacrylate monomers that simultaneously etch and prime the dentin, so that the disrupted smear layer is incorporated into the hybrid layer [20]. Recently, one-step self-etch adhesives have been developed that combine the conditioner, primer, adhesive resin and water into a single application. Self-etch systems are in general less technique sensitive than etch-and-rinse systems that utilize separate etching and rinsing steps [19]. When bonded to primary dentin [21], significant differences were found in bond strength between etch-and-rinse and two-step self-etch adhesives.

Glass Ionomer Restorative Materials (GIC)

Glass ionomer restorative materials have been available since the early 1970s and were derived from silicate cements and polycarboxylate cements (Table 3). GICs are classical water-based cements with an acid/base setting reaction of aluminium

silicate glass and a polyalkenoic acid [22, 23, 24]. Like many dental cements, the properties of glass ionomers are critically dependent upon the powder/liquid ratio. The powder/liquid ratio should be high in order to optimize strength and solubility, but there should be sufficient free polyacid available to form a bond with the tooth substance. The variability of material properties with the powder liquid ratio and the difficulties of mixing by hand suggest that there are definite advantages to be gained by using the materials in the encapsulated form. One of the most important properties of these materials is their ability to adhere to both enamel and dentin, but the precise mechanism of bonding is still somewhat unclear. One theory is that polyacid molecules chelate with calcium at the tooth surface. Support for this mechanism stems from the fact that the formation of the interfacial calcium polyalkenoate salt would involve a reaction similar to that thought to occur during the setting of the cement. Also, the significantly greater bond achieved with enamel than with dentin suggests that it is the calcium of the tooth substance that is involved in bond formation. Another theory is that the outer layers of apatite on the tooth surface become solubilized in the presence of acid. As more apatite dissolves and as the cement begins to set the pH begins to rise. This may cause a re-precipitation of the complex mixture of calcium phosphate (from apatite) and calcium salts of the polyacid onto the tooth surface. Thus, the polyacid chains would be bound to a re-precipitated layer on the tooth surface. This mechanism could operate on enamel and dentin surfaces and thus could also be supported by bond strength data. It has been suggested that in the case of dentin there may also be some bonding between carboxylic acid groups of the cement and reactive groups within collagen, either by hydrogen bonding or by metallic ion bridging. The fluoride release and the ion-exchange adhesion with both tooth structures have been reported to be the main advantages of this particular group of materials (25). However, the evidently low water resistance, moisture susceptibility, and inferior flexural strength characteristics lead to the development of several modifications in the field of conventional GICs [26, 27, 28]. Metal reinforced glass ionomer cements were formulated to improve the mechanical properties of the cements and to increase their wear resistance [29, 30, 31]. Further development of glass ionomer cements focused on a higher powder-to-liquid ratio, a lower water content, and smaller glass particles leading to high viscosity glass ionomer cements, which should be packable like amalgam and reveal enhanced flexural strength characteristics. Clinical studies, however, have not proved the results of the previous promising in vitro studies [32, 33].

Giomers

This group of materials is more correctly described as composites with active filler particles. The material unites the chemistries of composite and GIC in an effort to combine the advantages of both materials, whilst minimizing the limitations of each. The material is composed of pre-reacted GIC particles within a resin matrix. Giomers can be subdivided into two distinct groups of materials, namely those in which the glass ionomer particles have been surface reacted and those which have been fully reacted. Surface pre-reacted glass ionomer giomers are suitable for composite indications, whilst fully pre-reacted glass ionomer giomers are used in dentin adhesive systems, fissure sealants, and as restorative material for nonloaded-bearing areas [34].

Compomers

Compomer is a polyacid-modified composite resin [35, 36, 37]. Compomer is made predominantly from resin composite (90%) with the addition of a polyacid-modified molecule similar to that found in traditional GIC. Compomers are initially light-cured, but subsequently absorb water, allowing for an acid-based reaction to set the polyacid-modified molecule. Consequently, the material shrinks, initially due to polymerization contraction, and expands subsequently as water is absorbed. The addition of a polyacid-modified molecule makes the material more hydrophilic. Compomers are, therefore, relatively easy to handle and apt for preparation. A dentin-bonding agent is required for their successful placement. Physically, their properties are similar to those of a composite. The wear rates and fracture resistance are less than for a composite. Compomers and composites have the same advantages (38). Additional advantages of compomers include fluoride release and ease of handling [34].

Resin-modified glass ionomer

The chemistry of GIC has been incorporated into another modern material, namely the resin-modified glass ionomer cement (RMGIC). RMGICs most closely resemble conventional glass ionomer cements and are capable of setting by an acid-base mechanism. This is supplemented by a free radical polymerization reaction involving the monomer 2-hydroxyethyl methacrylate. The actual bonding mechanism of RMGIs to tooth tissues has been recently determined to be twofold, by micromechanical interlocking [39, 40] and by chemical interaction [41, 42]. Micromechanical interlocking is achieved by impregnation of a partially demineralized layer on the top of the dentin substrate with a high-molecular-weight polycarboxyl-based polymer [18] similar to the hybrid layer in resin-based interfaces. Chemical interaction in the form of gel-phase between polyalkenoic acids and calcium was demonstrated not only on hydroxyapatite blocks [41] but also on enamel and dentin [42]. The physical presence of the gel-phase attached to the tooth surface suggests that the resulting calcium polycarboxylate salt is stable in this particular

system [43]. RMGICs develop self-adhesion to tooth tissue, which is enhanced if the tooth surface is conditioned and left moist prior to application of the material. The resin-based systems are command-cured, although it is important to understand that the acid-base reaction for the traditional glass-ionomer cement proportion of the material (ca 80%) is really how the material cures and sets (44). This reaction is water dependent and takes several days for the process to be complete. More correctly, therefore, resin-modified glass-ionomer cements are initially photostabilized rather than light-cured. RMGICs are able to release fluorides. The materials have, however, disadvantages linked to their inferior properties when compared with composites, the most important being poor structure strength and wear rate, marginal chipping, and exogenous discoloration.

Tooth-colored materials have been used more and more frequently for restoration of primary dentition in recent years [45, 46, 21, 26, 32, 33, 35–38]. The findings of individual authors are, however, different and further well-founded clinical trials and experimental studies are necessary. Opinions on the decision-making process when choosing a restorative material are very similar and can be formulated in the following way: To receive reliable results with esthetic material restoration in primary dentition one must have three conditions in place: isolation, cooperation, and time. Microscopic structure peculiarities of the primary enamel and dentin and time until tooth exfoliation have to be taken into consideration too. As regards clinical application, composite resin can be used for restoration of primary incisors for class III, IV and V, and of primary molars for class I and II cavities if the patient is cooperative, because a resin composite material requires more time and more steps with great technique sensitivity to be effective. The conventional hand-mixed powder and water glass-ionomer cements have limited application and are used only as luting cements for preformed metal crowns and as temporary dressings. Resin-modified glass ionomers are becoming very popular in pedodontic practice, especially for class I and II restorations in primary molars. They can also be used for class V cavities and atraumatic restorative techniques or as a base material in deep-cavity preparations. Compomers need acid etching and bonding, both procedures which demand increased time and good patient compliance. They have been shown to be durable over a three-year period when used in approximal primary molar cavities. Therefore, they can be used in class I and II primary molar cavities and in class V cavities. One has to state, however, that the longevity of tooth-colored materials has not been found higher than that of amalgam [46]. That is why dentists should consider the diagnosis, ease of material placement, oral hygiene, caries risk, esthetic demands, and financial aspects when choosing a restorative material for primary teeth [47].

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COMPLETE DUPLICATION OF THE URINARY BLADDER AND THE URETHRA IN A WOMAN – A CASE REPORT WITH A REVIEW OF ARTICLES

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ABSTRACT

A complete duplication of the urinary bladder and the urethra is a rare congenital anomaly which is often associated with other congenital malformations. There are only few articles in the literature on this rare topic. This anomaly is presented in early age. The authors present the case of a 52-year-old woman in whom this anomaly was detected by laparoscopy from gynaecological indication. After 10 years from the diagnosis of the anomaly she is referred to the Department of Urology.

ABBREVIATIONS USED

PID – pelvic inflammatory disease
IVP – intravenous urogram
USG – ultrasonography
ATB – antibiotic
CT – computer tomography

INTRODUCTION

A complete duplication of the urethra and the bladder (CDBU) is a rare congenital anomaly which is often associated with other congenital malformations [2, 4, 7, 8, 10, 11, 12]. There are few articles in the literature on this rare topic [2–13]. A child with such an anomaly should be followed up immediately after the diagnosis to prevent possible complications. Anatomical disorders of the bladder are also associated with functional disorders, which is the potential cause of complications related to this congenital anomaly.

CASE REPORT AND MANAGEMENT

A 52-year-old woman was referred to the Department of Urology from the regional Gynaecology Department where she had been an inpatient for colpitis and had undergone extraction of an intrauterine device (IUD).

Table 1

Data for the separate urodynamics of each of the bladders (Pura max – maximal urethral pressure, Qmax – maximal flow rate, Qave – average flow rate, EMG – electromyogram of anal sphincter, Pbla – bladder pressure, Pdet – detrusor pressure, Pabd – abdominal pressure)

	Left bladder	Right bladder
Normal sensation /Pbla	179 ml/18 cm H2O	228 ml/11 cm H2O
Maximal capacity /Pbla	260 ml/27 cm H2O	292 ml/15 cm H20
Compliance	11 ml/cm H20	15.7 ml/cm H20
Uninhibited detrusor contraction	No	No
Functional length of urethra	27 mm	28 mm
Pura max	51 cm H20	51 cm H20
Qmax	11 ml/s	34 ml/s
Qave	4 ml/s	9 ml/s
Pdet/Pabd during voiding	45 cm H20/174 cm H2O	17 cm H20/89 cm H2O
Voiding time	53s	33s
EMG	Physiological finding	Physiological finding
Post-voiding residuum (ml)	38 ml	0 ml
Note:	During voiding, a low Pdet and a high Pabd were seen, suggesting thea low detrusor contractility with an insufficient evacuation bladder ability. The curve of micturition was irregular and flat.	The voiding curve was continuous in comparison with left bladder.

In 1996, when she was 43 years old, a duplication of the bladder was diagnosed by laparoscopy for a gynaecological indication. No other malformations were detected. At that time she was experiencing pelvic pain syndrome (PID) manifested by chronic idiopathic abdominal and pelvic pain and recurrent colpitis. These problems led to the indication for laparoscopy. Apparently, the first manifestation of the anomaly was recurrent interstitial bilateral nephritis at the age of 20 years. In spite of the late detection of the anomaly, additional screening including urological examination was not performed at the time. In May 2006, ten years after the discovery of the double bladder, a urological examination was completed during hospitalisation at the Department of Gynaecology as a consequence of painful voiding.

Abdominal ultrasonography (USG) confirmed the duplication of the bladder and showed cystolithiasis in the left bladder. An accidental finding from the abdominal USG was liver haemangioma. A subsequent intravenous urogram (IVP) showed normal excretory function of both kidneys with filling of the two separate bladders, each with one normally located ureter, but on the right side with a saccular dilatation indicating ureterocele.

According to the IVP cystogram, the bladder appeared duplicated rather than as a septum bladder, and the differen-

tial diagnosis was focused on this aspect. The urine culture confirmed significant bacteriuria of Streptococcus alpha haemolyticus and Enterococcus species, and antibiotic therapy was started. In the results provided by the Regional Health Institute, there was no mention of a duplication of the urethra. This anomaly was diagnosed by physical examination (Figure 1). The renal scan did not show passage obstruction. The voiding cystourethrogram showed a complete duplication of the bladder in the sagittal plane, with two stones in the left bladder where post-voiding residuum was significant (Figures 2, 3, and 4). However, vesicoureteral reflux was not confirmed by x-ray examination.

After repeated therapy for bacteriuria, cystoscopy was performed under epidural anaesthesia, which showed trabeculisation of the left bladder and ureteric plica with diverticulum at its proximal end. At this time, three calculi of 1.5 – 2.5 cm were detected in the left bladder. Using the intravenous indigo carmine, the ureteric orifice was detected just inside the proximal region of the ureteric plica**.** The cystoscopic view from the right bladder was dominated by pseudodiverticula, and the ureteric orifice was seen in one of these. The bilateral retrograde pyelography indicated in terms of IVP findings and for clinical purpose showed free passage on the right side, but there was a hypotonic ureter with a cut in the pelviureteral junction on the left side and an image of hydronephrosis. The cystolithiasis was fragmented by electrohydraulic lithotripsy and completely evacuated. In the postoperative period, fever and a positive urine culture were observed. After repeated targeted antibiotic therapy and extraction of the catheters, the patient had urine retention in her left bladder. The patient was discharged from the hospital on the 4th postoperative day after therapy with a permanent catheter, which was extracted during the check-up. The post-voiding residuum was 10 ml in the right bladder and 45 ml in the left bladder. The positive urine culture from the urine of the catheterised left bladder persisted, although the patient did not report any symptoms. During the check-up, we performed a computed tomography (CT) scan of the kidneys and the pelvis, which showed small cysts of 6 mm to 8 mm in the kidneys, and a polycystosis of the liver of 10 mm to 60 mm. The CT of the pelvis confirmed the double bladder and showed thickening of the bladder wall due to a chronic cystitis. We also completed a separate urodynamic examination of both bladders (Figures 5 and 6), whose results are shown in Table 1. During voiding, a low detrusor pressure (Pdet) and a high abdominal pressure (Pabd) were seen in the left bladder, suggesting a low detrusor contractility with an insufficient evacuation bladder ability. The voiding curve was irregular and flat, but the bladder outlet obstruction (BOO) was not confirmed.

The residual urine caused by the evacuation failure increases the risk of recurrent infections and formation of bladder stones. The urodynamic parameters of the right bladder, in comparison with the left bladder, were acceptable, and full evacuation was well-kept.

Except for the liver cysts, no other malformations were detected in the abdominal cavity and in the pelvis. At present, the patient is without symptoms, the urine culture of the separately catheterised urethras is free of bacteria, and the post-voiding residuum of 50 ml in the left bladder is acceptable. The evacuation function of the right bladder is complete. The patient is periodically checked as an outpatient.

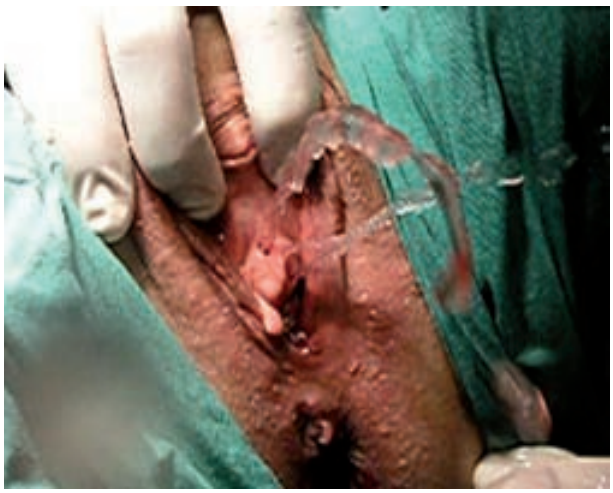

Figure 1
An examination of the vulva showed a double external orifice of the urethra and double flow during micturition

DISCUSSION

The urinary bladder and the urethra evolve from the endodermal element of the cloaca during embryogenesis. From the cloaca, the allantoideus duct (allantois) runs in the ventrocranial direction. Laterally in the cloaca, the Wolf's ducts end and later form the ureters. In the 2nd month of embryonic development, the urorectal septum divides the cloaca into a dorsal anorectal sinus and a ventral urogenital sinus. Later in embryonic development, the urinary bladder forms in the urogenital region, as well as the urethra and some of the genital organs. The caudal region of the urorectal septum later connects with the membrane of the cloaca in the 7th week of development. The membrane of the cloaca is divided into a ventral urogenital membrane and a dorsal anal membrane. The primitive urogenital orifice forms by the perforation of the ventral membrane. The membrane of the cloaca coalesces with the urorectal septum to create the perineum. The cranial region of the urogenital sinus forms the vesicourethral

Table 2
Reported cases of complete duplication of the urethra and the bladder (CDBU) with a review of their management (AU – accessory urethra, AB – accessory bladder)

Case No.	Reference	Year	Age	Sex	Associated anomaly	Treatment
2.	Voigt et al.	2005	1 d	M	Duplication of external genitalia; hypoplastic left kidney; bilateral bifid scrotums; imperforate anus; mass on perineum (teratoma)	Loop colostomy; removal of teratoma; left cystectomy and left penectomy; reconstruction of rectum
3.	Bae et al.	2005	7 yrs	M	None	Excision of AB and AU
4.	Gastol et al.	2000	1 yr 1 m 2 d	F F F	Duplication of vagina and uterus, symphysis diastasis; abdominal hernia Duplication of vagina and uterus; anal atresia with colon duplication Duplication of vagina and uterus; cloacal exstrophy with persistent urogenital sinus	Iliac osteotomy, approximation of symphysis and excision of hernia Excision of common wall of 2 descending colons and posterior sagittal anorectoplasty Reconstruction of cloacal exstrophy with anastomosis of bladders and vaginas
6.	Cheng et al.	1996	6 yrs	M	Accessory epispadiac meatal fossa	Excision of AU and AB, reconstruction of gland
7.	Ciftci et al.	1995	2 d	M	Incomplete diphallus, meningocele, imperforate anus	Transverse loop colostomy
8.	Goh et al.	1995	1st week	F	Duplication of vagina, uterus; stenosed anterior ectopic anus; double-outlet right ventricle; left uretrovesical junction obstruction, right vesicoureteral reflux, extrinsic duodenal obstruction	Vesicostomy; anorecto-urethro-vaginoplasty via the posterior approach; excision of bladder septum with reimplantation of right ureter; removal of vesicostomy; nephrectomy on the left
10.	Dajani et al.	1992	14 yrs	M	Chordee; congenital dislocation of right hip	Excision of AB and AU; correction of chordee
11.	Kapoor et al.	1986	1 d	F	Duplication of external genitalia, ventriculoseptal defect, malrotation of the gut, ectopic anal opening, maldescended left kidney, rachischisis of the lumbar spine and sacrum, umbilical hernia	Patient died of septicaemia
12.	Esham et al.	1980	7 yrs	F	Coarctation of aorta, ventral septal defect, duplication of colon with 2 appendices	Excision of diverticula from the left bladder, removal of the common septum
13.	Dunetz et al.	1985	14 d	M	Chordee	Excision of AB and AU, correction of chordee
14.	Varga et al.	2007	52 yrs	F	None	Electrohydraulic lithotripsy

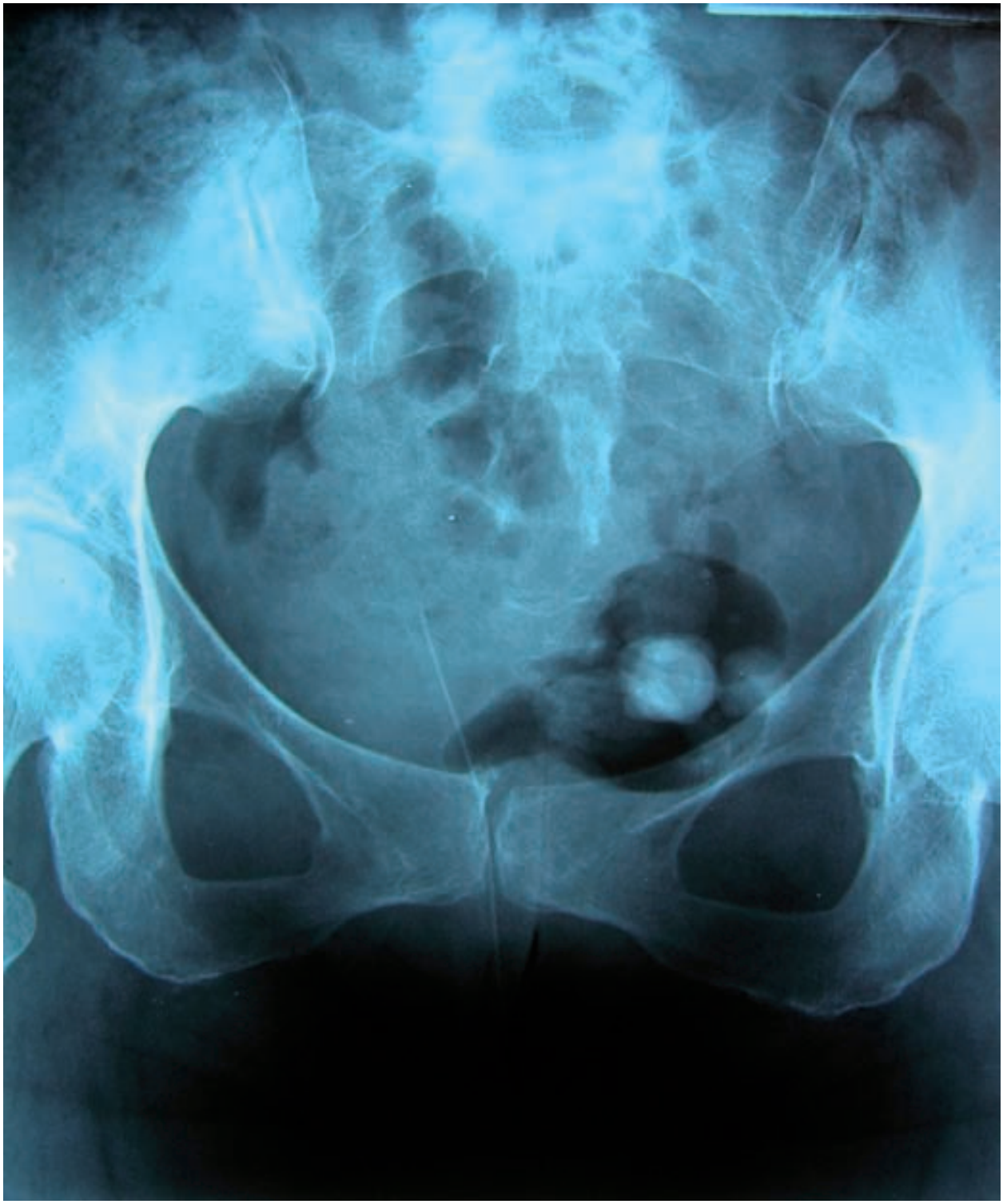

Figure 2
An X-ray examination of the pelvis with contrast calculi

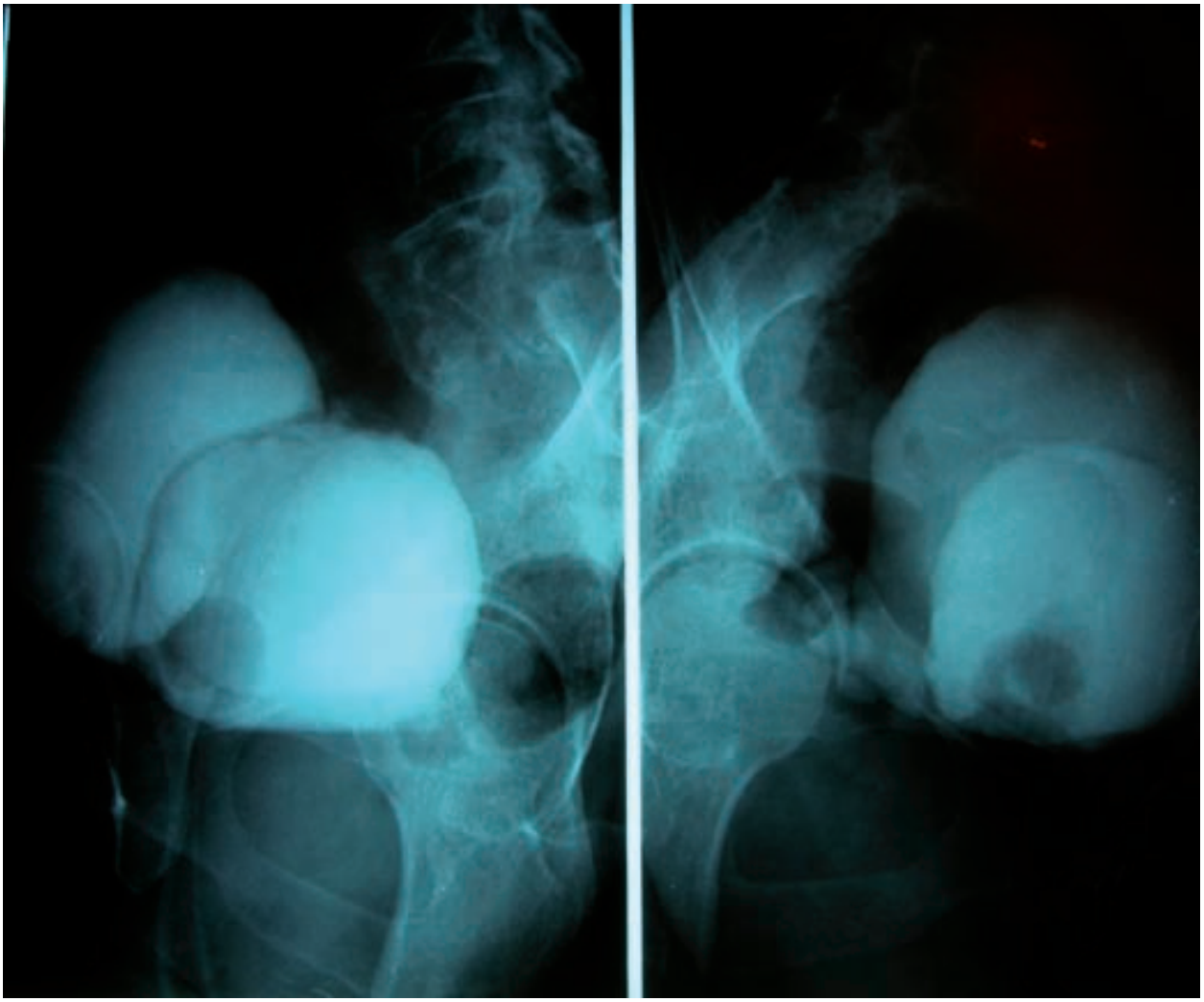

Figure 3
Cystography in the side projection, showing a complete duplication of the bladder

basement from which the urinary bladder evolves; a woman's urethra (or the prostate urethra in men) forms from the caudal region [1].

Failure of this developmental programme can lead to anatomical anomalies of the lower urinary tract [2, 8, 11]. The complete duplication of the urethra and the bladder in women is the result of a failure to form the vesicourethral basement of the urogenital sinus [1]. This anomaly, found more commonly in males than in females [12], is not only a latent "cosmetic" defect, but results in functional defects which may manifest clinically as pelvic pain disease (PID).

From the cases with CDBU reported in the literature, we illustrate the oldest patient in whom the anomaly was initially detected (Table 2).

CONCLUSION

This rare anomaly requires follow–up from the time of the first diagnosis because of the late complications that can result from bladder function failure, particularly evacuation of the bladder. In this time of neonatal ultrasound screening, this anomaly should not escape our notice in newborns.

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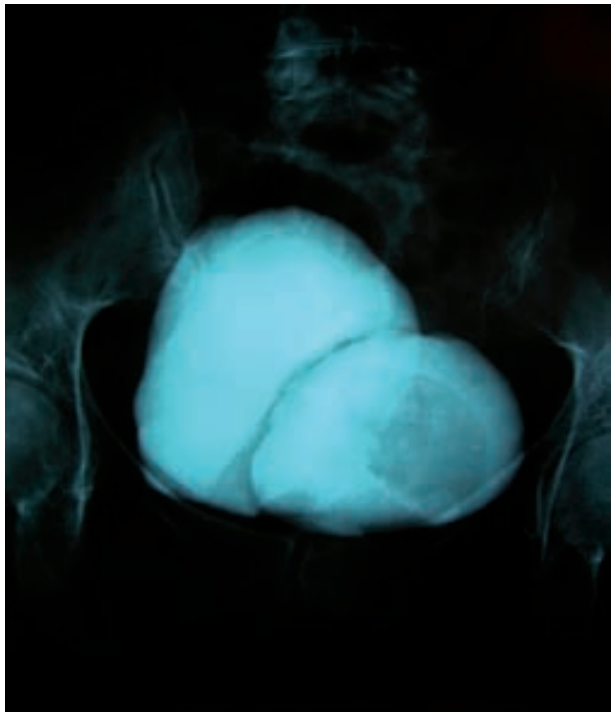

Figure 4
Cystography in the anteroposterioric projection, showing the complete duplication of the bladder

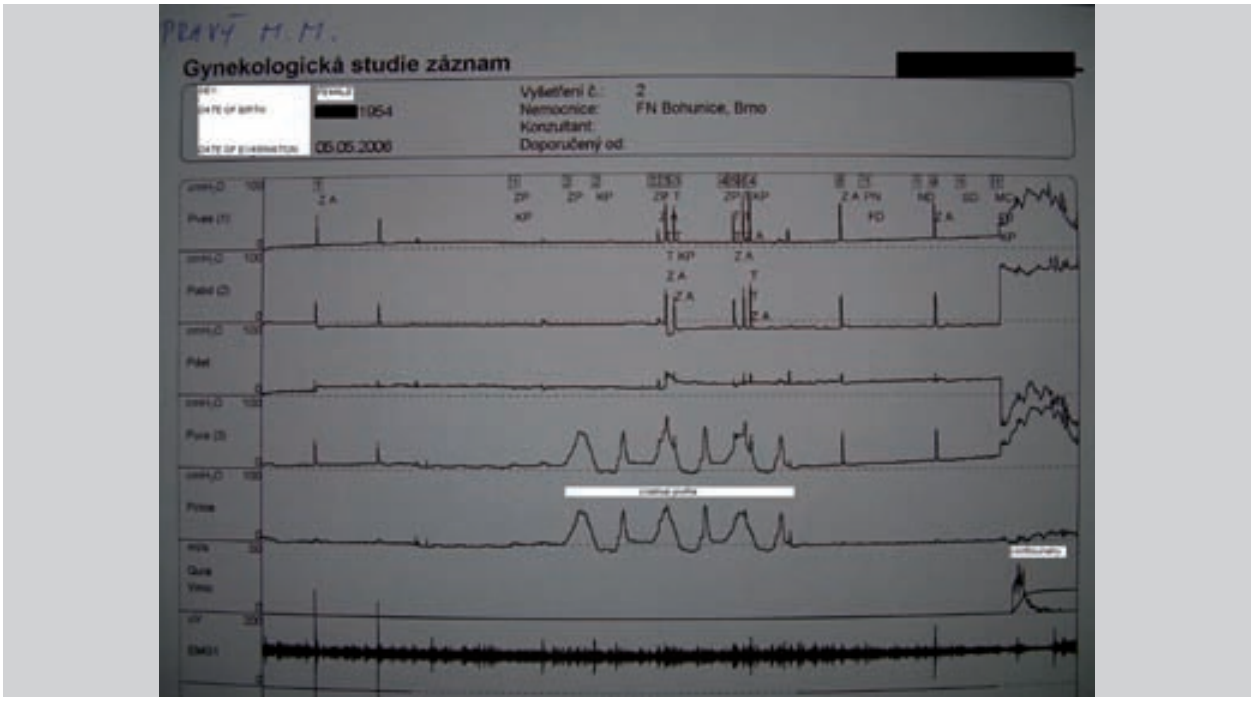

Figure 5
Image of the compresssed urodynamics curve of the right bladder

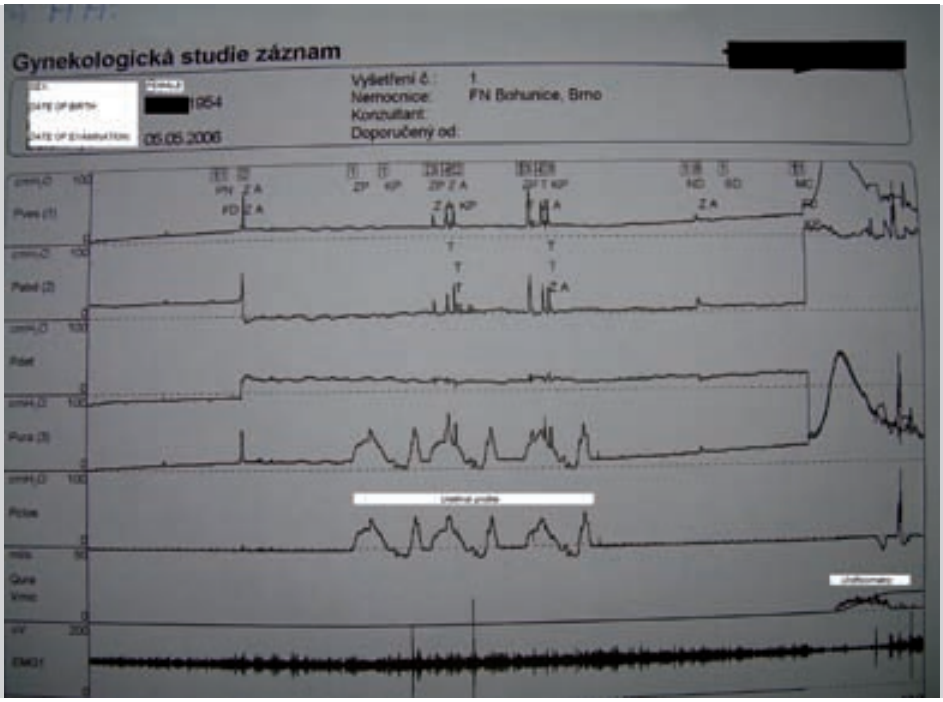

Figure 6
Image of the compressed urodynamics curve of the left bladder

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EVALUATION OF HGF AND ENDOSTATIN IN THE BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA AND THE EFFECT OF PERIPHERAL BLOOD ADMIXTURE

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KEY WORDS

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HGF
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ABSTRACT

The aim of our study was to evaluate the concentration changes in the proangiogenic hepatocyte growth factor (HGF) and antiangiogenic endostatin during the sampling of large bone marrow volumes in patients with multiple myeloma. HGF and endostatin concentrations were measured in the 1st ml, the 5th ml, the 19th ml, the 30th ml, and the 45th ml of the sample. Concentrations of HGF, which is produced by bone marrow and myeloma cells, decreased significantly in the course of BM sampling due to the admixture of peripheral blood. Endostatin concentrations did not change significantly during the procedure of sampling, as endostatin is mainly secreted by the parenchymatous organs such as liver, lungs, and others. In conclusion, the admixture of the peripheral blood could be partly responsible for conflicting results of studies of HGF in multiple myeloma. Standardisation of HGF sampling is required for valid comparisons between different studies.

INTRODUCTION

Continually, new prognostic and predictive factors are being discovered that contribute to our understanding of the pathogenesis of multiple myeloma (MM) and to the development of evidence-based approaches in MM treatment. Presently, there are tests that are used routinely and/or for research purposes in MM. Various parameters are determined from the bone marrow (BM), and sometimes they require sampling of large volumes of BM. Angiogenesis plays a key role in myeloma pathogenesis [1]. The evaluation of angiogenesis is usually done from BM. However, there is concern that concentrations of angiogenic parameters may vary during the sampling of large volumes of BM due to increasing admixture of peripheral blood, as has been described for other types of studies, e.g. morphology assessment [2] and S-phase DNA content measurement [3]. The aim of our study

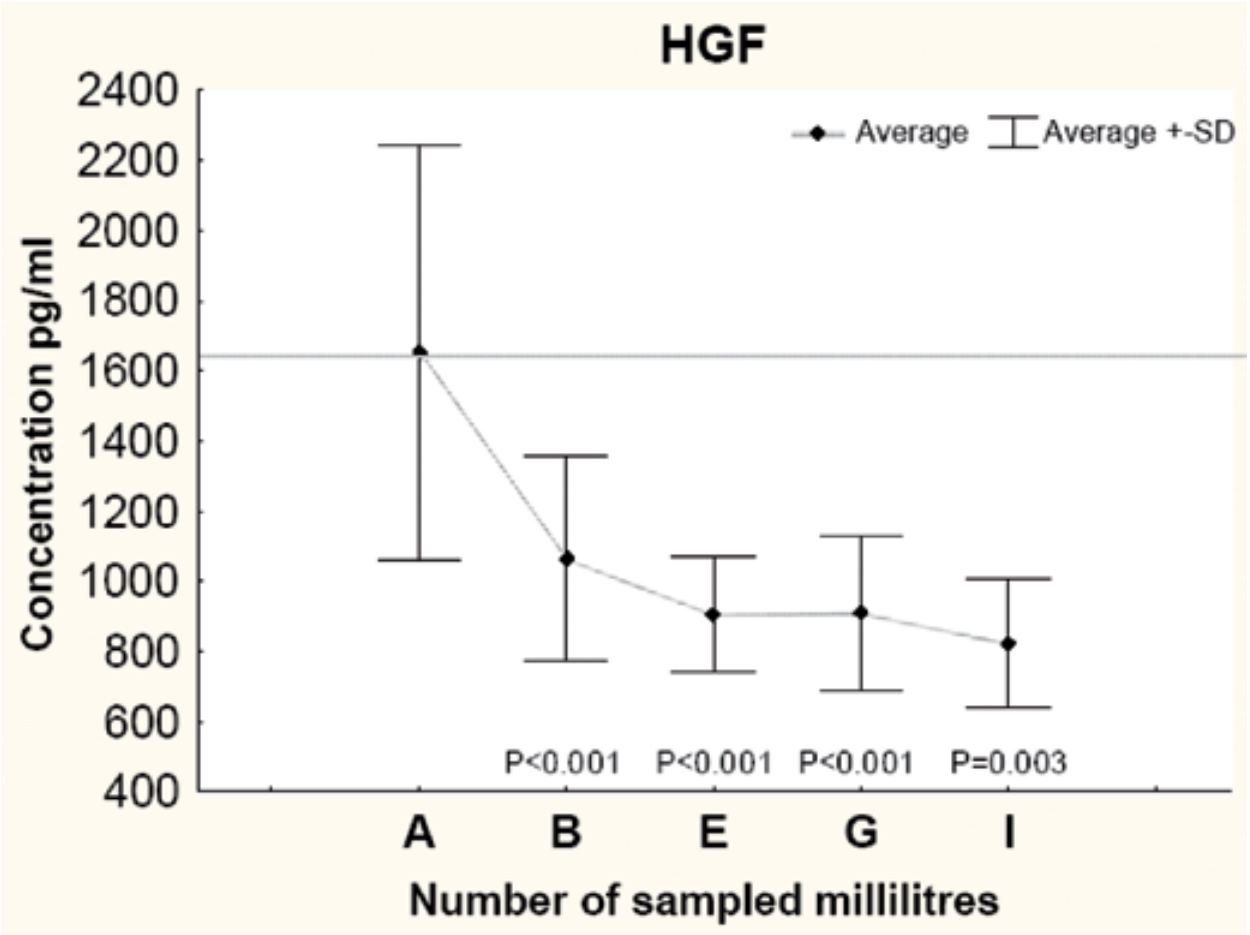

Figure 1A
Concentrations of HGF in pg/ml; p levels mean statistical difference between concentrations in 1st (A), 5th (B), 19th (E), 30th (G), and 45th (I) millilitre

was to evaluate the fluctuations in the concentrations of two molecules related to the formation of new vasculature – the proangiogenic hepatocyte growth factor (HGF) and the anti-angiogenic endostatin during the sampling of large BM volumes in patients with MM.

MATERIALS AND METHODS

BM was sampled from a standard sternal puncture after obtaining an informed consent. The maximum volume of BM sampled for diagnostic and research purposes was 45 ml. We tested samples from 9 men and 6 women. The type of the monoclonal protein produced was IgG in 11 patients and IgA in 4 patients. The average age of the patients was 64.8 years. HGF and endostatin concentrations were measured in the 1st ml, the 5th ml, the 19th ml, the 30th ml, and the 45th ml of the sample. ELISA kits HGF (Biosource, USA) and Endostatin (Chemicon, USA) were used according to the manufacturers' instructions. Statistical evaluation was carried out using Statistica software (StatSoft, USA).

RESULTS AND DISCUSSION

We sampled 45 ml of BM in eleven patients, 30 ml of bone marrow in two patients, and 5 ml of BM in two patients. Sampling of large BM volumes was well tolerated in all the patients. The concentration of HGF decreased progressively during the BM sampling (Figure 1A). Endostatin concentrations did not change significantly during the procedure of sampling (Figure 1B). HGF and endostatin are important factors thought to be involved in the angiogenesis in MM. Serum and BM levels of HGF have been reported to be higher in

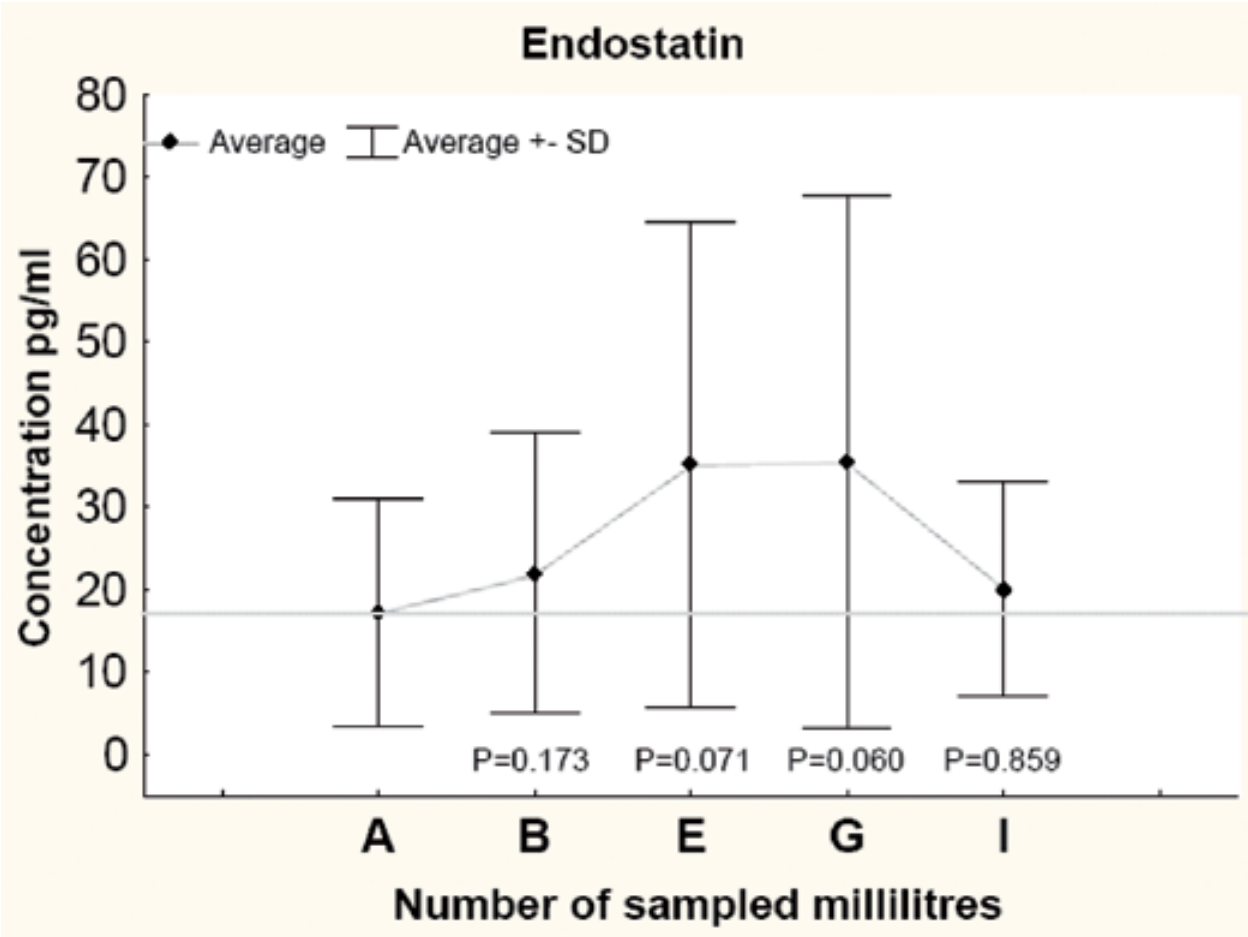

Figure 1B
Concentrations of endostatin in pg/ml; p levels mean statistical difference between concentrations in 1st (A), 5th (B), 19th (E), 30th (G), and 45th (I) millilitre

myeloma patients than in healthy controls [4]. However, the role of HGF in MM has yet to be fully elucidated. Its concentration was decreased after the treatment according to some reports [5], while according to others its level was not changed after the treatment [5]. Our study showing that HGF levels decrease significantly in the course of BM aspiration could at least partly explain the discrepancies between the various reports. The reason for the drop is probably the increased content of peripheral blood in the BM aspirate, because HGF is mainly produced by myeloma and BM stromal cells [6]. Endostatin is a terminal fragment of type XVIII collagen that arrests cell proliferation and is a potent inhibitor of tumour growth [7]. There are reports that the levels of endostatin are higher in myeloma patients compared to a healthy control [8]. Our results did not show statistically significant variations in BM endostatin levels in different phases of sampling. An explanation of this fact could be that endostatin is not produced in the BM but mainly in parenchymatous organs such as the liver, brain and lungs [7], and thus the admixture of peripheral blood does not alter BM endostatin concentrations. In conclusion, HGF levels decrease progressively during the sampling of BM. Therefore, it is best to evaluate HGF levels always in the same phase of sampling, preferably from the first portion. Standardisation of HGF sampling is required if different studies are to be compared.

ACKNOWLEDGEMENT

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The 53rd Students Medical Conference, Brno, 19 May 2009

More than 50 under- and postgraduate students gathered at the 53rd Students Medical Conference to present the results of their experimental work carried out at various departments and clinics of the Faculty of Medicine, Masaryk University, in Brno, Czech Republic. The conference was organised by the medical students association "Spolek mediků" under the patronage of the Dean of the Faculty, Prof. Jan Žaloudík, MD, PhD. Medical student conferences in Brno are held annually and have one of the longest traditions amongst the medical faculties in the Czech Republic. The presentations were structured into four sections: (1) theoretical and preclinical disciplines, (2) internal medicine and (3) surgical disciplines, and (4) a separate section for postgraduate students. The quality of the presentations was evaluated by committees consisting of the authorities of the respective disciplines and the best students awarded.

Supplements
The 53rd Students Medical Conference, Brno, 19 May 2009

"The tutors and supervisors of today's student participants very often began their scientific careers at the student conferences. Some of today's students will become teachers and researchers themselves later and will thus contribute to the continuum of scientific and academic life of our *alma mater*" said the Dean of the Faculty, Prof. Jan Žaloudík, MD, PhD. To help the awarded students to start their scientific careers not only by oral presentation but also with a chance to publish their work for the first time, the media partner to the conference – Scripta Medica – launched a new section dedicated to student research work.

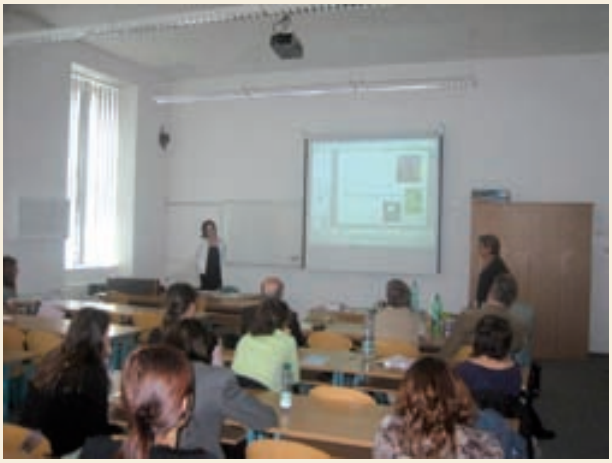
FINALISTS OF THE 53rd STUDENTS MEDICAL CONFERENCE

Theoretical and preclinical disciplines:

1. Kazda T, Tichý O: Changes of contractility of isolated cardiac muscle preparations induced by levosimendan in combination with negatively inotropic substances (Dept. of Physiology, supervisor: Prof. Marie Nováková, MD, PhD)
2. Procházková A: The presence of Candida dubliniensis in vulvovaginal mycoses (Dept. of Microbiology, supervisor: Filip Růžička, MD, PhD)
3. Trizuljak J: Mannose-binding lectin (MBL) deficiency in recurrent infections (Dept. of Clinical Immunology and Allergology, supervisor: Vojtěch Thon, MD, PhD)

Internal medicine disciplines:

1. Hošťálková P, Hnojčíková M: Incidence of epileptic activity and epilepsy in children with ADHD syndrome and the relationship between epileptic activity focus localisation and nuclear symptoms of ADHD (Dept. of Paediatric Neurology, supervisor: Assoc. Prof. Hana Ošlejšková, MD, PhD)
2. Kazda T: Stereotactic irradiation of secondary brain tumours at the Department of Radiation Oncology of Masaryk Memorial Cancer Institute (Dept. of Radiation Oncology of Masaryk Memorial Cancer Institute, supervisor: Ludmila Hynková, MD)
3. Gajdošíková M, Galková L, Gregorová Z: Mortality of patients with chronic heart failure monitored in specialised outpatient clinics (Dept. of Cardiology, Brno University Hospital, supervisors: Ondřej Ludka, MD, PhD and Prof. Jindřich Špinar, MD, PhD)

Surgical disciplines:

1. Mlynárová D: Obstetric injuries complicating extraction techniques during delivery (Dept. of Obstetrics and Gynaecology, supervisor: Radan Doubek, MD)
2. Urík M: Foreign bodies in paediatric otorhinolaryngology (Dept. of Paediatric Otorhinolaryngology, supervisor: Prof. Ivo Šlapák, MD, PhD)
3. Lukášová O: Measurement of skin elasticity in patients with large inborn pigment naevi (Dept. of Burns and Reconstructive Surgery, supervisor: Jitka Vokurková, MD, PhD)

Postgraduate section:

1. Trávníčková Z: Functional characterisation of B lymphocytes using ELISPOT in patients with common variable immunodeficiency (CVID) (Dept. of Clinical Immunology and Allergology, supervisor: Vojtěch Thon, MD, PhD)
2. Mráz M, Malinová K, Mayer J, Pospíšilová Š: MicroRNA expression in patients with chronic lymphatic leukaemia (CLL) and 17p13 and 22q11 deletions (Centre of Molecular Biology and Gene Therapy and Dept. of Internal Haematooncology, supervisors: Prof. Jiří Mayer, MD, PhD, and Dr. Šárka Pospíšilová, PhD)
3. Ondráčková B: Direct medical costs in patients with acute heart failure (Depts. of Pharmacology and Internal Cardiology, Brno University Hospital, supervisor: Prof. Jindřich Špinar, MD, PhD)

Assoc. Prof. Kateřina Kaňková, MD, PhD, on behalf of the organisers ■