Multiple myeloma

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1. Introduction

(a) Importance and Direction of the Lesson

This lesson on the subject "Theoretical Basics of Clinical Medicine" will focus on hemato-oncology. The goal of the lesson is to demonstrate comprehensive care for an oncology patient.

In this lesson, you will utilize knowledge of pathophysiology for differential diagnosis. You will also gain initial experiences in managing common complications arising from oncology treatments and familiarize yourself with the integration of the latest findings in tumor biology with modern diagnostic and therapeutic approaches in hemato-oncology. The sample diagnosis for the lesson will be multiple myeloma.

(b) Multiple myeloma

Multiple myeloma is the second most common malignancy of the hematopoietic system. It originates from malignant plasma cells that cause organ damage to the affected individual through their activity. Cells of multiple myeloma typically produce monoclonal immunoglobulin (paraprotein), which can be detected in the patient's blood. Organ damage in multiple myeloma is known by the English acronym **CRAB** (hyper**C**alcemia, **R**enal involvement, **A**nemia, **B**one lesions).

The diagnosis of multiple myeloma relies on examining the bone marrow, confirming the presence of clonal neoplastic plasma cells. In addition to routine tests (biochemical blood tests, complete blood count, coagulation studies), whole-body imaging (CT, MRI, PET/CT) is performed to detect bone lesions associated with this disease.

The treatment of multiple myeloma is predominantly pharmacological. Until recently, multiple myeloma was considered an incurable disease. However, the current perspective on this condition acknowledges the possibility of a cure in approximately 20% of patients with low-risk disease.

Structure of the lesson:

- Introduction
- Anemia – differential diagnosis
- Fever in immunocompromised patient
- Acute renal failure – differential diagnosis
- Monitoring of minimal residual disease and its significance
- Personalized Medicine in Hemato-Oncology

2. Anaemia – differential diagnosis

(a) Introduction

Anaemia is precisely defined as a reduced amount of erythrocyte mass in blood. However, determining this parameter is technically demanding, so in practice, we almost always rely on surrogate parameters that correlate with erythrocyte mass – particularly haemoglobin concentration, red blood cell count, or hematocrit. These values are less accurate because they depend on hydration status, but they are usually sufficient to raise suspicion of anaemia and guide further investigation. Anaemia is diagnosed when blood haemoglobin concentration reaches < 130 g/L in adult males or < 120 g/L in adult non-pregnant females. The severity of anaemia is graded as follows: mild anaemia – normal to 110 g/L, moderate anaemia – 110 to 80 g/L, and severe anaemia – <80 g/L. In pregnant women, especially in the later stages of pregnancy, these values are slightly lower due to their physiological hypervolemia and relative dilution of blood.

(b) Causes of anaemia

Anaemia in some form affects about a quarter to a third of the world's population, making it **one of the most common chronic diseases overall** – which means every medical doctor encounters it in their clinical practice. Erythrocytes, like other blood cells, are produced in bone marrow. Differentiation of hematopoietic stem cells into erythrocytes and their release into peripheral blood is primarily regulated by **erythropoietin**, a hormone secreted by the kidney interstitium in response to hypoxemia. **Androgens** also contribute to erythropoiesis, while **estrogens** have a slightly inhibitory effect (hence lower haemoglobin reference ranges in women). Synthesis of haemoglobin, the dominant protein in erythrocyte cytoplasm, is closely associated with erythrocyte maturation but also depends on other key factors, particularly the availability of divalent iron, an essential component of the haemoglobin molecule. Iron activates transcription factors that induce enzymes for heme and haemoglobin synthesis. Iron is transported in the blood bound to transferrin, and its reservoirs include not only bone marrow but also spleen and liver cells. Physiologically, erythrocyte production in the bone marrow is in a precisely controlled equilibrium with their destruction in the spleen or liver after approximately 90 days of circulation.

Although there are many steps in this complex process where dysfunction can occur, we can simplify our considerations of the origin of anaemia into two major groups: anaemia due to **decreased erythrocyte production** and anaemia due to **erythrocyte loss or increased destruction**. *(Fig. 4.1)*

(a) Anaemia Due to Decreased Erythrocyte Production

Anemia due to decreased production is characterized by normal or decreased count of **reticulocytes** which represent the penultimate stage of red cell development. The cause of these anaemias lies either in the deficiency of erythroid lineage precursors or in disorders of their maturation.

The most common cause of such anaemia is iron deficiency (**sideropenia**). As mentioned earlier, iron is critically important for haemoglobin synthesis and erythrocyte differentiation. Its deficiency leads to anaemia with small erythrocytes (**microcytes**) that contain relatively little haemoglobin (hypochromic anaemia). This condition can occur due to inadequate iron intake or malabsorption, as well as chronic blood loss (e.g., gastrointestinal tract, urinary tract, gynaecological bleeding, etc.).

Iron is also an important growth factor for bacteria, so systemic inflammation leads to a decrease in transferrin and thus in the availability of iron in blood plasma. Moreover, the cytokine **hepcidin** further reduces the amount of iron released from stores. Therefore, erythrocytes in chronic inflammatory conditions (chronic infection, neoplastic or autoimmune diseases) have a similar morphology to those in absolute iron deficiency. This type of anaemia is called **anaemia of chronic disease** (ACD). The same category also includes anaemia associated with kidney-damaging diseases (e.g., interstitial nephritis) that result in decreased erythropoietin levels. Defective erythrocyte and haemoglobin production in bone marrow may also be related to deficiencies of other substances necessary for proper cell maturation in bone marrow. In particular, **vitamins B9** (folic acid) and **B12** (cobalamin) play a crucial role in DNA synthesis. Folic acid deficiency is usually nutritional, while cobalamin deficiency can be either nutritional (found only in animal products and fortified foods) or due to malabsorption in the terminal ileum. The latter occurs for various reasons such as surgical resection of the intestine or stomach or autoimmune destruction of the gastric parietal cells (i.e., atrophic gastritis), resulting in decreased production of intrinsic factor, a protein necessary for B12 transport into the blood (**pernicious anaemia**). Other factors contributing to malabsorption include chronic inflammation or certain medications (proton pump inhibitors, metformin). The resulting anaemia is characterized by large erythrocytes (megaloblasts), which are numerically deficient and partially undergo lysis in the bone marrow.

Disorders of erythroid lineage division or maturation in bone marrow can be either congenital or acquired. There are several predominantly monogenic diseases associated with erythropoiesis failure or dysfunction, such as Fanconi anaemia or Diamond-Blackfan anaemia. However, acquired disorders, such as myelodysplastic syndromes (MDS), or the effects of myelotoxic drugs (e.g., cytostatics), are much more common. Another cause of anaemia may be the suppression of healthy hematopoiesis due to the infiltration of bone marrow by malignant cells (leukaemia, multiple myeloma, lymphomas, metastatic infiltration by solid tumours, etc.). These disorders are often accompanied by deficiencies in other blood cell lineages.

(b) Anaemia Due to Erythrocyte Loss or Increased Destruction

If hematopoiesis is unaffected, any loss of erythrocytes is followed by increased secretion of erythropoietin due to systemic hypoxia. This leads to compensatory production of new erythrocytes, which can be observed through an increased number of reticulocytes in the peripheral blood.

The most common cause of this type of anaemia is **acute haemorrhage**. Since bleeding results in the loss of relatively equal amounts of erythrocytes and plasma, the immediate blood count (in minutes) after bleeding may appear completely normal. The body's homeostatic mechanisms begin compensating for the loss of blood volume by mobilizing extracellular fluid within hours. It takes a significantly longer time to replenish the lost erythrocytes, resulting in relative anaemia.

Hemolytic anemias, that is anemias caused by erythrocyte destruction, can be classified into corpuscular and extracorpuscular based on their pathophysiology. In corpuscular hemolytic anaemias, the primary pathology lies within the erythrocyte itself, whereas in extracorpuscular hemolytic anaemias, the pathology occurs outside of the erythrocyte. **Corpuscular hemolytic anaemias** are predominantly congenital disorders. Among the causes are **abnormal haemoglobin folding** (sickle cell anaemia, thalassemia, etc.), **abnormal erythrocyte shape** due to cytoskeletal disorders (hereditary spherocytosis, elliptocytosis, etc.), **or reduced resistance to oxidative stress** which leads to hemolysis upon exposure to triggering factors (e.g., favism). These diseases are not very common in the European population.

Extracorpuscular hemolysis can be either **immune** or **non-immune**. The most common cause of immune extracorpuscular hemolytic anaemia is the presence of antibodies against one's erythrocytes. These antibodies are most commonly of the IgG class and cause erythrocyte destruction in the spleen upon binding. IgM autoantibodies can cause complement fixation and hemolysis within the blood vessels. Diagnosis of autoimmune hemolytic anaemia (AIHA) is confirmed by a **direct antiglobulin test (Coombs test)**. Non-immune extracorpuscular hemolytic anaemias are associated with **mechanical damage to erythrocytes** (artificial heart valves, hemodialysis, extensive burns, microangiopathic hemolytic anaemias, etc.), **chemical agents** (phospholipases in snake venom), or **infectious causes** (malaria, sepsis).

Hemolysis can further be divided into **extravascular** and **intravascular** based on the site of occurrence. Extravascular hemolysis occurs due to increased sequestration of erythrocytes by reticuloendothelial cells, particularly splenic macrophages. Intravascular hemolysis takes place directly within the blood vessels. The consequences of this process are more severe because the released phospholipids from destroyed erythrocytes have strong thrombogenic effects, free haemoglobin is toxic to renal tubular cells and heme tetrapyrroles can bind to free nitric oxide and cause generalized vasospasms.

During hemolysis, substances normally present in erythrocyte cytoplasm, such as **lactate dehydrogenase** and **free haemoglobin**, appear in the patient's plasma. Haemoglobin is proteolytically degraded, releasing free heme which is further metabolized to **unconjugated bilirubin**. A fraction of free haemoglobin physiologically binds to a specialized plasma protein called haptoglobin, and this complex is sequestered by the liver, leading to a decrease in circulating haptoglobin.

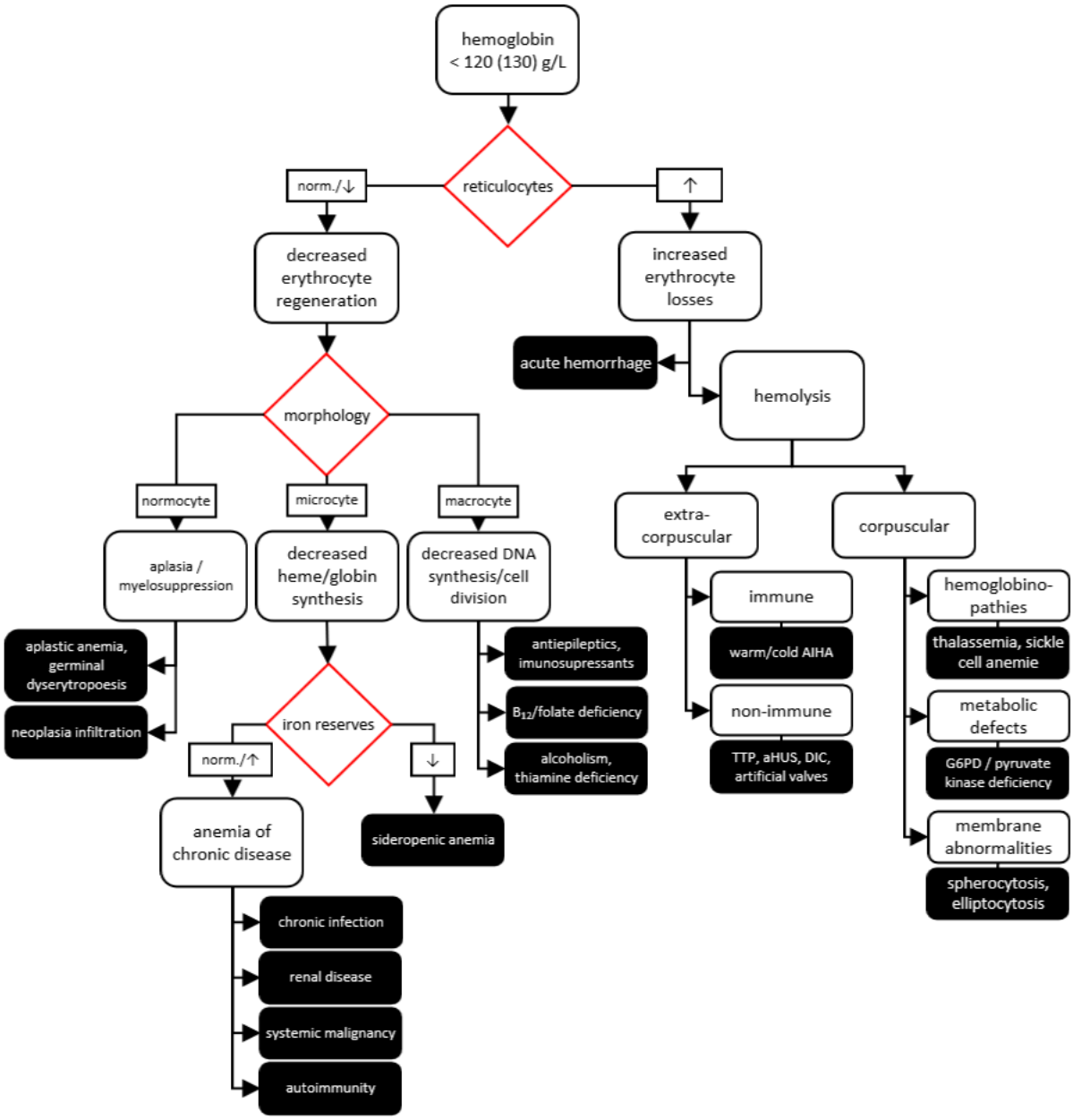

Fig 3.1. Anemia diagnostic algorithm

3. Fever in immunocompromised patient

(a) Introduction

Immunocompromised patients have one of the components of immunity reduced (cellular/humoral, specific/non-specific). Infections in these patients are often caused by **opportunistic pathogens** (microorganisms with low virulence), these are more severe, **rapidly progressing** and tend to persist. In patients with immunodeficiency, an early diagnosis of infection and **immediate initiation of** appropriate **treatment** is very important. In these patients, fever is often the only sign of infection.

The most common groups of immunocompromised patients include diabetics, oncological patients, critically ill patients (ICU), patients using immunosuppressive and biological treatment (rheumatology - corticotherapy, IBD), and patients after transplantation or splenectomy.

(b) Opportunistic pathogens

As opportunistic, we refer to those types of microbes that can **cause infection only in people with reduced defence mechanisms**. In contrast, obligate pathogens can cause disease even in healthy individuals. In case of **bacterial infections**, these are often infections originating from the patient's **flora**, most often intestinal primarily symbiotic pathogens - *E. coli*, enterococcus spp., *Klebsiella pneumoniae*, and anaerobes are less common. Pneumonia is usually caused by **atypical microbes** - viruses, *Mycoplasma pneumoniae*, *Pneumocystis carinii*, and Legionella.

We can also encounter **fungal infections** in these patients, whether it is very common **candidiasis**, or **invasive mycosis** occurring in patients with severe immunodeficiency, most often affecting the **lung parenchyma**. Less often, we encounter fungal infections of the abdominal parenchymatous organs or the central nervous system. These are most often caused by species of aspergillus, cryptococci or fungi from the Mucor class - *Cunninghamella echinulata*, *Mucor piriformis*, and *Absidia corymbifera*.

Viral infections in severely immunocompromised patients are often caused by **herpes viruses** - CMV (pneumonia, colitis, optic nerve infection), **generalized** EBV, HSV or VZV infections. Obligate virus infections often have an **atypical course** - severe **pneumonia** during influenza, SARS-CoV-2, and RSV. Antivirals or, in the case of EBV, anti-CD20 antibodies (rituximab) are used in treatment.

i. Nosocomial infections

Infections arising **in connection with hospitalization** are referred to as nosocomial. These infections can be exo- or endogenous, they are often **respiratory** or **gastrointestinal** infections, a specific group is represented by **catheter-related** infection (CRBSI - catheter-related bloodstream infection). These infections are often caused by strains of bacteria **resistant to standard antibiotic therapy**.

Enterococci resistant to vancomycin (VRE) or to the next line of therapy - **linezolid** (LRE) is among the most important, for which the stock antibiotic tigecycline remains a therapeutic option. Other frequently appearing strains are referred to as **ESBL** (extended-spectrum b-lactamase). These are bacteria that **hydrolyze** most **penicillins and cephalosporins**. For these, carbapenems or higher-generation cephalosporins can be used in therapy in combination with beta-lactam inhibitors (e.g., ceftazidime/avibactam). The next chapter is represented by ***Pseudomonas aeruginosa*** (PSAE) infections, which are almost **exclusively nosocomial**. Due to its natural **ability to produce a wide range of proteolytic enzymes**, this bacterium is **primarily resistant** to many commonly used antibiotics, exceptionally in repeatedly hospitalized and ATB-treated patients we can encounter carbapenem-resistant PSAE, with which our therapeutic options are severely limited.

The most effective prevention of the emergence of resistant strains of microbes in the hospital environment remains **rational antibiotic therapy** - using ATB therapy only in **highly suspected** or **proven bacterial infections**, taking **materials for culture examinations** before starting antibiotic therapy (cultivation with verification of sensitivity enables a step-down from broad-spectrum ATB therapy, which often leads to selection pressure on resistant strains). Furthermore, strict adherence to the **principles of asepsis** and **hand hygiene** is necessary.

ii. Febrile neutropenia

Febrile neutropenia is a **life-threatening** condition. It is defined as a decrease in the number of **neutrophil granulocytes below 1.0 x 10^9** and the onset of **fever above 38.0 °C** and, at the same time, **no focus of infection was found** (then the condition is referred to as infection of a specific organ in neutropenia, e.g., pneumonia in neutropenia). This state presents one of the urgent conditions in haematology, and with every hour of delay in the administration of ATB, mortality increases exponentially. In the case of febrile neutropenia, it is necessary to **search for the etiology** of the infection, to take at least blood for culture (hemoculture) and, if necessary, PCR examination before starting antibiotic therapy, urine for culture and perform a basic imaging examination - X-ray of the heart and lungs, paranasal sinuses, OPG, ultrasonography of abdomen and pelvis. In therapy, we use a combination of **broad-spectrum antibiotics** and possibly also antifungals as mentioned further.

iii. Infection Prevention

To reduce the risk of infection, we try to **isolate** immunocompromised patients and we educate patients about a **suitable diet** (exclusion of foods with a higher risk of fungal contamination). Furthermore, in neutropenic patients, we apply **granulocyte colony-stimulating factors** (except for myeloid malignancies, in which, in case of life-threatening infections, it is possible to use infusions of donor granulocytes), in case of a drop in the level of IgG below 5 g/l, as a marker of specific humoral immunity, we administer **infusions of immunoglobulins**. As part of **pneumocystis pneumonia prophylaxis**, at-risk patients take cotrimoxazole 480 mg once a day. Fluconazole or posaconazole are most often used to prevent **fungal infection**s.

(c) Therapy

The therapeutic procedure for febrile neutropenia is summarized in *Figure 5.1.*

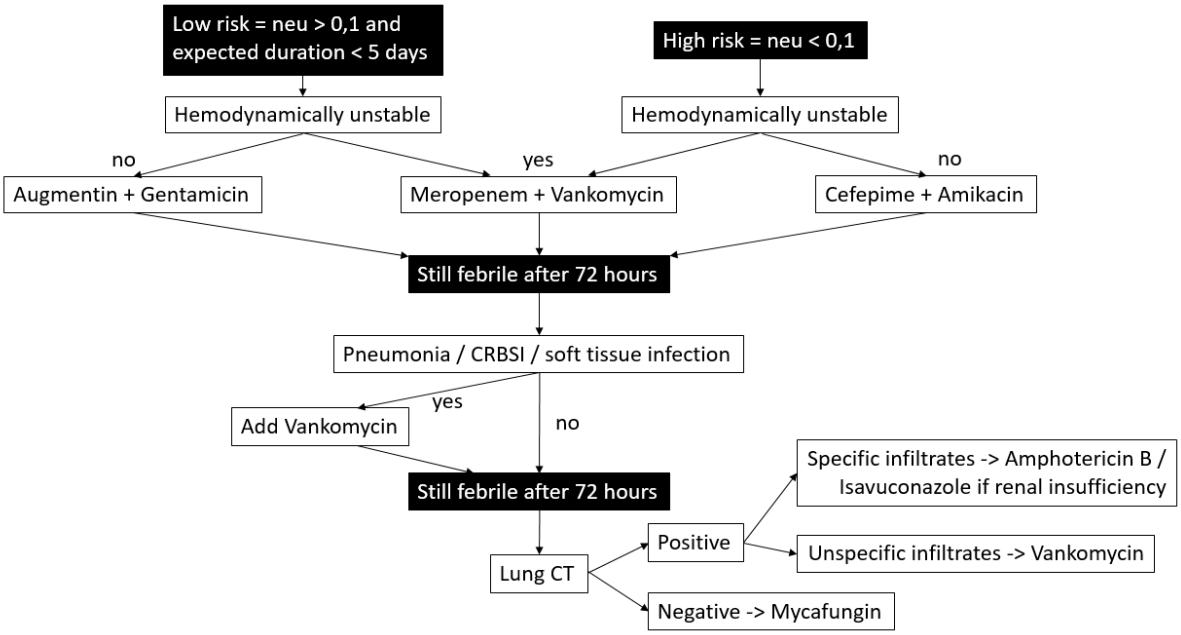

Fig. 3.1 Therapeutic schema for febrile neutropenia

4. Acute renal failure – differential diagnosis

(a) Introduction

Acute renal failure is **one of the most common issues** encountered by internists. This diagnosis encompasses a **wide spectrum of causes** yet leads to **very similar metabolic consequences**. Many causes of acute renal failure can be reversible with prompt and appropriate treatment.

(b) Pathophysiology of Renal Failure and Basic Differential Diagnosis

i. Basic Classification

The classical model divides acute renal failure and its causes into **prerenal, renal, and postrenal**. The prerenal part of the chain includes all processes and events that do not directly affect the renal glomeruli or tubules. Disorders occurring at the glomerular membrane, within the urinary space between the glomerulus and Bowman's capsule, in the renal tubules, or interstitium (excluding vascular flow

disturbances) are classified as renal. Problems from the renal calyces to the end of the ureter are referred to as postrenal.

ii. Prerenal Processes and Their Impairment

For proper kidney function, **adequate perfusion** is necessary. The incoming blood is filtered through the glomerular membrane and oxygenates and nourishes the interstitium. Both **filtration** and **nourishment** require sufficient perfusion **pressure** and **blood volume** flowing into the entire organ. Both processes normally occur if the mean arterial pressure is at least 65mmHg (approximately 90mmHg systolic blood pressure). Autoregulatory mechanisms exist within the kidney between the two circulations. When blood pressure or blood volume decreases, these mechanisms protect the renal parenchyma from ischemia and hypoxia at the expense of reduced glomerular filtration and renal clearance, which are fully reversible if decreased perfusion does not last too long.

In prerenal failure, the **urine sediment is normal**, **proteinuria is absent**, and a detailed urine examination shows a fractional excretion of sodium (EF Na+) of less than 1%. Often, a **reduced fluid intake or increased fluid loss** (sweating, diarrhoea, vomiting, diuretics) can be identified in the patient's history, and **signs of dehydration** may be present during physical examination.

The treatment principle for prerenal failure is to **restore kidney perfusion and oxygenation**. In many cases, hydration of the patient leading to volume replacement is sufficient. In more severe cases, vasopressors may be required for hemodynamic stabilization. The causal treatment of the condition leading to decreased perfusion (e.g., heart failure, bleeding, shock, fluid losses due to diarrhoea, etc.) is essential. Mechanical obstruction of the renal artery is typically managed through surgical or radiointerventional approaches.

iii. Renal Causes

Damage to the glomerulus can involve either the **glomerular capillaries or the filtration membrane itself**. The result is similar in both cases - impaired glomerular filtration. The etiology can be diverse, ranging from damage by microthrombi to inflammatory injuries (glomerulonephritis and vasculitis) or amyloid infiltration. Damaged glomerular membranes can also allow bigger amounts of substances (and substances normally unable to) to pass to and damage the renal tubules.

Damage of **tubular cells** can be related to **direct toxic effects** of substances (drugs, toxins) in the primary urine or may result from **precipitation and subsequent luminal obstruction** by substances that pass through the glomerular membrane (myoglobin, free light chains of immunoglobulins, urates, oxalates, etc.). Tubular cells can also be damaged during prolonged prerenal failure, leading to their **hypoxia and necrosis**. When renal tubules are damaged, the ability to concentrate urine and reabsorb or excrete individual substances decreases, and this condition persists until the renal tubules are

repaired. Clinically, this phenomenon is manifested by transitioning from oliguria to the polyuric phase of acute renal failure, during which waste products remain elevated in the blood while the body produces several litres of unconcentrated urine daily, leading to water and mineral losses.

When the **interstitium** is affected, similar consequences occur as with tubular damage. Transport of substances between tubular cells and interstitial capillaries fails, and the osmotic gradient in the kidney medulla is disrupted. Interstitial inflammation, vasculitis, amyloid deposition, and other conditions can be considered as possible etiologies.

The renal cause may be suggested by the **patient's medical history** with symptoms of infection, use of nephrotoxic medication, intoxication with nephrotoxic substances, or a known systemic disease affecting the kidneys. **Blood biochemical tests** may show signs of inflammation, elevated uric acid, and creatine kinase (a marker of rhabdomyolysis). Unlike prerenal failure, the **urine sediment is never normal in renal failure**, and proteinuria, hematuria, squamous or cylindrical epithelial cells, leukocyturia, and bacteriuria may be present in the urine. The fractional excretion of sodium (EF Na+) is >1%. Renal ultrasound can exclude obstruction but may reveal damaged architecture of the kidney parenchyma.

The treatment of renal failure consists of **eliminating the underlying cause** (discontinuing exposure to nephrotoxic substances, managing autoimmune or infectious inflammation, treating systemic diseases, etc.).

However, in many cases, the exact cause may not be initially known, or its removal may take some time. In such cases, **nonspecific therapeutic interventions** play a significant role in preventing further kidney damage. This nonspecific therapy primarily involves increased hydration and maintaining adequate diuresis, potentially with the use of diuretics. In the polyuric phase, it is essential to adequately replace ion and fluid losses.

If renal failure is severe, and its metabolic consequences are life-threatening, acute hemodialysis may be necessary. **Indications for acute hemodialysis** include hyperkalemia (>6.5 mmol/l) or with EKG changes, pH <7.2, hypervolemia, and fluid retention unresponsive to diuretics, or uremia >40 mmol/l or earlier signs of uremic syndrome. The value of creatinine does not play a role in the decision for acute hemodialysis.

iv. Postrenal Pathology

In postrenal renal failure, **urine outflow is obstructed** at any level from the renal calyces to the urethra. Gradual pressure build-up within the bladder, ureters and calyces worsens the kidney's filtration ability, and urine leakage into the interstitium leads to further restrictions in excretion and reabsorption. Due to the large functional reserves of the kidneys, postrenal renal insufficiency becomes evident only when

more than 50% of the renal parenchyma is compromised. For example, unilateral obstruction in one kidney with an otherwise healthy second kidney may not show signs of renal failure.

Oliguria to anuria is present. In cases of obstruction at the level of the **urinary bladder and below**, a palpable, painful distended urinary bladder may be observed. The definitive confirmation of the diagnosis is through an ultrasound (UZV) examination, which demonstrates bilateral dilation of the calyceal-pelvic systems. In most cases, the obstruction is mechanical. If the **obstruction is close to the kidneys**, its cause may be visible on **ultrasound**; if not, further investigation using **CT scans** is needed. The urine sediment may be normal but if the obstruction is intraluminal hematuria and epithelial cells may be present. Stagnant urine provides an excellent substrate for bacteria, so leukocyturia and bacteriuria may also be founnd.

The treatment of postrenal renal failure **aims to restore urine flow**. This form of treatment is often achieved **surgically** (ureteral stenting, catheter placement in different parts of the urinary system). **Pharmacologically**, it may involve influencing the obstruction, such as with benign prostatic hyperplasia or neuromuscular dysfunction of the urinary bladder. In the case of postrenal failure, **diuretics are contraindicated** as their use would worsen the existing situation.

Tab.6.1 Differential diagnostics of acute renal failure

Prerenal	Renal	Postrenal

• Dehydration
• Hypotension
• Shock
  ○ Cardiogenic
  ○ Hypovolemic (including hemorrhagic)
  ○ Distribution (anaphylaxis, sepsis, SIRS)
  ○ Obstructive
• Stenosis/thrombosis/embolism/extramural obstruction of the renal artery
	• Ischemic damage
	• Nephrotoxic medication
	• Intoxication by nephrotoxic compounds
	• Urate nephropathy
	• Rhabdomyolysis (myoglobin)
	• Microangiopathic hemolytic anaemia
	• Glomerulonephritis
	• Vasculitis
	• Systemic lupus
	• Tubulointerstitial nephritis
	• Pyelonephritis
	• Cast nephropathy
		• Urethral blockade (foreign body, tumour, hematoma, coagulum)
		• Prostate hyperplasia
		• Obstructed urine catheter
		• Urinary bladder tamponade
		• Neuromuscular impairment of the urinary bladder

	• Amyloid deposition • Contrast-induced nephropathy • Renal tumours • Renal trauma	• Bilateral ureteral blockage • Unilateral ureteral blockage with a solitary kidney

5. Monitoring of minimal residual disease and its significance

(a) Introduction

Minimal residual disease (MRD) is a small amount of cancer cells that persist in the patient's body even after achieving **disease remission**. These cells are not detectable by conventional diagnostic methods.

Biologically, MRD cells are an extremely interesting cell population. MRD cells can be resistant to administered treatment, and therefore, they can regrow the cancer population and cause **disease relapse**. On the other hand, there are cases of persistent stable MRD, suppressed by the patient's **anti-tumor immunity** or by the effect of **maintenance therapy**. In some cases, the presence or absence of MRD influences crucial treatment decisions for a specific patient.

(b) Methods of MRD Monitoring in Hematological Malignancies

By classical microscopic examination of bone marrow, 500 nuclear cells are evaluated. Although microscopic examination still plays an indispensable role in the diagnosis of hematologic diseases, it is not suitable for detecting MRD. This is due to the relatively small number of cells examined and the fact that tumour cells sometimes have identical morphology to their physiological counterparts.

To detect MRD in a specific patient, we need to **know the characteristics of tumour cells during the initial diagnosis** when there is an abundance of them in the bone marrow. Only then can we use sensitive methods to capture the minimum number of tumour cells among the vast quantities of physiological hematopoietic cells.

Flow cytometry is a highly effective method for MRD detection. The principle of flow cytometry involves the passage of individual cells from the examined suspension through a narrow capillary. A laser beam is directed at the individual cells in the capillary. The examined cell suspension is mixed with a **mixture of monoclonal antibodies targeting specific antigens on the cells**. These monoclonal antibodies are labelled with different **fluorochromes** that emit **light of varying wavelengths** when illuminated by the laser beam. This emitted light is captured by the sensitive detectors of the instrument.

Currently, by appropriately setting the laser and combining antibodies with fluorochromes, we can detect up to 15 antigens in a single test tube within the cell population. Tumor cells have **antigenic characteristics that differ from physiological hematopoiesis**. The appropriate **combination of antibodies** and fluorochromes can detect tumour cells with a sensitivity of up to 10-6 (1 tumour cell in 1,000,000 healthy cells). However, we are not able to achieve such sensitivity for all hematologic malignancies. The differences are determined by the antigenic distinctiveness of tumour cells from their physiological counterparts.

Another suitable methodology for MRD detection is **PCR (polymerase chain reaction)** analysis. In the examination of MRD in lymphoid malignancies, the detection of **clonal rearrangements of immunoglobulin heavy chains (IgH) or T-cell receptors (TCR)** is often used. During lymphocyte development, rearrangements occur in the VDJ segment of IgH and TCR. **These rearrangements are unique to each B or T lymphocyte clone**, including those corresponding to the tumour population. Another option is to monitor **specific fusion genes** (i.e., BCR-ABL, PML-RARA, ETV6-RUNX1, CBFB-MYH11, etc.) or **mutations in typical genes** for the patient's specific disease (i.e., NPM1). The absence of these abnormalities in healthy hematopoiesis can be utilized for MRD detection.

(c) The significance of MRD monitoring in multiple myeloma

In the case of multiple myeloma, flow cytometry is used for MRD monitoring. Current protocols achieve a **detection limit of 10^{-6}** for multiple myeloma. Due to its immense sensitivity and lower cost, this method is preferred over PCR-based methods in the Czech Republic.

The absence of MRD in patients with multiple myeloma is a strong independent indicator of good prognosis, although it does not guarantee a cure. Achieving MRD negativity is currently considered the most significant therapeutic goal for patients with this disease.

(d) The significance of MRD monitoring in other hematologic malignancies

The significance of MRD varies for different hematologic malignancies. One of the most significant analyses of MRD is in the case of **acute lymphoblastic leukaemia** of adult age. The persistence of MRD after induction therapy is a clear indication for directing the patient towards **allogeneic hematopoietic cell transplantation**. Allogeneic stem cell transplantation has the potential to eliminate even chemo-resistant residual leukemic cells through the **"graft-versus-leukemia" effect**. A comprehensive discussion of MRD in individual hematologic malignancies is the subject of ongoing research and goes far beyond the scope of this chapter.

(e) New perspectives in the assessment of MRD in haematological malignancies

The field of MRD assessment in haematology-oncology is relatively young. Soon, three main directions of research need to be pursued.

Firstly, it is necessary to **link the MRD assessment results** at specific stages of disease treatment with specific changes in treatment strategies. For example, achieving MRD negativity at a certain treatment phase should lead to the de-escalation of therapy without an increased risk of disease progression (new activities). Conversely, in the case of MRD positivity, a precisely defined treatment change should follow to achieve MRD negativity.

Secondly, the methodology for **MRD assessment needs to be optimized** to be more easily applicable in routine laboratory practice. Currently, high-quality MRD analysis is predominantly performed in highly specialized university laboratories.

Thirdly, there is a need to **explore the use of new methods that are more sensitive** and ideally **do not require invasive procedures** (such as peripheral blood examination compared to bone marrow examination).

6. Personalized Medicine in Hemato-Oncology

(a) Introduction

For decades, it has been known that different types of hematologic malignancies require distinct treatment protocols. Furthermore, the population of patients who develop a specific histological type of disease can significantly differ in terms of age, physical condition, and comorbidities. The current trend in hemato-oncology is moving towards even greater personalization, which considers other factors, such as molecular aspects of the pathogenesis of a specific disease in a particular patient.

(b) Tumor stage

One of the fundamental aspects of individualized patient treatment is the assessment of the **extent of the tumour** using a **staging system**. This form of treatment individualization is particularly crucial in solid tumour oncology. Staging in solid tumours is determined using the **TNM classification** (T = tumour size and extent, N = lymph node involvement, M = presence of distant metastases). The size, anatomical location, and absence of tumour metastasis determine the possibility of complete surgical resection, leading to curative treatment. On the other hand, diseases to a greater extent require systemic anti-cancer therapy.

Hematologic malignancies are, in most cases, disseminated diseases that require systemic treatment. Staging is often expressed in terms of the **degree of impact on healthy hematopoiesis** (e.g., Rai and Binet staging in chronic lymphocytic leukaemia) or the **extent of organ damage** (e.g., Durie-Salmon staging in multiple myeloma). In both mentioned cases, the disease stage is not the determining factor for treatment but influences the prognosis of the disease. **The anatomical extent** of the disease affects the intensity of treatment in **malignant lymphomas**. For example, localized stages of certain lymphomas require less aggressive systemic treatment combined with localized radiation therapy.

(c) Tumor biology

In hemato-oncology, the severity of the disease is reflected more by **specific prognostic biomarkers**. These often include the presence of recurrent genetic abnormalities or levels of certain proteins in the blood.

In some cases, the **presence or absence of these prognostic biomarkers leads to adjustments in the treatment strategy.** In multiple myeloma, for example, biomarkers such as beta-2 microglobulin, albumin, lactate dehydrogenase, and high-risk cytogenetic abnormalities in malignant plasma cells (del(17p13), t(4;14), and t(14;16)) are considered.

In other cases, the **presence of certain biomarkers** allows the use of **targeted therapies** that **directly affect the specific tumour clone** in the patient. By targeting surface molecules detectable by flow cytometry, monoclonal antibodies can be directed at the tumour clone. An example of this approach is the treatment of acute lymphoblastic leukaemia with atypical CD20 antigen expression on leukemic cells using the monoclonal antibody rituximab (anti-CD20).

Based on the presence of pathologically activated tyrosine kinases detected by PCR, targeted inhibitors can be used. For example, about one-third of patients with acute myeloid leukaemia have internal tandem duplications (ITDs) in the FLT3 tyrosine kinase. This mutation leads to autonomous and sustained activation of the kinase, resulting in uncontrolled proliferation of leukemic blasts. After detecting this mutation, the administration of a specific FLT3 inhibitor, such as midostaurin, is indicated in addition to the standard treatment regimen. Another example is the treatment of acute lymphoblastic leukaemia with atypical presence of the Philadelphia chromosome and the BCR-ABL fusion gene. These patients are treated with tyrosine kinase inhibitors (imatinib, dasatinib) in addition to the standard acute lymphoblastic leukaemia therapy.

The presence of **other molecular targets**, such as transcription factors, can also be therapeutically influenced. The translocation t(15;17) with the PML-RARα fusion gene in patients with **acute promyelocytic leukaemia (APL**). The formation of the PML-RARα fusion protein leads to a block in the differentiation and maturation of leukemic promyelocytes. By using high doses of retinoic acid as treatment, this block can be bypassed, resulting in the maturation of leukemic promyelocytes.

In patients with multiple myeloma and the t(11;14) translocation, there is overexpression of the anti-apoptotic protein BCL-2. These patients can be effectively treated with the BCL-2 inhibitor venetoclax.

(d) Patient`s performance status

It is always necessary to **assess the overall condition of the patient, not just their age.** Various classification systems are used to assess the overall condition of oncology patients, with the most well-known being the **Karnofsky Performance Status or the ECOG (Eastern Cooperative Oncology Group) Performance Status**. Both systems express how the tumour affects the **patient's daily activities** (Table 9.1). A poor overall condition of the patient can be a limitation for oncological treatment,

especially for aggressive treatment approaches such as intensive chemotherapy or hematopoietic stem cell transplantation. However, if the overall condition is primarily influenced by the disease activity in an otherwise healthy patient, it may not be an obstacle to aggressive treatment. Making decisions in these cases requires considerable **clinical experience from the treating oncologist and ideally involves interdisciplinary team consensus.**

(e) Comorbidities

When choosing oncology therapy, the **typical side effects** of anticancer drugs must be taken into account concerning **the patient's comorbidities** (e.g., cardiotoxicity of anthracyclines in a patient with heart failure, high doses of glucocorticoids in a diabetic patient, etc.). Another important aspect is the **pharmacokinetics** of drugs in **relation to comorbidities.** For example, lenalidomide, one of the key drugs in multiple myeloma, is predominantly eliminated by the kidneys. Impaired kidney function can lead to the accumulation of lenalidomide in the blood plasma, resulting in its most common side effects (leukopenia, anaemia, thrombocytopenia, seizures, and diarrhoea).

(f) Patient`s preference

The most important aspect when choosing oncology treatment is the decision of the well-informed patient. After all, it is their health, and they should have the opportunity to feel a sense of shared responsibility in the treatment process. In this case, **it is crucial to explain to the patient the nature of their illness and the chosen treatment.** Furthermore, it is important to explain **why** the treatment was chosen, **how long** it will last, **what is expected** from the patient, what possible **complications** may arise, and if other **alternatives** exist. The common phrase, *"I don't understand it, doctor, I'll leave it up to you,"* should stimulate the physician to provide a more suitable form of explanation rather than assuming absolute control over the patient's fate. **The physician should inform the patient sensitively but truthfully.**

(g) New perspectives in personalized oncology treatment.

New perspectives lie in the detailed understanding of the oncogenesis process of individual diseases and subsequently targeting this process through precision therapy. It may be a question of the near future that we will not pay as much attention to the histological type of the tumour, but rather to the deregulated signal pathway shared, for example, in breast, ovarian, or colon carcinoma. This approach requires close collaboration between clinical oncology teams and molecular biology teams in so-called "**tumor boards.**"

Tab. 9.1 - The performance status of the patient-scoring systems

Karnofsky performance status	**Karnofsky - grade**	**ECOG - score**	**ECOG performance status**
Ability to perform normal activities without the need for special care. Normal, without difficulties, and signs of illness.	100	0	Fully active, able to engage in all normal activities without limitations.
Ability to perform normal activities with mild signs or symptoms of illness	90	1	Limitation in physically demanding activities, ambulatory, able to perform light work such as household chores or office work.
Ability to perform normal activities with increased effort and presence of signs or symptoms of illness.	80		
Unable to work, able to carry out normal daily activities at home and take care of most personal matters with varying degrees of assistance. Self-care is possible, but unable to engage in normal activities or work.	70	2	Ambulatory, able to take care of oneself but unable to perform any work, spending more than 50% of the day out of bed.
Able to take care of the majority of their needs but occasionally requires assistance	60		
Requires significant assistance and frequent medical care.	50	3	Capable of limited self-care, bedridden for more than 50% of the day.
Unable to care for oneself, requiring institutional care or hospitalization, with the condition deteriorating rapidly. Incapacitated and in need of specialized care and assistance.	40		
Severely incapacitated, hospitalization is indicated, immediate death is not imminent	30	4	Completely incapacitated, unable to care for oneself, bedridden or confined to a chair.
Very ill, hospitalization is necessary, requiring essential active supportive care.	20		
Moribund; fatal processes progressing rapidly	10		
Dead	0	5	Dead