Review article

Pneumonia in patients after hematopoietic stem cell transplantation

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ABSTRACT

Pneumonia is one of the most frequent cause of death after hematopoietic stem cell transplantation (HSCT). The objective of this review is to present various aspects of pneumonia in this group of patients, with focus on invasive pulmonary aspergillosis and cytomegalovirus disease, being the most frequent etiological causes of pneumonia after HSCT. The review is aimed at practical approach to diagnostic and therapeutic management of pneumonia after HSCT with special attention to: definitions of infections and level of diagnosis of upper and lower respiratory tract infections, including issues specific for invasive fungal disease, pneumocystosis, cytomegalovirus disease, community acquired respiratory viral infections and bacterial pneumonia. Another topics analyzed in the review are: epidemiology and risk factors for development of infection and risk of death due to pneumonia; invasive and non-invasive diagnostics, including imaging and laboratory biomarkers; methods of pharmacological and environmental prophylaxis and specific targeted therapy of pneumonia after HSCT.

Key words: pneumonia, invasive fungal disease, invasive fungal infection, pulmonary aspergillosis, chemotherapy, hematopoietic stem cell transplantation, amphotericin B lipid complex

IMMUNOSUPPRESSION AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

A hematopoietic stem cell transplantation (HSCT) is used in patients with haematological, oncological and immunological diseases as well as metabolic and autoimmune disorders. The indications for HSCT are expanding steadily, with HSCT being used to treat a growing number of disorders [1, 2]. Among all therapies used currently, it is HSCT which achieves probably the strongest immunosuppressive effect in the human body. Immunosuppression also occurs, albeit to a lesser degree, in recipients of solid organ transplants (SOT), patients treated for cancer and patients with primary and acquired immunodeficiencies. Immunosuppression is associated with disabling the defence mechanisms of the immune system, including the ability to generate mediators, antibodies and immune system cells [3].

Infectious complications are one of the major factors affecting mortality in patents after a hematopoietic stem cell transplantation. Thus, infection-related complications may void the therapeutic effect of transplantation which determines whether the primary disease is cured or not [4]. Key infectious complications that increase the risk of mortality after HSCT include primarily fungal infections, and to a lesser extent, infections caused by multi-resistant bacteria and viruses [5, 6]. Major causes of mortality among onco-haematological patients in intensive care units include:

- invasive pulmonary aspergillosis
- bacterial pneumonia
- sepsis [7], particularly in a developing multiple organ failure [8].

The purpose of this paper is to present an overview of pneumonia in patients after HSCT, with a focus on invasive pulmonary aspergillosis (IPA) and cytomegalovirus disease (CMV) as the most frequent causes of infections. This review demonstrates a practical approach to diagnostic and therapeutic strategies in pneumonia after HSCT.

DEFINITIONS

Post-HSCT phases. There are 3 post-transplant phases which differ by the clinical manifestation, immunological disorders and associated risk factors. These are:

- early or neutropenia phase (pre-engraftment period) – from the HSCT until haematological reconstitution (engraftment), which usually occurs around Day +30
- intermediate phase – from Day +30 until Day +100
- late phase – from Day +100 until immunological reconstitution which takes place approx. 1–2 years after HSCT.

Types of infectious complications. There are three types of infection-related complications:

- fever of unknown origin (FUO)
- clinically documented infections (CDI)
- microbiologically documented infections (MDI).

An infection usually presents with clinical signs, of which fever is the most frequent. CDI is diagnosed when symptoms of an infection are present but the etiological factor has not been identified.
Neutropenic fever: fever (body temperature > 38°C occurring twice or > 38.3°C occurring once) in a patient with neutropenia (absolute neutrophil count < 0.5×10^9/l).
Bacteraemia: presence of bacteria in blood. In case of presence of Gram-negative bacteria, *Staphylococcus aureus*, *Candida spp*. or *Listeria monocytogenes,* a single positive blood test is sufficient, and for all other bacteria – presence must be confirmed by two culture tests [9]. When the blood culture test is positive, infection may be diagnosed based on the detected etiological factor even though symptoms are not present.
Invasive fungal disease (IFD): clinical symptoms produced by the affected organ, with concurrent detection of fungi in the tissue sample from the affected organ. IFD may be diagnosed as possible, probable or proven in accordance with the criteria proposed by EORTC [10]. IPA and invasive mucormycosis (IM) are the most frequently diagnosed fungal infections.
Viral infections:

- symptomless viremia – a situation in which a virus, its proteins or nucleic acid are isolated in a symptomless patient
- symptomatic infection – a situation in which a virus is detected and the patient has fever and/or other clinical symptoms
- invasive viral infection – a situation in which the affected organ produces clinical symptoms and a virus is confirmed to be present in the tissue sample of the affected organ.

In case of a VZV infection (*varicella-zoster virus*; chickenpox or zoster) a clinical diagnosis is sufficient to classify the disease.
Graft versus host disease (GVHD) is defined based on standard criteria for acute GVHD (aGVHD) and chronic GVHD (cGHVD). Regardless of the consensus introduced by NIH (National Institutes of Health, USA), cGVHD continues to be divided into a limited and an extensive form.
Infection-related mortality occurs when the patient was treated for an infection or an infection was detected in a posthumous test.
Infection-related mortality should be distinguished from death due to progression of the cancer disease or death due to toxicity.

PULMONARY COMPLICATIONS: INFECTION-RELATED AND NON-INFECTION RELATED

Patients after HSCT are at a risk of developing a number of complications, of which pulmonary complications are highly frequent and life-threatening. They occur at a specific point of time and have a particular ethiology which cannot always be established (fig. 1) [11].

Non-infectious complications

In case of the engraftment syndrome (EG), the standard strategy is to discontinue granulocyte colony-stimulating factor (G-CSF) and to initiate glucocorticosteroids. Idiopathic pneumonia syndrome (IPS) is diagnosed by way of elimination and based on absence of infectious factors.

Bronchiolitis obliterans (BO) is a form of pulmonary cGVHD which develops later, subsequent to Day +100. Chronic GVHD occurs in up to 10% patients [12].

When diagnosing COP (cryptogenic organizing pneumonia, previously called BOOP – bronchiolitis obliterans organizing pneumonia), it is obligatory to perform bronchoalveolar lavage (BAL), followed by a pulmonary biopsy.

In pulmonary complications related to cGVHD such as BO and COP, it is necessary to initiate therapy as soon as possible because an early initiation provides a chance for success.

Pulmonary infectious complications

This family of diseases always poses a direct threat to the life of patients after HSCT due to their deep immunosuppression and risk of rapid progression, leading to respiratory syndrome and multiple organ failure. Despite prophylactic strategies and advancing techniques for diagnosing and treating pulmonary infections, pneumonia remains the key cause of post-HSCT mortality unrelated to progression of the primary disease. Infectious complications occur more frequently in recipients of allo-HSCT compared to recipients of auto-HSCT. This is attributable to immunosuppressive therapy received by the patient and the possibility of GVHD.

In addition, medication that impairs the function of B and T lymphocytes (e.g. rituximab or purine analogues) also make patients susceptible to opportunistic infections.

PNEUMONIA IN PATIENTS AFTER HSCT

Pneumonia involves development of new, or progression of existing pulmonary infiltrates which are caused by the inflammation of lung parenchyma. Pneumonia is not identical with a lower respiratory tract infection (LRTI). Pneumonia may be evidenced by clinical or radiological examinations.

FIGURE 1.
Pulmonary complications after allo-HSCT.

Post-transplant phases	**Phase I (Days 0–30)**	**Phase II (Days 30–100)**	**Phase III (Days > 100)**
Factors that undermine the immune system:	neutropenia, damaged mucous membranes, central catheters, acute GVHD	impaired cellular immunity, acute GVHD	impaired cellular and humoral immunity, chronic GVHD
Infectious complications	ES, VOD, DAH, IPS	BO / BOOP, COP, IPS	BO / BOOP, COP
Infectious complications: fungal	*Aspergillus*, *Candida*	*Aspergillus*, *Candida*, *Pneumocystis jiroveci*	*Aspergillus*, *Pneumocystis jiroveci*
bacterial	Gram-positive, Gram-negative	Gram-positive, Encapsulated bacteria, Gram-negative	Encapsulated bacteria
viral	*cytomegalovirus*, *respiratory tract viruses*, *adenovirus*	*cytomegalovirus*, *respiratory tract viruses*, *adenovirus*	*respiratory tract viruses*

BO – *bronchiolitis obliterans*; BOOP – *bronchiolitis obliterans organizing pneumonia*; COP – *cryptogenic-organizing pneumonia*; DAH – *diffuse alveolar haemorrhage*; ES – *engraftment syndrome*; GVHD – *graft versus host disease*; IPS – *idiopathic pneumonia syndrome*; VOD – *veno-occlusive disease*.

It requires the following:

- determination of the epidemiological background of the disease (e.g. hospital-acquired, non-hospital-acquired, aspiration, other)
- determination of the progression rate (e.g. acute, subacute, chronic)
- determination of the radiological pattern (e.g. lobar, interstitial, bronchopneumonia, lung abscess, pneumonia and pleuritis).

Pneumonia in post-HSCT patients is predominantly caused by microbiological factors. Where the cause of the infection is not infection-related, the term „pneumonia" should not be used.

Pneumonia may develop at any point of time after HSCT; the median time to pneumonia is 66 days (across a range of 0 days to 2 years, in a 2 year follow-up period) after HSCT. When judging by the time to pneumonia after HSCT, fungal and viral pneumonia develop earliest, with the median times to onset being 44 and 48 days, respectively. Bacterial pneumonia develops later; the median time is 113 for Gram-negative bacteria and 120 for gram-positive bacteria [13].

Risk factors for pneumonia include (based on multiple-factor analysis) [13]:

- HLA mismatch (2.3-fold risk increase)
- aGVHD or cGVHD (2.1-fold risk increase)
- second HSCT (1.6-fold risk increase)
- male sex (1.6-fold risk increase).

Epidemiology and etiology

Cumulative frequency of pneumonia in patients after allo-HSCT varies across medical centres within a 20–80% range, with median frequency reaching 40% in patients up to 3 years after HSCT, while the majority of pneumonia incidents occur in the early post-transplantation period [6, 9, 13]. Pneumonia that occurs after HSCT is caused by a number of different factors. In the early post-transplantation phase (up to Day +30), bacterial and fungal causes prevail with viral pneumonia occurring less frequently. Between Day 30 and 100, viral pneumonia, including mostly caused by CMV, is predominant. Further into the post-transplantation period, pneumonia caused by all possible pathogens occurs. In addition, mixed-ethiology infections are also frequent. It is much more difficult to determine the cause of pneumonia in the later post-transplantation phase. Such a determination is only successful in 45% cases [14].

When analysing patients after HSCT in Spain in the first 24 months from the procedure, bacterial pneumonia was found to be the most frequent type (44.4% cases), followed by fungal pneumonia (29.2%) and viral pneumonia (19.4%). The origin of pneumonia was mixed in 7.1% cases, and presence of bacteria and/or fungi and/or viruses was detected [13]. *Aspergillus spp.* (15.4%) and CMV (15.4%) were found to be the most frequent etiological factors in all diagnosed pneumonia cases. Among bacterial infections, *Escherichia coli* (8.9%), *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* (5% each) were among the most frequent causes of pneumonia. *Aspergillus spp*. was responsible for 83% of all fungal infections, followed by mucormycosis and fusariosis. Most IFDs were diagnosed as probable. CMV was the main cause of viral pneumonia after allo-HSCT [13].

In a study conducted in Sweden, the most frequent etiological factors included viruses (53%), while bacteria and fungi demonstrated a comparable frequency (approx. 23% each). CMV infections prevailed (28%), and the most frequent microorganisms which caused fungal infections were *Aspergillus fumigatus* (9.5%) and *Pneumocystis jiroveci* (7.5%) [15].

The incidence of pneumonia by etiological factors was assessed as follows: pneumonia due to *Aspergillus* species*:* 89 cases per 1000 allo-HSCTs annually, pneumonia due to CMV: 56 cases per 1000 allo-HSCT annually, pneumonia due to *Escherichia coli:* 32 cases per 1000 allo-HSCT annually and pneumonia due to *Pseudomonas aeruginosa* and *Streptococcus pneumoniae:* 18 cases each per 1000 allo-HSCT annually [13].

Pneumonia-related mortality

Mortality rate after allo-HSCT unrelated to progression of the primary disease was 20.4% over a 2-year follow-up period. The leading causes of death were GVHD (47%) and infectious complications (39%). Mortality rate reached 46% in patients who have had at least 1 pneumonia episode and 13% in patients without a single episode.

The overall one-year survival rate was 47% for patients with at least one episode of pneumonia and nearly 83% for patients who have not had pneumonia at all [13].

Risk factors for fatality after allo-HSCT included:

- at least one episode of pneumonia (5.1-fold risk increase)
- cGVHD (2.9-fold risk increase).

In a group of patients with at least one episode of pneumonia, risk factors for fatality included:

- proven or probable pulmonary IFD (4-fold risk increase)
- aGVHD or cGVHD (5-fold risk increase)
- occurrence of pneumonia in the first 100 days following HSCT (3.5-fold risk increase).

Risk factors for fatality in patients with an ongoing pneumonia include: acute respiratory syndrome (6.7-fold risk increase) and septic shock (2.7-fold risk increase). Survival rate across a group of patients with pneumonia who require intensive care is assessed at 23% [13].

According to the Swedish study pneumonia was the leading cause of death in patients after allo-HSCT, accounting for 5.7% of all fatalities among patients up to Day +100 [16]. When pneumonia developed, the mortality rate was 13% [15]. In a multi-scenario analysis, three factors were found to significantly affect mortality in pneumonia: year in which HSCT was performed, T-cell depletion in the graft and bacteraemia in one study [16], and repeat transplantation, myeloablative conditioning and bacteraemia in another study [15].

Diagnosing pneumonia

Pneumonia is diagnosed when clinical and radiological criteria are met. Pneumonia diagnosis is also possible when microbiological criteria are satisfied.

Clinical symptoms

Pneumonia most typically presents with cough, fever and dyspnoea. In order to diagnose pneumonia in post-HSCT patients, the following assessments are made:

- radiological assessment (chest X-ray and CT)
- microbiological assessment (blood culture tests)
- assessment of the sample collected from the respiratory tract (to detect bacteria, mycobacteria, fungi and viruses using standard culture and staining techniques)
- galactomannan test (GM)
- other targeted tests.

Invasive pulmonary aspergillosis should be suspected when clinical symptoms of pneumonia show in a neutropenic patient with fever, the test result for GM is positive and imaging studies reveal new infiltrates.

Clinical and radiological criteria

- fever (body temperature > 38°C)
- cough
- dyspnoea
- expectoration of sputum
- abnormalities on auscultation.

Radiological criteria

Pulmonary X-ray or computed tomography (CT) study that confirms presence of new or progressing parenchymal (vesicular) or interstitial infiltrates or cavities in pulmonary parenchyma which cannot be attributed to non-infectious factors [13].

The most typical radiological abnormalities in an invasive fungal disease of the lungs include nodular lesions (particularly those measuring > 1 cm in diameter) and a halo sign in high-resolution computed tomography (HRCT) scans (tab. 1). Other abnormalities identified by HRCT may occur due to inflammatory processes caused by a variety of factors [17].

TABLE 1.
Typical pulmonary radiological lesions occurring in infectious diseases.

Infection	Radiological lesions
Aspergillus	Infiltrates, nodules, halo sign, air trappings [18]
Mucor	Reverse halo sign [19]
Pneumocystis jiroveci	Ground-glass infiltrates, small nodules, consolidations, interstitial lesions [20, 21]
CMV (cytomegalovirus disease)	Ground-glass infiltrates, small nodules, consolidations, interstitial lesions [22], halo sign [20]
Parainfluenza	Bilateral ground-glass opacities, focal infiltrates [23]
RSV (*Respiratory Syncytial Virus*)	Small nodules and tree-in-bud patterns, ground-glass opacity areas and infiltrates [23, 24]
Adenovirus	Tree-in-bud patterns, multiple small nodules, bronchial wall thickening [23]
Metapneumovirus	Bilateral ground-glass infiltrates, nodules [23]

Viral pneumonia that follows HSCT usually leads to new abnormalities, such as diffuse or localised (extensive or focal) ground-glass densities with varying intensity, bronchial wall thickening and multiple small nodules [17].

Patients in whom the cause of pneumonia has not been identified and/or those who do not improve on empirical therapy must undergo a bronchoscopy with BAL.

Biopsy should be considered when pulmonary abnormalities progress but the underlying cause has not been identified. It is performed after non-invasive diagnostic tests have been con-

ducted and pneumonia is suspected to have a non-infectious origin [13]. Diagnostic assessments based on BAL in patients after allo-HSCT proved to be useful in identifying the pathogen in 63% cases [15].

Microbiological criteria

Isolating or detecting microorganisms (except viruses) in blood without establishing another extrapulmonary focus of infection and/or identifying a microorganism in a representative sample from the respiratory tract (sputum, BAL liquid and bronchial epithelium sample). Detection of *Streptococcus pneumoniae* or *Legionella pneumophila* in a urine culture test can confirm the respective cause of pneumonia. Detection of *Mycobacterium tuberculosis*, *Aspergillus spp.*, *Pneumocystis jiroveci*, *Legionella pneumophila* or *Nocardia species* in any sample from the respiratory tract provides evidence of proven or probable pneumonia [13]. In order to identify pathogens in a sample from the lower respiratory tract one may perform direct microscopic studies, smear tests, culture tests, immunofluorescence studies, detection of certain antigens and polymerase chain reaction (PCR) studies.

INVASIVE FUNGAL DISEASE: INVASIVE ASPERGILLOSIS

Ethiology and epidemiology

Fungal infections are the key pulmonary complications that contribute to post-HSCT fatality [5–8]. IPA is the most frequent IFD in HSCT recipients; it occurs in 5–20% patients after allo-HSCT and 1–5% patients after auto-HSCT. Over the last years, the incidence of infections caused by *Aspergillus spp.* has decreased owing to antifungal prophylaxis (based on voriconazole and posaconazole).

IFD diagnosis

Depending on the certainty of diagnosis, IFDs are currently classified as:

- proven
- probable
- possible (fig. 2).

Diagnostic methods

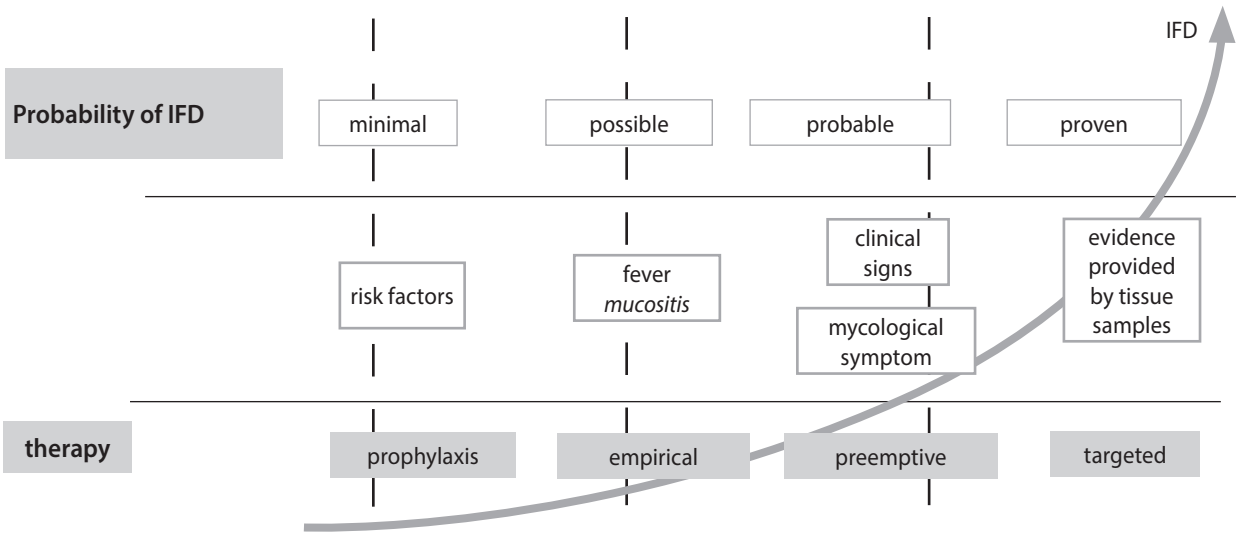
Presence of IFD is evidenced by clinical and microbiological data. Table 2 shows diagnostic criteria for high-risk patients along with risk factors and clinical signs of the disease [25].

TABLE 2.
Criteria for diagnosing IFD.

Criteria	Characteristics
Risk group	• high risk: allo-HSCT, AML, ALL, SAA, MDS, CGD, SCID • low risk: auto-HSCT, patients receiving chemotherapy due to other cancers
Clinical data	• suggestive radiologic features: Lung HRCT, sinus CT/ MRI, abdominal CT/MRI, CNS MRI (ultrasound scan is considered insufficient) • and/or persistent or recurrent neutropenic fever despite empirical therapy based on broad-spectrum antibiotics provided at least 96 h • and/or clinical signs of septic shock in a neutropenic patient
Microbiologic data	• markers: GM • and/or result of a histopathological examination: positive for fungi • and/or culture test for fungi conducted on blood and/or biological material from previously sterile sites

ALL – acute lymphoblastic leukaemia; AML – acute myeloid leukaemia; CGD – chronic granulomatous disease; GM – galactomannan; HRCT – high resolution computed tomography; CT – computed tomography; MRI – magnetic resonance imaging; MDS – myelodysplastic syndrome; CNS – central nervous system; SAA – severe aplastic anaemia; SCID – severe combined immunodeficiency.

FIGURE 2. Defining invasive fungal disease.



Fungal infections are diagnosed using invasive and non-invasive methods (tab. 3). At present, GM is the only marker commonly used in Poland for diagnosing an invasive fungal disease; a positive test result is obtained in case of aspergillosis. It is recommended to test blood serum and, whenever possible and practicable in given clinical conditions, also other body fluids for GM.

TABLE 3.
Diagnostic options in invasive pulmonary aspergillosis.

Diagnostic methods	Types of examinations
Classical methods (invasive)	direct examination, culture tests
Non-invasive methods (indirect)	• test for biomarkers: GM, mannan, β-G-glucan • imaging studies
Molecular methods	PCR

GM – galactomannan; PCR – polymerase chain reaction

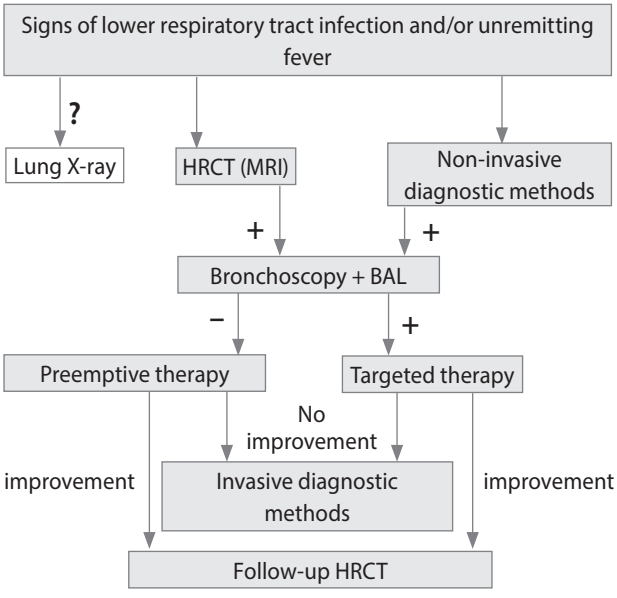
Pulmonary symptoms are detectable when the disease reaches at least a moderate stage.

The clinical decision must be made on the basis of:

- risk factors
- onset of symptoms
- severity of symptoms
- site
- progression to organs.

Although non-invasive testing methods (imaging studies and biomarkers testing) may seem attractive, it should be emphasized that only invasive diagnostic methods lead to a proven diagnosis. Figure 3 shows the typical diagnostic and therapeutic path when suspecting pulmonary aspergillosis.

FIGURE 3.
Diagnosing pneumonia after allo-HSCT (acc. to [26]).



Treating invasive pulmonary aspergillosis

Drug therapy

Due to the ethiology and epidemiology of pulmonary IFD, voriconazole or amphotericin B (AMB) are the first-line therapy and amphotericin B is the second-line therapy according to all recommendations [27, 28]. Use of voriconazole is associated with a risk of adverse effects for patients, a risk of lacking efficacy against mucormycosis and development of resistance to azoles among fungal species. Recently isavuconazole, a new azole antifungal agent against IPA and IM was registered [29]. The second and third-line therapy in invasive pulmonary aspergillosis is based on amphotericin B, echinocandins or combination therapy.

Surgical treatment

Surgical treatment is an important therapy in IPA; its use in this disease is optional. It is recommended by ECIL (European Conference on Infections in Leukemia) under the following scenarios:

- fungal focus present in an immediate vicinity of major blood vessels
- haemoptysis
- presence of a localised extrapulmonary lesion, including in the central nervous system [6].

Despite the optional nature of this recommendation, mostly owing to the fact that such conditions are usually preceded by stages of the disease which are treated with antifungal agents. It should be stressed that surgical treatment in IPA is indicated in case of direct threat to the life of the patient [8–10].

Prophylaxis

Patients after allo-HSCT and patients receiving immunosuppressive therapy due to GVHD are recommended to be treated with the new azoles (posaconazole, voriconazole) where neutropenia is anticipated to last > 14 days.

Another way to manage the disease is to use preemptive strategy based on screening tests. Ferritin concentrations above 1000 ng/ml prior to HSCT are another significant risk factor for IFD.

Role of amphotericin in treating invasive pulmonary aspergillosis

According to epidemiologic data, the most common single etiological factor for pneumonia in patients after HSCT is *Aspergillus spp*. [13–15]. Therefore, the diagnostic and therapeutic strategy should always target fungal pathogens. It was also demonstrated that in 13% cases fungal infections are caused by multiple

factors, with aspergillosis typically co-occurring with mucormycosis [30]. It is therefore justified to use amphotericin whenever voriconazole is ineffective or not tolerated, which is mostly due to co-occurring invasive mucormycosis showing resistance to voriconazole.

Both liposomal amphotericin B (LAMB) and amphotericin B lipid complex (ABLC) are recommended to be used in pulmonary aspergillosis and mucormycosis, but one needs to note that the liposomal form penetrates the pulmonary tissue more effectively and achieves lung tissue concentrations which are 10 times higher (tab. 4).

TABLE 4.
Comparison between relative tissue concentrations and recommendations for amphotericin formulations.

Characteristics	LAMB	ABLC	Source
Molecule size	0.08 µm	1.6–11 µm	[31]
Relative concentration in lung tissue	1	10	[32]
Relative concentration in kidneys	1	1	[32]
Relative concentration in the CNS	1	0.2	[32]
ECIL recommendation for IA - 1st line	BI	BII	[29]
ECIL recommendation for IM - 1st line	BII	BII	[33]
ECIL recommendation for IA - 2nd line	BII	BII	[29]
IDSA recommendation for IA - 2nd line	all	all	[34]

ABLC – amphotericin B lipid complex; LAMB – liposomal amphotericin B; ECIL – European Conference on Infections in Leukemia; CNS – central nervous system

ABLC has the biggest molecule among all lipid AMBs. For this reason, the ABLC molecule is quickly captured by the circulating phagocytes and macrophages, and transported to the focus of infection. Relative to the conventional form, ABLC results in a lower serum concentration of amphotericin which is reflected by a higher volume of distribution and improved drug clearance. In addition, ABLC therapy contributes to a significantly higher drug concentration in the lungs relative to therapies based on other forms of amphotericin [35]. These findings suggest that ABLC may have a quicker onset of action and achieve higher concentrations in the cells of the reticuloendothelial system than LAMB and the conventional form [36]. The recommended therapeutic dose of ABLC is 5 mg/kg of body weight/24 h [28].

Based on *in vitro* studies comparing amphotericin B formulations, ABLC was found to have a stronger antifungal activity than LAMB [37]. Due to its pharmacokinetic profile, ABLC enables a quick and targeted deposition of the drug in certain tissue including lungs, liver and spleen which are typically affected by invasive fungal diseases [36].

INVASIVE PULMONARY MUCORMYCOSIS

The incidence of mucormycosis is growing, which is probably attributable to the use of voriconazole for prophylaxis. IM is difficult to diagnose, with no non-invasive methods available at present to diagnose this disease. When IM is suspected, invasive diagnostic methods should always be used. On detection of IM presence one should always strive at removing the affected tissue by surgical methods as much as possible. IM is also treated with pharmacotherapy based on amphotericin B (ABLC or LAMB), with posaconazole used as the second line of treatment. Isavuconazole presents a new therapeutic possibility.

FUSARIOSIS AND SCEDOSPORIOSIS

Fungal pneumonia is relatively rarely caused by *Fusarium* and *Scedosporium* which account for 0.5–2% of all proven IFDs. The clinical manifestation of *Scedosporium* infection may resemble that of IPA. A prolonged neutropenia is a risk factor for unsuccessful treatment. Voriconazole and amphotericin B are used for treatment of the disease. Surgical intervention may also be considered.

PNEUMONIA CAUSED BY PNEUMOCYSTIS JIROVECI

Pneumocystis jiroveci pneumonia (PJP) is a fungal infection which may develop in immunosuppressed patients. Routine use of co-trimoxazole for prophylaxis helped to significantly reduce the incidence of PJP. On the other hand, not using PJP prophylaxis is a key risk factor for this disease, as is receiving immunosuppressive therapy due to GVHD. PJP prophylaxis is based on biseptol taken 2–3 times a week for up to 6 months after allo-HSCT [38]. PJP is treated with co-trimoxazole or, alternatively, trimethoprim with dapsone, or primaquine with clindamycin, or atovaquone.

CMV pneumonia

CMV activity

CMV produces direct (organ-related) and indirect effects.
CMV symptoms that affect body organs include:

- pneumonia
- gastroenteritis
- encephalitis
- hepatitis
- chorioretinitis
- suppression of bone marrow.

Indirect effects include suppression of the immune system and facilitated development of other infections, including mostly fungal diseases. Other indirect effects include:

- bacterial infections
- vasculopathy
- graft failure
- GVHD, particularly in recurrent CMV infections.

Criteria for diagnosing CMV disease

Cytomegalovirus disease may be detected by means of a test with sufficient sensitivity and specificity performed on an affected organ, a biopsied sample or material obtained from the affected organ using invasive methods. Only chorioretinis secondary to a CMV infection can be diagnosed with sufficient certainty based on the typical symptoms in a funduscopic examination.

Definition of CMV pneumonia

According to the latest definitions of CMV disease developed in 2017, CMV pneumonia can be diagnosed as proven or probable as follows [39]:

1. Proven CMV pneumonia is diagnosed when clinical signs of pneumonia are present including new infiltrates evidenced by imaging studies, hypoxia, tachypnoea and/or dyspnoea with a concurrent detection of CMV in lung tissue using a virus isolation test, a quick culture test, a histological or immunohistochemical examination or a DNA hybridisation technique.
2. Probable CMV pneumonia is diagnosed when CMV is detected in BAL fluid using a virus isolation test, a quick culture test or the CMV-DNA detection method and clinical signs of pneumonia co-occur. The certainty of CMV pneumonia diagnosis increases with the growing number of virus copies. On the other hand, absence (CMV-DNA) of virus replication in the BAL fluid carries a negative predictive value (NPV) reaching nearly 100%.
3. Probable diagnosis. A high viral load established using a quantitative PCR method performed on tissue from the affected organ may attest to a CMV infection, therefore the term "possible CMV" was introduced which is particularly useful when no CMV-DNA is present in blood [39]. CMV pneumonia is not correlated with the presence and intensity of CMV-DNA viremia in peripheral blood. Presence of CMV-DNA-emia is not a prognostic factor for CMV pneumonia [22].

Diagnostic methods

Serological tests (IgG or IgM) help to identify the risk of CMV infection but are not useful in diagnosing the CMV infection or disease [40, 41]. At present, the key diagnostic methods related to CMV include tests that detect antigens (pp65, antigenemia tests), DNA or mRNA. In case of immunosuppressed patients after HSCT who are unable to generate antibodies, it is recommended to diagnose CMV infection by testing peripheral blood for CMV-DNA, CMV-RNA or CMV antigens using quantitative techniques [42]. Monitoring CMV-DNA in blood informs the choice of a diagnostic and therapeutic strategy targeting CMV disease after HSCT (tab. 5).

TABLE 5.
Possible therapeutic strategies in CMV infections.

Diagnosis	Clinical signs	Therapeutic strategy
Absence of CMV-DNA	none	prophylaxis
CMV-DNA-emia	none (or fever only)	preemptive therapy
CMV disease	yes	targeted therapy against CMV

Therapy

Antiviral medication is used as chemoprophylaxis, preemptive therapy or symptomatic therapy in a CMV disease. Ganciclovir, valganciclovir, foscarnet and cidofovir are antiviral agents which are currently used against CMV. New drugs intended as prophylaxis against CMV disease include: brincidofovir, letermovir and maribavir [43–45]. Ganciclovir and intravenous immunoglobulins (IVIG) with a high anti-CMV titre are the first--line therapy. However, recent studies do not support efficacy of IVIG [46]. The therapy should be maintained for at least 2 weeks and discontinued only when CMV is confirmed to be absent. An elevated CMV-DNA titre one week into therapy should not be interpreted as a sign of resistance and does not require the therapy to be modified. If CMV is still present after two weeks of therapy, supportive treatment should be instituted [4, 42]. Recipients of allo-HSCT from an unrelated donor may need to receive repeat or prolonged preemptive therapies. Patients after auto-HSCT from the high risk group may potentially benefit from CMV monitoring and preemptive therapy.

CMV resistance

CMV resistance against ganciclovir occurs rarely among patients after HSCT, however the issue is gradually becoming more serious. Two types of resistance are distinguished: clinical and genetic. A clinical resistance is a situation in which there is no improvement after 14 days of therapy. A genetic resistance is established on the basis of tests for UL97 mutation and other.

VIRAL INFECTIONS OF THE RESPIRATORY TRACT

Ethiology and epidemiology

Pneumonia and other occasional (external) infections of the lower respiratory tract are caused by a group of community-

-acquired respiratory viral pathogens (CARV), including influenza A and B virus, parainfluenza virus, respiratory syncytial virus (RSV), MPV (metapneumovirus) and RV (rhinovirus), as well as the less known *Coronavirus* and *Bocavirus*.

Key factors which contribute to disease progression into lower respiratory tract include lymphopenia and a therapy based on glucocorticosteroids. A respiratory tract obturation which accompanies parainfluenza virus and RSV infections is another important factor which is favourable to disease progression [47, 48]. It is estimated that approx. 1/3 of acute respiratory tract infections (pneumonia, bronchitis) are caused by CARV. CARV may cause pneumonia in approx. 30% cancer patients, with mortality risk reaching 25%.

Diagnostic methods

A nasopharyngeal swab or lavage should be used to diagnose CARV infections of the upper respiratory tract. A nucleic acid amplification based-technique (NAT) using PCR method is considered to the best diagnostic tool. At present, these infections are predominantly diagnosed by viral DNA or RNA tests using Multiplex PCR which detects several viruses at the same time.

Upper respiratory tract infection (URTI)

An URTI is diagnosed when a virus is present in the nasopharyngeal swab specimen or sputum, the patient shows clinical signs of an upper respiratory tract infection and no new pulmonary infiltrates are identified [49].

Lower respiratory tract infection (LRTI)

This infection is diagnosed with three levels of certainty: possible, probable and proven. A possible LRTI is diagnosed when a virus is found in the nasopharyngeal swab specimen or sputum, new pulmonary infiltrates are identified (without a confirmation of virus presence in the lower respiratory tract), and the patient has or does not have clinical signs of an upper respiratory tract infection (such as cough, wheezing, rales, tachypnoea, dyspnoea, hypoxia).

A probable LRTI is diagnosed when a virus is detected in the bronchoalveolar lavage (BAL) fluid or a biopsy specimen from the patient's lungs, the patient shows clinical signs of a LRTI but there are no new pulmonary infiltrates discovered.

A proven LRTI is diagnosed when a virus is detected in the BAL fluid or a biopsy specimen from the patient's lungs, new pulmonary infiltrates are identified and the patient shows or does not show clinical signs of a LRTI [49].

Prophylaxis and therapy

The key measures to contain an infection (to stop it from spreading) include hand hygiene, contact isolation and wearing protective masks. Influenza virus infections are recommended to be treated with oseltamivir (or other neuraminidase inhibitors) and RSV infections – with ribavirin. In addition, IVIG are used. Ribavirin is also applied in parainfluenza virus and metapneumovirus infections, but the evidence of its efficacy is not conclusive and there is a risk of toxicity [50].

BACTERIAL PNEUMONIA

Bacterial pneumonia occurs in 20–50% neutropenic patients [11]. The standard of care for patients with neutropenic fever who are suspected of pneumonia is an empirical therapy with broad-spectrum antibiotics. According to IDSA (Infectious Diseases Society of America), patients in whom neutropenia persists for more than 7 days should receive fluoroquinolone as prophylaxis. This reduces the incidence of gram-positive bacterial infections but increases resistance to fluoroquinolone. Pneumonia caused by *Nocardia spp.* (a Gram-positive bacterium) is rare but should be taken into consideration in case of patients who have not shown improvement on β-lactam antibiotics or fluoroquinolone. Cotrimoxazole (TMP-SMX) is the treatment of choice in this situation [11]. Pneumonia caused by *Mycobacterium tuberculosis* is very rare in non-endemic areas (< 0.25%) [11]. The disease should be diagnosed and treated using the same methods as those intended for tuberculosis.

Prophylaxis

Respiratory tract infections and pneumonia may be prevented using environmental (non-pharmacological) and pharmacological methods. Key environmental methods include:

- hand hygiene
- contact isolation
- wearing protective masks.

Pharmacological prophylaxis includes:

1. **Antifungal prophylaxis**. Until recently, fungal prophylaxis was mostly based on fluconazole (400 mg/24 h for adult patients) which was administered from the start of the conditioning therapy until the end of the immunosuppressive therapy (including therapy used due to GVHD). At present, neutropenic patients are mostly recommended to be treated with posaconazole, micafungin and fluconazole, and patients receiving immunosuppressive therapy and those suf-

fering from GVHD after allo-HSCT – with posaconazole and voriconazole [9].

2. **Antiviral prophylaxis.** Reactivation of CMV is usually prevented by preemptive strategies that involve CMV-DNA-emia monitoring using PCR (or antigenemia pp65 testing) from Week 2 till approx. Day +100, and use of anti-CMV drugs (ganciclovir, valganciclovir) in case of high or rising levels of viremia. Given that CMV could be transmitted in leucocytes from the blood donor, it is a standard practice to transfuse leukoreduced and irradiated blood components [9].
3. **Antibacterial prophylaxis**. At present, fluoroquinolone, including ciprofloxacin and levofloxacin, are used most commonly. Some medical centres use cotrimoxazole (trimethoprim-sulphametoxazol) only. Many paediatric medical centres use oral penicillin (amoxicillin) [9]. In order to prevent pneumonia in patients with GVHD after allo-HSCT, particularly patients with hypogammaglobulinemia, antibiotics which are effective against encapsulated bacteria must be used.
4. In addition, co-trimoxazole should be used to prevent PJP, toxoplasmosis, listeriosis and nocardiosis. Aciclovir or valaciclovir are recommended to be administered to VZV-seropositive patients for at least one year after allo-HSCT or until completion of immunosuppressive therapy.

CONCLUSIONS

Any disease affecting pulmonary parenchyma in a patient after HSCT may involve a risk of infectious and non-infectious complications. In each case, infectious factors need to be considered and tested as the first step because their presence carries a risk of a rapid development of a pulmonary disease. Besides, the likelihood of an infectious disease is higher. Non-infectious diseases should only be considered as a second step. They typically have a common cause which is impaired endothelium, and chiefly develop in the early post-HSCT phase. In the later phase after allo-HSCT, chronic pulmonary forms of GVHD should be taken into consideration when making a differential diagnosis.

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