Epidemiology of most frequent infectious complications in immunocompromised patients:

focusing on bacteraemia, CMV and HHV-6 infections in haematological patients, and following autologous stem cell transplantation

PhD Thesis

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ORVOSI HETILAP **157:**(35) pp. 1403-1409. (2016) **IF: 0,291**

II. Piukovics Klára, Terhes G, Lazar A, Timar F, Borbenyi Z, Urbán E. Evaluation of Bloodstream Infections During Chemotherapy-Induced Febrile Neutropenia in Patients with Malignant Hematological Diseases: Single Center Experience.
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III. Piukovics Klára, Borbényi Z, Rajda C, Csomor A, Deák J, Terhes G. Monitoring Human Herpesvirus-6 in Patients with Autologous Stem Cell Transplantation.
IN VIVO **28:**(6) pp. 1113-1117. (2014) **IF: 0,974**

IV. Terhes G, **Piukovics Klára,** Urbán E, Nagy E. Four cases of bacteraemia caused by *Fusobacterium nucleatum* in febrile, neutropenic patients.
JOURNAL OF MEDICAL MICROBIOLOGY **60:**(7) pp. 1046-1049. (2011) **IF: 2,502**

V. Piukovics Klára, Terhes G, Gurbity-Pálfi T, Bereczki Á, Rárosi F, Deák J, Borbényi Z, Urbán E. Cytomegalovirus infection in patients with haematological diseases and after autologous stem cell transplantation as consolidation: a single-centre study.
ANNALS OF HEMATOLOGY 2016 Oct 11. [Epub ahead of print] **IF: 3,022**

VI. Klára Piukovics, Viktória Bertalan, Gabriella Terhes, Ágnes Báthori, Edit Hajdú, Gyula Pokorny, László Kovács, Edit Urbán. Fatal cases of disseminated nocardiosis-challenges to physicians and clinical microbiologist-case report.
ACTA MICROBIOLOGICA ET IMMUNOLOGICA HUNGARICA **IF: 0,568**
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VII. Tóth Eszter, Hajdú Edit, **Piukovics Klára,** Borbényi Zita, Nagy Erzsébet. Véráram fertőzést okozó baktériumok előfordulása acut leukémiás betegeknél.
BULLETIN OF MEDICAL SCIENCES/ORVOSTUDOMÁNYI ÉRTESÍTŐ **80:** pp. 32-34. (2007)

VIII. Hajdú Edit, Terhes Gabriella, Bertalan Viktória, **Piukovics Klára**, Nagy Erzsébet. Fatalis kimenetelű nocardiosis immunsupprimált betegeknél.
INFEKTOLÓGIA ÉS KLINIKAI MIKROBIOLÓGIA **15:** pp. 46-49. (2009)

LIST OF ABSTRACTS RELATED TO THE SUBJECT OF THIS THESIS

I. Piukovics Klára, Terhes G, Palfi T Gurbity, Marton I, Bereczki A, Borbenyi Z, Deak J, Urban E. Monitoring Cytomegalovirus infection by quantitative real-time PCR method following autologous peripheral stem cell transplantation.
BONE MARROW TRANSPLANTATION **50:**(Suppl. 1.) p. 5576. (2015)
41st Annual Meeting of theEuropean-Society-for-Blood-and-Marrow-Transplantation. Istanbul, Törökország: 2015.03.22 -2015.03.25.

II. Urbán Edit, Terhes Gabriella, Borbényi Zita, Lázár Andrea, **Piukovics Klára.** Magyar Infektológiai és Klinikai Mikrobiológiai Társaság (szerk.)
Véráram infekciók előfordulása lázas neuropéniás hematológiai betegek között a SZTE ÁOK Hematológiai Osztály beteganyagában
Konferencia helye, ideje: Nyíregyháza, Magyarország, 2015.09.24-2015.09.26. 2015. 1 p.

III. Piukovics Klára, Terhes G, Deak J, Borbényi Z. CYTOMEGALOVIRUS INFECTION IN HEMATOLOGICAL PATIENTS FOLLOWING AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION
ANTICANCER RESEARCH **34:**(10) p. 6114. (2014)
EDITORIAL OFFICE 1ST KM KAPANDRITIOU-KALAMOU RD KAPANDRITI. Athens, Görögország: 2014.10.06 -2014.10.10.

IV. Piukovics Klára, Terhes G, Meszaros E, Hajdu E, Borbenyi Z, Nagy E. Fatal sepsis caused by Nocardia farcinica in a patient with haematological malignancy
BLOOD REVIEWS **21:** p. S144. (2007)

V. Piukovics Klára, Hajdu E, Terhes G, Soki J, Borbenyi Z, Nagy E. Bacteremia caused by *Achromobacter xylosoxidans* in a patient with haematological malignancy
BLOOD REVIEWS **21:** p. S145. (2007)

CONTENTS

ABBREVIATIONS

ABVD	adriamycin, bleomycin, vinblastine and dacarbazine
AML	acute myeloid leukemia
ANC	absolute neutrophil count
ASCT	autologous stem cell transplantation
A. xylosoxidans	*Achromobacter xylosoxidans*
BC	blood culture
B. cepacea	*Burkholderia cepacea*
BEAM	BiCNU®, etoposide, cytosine-arabinoside, melphalan
BK	human poliomavirus 1
BSIs	bloodstream infections
CDI	*Clostridium difficile* infection
CDV	cidofovir
CLABSI	central line-associated bloodstream infection
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
CNS	coagulase-negative staphylococci
CSF	cerebrospinal fluid
CT	computed tomography
CVCs	central venous catheters
CVID	common variable immunodeficiency syndrome
Dex	dexamethasone
DHAP	dexamethasone, cisplatin, cytarabine
dsDNA	double-stranded DNA

E. coli	*Eschericia coli*
ELISA	enzyme-linked immunosorbent assay
ESBLs	extended-spectrum beta-lactamases
FN	febrile neutropenia
F. nucleatum	*Fusobacterium nucleatum*
FUO	fever of unknown origin
GCV	ganciclovir
HHV-1	human herpesvirus 1 or herpes simplex 1 (HSV-1)
HHV-2	human herpesvirus 2 or herpes simplex 2 (HSV-2)
HHV-3	human herpesvirus 3 or varicella-zoster virus (VZV)
HHV-4	human herpesvirus 4 or Epstein-Barr virus (EBV)
HHV-5	human herpesvirus 5 or cytomegalovirus (CMV)
HHV-6	human herpesvirus 6
HHV-7	human herpesvirus 7
HHV-8	human herpesvirus 8 or Kaposi sarcoma-associated herpesvirus (or KSHV)
HL	Hodgkin lymphoma
HSCT	haematopoietic stem cell transplantation
HSV	herpes simplex virus
IgM, IgG	immunoglobulin M, immunoglobulin G
MDI	microbiologically documented infection
MM	multiple myeloma
MRI	magnetic resonance imaging
MRSA	methicillin resistant *Staphylococcus aureus*
NHL	non-Hodgkin lymphoma

NK	natural killer cells
N. farcinica	*Nocardia farcinica*
P. aeruginosa	*Pseudomonas aeruginosa*
PCRs	polymerase chain reactions
RBCs	red blood cells
R-FC	rituximab + fludarabine + cyclophosphamide
RSV	respiratory syncytial virus
SAA	severe aplastic anaemia
S. aureus	*Staphylococcus aureus*
SCT	stem cell transplantation
S. pneumoniae	*Streptococcus pneumoniae*
Thal	thalidomide
TNF	tumour necrosis factor
VGC	valganciclovir
VRE	vancomycin resistant enterococci
WBCs	white blood cells
WHO	World Health Organization

1. INTRODUCTION

1.1. INFECTIOUS COMPLICATIONS IN PATIENTS WITH HAEMATOLOGICAL DISORDERS

During the past few decades, significant developments have occurred in the treatment of malignant diseases and, because of these, the cancer-related death rate is decreasing [86]. Haematopoietic stem cell transplantation (HSCT) has been incorporated into the consolidating treatment of different types of lymphoproliferative disorders in an autologous setting, or allogeneic stem cell transplantation, as curative intent for acute leukaemias [85]. However, mainly because of the better immunosuppressive effects of these new drugs, neutropenia remains one of the most serious side effects of antineoplastic treatments [85]. Neutropenia may be associated with the malignancy itself or it may be present as a consequence of chemotherapy and this is the major risk factor for developing infectious complications in this patient group. The role of neutropenia in infections was recognised in the 1960's [15]. The majority of patients (about 30-60%) with neutropenia develop infectious complications and 13-37% of them may develop bacteremia [110, 112, 78, 93]. The most common sites and organs of infections in neutropenic patients are the respiratory tract (35-40%), (paranasal sinuses and lung infiltration), and bacteremia (15-35%), (central line-associated bloodstream infection, CLABSI). Skin, soft tissues and urinary tract are involved in 5-10% of cases, and also infections originating from the gastro-intestinal tract (neutropenic enterocolitis and perianal inflammation) and oro-pharynx occur in 5-10% of cases [85, 46].

The widespread use of more aggressive chemotherapy, and introduction of monoclonal antibodies and biological therapy in the treatment of malignant haematological diseases have produced a persistent and worsening immune-deficient state, and this may also contribute to the development of various infections. During the hospitalisation of these patients, the spectrum of colonising microorganisms on the skin and mucosal surfaces has radically changed because of therapeutic, surgical and other invasive interventions, hence the colonisation resistance of the normal flora has decreased [77, 47, 49]. In the majority of microbiologically documented infections in patients with cancer, the causative microorganisms are part of the endogenous flora. Only in a few cases might the infection arise from exogenous sources. Because of the above-mentioned factors, bacterial, viral and fungal infections are important causes of morbidity and mortality for patients with malignancies (Fig. 1) [111].

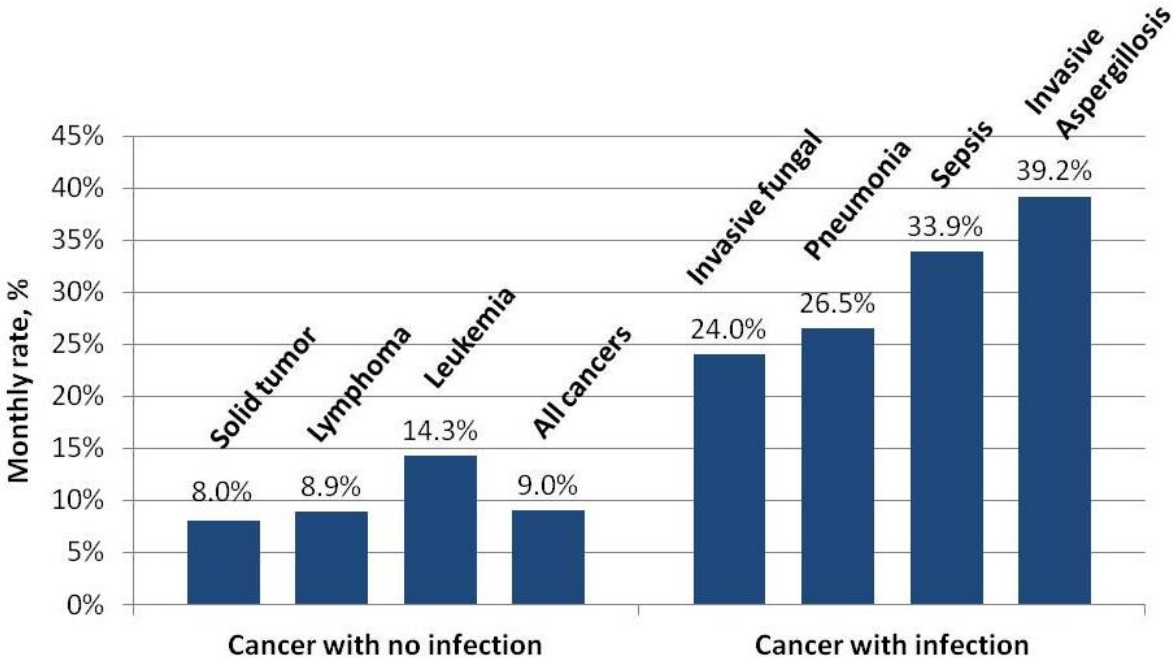

Figure 1. Increase in mortality rates among patients with cancer owing to various infections (source: Kuderer NM et al.: Mortality, morbidity, and cost associated with febrile neutropenia in adult cancer patients. Cancer. 2006 May 15;106(10):2258-66.)

The spectrum of pathogens causing infections in this patient group is constantly changing, and it is influenced by various factors including local epidemiology, the use of antibiotics, antiviral and antifungal agents, the use of catheters and various medical devices [85]. Viral infections are the most common cause of morbidity in cancer patients especially among pediatric patients. Viruses directly affect the cell-mediated immune system, thus they increase the risk of developing serious and life threatening infections. In the case of viral infections, the most important pathogens are the cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), respiratory syncytial virus (RSV), parainfluenza and influenza viruses, the human herpesvirus 6 (HHV-6), human poliomavirus 1 (BK virus), adenovirus and the human metapneumovirus. Regarding bacterial infections in patients with haematologic malignancy, the results of various international surveys are available. Earlier, mainly Gram-negative bacteria could be detected from infectious complications, while nowadays, Gram-positive bacteria are frequently associated with sepsis in this patient group and the role of atypical mycobacteria, intracellular pathogens and opportunistic bacteria is becoming evermore important due to developments in microbiological methods. The significance of bacterial infections in patients with haematologic malignancy is well characterised, while the incidence and consequence of viral infections are poorly described in the non-transplant haematologic patient group.

1.1.1. Bacterial infections in patients with haematologic diseases

In recent years cancer-related mortality has decreased substantially in patients with malignant haematological diseases because of the use of modern chemotherapeutic drugs, monoclonal antibodies, and stem cell transplantation (SCT). In spite of this, infectious complications such as bloodstream infections (BSIs) are major causes of morbidity and mortality among these patients [77, 36]. In the nineteenth century, the relevance of infection in morbidity and mortality of patients with acute leukaemia had already been described, while the role of neutropenia in infection was only recognised in the 1960's. Febrile neutropenia (FN) is one of the most important complications in patients undergoing anticancer therapy [93, 76]. During a neutropenic episode, a fever developed in 80% of patients with malignant haematological diseases [64, 46]. The occurrence of BSIs at the onset of FN accounts for 10-25 % of all febrile events. This incidence is higher (13-60%) in haematopoietic transplant recipients. In the 1960s and 1970s, BSIs mainly were caused by Gram-negative bacteria. In the mid-eighties, changes were observed in the spectrum of pathogens associated with bloodstream infections from Gram-negatives to Gram-positives because of the widespread use of fluoroquinolone prophylaxis [50, 119, 110, 103], the wide range of applications of central venous catheters (CVCs), severe mucositis and prolonged neutropenia associated with intensive chemotherapeutic regimens [50].

Patients with acute leukemia may have a high risk of infection as a consequence of both leukemic cell proliferation and infiltration in the bone marrow, and anti-leukemic treatment. Complete remission may be achieved with intensive chemotherapy in 80% of patients with AML (acute myeloid leukemia) below 60-65 years of age, and nearly 40% of cases may be cured with intensive post-remission consolidating therapy [22, 65]. The treatment regimens, mainly in patients with AML during and after the first course of induction therapy may produce long-lasting neutropenia that predisposes patients to infections [78]. The rate of infections increases with the severity and duration of neutropenia [36]. Probably more than 90% of febrile neutropenic episodes in AML patients are caused by infection. Madani *et al.* identified the site of infections in 81% of febrile events. Among neutropenic patients with AML, the rate of BSI was 37.8%, and 75.8 % of it was caused by Gram-positive cocci, while only 12.1% of BSIs were caused by Gram-negative bacilli [77]. There are several risk factors

for infections, which may present simultaneously, such as an injury of the normal muco-cutan barrier (mucosal surface and skin) and the use of intravascular catheters. On the basis of the predisposing factors and local epidemiologic data, the spectrum of the possible pathogens is already taking shape. At the same time, the use of antibiotic therapy with different indications (prophylactic, empiric or targeted therapy) has also had an impact on the selection of the spectrum of the pathogen and bacterial resistance [63]. Bacterial infections are frequently detected early in the neutropenic phase, while fungal infection occurs when the neutropenia is prolonged. The spectrum of pathogens and its epidemiology occasionally vary in this patient group, hence it is a good idea to survey local epidemiological data [97, 82].

Infectious complications in lymphoproliferative disorders. In ***patients with non-Hodgkin lymphomas*** infections originate from both the underlying disease and treatment of lymphoma. Infections are influenced by several factors, such as impaired cell-mediated and humoral immunity, hypogammaglobulinaemia, insufficient neutrophil function and complement activity, but the most important predisposing factor is neutropenia. During the initial phase of profound neutropenia, the dominating pathogens are bacteria, mainly Gram-positive cocci (*Staphylococcus* sp., *Streptococcus* sp. and *Enterococcus* sp.), are twice as likely as Gram-negative bacteria. The defect in humoral immunity owing to the lack of normal B-lymphocytes is associated with the inadequate production of IgM, IgG and IgA immunoglobulins, and due to this, opsonisation and phagocytic function are impaired. Because of these problems, encapsulated organisms (*Streptococcus pneumoniae, Haemophilus influenzae* and *Neisseria meningitidis*) may cause severe occasionally fatal infections in patients with a low level of immunoglobulins (see Table 1). Cell-mediated immune-deficiency often exists in malignant lymphoproliferative disorders, and in those patients treated with nucleoside analogues or monoclonal antibodies [82]. For these patients, the incidence of infection casued by intracellular pathogens such as *Salmonella* sp., *Mycobacterium* sp. and viral infections is also higher (see Table 1). The spectrum of infections and pathogens in lymphoproliferative disorders has been influenced by the introduction of new therapeutic drugs and monoclonal antibodies. Following the classic chemotherapeutic treatments, such as alkylating-containing regimens, infections are mainly caused by bacteria (such as *S. pneumoniae, H. influenzae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella pneumoniae*) (see Table 1). The introduction of purine analogues in the treatment of lymphoproliferative malignancies

(especially in chronic lymphocytic leukemia (CLL)) has resulted in a change of microorganisms in general [84]. Due to the persistent immunosuppressive state, opportunistic infections caused by *Nocardia* sp., *Mycobacterium* sp., systemic fungi and herpesviruses are frequently detected [60, 61, 4].

Table 1. Summary of the most common pathogens in patients with malignant lymphomas (adapted from Rolston KV)[82]

Bacterial pathogens	**Fungal pathogens**	**Viral pathogens**	**Parasites/Warm**
Streptococcus pneumoniae	*Aspergillus* sp.	HSV-1/2	*Toxoplasma gondii*
Haemophilus influenzae	*Zygomycetes* sp.	CMV	*Strongyloides stercoralis*
Neisseria meningitidis	*Fusarium* sp.	EBV	*i*
Nocardia sp.	*Pneumocystis jiroveci*	VZV	
Salmonella sp.	*Cryptococcus neoformans*	HHV-6	
Listeria monocytogenes	*Candida* sp.		
Campylobacter sp.	*Histoplasma capsulatum*		
	Scedosporium sp.		
Legionella sp.			
Capnocytophaga sp.			
Mycobacterium sp.			

With the introduction of SCT and novel agents (bortezomib, thalidomide, and lenalidomide) in the treatment of multiple myeloma (MM) in the past few decades, the survival of ***patients with multiple myeloma*** has improved [67, 14]. In parallel with higher survival rates, it has brought about complications of the disease, such as infections. In spite of this, the infectious complications have induced a significant morbidity and mortality in this patient group [87]. According to Augustson *et al.*, a higher rate of early death (45%) was observed in this patient group because of infection within 6 months from the time of diagnosis [9]. A higher risk of infection may be a result of antineoplastic therapy of MM, and impaired humoral and cellular immunity. B-cell dysfunction may result in hypogammaglobulinemia, and a decreased number and function of natural killer (NK) and CD8+ T-cells may lead to the development of infections [83]. In Sweden, a large population-based study was carried out between 1988 and 2004, and was followed up in 2007. Over 9253 patients with MM, and 4931 matched controls were recruited to estimate the risk of infections. They found that infection-related deaths were significantly higher in MM patients than in the control group, and the risk of infection increased mainly in the first six months from the time of diagnosis [14, 9]. Offidani and co-workers examined the infection rate in patients with a thalidomide-containing regimen, and they found that the infection rate was 42%, and that 19% of these infections were severe [88].

Because of persistent immunosuppression in patients, bacterial infections are among the major complications of ***haematopoietic stem cell transplant*** **(**HSCT) ***patients***. High dose chemotherapy supported by autologous stem cell transplantation is a widely used method for malignant haematological diseases - mostly multiple myeloma and lymphomas [51]. BSIs, gastrointestinal infections and pneumonia are the most frequent bacterial infections in this patient group. The incidence of BSIs after HSCT varied between centers, but the BSI rate is 5-10% for autologous HSCT recipients, while in an allogeneic setting, 20-30% of recipients had BSI [10]. During the pre-engraftment neutropenia, the incidence of BSIs is higher and the rate is influenced by the degree of oral and enteric mucositis and the use of a central venous catheter. During the non-neutropenic phase, BSIs are frequently detected in the case of Graft-versus-Host-Disease, hypoglobulinaemia, and the presence of central venous lines. *Staphylococci*, mostly *coagulase-negative staphylococci,* are the most frequent cause of BSI after HSCT, while the second most common pathogens are the members of *Enterobacteriaceae* family, mainly *E. coli* (*Eschericia coli*); and the third most frequent bacteria are *enterococci*. Unfortunately, in the case of these pathogens, we are confronted by

increasing resistance rates; namely, the methicillin-resistance of *S. aureus* (*Staphylococcus aureus/*MRSA), the production of ESBLs (extended-spectrum beta-lactamases) among Gram-negative bacteria and vancomycin resistant enterococci (VRE) are becoming more frequent after HSCT [10]. Retrospective studies showed that the rate of pneumonia in HSCT recipients is 15-25%; bacterial pneumonia (44%) are more frequent than fungal- (29%) and viral- (19%) pneumonia [45, 48]. The most frequent bacterial pathogens are *E. coli*, and *Pseudomonas aeruginosa* (*P. aeruginosa),* while because of the use of immunisation and prophylaxis *S. pneumoniae* are responsible for 5% of pneumonias [10]. In the case of gastrointestinal infections, CDI (*Clostridium difficile* infection) is the most frequent cause of diarrhoea after HSCT. During the first year after transplant, the rate of CDI is between 5.7% and 24.7%, and CDI is more frequently detected in the early phase of HSCT [2, 21].

1.1.2. Viral infections among haematological patients after chemo-immunotherapy and patients who had undergone autologous stem cell transplantation

Viral infections are an important cause of morbidity and mortality of patients with malignant haematological diseases; however the exact incidence mainly in the case of non-transplant patients is not yet well described. The main causes of this are the following: the microbiological diagnosis of a viral infection is difficult mainly because of the immunosuppression, serology is usually not adequate method to set up the diagnosis, while molecular techniques are the most sensitive methods. Because of the high cost, these methods, mainly validated, quantitative PCRs (polymerase chain reactions) are not widely available in certain countries. The most common viral infections in this patient group are CMV, EBV, HSV-1/2, and VZV; among the respiratory viruses, RSV, the influenza virus and parainfluenza virus infections are the best characterised. Moreover, the role of adenoviruses, metapneumovirus, BK virus and HHV-6 in patients with haematologic disorders is newly recognised [111].

Over 130 herpesviruses of the *Herpesviridae* family are known and have been isolated from several animals. *Herpesvirales* ordo contains three families, these being *Herpesviridae*, *Alloherepesviridae* and *Malacoherpesviridae*. The *Herpesviridae* family also contains three subfamilies, *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammaherpesvirinae*. Up until now, eight species of herpesviruses are known to infect human individuals, these being human herpesvirus 1 (HHV-1) or herpes simplex 1 (HSV-1), human herpesvirus 2 (HHV-2) or herpes simplex 2 (HSV-2), human herpesvirus 3 (HHV-3) or varicella-zoster virus (VZV),

human herpesvirus 4 (HHV-4) or Epstein-Barr virus (EBV), human herpesvirus 5 (HHV-5) or cytomegalovirus (CMV), human herpesvirus 6 (HHV-6A and HHV-6B), human herpesvirus 7 (HHV-7) and human herpesvirus 8 or Kaposi sarcoma-associated herpesvirus (HHV-8 or KSHV) (see Fig. 2). Besides these, Macacine herpesvirus 1 can cause an infection in human individuals [105].

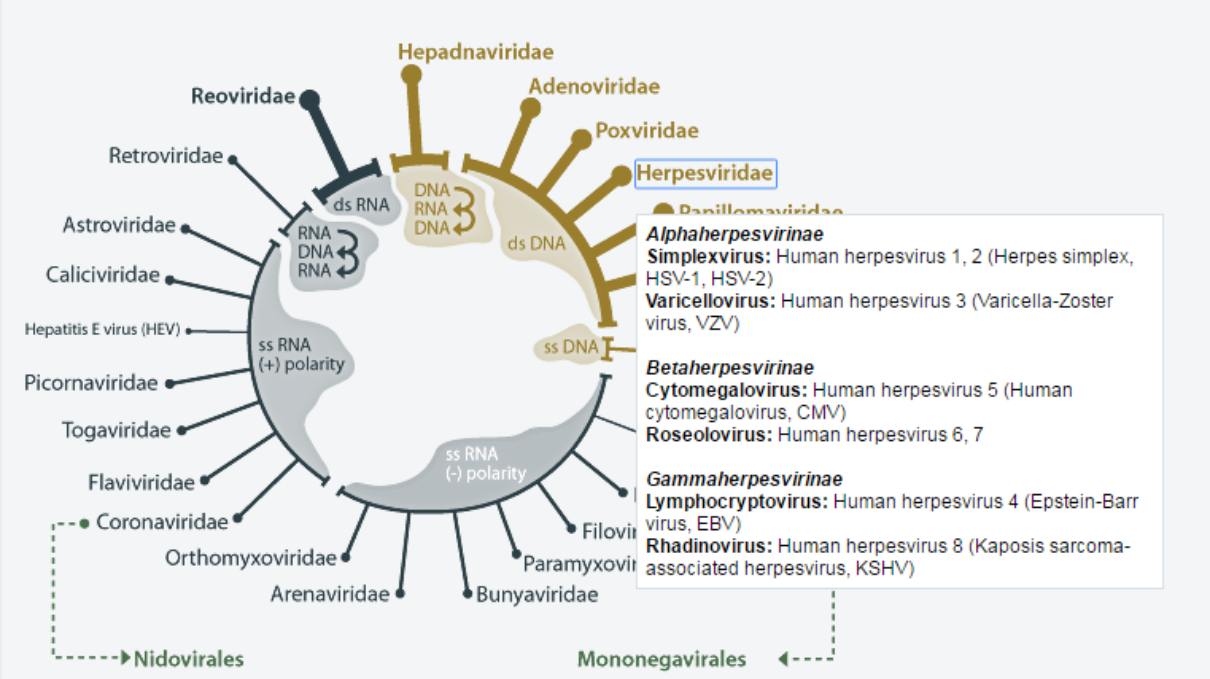

Figure 2. Taxonomy of herpesviruses
(source: https://www.antiviralintelistrat.com/1/Viral_Taxonomy)

The viral genome of these herpesviruses is double-stranded DNA (dsDNA) coated with icosahedral nucleocapsid. This nucleocapsid is covered by a pleomorph envelope. Between the envelope and nucleocapsid, tegument (matrix) can be observed (see Fig. 3.). On the basis of the microscopic morphology of herpesviruses, various species of herpesviruses could not be differentiated. Members of *Alphaherpesvirina*e including HSV-1, HSV-2, and VZV have a short life cycle and these viruses can spread rapidly and cause mainly mucocutaneous infection, while for *Betaherpesvirinae* (CMV, HHV-6 and HHV-7) the life cycle is slow; the infection is spread via saliva, genital secretes, blood or stem cell products, and these viruses may be latent in mononuclear cells. Gammaherpesvirinae including EBV and HHV-8 can establish latency in lymphoid cells and may cause a lytic infection in epithelial and fibroblast cells [79]. During infection, herpesviruses may enter the cells by endocytosis, the viral envelope fuses with the membrane of endocytotic vesicle and the viral nucleocapsid is finally

transported from the cytoplasm to the nucleus of the host cell. In the nucleus, the linear viral DNA will be circularised and DNA replication can begin. Viral gene expression is characteristic in herpesviruses and this process starts with the expression of immediate-early genes that code proteins regulating further gene expression. This is followed by the expression of early viral proteins that are necessary for DNA replication and protein phosphorylation; lastly late proteins are expressed, several of these being major structural proteins [79].

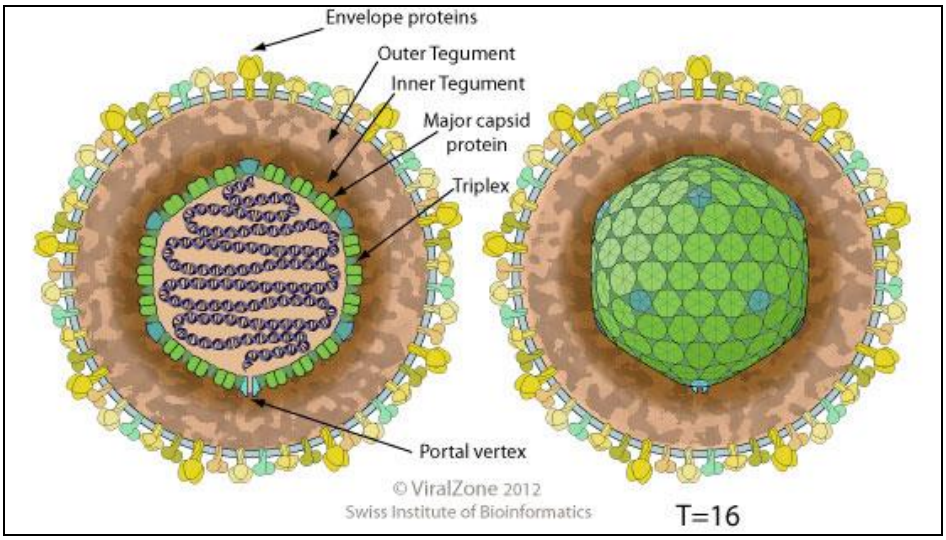

Figure 3. Structure of herpesviruses (source: http://viralzone.expasy.org/all_by_species/185.html)

Herpesviruses except VZV (airborne transmission) is mostly spread by direct contact, such as by infected saliva (CMV, EBV, HSV, and HHV-6). Sexual transmission (HSV, CMV, and HHV-8) and intrauterine infection (CMV, HSV, and VZV) can also occur. However, the majority of HSV infected infants acquire HSV infection at the time of delivery. CMV and EBV are spread via blood transfusion, while CMV, EBV and HHV-8 are also spread by organ transplantation [79]. Clinical syndromes caused by herpesviruses are similar, such as skin lesions, encephalitis, retinitis, hepatitis, mononucleosis and mononucleosis-like syndromes, Hence there is a need for clinical microbiology to establish a diagnosis [79].

The biology of human cytomegalovirus (CMV) and infections associated with this virus. CMV, formally designated human herpesvirus 5 (HHV-5), is a member of the *Betaherpesvirinae* subfamily of the *Herpesviridae* family. This is the largest member of

human herpesviruses that was first isolated from the salivary gland, while a description of the CMV disease was first reported in 1965 [79]. The structure of this virus is similar to that of HSV and VZV. The viral genome is linear dsDNA, which contains 164 non-overlapping open reading frames and it is completely sequenced. CMV DNA is located in a nucleoprotein core that is surrounded by a matrix protein and pp65 antigen. The CMV genome has a single replication origin and contains a DNA polymerase gene (*UL54*). Another important gene is *UL97*, which encodes phosphotransferase enzyme. This enzyme can phosphorylate ganciclovir to ganciclovir monophosphate, this step being essential for ganciclovir to inhibit viral replication. CMV has also several genes that can downregulate the host's immune response. CMV enters the host cell via endocytosis, and like other herpesviruses the viral core is transported from the cytoplasm to the nucleus where after the synthesis of DNA polymerase, viral replication occurs. The consequence of viral replication is the development of nuclear viral inclusions [79]. CMV infection as an endemic, seasonal variation could not be detected (i.e. it occurs all year round). After infection the virus remains dormant in monocytic/macrophage cells. In humans, the virus can infect various cell types including epithelial-, endothelial-, neuronal-, smoth muscle-cells and fibroblast. Seropositivity rate changes lie between 40% and 90%, and they exceed 90% in the adult population. CMV infection is often asymptomatic in a healthy individual, but sometimes a mononucleosis-like syndrome may occur in young adults. With cytomegalovirus mononucleosis, fever, lymphadenopathy and relative lymphocytosis like EBV mononucleosis may be observed, but the heterophil agglutinin test is negative, and a sore throat with enlarged tonsils is not characteristic; at the same time only low-level liver function abnormalities can be seen. In an immunocompromised host, CMV-related disease may involve almost any organ, but the most common are the lung and gastrointestinal tract [5, 23,111]. Interstitial pneumonia is one of the most frequent complications of CMV infection in immunocompromised hosts - mainly in haematopoetic stem cell transplants -, and with this complication is associated a high mortality even with aggressive antiviral therapy. Hepatitis and granulomatous hepatitis are usually associated with CMV mononucleosis and these may resolve fully. Another complication of CMV infection mainly in immunocompromised patients is meningoencephalitis with motor and sensory weakness. Myocarditis, thrombocytopenia and haemolytic anaemia are rare complications of CMV mononucleosis in otherwise healthy children or adults. In immunocompromised patients, the gastrointestinal manifestation of CMV infection may be present as ulcers in the oesophagus and colitis with severe explosive

watery diarrhoea. However, the incidence of CMV infection and disease is less clearly defined among patients with malignant hematological disorders [25, 52, 53]. Various studies have shown that the rate of severe CMV disease is higher among patients with cell-mediated immune-deficiencies following a stem cell and solid-organ transplant. However, CMV reactivation and infection are unusual infectious complications in patients with hematological diseases treated with conventional chemotherapy and these patients did not have stem cell transplantation [7, 86]. In stem cell transplant recipients, the rate of CMV reactivation is about 30-70% [6, 16]. In particular, this rate is much lower in autologous stem cell transplant recipients or in patients treated with high-dose chemotherapy than in an allogeneic setting.

The laboratory diagnosis of CMV infection is based on the culture of the virus taken from various body fluids or the detection of viral antigen or viral DNA or CMV specific antibodies. The first laboratory test to establish the diagnosis of CMV infection mainly in infants with cytomegalic inclusion disease involved the detection of large nuclear inclusion-bearing cells in the urine. Another diagnostic method is the culture of viruses in human fibroblast cell line (MRC-5 cells), but as this is quite a labour-intensive and time-consuming method, these methods are not frequently used nowadays. The direct detection of antigens such as CMV matrix protein pp65 in neutrophils using monoclonal antibody is frequently used in patients with immunosuppression. However molecular methods like real-time quantitative PCR have a higher sensitivity, therefore these techniques are more reliable diagnostic tools in immunocompromised patients. With the use of molecular methods, it is possible to commence antiviral treatment in order to prevent the development of CMV end-organ disease, so the use of the molecular method provides the basis of preemptive therapy. In immunocompetent hosts, the majority of laboratories use ELISA (an enzyme-linked immunosorbent assay) for the detection of cytomegalovirus specific IgM and IgG, but the value of IgM detection is questionable because IgM may be present in the case of primary infection, reinfection and reactivation as well. At the same time after acute infection, IgM may persist for several months and with other herpesvirus infections we may get a false positive IgM result. Thus IgG seroconversion is indicative of a primary CMV infection, while in the case of the presence of the cytomegalovirus specific IgM, performing the IgG avidity test is a useful alternative to establish the diagnosis of acute cytomegalovirus infection.

In the case of antiviral therapy we need to distinguish between antiviral prophylaxis (the routine administration of antiviral drug for a fixed period at the patient’s risk to prevent CMV

reactivation) and preemptive therapy (based on the detection of viral reactivation by the molecular method, a pp65 antigenemia assay or culture; therefore the early introduction of antiviral therapy could prevent a progression to the CMV disease). Three antiviral drugs [ganciclovir (GCV), foscarnet, cidofovir (CDV)] have been shown to be efficacious and have been approved in the treatment of CMV infection. The mechanism of action of these drugs are an inhibition of the viral DNA polymerase. Ganciclovir is a guanosine nucleoside analogue, and this was the first effective antiviral drug against CMV disease in human individuals [38]. The UL97 gene of CMV produces phosphotransferase, which converts GCV to GCV monophosphate, and it then is phosphorylated to GCV triphosphate. The triphosphorylated form of ganciclovir specifically inhibits the viral DNA polymerase. CMV resistance to GCV is developed by point mutation of the UL97 gene, and the another type of GCV resistance is a consequence of mutation in the viral DNA polymerase gene [74, 75]. Valganciclovir (VGC) is a prodrug of the GCV with a much higher bioavailability. Its oral form is equivalent to intravenous GCV. Foscarnet is a pyrophosphate analogue, and acts by direct binding to the viral DNA polymerase (CMV and other herpesviruses). Foscarnet is a treatment option for GCV-resistant CMV infection. It is administered intravenously, and it has a metabolic and nephrotoxic adverse reaction, such as renal failure, hypocalcaemia, hypomagnesaemia, hypophosphataemia [42], thus the close monitoring of serum creatinin and above-mentioned electrolytes levels, and supplementation of it are essential during therapy. Foscarnet resistant strains of CMV have been published because of the viral DNA polymerase gene mutation [27, 28]. Cidofovir (CDV) is a nucleotide analogue of cytosine with potent anti-CMV activity. The phosphorylation step is not necessary using a viral enzyme. CDV was found to be effective in the treatment of CMV infection in patients undergoing allogeneic stem cell transplantation. In a retrospective study, Ljungman *et al.* analysed 82 allogeneic stem cell transplant patients, and data were gathered concerning the toxicity and efficacy of CDV. The dose of CDV was 1 to 5 mg/kg once a week, and followed by maintenance therapy every second week. Twenty patients with CMV disease (16 with pneumonia) were treated with CDV, and 50% of them responded to CDV therapy (9 out of the 16 patients had pneumonia). CDV is also effective as a second-line therapy in relapsing cases after GCV or foscarnet treatment [70]. One of the most important side effects is proximal renal tubular damage.

Biology of human herpesvirus 6 (HHV-6) and infections associated with this virus. Human herpesvirus 6 (HHV-6) is a member of *Herpesviridae* family and it is grouped into roseoloviruses with Human herpesvirus 7 (HHV-7). This virus was first isolated from patients with AIDS and lymphoproliferative disease in 1986 [58, 100], and it is genetically related to CMV [1]. There are two variants of HHV-6, namely HHV-6A and HHV-6B; on the basis of sequence analysis of their genomes, the differences between these two types are 5% [120]. The seroprevalence is above 95% in adults, but current serologic tests cannot distinguish between HHV-6A and HHV-6B infections, hence precise data about their seroprevalence are not available [19]. HHV-6 have a wide cell tropism; and *in vitro* studies have confirmed the presence of this virus in the brain, tonsils, salivary glands, kidneys, liver, lymph nodes, endothelial cells, and monocytes/macrophages, but the virus may infect preferably activated $CD4^+$ T lymphocytes, and the infected cells display characteristic features such as cytomegalia, and ballooning. As regards HHV-6A, CD46 (a regulator of complement activation) is the major receptor for cell entry. This receptor can be found on all nucleated cells, while for HHV-6B, CD134, a member of tumour necrosis factor (TNF) receptor superfamily serves as a receptor for entry, and this receptor can be detected only on activated T lymphocytes [1]. Latency in monocytic/macrophage and bone marrow progenitor cells has also been confirmed; hence the virus can establish a lifelong infection characteristic of other herpesviruses [120]. Genomic HHV-6 DNA may be present as covalently integrated DNA in the subtelomeric region of the host chromosome [1]. A specific region of the viral genome including terminal direct repeats is responsible for the chromosomal integration [1].

The pathogenic role of this virus in exanthema subitum (roseola infantum) was first described in 1988 by Yamanishi [116] *et al.* [24]. The majority of HHV-6 infections, including primary infection, reactivation and reinfection are asymptomatic [1]. HHV-6 infection is mostly acquired early in life - in general between 6 months and 2 years of age. Primary infection mostly manifests itself as undifferentiated febrile illness within the first 2 years of life. HHV-6A may cause a primary infection in childhood, but sometimes it may be asymptomatic, and the etiologic role in infections is less well known. HHV-6B is known to be the causative agent of roseola infantum or exanthem subitum [19]. The typical symptom in exanthema subitum is a high fever for 3-4 days, and after this fever, measles-like or rubella-like macular or papular rashes appear [120]. Other symptoms, such as diarrhoea, respiratory symptoms, convulsions, cervical lymphadenomegaly, and anterior fontanelle bulging during HHV-6 infection may be

also observed, and with these unspecific symptoms HHV-6 infection is frequently underdiagnosed [120]. Because of the latency, HHV-6 can be reactivated in the case of immunosuppressive circumstances, such as following intensive chemotherapeutic treatment, solid organ, bone marrow and stem cell transplantations. The first case of fatal encephalitis following bone-marrow transplantation was described by Drobyski *et al*. in 1994 [41]. The risk of HHV-6 reactivation is associated with allogeneic (mainly cord blood) transplantation and rarely with autologous stem cell transplantation. In this case, the symptoms range from asymptomatic infection to severe manifestations (encephalitis, pneumonitis, hepatitis and myelosuppression), prolonged haematopoietic recovery and graft rejection. Encephalitis is one of the most frequent and life-threatening clinical complications of reactivation [19]. Reactivation has been documented by molecular techniques in 40-60% from peripheral blood and in 1-2% from cerebrospinal fluid associated with neurological signs [56, 112,117]. In addition to this, a diagnosis may be possible based on the detection of viral antigens or antibodies from various clinical specimens; however, the interpretation of results is complicated because of the high seroprevalence, immunosuppression and latent form of the virus [19]. On the basis of these, various aims have been decided in the virological diagnosis of HHV-6 infection, namely the detection of HHV-6A and HHV-6B infection, the determination of the status of this infection (latent or active or ciHHV-6 related), the determination of viral load, and the question of treating this infection [1]. Various laboratory methods are available for the detection of HHV-6 infections, these being serological assays and molecular methods. Serological assays (indirect immunofluorescence assays, ELISA and immunoblot) are relatively cheap and easy to carry out, and a large number of sera can be investigated simultaneously, but these assays have several limitations, two being that the presence of IgM and the rapid increase in the level of IgG are not specific for acute HHV-6 infection. At the same time other herpesvirus infections may cause false positive results; serologic findings may be atypical with immunocompromised patients and chromosomally integrated HHV-6 [1]. Because of various drawbacks of serological assays, they are useful in the case of the diagnosis of primary infection, the identification of HHV-6 in naïve subjects and seroprevalence studies [1]. Among direct methods, viral isolation is a reference method. However this method has a poor sensitivity and preparing the viral culture is time-consuming and quite expensive; hence this method is not recommended for diagnostic use. Immunohistochemistry techniques also have low sensitivities, so the use of these methods is limited. These days, molecular methods are widely available for diagnostic purposes like the

detection of HHV-6A and HHV-6B and the determination of viral load from various types of samples including plasma, cerebrospinal fluid and any other body fluids [1].

2. AIMS OF THE STUDY

The changing epidemiological patterns of infections in patients who suffer from hematological malignancy are characterised not only by the increasing incidence of some opportunistic pathogens, but also by the wide emergence of resistance to antimicrobials, especially in bacteria. In the practice of antimicrobial prophylaxis, it is crucial to continuously keep abreast of new epidemiological data in order to monitor the emerging antimicrobial resistances and define tailored prevention strategies. Based on findings of various international surveys and the lack of local data on the epidemiology of bloodstream and viral infections in patients with haematologic malignancy, the aims of this study were to establish the epidemiology of these diseases in patients at the Department of Haematology, University of Szeged in Hungary. In other words, we sought

1. To evaluate the occurrence of bacterial species causing bloodstream infections in febrile neutropenic episodes between 2005 and 2008.

2. To characterise bloodstream infections caused by “unusual” pathogens including *Fusobacterium* spp., *Nocardia* spp., and *Achromobacter* spp.

3. To present the results of CMV monitoring in patients with haematological diseases, and following autologous stem cell transplantation.

4. To determine the occurrence of HHV-6 infection in autologous stem cell transplant recipients.

3. MATERIALS AND METHODS

3.1. COMMONLY USED DEFINITIONS

***Febrile neutropenia*.** Febrile neutropaenia was defined if a single oral temperature was measured higher than 38.3 °C, or the temperature was 38.0 °C or higher for 1 hour. Neutropenia is defined as when the absolute neutrophil count (ANC) is less than 0.5xG/L or less than 1.0xG/L and it rapidly declines below 0.5xG/L [55].

Classification of febrile episodes. Three distinct groups of febrile episodes were distinguished in neutropenic patients. Microbiologically documented infection (MDI) means a positive culture result can be confirmed from different sites (blood or other specimens). Clinically documented infection means features of infections are diagnosed clinically or by radiographic examinations without positive microbiologic samples. Fever of unknown origin (FUO): means there is no evidence of positive microbiological results or no clinical/radiographic signs of infection could be detected [64, 82].

Polymicrobial bloodstream infection. BSI infection is defined as polymicrobial if more than one species of bacteria grew from BC (blood culture) on the same day. In a neutropenic setting, the rate of polymicrobial origin is roughly 15-25% of BSIs [64, 96]. The typical organ involvement of polymicrobial infections is predominantly the enterocolitis, perirectal and skin infection [96].

Bloodstream infections caused by rarely isolated bacteria, and the occurrence of infections on unusual localisation. About 10-15% of infectious complications of neutropenic patients appear in unusual localisation including central nervous system involvement (meningitis and brain abscess), septic arthritis, and septic involvement of the liver, spleen and kidneys caused by rarely isolated bacteria [43, 82, 92, 95].

3.2. BLOODSTREAM INFECTIONS IN PATIENTS WITH HAEMATOLOGIC MALIGNANCY

3.2.1. Patients

Between 2005 and 2008, 469 patients with febrile neutropenia (230 females and 239 males, median age 60 years) were examined at our department. The data collected from patient

documentation included the demographics of patients, diagnosis, febrile episodes, source of fever and source of infection, neutrophil count, and clinical significance of the isolated organism. Infectious complications were categorised into three groups, namely 1 - FUO, 2 - MDI, 3 - clinically documented infection. Definitions of febrile neutropenia, neutropenia, and polymicrobial BSI were used (see above). A single positive BC was considered significant if the isolated strain was a clinically relevant cause of the infection. Common skin contaminants (CNS (coagulase-negative staphylococci) and propionibacteria) were considered significant only if they were found in two consecutive BC samples or if there were concurrent skin, soft tissue or catheter–related infections. Microbiological results were retrieved from the Clinical Microbiology Laboratory information system. A medical database of patients was used to collect information on the haematologic diseases, presence of a febrile neutropenic episode, the duration of neutropenia, and the source of the infection.

3.2.2. Analysis of blood cultures

BCs were collected at the onset of a fever. In patients with central venous catheters, BCs were taken simultaneously from both central and peripheral veins. For the collection of blood culture, blood culture systems (BD Bactec, Beckton Dickinson, USA) including aerobic, anaerobic bottles and bottles for fungi were used. After the collection of blood, the bottles were immediately placed in an incubator, where they were incubated for 5-14 days, depending on the type of the putative pathogens. In the case of a positive signal produced by the instrument based on bacterial or fungal growth, microscopic examinations (Gram stained preparations) and culture were performed. For an aerobic culture, Columbia blood agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France), chocolate agar supplemented with PolyViteX (bioMérieux, Marcy L'Etoile, France), eosin-methylenblue (Lab M, UK) and Sabouraud Chloramphenicol (Bio-Rad, France) agars was inoculated, while for an anaerobic culture, Schaedler agar supplemented with 5% sheep blood (bioMérieux, Marcy l'Etoile, France) was inoculated. Plates were incubated at 37 °C for 24 hours in a 5% CO_2 incubator or 37 °C for 24 hours at one atmosphere or at 37 °C for 48 hours in an anaerobic cabinet (Concept 400; Ruskinn Technology Ltd., Bridgend, UK) under a gas composition of 85% N_2, 10% H_2 and 5% CO_2. From a pure culture, antibiotic susceptibility tests were performed on the basis of recommendations by the Clinical Laboratory Standard Institute [30-34].

3.2.3. Identification of bacteria and yeasts

For the precise identification of bacteria and yeasts, traditional biochemical tests or automated identification systems, such as VITEK®2 ID (bioMérieux, France), API 20 A (BioMérieux, France), or RapID™ ANA (bioMérieux, France) were applied on the basis of colony morphology, O_2/CO_2 requirement, and phase contrast microscopy findings. If the identification proved inconclusive using traditional or automated identification systems, the amplification of 16S rDNA was used. In short, nucleic acid from the colonies was purified using the QIAamp DNA mini kit (Qiagen) and the 16S rDNA was amplified using universal primers E8F (5'-AGAGTTTGATCCTGGCTCAG-3') and E533R (5'-TTACCGCGGCTGCTGGCA-3'). For amplification, the following cycling conditions were used: starting denaturation at 94ºC for 3 min; 35 cycles: denaturation at 94ºC for 15 s, annealing at 55.5ºC for 30 s, extension at 72ºC for 1 min; final extension at 72ºC for 10 min. The PCR product was purified using the High Pure PCR Cleanup Micro Kit (Roche, Germany) according to manufacturer's instructions and the purified PCR product was sequenced. The sequences obtained were then compared with those stored in GenBank using BLAST alignment software (http://www.ncbi.nlm.nih.gov/blast).

3.2.4. Antibiotic protocol

At the onset of fever, after the collection of BC samples, empirical broad-spectrum antibiotic treatment was started (piperacillin-tazobactam, cefepime, and imipenem or meropenem). The antibiotic dosage was modified according to the patient's renal function. Patients were examined once a day by their physician to detect any potential source of infection. After 48-72 hours of observation, the patient's condition was re-evaluated. Changes in empiric antibiotic therapy depended on BC results and clinical response. In afebrile and culture-negative patients with a stable clinical state, empiric antibiotic treatment was continued until the ANC reached 0.5xG/L. Vancomycin was used in patients with central venous devices, persistent fever and hypotension. On days 4-5, in patients with a persistent fever that suggested a fungal infection on the basis of clinical signs and CT (computed tomography) scans, amphotericin-B was initiated.

3.3. MONITORING THE PRESENCE OF CMV IN PATIENTS WITH HAEMATOLOGIC DISEASES

3.3.1. Patients and sample collection

Between 2008 and 2014, 1238 plasma samples were collected from 271 patients with haematological diseases at our department (University of Szeged, Hungary). The clinical and demographic data of patients were gathered from the local medical computer-based database. The findings of serological investigations and CMV PCR were analysed retrospectively. Patients were divided into three groups according to the underlying disease (lymphoid and myeloid haematologic malignancies, and other haematologic diseases) and the first two categories were further divided into two subgroups: patients who underwent autologous stem-cell transplantation (ASCT) or not (non-ASCT). Patients in the ASCT group gave written informed consent for the transplant procedure. We also sought informed consent from patients with haematological diseases (non-ASCT) for the treatment according to the local policy of the hospital. The conditioning regimen for Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) patients was BEAM (BiCNU®, etoposide, cytosine-arabinoside, melphalan supplemented with 375 mg/m^2 of rituximab for CD20+ NHL patients); while for myeloma patients and with or without renal failure 200 mg/m^2 or 140 mg/m^2 of melphalan was used [26, 8, 11]. Out of 271 patients examined, 126 received unmanipulated autologous stem cells (2-7 x 10^6/kg, median: 4.94 x 10^6/kg, range: 2.73-7.15). All transplant patients were supported with filtered and irradiated blood products. Among patients, in the non-ASCT patient group undergoing purine-analogue-based chemotherapy, or anti-CD52 antibody or anti-thymocyte globulin, similarly filtered or leuko-depleted and irradiated red blood cell and platelet transfusions were used. Anti-infective prophylaxis for the ASCT patient group was fluconasole (100 mg once daily), and during the neutropenic phase, this was levofloxacin (500 mg once daily). Antiviral prophylaxis consisted of acyclovir (200 mg five times daily, and this was switched to 250 mg twice daily intravenously if the patient was unable to take solid oral dosage forms). The use of antiviral prophylactic agents was continued for 6 months post-ASCT.

CMV specific antiviral therapy was initiated on the basis of at least two consecutive CMV DNA viral copy numbers if the copy number was >1000 copies/ml in one sample or if the copy number was <1000 copies/ml, but the copy number indicated that there was an increasing tendency in two consecutive specimens.

3.3.2. Determination of the CMV serologic status and monitoring for CMV DNA viral load

Among non-ASCT patients with haematologic diseases, quantitative real-time CMV PCR from plasma was performed if febrile episodes of unknown origin without the presence of concomitant bacterial or fungal infections had arisen during antineoplastic treatment (especially the purine-analogue or monoclonal-antibody therapy associated with lymphopenia). In patients with ASCT, CMV PCR was carried out once a week until the 30th post-transplant day, and then non-regularly up to the 100th post-transplant day or during hospitalisation and occasionally during the stem cell harvesting period. For the determination of the CMV viral load, artus CMV RT PCR (Qiagen, Germany) was applied after nucleic acid isolation from plasma specimens was performed using the MagNa Pure Compact Nucleic Acid Isolation Kit I (Roche, Swiss). Nucleic acid isolation and quantitative real-time PCR were carried out according to the manufacturers' recommendations. A serologic assay using ETI-CYTOK-M reverse Plus CMV IgM EIA (DiaSorin, Italy) and ETI-CYTOK-G Plus CMV IgG EIA (DiaSorin, Italy) was performed in the ASCT group before the stem cell harvesting procedure, while in the non-ASCT patients these tests were carried out occasionally. CMV symptomatic infection was defined if CMV DNAemia was detected in two consecutive specimens along with the clinical suspicion of reactivation. CMV end-organ disease was defined by the presence of symptoms consistent with CMV infection and the simultaneous presence of molecular, histopathological, clinical or imaging findings that suggested CMV infection [69, 72, 73]. As for the statistical methods applied, we used statistical software IBM SPSS Version 22. Among patient groups, we used the Mann-Whitney U-test to compare the baseline characteristics. The age was compared across CMV PCR positive and CMV PCR negative groups via the Mann-Whitney U-test. The independence of CMV PCR groups and sex, CMV PCR groups and the initial diagnosis was tested by the chi-square test for independence. Here, a p-value of <0.05 was deemed statistically significant.

3.4. MONITORING THE PRESENCE OF HHV-6 IN PATIENTS WITH HAEMATOLOGIC DISEASES

3.4.1. Patients

Between 2010 and 2012, 35 consecutive patients that had undergone autologous peripheral stem cell transplantation were observed at our institution (namely, 19 females and 16 males with a median age of 60 years; range 22-71 years). The underlying diseases were multiple myeloma, Hodgkin lymphoma and non-Hodgkin lymphoma in 29, 2 and 4 cases, respectively.

One hundred and twenty-one anticoagulated (EDTA) blood and 2 cerebrospinal fluid (CSF) samples taken from the 35 patients were collected, which meant that at least 2 blood samples were taken from each patient (in 7 cases, only 1 specimen). All patients received antiviral prophylaxis against the herpes simplex virus-1/2 and varicella-zoster virus (acyclovir 400 mg twice daily), antibacterial (levofloxacin 500 mg once a day) and antifungal drugs (fluconazole 150 mg once a day) for the prevention of infection. Engraftment was defined if the neutrophil and platelet count was more than 0.5 and 20xG/L on two consecutive days without any support, respectively.

3.4.2. Molecular detection of HHV-6

Plasma specimens and, in the case of neurological manifestations, CFSs were tested for the presence of CMV, HSV-1/2, EBV DNA using real-time PCR (artus CMV LC PCR Kit; Qiagen, Hilden, Germany, artus HSV-1/2 LC PCR Kit; Qiagen and artus EBV LC PCR Kit; Qiagen). HHV-6 nested PCR was performed with the use of Dream Taq Green PCR Master mix (2x) (Thermo Scientific; Waltham, MA, USA) containing a 2x DreamTaq Green buffer, 0.4 mM dNTP, 4 mM $MgCl_2$. The primers were derived from the immediate early gene locus of two variants of the HHV-6 strain (U1102) (115). Ten microliters of 1x DreamTaq Green PCR Master mix, 0.4 µl of 25 mM $MgCl_2$, 0.3 µl of 25 pmol HHV6-F out primer(5'-TTC TCC AGA TGT GCC AGG GAA ATC C-3'), 0.3 µl of 25 pmol HHV6-R out primer (5'-CAT CAT TGT TAT CGC TTT CAC TCT C-3'), 4 µl of H_2O, and 5 µl of a purified DNA template were used for PCR. For the next PCR, 10 µl of 1x Dream Taq Green PCR Master mix, 0.4 µl of 25 mM $MgCl_2$, 0.3 µl of 25 pmol HHV6-F in primer (5'- AGT GAC AGA TCT GGG CGG GCC CTA ATA ACT T-3'), 0.3 µl of 25 pmol HHV6-R in primer (5'- AGG TGC TGA GTG ATC AGT TTC ATA ACC AAA-3'), 4 µl of H_2O and 5 µl template (from the samples amplified in the course of the previous PCR) were prepared. The amplified PCR product was detected using gel electrophoresis and two variants of HHV-6 were identified on the basis of the size of the amplified products (variant A gave 195 bp, while variant B gave 423 bp of PCR products).

4. RESULTS

4.1. BLOODSTREAM INFECTIONS IN PATIENTS WITH HAEMATOLOGIC MALIGNANCY

Between 2005 and 2008, 1361 patients were hospitalised in the haematology department because of various haematological diseases, and the average number of cases/year was 340. Alltogether 812 febrile episodes were recorded in 469 (34.5%) patients and blood was collected for microbiological culture. Of the 469 patients, 128 (27.3%) had acute myeloid leukaemia, 85 (18.1%) non-Hodgkin's lymphoma, 66 (14.1%) multiple myeloma, 64 (13.6%) chronic lymphocytic leukaemia, 41 (8.7%) acute lymphoblastic leukaemia and 85 (18.1%) others (Hodgkin's lymphoma, myelodysplastic syndrome, chronic myeloprolipherative disorders, etc.). Altogether 3714 blood culture bottles, 7.9 bottles/patient (ranging from 2-12) were sent to the laboratory. In 126 (27%) cases of the 469 patients, only one pair of blood culture bottles was taken for an analysis of febrile episodes. Clinically documented infections were observed in 430 cases (52.95%) of 812 febrile episodes. The majority of them were localised to the lungs (39.5%). Colitis and skin and soft tissue infections were the second and third most common types of infection.

Using a microbiological culture, 759 (20.4%) of 3714 blood culture bottles gave positive signals. From the majority of positive blood culture bottles (509 bottles (67.1%)), Gram-positive bacteria were detected. Among Gram-positive bacteria, the most frequent isolates were coagulase-negative staphylococci (65%), *S. aureus* (10%), *Enterococcus* spp. (6.7%), *P. acnes* (5.7%), beta-hemolytic streptococci (3.1%), *S. pneumoniae* (2.8%), alfa-hemolytic streptococci (2.4%) *Clostridium* spp. (1.4%), and others (3%) (including *Listeria monocytogenes*, *Nocardia farcinica*, *Gemella* spp., *Micrococcus* spp., *Brevibacterium* spp., and Gram-positive non-identified bacteria) (See Table 2).

Table 2. Gram-positive bacteria isolated from blood cultures between 2005 and 2008.

Isolated strain	Number of isolated strains per annum				Total
	2005	2006	2007	2008	N=509 (%)
Coagulase-negative staphylococci	94	73	86	78	331 (65)
S. aureus	25	6	11	9	51 (10)
Enterococcus spp.	6	9	5	14	34 (6.7)
P. acnes	13	10	2	4	29 (5.7)
Beta-hemolytic streptococci	10	5	1	0	16 (3.1)
S. pneumoniae	5	0	0	9	14 (2.8)
Alfa-hemolytic streptococci	0	7	1	4	12 (2.4)
Clostridium spp.	2	4	0	1	7 (1.4)
Others (*L. monocytogenes, N. farcinica, Gemella* spp., *Micrococcus* spp., *Brevibacterium* spp. etc.)	4	6	2	3	15 (3)

Gram-negative bacteria were isolated from 250 (32.9%) blood culture bottles. A high prevalence of *E. coli* (52%) could be detected in these specimens, while 14% of samples contained *P. aeruginosa*, 9.6% *Klebsiella* spp., 8% *Enterobacter* spp., 3.6% *Citrobacter* spp., *Stenotrophomonas maltophilia* 2%, *Acinetobacter* spp., and 1.6% *Fusobacterium* spp. (see Table 3).

Only 6 bottles proved positive for fungi during the period in question; in 2 cases, *Candida albicans* and also in 2 bottles, *Candida tropicalis* was detected, while 2 other bottles were positive for *Cryptococcus* spp.

Table 3. Gram-negative bacteria isolated from blood culture bottles between 2005 and 2008.

Isolated strain	**Number of isolated strains per annum**				**Total**
	2005	**2006**	**2007**	**2008**	**N=250 (%)**
E. coli	22	38	41	29	130 (52)
P. aeruginosa	6	6	10	13	35 (14)
Klebsiella spp.	0	10	6	8	24 (9.6)
Enterobacter spp.	7	3	0	1	20 (8)
Citrobacter spp.	2	3	2	2	9 (3.6)
S. maltophilia	0	0	2	3	5 (2)
Acinetobacter spp.	0	1	1	2	4 (1.6)
Fusobacterium spp.	0	2	2	0	4 (1.6)
Others (*H. influenzae, A. xylosoxidans, P. mirabilis, Salmonella* spp., *Neisseria* spp., Gram-negative non-identified rods etc.)	5	4	5	5	19 (7.6)

Among Gram-positive isolates, coagulase-negative staphylococci were identified in 331 cases. These blood culture samples were collected from 161 febrile episodes of 149 patients.

In 50 febrile neutropenic episodes, CNS played a role as a causative agent of fever because of the coexistence of skin, soft tissue and central venous catheter-related infections. As for the remaining 111 cases, contamination might have been the source of CNS. Among rarely isolated pathogens, *Listeria* sp. was identified from one patient with AML due to a second relapse in 2006. The patient had clinically documented pneumonia, and was treated with ampicillin. *Nocardia farcinica (N. farcinica)* was also detected among rare pathogens in a 37-year-old man diagnosed with large granular lymphocytic leukaemia. He underwent 6 cycles of combined chemotherapeutic treatment (Cytoxan, Vincristine, and Prednisone), and later had long-term steroid therapy due to Coombs positive haemolytic anaemia, and active haemolytic events. He was hospitalised due to several febrile episodes six months before present admission, but infectious agents could not be detected. Upon admission, the patient complained of general fatigue, fever, and vomiting. After hospital admission, headache and dizziness commenced. A neurological examination did not reveal any signs of meningitis. The serum laboratory results displayed a normal level of procalcitonin, liver and kidney function, elevated leukocyte count (34.2xG/L) with 80 % neutrophil cells, mild anaemia (haemoglobin level 113 g/L) and a normal platelet count (339xG/L). Three days later CT was performed because of central type paresis of facial and hypoglossal nerves. The CT scan revealed multiple lesions (6-13 mm in diameter) with perifocal edema. A stereotactic core biopsy from lesions could not be carried out because of a deterioration in the patient's clinical status. Blood and urine samples for bacteriological culture were collected, and empiric antibiotic therapy (imipenem-cilastatin 500 mg every 6 hours) was started. Two blood culture bottles gave a positive signal after 52 h and 65 h of incubation; and in the Gram-stained preparations, Gram-positive branching filaments could be observed (Fig. 4A). After 24 h of incubation in air containing 5% CO_2, small orange colonies had grown (Fig. 4B). Because of the unsuccessful identification of the isolated strains using a commercial kit (VITEK 2 GP ID card, BioMérieux), 16S rDNA sequencing was performed. On the basis of sequence analysis of the amplified PCR product, *N. farcinica* was identified.

In 2006 and 2008, *Burkholderia cepacea* (*B. cepacea*) was identified in two patients. One of them was observed with AML, and did not receive any chemotherapeutic treatment. A blood culture sample was taken from a peripheral vein. Here, the patient was treated empirically with levofloxacin. The other patient suffered from relapsed Hodgkin lymphoma, and was treated according to the ESHAP chemotherapeutic regimen (which involves a combination of

high dose cytosine-arabinoside, methylprednisolone, cysplatin and etoposide) through the central venous line. Two pairs of blood culture samples were taken following a febrile episode from a catheter, and *B. cepacea* was grown from each sample.

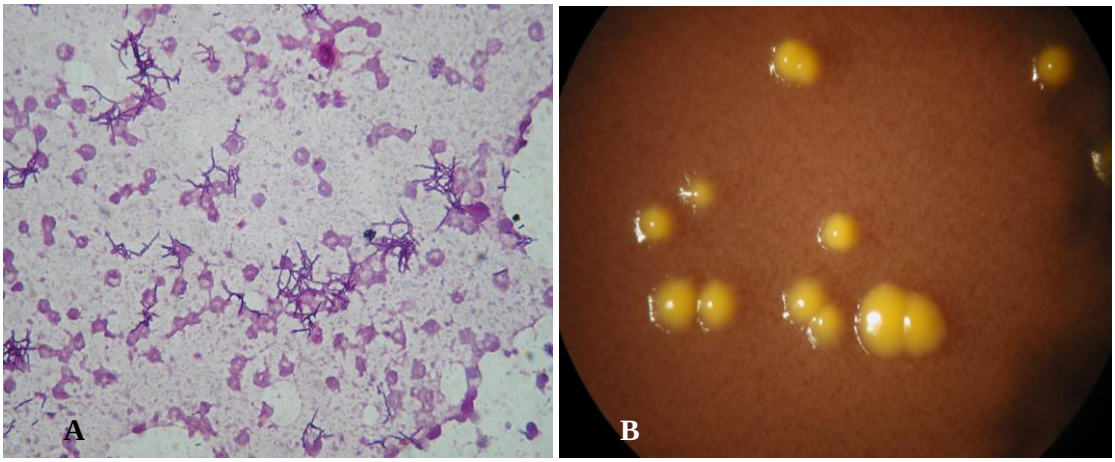

Figure 4. Microscopic results of blood culture of patients with *N. farcinica* sepsis (mag. 1 000x) **(A)** and colony morphology of *N. farcinica* on chocholate agar **(B)** (source: Terhes G.).

Between 2005 and 2008, four cases of bacteremia caused by *Fusobacterium nucleatum* (*F. nucleatum*) were detected in patients with acute leukemia at our hospital. The first case was a 51-year-old woman who was admitted to the Haematology Unit with acute myelomonocytic leukaemia; therefore cytosine arabinoside (2200 mg per day), mitoxantrone (15 mg per day) and etoposide (220 mg per day) therapies were given over five days. At the end of the chemotherapy, the patient developed a fever and oropharyngeal mucositis of World Health Organization (WHO) grade 3. The second case was a 71-year-old woman suffering from acute myeloid leukaemia, who was admitted to our department with a febrile episode, mucositis WHO grade 1 (soreness and erythema) and the possibility of perianal abscess. Laboratory investigations identified neutropenia and a high C-reactive protein level. Chest radiography revealed pulmonary infiltration in the upper right side of the lung. The third case was a 46-year-old man diagnosed with acute pre-B-cell lymphoblastic leukaemia. In his second remission, after consolidation therapy (high dose cytosine arabinoside and mitoxantrone), he developed a fever and neutropenia without mucositis despite the chemotherapeutic treatment. The fourth case was a 61-year-old woman with acute myeloid leukaemia, who had been admitted to the Haematology Unit because of a fever, cough and dyspnea. Her C-reactive protein level was elevated, and her absolute neutrophil count was 0.2xG/L. She had severe oropharyngeal mucositis of WHO grade 3, while chest radiography

showed pulmonary infiltration on the left side of the lung. During the febrile period, at least one pair of blood samples was taken in aerobic and anaerobic bottles from all patients. In each case, only anaerobic bottles gave positive signals after 35-87 hours of incubation. On the basis of a microscopic examination, because of the characteristic morphology (see Fig. 5.), the possibility of bacteraemia caused by *Fusobacterium* sp. had arisen and empiric antibiotic therapy was started immediately.

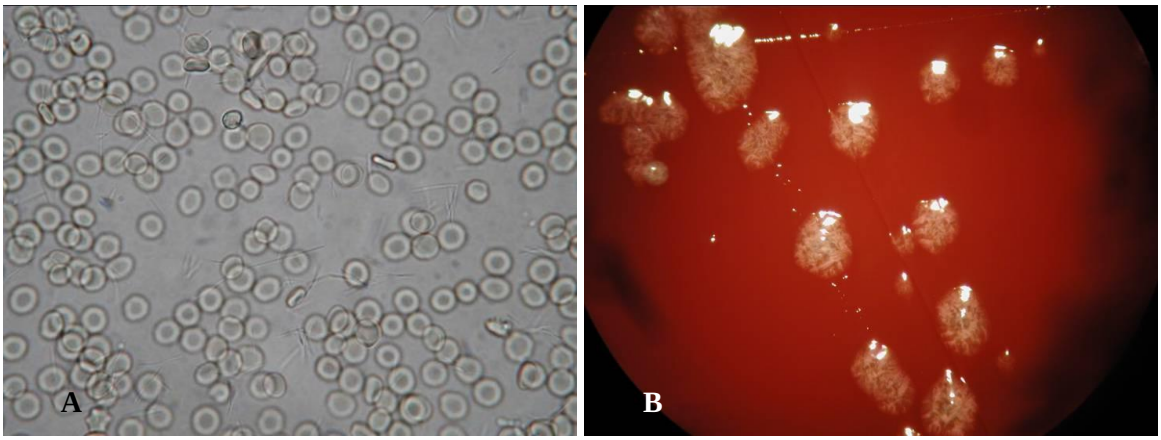

Figure 5. Characteristic microscopic findings concerning *F. nucleatum* bacteraemia (mag. 1000x) **(A)**, and colony morphology of *F. nucleatum* on anaerobic blood agar **(B)** (source Terhes G.).

Another rarely isolated pathogen from blood culture was *Achromobacter xylosoxidans* (*A. xylosoxidans*). A 41-year-old woman was admitted to our department because of acute promyelocytic leukaemia with disseminated intravascular coagulation. After the induction treatment she had febrile neutropenia. Empirical antibiotic therapy was started after taking a blood culture sample. Her clinical state was stable. A few days later, dyspnea and abdominal pain developed. Chest radiography showed pulmonary infiltration in the right side of the lung and interstitial pneumonia. A blood culture sample was taken again before changing antibiotics (carbapenem), and she was transferred to the Intensive Care Unit because of respiratory failure. She died of progressive pneumonia and acute respiratory distress syndrome a few hours later. During the febrile period, blood cultures were taken from the patient and a positive signal was generated in the case of 4 bottles. Phase contrast microscopic examination and acridine orange staining revealed long bacilli, which proved to be Gram-negative in a Gram-stained smear. After 24 hours of incubation, greyish colonies could be observed on the surface of sheep blood agar and chocolate agar as well. The amplified partial 16S rDNA PCR product of this strain was sequenced, and the generated nucleotide sequence

displayed 100% homology with *A. xylosoxidans* strain L2 2 by using the BLAST program (Fig. 6).

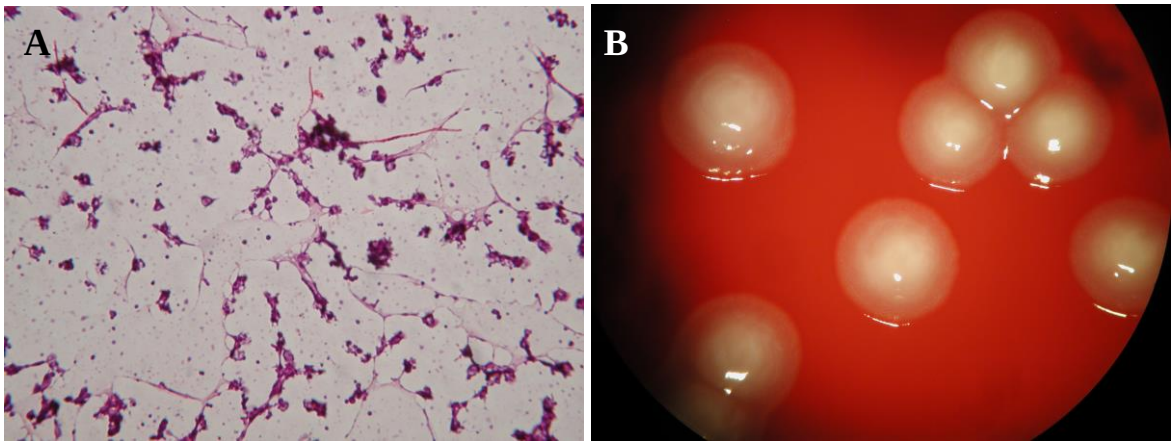

Figure 6. Characteristic microscopic results concerning *A. xylosoxidans* bacteraemia (mag. 1 000x) **(A)**, and colony morphology of *A. xylosoxidans* on blood agar **(B)** (source: G Terhes).

4.2. MONITORING THE PRESENCE OF CMV IN PATIENTS WITH HAEMATOLOGIC DISEASES

Between 2008 and 2014, 271 patients with haematological malignancies were tested for the presence of CMV DNA. In total, 1238 PCRs were carried out. The median age of the patients was 57 years (range 42-72 years) and 67% of the patients tested were above 50 years of age. The male to female ratio was 0.99; that is 135 men and 136 women. Below in Table 4, the patients' characteristics are shown. A CMV serological assay was performed for 204 patients. Here, 154 (75.5%) of the 204 patients had CMV specific IgG, while in the case of the remaining 50 (24.5%) patients the CMV IgG tests proved to be negative.

Table 4. Demographics and types of haematological malignancies of the patients examined.

Variables		
All patients	271	
Mean age (yr)	57 (range 42-72)	
Sex		
Men	135	
Women	136	
Types of underlying diseases	**Number of patients (%)**	**Number of patients underwent ASCT**
Lymphoid malignancies		
Non-Hodgkin lymphoma	68 (25.1%)	37
Hodgkin lymphoma	23 (8.5%)	18
Multiple myeloma	80 (29.5%)	71
Chronic lymphocytic leukemia	39 (14.4%)	0
Acute lymphoblastic leukemia	14 (5.2%)	0
Myeloid malignancies		
Acute myeloid leukemia	23 (8.5%)	0
Myelodysplastic syndrome	3 (1.1%)	0
Others[a]	21 (7.7%)	0
Total	**271 (100%)**	**126 (46.5%)**

[a]Severe aplastic anaemia (n=5), autoimmune disease (n=6), common variable immunodeficient syndrome (n=2), fever of unknown origin (n=8)

During the given period, 1238 plasma specimens were tested for the presence of CMV DNA using RT PCR. Out of the total plasma samples examined, 118 (9.5%) were positive, and these positive specimens were collected from 66 (24.4%) patients. 126 patients (46.5%) underwent autologous stem cell transplantation, while 145 (53.5%) of the patients examined with different malignant or non-malignant hematological diseases did not have stem cell transplantation (non-ASCT group). As regards the use of BEAM (55 patients), 13 (23.6%) specimens from patients proved to be positive for CMV DNA, while a higher CMV positivity rate (39.4%) was observed when patients were treated with melphalan (71 patients).

Twenty-four (16.6%) of 145 non-ASCT patients had CMV PCR positive specimens. Among the 24 CMV PCR positive patients, 6 of them had long-term steroid therapy as part of an ALL protocol and because of a severe haemolytic event. Before the CMV PCR positive results, 1 T-ALL patient was treated with clofarabine because of a second relapse and 3 patients were treated with a GMALL protocol. Two severe aplastic anaemia (SAA) patients were treated with anti-thymocyte globulin + cyclosporine. Four of the 10 CLL patients were given alemtuzumab (an anti-CD52 antibody), 3 patients were treated with rituximab+fludarabine+ cyclophosphamide (R-FC), 2 patients were on rituximab+bendamustine and only 1 elderly woman was treated using chlorambucil monotherapy. R-FC and alemtuzumab were used for two relapsed PTCL patients (angioimmunoblastic subtype) before positive PCR. Two patients with common variable immunodeficiency syndrome (CVID) received immunoglobulin substitution regularly. The relapsed AML patient was treated with a fludarabine-containing regimen, while in the case of MM patients bortezomib-based therapy was used. Among non-ASCT patients with positive CMV PCR results, 10 patients were asymptomatic, 14 had symptomatic reactivation, while 2 had CMV disease (1 ALL patient had colitis confirmed with colonoscopy and histology, while the other patient with common variable immunodeficient syndrome had CMV hepatitis). The most common clinical signs and symptoms of reactivation were fever (58%), diarrhoea (33%), pancytopenia (12.5%), and liver function abnormality (8.3%). Out of the 271 patients examined, 126 (46.5%) underwent autologous stem cell transplantation. High-dose chemotherapy with stem cell support was applied in patients with MM (n=71), aggressive NHL (n=37) and HL cases (n=18). Below in Table 5, the patient’s characteristics are listed.

Table 5. Characteristics of patients who underwent autologous stem cell support.

Variables	Number of patients (%)
Non-Hodgkin lymphoma	37 (29.4)
DLBCL	13
FL	4
PTCL	8
MCL	12
Hodgkin lymphoma	18 (14.3)
Multiple myeloma	71 (56.3)
Total	126 (100%)

DLBCL-Diffuse Large B-Cell Lymphoma; FL-Follicular Lymphoma; PTCL-Peripheral T-Cell Lymphoma; MCL-Mantle Cell Lymphoma

In the ASCT group, 42 (33.3%) patients had CMV PCR samples that proved positive. CMV reactivation was asymptomatic in 34 (81%) cases, in which spontaneous resolution could be observed, while 8 (19%) patients had symptomatic reactivation. All of these patients had a fever, 4 patients had cytopenia, prolonged engraftment, and only 1 patient had CMV pneumonia (and the post-mortem diagnosis was confirmed histologically). The majority of positive CMV PCR results (45.2%) were observed in the ASCT group between 20-40 days after transplantation, while 5 patients had a positive PCR result between 10-20 days after transplantation, and only 3 specimens proved to be positive 100 days after transplantation. The viral load in PCR positive specimens was under 1000 copies/ml in 87 (72%) cases. No significant differences could be observed between the CMV PCR positive and negative groups according to age, sex and initial diagnosis ($p=0.987$, $p=0.411$, $p=0.416$), respectively.

A total of 24 patients received antiviral therapy (intravenous ganciclovir 5 mg/kg twice daily or oral valganciclovir 900 mg daily) until resolution of the clinical signs and after two consecutive negative or decreasing CMV PCR results.

4.3. MONITORING THE PRESENCE OF HHV-6 IN PATIENTS WITH HAEMATOLOGIC DISEASES

Four out of 35 (11.4%) of patients tested using the PCR method post-transplant had HHV-6 positivity in the peripheral blood or CSF. Two patients had HL and two patients suffered from MM. The patients with MM were given a combination of thalidomide (Thal) and dexamethasone (Dex); in HL patients, a combination treatment of ABVD (Adriamycin, Bleomycin, Vinblastine and Dacarbazine) and DHAP (Dexamethasone, Cisplatin, Cytarabine) was applied. The HHV-6 variant A was detected only in MM patients, while the HHV-6 variant B was amplified from HL. In the case of 1 MM patient with HHV-6 variant A, CMV DNA was also detected with a low copy number (273copies/ml) from the plasma specimen using real-time PCR. The patients' characteristics are summarised in Table 6. Three of the 4 patients did not show clinical signs of infection at the time of sample collection, while patient 4 had limbic encephalitis.

Table 6. Characteristics of patients with HHV-6 PCR positive results.

Patients Age in years and sex	Underlying disease	Pretransplant treatment	Conditioning regimen	$CD34^+$ 10^6/kgw of cells	Engraftment of neutrophil/ platelet (post transplant days tratransplant days)	Type of HHV-6	Coexistence of other viral DNAemia	Clinical signs of infection	Bacterial/fungal infections
(1) 19/M	HL	ABVD DHAP ICE	BEAM	5.55	8/12	B	-	-	-/-
(2) 61/M	MM	Thal-Dex	HD Mel	5.56	10/10	A	-	-	-/-
(3) 56/F	MM	Thal-Dex	HD Mel	5.33	10/10	A	CMV	-	-/-
(4) 32/M	HL	ABVD DHAP	BEAM	5.56	8/12	B	-	Lymbic encephalitis**	-/-

HL: Hodgkin Lymphoma; MM: multiple myeloma; M: male; F: female; CMV: cytomegalovirus; **: identified from cerebrospinal fluid and MRI findings; Thal: thalidomide; Dex: dexamethasone; ABVD: Adriamycin, Bleomycin, Vinblastine and Dacarbazine; DHAP: Dexamethasone, Cisplatin, Cytarabine Q3-4w; ICE: Ifosfamide, Carboplatin, Etoposide; BEAM: Carmustine (BiCNU), Etoposide, Arabinoside and Melphalan and HD Mel: high dose Melphalan.

Patient 4, a 32- year-old man with stage II/A Hodgkin lymphoma, nodular sclerosis subtype, EBV-negative), was admitted to our haematology department in 2011. The patient had been treated with six cycles of chemotherapy according to the ABVD protocol that conferred complete remission (18). Four months later, the positron emission computer tomography (PET CT) showed an early relapse in his right supraclavicular and upper mediastinal lymph nodes, hence treatment with the DHAP regimen was started. After receiving a second course of DHAP, a peripheral blood stem cell harvest was performed ($5.56x10^6$/ body weight; CD34 positive cells). The patient was conditioned according to the BEAM protocol (26) followed by

autologous stem cell infusion. On post-transplant day 17, the patient was referred to a neurologist because of confusion. Elevated liver enzymes and differences in the kidney enzyme levels were observed, while fever appeared and disappeared periodically. The first neurological examination did not reveal any focal neurological signs. The patient was vigil, disoriented in time and space but oriented in own data; however, he was psychomotorically slow. No signs of neck-stiffness and meningeal irritation could be observed. Cranial nerves without abnormalities, normal muscle tonus and strength and distally decreased vibration sensation in both legs were noted. Minimal symmetrical hyporeflexia in the Achilles and plantar deep tendon reflexes and insecurity with the Romberg test could be seen. Otherwise, tendon reflexes, coordination and cerebellar tests proved to be normal. The neurological examination pointed to a polyneuropathy (most probably drug-induced) and there was a suspicion of hepato-renal insufficiency and metabolic encephalopathy. In November, 2012, because of deterioration in the patient's orientation and his febrile state, a suspicion of infectious origin of this condition arose. After some unsuccessful lumbar punctures, on the basis of findings during the MRI scan of the brain, the possibility of viral encephalitis, most probably herpes-encephalitis caused by HHV-6, in the temporal lobes was assumed (figs. 7A-7B).

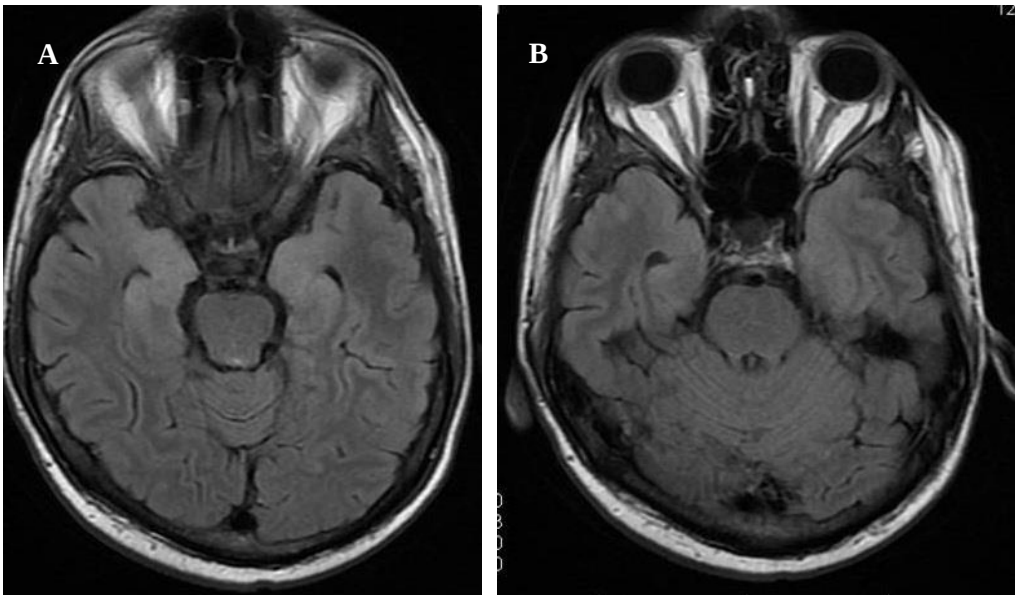

Figure 7. Magnetic resonance imaging (MRI) of the brain on 19th post-transplant day: fluid attenuated inversion recovery (FLAIR) showing bilateral encephalitis in the temporal lobe, mainly in the region of the hippocampus **(A)**. Diffusion weighted images with signal hyperintensities **(B).**

The patient was treated with gancyclovir, steroid and dexamethasone therapy intravenously. The patient's condition improved after the introduction of the therapy. His status was vigil and partially disoriented; and he had slow psychomotorium. Pupil dilatations on both sides were reactive to light. His muscles were hypotonic over all extremities. No pathological reflexes or focal neurological signs could be observed. Lumbar puncture was then performed with minimal artificial bleeding (24.661M/lof red blood cells (RBCs), 20M/l of white blood cells (WBCs) and 1.22g/l of total protein). Cytology in CSF revealed an excessive amount of RBCs with a mixed cell count of WBCs dominated by segmented neutrophils. Real-time PCR tests proved negative for HSV-1/2, CMV, EBV and enteroviruses (Enterovirus RNA Kit, DiaPro; Milan, Italy) from plasma and cerebrospinal fluid samples. HHV-6 nested PCR was carried out and HHV-6 specific nested PCR gave positive results for the HHV-6 B variant in both sample types (Fig. 8).

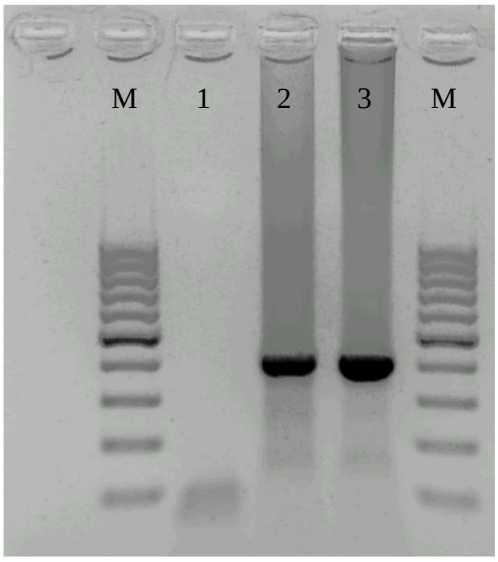

Figure 8. Detection of HHV-6 specific PCR product by agarose gelelectrophoresis (M-GeneRuler 100 bp DNA ladder; 1, negative control; 2, positive control (HHV-6 B); 3, HHV6B PCR product from a cerebrospinal fluid sample).

Three days later, a deterioration in the patient's condition was observed due to epileptic seizures. Partial seizures with oral and manual automatisms, confusion, and repetitive jerking movements in both extremities could be seen. Levetiracetam (500mg twice daily intravenously) was started and 2 days later the dose was increased to 1000mg twice daily and clobazam (10mg orally, 3 times a day) was introduced. In December, 2012, a lumbar puncture was performed; his CSF was water clear with 1M/l of RBCs, 1M/l of WBCs and 0.62g/l of total protein. Cytology testing indicated 1-2 RBCs, 18 lymphocytes and 2 macrophages.

HHV-6 nested PCR gave a positive result but the CSF was negative for HSV-1/2, CMV, EBV, and enteroviruses. Parenteral gancyclovir (500mg twice daily) therapy was given for 21 days and followed by oral valgancyclovir (450mg twice daily) for four weeks. His mental and neurological state recovered and could be discharged from the transplant unit on 58th post-transplant day (Fig. 9).

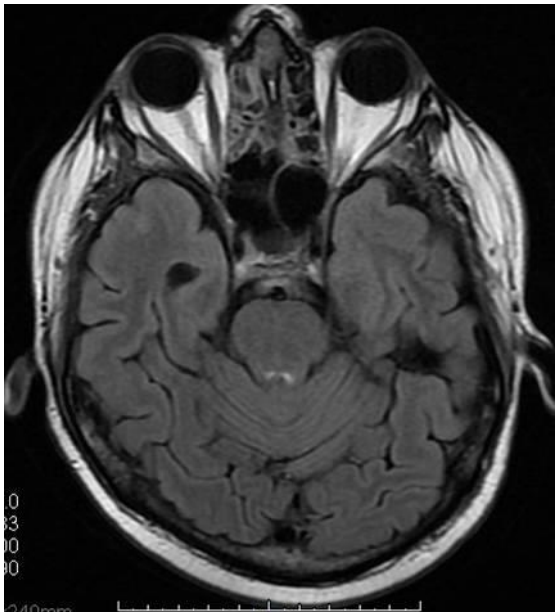

Figure 9. Signal hyperintensities resolved on both sides of temporal lobe, consequent atrophia with dilatation of the sulcus and chambers.

5.DISCUSSION

5.1. BLOODSTREAM INFECTIONS IN PATIENTS WITH HAEMATOLOGIC MALIGNANCY

Although major advances in the care of cancer patients over the past several decades have resulted in improved survival rates, infectious complications remain a significant cause of morbidity and mortality. To successfully identify, treat, and prevent infections, a comprehensive understanding of risk factors that predispose to infection and of commonly encountered pathogens is necessary. In addition, clinicians must keep abreast of the changing epidemiology of infections in this population. As therapeutic modalities continue to evolve, as established pathogens become increasingly drug resistant, and as new pathogens are discovered, successful management of infections will continue to present challenges in the years to come. Febrile neutropenia is the most common complication of chemotherapy in patients with haematologic malignancy. This may have an influence on the chemotherapy and dose reduction applied; moreover, treatment delays may be observed if febrile neutropenia is present. These have an unfavourable long-term outcome in otherwise curable malignancy [62]. In patients with haematologic malignancy, bloodstream infections are the most important bacterial infections. Despite developments in microbiological diagnosis and antimicrobial therapy, these infections are responsible for the large proportion of nosocomial infections worldwide. In the early 1960s, the importance of bloodstream infection in neutropenic patients had been recognised, hence empirical treatment protocols were established for mainly Gram-negative bacteria, because at that time, the most common pathogens associated with bloodstream infections were Gram-negative bacilli [62]. Later, the spectrum of pathogens associated with BSI shifted from Gram-negative to Gram-positive bacteria due to the increased use of antibiotic prophylaxis and indwelling catheters allowing colonisations and infections. Nowadays, the most common pathogens isolated from the blood are coagulase-negative staphylococci and various antibiotic-resistant bacteria including multidrug resistant Gram-negative bacteria and VRE. In the majority of cases, the source of these infections is unknown in spite of various efforts to find them. The recognition of changes in the epidemiology of BSIs is very important in order to modify the antibiotic policy, because on the basis of these findings, we can reduce the infection-related morbidity and mortality [95, 104]. In our retrospective survey, during the 4-year study period, the incidence of bacteraemia was 20.4%. Similar findings were reported in the literature;

Klastersky *et al*. showed that the incidence of bacteraemia was 23% in cancer patients, while Viscoli *et al*. found that bacteraemia occurred in 29% of patients with febrile neutropenia [64, 109]. Our findings were correlated with the above-mentioned literature data, because from the majority of blood culture bottles (13.7%), Gram-positive bacteria were isolated. Sixty-five percent of Gram-positive bacteria belonged to coagulase-negative staphylococci. However, in a study by Winston *et al.* in North America, Gram-negative bacteria (55.6%) were responsible for the majority of bacteraemia in febrile neutropenic patients [114]. At the same time, other researchers from Italy and France demonstrated that the most important isolates in neutropenic patients are Gram-positive bacteria, including coagulase-negative staphylococci or streptococci, while Gram-negative organisms including *E. coli* or *Klebsiella* spp., *P. aeruginosa* constituted a smaller portion of the isolates [40, 35]. In our case, the most frequently used empiric treatment in this patient group is piperacillin/tazobactam, or if the patient has colitis or the possibility of abdominal infection arises, imipenem or meropenem are frequently used antibiotics. Thus the increased incidence of Gram-positive bacteria can be accounted for by the empiric antibiotic treatment applied, while the presence of coagulase-negative staphylococci may be attributed to the frequently used central venous catheters. The incidence of bacterial species in blood cultures can be influenced by the chemotherapy applied. In our case, 30% of patients with acute leukaemia received high dose Ara-C chemotherapy, and 15% and 12% of patients were treated with fludarabine and Ara-C plus idarubicin, respectively. On the basis of literature data, an increasing prevalence of Gram-positive cocci in febrile neutropenic patients could be observed after high-dose cytarabine chemotherapy, and this was confirmed by our results [35]. Cordonnier *et al*. showed that the prevalence of staphylococci is higher than the prevalence of streptococci and enterococci in febrile neutropenic patients [35]. Similarly, our results bore this out, because among Gram-positive bacteria the majority of the isolated strains were coagulase-negative staphylococci, 6.7% and only 2.4% of Gram-positive bacteria belonged to *Enterococcus* spp. and beta-hemolytic streptococci, respectively. 331 blood culture samples proved to be positive for coagulase-negative staphylococci, which had been collected from 161 febrile episodes of 149 patients. In 50 febrile neutropenic episodes, coagulase-negative staphylococci were identified as the cause of fever, and these were confirmed with the coexistence of skin, soft tissue and central venous catheter-related infection. According to the clinical data and examination of the manifestations, the cause of the remaining 111 cases was probably due to contamination.

In the case of blood cultures that had positive results, Gram-negative bacteria were cultured, and the majority of these proved to be positive for *E. coli* (52%). The second most common isolate was *P. aeruginosa* (14%), while the third was *Klebsiella* spp (9.6%). Similar findings were obtained by Ramphal [95]. In this review, the results of four articles were analysed, and among Gram-negative organisms, the key pathogens were also *E. coli*, *Klebsiella* spp. and *P. aeruginosa* [95].

Among rarely isolated bacteria, *A. xylosoxidans*, *B. cepacia* are usually associated with catheter-related sepsis, while *S. maltophilia* usually causes nosocomial bacteraemia [92]. However, the possible source of *H. influenzae, Neisseria* spp., *Gemella* spp. is the damaged oral mucosa. The identification of the isolated strain from bloodstream infection using traditional biochemical methods or automatic identification systems is sometimes unsuccessful because of the low biochemical activity of the isolated strain or if the isolated species cannot be found in the library of the automatic identification system, the result of identification will be misleading. In these cases, molecular methods like universal bacterial PCRs and sequencing or the use of MALDI-TOF, provide the opportunity to obtain the correct species name, as we saw in the case of bacteraemia caused by *Nocardia* sp., and *A. xylosoxidans*. Because of the fastidious or slow-growing nature of some rarely isolated bacteria, special nutriments and culture conditions are required for a successful culture test. Thus in the case of the possible presence of these opportunistic pathogens, the past medical history of the patients and communication between clinicians and microbiologists are essential to help confirm the presence of these bacteria. This situation may be observed in the case of nocardiosis in patients with cancer. Nocardiosis is most frequently described as an opportunistic infection in immunocompromised patients, especially in patients with depressed cell-mediated immunity. Pulmonary nocardiosis is the most common clinical presentation of this infection, while among the extrapulmonary forms, central nervous system (CNS) involvement is quite common [20, 107, 113]. Nocardia bacteraemia is a rarely described clinical entity and in the majority of patients (64%) concurrent pulmonary, cutaneous (28%) and CNS involvements (19%) may be observed [113]. In the case of disseminated nocardiosis, two or more organs are infected, and in the majority of cases, due to airway transmission the lung is also affected. Several publications described case reports about the presence of *N. farcinica* in various infections, but according to Christidou *et al*. up to 2004, only 11 English publications were found where *N. farcinica* bacteraemia was described [29].

The majority of patients were male and had one or more predisposing factors, and the lung was the most common primary site of infection. The mortality rate was 41.7%, and a similarly poor outcome associated with *N. farcinica* was previously reported by Torres *et al.* [106]. General treatment recommendations for nocardiosis are difficult to provide due to the lack of controlled trials and variable *in vitro* antibiotic susceptibility patterns [113]. What is more, it is difficult to perform antimicrobial susceptibility testing because of the slow growing nature of several *Nocardia* sp., inoculum consistency and interpretation of the cut-off [101]. The treatment of *N. farcinica* infections is also problematic, because the majority of clinical isolates are multi-resistant, hence in our case the isolated strain was susceptible only to imipenem and amikacin.

Because of the possible presence of unusual pathogens, such as anaerobic bacteria in bloodstream infections, the use of various blood culture bottles including anaerobic bottles should be considered. These confirmed those cases where a microbiological investigation revealed the presence of *F. nucleatum* in the blood cultures of four patients. In three cases, only one anaerobic bottle gave a positive signal. All the patients had clinical symptoms of sepsis; since the isolated *F. nucleatum* is not a member of the skin flora, its role as a possible contaminant was excluded. The mean number of days required for the blood culture bottles to become positive was 2.6 days (range 35 to 87 h). All four *F. nucleatum* strains proved to be susceptible to all the anti-anaerobic antibiotics tested (penicillin, amoxicillin/clavulanic acid, clindamycin, cefoxitin, imipenem and metronidazole). In each case, the underlying disease was haematological malignancy, acute myeloid or lymphoblastic leukaemia. Two patients (cases 1 and 3) received chemotherapy before the development of a febrile period, one of them had confirmed oropharyngeal mucositis, while with the other, there were no clinical symptoms of this, but the therapy used is known to make patients susceptible to the development of oral mucositis. In case 4, the patient had severe oropharyngeal mucositis without previous chemotherapy, while in case 2, the peritonsillar region and the soft palate were edematous. Fanourgiakis et al. [43] reported fifteen cases of bacteraemia caused by *Fusobacterium* spp. over a period of 6.5 years. Among these, thirteen patients were neutropenic, their mean age was 53 years, and the majority of them had underlying haematological malignancies and oral postchemotherapy mucositis. As oral mucositis was observed in almost all the patients, and this was the most likely source of the bacteraemia. This study also demonstrated that a relatively long incubation period (about 5 days) was

sometimes necessary to get positive signals in the case of anaerobic bottles. On the basis of literature data, in the 1960s and 1970s, the incidence of anaerobic bacteraemia increased due to improvements in anaerobic laboratory culture methods; and later this was followed by a decrease. Then between 1993 and 2004, the mean incidence of anaerobic bacteraemias once again increased [89].

5.2. MONITORING THE PRESENCE OF CMV IN PATIENTS WITH HAEMATOLOGIC DISEASES

Because of the increasing number of drugs associated with severe and prolonged cell-mediated immunodeficiency, CMV infection is a major opportunistic infection among patients with malignant haematological diseases. The vast majority of publications have presented data about the occurrence of CMV reactivation and disease in haematological patients following stem cell transplantation - predominantly in an allogeneic setting-, hence little information is available about patients treated for haematological diseases without stem cell support [7, 25, 52, 86]. In our study, we retrospectively analysed epidemiological data and the incidence of CMV infection in patients with malignant haematological diseases over a 6-year period. In the ASCT group, a higher rate of CMV infection (33.3%) was observed than that in the non-ASCT group (16.6%). Similarly, a higher rate of CMV infection (26-39%) was observed in autologous transplantation recipients in other international studies, mainly in patients who had received $CD34^+$ selected autograft [44, 98]. Holmberg *et al.* also reported higher reactivation rates (22.6%) in patients who had received $CD34^+$ selected autograft, than those in unselected cases (4.2%) during the first 100 post-transplant days [54]. However, a low risk of CMV infection was observed earlier in patients undergoing autologous stem cell transplantation [111]. In patients who had received pre-emptive anti-CMV treatment, CMV disease was not observed in association with ASCT [99, 37]. CMV pneumonia was identified in 2% of patients with ASCT and these were associated with CMV seropositivity before transplantation. Marchesi *et al.* also examined the rate of CMV reactivation after ASCT and he found that 11% of patients had required antiviral treatment because of CMV infection or end-organ diseases [81]. The majority of CMV reactivation occurred in patients with lymphoma (16%), while CMV PCR positivity was detected in 8% of myeloma patients [81]. Al-Rawi *et al.* (2015) examined CMV reactivation with a CMV pp65 antigenemia test, and observed a CMV reactivation in 37 out of 210 patients (17.6%), and 94.6% of patients with a

positive antigenemia assay were treated with anti-CMV therapy. Due to the anti-CMV therapy, symptomatic CMV infection or end-organ disease was not observed, and the mortality was 29.7% in this group [3]. In our case, the majority of ASCT patients had asymptomatic reactivation. Eight patients (19%) suffered from symptomatic reactivation, while only 1 patient had CMV disease with 6225 copies/ml; and in this case, interstitial pneumonia and acute respiratory distress syndrome were demonstrated after a chest radiographic examination. The patient's condition rapidly declined and the subject died two days later. The postmortem histological examination confirmed the diagnosis. As data published previously showed, the incidence of CMV pneumonia is 2-9% in ASCT recipients, with an extremely high mortality rate [7, 66, 68]. Marchesi and colleagues found a significantly higher transplant-related mortality rate in CMV reactivated patients (8.4%+/-4.7% versus 1.7%+/-0.8%; p=0.047), while sex, diagnosis (NHL versus MM) and types of conditioning regimen did not display any significant differences [81]. Likewise in our observations, for ASCT and non-ASCT patients, no observed significant differences were found between the CMV PCR positive and negative groups according to age, sex (p=0.671, p=0.411), and mortality rate (p=0.429). On the basis of a statistical analysis, in the ASCT group, regarding the types of malignancies (MM, NHL, HL), no difference could be detected in the rate of CMV reactivation at a significance level of 5%, while differences were confirmed at a significance level of 10% (p=0.076 versus p=0.038). NHL patients with a B-cell phenotype who had had a transplant were treated with rituximab as part of a pretransplant induction therapy, while only thirteen patients were treated with rituximab as part of a conditioning therapy. In a comparison of two types of conditioning treatment (BEAM+R-BEAM versus melphalan), significant differences were found to exist on the prevalence of CMV reactivations (23.6% versus 42.3%; p=0.029). For R-BEAM, only one patient with NHL had a CMV positive PCR result. Because of the low number of cases, we did not look for a correlation between R-BEAM versus BEAM and CMV reactivation. Jain *et al.* (2016) analysed the results of CMV PCR in ASCT patients. In that study, 24% of patients were treated with rituximab as part of a pretransplant induction therapy or conditioning treatment. The rate of CMV PCR positivity was 2.9% in the case of the 239 patients who were examined. CMV viraemia was detected in seven patients, and three of them were treated with rituximab so this treatment for patients was not a significant risk factor for developing symptomatic CMV reactivation (p=0.34) [57]. Even though the number of patients with NHL was lower in our study, we obtained similar findings. In the case of patients with MM, no

significant differences were seen between the type of pretransplant induction treatment (bortezomib versus thalidomide) and a CMV positive/negative group (45% vs 46.7%; p=0.912). According to Marchesi et al, with MM patients treated with tandem ASCT, CMV reactivation after the first ASCT was more common when the induction treatment contained novel agents (odds ratio [OR]: 9.897; 95% confidence interval [CI]); p=0.021) [80]. It seems that high dose of dexamethasone applied in cases of patients undergoing this type of induction treatment has a detrimental effect on cell-mediated immunity. Still, proteasome inhibitors and immunomodulators may have an adverse effect on the T cell (NK, CD4+ and CD8+) count and function [12, 80, 90]. In our study, the majority of patients with lymphoid malignancies had CMV reactivation in the non-ASCT group, while the number of patients with myeloid malignant disease who suffered from a CMV infection was low (just 1 case). In the non-ASCT group, fourteen patients had symptomatic CMV infections, and among these, CMV disease was observed in two cases (colitis and hepatitis). Similarly, the authors of several publications said that the risk for reactivation is highest among patients with lymphoproliferative diseases and those who were being treated for decreasing T-cell function with a drug such as alemtuzumab [5, 52, 59]. Han et al obtained similar findings when they examined CMV reactivation using a CMV pp65 antigenemia assay in patients with malignant haematological diseases [52]. They demonstrated that a significantly higher rate of reactivation could be detected in lymphoid malignancies than in myeloid ones. Another centre (Peter MacCallum Cancer Center in Melbourne, Australia) examined the presence of CMV DNA taken from whole blood in patients undergoing various chemotherapeutic regimens [hyper-CVAD (cyclophosphamide, vincristine, doxorubicin, dexamethasone, methotrexate and cytarabine), fludarabine-containing regimens, alemtuzumab] and ASCT. The rate of CMV reactivation was 4.6, 4.2, 9.7, 2.6 and 50% in patients that had a fludarabine-based therapy, ASCT, hyperCVAD, rituximab treatment and alemtuzumab treatment, respectively [7]. Likewise, in our non-ASCT patient group with CMV reactivation, five patients were treated with alemtuzumab, two with a fludarabine-based therapy, and those who had the hyper-CVAD therapy did not have reactivation. Our findings are consistent with those found in observations published earlier [7, 13, 52, 99].

5.3. MONITORING THE PRESENCE OF HHV-6 IN PATIENTS WITH HAEMATOLOGIC DISEASES

Autologous peripheral stem cell transplantation is a widely used procedure in the treatment of multiple myeloma, Hodgkin and non-Hodgkin lymphoma [102]. In this patient group, viral infection is one of the most important causes of post-transplant morbidity and mortality. In this setting, HHV-6 encephalitis represents a very rare type of viral infection and it is a severe life-threatening complication in ASCT recipients. Pagter *et al*. summarised several studies in which they analysed HHV-6 reactivation during haematopoietic stem cell transplantation. Eighteen studies were examined and, in the case of twelve of these, one month after haematopoietic stem cell transplantation, HHV-6 reactivation occurred. The incidence of HHV-6 reactivation ranged from 28% to 78% in allogeneic and autologous patients. Delayed platelet engraftment displayed a significant association with the presence of HHV-6 DNA [39]. Imbert-Marcille *et al*. studied 846 peripheral blood samples obtained from 92 consecutive patients (526 from autologous and 320 from allogeneic transplant recipients) for HHV-6 DNA; 18.3% of these samples gave positive results. Similar to other studies, the incidence of active HHV-6 infection was 42.5 % after autologous transplantation (27/64) and also allogeneic (12/28) transplantation [56]. Ljungman *et al*. also confirmed that high HHV-6 viral load was associated with the development of HHV-6 disease, myelosuppression and prolonged engraftment, mainly in megakaryocytic and erythroid cell lineages [71]. In addition to this, only patient 4 displayed clinical signs of infection due to HHV-6 reactivation. In our study, the number of HHV-6 positive samples was lower than that in previous studies; however, the number of patients analysed was also lower. Delayed engraftment was not observed in our study. In immunocompromised patients with signs of encephalopathy, a change in mental status and loss of short-term memory may help us to differentiate several causes not only intra-cerebral bleeding from thrombocytopaenia, direct toxicity of drugs, electrolite and other metabolic disturbances but infectious complications as well. Bommer *et al*. described encephalitis and pneumonitis caused by HHV-6 in a young patient with relapsed Hodgkin's lymphoma that had undergone autologous stem cell transplantation. In that case, mental disturbances and convulsion were developed without abnormality in the first MRI image of the brain on the twelfth post-transplant day, while a CT scan of the chest revealed diffuse interstitial pneumonia on both sides of the lung. Bronchoalveolar lavage and CSF were positive for HHV-6 DNA. The patient was successfully treated with foscarnet followed by oral valgancyclovir for another six weeks [17]. We also had a patient with encephalitis due

to HHV-6 reactivation shortly after transplantation. Multiple EDTA blood and two CSF specimens taken from this patient gave positive HHV-6 PCR results, but no viral or bacterial pathogens were detected as a cause of encephalitis. Because of confirmed HHV-6 encephalitis, parenteral gancyclovir was started followed by oral valgancyclovir. During this course of treatment, the neurological signs were resolved successfully with minimal short-term memory dysfunction; and his haematological disease is now in complete remission. Similar to our findings, Imbert-Marcille *et al*. confirmed that active HHV-6 infection frequently occurred three or four weeks after transplantation [56]. However, lacking randomised clinical trials, treatment with gancyclovir, foscarnet or cidofovir is recommended for patients with HHV-6 encephalitis [118, 94]. In our case, gancyclovir treatment resolved the neurological signs with minimal sequelae.

6. CONCLUSIONS

In this study our key aim was to evaluate the local epidemiology of most frequent infectious complications in immunocompromised patients, especially bacteraemia, CMV and HHV-6 infections in haematological patients, and following autologous stem cell transplantation.

1. We evaluated the local occurrence of bacterial species that caused bloodstream infections in febrile neutropenic episodes between 2005 and 2008. In this study period among patients with febrile neutropenic episodes using microbiological culture, 20.4% of BC samples were positive, and in 67.1 % of positive BC samples Gram-positive bacteria were detected. The most frequent Gram-positive isolates were CNS (65%), *S. aureus* (10%) and the *Enterecoccus* species (6.7%). Here, CNS was identified in 331 cases and it was collected from 161 febrile neutropenic episodes of 149 patients. In 50 FN episodes, CNS was relevant as a causative agent of fever, because of the coexistence of skin, soft tissue and central venous catheter-related infections. The remaining 111 cases that had a positive BC result for CNS were presumed to be due to contamination, hence it is necessary to stress importance of a well-timed and precise sampling of a BC to avoid contamination. Gram-negative bacteria were isolated in 32.9 % of the positive BC results, and a high prevalence of *E. coli* (52%) was detected. This can be explained by the widespread use of indwelling catheters and more toxic high-dose chemotherapeutic regimens that can cause severe mucosal damage.

2. In this study we characterised bloodstream infections caused by “unusual” pathogens and described some instructive cases that were caused by these interesting, rarely isolated pathogens. The identification of rarely isolated pathogens (*A. xylosoxidans, B. cepacia, F. nucleatum* and *N. farcinica*) not only required the use of traditional biochemical methods and automatic identification systems, but also special conditions for a successful culture; and in these cases bacterial PCRs and sequencing or MALDI-TOF led us to determine the exact microbial species.

3. Out of the 271 patients with haematological malignancies all were tested using the CMV DNA method, and in 66 cases (24.4%) positive results were detected. Among CMV PCR

positive patients, 42 underwent ASCT. In the non-ASCT patient-group the risk of reactivation was highest among patients with lymphoproliferative disorders, and those that had received treatment associated with decreasing T-cell function (purine analogues, alemtuzumab). In the ASCT group according to the type of underlying disease (MM versus NHL patients), borderline significant differences were detected in the rate of CMV reactivation, and by comparing the type of conditioning regimen, it was found that significant differences exist across BEAM+R-BEAM versus Melphalan ($p=0.029$) regimens.

4. After ASCT, 11.4% of the patients examined were found to be positive for HHV-6 after taking a sample of PB or CSF and applying the PCR method. Only one patient had been diagnosed with limbic encephalitis caused by HHV-6, and he was treated successfully with GCV. However without a randomised clinical trial, GCV, Foscarnet or CDV is recommended for patients with HHV-6 encephalitis.

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REFERENCES

1. Agut H, Bonnafous P, Gautheret-Dejean A. Laboratory and Clinical Aspects of Human Herpesvirus 6 infections**.** Clin Microbiol Rev 2015; 28(2): 313–35. Published online 2015 Mar 11. doi: 10.1128/CMR.00122-14

2. Alonso CD, Dufresne SF, Hanna DB, Labbe AC, Treadway SB, Neofytos D, Belanger S, Huff CA, Laverdiere M, Marr KA. *Clostridium difficile* infection after adult autologous stem cell transplantation: A multicenter study of epidemiology and risk factors. Biol Blood Marrow Transplant 2013; 19(10): 1502–8.

3. Al-Rawi O, Abdel-Rahman F, Al-Najjar R Cytomegalovirus Reactivation in Adult Recipients of Autologous Stem Cell Transplantation: a Single Center Experience. Mediterr J Hematol Infect Dis 2015; 24;7(1):e2015049. doi: 10.4084/MJHID.2015.049.

4. Anaissie EJ, Kontoyiannis DP, O'Brien S, Kantarjian H, Robertson L, Lerner S, Keating MJ. Infections in patients with chronic lymphocytic leukemia treated with fludarabine. Ann Intern Med 1998; 1; 129(7): 559-66.

5. Angarone M. Epidemiology and prevention of viral infections in patients with hematologic malignancies. Infectious Disorders-Drug Targets 2011; 11 (1): 27-33.

6. Asano-Mori Y, Oshima K, Sakata-Yanagimoto M, Nakagawa M, Kandabashi K, Izutsu K, Hangaishi A, Motokura T, Chiba S, Kurokawa M, Hirai H, Kanda Y. High-grade cytomegalovirus antigenemia after hematopoietic stem cell transplantation. Bone Marrow Transplant 2005; 36(9): 813-9.

7. Ashley P Ng, Worth L, Chen L, Seymour JF, Prince HM, Slavin M, Thursky K. Cytomegalovirus DNAemia and disease: incidence, natural history and management in settings other than allogeneic stem cell transplantation. Haematologica 2005; 90(12): 1672-9.

8. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, Casassus P, Maisonneuve H, Facon T, Ifrah N, Payen C, Bataille R. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. N Engl J Med 1996; 335: 91–7.

9. Augustson BM, Begum G, Dunn JA, Barth NJ, Davies F, Morgan G, Behrens J, Smith A, Child JA, Drayson MT. Early mortality after diagnosis of multiple myeloma: analysis of patients entered onto the United kingdom Medical Research Council trials between 1980 and 2002--Medical Research Council Adult Leukaemia Working Party. J Clin Oncol 2005; 23(36): 9219-26.

10. Balletto E, Mikulska M. Bacterial infections in hematopoietic stem cell transplant recipients. Mediterr J Hematol Infect Dis 2015; 7 (1): e2015045

11. Barlogie B, Hall R, Zander A, Dicke K, Alexanian R. High-dose melphalan with autologous bone marrow transplantation for multiple myeloma. Blood 1986; 67 (5): 1298-1301.

12. Basler M, Lauer C, Beck U, Groettrup M. The Proteasome Inhibitor Bortezomib Enhances the Susceptibility to Viral Infection. J Immunol 2009; 183; 6145-50.

13. Bilgrami S, Aslanzadeh J, Feingold JM, Bona RD, Clive J, Dorsky D, Edwards RL, Tutschka PJ. Cytomegalovirus viremia, viruria, and disease after autologous peripheral blood stem transplantation: no need for surveillance. Bone Marrow Transplant 1999; 24: 69-73.

14. Blimark C, Holmberg E, Mellqvist UH, Landgren O, Björkholm M, Hultcrantz M, Kjellander C, Turesson I, Kristinsson SY. Multiple myeloma and infections: a population-based study on 9253 multiple myeloma patients. Haematologica 2015; 100 (1): 107–13.

15. Bodey GP, Buckley M, Sathe YS, Freireich EY. Quantitative relationship between circulating leukocytes and infections in patients with acute leukemia. Ann Intern Med 1966; 64; 328-40.

16. Boeckh M, Ljungman P. How we treat cytomegalovirus in hematopoietic cell transplant recipients. Blood 2009; 113 (23): 5711–9.

17. Bommer M, Pauls S, Greiner J. Challenging complications of treatment-human herpesvirus 6 encephalitis and pneumonitis in a patient undergoing autologous stem cell transplantation for relapsed Hodgkin's disease: a case report. Virol J 2009; 6: 111.

18. Bonadonna G, Zucali R, Monfardini S, De Lena M, Uslenghi C. Combination chemotherapy of Hodgkin's disease with adriamycin, bleomycin, vinblastine and imidazole carboxide versus MOPP. Cancer 1975; 36: 252-9.

19. Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. Clin Microbiol Rev 1997; 10: 521-67.

20. Brown-Elliott B, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin Microbiol Rev 2006; 19: 259-82.

21. Bruminhent J, Wang ZX, Hu C, Wagner J, Sunday R, Bobik B, Hegarty S, Keith S, Alpdogan S, Carabasi M, Filicko-O'Hara J, Flomenberg N, Kasner M, Outschoorn UM, Weiss M, Flomenberg P. *Clostridium difficile* colonization and disease in patients undergoing hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2014; 20(9): 1329–34.

22. Burnett AK. The treatment of AML: current status and novel approaches. Hematology 2005;10 S 1:50-3.

23. Busca A. Viral infections in patients with haematological malignancies. Leukemia 2012; S.1:S24-S25.

24. Caserta MT, Mock DJ, Dewhurst S. Human Herpesvirus 6. Clinical Infectious Diseases 2001; 33: 829–33.

25. Chemaly RF, Torres HA, Hachem RY, Nogueras GM, Aguilera EA, Younes A, Luna MA, Rodriguez G, Tarrand JJ, Raad II. Cytomegalovirus pneumonia in patients with lymphoma. Cancer 2005; 104(6): 1213-20.

26. Chopra R, Linch DC, McMillan AK, Blair S, Patterson KG, Moir D, Richards JD, Cervi P, Kinsey S, Goldstone AH. Mini-BEAM followed by BEAM and ABMT for very poor risk Hodgkin's disease. Br J Haematol 1992; 81(2): 197-202.

27. Chou S, Marousek G, Parenti DM, Gordon SM, LaVoy AG, Ross JG, Miner RC, Drew WL. Mutation in region III of the DNA polymerase gene conferring foscarnet resistance in cytomegalovirus isolates from 3 subjects receiving prolonged antiviral therapy. J Infect Dis 1998; 178: 526-30.

28. Chou S, Marousek GI, Van Wechel LC, Li S, Weinberg A. Growth and drug resistance phenotypes resulting from cytomegalovirus DNA polymerase region III mutations observed in clinical specimens. Antimicrob Agents Chemother 2007; 51: 4160-2.

29. Christidou A, Maraki S, Scoulica E, Mantadakis E, Agelaki S, Samonis G. Fatal *Nocardia farcinica* bacteremia in a patient with lung cancer. Diagn Microbiol Infect Dis 2004; 50: 135-9.

30. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15 (ISBN 1-56238-556-9)

31. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. CLSI document M100-S16 (ISBN 1-56238-588-7)

32. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement. CLSI document M100-S17 (ISBN 1-56238-625-5)

33. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Eighteenth Informational Supplement. CLSI document M100-S18 (ISBN 1-56238-653-0)

34. CLSI (2007). Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria, approved standard, 7th edn, M11–A7 (ISBN 1–56238–626–3) Wayne PA: Clinical and Laboratory Standards Institute.

35. Cordonnier C, Buzyn A, Leverger G Herbrecht R, Hunault M, Leclercq R, Bastuji-Garin S. Club de Réflexion sur les Infections en Onco-Hématologie. Epidemiology and risk factors for gram-positive coccal infections in neutropenia: toward a more targeted antibiotic strategy. Clin Infect Dis 2003; 36: 149-58.

36. Crawford J, Dale DC, Lyman GH. Chemotherapy-induced neutropenia: risks, consequences, and new direction for its management. Cancer 2004; 100 228-37.

37. Crippa F, Holmberg L, Carter RA, Hooper H, Marr KA, Bensinger W, Chauncey T, Corey L, Boeckh M. Infectious complications after autologous CD34-selected peripheral blood stem cell transplantation. Biol Blood Marrow Transplant 2002; 8(5): 281-9.

38. Crumpacker CS. Ganciclovir. N Engl J Med 1996; 335: 721-9.

39. de Pagter PJ, Schuurman R, Meijer E, van Baarle D, Sanders EAM, Boelens JJ. Human herpesvirus type 6 reactivation after haematopoietic stem cell transplantation. J Clin Virol 2008; 43: 361-6.

40. Del Favero A, Menichetti F, Martino P, Bucaneve G, Micozzi A, Gentile G, Furno P, Russo D, D'Antonio D, Ricci P, Martino B, Mandelli F; Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) Infection Program. A multicentre, double-bind, placebo-controlled trial comparing piperacillin-tazobactam with and without amikacin as empiric therapy for febrile neutropenia. Clin Infect Dis 2001 ; 33: 1295-1301.

41. Drobyski WR, Knox KK, Majewski D, Carrigan DR. Brief report: fatal encephalitis due to variant B human herpesvirus-6 infection in bone marrow-transplant recipient. N Engl J Med 1994; 330: 1356-60.

42. Erice A, Chou S, Biron KK, Stanat SC, Balfour HH Jr, Jordan MC. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. N Engl J Med 1989;320: 289-93.

43. Fanourgiakis P, Vekemans M, Georgala A, Daneau D, Vandermies A, Grenier P, Aoun M. Febrile neutropenia and *Fusobacterium* bacteremia: clinical experience with 13 cases. Support Care Cancer 2003; 11: 332–5.

44. Fassas AB, Bolaños-Meade J, Buddharaju LN, Rapoport A, Cottler-Fox M, Chen T, Lovchik JC, Cross A, Tricot G. Cytomegalovirus infection and non-neutropenic fever after autologous stem cell transplantation: high rates of reactivation in patients with multiple myeloma and lymphoma. Br J Haematol 2001; 112 (1): 237-41.

45. Forslöw U, Mattsson J, Ringden O, Klominek J, Remberger M. Decreasing mortality rate in early pneumonia following hematopoietic stem cell transplantation. Scand J Infect Dis 2006; 38(11-12): 970-6.

46. Freifeld AG, Bow EJ, Sepkowitz KA, Boeckh MJ, Ito JI, Mullen CA, Raad II, Rolston KV, Young JA, Wingard JR, *et al*. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 Update by the Infectious Diseases Society of America. Clin Infect Dis 2011; 52: 427-31; http://cid.oxfordjournals.org/content/52/4/e56.full.pdf+html

47. Gencer S, Salepci T, Ozer S. Evaluation of infectious etiology and prognostic risk factors of febrile episodes in neutropenic cancer patients. J Infect 2003; 47(1): 65-72.

48. Gentile G, Micozzi A, Girmenia C, Iori AP, Donati PP, Capria S, Martino P. Pneumonia in allogenic and autologous bone marrow recipients. A retrospective study. Chest 1993; 104 (2): 371-5.

49. Giustinetti G, Mikulska M. Bloodstream infections in neutropenic cancer patients: A practical update. Virulence 2016; 7: 280-97.

50. Gonzalez-Barca E, Fernandez-Sevilla A, Carratala J, Granena A, Gudiol F. Prospective study of 288 episodes of bacteremia in neutropenic cancer patients in a single institution. Eur J Clin Microbiol Infect Dis 1996; 15: 291-6. PMID: 8781879; http://dx.doi.org/10.1007/BF01695660

51. Gratwohl A. Activity survey and historical perspective of autologous stem cell transplantation in Europe. Semin Hematol 2007; 44(4): 220-6.

52. Han XY. Epidemiologic analysis of reactivated cytomegalovirus antigenemia in patients with cancer. J Clin Microbiol 2007; 45(4): 1126-32.

53. Hermouet S, Sutton CA, Rose TM, Greenblatt RJ, Corre I, Garand R, Neves AM, Bataille R, Casey JW. Qualitative and quantitative analysis of human herpesviruses in chronic and acute B cell lymphocytic leukemia and in multiple myeloma. Leukemia 2003; 17(1): 185-95.

54. Holmberg LA, Boeckh M, Hooper H, Leisenring W, Rowley S, Heimfeld S, Press O, Maloney DG, McSweeney P, Corey L, Maziarz RT, Appelbaum FR, Bensinger W. Increased incidence of cytomegalovirus disease after autologous CD34-selected peripheral blood stem cell transplantation. Blood 1999; 94(12): 4029-35.

55. Hughes WT, Armstrong D, Bodey, Bow EJ, Brown AE, Calandra T, Feld R, Pizzo PA, Rolston KV, Shenep JL, Young LS. Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Inf Dis 2002; 34: 730–51.

56. Imbert-Marcille BM, Tang XW, Lepelletier D, Besse B, Moreau P, Billaudel S, Milpied N. Human Herpesvirus 6 infection after autologous or allogeneic stem cell transplantation: A single-center prospective longitudinal study of 92 patients. Clin Infect Dis 2000; 31: 881-6.

57. Jain T, John J, Kotecha A, Deol A, Saliminia T, Revankar S, Chandrasekar P. Cytomegalovirus infection in autologous stem cell transplant recipients in the era of rituximab. Ann Hematol 2016; 95(8):1323-7. doi: 10.1007/s00277-016-2700-4.

58. Josephs SF, Salahuddin SZ, Ablashi DV, Schachter F, Wong-Staal F and Gallo RC. Genomic analysis of human B-lymphotropic virus (HBLV). Science 1986; 234: 601-3.

59. Keating MJ, Flinn I, Jain V, Binet JL, Hillmen P, Byrd J, Albitar M, Brettman L, Santabarbara P, Wacker B, Rai KR. Therapeutic role of aleumtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood 2002; 99: 3554-61.

60. Keating MJ, O'Brien S, Kantarjian H, Plunkett W, Estey E, Koller C, Beran M, Freireich EJ. Long-term follow-up of patients with chronic lymphocytic leukemia treated with fludarabine as a single agent. Blood 1993; 81(11): 2878-84.

61. Keating MJ, O'Brien S, Lerner S, Koller C, Beran M, Robertson LE, Freireich EJ, Estey E, Kantarjian H. Long-term follow-up of patients with chronic lymphocytic leukemia (CLL) receiving fludarabine regimens as initial therapy. Blood 1998; 92(4): 1165-71.

62. Keng MK, Sekeres MA. Febrile neutropenia in hematologic malignancies. Curr Hematol Malig Rep 2013; 8: 370–8.

63. Kern WV, Klose K, Jellen-Ritter AS, Oethinger M, Bohnert J, Kern P, Reuter S, von Baum H, Marre R. Fluoroquinolone resistance of *Escherichia coli* at a cancer center: epidemiologic evolution and effects of discontinuing prophylactic fluoroquinolone use in neutropenic patients with leukemia. Eur J Clin Microbiol Infect Dis 2005; 24 (2): 111–8.

64. Klastersky J, Ameye L, Maertens J, Georgala A, Muanza F, Aoun M, Ferrant A, Rapoport B, Rolston K, Paesmans M. Bacteremia in febrile neutropenic cancer patients. Int J Antimicrob Ag 2007; 30S: S51-S59.

65. Koistinen P, Räty R, Itälä M, Jantunen E, Koivunen E, Nousiainen T, Pelliniemi TT, Remes K, Ruutu T, Savolainen ER, Siitonen T, Silvennoinen R, Volin L, Elonen E. Finnish Leukaemia Group. Long-term outcome of intensive chemotherapy for adults with de novo acute myeloid leukaemia (AML): the nationwide AML-92 study by the Finnish Leukaemia Group. Eur J Haematol 2007; 78(6): 477-86.

66. Konoplev S, Champlin RE, Giralt S, Ueno NT, Khouri I, Raad I, Rolston K, Jacobson K, Tarrand J, Luna M, Nguyen Q, Whimbey E. Cytomegalovirus pneumonia in adult autologous blood and marrow transplant recipients. Bone Marrow Transplant 2001; 27(8): 877-81.

67. Kumar SK, Dispenzieri A, Lacy MQ, Gertz MA, Buadi FK, Pandey S, Kapoor P, Dingli D, Hayman SR, Leung N, Lust J, McCurdy A, Russell SJ, Zeldenrust SR, Kyle RA, Rajkumar SV. Continued improvement in survival in multiple myeloma: changes in early mortality and outcomes in older patients. Leukemia 2014; 28 (5): 1122-8.

68. Ljungman P, Biron P, Bosi A, Cahn JY, Goldstone AH, Gorin NC, Link H, Messina C, Michallet M, Richard C, et al. Cytomegalovirus interstitial pneumonia in autologous bone marrow transplant recipients. Infectious Disease Working Party of the European Group for Bone Marrow Transplantation. Bone Marrow Transplant 1994; 13 (2): 209-12.

69. Ljungman P, de la Camara R, Cordonnier C, Einsele H, Engelhard D, Reusser P, Styczynski J, Ward K. European Conference on Infections in Leukemia. Management of CMV, HHV-6, HHV-7 and Kaposi-sarcoma herpesvirus (HHV-8) infections in patients with haematological malignancies and after SCT. Bone Marrow Transplant 2008; 42: 227-40.

70. Ljungman P, Lambertsengli Delliers G, Platzbecker U, Matthes-Martin S, Bacigalupo A, Einsele H, Ullmann J, Musso M, Trenschel R, Ribaud P, Bornhäuser M, Cesaro S, Crooks B, Dekker A, Gratecos N, Klingebiel T, Tagliaferri E, Ullmann AJ, Wacker P, Cordonnier C. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. Blood 2001; 97: 388-92.

71. Ljungman P, Wang FZ, Clark DA, Emery VC, Remberger M, Ringdén O, Linde A. High levels of human herpesvirus 6 DNA in peripheral blood leucocytes are correlated to platelet engraftment and disease in allogeneic stem cell transplant patients. Br J Haematol 2000; 111: 774-81.

72. Ljungman P, Griffiths P, Paya C. Definitions of Cytomegalovirus Infection and Disease in Transplant Recipients. Clin Infect Dis 2002; 34 (8): 1094-7. doi: 10.1086/339329

73. Ljungman P, Morgan H, Boeckh M. Cytomegalovirus in Hematopoietic Stem Cell Transplant Recipients. Hematol Oncol Clin North Am 2011; 25(1):151-69. doi:10.1016/j.hoc.2010.11.011.

74. Lurain NS, Spafford LE, Thompson KD. Mutation in the UL97 open reading frame of human cytomegalovirus strains resistant to ganciclovir. J Virol 1994; 68: 4427-31.

75. Lurain NS, Thompson KD, Holmes EW, Read GS. Point mutations in the DNA polymerase gene of human cytomegalovirus that result in resistance to antiviral agents. J Virol 1992; 66: 7146-52.

76. Lyman GH, Michels SL, Reynolds MW, Barron R, Tomic KS, Yu J. Risk of mortality in patients with cancer who experience febrile neutropenia. Cancer 2010; 116: 5555-5563

77. Madani TA. Clinical infections and bloodstream isolates associated with fever in patients undergoing chemotherapy for acute myeloid leukemia. Infection 2000; 28: 367-73.

78. Malagola M, Peli A, Damiani D, Candoni A, Tiribelli M, Martinelli G, Piccaluga PP, Paolini S, De Rosa F, Lauria F, Bocchia M, Gobbi M, Pierri I, Zaccaria A, Zuffa E, Mazza P, Priccolo G, Gugliotta L, Bonini A, Visani G, Skert C, Bergonzi C, Roccaro AM, Filì C, Fanin R, Baccarani M, Russo D. Incidence of bacterial and fungal infections in newly diagnosed acute myeloid leukaemia patients younger than 65 yr treated with induction regimens including fludarabine: retrospective analysis of 224 cases. Eur J Haematol 2008; 81 (5): 354-63.

79. Mandell, Douglas, and Bennett's. Principles and Practice of Infectious Diseases. Elsevier Saunders. 8th ed. 2015 ISBN: 978-1-4557-4801-3.

80. Marchesi F, Pimpinelli F, Dessanti ML, Gumenyuk S, Palombi F, Pisani F, Romano A, Spadea A, Maschio M, Ensoli F, Mengarelli A. Evaluation of risk of symptomatic cytomegalovirus reactivation in myeloma patients treated with tandem autologous stem cell transplantation and novel agents: a single-institution study. Transpl Infect Dis Off J Transplant Soc 2014; 16: 1032–8.

81. Marchesi F, Pimpinelli F, Gumenyuk S, Renzi D, Palomb F, Pisani F, Romano A, Spadea A, Papa E, Canfora M, Ensoli F, and Mengarelli A. Cytomegalovirus reactivation after autologous stem cell transplantation in myeloma and lymphoma patients: a single-center study. World J Transplant 2015; 5(3): 129-36.

82. Maschmeyer G, Rolston KV. Infections in Hematology. Springer 2015. ISBN 978-3-662-44000-1 (eBook)

83. Mills KH, Cawley JC. Abnormal monoclonal antibody-defined helper/ suppressor T-cell subpopulations in multiple myeloma: relationship to treatment and clinical stage. Br J Haematol 1983; 53: 271–5.

84. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches. Best Pract Res Clin Haematol 2010; 23 (1): 145-53.

85. Nesher L, Rolston KV. The current spectrum of infection in cancer patients with chemotherapy related neutropenia. Infection 2014; 42 (1): 5-13. doi: 10.1007/s15010-013-0525-9.

86. Nguyen Q, Estey E, Raad I, Rolston K, Kantarjian H, Jacobson K, Konoplev S, Ghosh S, Luna M, Tarrand J, Whimbey E. Cytomegalovirus pneumonia in adults with leukemia: an emerging problem. Clin Infect Dis 2001; 32: 539-45.

87. Nucci M, Anaissie E. Infections in patients with multiple myeloma in the era of high-dose therapy and novel agents. Clin Infect Dis 2009; 49 (8): 1211-25. doi: 10.1086/605664.

88. Offidani M, Corvatta L, Polloni C, Gentili S, Brioni A, Visani G, Galieni P, Brunori M, Alesiani F, Catarini M, Centurioni R, Samori A, Blasi N, Ferranti M, Fraticelli P, Mele A, Rizzi R, Larocca F, Leoni P. Infectious complications in patients with multiple myeloma treated with new drug combinations containing thalidomide. Leuk Lymphoma 2011; 52 (5): 776-85.

89. Ortiz E, Sande MA. Routine use of anaerobic blood cultures: are they still indicated? Am J Med 2000; 108: 445–7.

90. Paliogianni F, Ahuja SS, Balow JP, Balow JE, Boumpas DT. Novel mechanism for inhibition of human T cells by glucocorticoids. Glucocorticoids inhibit signal transduction through IL-2 receptor. J Immunol 1993; 151(8): 4081-9.

91. Perola O, Nousiainen T, Pentikäinen J, Laatikainen A, Katila ML. Infections and bacterial colonization during cytotoxic therapy in patients with acute leukemia. Eur J Clin Microbiol Infect Dis 2005; 24 (11): 766-8.

92. Picazo JJ. Management of the Febrile Neutropenic Patient: A Consensus Conference. Clin Infect Dis 2004; 39: 1-6.

93. Pizzo PA. Management of fever in patients with cancer and treatment-induced neutropenia. N Engl J Med 1993; 328: 1323-32.

94. Pritchett JC, Naesens L, Montoya JG. 2014. Treating HHV-6 infections—the laboratory efficacy and clinical use of anti-HHV-6 agents, p311–331. *In* Flamand L, Lautenschlager I, Krueger G, Ablashi D (ed), Human herpesviruses HHV-6A, HHV-6B & HHV-7, 3rd ed. Elsevier, Amsterdam, Netherlands.

95. Ramphal R. Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clin Infect Dis 2004; 39: 25-31.

96. Rolston KV, Bodey GP, Safdar A. Polymicrobial infection in patients with cancer: an underappreciated and underreported entity. Clin Infect Dis 2007; 45: 228–33.

97. Rolston KV. Challenges in the treatment of infections caused by gram-positive and gram-negative bacteria in patients with cancer and neutropenia. Clin Infect Dis 2005; 40 Suppl 4: S246–52.

98. Rossini F, Terruzzi E, Cammarota S, Morini F, Fumagalli M, Verga L, Elli E, Verga M, Miccolis I, Parma M, Pogliani EM. Cytomegalovirus infection after autologous stem cell transplantation: incidence and outcome in a group of patients undergoing a surveillance program. Transpl Infect Dis 2005; 7 (3-4): 122-5.

99. Ruell J, Barnes C, Mutton K, Foulkes B, Chang J, Cavet J, Guiver M, Menasce L, Dougal M, Chopra R. Active CMV disease does not always correlate with viral load detection. Bone Marrow Transplant 2007; 40(1): 55-61.

100. Salahuddin SZ. The discovery of human herpesvirus type 6, p. 3-8. 1992. In Ablashi, D.V., Krueger, G.R.F. & Salahuddin, S.Z. (ed.), Human herpesvirus 6: epidemiology, molecular biology, and clinical pathology, Vol. 4. Elsevier Biomedical Press, Amsterdam, The Netherlands.

101. Schlaberg R, Fisher MA, Hanson KE. Susceptibility profiles of *Nocardia* isolates based on current taxonomy. Antimicrob Ag and Chemother 2014; 58: 795-800.

102. Schmitz N, Buske C, Gisselbrecht C. Autologous stem cell transplantation in lymphoma. Semin Hematol 2007; 449: 234-45.

103. Sinkó J, Cser V, Konkoly Thege M, Masszi T. Gram-negatív bacteriaemia neutropeniás hematológiai betegekben. Tapasztalatok fluorokinolon profilaxis alkalmazása kapcsán. Gram-negative bacteremia in neutropenic patients with hematologic disorders. Experiences with prophylactic use of fluoroquinolones. Orv Hetil 2011; 152:1063-1067. DOI: http://dx.doi.org/10.1556/OH.2011.29150

104. Syrjala H, Ohtonen P, Kinnunen U, Raty R, Elonen E, Nousiainen T, Jantunen E, Remes K, Itala-Remes M, Silvennoinen R, Koistinen P, Finnish Leukemia Group. Bloodstream infections during chemotherapy-induced neutropenia in adult patients with acute myeloid leukemia: treatment cycle matters. Eur J Clin Microbiol Infect Dis 2010; 29: 1211-8.

105. Takács Mária. Klinikai és járványügyi virológia. VOX MEDICA Kiadói Kft. 2010 ISBN 9789639740204

106. Torres OH, Domingo P, Pericas R, Boiron P, Montiel JA, Vazquez G. Infection caused by *Nocardia farcinica*: case report and review. Eur J Clin Microbiol Infect Dis 2000; 19: 205–12.

107. Valerio Minero M, Marín M, Cercando E, Rabadán MP, Bouza E, Munoz P. Nocardiosis at the turn of the century. Medicine 2009; 88: 250-61.

108. Viscoli C, Castagnola E. Planned progressive antimicrobial therapy in neutropenic patients. Br J Haematol 1998; 102 (4): 879-88.

109. Viscoli C, Cometta A, Kern WV, Bock R, Paesmans M, Crokaert F, Glauser MP, Calandra T. International Antimicrobial Therapy Group of the European Organization for Research and Treatment of Cancer. Piperacillin-tazobactam monotherapy in high-risk febrile and neutropenic cancer patients. Clin Microbiol Infect 2006; 12: 212–16.

110. Viscoli C, Varnier O, Marchetti M. Infections in patients with febrile neutropenia: epidemiology, microbiology and risk stratification. Clin Infect Dis 2005; 40: 240-5.

111. Wade JC. Viral infections in patients with haematological malignancies. Hematology Am Soc Hematol Educ Program. ASH Education Book 2006; 368-74. doi: 10.1182/asheducation-2006.1.368

112. Wang FZ, Linde A, Hagglund H, Testa M, Locasciulli A, Ljungman P. Human herpesvirus 6 DNA in cerebrospinal fluid specimens from allogeneic bone marrow transplant patients: does it have clinical significance? Clin Infect Dis 1999; 28: 562-8.

113. Wilson JW. Nocardiosis: Updates and Clinical Overview. Mayo Clin Proc 2012; 87: 403-7.

114. Winston DJ, Lazarus HM, Beveridge RA, Hathorn JW, Gucalp R, Ramphal R, Chow AW, Ho WG, Horn R, Feld R, Louie TJ, Territo MC, Blumer JL, Tack KJ. Randomized, double-blind, multicentre trial comparing clinafloxacin with imipenem as emprirical monotherapy for febrile granulocytopenic patients. Clin Infcet Dis 2001; 32: 381-90.

115. Yalcin S, Karpuzoglu T, Suleymanlar G, Mutlu G, Mukai T, Yamamoto T, Isegawa Y and Yamanishi K. Human herpesvirus 6 and human herpesvirus 7 infections in renal transplant recipients and healthy adults in Turkey. Arch Virol 1994; 136: 183-90.

116. Yamanishi K, Okuno T, Shiraki K, Takahashi M, Kondo T, Asano Y, Kurata T. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. Lancet 1988; 1(8594): 1065-7.

117. Zerr DM, Corey L, Kim HW, Huang ML, Nguy L and Boeckh M. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. Clin Infect Dis 2005; 40: 932-40.

118. Zerr DM, Gupta D, Huang ML, Carter R, Corey L. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002; 34: 309-17.

119. Zinner SH. Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. Clin Infect Dis 1999; 29:490-4; PMID:10530434; http://dx.doi.org/10.1086/598620

120. Zuckermann AJ. Principles and Practice of Clinical Virology. John Wiley and Sons Ltd 2009.

LIST OF FIGURES

LIST OF TABLES

APPENDICES I-VI

I.

II.

III.

IV.

V.

VI.