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VIRAL INFECTIONS IN IMMUNOSUPPRESSED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES

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Viral infections in immunosuppressed patients with hematological malignancies

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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ABSTRACT

Acute or reactivated viral infections are common in patients who are immunosuppressed because of hematopoietic stem cell transplantation (HSCT) or chemotherapy due to hematological malignancies. The severity of the immunosuppression, the type of immune functions that are affected by various therapeutic interventions, as well as underlying hematological malignancy contributes to viral susceptibility and clinical outcome of the infection. Furthermore, in patients undergoing HSCT, the serostatus of viruses with reactivation capacity in both the recipient and the donor must be considered, as well as the sociodemographic and genetic characteristics.

Here we have studied DNA viruses that can cause clinical events in patients with malignant hematological diseases. Foremost, we evaluated whether it is advisable to continuously screen for the presence of these viruses during illness or chemotherapy in children and adults. In papers I and II we studied the presence of human adenovirus (HAdV) in the blood of patients undergoing HSCT. In papers III and IV, the presence of parvovirus B19 (B19V) in the bone marrow of children with various malignancies was studied, while paper V focused on the presence of B19V in the blood of adults and children undergoing HSCT. Paper VI focused on human herpesvirus 6 (HHV-6), polyoma BK virus (BKV) and B19V in the blood of adult patients undergoing chemotherapy for non-transplanted hematological malignancies.

In these retrospective studies, blood and/or bone marrow samples were analyzed by quantitative real-time PCR for DNA representing HAdV, B19V, HHV-6 and BKV. Clinical and laboratory data were obtained from medical records. The proportion of patients with HAdV infection was relatively small, and asymptomatic infections did not occur. On the other hand, HAdV DNA loads >15,000 copies/mL in blood were associated with morbidity and mortality. Furthermore, findings of B19V in bone marrow of children undergoing treatment for acute lymphoblastic leukemia (ALL), were associated with prolonged chemotherapy. Neither B19V, HHV-6A, 6B nor BKV was common in the blood of adult patients with hematological malignancies who were immunosuppressed due to chemotherapy.

In general, screening for these viruses in the patient groups presented may not be indicated at the current state. However, testing for HAdV should be performed generously when unexpected symptoms occur, even if they are not typical for the virus. B19V infection is almost always linked with some degree of cytopenia, and if unexpected cytopenia occurs in children undergoing chemotherapy, B19V infection should be tested for. Patients with primary infections normally suffer from more severe clinical disease as compared to those with reactivated infections. Thus, knowledge of viral serostatus in HSCT recipients and donors should be taken into account in diagnostic considerations.

Overall, the diagnostic value of direct viral detection in blood and/or bone marrow samples of immunosuppressed patients with hematological malignancies is of considerable importance. Hopefully, a broad spectrum of novel antiviral compounds as well as novel procedures for

adoptive cell therapy will be developed for these viral infections. Whether novel interventions will be used as pre-emptive therapy, or as symptomatic treatment, there will be an urgent need to monitor viral load. The present thesis can thus inform the field of clinically relevant viral infections and how to monitor these in selected patient categories that can be targeted for future therapeutic clinical interventions.

LIST OF SCIENTIFIC PAPERS

I. **Igge Gustafson**, Anna Lindblom, Zhibing Yun, Hamdy Omar, Liselotte Engström, Ilona Lewensohn-Fuchs, Per Ljungman, Kristina Broliden. **Quantification of adenovirus DNA in unrelated donor hematopoietic stem cell transplant recipients.** Journal of Clinical Virology, 2008; 43: 79–85

II. Lars Öhrmalm, Anna Lindblom, Hamdy Omar, Oscar Norbeck, **Igge Gustafson**, Ilona Lewensohn-Fuchs, Jan-Erik Johansson, Mats Brune, Per Ljungman and Kristina Broliden. **Evaluation of a surveillance strategy for early detection of adenovirus by PCR of peripheral blood in hematopoietic SCT recipients: incidence and outcome.** Bone Marrow Transplantation, 2011; 46, 267–72

III. Anna Lindblom, Mats Heyman, **Igge Gustafsson**, Oscar Norbeck, Tove Kaldensjö, Åsa Vernby, Jan-Inge Henter, Thomas Tolfvenstam and Kristina Broliden. **Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy.** Clinical Infectious Diseases, 2008; 46: 528-36

IV. **Igge Gustafsson**, Tove Kaldensjö, Anna Lindblom, Oscar Norbeck, Jan-Inge Henter, Thomas Tolfvenstam and Kristina Broliden. **Evaluation of parvovirus B19 infection in children with malignant or hematological disorders.** Clinical Infectious Diseases, 2010; 50(10): 1425-26

V. Lars Öhrmalm, **Igge Gustafson**, Anna Lindblom, Oscar Norbeck, Jan-Erik Johansson, Mats Brune, Per Ljungman and Kristina Broliden. **Human parvovirus B19 in pediatric and adult recipients of allogeneic hematopoietic stem cell transplantation.** Bone Marrow Transplantation, 2013; 48, 1366–67

VI. **Igge Gustafsson**, Carl Aust, Zhibing Yun, Kristina Broliden, Lars Öhrmalm. **Presence of human herpesvirus type 6, polyoma BK virus and parvovirus B19V in non-transplanted patients with hematological malignancies and neutropenic fever.** In manuscript, 2020.

CONTENTS

LIST OF ABBREVIATIONS

ADCC	Antibody-dependent cell-mediated cytotoxicity
ADE	Antibody dependent enhancement
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APC	Antigen presenting cell
ATG	Anti-thymocyte globulin
B19V	Human parvovirus B19
BCR	B cell receptor
BKV	BK polyomavirus
CAR	Coxsackie adenovirus receptor
CD46	Cluster of differentiation 46
CDM	Chronic myeloproliferative disorder
CLD	Chronic lymphocytic disorder
CLL	Chronic lymphocytic leukemia
CLR	C-type lectin receptor
CML	Chronic myeloid leukemia
CpG	Cytidine-phosphate-guanosine
CRP	C-reactive protein
CTL	Cytotoxic T lymphocyte
CyA	Cyklosporin A
DC	Dendritic cells
DSG2	Desmoglein-2
dNTP	Deoxyribonucleotide triphosphate
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
EBV	Epstein-Barr virus
ECIL-4	The fourth European Conference of Infections in Leukemia
ELISA	Enzyme-linked ImmunoSorbent Assay
ELIspot	Enzyme-linked ImmunoSpot
EPO	Erythropoietin

FUO	Fever of unknown origin
G-CSF	Granulocyte colony-stimulating factor
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GvHD	Graft-versus-host disease
HAdV	Human adenovirus
HCMV	Human cytomegalovirus
HHV-6A	Human herpesvirus type 6A
HHV-6B	Human herpesvirus type 6B
HLA	Human leukocyte antigen
HSC	Hematological stem cells
HSCT	Hematopoietic stem cell transplantation
iciHHV-6A, 6B	inherited chromosomally integrated HHV-6A, 6B
Ig	Immunoglobulin
IFN	Interferon
IL	Interleukin
IRF	Interferon regulatory factor
IVIG	Intravenously administered immunoglobulin
KIR	Killing inhibition receptor
MC	Myeloablative conditioning
MDS	Myelodysplastic syndrome
MHC	Major histocompatibility complex
MUD	Matched unrelated donor
NHL	Non-Hodgkin lymphoma
NK	Natural killer
NKT	Natural killer T cells
NLR	NOD-like receptor
NOD	Nucleotide-binding and oligomerization domain
NS1	Non structural protein
PAMP	Pathogen associated molecular pattern
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-Buffered Saline

PBSC	Peripheral blood stem cell
PBSCT	Peripheral blood stem cell transplantation
PC	Pentameric glycoprotein complex in HCMV (ligand)
PCR	Polymerase chain reaction
qPCR	Quantitative PCR (also known as real-time PCR)
PRR	Pattern recognition receptor
PTLD	Post-transplant lymphoproliferative disease
RAEB	Refractory anemia with excess of blasts – an AML type
RIC	Reduced intensity conditioning
RLR	Retinoic acid-inducible gene I like receptor
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
STING	Stimulator of interferon genes
TBI	Total body irradiation
Th1	T helper 1
TLR	Toll like receptor
TNF	Tumor necrosis factor
VP	Viral protein

1 INTRODUCTION

1.1 VIRUSES IN THE THESIS

One cannot help but admire the strategies that various viruses have developed to invade a host cell and then avoid the host´s defense. Below is a brief description to understand how cunning viruses are, and how difficult it is to fight them.

The viral taxonomy is constantly evolving, the name of a virus can change over time. Table 1 describes the current taxonomy that the International Commission on Taxonomy of Viruses (ICTV) (1) has assigned to the viruses we report. In this thesis, the synonyms of the viruses used are those used in the papers (bold text).

Table 1. Taxonomy of viruses

Order	Rowavirales	Piccovirales	Sepolyvirales	Herpesvirales		
Family	Adenoviridae	Parvoviridae	Polyomaviridae	Herpesviridae		
Subfamily	---	Parvovirinae	---	Gammaherpes-virinae	Betaherpesvirinae	
Genus	Mastadeno-virus	Erythroparvo-virus	Betapolyoma-virus	Lymphocrypt o-virus	Cytomegalo-virus	Roseolovirus
Species	Human mast-adenovirus A-G	Primate erythro-parvovirus 1	Human polyoma-virus 1	Human gamma herpesvirus 4	Human beta-herpesvirus 5	Human betaherpes-virus 6A, 6B
Synonyms	Human adeno-virus A-G	Human parvo-virus B19	BK polyoma-virus	Epstein-Barr virus	Cytomegalo-virus	Human herpesvirus 6A, 6B
Abbreviations	**HAdV**	**B19V**	**BKV**	**EBV**	**HCMV**	**HHV-6A, HHV-6B**

Just for fun. The shape of the capsids of the viruses discussed here are assumed to be icosahedral, i.e. a closed form of 20 equilateral triangles. According to the Caspar-Klug theory, the 20 triangles are based on 60 subunits. The T number indicates the number of proteins that create one of the 60 subunits. T = 1 means one protein in each subunit, i.e. a total of 60 proteins form the capsid, T = 2 means two proteins each, a total of 120 proteins in the capsid, etc. The higher T-numbers, consist of different numbers of hexagons and always 12 pentagons (2). HAdV: T=25; HCMV, EBV, HHV-6A, 6B: T=16; BKV: T=7; B19V: T=1.

If the classic black and white football, designed for the 1970 World Cup, was a giant virus, it might be T=7.

1.1.1 Human adenovirus (HAdV)

1.1.1.1 Structure

HAdVs are non-enveloped double-stranded linear DNA viruses, 26-45 kB in length, carrying approximately up to 40 genes. The viral capsid consists of three major proteins; 240 hexons – hexagon-shaped protein, 12 pentons – pentagon-shaped proteins, and 12 fiber proteins. In addition, there are several smaller proteins. The hexons and pentons form an icosahedral with 20 triangular faces, 30 edges and 12 vertexes (3-5).

The fiber proteins, one attached to each vertex, contain three structural domains

1. The knob - the essential part in the end of the fiber that binds to a host cell receptor
2. The shaft - that varies in length between different HAdV types and thus enables different interactions with host cells
3. The binding site to the pentons vertex (4-6).

1.1.1.2 Cell receptors

The cell receptors for HAdV are:

- CAR, the coxsackie adenovirus receptor to which most HAdVs bind
- CD46 - preferred by HAdV species B
- Desmoglein-2 (DSG2) – also preferred by species B
- α2,3-linked sialic acid- a cell adhesion protein that some HAdV species D use alone and others in combination with CD46 (6)

1.1.1.3 Replication

HAdVs start their DNA replication with transcription of proteins, partly for further efficient viral DNA replication, but also to block host cell defense mechanisms, such as interferon production, MHC class I formation and translocation, and cell apoptosis. This is followed by replication to produce mature virions and capsid proteins (5-7).

1.1.1.4 Species and types

In July, 2019, HAdV had been assigned 103 genotypes (8). Here, 67 different types of HAdV are considered, characterized within seven species, A-G (5). The types 1-51 were identified by classic serum neutralization, which aimed to test the humoral immunity to the hexon, together with hemagglutination inhibition, in which the fiber protein was identified. Since serologic determinants represent less than 5-6% of the total genome, different genotypes may occur within the same serotype (7). The newer types 52-67 were identified by genomic sequencing. New types of HAdVs arise partly through recombination between two or more

previous genotypes (5, 7). For example, recombination of HAdV-11 and HAdV-14 resulted in the HAdV-55 genotype (9). New genotypes may lead to symptoms that do not occur with the original serotype (7). The clinical significance of genotype 68-103 is not clear in the literature, which is also confirmed in a compilation of HAdVs from 2019 (10).

Co-infections with at least two HAdVs with highly similar nucleotide sequences, at recombination hotspots in the genome, are possible, especially in immunocompromised patients, where an infection can last for a long time (4, 7).

1.1.1.5 Transmission

Transmission routes are person to person – inhalation of aerosolized infected droplets, direct conjunctival inoculation, fecal-oral – through water, through environmental surfaces and instruments (fomites) (4, 5).

At about 10 years of age, most children have been infected with some type of HAdV (11). Severe local outbreaks as well as nosocomial infections have been reported. HAdVs are not zoonotic, and only a few animal models can be used to study HAdVs (4).

HAdVs are stable at low pH, can remain infectious in room temperature for up to 3 weeks and are highly resistant to physical and chemical agents. Sodium hypochlorite[1] (500 ppm) for 10 min or 70% ethanol for at least 1 minute can be used to inactivate HAdVs on surfaces (4, 5).

1.1.1.6 Tissue tropism, infection and persistence

Depending on HAdV type, the incubation period can be 2-14 days, and the range of clinical manifestations is wide (4, 5) see Table 2. Probably due to an immature adaptive immune system, the primary infection occurs mainly during the first five years of life (5).

The diversity of clinical manifestations shows that HAdV tropism varies by viral type. After primary infection, latent viruses, i.e. viruses that express viral proteins but not virions, persist in e.g. lymphocytes from the tonsils or the intestine and lung epithelial cells (5).

[1] Klorin

Table 2. HAdV and clinical manifestations (4, 5, 7, 12, 13).

HAdV species	A	B1	B2	C	D	E	F	G
Types	12, 18, 31, *61*	3, 7, 16, 21, 50, *55, 66*	11, 14, 34, 35	1, 2, 5, 6, *57*	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, *53, 54, 56, 58-60, 62-65, 67*	4	40, 41	52
Common HAdV types that cause different illnesses in individuals								
Immunocompetent individuals		3, 7, 21		1, 2, 5		4	41	
Immunocompromised patients	12, 31	3, 16	11, 34, 35	1, 2, 5				
HAdV types and association to diseases								
Respiratory diseases								
Upper respiratory tract	18	All	All	1, 2, 6	15, 19, 29, 30, 37	4		
Lower respiratory tract	12	3, 7, 16, 21	14, 35	1, 2, 5	8, 19, 39, *56*	4		
Pertussis syndrome				5				
Acute respiratory diseases		7, 21, 55	14			4		
Conjunctivitis								
Ocular infections unspecified			14	5, 6	10, 15, 23, 24			
Acute (*hemorrhagic)		3, 7	*11	1, 2		4		
Pharyngoconjunctival fever		3, 7						
Epidemic keratoconjunctivitis					8, 9, 17, 19, 37, *53, 54, 56*			
Other infections and conditions								
Disseminated diseases	31		11, 34, 35	1, 2, 5			40	
Gastroenteritis	12, 18	3, 7		2	9, 13, 20, 25-28, 32, 33, 36, 38, 39, 42, *65*, *67*		40, 41	*52*
Hemorrhagic cystitis		7	11, 34, 35					
Other illnesses in the genitourinary tract		21		1	8, 37	4		
Hepatitis		3, 7		1, 2, 5				
Myocarditis		7, 21		2, 6				
CNS infections		7		2, 5				
Venereal diseases				2				
Obesity or adipogenesis		31		5	9, 36, 37			

Italicized numbers represent virus types determined by genotyping.

1.1.2 Epstein-Barr virus (EBV), Human cytomegalovirus (HCMV), Human herpesvirus 6A and 6B (HHV-6A) and (HHV-6B)

This thesis includes studies on HHV-6A and HHV-6B in paper VI. Herpes viruses in general are fascinating and often occur in patients discussed in the other papers, e.g. paper I. To understand the challenges of these viruses, several of them are briefly presented here.

1.1.2.1 Structure

The four viruses are large enveloped double-stranded linear DNA viruses with an icosahedral capsid protected by a proteinaceous layer, the tegument. The envelope is a lipid bilayer that contains various proteins, e.g. three proteins conserved throughout the herpesviridae, and also species-unique proteins. The proteins are involved in binding to target cells - viral tropism - and ensure that the virus enters the cell (14-20).

1.1.2.2 Entry into the cell

The four viruses have slightly different strategies for binding to cells. Common between the viruses is the fusion machinery. Two conserved proteins, which form a dimer, regulate a third conserved protein, which activates the fusion machinery, ensuring entry of the virus either via direct fusion with the cell membrane or via the endosome membrane through endocytosis. The dimer often - but not always - forms complexes with species-specific proteins (15) and this determines viral tropism (21, 22)
Several different cell proteins, receptors and co-receptors that facilitate the entries of the viruses, have been identified. New cellular proteins used by the viruses are still being discovered (14, 17, 20, 21, 23-26).

EBV: The entry into B cells is by endocytosis (14, 15). The exact mechanism for the virus to enter epithelial cells is less well known. Direct fusion between virus and cell membrane is an accepted route, but it is possible that entry can also occur via endocytosis (21, 23).

HCMV: The conserved regulatory dimer, see above, forms either trimer or pentamer complexes with species-specific proteins. Upon formation of a trimer, the virus can enter fibroblasts by direct fusion. In the formation of pentamers, the virus enters many cell types through endocytosis (17, 21, 24).

HHV-6A, 6B: HHV-6 consists of two separate viruses, type 6A and type 6B with a 90% identic genome (27) entering the cells via different receptors. HHV-6A enters via CD46 - also preferred by HAdV species B - and HHV-6B enters via CD134, which is part of the TNF-receptor superfamily (19, 28, 29). Serologic assays cannot differ the species (20).

1.1.2.3 Tropism

EBV: It is discussed whether the virus at the time of transmission passively passes (transcytosis) or infects the oropharyngeal epithelial cells before infection of B cells in the underlying lymph tissue, i.e. tonsils and adenoids (Waldeyer's ring). It is clear, however, that epithelial cells in the throat can be infected with actively replicating virus present in the B cells located in Waldeyer's ring (30, 31).

HCMV: The virus thrives in all tissues with epithelial cells, endothelial cells, hematopoietic cells (monocytic lineage), fibroblasts, smooth muscle cells, hepatocytes, neuronal cells or trophoblasts. Infection of epithelial cells occurs mainly due to transmission between hosts, while other cells become infected due to systemic spread within the infected host. HCMV can proliferate efficiently in fibroblasts and smooth muscle cells. Different tropism for e.g. endothelial cells, macrophages and dendritic cells are due to differences in the HCMV strain. (32).

HHV-6A, 6B: Both species infect essentially the same cells; CD4+ T cells, CD8+ T cells, monocytes, macrophages, hematopoietic cells in the bone marrow, epithelial cells in the kidneys and salivary glands, endothelial cells, astrocytes, oligodendrocytes, microglial cells, and gametes (19). Unique properties of HHV-6A, 6B include that a DNA sequence in the viral genome corresponds to the DNA sequence of human telomeres. By homologous recombination (i.e. two identical DNA sequences are exchanged between genomes) in an infected cell, the virus can be incorporated into the telomeres (33).

1.1.2.4 Transmission

These four species of herpesviruses are human specific, and transmission occurs mostly from person to person through body secretions (34). They have the ability to use monocytes/macrophages to spread the infection within the host (35).

EBV: The transmission is through saliva. Worldwide, more than 90% of the adults are infected (34). In the primary infection, large amounts of viruses derived from oropharyngeal epithelial cells (36) can be shed between one month and three years (31). In the latent stage, virus shedding is constant and fluctuates over time in the individual (30). Transmission via blood products is a possible, but not common route. Transmission is also possible in patients undergoing hematopoietic stem cell transplantation. The transmission route for young children is not known, but transmission from infected caregivers, siblings, etc. is possible. There is no evidence of transmission via fomites (31).

HCMV: The transmission is through exposure to infected body secretions such as saliva, urine, feces, semen, blood (e.g. transfusions and transplants) etc, which may come in contact with mucosal epithelial cells – or endothelial cells – of an uninfected person (37). Seroprevalence increases with age (38) and more than 80% are infected worldwide (39). In

connection with the primary infection, infants and young children can continuously shed HCMV for several years, roughly speaking, while adults can shed for up to half a year.

After primary infection, intermittent shedding can occur in both children and adults (37). HCMV can be transmitted through the placenta from mother to fetus and by breastfeeding – 30-40% of HCMV-positive women who breastfeed transmit the virus to their children (32). Reinfection with a new strain of HCMV may occur (40). Viable HCMV can survive on different surfaces between 1-6 hours (41).

HHV-6A, 6B: The transmission is via intermittent viral shedding from saliva. At least 90% of adults worldwide are infected (42). The virus can be transmitted from woman to fetus via the placenta. Through virus-infected gametes – eggs or sperm – the virus can be transmitted to the fetus during pregnancy (Mendelian inheritance), where a complete viral genome is integrated into chromosomes in all cells, i.e. inherited chromosomally integrated HHV-6A, 6B (iciHHV-6A, 6B) (43, 44). In these cases, viral load of HHV-6A, 6B DNA in whole blood exceed 5.5 log10 copies/mL. To confirm iciHHV-6A, 6B, tissues normally not infected with the viral DNA, e.g. hair roots, can be analyzed (44). About 1% are infected at birth (19).

1.1.2.5 Infection, persistence and reactivation in immunocompetent individuals

Herpesviruses remain in the host for the rest of the host's life, mostly in latency. EBV and HCMV persist as extrachromosomal episomes, (i.e. circular DNA) in the cell nucleus (45-47) while HHV-6A, 6B are integrated to the telomeres, see above.

EBV: Primary infection. In children most infections are asymptomatic or have nonspecific symptoms (34). In adolescent and adults, infectious mononucleosis, with the classical symptoms of fever, pharyngitis, cervical lymphadenopathy and fatigue are common symptoms (31). Approximately 90% of patients with infectious mononucleosis have EBV as a cause. CMV, HHV-6B and HAdV (and some other pathogens which are not discussed further here) can also mimic the symptoms (48).

Persistent latency. When B cells become infected, they are activated to proliferating blasts and migrate to the lymph nodes, where they are transformed into dormant memory-B cells. These cells spread to the peripheral circulation, where they divide and at the same time also replicate viral DNA, which is transferred to the daughter cells. Resting memory-B cells do not express viral proteins on the cell surface and divide as normal memory-B cells, which means that the host´s immune system cannot distinguish infected memory-B cells from uninfected. It is unclear which signal causes these memory-B cell to differentiate into plasma cells, but it is the differentiation itself that initiates the viral replication (30, 31). Epithelial cells are also replication sites during latency, but whether the cells can also harbor the virus through persistence is unclear (30).

Malignancies. The EBV genome encodes for latent proteins that can cause cell proliferation in B cells and there is convincing evidence associating EBV with Hodgkin lymphoma,

Burkitt lymphoma and lymphoma in immunosuppressed individuals such as post-transplant lymphoproliferative disease (PTLD) (30).

HCMV: Primary infection. The primary infection can occur already *in utero* and up to adulthood. Fetuses can die or suffer from serious complications. In children, adolescents and adults, the infection is often asymptomatic although non-specific symptoms (40) or symptoms such as infectious mononucleosis may occur (48).

Persistent latency and reactivation. In immunocompetent individuals, the virus reactivates intermittently and triggers the adaptive immune system to regain control of viral replication (49). HCMV has the ability to spread to all organs, via blood, but the immune system prevents serious diseases. Even at a so-called quiet stage, at least 1% of the peripheral T cells control HCMV, which means that the immune system uses more resources for this virus than for any other virus (40). There are many indications that events in the normal cell cycle may be sufficient to trigger virus activation (50).

HHV-6A, 6B: Primary infection. Most often the infection occurs in early childhood, where HHV-6B presents high fever followed by rash (exanthema subitum = roseola infantum = sixth disease) as the most common symptoms (51). Less is known about primary HHV-6A infection, but it may be associated with fever, without the rash (44, 52). The amount of HHV-6A, 6B DNA in various body fluids is elevated in an acute infection, but the viral load is never as high as in individuals with iciHHV-6A, 6B, see above (44).

Persistent latency. HHV-6B, 6A prefer to persist in latency in monocytes and macrophages in peripheral blood, but can persist in hematopoietic progenitor cells as well as other cells including T cells and probably neuronal cells (19, 53, 54). It is not entirely clear whether iciHHV-6A, 6B can produce active virions (54).

Reactivation. Some drugs may reactivate or increase the replication of viral DNA, which may cause eosinophilia and rash symptoms. Examples of drugs are vancomycin (antibiotics), carbamazepine and phenytoin (antiepileptics), ibuprofen and naproxen (NSAID and, allopurinol (anti hyperuricemia) (44).

1.1.3 Human parvovirus B19 (B19V)

1.1.3.1 Structure

There are three distinct genotypes of B19V; 1, 2 and 3. Genotype 2 is not in circulation, and genotype 3 is endemic in some areas as Ghana, Brazil and India (55). Here we discuss genotype 1.

B19V is a non-enveloped single stranded linear DNA virus, 5.6 kB in length, carrying 5 genes. The icosahedral capsid consists of 60 protein structures (VPs), divided into VP1 and

VP2 in a ratio of about 1:20. A large part of the genome consists of the non-structural NS1 protein. The genome also encodes two small non-structural proteins (55).

Capsid proteins. The VPs are immunogenic and CD4+ T cells can be triggered by VP epitopes. IgM and IgG antibodies are directed against VPs. VP1 is involved in the binding of the virus to the cell and enables subsequent endocytosis. VP2 is involved in the production and installation of new capsids, consisting of VP1 and VP2, inside the cell (55).

The non-structural NS1 protein. The most essential and multi-functional protein in B19V is the NS1 protein, which is involved in viral DNA replication and folding, and also in the packaging of DNA into capsids, by regulating e.g. the cell cycle and various cell genes. NS1 is also thought to affect the cell's defense system (56).

1.1.3.2 Cell receptors

Using the cell protein Ku80, B19V attaches to the cell glycoprotein globoside (synonymous with erythrocyte P antigen), which activates the viral protein VP1 to prepare for endocytosis. During internalization, B19V need to interact with the cellular protein α5β1 integrin. If this interaction does not work, the virus detaches and repeats the procedure (57).

1.1.3.3 Tropism

The virus infects different stages of erythroid progenitor cells in bone marrow and in fetal liver (58, 59).

1.1.3.4 Transmission

The transmission is via the respiratory route probably through droplets, but no specific respiratory symptoms are present. The virus can also transmit via blood products or through transplantation. Also, transmission from infected surfaces is possible. In pregnancy B19V can be transmitted from woman to fetus via the placenta. Seroprevalence rates vary around the world, but increases with age, and in general more than 50% of a population has been infected (55).

1.1.3.5 Infection

Primary infection. The typical clinical manifestation in children, 5 to 15 years, are symptoms such as fever and red girth-shaped rashes also called erythema infectiosum or the fifth disease. In both children and adults, stomach ache, nausea, diarrhea and arthralgia may occur. Infection before the 20th week of pregnancy can lead to hydrops fetalis and fetal death (55).

In more severe cases, B19V can cause hemolytic anemia as well as thrombocytopenia, neutropenia, granulocytopenia and even pancytopenia (60).

Persistent infection. B19V DNA can persist lifelong in skin, synovium, tonsil liver, bone marrow, colon, heart, lymphoid-, testicular-, and thyroid tissues (60, 61). B19V DNA, together with other genotypes of parvovirus, have been discovered in skeletons from Finnish World War II victims (62). However, the immune response against B19V is strong. B19V specific T cells together with neutralizing antibodies, directed against some epitopes on VP1 and VP2, confer lifelong protection against reinfections (55).

1.1.4 BK polyomavirus (BKV)

1.1.4.1 Structure

BKV is a non-enveloped double-stranded circular DNA virus. The capsid is icosahedral, consisting of three viral proteins (VPs), where VP1 is the most common protein. Inside the capsid there are three additional proteins (63). BKV has at least five genotypes which are completely distinct serotypes – Ib1, Ib2, II, III, IV (64).

1.1.4.2 Tropism

The different genotypes may have different tropism (64). At least BKV I enters the urinary epithelium via endocytosis (63). BKV has been found in other cells as well, e.g. peripheral leukocytes, fibroblasts, lymphoid tissues as tonsils etc. (63, 65, 66).

1.1.4.3 Transmission

Transmission occurs from human to human in early childhood, according to seroconversion, but the exact route is not known (63). Transmission via respiration droplets has been suggested (66), but it is uncertain whether the virus causes respiratory infections (67), although it is detected in saliva (68). Also, transmission via the fecal-oral route has been suggested (63). Some immunocompetent individuals shed low levels of virions in the urine (69, 70). In addition, other routes e.g. as via placenta or via infected water have been discussed (63).

Seroprevalence rates vary between different populations, but in Europe approximately 60-85% of the population have antibodies to the virus (63).

1.1.4.4 Infection

Primary infection. Symptoms are absent or mild in the immunocompetent person (63).

Persistent infection. During latency, BKV is found in the urinary tract and kidneys. Immunocompetent individuals do not reactivate the virus, but in immunosuppressed patients, viral reactivation can cause symptoms and disorders from the urinary tract and kidneys, e.g. hemorrhagic cystitis and polyomavirus-associated nephropathy (63).

1.2 INNATE AND ADAPTIVE IMMUNE RESPONSES IN VIRAL INFECTIONS

When the external barriers do not prevent virus entry, the first line of the body's defense system is innate cells, e.g. dendritic cells (DCs), macrophages, monocytes, granulocytes and NK cells, as well as epithelial cells and endothelial cells. The complement system and other acute phase proteins also belong to the defense system, but this is not discussed further here. To eliminate, or at least minimize, an infection, the next line of defense, consisting of T cells and B cells, is also needed, but is discussed here very briefly. An overview of the hematopoietic and immune system is presented in Figure 1.

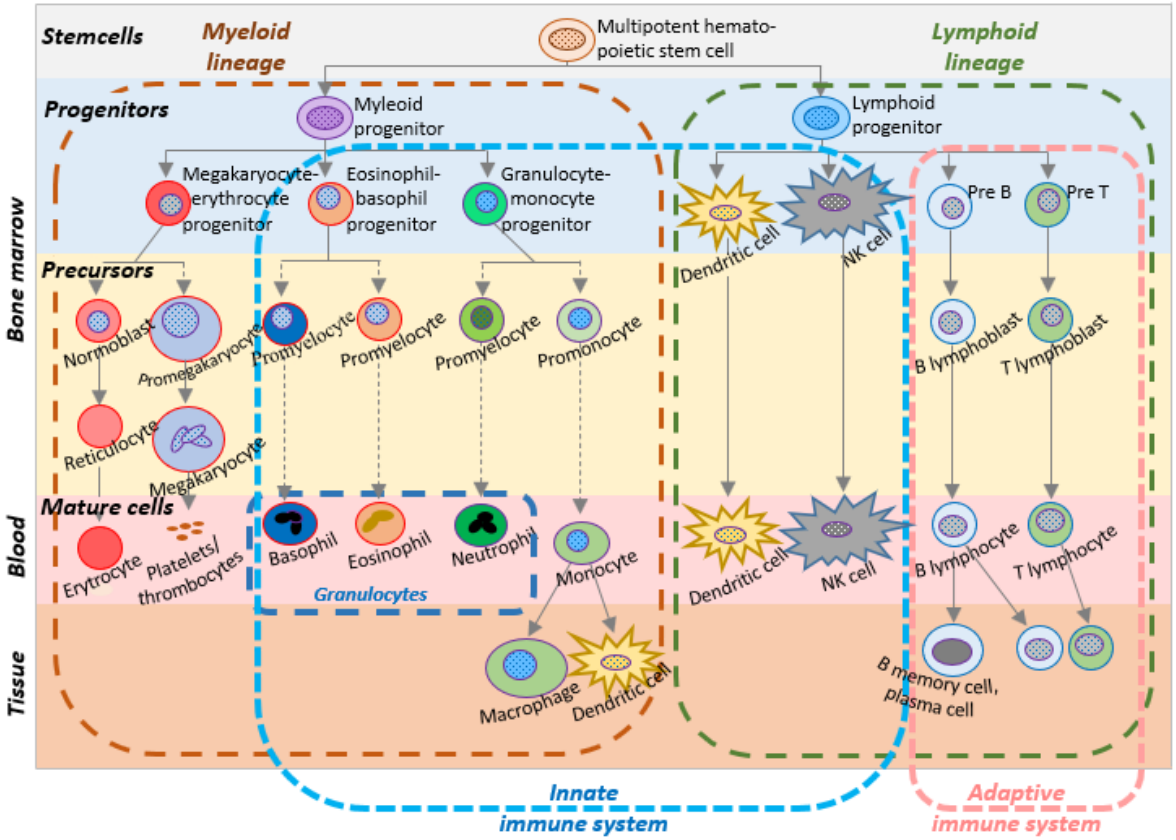

Figure 1. An overview of the hematopoietic and immune system.

All nuclear-containing body cells express on their surfaces, major histocompatibility class I (MHC-I) molecules that present peptides produced in the cell. Antigen presenting cells (APCs) i.e. DCs, macrophages, B cells and Langerhans cells have the ability to present peptides from endocytosed or phagocytosed antigens via MHC-II (71).

Viruses have specific conserved structures – pathogen associated molecular patterns (PAMPs), which differ from host molecules. In viruses, envelope proteins, double stranded (ds) RNA, single stranded (ss) RNA and DNA sequences – especially containing unmethylated cytidine-phosphate-guanosine (CpG) motifs – form PAMPs (72). Many cells in the defense system have pattern recognition receptors (PRRs) that can recognize PAMPs. When detected, the viruses are endo- or phagocytosed (73-75).

1.2.1 Virus-recognizing PRRs in humans

1.2.1.1 Toll-like receptors (TLRs)

Six of 10 different transmembrane TLRs in humans can detect viruses and are expressed in monocytes, macrophages, DCs, neutrophils, B cells, T cells, fibroblasts, endothelial cells and epithelial cells. TLR2 and TLR4, expressed at cell membranes, are activated by viral envelope glycoproteins. Intracellular TLRs expressed in the endosomal membranes are TLR3 - activated by dsRNA, TLR 7 and 8 - activated by ssRNA and TLR9 - activated by DNA with unmethylated CpG. Activation of TLRs initiate the production of various cytokines, often type I interferon (IFN) (72, 74-76).

1.2.1.2 C-type lectin receptors (CLRs)

CLRs are mostly transmembrane receptors on cell membranes that interact with other intracellular PRRs and support antiviral actions. They are expressed on DCs, macrophages, monocytes and Langerhans cells. Soluble CLRs in the bloodstream act together with the complement system. Unfortunately, some viruses have the ability to exploit transmembrane CLRs, often DC-SIGN, to enter cells, avoid antiviral machinery and transmit to other cells to spread the infection (77, 78).

1.2.1.3 Retinoic acid-inducible gene I (RIG-I) like receptors (RLRs)

RLRs are cytoplasmic receptors, in myeloid and epithelial cells, as well as in cells of the central nervous system. They bind dsRNA or ssRNA from replicating RNA viruses and also bind small RNA fragments encoded from EBV. When activated, RLRs initiate signaling pathways, resulting in production of various cytokines, e.g. type I IFN (76, 79).

1.2.1.4 Nucleotide-binding and oligomerization domain (NOD)-like receptors (NLRs)

NLRs are intracellular receptors where at least two out of 22 members can detect viral RNA. NLRs contribute to IFN production (80, 81).

1.2.1.5 DNA-sensors

DNA sensors, e.g. stimulator of interferon genes (STING), are intracellular molecules in DCs, macrophages and fibroblasts that have the ability to detect cytoplasmic dsDNA and stimulate type I IFN production (82).

1.2.2 Interferon (IFN)

1.2.2.1 Type I IFN

When viruses bind to type I IFN-initiating PRRs, interferon-regulating factors (IRFs) are upregulated, inducing transcription of type I IFNs, which have paracrine and autocrine functions. Type I IFNs stimulate proliferation, expansion and differentiation of the cells of the immune system. Many non-immunological cells have type I IFN receptors on the cell surface, which increases the transcription of IFN-dependent genes. These affect the synthesis of cell proteins, inhibit cell growth and induce apoptosis which result in an inhospitable environment for viruses (77, 83).

1.2.2.2 Type II IFN

IFN-γ is a type II IFN which is produced by NK cells, NKT cells (not discussed further), activated CD8+ T cells and CD4+ T cells polarized as T helper 1 (Th1) cells. For example, IFN-γ initiates Th1 cell differentiation, stimulates macrophages and B cells and increases the transcription of MHC molecules in different cells (84).

1.2.3 Dendritic cells (DCs)

Virus uptake, or viral protein and peptide uptake, affects several functions of DCs, such as upregulation of MHC-II to present viral peptides (71), and also make MHC-I available to these peptides, whether DCs are infected or not (cross-presentation) (85).

With MHC-bound peptides and various DC-produced cytokines, both CD8+ T cells and CD4+ T cells are activated. Type I IFN together with certain cytokines stimulates NK cells, and type I IFN together with other cytokines activates B cells into plasma cells and enhances antibody production (86). The Conventional DCs (cDCs) or myeloid DCs (mDCs) are found mainly in barrier and lymphoid tissues. Immature cDCs located in the mucosa strongly

express CLRs (e.g. DC SIGN) in their cell membrane. In the cytoplasm, TLR3 and TLR9 are expressed which detect viral RNA. When activated, the cDCs migrate to nearby lymph nodes and present the antigen to naive T cells. cDCs produce cytokines, e.g. type I IFN and type II IFN (87, 88). Plasmacytoid DCs (pDCs) express cytoplasmic TLR7 and TLR 9 as PRRs. They circulate mostly in the bloodstream and when activated they produce large amounts of type I IFN (89).

Other DCs, e.g. monocyte derived DCs, Langerhans cells etc. are not further discussed here.

1.2.4 Monocytes and macrophages

Monocytes are non-dividing receptor-rich cells with high phagocytic and antigen presenting capacity via MHC-II. When monocytes encounter viruses they activate and transform into macrophages (35). Antigen uptake by monocytes and macrophages occur approximately the same way as in DCs (78). There are several phenotypes of macrophages. The “classical” main groups are M1 macrophages that respond to infectious agents and M2 macrophages involved in tissue repair, the latter not further discussed here even though there is no clear boundary between M1 and M2. M1 macrophages are activated by antigens, cytokines, e.g. type II IFN and TNF-α (90) and by direct co-stimulatory contact (CD40) of Th1 cells which increase a non-specific phagocytosis of antigens and matrix debris (90, 91). Macrophages stimulate CD4+ T cells into Th1 cells by direct co-stimulatory contact (e.g. CD80) and produce various cytokines that contribute to inflammation (90).

1.2.5 Natural killer (NK) cells

About 5-10% of lymphocytes are NK cells, which without prior antigen presentation destroy defective cells using cytotoxic granules (71). Type I IFN and DC- or macrophage-derived cytokines stimulate NK cells to develop and mature (89, 92). To avoid destroying healthy cells, NK cells have killer inhibition receptors (KIR) that recognize cells with sufficient expression of MHC I on the cell surfaces. NK cells destroy other cells when they downregulate their MHC-Is, when they produce stress molecules due to infection and when the NK cells´ antibody receptors (Fc-γ-receptors) detect antibodies bound to the cells - a process called antibody-dependent cell-mediated cytotoxicity (ADCC) (71, 93). NK cells produce various cytokines e.g. type II IFN to enhance response from other immune cells (94). NK cells are usually considered to be part of the innate immune system. However, some NK cells are considered to have memory-like functions. The "memory" consists of these NK cells being activated by e.g. certain combinations of cytokines to which it has previously been exposed, or via the actual activating of the NK cell receptor itself – it is not bound to any specific antigen. As a result of this adaptation, it can be said that memory-like NK cells are part of the adaptive immune system (95).

1.2.6 T cells

1.2.6.1 Naive T cells

Naive T cells circulate in the bloodstream and lymphoid tissues and when they encounter APCs in the lymph nodes, they can be activated if they: 1) detect antigen presented on the APCs MHC-I or MHC-II, 2) are stimulated by special co-stimulating molecules on the APCs, e.g. CD80 or CD86 and 3) can interpret the cytokines that APCs express and differentiate based on them (96, 97).

1.2.6.2 CD4+ T cells

After antigen priming via MHC-II mainly DCs and NK cells stimulate CD4+ T cells with IL-12 or type II IFN, respectively, to differentiate into Th1 cells. Th1 cells in turn activate DCs, macrophages and B cells through direct contact with their CD40 receptors (91) and produce various proteins as IL-2, type II IFN and lymphotoxin α (LTα) – the latter not further discussed here. IL-2 is important for activation of CD8+ T cells (98), activation of B cells (99) and CD4 T cell memory (100, 101). Type II IFN activates e.g. macrophages (102). Other CD4+ T cell subsets are not further discussed here.

1.2.6.3 CD8+ T cells

After antigen priming via MHC-I and activation with type I IFN from DCs, IL-2 from Th1 cells together with IL-12 from APCs, stimulate naive CD8+ T cells to differentiate and replicate to large numbers of cytotoxic T cells (CTLs), enter the bloodstream, and transport to the site of infection. In a feed-forward loop, CD8+ T cells help to stimulate DCs, where CD4+ T cells also are involved, see above. When the infection is cleared, most CTLs die, and only a small amount remains, which will be included in the group of memory cells. CTLs produce type II IFN, TFN-α and the cytotoxic proteins perforin and granzyme B. To prevent tissue damage during viral infection, a subset of CTLs also produce IL-10 (98).

1.2.6.4 Memory T cells

In various studies, attempts have been made to define memory T cells without a uniform definition being achieved. Briefly, they are long-living and can reside in lymphoid and non-lymphoid tissues. Residing CTLs have a lower activation threshold than naive CD8+ T cells and can be activated without help from Th1 cells. T cell memory appears to be a heterogeneous set of cells, which can act differently based on different antigens (103).

1.2.7 B cells

Despite extensive maturation steps in the bone marrow, the “conventional” B cells, with their functional immunoglobulin (Ig) M (IgM) B cell receptors (BCRs), and ability to capture viruses and present antigen peptides on MHC-II or MHC-I, are not fully mature when they enter the bloodstream. The antigen-presenting B cells mature fully in a 7-day machinery for proliferation and differentiation into isotype-specific IgG-producing plasma cells and IgG-isotype-switched memory B cells, after direct co-stimulatory contact (CD40) with cytokine secretory (IL-2) Th1 cells in secondary lymphoid tissues such as the spleen and lymph nodes.

Memory B cells migrate between circulation and secondary lymphoid organs. When an antigen is detected, they must be stimulated by Th1 cells to differentiate into plasma cells and produce isotype-specific antibodies, but do not go into an extensive cell proliferation (104, 105).

Antibodies (i.e. immunoglobulins) are either BCRs on the B cell surface or secreted outside the cell and aim to bind to antigens, activate macrophages and other immune cells and activate the complement system (104). Neutralizing antibodies, usually IgG or IgA, are produced in a primary e.g. viral infection, and when a reinfection occur, they bind to the viral antigen and prevent the virus from attaching to cell receptors (71).

A minor subset of B cells derived from the bone marrow, located in the marginal zone of the spleen, functions as innate cells and recognize carbohydrate or glycolipid antigens by either BCRs or TLRs. They can produce IgMs against antigen within 1-3 days, without being activated by Th1 cells. Another subgroup, called B1 cells and derived from the fetal liver, works in a similar way (104, 105).

1.2.8 Summary

1.2.8.1 Activated APCs

DCs present antigens to naive T cells, which are activated and become Th1 cells. Macrophages present antigen to Th1 cells, which activate CD8+ T cells to CTLs, which in turn kill the activated macrophages and infected cells. Both DC and macrophages can be used to transmit viruses to other cells. B cells present antigens to Th1 cells, which stimulates the B cells to produce antibodies.

1.2.8.2 Immunity

Virus-specific proteins determine which cell receptor to bind and infect a specific cell type via endocytosis, phagocytosis or membrane fusion. Memory B cells and memory T cells can recognize a previously known virus. The former immediately begin to produce antibodies, the latter take some time and begin to act when viral antigens are presented by MHC molecules,

meaning that viruses have already had time to infect cells. Immunity means that neutralizing antibodies secreted from activated memory B cells prevent virus-specific proteins from attaching to cell receptors.

1.3 HEMATOLOGICAL MALIGNANCIES

The classifications of hematological diseases have changed a lot over the years. Sometimes a disease has been a lymphoma and then a leukemia and then again, a… - very confusing. Below is an attempt to present the various hematological malignancies that currently apply. At the end of this section, Figure 2 presents an overview of hematological malignancies.

1.3.1 Acute lymphoblastic leukemia (ALL)

ALL is characterized by a malignant proliferation of lymphoblasts in the blood and bone marrow. ALL is often formed *de novo*, but a chronic myeloid leukemia (see below) can turn into an ALL. Note, despite the name of the chronic disease, ALL never involves cells from the myeloid lineage. ALL is classified into three main types. The most common is precursor-B ALL (formerly B-lymphoblastic lymphoma). Others are T cell ALL (formerly T-lymphoblastic lymphoma), and the relatively rare Burkitt leukemia (same as Burkitt lymphoma, formerly B cell ALL) (106, 107).

1.3.2 Acute myeloid leukemia (AML)

Characteristic of AML is that at least 20% of the nucleated cells in the blood or bone marrow are proliferating malignant myeloid precursors. The disease never involves cells from the lymphoid lineage. Chronic myeloid leukemia (see below) and myelodysplastic syndrome can turn into AML. There are many different types of AML, and there are two different classification systems. The old classification – French-American-British classification (FAB) was based on the appearance of the cells (cell morphology) that labeled the different types of AML with numbers, AML-M0, AML-M1… … AML-M7. The newer classification – the WHO classification – divides the types of AML into 5 classes based on morphology, genetic changes, previous therapies e.g. chemotherapy (such as busulfan or cyclophosphamide (alkylating agents) or etoposide (topoisomerase II inhibitors)) and/or radiation therapy etc. (106-108).

1.3.3 Myelodysplastic syndrome (MDS)

In MDS, heterogeneous dysplastic changes occur in myeloid cells and there may, but need not, be an increase in blasts. Recent studies point to a disorder in a hematopoietic stem cell. The cytopathologic picture is abnormal erythrocytes, neutrophils and/or megakaryocytes. There are 6 types of MDSs, based on different dysplastic changes, e.g. refractory anemia with excess of blasts (RAEB), an aggressive type with high risk of converting to AML (107, 109, 110).

1.3.4 Chronic lymphoproliferative disorder (CLD).

The malignancy originates from relatively mature lymphoid cells, not from stem cells.

The most common type is chronic lymphocytic leukemia (CLL), also known as small lymphocytic lymphoma, which is characterized by mature-appearing B cells that infiltrate lymphatic organs, including blood and bone marrow. Some patients with CLL have hypogammaglobulinemia, which may increase susceptibility to infection. CLD also includes hairy cell leukemia, a rare form that originates in B cells, with primary sites in the blood, bone marrow and spleen. Monocytopenia is always present and pancytopenia is common. Other rare CLDs are prolymphocytic leukemia, an aggressive disease, mostly B cell type, and large granulated lymphocyte leukemia, the cell type being T- or NK cells (107, 108).

1.3.5 Chronic myeloproliferative disorder (CMD).

The disease originates in immature early stem cells before differentiation into myeloid or lymphoid stem cells. Tyrosine kinase-dependent growth receptors are constantly activated, forcing cells in different stages of maturation to proliferate. CMDs can turn into acute leukemias. CMD includes chronic myeloid leukemia (CML), characterized by high proliferation of neutrophils and their precursors in the bone marrow and blood, polycythemia vera, which affects the erythrocytic lineage, essential thrombocythemia and chronic myelofibrosis, which is characterized by marrow fibrosis (106-108).

1.3.6 Myeloma

Myeloma is a lymphoproliferative disorder in which malignant monoclonal plasma cells (from the B cell line), located in the bone marrow, produce large amounts of monoclonal immunoglobulins. The disease does not involve lymph nodes (106, 107).

1.3.7 Lymphoma

The disease begins in a lymph node or in a lymphoid tissue with cells that are sometimes morphologically and immunophenotypically identical to those seen in leukemias. The lymphomas are divided into two main groups, Hodgkin- and non-Hodgkin lymphoma (NHL). Hodgkin lymphoma, with few subtypes, originates from lymph nodes and spreads to adjacent nodes. NHL, with many different subtypes, originates and spreads between lymph nodes and/or lymphoid tissues in a rather unpredictable manner.

Different classification systems for lymphomas have been used over the years and the classification have varied greatly. Lymphomas will not be further discussed in this thesis (107).

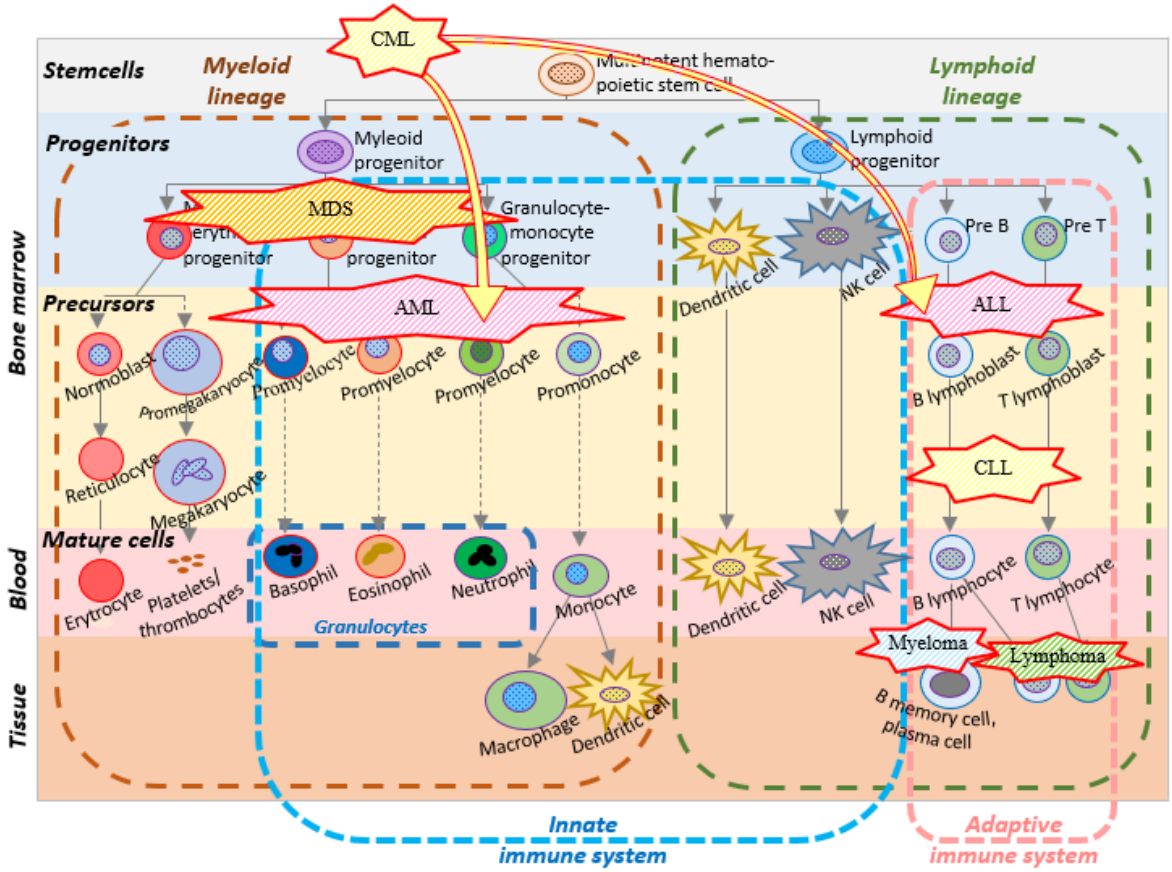

Figure 2. An overview of hematological malignancies.

Abbreviations: ALL: Acute lymphoblastic leukemia; AML: Acute myeloid leukemia; CLL: Chronic lymphocytic leukemia; CML: Chronic myeloid leukemia; MDS: Myelodysplastic syndrome.

1.4 HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

1.4.1 Allogenic grafts

Every individual has four haplotypes of human leukocyte antigen (HLA), two each from father and mother, respectively. Optimal is if the donor and the recipient have identical HLA. A matched unrelated donor (MUD) is a donor not related to the recipient, but where HLA matches well enough.

1.4.1.1 Source of grafts

Peripheral blood stem cells (PBSCs). The donor's bone marrow is stimulated to increase the production of stem cells into the bloodstream, where they can be harvested from a peripheral blood vessel for 3-5 hours (111).

Bone marrow is taken from the donor´s hip bone. The method is currently used for patients who have non-malignant diseases, or if the donor is too young to be able to lie still for as long as a peripheral harvest takes (111). Cells from bone marrow give less risk of GvHD and of transplant-related death (112).

Umbilical cord cells from the placenta are recovered at the birth of a sibling or retrieved from an umbilical cord bank. The cells are immature and can be used when it is difficult to match the HLA between the recipient and the donor. In special cases, cells from two umbilical cord donors can be combined (111).

1.4.2 Conditioning

Conditioning before HSCT is performed for two purposes: Reduce the amount of tumor cells and depress the recipient's immune system so that the donor's cells can engraft. A myeloablative conditioning (MC) consists of alkylated agents[2], often supplemented with total body irradiation (TBI). The patient's hematopoiesis is completely eliminated and cannot recover. A less toxic but still immunosuppressive regimen is the reduced intensity conditioning (RIC). Very simplified, RIC differs from MC in that the alkylating agents or TBI are reduced by about 30%. To prevent graft rejection and to prevent GvHD, patients often receive antibodies directed against T cells during conditioning. The exact conditioning regimen varies between patients and is based on the underlying diagnosis, age, comorbid factors etc. (113).

[2] Alkylating agents attach alkyl groups (C_nH_{2n+1}) covalently to nucleic acid bases of DNA (usually guanine), leading to cross-linking of DNA, causing strain breakdown and inhibition of replication.

1.4.3 T cell depletion

Anti-thymocyte globulin (ATG) is purified IgG fractions of sera from pathogen-free rabbits, horses or sometimes goats, who have been immunized with human thymocytes or T cell lines. The effects of ATG are depletion of circulating T cells and interference with B cells, NK cells and dendritic cells (114, 115).

Alemtuzumab is an antibody directed against the protein CD52, expressed on both T- and B cells, but not on hematopoietic stem cells. When alemtuzumab is bound to the lymphocyte, either NK cells will destroy the lymphocytes through antibody-dependent cellular cytotoxicity, or the complement system is activated, which destroys the cells through osmotic lysis (116).

1.5 THE IMMUNOSUPPRESSED PATIENT

The impressive and complex immune system sometimes fails with its replication, signaling and self-regulation, which is not surprising given all the components and sub-steps involved. Unfortunately, failures can lead to the development of serious hematological diseases, as described above.

Therapies for hematological malignancies and stem cell transplantation aim to suppress or eliminate defective cells to replace them with functional cells. An undesirable effect of the treatment is that all parts of the hematopoietic system are affected, i.e. even the cells that functioned properly, which in turn means that the immune system becomes dysfunctional and patients become immunosuppressed.

Immunosuppression may increase the susceptibility to infections and contribute to increased morbidity and mortality, compared to individuals with a functioning immune system.

In viral infections, many symptoms depend on the immune system's response to the virus. However, diagnosing a viral infection only by interpreting the symptoms is a challenge, especially in immunosuppressed patients. The immunosuppressed patient does not always express the symptoms expected from a particular virus. In addition, the symptoms may debut later than expected and they may also be misinterpreted as symptoms of a treatment or the underlying disease, e.g. malignancy. This means that it is not possible to rely solely on symptomatology to diagnose a viral infection.

In the section on viruses above, a brief review has been made regarding viral tropism. This means that organs that contain these cells can be affected by a viral infection, and that the infection becomes more ruthless if the patient is immunosuppressed. In the literature, there are a variety of descriptions of various serious viral infections that can occur in immunosuppressed patients. In this introduction, we do not go on to describe the infections themselves, but we are content to state that viral infections in immunosuppressed patients are of evil, and should be detected early.

1.6 PRINCIPLES FOR ANALYSIS METHODS IN THIS THESIS

Sample material that has been analyzed is:

- *Whole blood*: All blood components remain, i.e. cells – erythrocytes, leukocytes and thrombocytes - as well as proteins and coagulation factors.
- *Blood plasma*: All cells have been removed, but proteins - including immunoglobulins - and coagulation factors as well as fibrinogen remain
- *Serum*: All cells and coagulation proteins have been removed, but other proteins such as immunoglobulins remain.
- *Bone marrow*: A soft tissue found in the hollow parts of the hip bone and sternum, containing hematopoietic stem cells.

1.6.1 Polymerase chain reaction (PCR)

PCR is a method that allows a predetermined DNA sequence to be copied, i.e. amplified, in large quantities (117, 118). The method can also be used for RNA, but this will not be discussed further here.

To perform a DNA PCR, the following components are needed

- a copier for the DNA sequence – a DNA polymerase
- a start point for the copying – a primer
- building blocks for creating copies of DNA – deoxyribonucleotide triphosphates (dNTPs)
- suitable equipment and environment for the above

1.6.1.1 DNA polymerase

DNA polymerases have the ability to read DNA and build a complementary new DNA sequence while reading. The bonds between the nucleotides in DNA are strictly regulated. In one direction there is always a free hydroxyl group (3 'end) and in the other a free phosphate group (5' end). The DNA polymerases always start at the 3´end, and read towards the 5´end. Since the creation of the new DNA sequence is complementary, the new DNA strand is formed in a 5´-3´direction. The DNA polymerases used in PCR are often so-called Taq-polymerases, heat-resistant enzymes derived from the bacteria, *Thermus aquaticus* (119).

1.6.1.2 Primers

To know where to start amplifying, the DNA polymerases needs starting points, primers. The primers consist of about 20 nucleotides and are complementary bound to the DNA sequences to be copied. Because PCR technology produces double-stranded DNA (dsDNA), hybridized to single-stranded DNA (ssDNA), the primers must fit both forward and reverse DNA sequences, and be placed at the 3´end of both.

1.6.1.3 dNTPs

DNA consists of nucleotides and during reading, the DNA polymerases capture the free dNTPs to create new DNA sequences.

1.6.1.4 The PCR cycles

1. *Denaturation*. Upon heating, the dsDNA is denaturated and divided into (ssDNA). The DNA polymerase will not get affected of the heat, since it is heat-resistant.
2. *Hybridization*. The temperature drops, and the primers anneal to complementary nucleotides in the ssDNA.
3. *Elongation of DNA*. The temperature adapts to an optimal working temperature for the DNA polymerase, which makes it possible to build DNA copies from dNTPs.

These 3 steps are repeated for 30-50 cycles, and DNA is amplified for each cycle.

4. *Detection*. The PCR products (amplicons) can be detected with different techniques
   a. Size separation via gel electrophoresis (not further discussed here).
   b. Signals from sequence-specific, reporter-labeled probes. In a real-time PCR, a probe will hybridize the ssDNA in step 2 above. The probe consists of complementary nucleotides placed between the nucleotides for the forward and reverse primer, a fluorescent reporter dye in its 5´end and a quencher in its 3´end. The quencher´s function is to prevent the reporter from sending a fluorescent signal until the DNA polymerase arrives and cleaves the probe. When cleaved, the signal is sent, and the number of signals is directly related to the number of DNA copies.

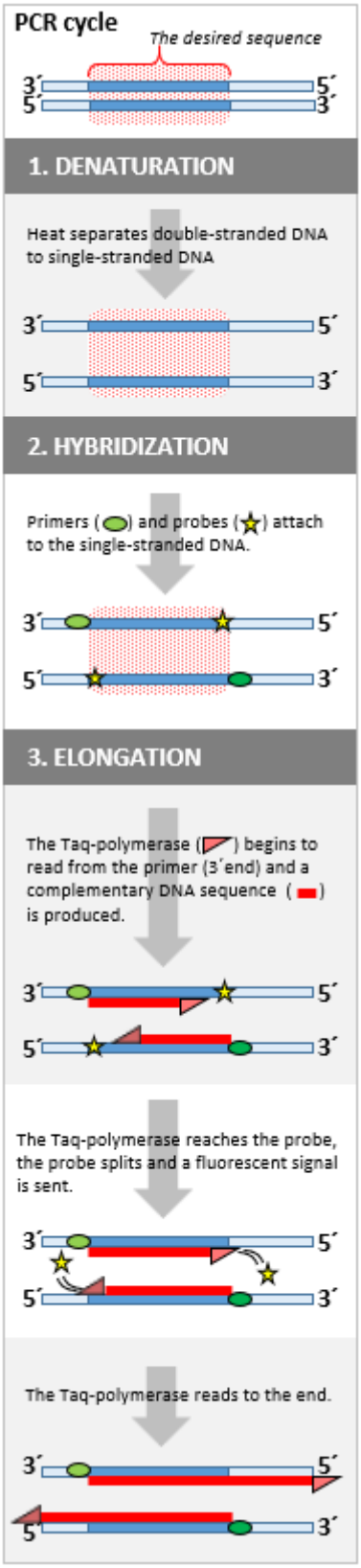

Figure 3.

1.6.2 Nested PCR

Since primers bind to complementary nucleotides throughout the whole DNA, incorrect DNA sequences may be amplified. To avoid this, the amplification can be performed in two steps with two different sets of primers, consisting of different nucleotide sequences, so-called nested PCR (120). The first set of primers - outer primers - bind outside the DNA sequence to be copied. The second set of primers - inner primers - binds more specifically to the requested sequence. The specificity of the PCR and the sensitivity of the assay increase.

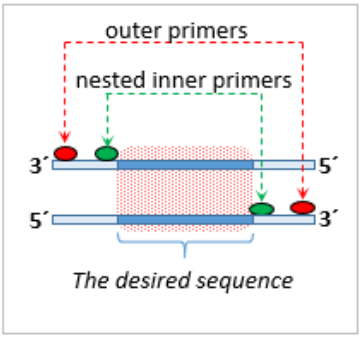

Figure 4.

1.6.3 Enzyme-Linked ImmunoSpot Assay (ELISpot)

Each antibody targets, and binds specifically to, its unique protein. If a traceable molecule is bound to the antibody, the antibody-protein complex can be detected.

All cell types express their own unique proteins on the cell surface. Protein expression can vary if the cell is activated by a stimulus or at rest. With antibodies directed against the cell surface proteins, information can be obtained about the cell type and whether the cells are activated. These principles are used in various laboratory methods – immunoassays.

If the presence or amount of *proteins already produced* is to be analyzed, the classical method Enzyme-Linked ImmunoSorbent Assay (ELISA) can be used (121). If the number of cells that have an *ongoing protein production* is to be examined, an ELISpot can be selected (122). ELISA will not be further discussed in this thesis.

Basic principles of immune assays are presented in Figure 5.

1.6.3.1 The procedure of ELISpot

1. *Preparing and coating*. The inside of the wells of a culture plate is coated with primary antibodies, which bind specifically to a unique cell surface protein secreted from activated cells from the cell type to be analyzed.
2. *Activating cells*. In the wells, a cell mixture (e.g. a blood sample) is added together with a stimulus, (e.g. an antigen) which activates the desired cells.
3. *Cell surface protein excreted*. Once activated, cell surface proteins are formed that attach to the primary antibodies.
4. *Removing excess cells*. The wells are rinsed, and cells that are not bound to the primary antibodies disappear – only activated cells of the desired cell type remain in the well.

5. *Labeling remaining cells*. New secondary antibodies, directed against the coated primary antibodies, are added to the well, followed by a chromogenic substrate (i.e. color marker) which attaches to the secondary antibodies.
6. *Detection*. The primary-antibody + cell + secondary-antibody + color-marker complex can be detected as spot forming cells in an ELISpot reader.

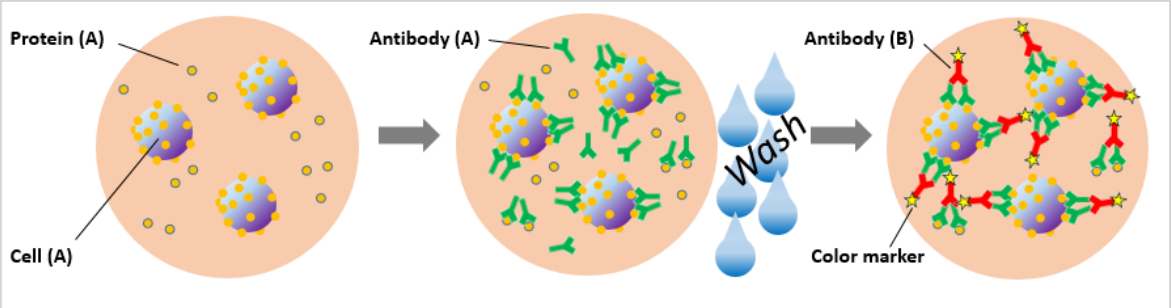

Figure 5. Basic principles of immune assays.

2 AIMS OF THE THESIS

The overarching aim of this thesis was to contribute additional knowledge about DNA viruses that cause unexpected events in patients with hematological diseases – mostly malignancies – and to evaluate whether it is advisable to continuously screen for these viruses. Two papers focused mainly on HAdV, three papers on B19V while the sixth paper focused on HHV- 6, BKV and B19V.

I. Paper [*Quantification of adenovirus DNA in unrelated donor hematopoietic stem cell transplant recipients*] aimed to evaluate the extent to which HAdV DNA is detected in patients undergoing HSCT with unrelated donors and whether screening for HAdV DNA in peripheral blood, along with routine screening for HCMV, is advisable.

II. Paper [*Evaluation of a surveillance strategy for early detection of adenovirus by PCR of peripheral blood in hematopoietic SCT recipients: incidence and outcome*] aimed to prospectively evaluate the prevalence of HAdV with means of repeated sampling during one year, in patients who had undergone allogeneic HSCT, regardless of donor status, and also to evaluate the risk factors that affect the occurrence of HAdV.

III. Paper [*Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy*] aimed to evaluate the extent to which B19V is prevalent in the bone marrow in children with cytopenia where ALL is the baseline diagnosis. We were also interested in whether, and if so, B19V affected the patients´ therapy for leukemia.

IV. Paper [*Evaluation of parvovirus B19 infection in children with malignant or hematological disorders*] aimed to investigate the extent to which B19V DNA is detected in bone marrow from children with malignant diagnoses other than ALL, whether infection with B19V also affected these patients´ condition and therapy, and if screening for B19V would be recommended for episodes of unexpected cytopenia.

V. Paper [*Human parvovirus B19 in pediatric and adult recipients of allogeneic hematopoietic stem cell transplantation, 2013*] aimed to investigate whether B19V is detected in serum during the first year post-HSCT in pediatric and adult patients.

VI. Paper [*Presence of human herpesvirus type 6, polyoma BK virus and parvovirus B19V in non-transplanted patients with hematological malignancies and neutropenic fever*] aimed to investigate the extent to which HHV-6, BKV and B19V are detected in adult patients with hematologic malignancies - not undergoing HSCT – but who had neutropenic fever.

3 PATIENTS, MATERIALS AND METHODS

All studies have ethical permissions from the Regional Ethical Review Board in Stockholm.

3.1 PAPER I *[QUANTIFICATION OF ADENOVIRUS DNA IN UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS]*

3.1.1 Patients

All 40 patients - 27 adults and 13 children – who underwent allogeneic HSCT with matched unrelated donors at the Karolinska University Hospital, Huddinge, during January – December 2004, as a result of an underlying hematological disease, were included in this retrospective study, where DNA from HCMV, HAdV and/or EBV was evaluated for one year after the transplantation. Thirty patients (of whom six were children) had a hematological malignancy as the underlying diagnosis. One of these patients died before any samples were taken. The patients were treated before HSCT, and monitored after HSCT, in accordance with the standard programs of Karolinska University Hospital, Huddinge (123). All patients underwent T cell depletion *in vivo* before HSCT, with either anti-thymocyte globulin (ATG) or alemtuzumab.

3.1.2 Sample collection

According to the clinical routines, all patients were screened for the presence of HCMV DNA after HSCT at least once a week for 12 weeks, followed by scheduled controls approximately 6, 9 and 12 months after HSCT. If an infection was suspected, analyses for DNA from HCMV, HAdV and/or EBV were performed immediately. To exclude asymptomatic viremia in patients not controlled for DNA from HAdV or EBV, three blood samples per patient, from the beginning, mid and end of the year, were tested retrospectively.

3.1.3 Methods

DNA for the viruses was analyzed with quantitative PCR (qPCR) at the Department of Clinical Virology, Karolinska University Hospital, where HCMV and EBV were analyzed according to applicable laboratory standard. Initially, the presence of HCMV DNA was quantified in leukocytes, but during the final three months of the study, the method was changed to whole blood analysis. Plasma or serum was used for assays for EBV DNA, and serum was used for HAdV DNA. The qPCR assay for detection of HAdV DNA was based on amplification of a conserved region of the HAdV hexon (124). The template was supplemented with a newly designed probe to minimize mismatch between nucleotides in HAdV subgroup C and the PCR amplicon. The sequence of the probe is reported in this paper. Clinical data were obtained from the patients´ medical records.

3.2 **PAPER II** [*EVALUATION OF A SURVEILLANCE STRATEGY FOR EARLY DETECTION OF ADENOVIRUS BY PCR OF PERIPHERAL BLOOD IN HEMATOPOIETIC SCT RECIPIENTS: INCIDENCE AND OUTCOME*] **AND PAPER V** [*HUMAN PARVOVIRUS B19 IN PEDIATRIC AND ADULT RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION*]

3.2.1 Patients

A total of 97 patients - 77 adults and 20 children – out of 101[3] patients who underwent allogeneic HSCT, with matched unrelated or related donors, at the Karolinska University Hospital, Huddinge and Sahlgrenska University Hospital, Gothenburg, during a period for 1.5 years (March 2006 – September 2007), were included in a prospective study.

The patients were treated before HSCT, and monitored after HSCT, in accordance with the standard programs of Karolinska University Hospital, Huddinge and Sahlgrenska University, Gothenburg (123). T cell depletion *in vivo* were performed in 47 adults and 15 children before HSCT, with either anti-thymocyte globulin (ATG) or alemtuzumab.

3.2.2 Sample collection

According to the HCMV monitoring program, all patients were screened for the presence of HCMV DNA, and for this study simultaneously for HAdV DNA and B19V, at least once a week for 9 weeks after HSCT. Thereafter, scheduled analyses were performed approximately around 6, 9 and 12 months after HSCT. If infection was suspected, DNA analyses were performed immediately.

3.2.3 Methods, paper II

DNA for HAdV was analyzed in plasma with the same qPCR method as described in paper I, at the Department of Clinical Virology, Karolinska University Hospital. Clinical data were obtained from the patients´ medical records.

For twelve randomly selected adult patients, a supplemental ELISpot assay was performed to evaluate the presence of HAdV-specific T cells. Here, the method is briefly described:

Day 1, 96-well culture plates were prepared and coated with antihuman IFN-γ monoclonal antibodies in order to detect cells with IFN-γ release, here T cells.

Day 2, PBMCs, isolated from blood samples, together with a suspension of HAdV antigen, were added to the wells.

[3] Four patients were excluded as a result of too few samples (< 5).

Day 3, spot-forming cells were detected with an ELISpot reader by the addition of biotinylated[4] anti-IFN-γ monoclonal antibodies followed by a color substrate.

The more detailed protocol with information on the type of monoclonal antibodies, substrates, manufacturing companies, etc. is presented in Paper II.

Risk factors for HAdV infection were evaluated statistically with a multiple logistic regression model.

3.2.4 Methods, paper V

DNA for B19V was analyzed in serum with a qPCR (125) at the Department of Clinical Virology, Karolinska University Hospital.

3.3 PAPER III [*PARVOVIRUS B19 INFECTION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IS ASSOCIATED WITH CYTOPENIA RESULTING IN PROLONGED INTERRUPTIONS OF CHEMOTHERAPY*] AND PAPER IV [*EVALUATION OF PARVOVIRUS B19 INFECTION IN CHILDREN WITH MALIGNANT OR HEMATOLOGICAL DISORDERS*]

3.3.1 Patients

A total of 240 children underwent investigations, for proven or suspected malignancies, at the Pediatric Oncology Unit, Karolinska Hospital, Stockholm, during a period for 5.5 years (1995-2000). Paper III includes 117 children with ALL, and paper IV includes 123 children with hematological diseases, other than ALL, and children with solid tumors – primarily neuroblastoma and sarcoma.

3.3.2 Sample collection

According to the clinical routines, bone marrow from the patients was analyzed when malignancy occurred or was suspected. Retrospectively, samples not excluded on technical or logistic grounds, were analyzed to investigate the presence of B19V DNA.

[4] Biotin forms the bond for the color marker.

3.3.3 Methods

All bone marrow samples were analyzed with a qualitative nested PCR representing the non-structural NS protein of B19V (126). Samples positive for the NS protein were further analyzed in our group with qPCR (125) performed at the Department of Clinical Virology, Karolinska University Hospital. In some cases, the samples had been thawed and frozen on several occasions, making it difficult to replicate a positive result from the nested PCR. In other cases, the samples contained so much B19V DNA that they had to be diluted several times. In those patients where only one of several samples was positive on the nested PCR, all samples were re-analyzed in the more sensitive qPCR. In some cases, B19V DNA was also detected in some of these samples. All samples that tested positive for B19V DNA in either the nested PCR or the qPCR were included in the final assay. Clinical data were obtained from the patients´ medical records, and in paper II also from the Nordic Childhood Leukemia registry (NCLR).

In paper III several statistical analyses were performed to evaluate if the results could be generally valid. Patients with the presence of B19V DNA were compared to patients without B19V DNA. To evaluate how B19V was linked to age and affected the number of days without treatment, the Mann-Whitney *U*-test was used. The association of B19V to age at diagnosis, gender, treatment protocol and possible delays in therapy, was evaluated using a multivariable linear regression model. To analyze if the need for extra bone marrow examinations and extra blood transfusions was limited to only the relatively few patients diagnosed with B19V DNA, a logistic and Poisson regression with exact estimates was used.

3.4 PAPER VI [*PRESENCE OF HUMAN HERPESVIRUS TYPE 6, POLYOMA BK VIRUS AND PARVOVIRUS B19V IN NON-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND NEUTROPENIC FEVER*]

3.4.1 Patients

A total of 79 adult non-transplanted patients with different hematological malignancies, who were treated at the Karolinska University Hospital, Stockholm, and had at least one episode of neutropenic fever during a period for 2 years (February 2013 – February 2015) were included in the study.

3.4.2 Sample collection

According to the clinical routines, blood samples were collected when the patient arrived at the hospital, and were here retrospectively analyzed for DNA from HHV-6A or 6B, BKV and B19V.

3.4.3 Methods

Quantification of HHV-6A or 6B (127) and B19V (125) DNA have been previously described. For analysis of BKV DNA the commercial BKV ELITe MGB ® Kit was used (128). All analyzes were according to the applicable laboratory standard, at the Department of Clinical Microbiology, Karolinska University Hospital.

4 RESULTS AND DISCUSSION

4.1 **PAPER I** [*QUANTIFICATION OF ADENOVIRUS DNA IN UNRELATED DONOR HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS*] **AND PAPER II** [*EVALUATION OF A SURVEILLANCE STRATEGY FOR EARLY DETECTION OF ADENOVIRUS BY PCR OF PERIPHERAL BLOOD IN HEMATOPOIETIC SCT RECIPIENTS: INCIDENCE AND OUTCOME*]

Infections with human HAdVs are not uncommon in patients undergoing HSCT (4, 129-131). When the samples were collected for the first study, the Department of Clinical Virology, Karolinska University Hospital in Huddinge, had just launched the PCR assay for HAdV DNA.

4.1.1 Papers I and II

PCR. The specificity of PCR depends on how unique the gene sequence to be analyzed is and how well the method's primers and probes are designed. In the first study a newly designed probe was used to minimize mismatch between nucleotides in HAdV subgroup C and the PCR amplicon. At the time for the first study, there were no accepted limits for when viral DNA loads were “high”, but we considered > 10,000 copies/mL as "high".

When the presence of viral DNA is analyzed by PCR, only specific DNA sequences are searched, i.e. the entire genome is not searched for. It may be unclear if the DNA sequence sought comes from a viable virus or if viral residues are captured. Low levels of viral DNA detected in blood can also be derived from latent infections. This means that DNA detected by PCR does not automatically indicate an ongoing infection. An evaluation of the patient's general condition must always be included in the assessment. The general condition of all patients with high viral load was evaluated using medical records.

4.1.1.1 Presence of HAdV DNA

There is no effective treatment for HAdV, but it may still be important to diagnose the virus, not for the treatment itself but to rule out other pathogens. In the first study, with patients undergoing HSCT with matched unrelated donors, we found HAdV DNA at least on one occasion in 6 of 39 (15%) patients tested. For comparison, the presence of HCMV and EBV viremia was also evaluated, and according to previous studies, high loads of DNA from HCMV and EBV are associated with morbidity and mortality.

For 27 of 29 patients tested – with underlying hematological malignancies – DNA from HAdV, HCMV and/or EBV was detected. High DNA loads (> 10,000 copies/mL) from either of the viruses were detected in 11 patients, of whom 8 died in the first year after HSCT. Three of the deceased patients had high levels of HAdV DNA and in at least two patients, infection with HAdV was considered to contribute to the fatal outcome. High levels of viral

DNA were not detected in any of the 10 patients with a non-malignant hematological diagnosis.

Although three samples per patient were retrospectively analyzed from patients without symptoms of HAdV infection, and no DNA was present, it was unclear whether transient asymptomatic viremia could occur, which the patient himself cured. This, together with the fact that high levels of HAdV DNA appeared to be associated with serious or fatal outcomes, led to the initiation of study 2.

In the second study two years later, we found that in patients undergoing HSCT with unrelated donors, HAdV DNA in blood could be detected at least on one occasion in 3 of 59 (5%) patients tested. In the total study population of 97 participants, HAdV DNA was present in 5 patients. None of them had high viral DNA loads (> 10.000 copies/mL) of HAdV. None of the patients developed symptoms associated with HAdV.

Table 3. Comparison between positive cases in study 1 and study 2

Patient no. *(Diagnose)*	Age at HSCT *(Graft)*	Conditio-ning/ *(T cell depletion)*	Maximum load viral DNA, [copies/mL]			Days to death post-HSCT
			HAdV *(serotype)*	CMV *(lc/wb)*	EBV	
Study 1						
Unrelated donors						
7. *(MDS/RAEB)*	13 *(BM)*	RIC *(yes)*	15,000 *(HAdV 5)*	< 500 *(lc)*	90,000	94
10. *(Myeloma)*	49 *(PBSC)*	RIC *(yes)*	$> 10^6$ *(HAdV 2)*	200,000 *(wb)*	450,000	82
12. *(NHL-B)*	48 *(BM)*	RIC *(yes)*	$> 10^6$ *(HAdV 35)*	< 500 *(wb)*	0	40
13. *(ALL pre-B)*	24 *(cord)*	MC *(yes)*	< 500 *(HAdV 2)*	3,500 *(wb)*	< 500	alive
22. *(CML)*	53 *(PBSC)*	MC *(yes)*	< 500 *(HAdV 12)*	0 *(lc)*	0	alive
36. *(SAA)*	8 *(BM)*	MC *(yes)*	< 500 *(NA)*	0 *(wb)*	0	alive
Study 2						
Unrelated donors						
2. *(MDS)*	32 *(PBSC)*	MC *(yes)*	< 500 *(HAdV 3)*	5,400 *(wb)*	1,200	alive
3. *(MDS)*	14 *(PBSC)*	MC *(yes)*	< 500 *(HAdV 2)*	800 *(wb)*	< 500	106
5. *(Hurlers')*	1,5 *(BM)*	RIC *(yes)*	9,000 *(HAdV 1)*	0 *(wb)*	120,000	alive
Related donors						
1. *(MDS)*	42 *(BM)*	RIC *(yes)*	< 500 *(NA)*	14,000 *(wb)*	0	alive
4. *(MDS)*	12 *(BM)*	MC *(no)*	830 *(HAdV 31)*	37,000 *(wb)*	< 500	166

Abbreviations: MDS: Myelodysplastic syndrome, NHL: Non-Hodgkin lymphoma, ALL: Acute lymphoblastic leukemia, CML: Chronic myeloid leukemia, SAA: severe anaplastic anemia, BM: Bone marrow, PBSC: Peripheral blood stem cells, MC: Myeloablative conditioning, RIC: Reduced intensity conditioning, lc: Leukocytes, wb: Whole blood, NA: Not analyzed.

It is interesting that two studies, conducted relatively closely in time, in which patients followed the same treatment protocol and the same methods were used, differed regarding the presence of HAdV DNA. We do not know what caused the differences, but one explanation may be that the number of participants in both studies was too small to cover the normal different individual variations that occur in people. Alternatively, the initial hematological diagnoses were too heterogeneous, and the number of patients with each diagnosis too few, for the results to be representative. A total of 8 different serotypes from species A, B1, B2, C were present in the studies - none of them uncommon in immunocompromised patients, see Table 2. None of the studies considered co-infections with bacteria or fungi. One conclusion, however, is that high levels of DNA from HAdV, CMV or EBV are associated with fatal outcome.

4.1.1.2 Risk factors

In the second study with a total of 97 patients, 15 of 84 (18%) patients with an underlying hematological malignancy were diagnosed with myelodysplastic syndrome (MDS). Four of these patients presented HAdV DNA in blood, and in two of these, bone marrow was the source of stem cells.

Risk factors for the development of HAdV infection have previously been reported to be low age, unrelated donors, total body irradiation, donor HAdV antibodies, *in vitro* or *in vivo* T cell depletion. In a large study, the virus was isolated exclusively in recipients of T cell-depleted grafts (132). In our second study, statistical analysis concluded that the underlying diagnosis and type of graft – MDS and bone marrow, respectively – were two independent significant risk factors for presenting HAdV DNAemia. Factors that did not increase the risk of HAdV infection were the type of preconditioning, *in vivo* T cell-depletion, TBI, unrelated donors and GvHD. A statistical analysis of risk factors was never done in the first study, partly because the number of participants was low. When the risk factors MDS and bone marrow had been determined, a comparison was made with the first study to see the impact of these factors:

- *MDS.* In the first study, 6 of 30 (20%) patients with hematological malignancies were diagnosed with MDS, of which 5 died. One of the deceased patients received stem cells from bone marrow and the DNA loads of HAdV as well as EBV were high. The other patients with MDS received PBSC, and in 2 of the deceased, large amounts of DNA from HCMV and EBV were detected in blood. DNA from HAdV was not present. However, MDS is a heterogeneous diagnosis with many subtypes, which were not taken into account when evaluating risk factors.

- *Bone marrow.* In the first study, 5 of 30 (17%) patients with underlying hematological malignancies received bone marrow as graft. In 3 out of 5 patients, high loads of viral DNA were detected in blood. All three died. In addition to the patient with MDS, as

mentioned above, the other two patients with diagnoses other than MDS presented high viral DNA loads of HAdV and HCMV, respectively, in blood.

It is important to remember that discussions about risks are usually about the group, not about the individual, and risk factors are events that involve increased risk. The absence of one or more risk factors does not necessarily mean that the risk does not exist. In medicine, risk factors are based on current knowledge. New knowledge is constantly being added. Each patient must always be evaluated individually.

4.1.1.3 Screening.

None of the studies provided a basis for the introduction of HAdV DNA screening in blood post-HSCT, but analysis of HAdV DNA in blood was considered valuable to exclude possible infections in patients who do not respond as expected to the treatment given after HSCT.

4.1.1.4 HAdV specific T cells

HAdV-specific T cells develop during childhood and are thought to have a cross-reactivity between different serotypes of HAdV. Since the patient's own immune system is suppressed, it is mainly the presence of the donor's HAdV-specific T cells that is of interest here. With ELISpot, the presence of HAdV-specific T cells was analyzed in twelve randomly selected patients. In parallel a chimeric analysis for mature T cells was performed, to determine the establishment of the graft. Unfortunately, none of the patients in whom HAdV DNA was detected were included. The group was heterogeneous with different hematological diagnoses, both related and unrelated donors, both T cells-depleted and not T cells-depleted, myeloablative conditioning (MC) and reduced intensity conditioning (RIC), and both PBSC and bone marrow as grafts. The results are presented below in Table 4, and it is difficult to draw any other conclusions than that HAdV-specific T cells were present in 7 of 12 patients.

4.1.1.5 Clinical recommendations for HAdV

In 2011, the fourth European Conference of Infections in Leukemia (ECIL-4) recommended weekly monitoring with qPCR of HAdV in patients with at least one risk factor. Risk factors for children and adults are unrelated HSCT with a cord blood graft, severe graft-versus-host disease and lymphopenia. Additional risk factors for adults are treatment with alemtuzumab, and for children, T cell depletion associated with the HSCT. Generally, children have an increased risk of HAdV infections (133).

Papers I and II are included in the reference list of the ECIL-4 guideline, but the risk factors from Paper II were not taken into consideration.

Table 4. Relations between patients and results from ELISpot and chimerism.

Patient no. *(Diagnose)*	Donor *(Graft)*	Conditioning *(T cell depletion)*	ELISpot result, number of spots *(Chimerism **donor** CD3 %)*					
			4 weeks		8 weeks		12 weeks	
1. (AML)	MUD *(PBSC)*	MC *(yes)*	*NA*	*99,99*	*NA*	*99,08*	**80**	*99,83*
2. (AML)	MUD *(PBSC)*	RIC *(yes)*	**20**	*99,47*	**34**	*99,80*	**25**	*83,66*
3. (Lymph.)	MUD *PBSC)*	RIC *(no)*	*no spots*	*99,99*	**30**	*99,01*	**32**	*99,80*
4. (AML)	MUD *(BM)*	MC *(no)*	**24**	*99,92*	**36**	*99,99*	*no spots*	*99,90*
5. (MDS)	MUD *(PBSC)*	MC *(no)*	**87**	*82,00*	*no spots*	*95,20*	*no spots*	*88,50*
6. (AML)	Sibling *(BM)*	MC *(no)*	**111**	*96,56*	**38**	*99,03*	**32**	*97,10*
7. (ALL)	Sibling *(BM)*	MC *(no)*	*NA*	*95,64*	*no spots*	*96,14*	**30**	*99,99*

Abbreviations: AML: Acute myeloid leukemia, Lymph: lymphoma, MDS: Myelodysplastic syndrome, ALL: Acute lymphoblastic leukemia, MUD: Matched unrelated donor, BM: Bone marrow, PBSC: Peripheral blood stem cells, MC: Myeloablative conditioning, RIC: Reduced intensity conditioning, NA: Not analyzed.

Cut off value for ELISpot confirming the presence of HAdV specific T cells is 20 spots or more. Chimerism status is an analysis of the proportion of stem cells derived from the recipient and donor, respectively. The table shows the chimerism of the donor.

4.2 **PAPER III** *[PARVOVIRUS B19 INFECTION IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IS ASSOCIATED WITH CYTOPENIA RESULTING IN PROLONGED INTERRUPTIONS OF CHEMOTHERAPY]*, **PAPER IV** *[EVALUATION OF PARVOVIRUS B19 INFECTION IN CHILDREN WITH MALIGNANT OR HEMATOLOGICAL DISORDERS]* **AND PAPER V** *[HUMAN PARVOVIRUS B19 IN PEDIATRIC AND ADULT RECIPIENTS OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION]*

It has been shown that B19V DNA is present in children with hematological malignancies, i.e. during the so-called maintenance treatment, (134-137) and also in other malignancies (138, 139) and is connected to increased need for transfusions, extra bone marrow tests and also interruptions in the ongoing chemotherapy. In immunocompetent individuals without malignancies, bone marrow persistence of B19V may exist, but is not common after the primary infection (140, 141).

4.2.1 Papers III and IV

4.2.1.1 PCR

The analysis of the extensive material (726 bone marrow samples) started with nested qualitative PCR with sequences from the essential protein NS1 as primer. When B19V DNA

was detected in a patient, complementary analysis was performed for all samples from that patient, using a more sensitive qPCR method (125). A clinical evaluation using medical records was performed for all patients who presented B19V DNA in bone marrow.

4.2.1.2 Presence of B19V DNA

In studies 3 and 4 bone marrow samples from 240 children, collected under 5.5 years were tested in retrospective. In total, B19V DNA was detected in 27 patients. According to the medical records only one patient had symptoms typical of B19V infection. No analysis was performed to evaluate the presence of B19V DNA in peripheral blood.

The most common diagnosis for bone marrow sampling was ALL and the results are presented in study 3. Other diagnoses for bone marrow sampling were AML, NHL, other hematological malignant and non-malignant disorders as well as solid tumors such as neuroblastoma and sarcoma, which are presented in study 4. The number of patients within each diagnosis and the presence of B19V DNA are presented in Table 5.

Table 5. Patients positive for B19V DNA

Diagnoses	No. of patients	No. of samples tested	No. of patients with B19V DNA *(%)*
Study 3			
ALL	117	497	18 *(15 %)*
Study 4			
Other than ALL	123	229	9 *(7 %)*
AML	*20*	*78*	*2*
NHL	*12*	*25*	*3*
Solid tumors	*31*	*59*	*2*
Non-malignant hematological disease	*41*	*43*	*2*
MDS, Hodgkin	*9*	*13*	--
Unspecific diagnoses	*10*	*11*	–
Total	240	726	27 *(11 %)*

Abbreviations: ALL: Acute lymphoblastic leukemia, AML: Acute myeloid leukemia, NHL: Non-Hodgkin lymphoma, MDS: Myelodysplastic syndrome.

The treatment protocols for ALL have varied over the years, but generally after a thorough examination of the disease, an induction phase begins with high-dose cytostatic and cortisone, currently for about a month, followed by maintenance treatment with varying

chemotherapies for about 2.5 years (142). Compared to the induction phase, the maintenance phase is less toxic and severe cytopenia is not expected.

In the group with ALL, B19V DNA was detected in seven patients during maintenance therapy. A statistically significant association could be found between the presence of viral DNA and an increased number of episodes of unexpected cytopenia requiring erythrocyte- and/or thrombocyte infusions and more days of treatment discontinuation, compared with patients without B19V DNA in bone marrow. In four of these patients, episodes of cytopenia were so severe that relapse in ALL was suspected. The viral load was higher in patients in the maintenance phase compared to the other patients. However, both the underlying disease and the toxic effect of chemotherapy can affect the number of cells in the bone marrow. Because the number of cells was not counted, it is difficult to draw any conclusions based on viral load.

No statistically significant conclusions could be drawn for the 6 patients who were in the diagnosis and induction phase, nor for the 5 patients who had completed their chemotherapy.

The non-ALL group included heterogeneous diagnoses that require different therapies. In eight of nine patients, B19V DNA was detected in the first bone marrow sample, which was performed as part of the diagnostic examination. Because bone marrow punctures are invasive, no further sampling was performed for the majority when the diagnosis was made, unless it was absolutely necessary. None of the patients were immunosuppressed and none had received any previous blood products. B19V serology was not examined. During the following six months after diagnosis, seven of the patients had several episodes of fever and prolonged transfusion-requiring cytopenia. Complementary bone marrow punctures were performed in four patients with hematological malignancies, where B19V DNA was present for up to two years. Because the number of underlying diagnoses in the non-ALL group was broad-spectrum, statistical evaluation was not possible.

4.2.1.3 Serology

In 2002 Heegaard et al, showed that children with ALL could seroconvert with respect to B19V, both from IgG-negative to IgG-positive as well as the opposite, i.e. losing the antibodies. Upon conversing to IgG-positive, B19V DNA was detected in peripheral blood, and the infection resulted in anemia and thrombocytopenia (143).

In the first study, serology testing was performed for 38 patients with ALL. The limited serological evaluation showed that B19V IgG was present in 15 patients where B19V DNA could

Table 6. Serology for patients in study 3.

	B19V DNA positive *(n=18)*	B19V DNA negative *(n=99)*
IgG-positive *(n=15)*	none	15
IgG-negative *(n=23)*	5	18
Serology not tested *(n=79)*	13	66

not be detected in bone marrow, see Table 6. The result is interesting and may indicate that the immunogenic effect of B19V remains in some patients with ALL, but the number of patients in this study is too small to draw such conclusions.

4.2.2 Paper V

To evaluate whether B19V infection occurs in patients undergoing HSCT, 97 patients (77 adults, 20 children) from study 2 were analyzed by qPCR for the possible presence of B19V DNA in peripheral blood. Samples were collected weekly for the first nine weeks post-HSCT and then on return visits approximately week 12, 26 and 52.

B19V DNA was detected in two adults and one child undergoing HSCT with a matched unrelated donor (MUD) with peripheral blood stem cells (PBSC) and T cell depletion. When B19V DNA was detected, the child had fever, but none of the adults reported any symptoms. This was consistent with previous observations where a low prevalence of B19V infections has been reported post-HSCT (144).

4.2.3 Clinical recommendations

From an academic point of view, it is interesting to know whether the presence of B19V is a primary or reactivated infection, which the studies 3 and 4 could not determine. From a clinical perspective, it is more important to know if infectious B19V is present, and that the methods used to detect the virus are reliable.

If unexpected cytopenia occurs in a child with hematological malignancy, the current clinical practice at Karolinska University Hospital is, based on several studies - our studies included, that blood samples and even bone marrow - if available – are checked for presence of B19V DNA.

For children with hematological malignancies, there is no exact viral DNA load of B19V indicating a clinically relevant viremia. The assessment of a threshold value is also complicated by the fact that the patients´ immune status are affected by the treatment and the underlying disease. For comparison, it can be stated that in the acute phase, and before the onset of symptoms, the level of B19V DNA in serum in an immunocompetent individual can reach > $1x10^{10}$ copies/mL (55). The level of viral DNA decreases quite rapidly, but a low level can remain for several years as a sign of a persistent infection (145).

At what B19V DNA level the infection should be considered as “recent” or “past” is discussed, and a suggestion is a cut-off level of about $1x10^4$ copies/mL. If there is uncertainty regarding the interpretation of levels around $1x10^4$ copies/mL, it is recommended that the analysis be supplemented with serology (146). It is not possible to find information in the literature on how different levels of B19V DNA in bone marrow should be interpreted.

In adults with hematological malignancies or who have undergone HSCT, and where unexpected cytopenia occur, there is currently no general protocol for B19V DNA testing.

4.3 PAPER VI [*PRESENCE OF HUMAN HERPESVIRUS TYPE 6, POLYOMA BK VIRUS AND PARVOVIRUS B19V IN NON-TRANSPLANTED PATIENTS WITH HEMATOLOGICAL MALIGNANCIES AND NEUTROPENIC FEVER*]

Paper I, II and V include adults who were immunosuppressed as a result of HSCT. Paper VI includes adults with hematological malignancies, who have *not* undergone HSCT, and in whom immunosuppression was due to chemotherapy. Because the conclusions in the literature are not entirely consistent regarding the prevalence of HHV-6A, 6B and BKV DNA in blood in adult immunosuppressed patients *not* undergoing HSCT, the presence of these viruses was studied when patients were diagnosed with neutropenic fever. As neutropenia occurred, analyses of B19V DNA in blood were included.

None of the patients presented DNA from HHV-6A. In 14 of 79 (18%) patients, DNA from any of the other viruses was present, see Table 7. One patient was diagnosed with “fever of unknown origin” (FUO). For the other patients, alternative explanations for the fever were found.

A high viral load in blood of HHV-6B DNA (2,4 x 10^5 copies/mL) was detected in a patient with a blood neutrophil count of <0.1x10^9/L. This may indicate chromosomally integrated HHV-6B DNA rather than viral reactivation.

Because analyzes were not performed on the patients´ bone marrow, this study does not clarify whether HHV-6B, BKV or B19V in any way contributed to these patients´ neutropenia. Paper III and IV presented B19V-associated cytopenia in children undergoing chemotherapy and BKV may cause cytopenia in kidney-transplant recipients (147). Whether infectious B19V and/or HHV-6B is present in bone marrow of adults undergoing chemotherapy is less well known. There are case reports in the literature on infectious virus, but it is difficult to find studies evaluating infectious B19V or HHV-6B in bone marrow. Whether this is a consequence of the lack of findings not being considered interesting, or the fact that studies have not been carried out, is unclear.

On one hand, it is a pity that the bone marrow was not tested in this study. On the other hand, it is doubtful to perform bone marrow puncture, an invasive method, on patients with neutropenic fever, unless the results of the tests are essential for the patient's treatment. There is currently no *general* recommendation for bone marrow analysis when neutropenic fever occurs in adult patients undergoing chemotherapy.

Table 7. Viral DNA in adult patients with non-transplanted hematological malignancies.

Patient no.	Maximum load viral DNA, [copies/mL]		
	HHV-6B	BKV	B19V
ALL			
D.	30,000	-	-
L.	2,900	21,800	-
K.	-	14,500	-
M.	-	6,600	-
I.	-	< 500	-
G.	-	650	< 500
F.	-	-	< 500
AML			
C.	240,000	-	-
B.	600	-	-
A.	< 500	< 500	-
J.	-	-	< 500
NHL			
E.	< 500	< 500	-
H	-	1,350	-
N.	-	< 500	-

Abbreviations: ALL: Acute lymphoblastic leukemia, AML: Acute myeloid leukemia, NHL: Non-Hodgkin Lymphoma.

5 CONCLUSIONS AND FUTURE PERSPECTIVES

The aim of these studies was to understand the prevalence of HAdV and B19V infections in different groups of immunosuppressed patients, and whether established screening programs for these infections, as well as for infections with HHV-6A, 6B and BKV, may be of clinical value.

Diagnosis of viral infections

The proportion of patients with HAdV infection in our material was relatively small, compared to the literature, and asymptomatic infections did not occur. On the other hand, HAdV DNA loads >15,000 copies/mL in blood were associated with morbidity and mortality. Findings of B19V in bone marrow from children undergoing treatment for ALL were associated with discontinuation of treatment, leading to prolonged chemotherapy. Neither B19V, HHV-6A, 6B nor BKV was common in the blood of adult patients with hematological malignancies who were immunosuppressed due to chemotherapy. Overall, general screening for these viruses, in the patient groups presented, does not seem to be indicated according to these studies. However, testing for HAdV should be performed generously when unexpected symptoms occur, even if they are not associated with common signs of the virus infection. B19V infection is almost always linked to some form of cytopenia, and if unexpected cytopenia occurs in children undergoing chemotherapy, B19V infection should be ruled out.

While analyzing the present studies, the challenges that many clinicians face also emerged. One of the challenges is to understand if unexpected symptoms are due to the underlying disease or an ongoing therapy, or should be related to pathogens - viruses, bacteria or fungi. At present, there is no rapid general analysis that can determine the type of pathogen that causes an infection. This can result in excessive antibiotic use and subsequent risk of antibiotic resistance. However, each group of pathogens, bacteria, viruses or fungi, must be tested separately, based on the clinical assessment. Another challenge is to identify if an infection is systemic or local - should samples be taken from blood, tissue or both? Reactivated infections with B19V and HAdV often begin at a tissue site and cannot always be detected in blood until the infection becomes apparent and symptoms, sometimes severe, have occurred. Yet another challenge, when the type of pathogen is to be determined, e.g. a virus, is to pre-define which virus to test for since each test is specific. Molecular methods are relatively fast and for respiratory viruses there are methods for multi-testing, i.e. one can test for several viruses simultaneously. However, tests for blood-borne viruses and viruses in tissues are still based on the clinician deciding in advance which virus to test for. If the clinician assessed the diagnosis correctly, the quantitative value of the viral load may be indicative. However, if the assessment was incorrect regarding which virus to test for, the pre-selected PCR will not provide further information. This means that a patient may have a fulminant viral infection, but if the initial assessment (guess) regarding the virus was incorrect, the analysis will have no major value.

Targeted microbiological examinations to cover unclear or rare pathogens are available today, but are not routinely used in the clinic. For example, there are possibilities to analyze all nucleic acids in a sample material by so-called metagenomics, and then compare any non-human sequences with databases of different pathogens. The method sounds simple, but requires skilled bioinformatics and clinical understanding, as large amounts of "noise" are often generated. Convenient and cost-reduced assays for clinical use are thus highly needed.

The patient´s immune system

Although the methods of analysis are becoming more advanced, there is a need to increase the understanding of how the human immune system reacts to various infections. This is because it is unclear why some patients seem to suffer more from viral infections than others, even though the external conditions seem to be the same.

Perhaps at some point in the future, the individual will be able to get an accurate description of their own immune system - much in the same way that an individual's e.g. blood type can be determined today. Perhaps it will also be clarified why some individuals remain asymptomatic, while others can become very ill from the same pathogen. With such knowledge, different individuals can take different measures to protect themselves against infection, sampling and treatment would be individually focused and perhaps thus more effective and contribute to reduced morbidity.

Antiviral therapies

According to the recommendations of ECIL-4, prophylactic treatment of HAdV is not recommended. The document points out T cell depletion as one of the risk factors for HAdV infection. More risk factors are stated in the document but are not discussed further here. For patients with viremia and at least one risk factor, preemptive treatment with cidofovir, a cytidine[5] analogue, is recommended, even if the viremia does not appear to be clinically significant. When an infection with HAdV occurs, it is recommended to reduce the immunosuppressive treatment, if possible. If this is not possible, or if the infection persists, treatment with cidofovir is recommended. The document states that no clinical trials have been performed that clearly support the effect of cidofovir as an antiviral medication. Treatment with donor-derived HAdV-specific T cells are also discussed, which should primarily be seen as an experimental treatment (133).

There are no specific antiviral drugs for the treatment of B19V infection. However, if a severe B19V infection is diagnosed, intravenously administered immunoglobulin intravenously (IVIG) is recommended. Neutralizing antibodies against B19V are a natural component of the IVIG preparation and can effectively lower the B19V load in

[5] Cytidine is a nucleoside, i.e. a cytosine molecule linked to the sugar molecule ribose.

immunosuppressed patients (55). A vaccine against B19V, consisting of viral capsid proteins, has been tested in clinical trials. Neutralizing antibodies to VP1 and VP2 were detected as a result of the vaccine (148, 149). No evaluation has been made as to whether the vaccine can prevent reactivation of B19V infection. However, the trial was discontinued due to unexplained cutaneous events (148, 150).

Regarding infection with BKV and based on current data, the Swedish reference group for antiviral therapy (RAV) does not recommend cidofovir as standard treatment for BKV. RAV states, however, that it cannot be ruled out that a certain effect may exist. Attempts have been made to treat BKV with IVIG. The results have been contradictory, and IVIG cannot be recommended as a treatment (151). An ongoing phase 2 clinical trial is testing whether human monoclonal antibodies can neutralize all four genotypes of BKV. The first patient was included in August 2020 (152).

When HHV-6 is reactivated, it occurs mainly post-HSCT and symptoms associated are e.g. fever and rash, myelosuppression, GvHD and even pneumonitis; conditions that cause increased morbidity and mortality. There is currently no specific treatment for HHV-6 viremia. Immunotherapy with HHV-6-specific T cells has been tested in small studies. The most serious clinical complication of HHV-6B is encephalitis, which is a major contributor to increased morbidity and mortality (153). HHV-6-induced encephalitis, or its treatment, will not be further discussed here. Finally, increased knowledge about the clinical significance of the presence of viral DNA in the blood and/or bone marrow of immunosuppressed patients with hematological malignancies will be useful tools in future therapies. Hopefully in the future there will also be a wide range of new antiviral compounds. Another hope is that the treatment arsenal will also be expanded with effective adoptive cell therapy against viral infections. Whether such interventions, as mentioned above, will be used as prophylactic, preemptive or symptomatic treatment, there will be an urgent need to monitor viral loads. The current thesis can thus inform the field about clinically relevant viral infections in selected patient categories that can be targeted for future therapeutic clinical interventions.

6 POPULÄRVETENSKAPLIG SAMMANFATTNING

Virusinfektioner kan ge allvarliga, och t.o.m. livshotande, tillstånd hos patienter som genomgår transplantation med blodstamceller, s.k. hematopoetisk stamcellstransplantation (HSCT). Detsamma gäller för många patienter som erhåller behandling till följd av malign[6] blodsjukdom. Både HSCT och behandlingarna påverkar och försämrar patienternas immunförsvar, vilket innebär att de har en ökad risk för att få olika infektioner, t.ex. virusinfektioner.

Olika typer av virus innehåller ett för virustypen specifikt arvsanlag – här DNA. Med hjälp av diagnostiska metoder, exempelvis kvantitativ PCR, går det att analysera *om* virus-DNA finns i exempelvis ett blodprov. Med hjälp av metoden går det även att bedöma *hur mycket* DNA som finns, och därmed beräkna hur mycket virus som finns.

Målet med denna avhandling, har varit att utvärdera i vilken omfattning vissa virus bidrar till att försämra prognosen för de patienter som nämns ovan.

HAdV är ett virus som kan ge svår sjukdom hos patienter med nedsatt immunförsvar. I **delstudie I** utvärderades i efterhand hur mycket DNA från humant adenovirus (HAdV) som fanns i blodet hos 40 patienter som genomgått HSCT. Patienterna hade fått stamceller från icke-besläktade givare. Eftersom immunsystemets T celler kan störa återhämtningen efter en HSCT, hade patienternas T celler tagits bort före HSCT. T celler är dock viktiga för att bekämpa virusinfektioner. Hos sex av de 40 (15%) patienterna kunde HAdV DNA påvisas i blod, och alla hade symtom av olika slag. En av patienterna hade ca 15 000 DNA-kopior/mL blod och även mycket DNA från Epstein-barr virus (EBV). Två patienter hade miljontals DNA-kopior/mL blod, och en av dessa hade förutom DNA från HAdV även mycket DNA från EBV och cytomegalovirus (HCMV). Samtliga tre patienter avled inom några månader efter HSCT. De andra tre patienterna hade låga nivåer av HAdV DNA i blodet. För att utesluta att det fanns infektioner, som inte gav symtom (asymtomatiska infektioner), analyserades även blod från de patienter som inte uppvisat symtom på virusinfektion. HAdV DNA kunde inte hittas hos någon av dessa patienter.

I **delstudie II** följdes 97 patienter (77 vuxna och 20 barn) som genomgått HSCT. Patienterna provtogs regelbundet för att se om de hade DNA från HAdV i blodet. Samma PCR-metod användes som i delstudie I. Den här patientgruppen hade fått stamceller från både besläktade och icke-besläktade givare. Hos ungefär hälften av patienterna hade T cellerna tagits bort innan HSCT genomfördes. DNA från HAdV påvisades i blodet hos fem av 97 patienter (5%), och den högsta uppmätta mängden DNA från HAdV var 9 000 DNA-kopior/mL blod.

Detta resultat var på ett sätt förvånande, med tanke på resultatet från delstudie I, men å andra sidan visar det att man inte kan dra långtgående slutsatser från endast en delstudie.

[6] Med malign menas elakartad sjukdom, ofta tumörsjukdom av något slag.

I den andra delstudien undersöktes även om tolv slumpmässigt utvalda patienter efter HSCT hade T celler som specifika kunde angripa HAdV. Ingen av dessa tolv patienter hade DNA från HAdV i blodet. Hos sju av patienterna kunde HAdV specifika T celler påvisas, men så mycket mer slutsatser än så kunde inte dras.

I **delstudie III** och **IV** studerades i vilken omfattning DNA från parvovirus B19 (B19V) fanns i benmärg hos barn som undersökts, eller genomgått behandling, för maligna sjukdomar. B19V är känt för att bland annat påverka benmärgen och orsaka brist på olika blodceller. Analyser gjordes också för att bedöma om viruset försämrat prognosen för att tillfriskna.

I **delstudie III** undersöktes i efterhand samtlig tillgänglig benmärg från 117 barn med diagnosen akut lymfoblastisk leukemi (ALL). Behandling mot ALL pågår under flera år, och inleds med en tuff s.k. induktionsbehandling där bland annat cellgifter ges under flera veckor. Efter det följer flera års s.k. underhållsbehandling, där cellgifter också ges, men i lägre doser. Hos arton av de 117 barnen (15%), kunde DNA från B19V påvisas i benmärg. Hos sex barn i samband med induktionsbehandlingen, hos sju barn i samband med underhållsbehandlingen och hos fem barn efter avslutad behandling. Mängden B19V DNA i benmärg var högst hos de barn som genomgick underhållsbehandling. När DNA från B19V fanns i benmärgen såg man att det samtidigt var brist på olika celler i blodet. Dessa barn fick fler blodtransfusioner jämfört med övriga barn. Ibland hade bristen på blodceller tolkats som att sjukdomen förvärrats, eller börjat om igen. Flera av barnen var tvungna att avbryta eller skjuta upp behandlingen med cellgifter. Barnens underhållsbehandling påverkades alltså av att det fanns DNA från B19V i benmärgen. Endast ett barn hade symtom som kunde kopplas till infektion med B19V.

I **delstudie IV** undersöktes i efterhand benmärg från 123 barn med akut myeloisk leukemi, non-Hodgkins lymfom och s.k. solida tumörer[7]. Benmärg från barn som inte hade malign sjukdom, men ändå hade påverkan på blodet, undersöktes också. Inget av de 123 barnen hade dåligt immunförsvar. Hos nio av de 123 barnen (7%) hittade man DNA från B19V i benmärgen. Sju av barnen hade återkommande feberepisoder och så långvarig brist på blodceller att de behövde flera blodtransfusioner. Inget av barnen hade symtom som kunde kopplas till infektion med B19V.

I **delstudie V** undersöktes förekomsten av B19V DNA i blod hos de 97 patienter som ingick i delstudie II ovan. Hos tre patienter, ett barn och två vuxna, kunde man hitta DNA från B19V. Barnet hade till en början feber, men hos de vuxna fanns inga symtom som kunde kopplas till infektion med B19V. Hos samtliga tre patienter försvann B19V DNA spontant.

[7] Med solida tumörer menas tumörsjukdom som finns någon annanstans än i blodet.

I **delstudie VI** analyserades i efterhand om det fanns DNA från herpesvirus 6A eller 6B (HHV-6A, 6B), polyoma BK-virus (BKV) eller B19V i blod hos 79 vuxna, som på grund av behandling för malign sjukdom i blodet, hade brist på vissa immunceller (neutropeni) och dessutom hade feber. Funderingar fanns om HHV-6A eller 6B, BKV eller B19V kunde vara orsaken till själva febern? Ingen av de 79 patienterna hade DNA från HHV-6A. Små mängder DNA från B19V fanns hos några få patienter. Hos sex patienter fanns DNA från HHV-6B och hos nio patienter fanns DNA från BKV. Hos samtliga 79 patienter, utom en, kunde en rimlig förklaring ges till febern, som var en annan än att den skulle vara orsakad av något av de virus som analyserats.

Sammantaget är generell screening för HAdV, B19V, BKV eller HHV-6A, 6B inte aktuell för de patientgrupper som har studerats här. Om oväntade symtom uppstår hos patienter som genomgått HSCT, ska man dock vara generös med att testa om DNA från HAdV finns. Infektion med B19V är nästan alltid kopplad till någon form av brist på blodceller, och om oväntad brist på blodceller uppträder hos barn som genomgår behandling för malign sjukdom i blodet, bör B19V-infektion uteslutas.

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