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VIRAL INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

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ABSTRACT

The number of patients undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) is steadily increasing, and the outcome of this intervention is largely dependent on how well complications in the form of severe infections can be adequately diagnosed and controlled. Adenoviruses (AdV) have emerged as important causes of morbidity and mortality in these patients. Early diagnosis of the infection by detection of viral DNA may improve the prognosis. In **paper I** we evaluated a surveillance strategy for detection of AdV DNA by real-time PCR in a prospective study of hematological allo-HSCT recipients. In parallel with a routine cytomegalovirus surveillance program, plasma samples from 97 recipients were analyzed by quantitative PCR for detection of AdV DNA. A total of 5% of the patients had detectable AdV DNA in plasma. Only one patient had high titers and none developed AdV disease. Bone marrow as a source of stem cells and myelodysplastic syndrome as the indication for transplantation were independently associated with higher risk of acquiring AdV infection. We concluded that the strategy did not have a significant effect on the clinical outcome in our material, but given the sometimes high incidence of AdV infection and disease in other settings, we do not dismiss the idea of surveillance. With a somewhat different approach to improve the clinical care for patients undergoing immunosuppressive treatment, we investigated the etiology to febrile neutropenia. Chemotherapy-induced neutropenia is one of the major side effects of the treatment of maligancies, and the risk of infection is increased by the severity and duration of neutropenia. The empiric administration of broad spectrum antibiotics has substantially decreased the mortality rate of patients with febrile neutropenia, but in only approximately one-third or fewer of the fever episodes, bacterial infection is documented. It is likely that other pathogens, such as viruses, play an important role as etiological agents, and an overuse of antibiotics could be anticipated. Therefore, in **paper II** and **paper IV** we investigated the presence of common viral infections and febrile neutropenia in children with cancer as well as adult patients with hematological disorders. A broad range of respiratory viruses in nasopharyngeal aspirate (NPA) and viruses commonly reactivated in allo-HSCT recipients were sought for. With human rhinovirus (HRV) being the predominant virus, we found an viral agent in half of the cases in the pediatric cohort. Of these, 25% co-ocurred with a bacterial finding. Virus detcted in blood was a rare event. In the adult population, we detected a viral pathogen in 42% of the episodes of febrile neutropenia. This should be compared to 13% in afebrile neutropenic patients that were included as controls. In both groups, approximately half of the viruses were detected in blood. The predominant respiratory virus was HRV, whereas BK virus was the commonest finding in blood. In one-third of the virus-positive cases, a bacterial infection was documented. We furthermore found NPA being superior to a flocked nasal swab for collection of respiratory specimens (**paper III**). We concluded that the prevalence of viruses was high in neutropenic patients with fever, and it was higher than for neutropenic patients without fever. It is plausible that a number of the patients with febrile neutropenia suffer from viral infections, and are thus not helped by antibiotics. Unfortunately, the presence of virus could not function as a predictor for non-bacterial infection. The findings, however, warrants further research related to the earlier achievements with aim to identify patients where continuous empiric antibiotic treatment could be avoided.

LIST OF PUBLICATIONS AND MANUSCRIPTS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

I. ÖHRMALM L*, Lindblom A*, Omar H, Norbeck O, Gustafson I, Lewensohn-Fuchs I, Johansson JE, Brune M, Ljungman P, Broliden K. (*Equal contribution)
Evaluation of a surveillance strategy for early detection of adenovirus by PCR of peripheral blood in hematopoietic SCT recipients: incidence and outcome.
Bone Marrow Transplant. 2010 Apr 19. [Epub ahead of print]

II. Lindblom A, Bhadri V, Söderhäll S, ÖHRMALM L, Wong M, Norbeck O, Lindau C, Rotzén-Ostlund M, Allander T, Catchpoole D, Dalla-Pozza L, Broliden K, Tolfvenstam T.
Respiratory viruses, a common microbiological finding in neutropenic children with fever.
J Clin Virol. 2010 Mar;47(3):234-7. Epub 2010 Jan 6
.

III. ÖHRMALM L, Wong M, Rotzen-Ostlund M, Norbeck O, Broliden K, Tolfvenstam T.
Flocked nasal swab versus nasopharyngeal aspirate for detection of respiratory tract viruses in immunocompromised adults: a matched comparative study.
BMC Infect Dis. 2010 Nov 26;10(1):340. [Epub ahead of print]

IV. LARS ÖHRMALM, Michelle Wong, Carl Aust, Per Ljungman, Oscar Norbeck, Kristina Broliden, Thomas Tolfvenstam.
Virus association to fever in adult neutropenic patients with hematological disorders: a cross-sectional study.
In manuscript.

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LIST OF ABBREVIATIONS

AdV	Adenovirus
ALL	Acute lymphocytic leukemia
Allo	Allogeneic
AML	Acute myeloid leukemia
B19	Parvovirus B19
BAL	Bronchoalveolar lavage
BKHC	BKV-associated hemorrhagic cystitis
BKV	BK virus
cDNA	Complementary DNA
CLL	Chronic lymphocytic leukemia
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CR	Complete remission
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
ds	Double stranded
EBV	Epstein-Barr virus
EBV-LPD	EBV-associated lymphoproliferative disease
ELISA	Enzyme-linked immunosorbent assay
fNS	Flocked nasal swab
G-CSF	Granulocyte colony stimulating factor
GVHD	Graft versus host disease
HboV	Human bocavirus
HCoV	Human corona virus
HEV	Human enterovirus
HIV	Human immunodeficiency virus
HL	Hodgkin lymphoma
HLA	Human leukocyte antigen
HMPV	Human metapneumovirus
HRV	Human rhinovirus
HSCT	Hematopoietic stem cell transplantation
HSV	Herpes simplex virus
IF	Immunofluorescence
IFN-γ	Interferon gamma
Ig	Immunoglobulin
IL	Interleukin
LRTI	Lower respiratory tract infections
MAC	Myeloablative conditioning
MBL	Mannose-binding lectin
MDS	Myelodysplastic syndrome
MUD	Matched unrelated donor
NHL	Non-Hodgkin lymphoma
NK	Natural killer

NF-κB	Nuclear factor κB
NPA	Nasopharyngeal aspirate
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PIV	Parainfluenza virus
RIC	Reduced intensity conditioning
RLR	RIG-I-like receptor
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT	Reverse transcriptase
SARS	Severe acute respiratory syndrome
SLL	Small lymphocytic lymphoma
ss	Single stranded
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor alpha
UCB	Umbilical cord blood
URTI	Upper respiratory tract infections
URTS	Upper respiratory tract symptoms
VZV	Varicella-Zoster virus

1 INTRODUCTION

A beginning exists - at least for biological life on planet earth. About four billion years ago, lifeless molecules formed cells, the structural and functional units of all living organisms with the ability to reproduce themselves. At some later stage, relatively simple unicellular organisms became multicellular, and the cells began to differentiate to form organs working together in more complex organisms. All shapes of life were targets for invaders and, to be a lucky member of the fittest, defense mechanisms were developed in order to kill or live side by side with the trespassers [1]. Thus, the immune system that keeps you alive today is the result of a billion-year project – a diamond that was polished on the beaches of Rodinia and Pangæa.

Recently in this perspective, during World War II, the chemical warfare agent, nitrogen mustard, leaked out from a U.S. liberty ship after a German air raid in Italy. Autopsies of the victims revealed results suggesting a profound suppression of cell types that normally divided rapidly. With this knowledge, two pharmacologists set up an animal model where they treated cancer with mustard agents [2], and ever since, antineoplastic drugs have dominated the treatment of cancer in humans. A myth or not, the story reflects the time point of the discovery of antineoplastic chemotherapy [3]. Although the drugs have been refined, the adverse effects are severe; the carefully designed and frequently dividing immune cells are also affected and, subsequently, infections are the major cause of morbidity and mortality beside the cancer itself.

Even when taking the time span of the immune system's development into consideration, the excellence of the system is so inconceivable that neither Darwin's theory nor other, less scientific, explanations make the success comprehensible. Fortunately, that mystery is beyond the scope of this thesis which will mainly focus on certain infections in cancer patients being immunodeficient due to anti-cancer treatment. However, keeping a larger picture in mind will help us understand the magnitude of the problem that infections cause in immunocompromised individuals.

2 BACKGROUND

2.1 IMMUNOLOGY

As described in the introduction section, the immune system is the result of many years of evolution. One part, the non-specific, is rather static and retains the same properties during an individual's life. However, the other part, the specific immune system, has the ability to adapt to the current environment and so to say undergo an intra-individual evolution. In the following sections these two parts of the system are described in general and broad terms.

2.1.1 The non-specific immune system

The very first defense against infections would be the behavior; you do not go towards a sneezing or coughing person and you do not eat food that obviously been mishandled. However, avoiding any contact with pathogens is impossible. The body has to be in contact with its surrounding in order to exchange oxygen and carbon dioxide, reach nutrition and get rid of non-digestible food, eliminate ammonia, and to explore the environment. Thus, the respiratory and the gastro-intestinal epithelium, the urothelium, and the skin, respectively, are technically the outside of the body. Although these barriers are the first line of defense against pathogens and possess numerous of defense mechanisms [4-8], their cells are not usually addressed as "immunological", a title they undoubtedly deserve! The non-specific immune system is non-specific in the sense that it contains mechanisms that exist before infection. However, as it is developed to protect from, in principle, all non-self structures it is specific regarding the selection of self and non-self.

The cells that contribute to the rapid non-specific immune response are macrophages, neutrophils, and the natural killer (NK) cells. Macrophages and neutrophils use phagocytosis to eliminate their enemies, whereas the NK cells kill cells that cannot show that they are "self". Some macrophages are distributed in the tissues to screen the surrounding whereas others are recruited to the tissue upon infection. The classical signs of inflammation are caused by the innate response, and the pus that may appear mainly consists of sacrificed neutrophils. Upon viral infections, one important part of

the innate response is the production of type I interferons (Figure 1a). These molecules set the cells in an "antiviral state" that minimizes the viral spread until an adequate and specific (adaptive) immune response is reached (Figure 1b). It further stimulates NK cell activity and facilitates the survival of dendritic cells that, via their antigen presentation, are one of the bridges between the non-specific and specific immune system [9].

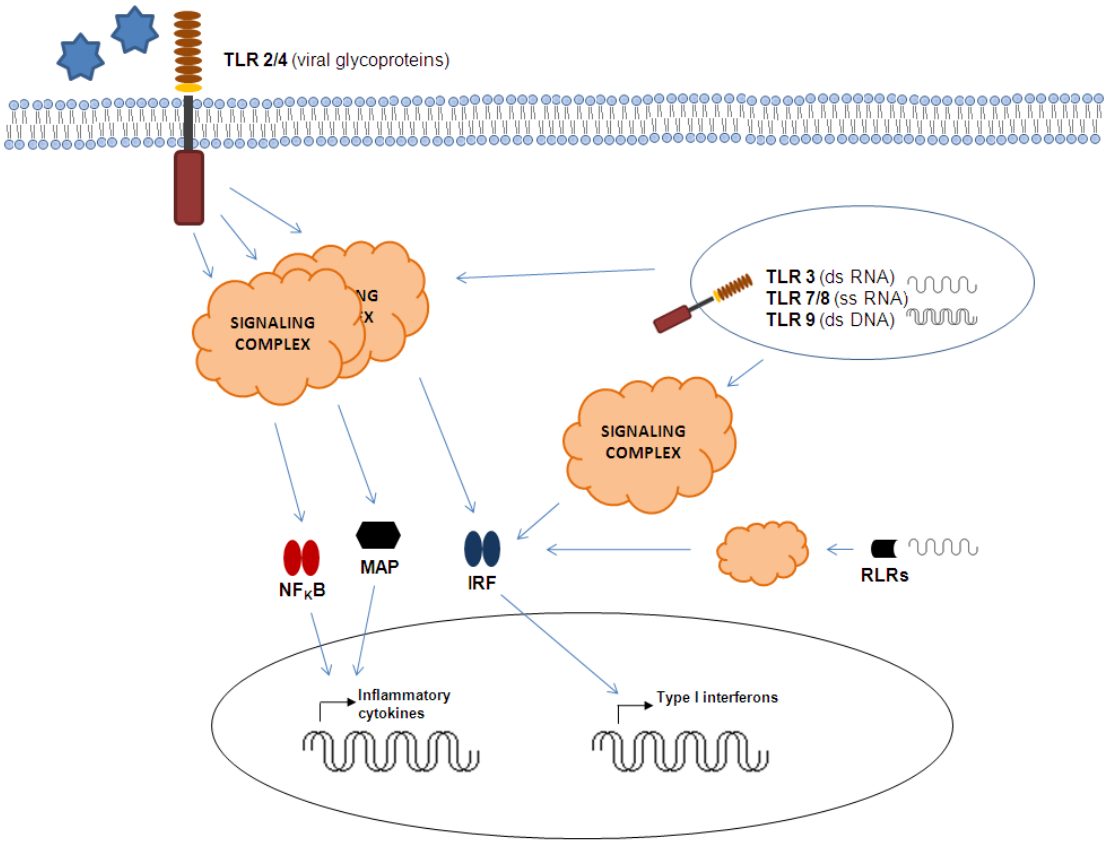

Figure 1a. A simplified schematic illustration of a host cell's recognition of, and immunological response to, viruses (blue stars). Membrane-bound Toll-like receptors (TLRs) on the cell surface can recognize certain viral protein structures, whereas TLRs in endosomal compartments are capable to detect different viral genomes. Other pattern recognition receptors are the RIG-I-like receptors (RLRs) that detect the viral genome in the cytoplasm. These two families of pattern recognition receptors comprise the front line of defense that the host possesses against viral pathogens. Among many other actions, the induced type I interferons increase the ability of uninfected host cells to resist the virus.

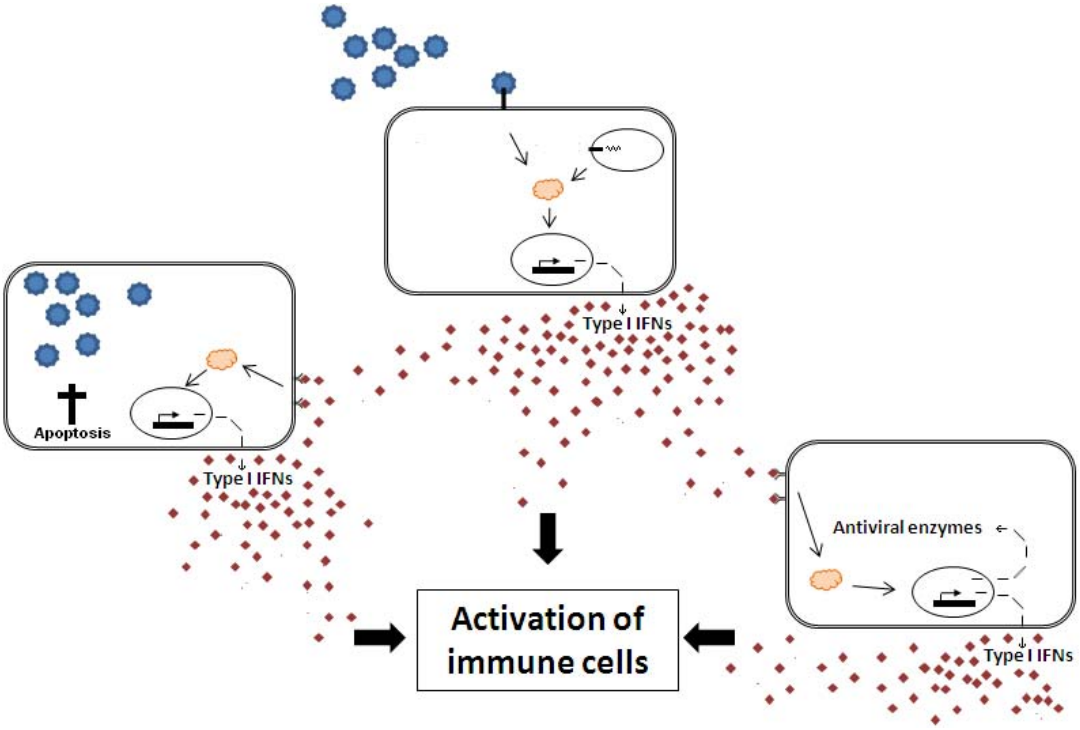

Figure 1b. Simplified schematic illustration of the action of type I interferons. Viruses recognized by a host cell, induce production of type I interferons which have their action on nearby cells. Uninfected cells (to the right) are induced to produce type I interferons and anti-viral peptides, whereas already infected cells (to the left) undergo programmed cell death, apoptosis. Type I interferons also induce other cells to produce interferons. Finally, the response activates cells from the non-specific immune system.

2.1.2 The complement system

A number of proteins circulate as precursors in the bloodstream, ready to be cleaved and thus activated. In their active form they are able to facilitate the ability of antibodies and phagocytic cells to eliminate pathogens. Specific actions are either direct or indirect. Lysis of membranes of pathogens, clumping of the pathogens, and changing of viruses' molecular structure are examples of direct actions independent of other mechanisms, whereas opsonization of pathogens and attraction of innate immune cells via chemotaxis enhance mechanisms from other parts of the immune system. Although it can be recruited and brought into action by the specific immune system, the complement system belongs to the non-specific part. The involved proteins do not adapt and the mechanism could therefore be addressed as a "non-specific humoral defense system". The proteins are produced by the liver and use three different pathways for activation; the classical, alternate, and the mannose-binding lectin (MBL)

pathways [9]. A mutation leading to an altered MBL production is suggested to be a risk factor for severe infections and fever in immunocompromised patients [10]. This is however controversial and a recent summary of the literature conclude that MBL could not be identified as an independent risk factor [11].

2.1.3 The specific immune system

The components of the specific immune system are lymphocytes and their products. The number of lymphocytes in the human body is huge – 1000 billions with a cumulative weight of 500 grams and the size of the liver! This part of the immune system first appeared in jawed vertebrates and is thus much younger than the innate part. The specific immune response could be divided into the humoral and cell-mediated immunity achieved by the B cells and the T cells, respectively.

The humoral response is mediated by antibodies produced by certain B cells. The antibodies recognize specific antigens on the invaders, and can have two actions; neutralization of the infectivity of the microbes, and opsonization of the microbes for elimination by other mechanisms. For obvious reasons, the humoral defense is the principle mechanism against extracellular microbes and their toxins. However, it plays an important role in the defense against intracellular infections as well.

The cellular immunity mediated by the T cells has its impact on intracellular pathogens such as viruses and some bacteria. Instead of using neutralizing antibodies, cytotoxic T cells promote destruction of microbes residing in phagocytes, but also induce lysis of infected cells. When a dendritic cell from the innate system shows an antigen in its receptor, it has to interact with the correct lymphocyte in order to initiate a proliferation. This “meeting” is made possible by the concentration of lymphocytes to the lymphoid tissues, but is still somewhat equivalent to finding a needle in a haystack.

A simple explanation does not exist why the B cells and the T cells can be uniquely designed for specific pathogens or infected cells. In summary, due to gene recombination, the receptors on these cells are unique for each single cell. Via selection processes, only the lymphocytes with two important features survive; the ability to “shake hands” with the host cells in a descent way, and to distinguish “self” from “non-

self". Thus, the high number of lymphocytes and their uniqueness explain why there is at least one specific lymphocyte for each pathogen circulating. Those who cannot remember the past are condemned to repeat it, and one most important feature of the adaptive immune system is its ability to "remember" past infections. Keeping a pool of memory cells enables a much faster specific response upon re-infection. The specific immune response is essential for elimination of viral infections [9].

2.1.4 Fever

The oldest known written reference to fever exists in inscriptions from the sixth century BC, with a flaming brazier that symbolized fever and the local warmth of inflammation. Roman military physicians also wrote of the resolution of fever in soldiers once pus was drained [12]. Fever is defined as an elevation of the body temperature above the normal range caused by a changed thermoregulatory set-point in hypothalamus. Just like a triggered thermostat, the brain sends signals to different mechanisms, such as shivering or vasoconstriction, in order to increase the body temperature. Molecules able to change the set-point, and thus induce fever, are called pyrogens and can be either endogenous (cytokines from the innate immune system) or exogenous (e.g. bacterial endotoxins). Most important endogenous pyrogens are interleukin (IL)-1, IL-6, and TNF-α, but other minor pyrogens, such as IL-8 and type II interferons, can also cause fever [13]. Also type I interferons, important cytokines in viral infections, are known to induce fever [14]. However, although it is not debated that infections in many situations cause fever, the classical mechanisms described above are debated [15].

Questions about the fever's benefit have generated considerable controversy during the years because of substantial data indicating potentiating and inhibitory effects of the response on resistance to infection. As a result, there is no consensus about the appropriate clinical situations in which fever or its mediators should be suppressed [16, 17].

Most people associate fever with infectious diseases, but also other conditions sometimes go with fever; neoplasms (e.g. lymphomas), inflammatory diseases (e.g. temporal arthritis), drug fevers (e.g. cytarabine). Differentiation between these types of

fever is one of the challenges within the clinical care of patients with febrile neutropenia.

2.2 CANCER IN CHILDREN

Cancer is a rare event in children compared to adults, but this fact is of course of little consolation to those approximately 300 children and their families who are affected in Sweden every year [18, 19]. During the last 40 years we have seen a dramatic improvement of the results from treatments of cancer in children and adolescents. With a great variation between the different diseases, in Scandinavia, four out of five of these patients survive at least five years after diagnosis [20]. In contrast to cancers in adults, the pediatric cancers often develop in embryonic precursor cells [21]. Approximately half of the malignancies are leukemia, and brain and spinal tumor. The remaining types are lymphomas, sarcomas, neuroblastomas, Wilms tumors, retinoblastomas, germ cell tumors, and epithelial tumors [22]. The success of cancer treatment in children can be attributed mostly to powerful combinations of chemo (and radio) therapies as well as advances within cancer surgery. Unfortunately, tougher treatments are associated with more severe side-effects and complications which have required improvements in intensive care of the child and increased knowledge about infections [22]. A form of solid tumor that was frequent in the children in study II are described here, while hematological malignancies are found in the next chapter.

2.2.1 Neuroblastoma

Neuroblastoma is a tumor that affects young children. The median age at diagnosis is about two years, but it also appears that children are born with the disease. The tumor develops in the sympathetic part of the autonomic nervous system, and neuroblastoma can thus occur in almost any part of the body; most commonly in the adrenal gland. Symptoms are often absent and the disease is instead detected by noticing a resistance. Sometimes the tumor secretes hormones that can cause diarrhea, sweating and other symptoms. Moreover, the tumor can press on other organs and thus cause symptoms. Simplified, the treatment is based on the classification of the disease; benign, moderate or aggressive. Surgery and observation is used in benign cases, whereas in more

advanced diseases treatment with chemotherapy, surgery, radiotherapy, autologous hematopoietic stem cell transplantation (HSCT), or 13-cis retinoic acid is employed.

2.3 HEMATOLOGICAL DISORDERS IN CHILDREN AND ADULTS

Generating 300 billions of blood cells daily, the production of red and white blood cells is of enormous proportion. Being a minority in the bone marrow, the hematopoietic stem cells have the capacity to replace the blood hundreds of times during a normal life span. Hematology is the subspecialty of internal medicine that deals with etiology, diagnosis, treatment, prognosis, and prevention of disorders of the blood and the blood-forming organs.

2.3.1 Disorders

Hematological disorders are divided into groups and subgroups by different classifications. In the sections below the most common disorders from our patient cohorts are described.

2.3.1.1 Lymphoma

Back in 1832 the pathologist Thomas Hodgkin described the Hodgkin lymphoma (HL). This lymphoma should be followed by several additional forms. Thirty years ago, a consensus was reached to denote these additional lymphomas as non-Hodgkin lymphoma (NHL) [23, 24].

2.3.1.1.1 Non-Hodgkin lymphoma

A number of various different classification systems exist for lymphoma. NHL is a heterogeneous group of malignancies which is distinguished from the far less common HL. All NHL originate from lymphocytes or their precursors. One classification of NHL is based on the degree to which the “NHL cells” mimic the normal lymphocytes in different compartments of the lymph node, in bone marrow, in thymus, spleen, or

other lymphoid organs. Both small lymphocytic lymphoma (SLL) and chronic lymphocytic leukemia (CLL) arise from prefollicular B cells, but manifest in different ways; if the disease, in addition to lymph nodes or other solid organs, involves blood, it is called CLL. CLL is the most common lymphoid malignancy in our part of the world, characterized by an increase in fairly normal lymphocytes. As a disease of the elderly the majority of patients are more than 50 years old and the median age at presentation is about 65 years. Both SLL and CLL have a slow progress and are considered incurable. The patients can, however, often live a fairly normal life, and therapy is generally aimed towards relief of symptoms. This is also true for follicular lymphoma, another NHL that is common in the Western Hemisphere. The cells of large cell lymphomas mimic the largest cells in a normal follicle, but do not form lymphoid follicles and are thus called diffuse large B cell lymphoma. This is an aggressive malignancy that responds well to chemotherapy; roughly half of the patients are cured. Another highly aggressive lymphoma is the Burkitt lymphoma which is mentioned here since it is hypothesized that it originates in the germinal center. It responds to chemotherapy but relapse is unfortunately rather common. Mantle cell lymphoma shares some features with SLL and CLL and is also incurable. However, it is more aggressive and has a median survival of 3-5 years. Marginal zone lymphomas normally have an indolent progress, but even with intensive treatment regimens the median survival of 3 years have been difficult to improve. Some lymphomas display T cell phenotypes, but T cell lymphomas are a minority of the NHL. The NHL above can be divided into high- and low-malignant NHL; diffuse large B cell lymphoma is the most common high-malignant, whereas SLL, CLL, and follicular lymphoma are common low-malignant NHL.

NHL mostly affects middle-aged and elderly, but also children have the disease, foremost originated in precursor B cells. Typically, NHL presents with painless swelling of one or several lymph nodes or other lymphoid tissues. Additional symptoms (B-symptoms) include night sweats, fever and weight loss. For indolent NHL, the clinical approach is expectation with regular check-ups until symptoms appear. Chemotherapy, sometimes together with radiography, is used for treatment of more aggressive and generalized disease [23, 24]

2.3.1.1.2 Hodgkin lymphoma

HL is a malignancy that arises in lymphoid tissues and account for less than 10% of all lymphomas. It is divided into two major groups; nodular lymphocyte predominance HL, and the far more common classical HL. Similar to NHL the majority of patients have painless swollen lymph nodes. Furthermore, depending on subtype, HL patients can show B-symptoms. The incidence is biphasic with peaks around 20 and 70 years of age. Depending on the stadium of the disease, the treatment consists of either or both of radio- and chemotherapy. Relapse can motivate autologous HSCT [23, 24].

2.3.1.2 Acute leukemia

Acute leukemia is characterized by a neoplastic proliferation of immature hematopoietic cells in the bone marrow. These blasts accumulate and consequently suppress the normal hematopoiesis which leads to anemia, neutropenia, and thrombocytopenia. Fatigue, infections, and bleeding disorders, respectively, are therefore common manifestations. The blasts enter the bloodstream in varying extent and organs can be infiltrated. Furthermore, extremely high levels can cause microcirculation disturbance. Acute leukemia can be classified into two categories where acute lymphocytic leukemia (ALL) is by far more common in children than in adults. The opposite relationship is true for acute myeloid leukemia (AML). Other clinically important distinctions between these groups are the treatment and the prognosis [25].

2.3.1.2.1 Acute myeloid leukemia

AML is a neoplasm of immature myeloid precursor cells, myeloblasts, and is further divided into seven subtypes based on differentiation. The incidence increases with age so that the median age of AML patients is about 65 years [26]. The prognosis is dependent on several factors such as age, cytogenic abnormalities in the leukemic blasts, history of bone marrow disorder, treatment-related AML, multidrug resistance, hyperleukocytosis at presentation, etc. Children and individuals over 60 years of age have a poorer prognosis. The treatments can be divided into two phases. The induction chemotherapy aim to eradicate the malignant cell and obtain so called complete

remission (CR). As blasts still exist after induction therapy, the postremission therapy is necessary to prevent relapse. This therapy can be of similar intensity as for the induction therapy or be more aggressive including high-dose chemotherapy, autologous HSCT, or allogeneic HSCT (allo-HSCT). The treatment of AML subtype M3, however, requires a totally different set of regimens [25].

2.3.1.2.2 Acute lymphocytic leukemia

As for AML, ALL can be divided into subtypes, L1-L3. ALL account for about 75% of all pediatric leukemias [27]. In contrast to AML in children, ALL has an excellent prognosis in the age between 2-9 years. Approximately one third of adults survive, but for patients older than 60 years of age the prognosis is worse. Infants also have a poor prognosis. Other factors also influence; cytogenetic abnormalities, immunophenotype, hyperleukocytosis at presentation, etc. Many drugs are active in ALL and most children are cured with standard chemotherapy - a success that is only partially achieved in adults. The regimen typically consists of a 2- to 3-year program including of a combination of several drugs, both intravenously and intrathecally. After first CR, high risk patients can be subjects for allo-HSCT [25].

2.3.1.3 Myelodysplastic syndrome

Myelodysplastic syndrome (MDS) refers to a heterogeneous group of acquired bone marrow failure disorders that have a tendency to progress to AML. It is characterized by peripheral blood cytopenias with morphologic evidence of dysplasia in the bone marrow progenitor cells. Due to hypercellularity in the bone marrow, the hematopoiesis is ineffective. The incidence increases with age, and MDS is thus a disease of the elderly. No other interventions than allo-HSCT have been shown to extend survival of MDS patients. Unfortunately, being a disease of the elderly, many of the MDS patients are not suitable for allo-HSCT and supportive care alone often remains as the only option [25].

2.3.2 Treatments

There is a great variety of treatment protocols for hematological disorders, and often a combination of two or more drugs are used. The names of the regimens are abbreviations based on the containing substances. Examples are R-CHOP, DA, BEA-COPP, ABCDV, MIME, etc. Described below are two regimens, DA and R-CHOP, which are commonly used for AML and NHL, respectively. The regimens include three groups of pharmacological agents with principally different immunosuppressive effects; antineoplastic drugs, monoclonal antibodies, and steroids. The abbreviated name **DA** stands for the substances **D**aunorubicin and **A**rabinofuranosyl Cytidine (Ara-C), while **R-CHOP** consist of five drugs; **R**ituximab, **C**yclophosphamide, **H**ydroxydoxorubicin, **O**ncovin (brand name), and **P**rednisone. DA can be administrated as follows: induction treatment including Ara-C given intravenously in bolus doses or by continuous infusion over a period of seven days, and daunorubicin given intravenously in bolus doses for 3 days. Upon CR, the consolidation could consist of one or more cycles of high-dose Ara-C. Other post-remission therapeutic options are allo-HSCT, autologous HSCT or low-dose maintenance therapy. R-CHOP is often administered in cycles of 4 weeks. A common treatment regimen is for at least 6 cycles.

2.3.2.1 Antineoplastic chemotherapy

Because cancer cells spend more time dividing than other cells, inhibiting cell division harms tumor cells more than other cells. The drugs described here have their primarily mode of action in altering cell division – acting antineoplastically.

Daunorubicin, an anthracycline, is a potent cytostatic agent which primarily mode of action is intercalation of DNA. This inhibits the transcription, replication, and DNA repair processes in the cancer cells as well as other rapidly dividing cells. In addition to its major use in treating AML, daunorubicin is also used to treat other malignancies such as neuroblastoma. Being a synthetic analogous of the nucleoside deoxicytidin, Ara-C, inhibits the DNA-synthesis. Like daunorubicin the cytotoxic effect is linked to the substance's ability to bind to DNA and inhibiting enzymes necessary for replication and transcription. One of the unique toxicities of cytarabine is cerebellar toxicity when

given in high doses. The mechanism of the alkaloid Oncovin (vincristine) is yet to be fully understood. A probable principle of vincristine's cytostatic effect is the substance's tubulin dimer binding capacity and the consequent metaphase mitosis arrest. Consequently, all the above mentioned agents are suppressing hematopoiesis which renders cytopenia and susceptibility to infection in the treated patients.

2.3.2.2 Monoclonal antibodies

Rituximab is a monoclonal antibody that can bind to CD20-antigen on pre-B and mature B-lymphocytes. CD 20 is present on all B-lymphocytes, malignant as well as normal cells, but not on hematopoietic stem cells. Thereby, the immune system is targeted for lysis of B-lymphocytes and the action is not myelosuppressive. The hematological side effects are thus primarily related to the reduced humoral responses. However, rituximab are seldom used as mono therapy in these patients.

2.3.2.3 Corticosteroids

Prednisone is a synthetic corticosteroid with effects on both the innate and adaptive immune response. The inflammation caused by the innate system is reduced due to a number of mechanisms, such as inhibition of the phospholipase cascade, and induction of a protein that inhibits the nuclear factor κB (NF-κB) (Figure 1a). By inhibiting the production of certain interleukins, prednisone alters the adaptive immune response in different ways. Pathways to an adequate humoral and cell-mediated response are affected. In contrast to the monoclonal antibodies this drug has a more general effect, but it is not antineoplastic.

2.3.2.4 Allogeneic hematopoietic stem Cell Transplantation

Allo-HSCT is a therapeutic procedure which has evolved enormously since its introduction over 50 years ago [28]. It was initially used as treatment for different immune deficiencies to add missing cell types, but is today also used as a cure for hematological malignant and non-malignant disorders. Examples of malignant

indications are chronic myeloid leukemia (CML), MDS, AML, and CLL [29], whereas non-malignant disorders could be bone marrow failure and congenital red cell disorders [30].

In the beginning, only bone marrow was used as stem cell source and is still today the major source used in children [31]. However, after stimulation of the donor with granulocyte colony stimulating factor (G-CSF) which mobilizes stem cells from the bone marrow into the peripheral circulation, they can be harvested from peripheral blood. This method is predominantly used in adults [32-34]. Umbilical cord blood (UCB) is also used where the recipient often is a child or an adult missing a suitable donor [35-37]. Each of these three sources of stem cells have their own advantages and disadvantages. Bone marrow and peripheral blood can be donated again if necessary, but collecting bone marrow is performed under general anesthesia and can be a painful procedure. Using peripheral blood is associated with a rapid hematological recovery and low relapse rate, but has increased risk for chronic graft versus host disease. UCB is better suited for HLA (human leukocyte antigen) mismatch, but only a small number of cells are collected in each unit, and it is associated with a higher rate of non-engraftment.

The donors of the graft are preferably HLA-identical siblings, but in the absence of such, HLA-matched unrelated donors are an option. Therefore, several registries are developed containing volunteer donors. A haplo-identical parent is also a possible donor [38]. A high grade of mismatch between donor and recipient increases the risk of graft versus host disease (GVHD), but also a greater effect of graft versus malignancy (leukemia). Therefore, the seemingly best matched donor, an identical twin, is more suitable in non-malignant disorders [39, 40].

The initial treatment prior allo-HSCT aims to eradicate the disease, suppress immune reactions towards the graft, and eliminate the recipient hematopoietic stem cells to make place for the graft in the bone marrow. This procedure is referred to as the conditional (or preparative) regimen. Myeloablative conditioning (MAC) and reduced intensity conditioning (RIC) are the two major types of regimens. MAC consists of high dose of chemotherapy, alone or together with radiotherapy [41-43], which aim to directly but also indirectly, via graft-vs-malignancy effect, cure the disease. The treatment itself is more toxic compared to RIC but is associated with a lower risk for

relapse of certain malignancies [44, 45]. RIC is less toxic and are therefore used in patients that may not tolerate MAC (e.g. high age, organ dysfunction) but also where MAC would not be superior RIC to cure the disease [46-50]. Conditioning with RIC relies on the graft-vs-malignancy effect in a higher extent than does MAC.

After transplantation engraftment, both the specific and non-specific immune system reconstitutes. This is however a tedious process that can take years [51-57], a period that the patient is extremely susceptible for almost all kinds of opportunistic infections. The skin and the mucous barriers as well as the innate immune system recover rapidly, and are not dependent on the compatibility of the donor and the recipient [58]. The engraftment, normally defined as a neutrophil count > 500 cells/mm^3 for three days, takes place approximately one to two weeks after allo-HSCT and total recovery of the granulocyte, platelet, and NK cell numbers is achieved within a month [59]. The recovery of the specific immune system takes around one year [60] but some parts take even longer [61]. The reconstitution of the T cells is dependent on two mechanisms; (1) the expansion of already mature T cells infused via the graft which is important for protection against infections as well as graft rejection [62], and (2) de novo generation of thymic-dependent T cells which is the most important mechanism when a T cell depleted graft have been used [63].

2.4 VIRAL DETECTION METHODS

Four principally different methods are used to detect viral infections:

1. Virus isolation
2. Antigen detection
3. Genome detection
4. Serology

The first relies on the viruses capability to replicate, thus viable viruses are required. The second and third can detect non-viable viruses and relies on detection of parts of the virus. The forth detects the immune response by the infected host as an indirect measure of an acute or past infection. With some exceptions, virus isolation, antigen detection methods, and serology are less frequently used today. Although they have several advantages, their limitations have made polymerase chain reaction (PCR), and foremost real-time PCR, the method of choice for viral detection in many settings (Table 1). The former methods are briefly presented below, whereas real-time PCR is described more in detail.

Table 1. Advantages and disadvantages of four different viral detection methods

Advantages	Disadvantages
Virus isolation	
- High sensitivity for several viruses	- Requires trained personnel
- Can detect other viruses than the expected when use of several cell lines	- Requires special equipment in special laboratories
- Not very sensitive for changes in the viral genome	- Time to detection is for most respiratory viruses about a week
- A positive result, indicate viable viruses	- Requires viable viruses
	- Some viruses are difficult or impossible to isolate
Antigen detection (here represented by IF)	
- Rapid	- A sufficient amount of epithelial cells are required
- Relatively inexpensive	- Needs trained personnel
- Has high sensitivity and specificity	- Validated antibodies for certain viruses are missing
Serology	
- Sensitive	- Time to result is >10 days when it requires a paired follow-up sample
- Indicate actual infection	
- Capable to detect previous infections	
Real-time PCR	
- Sensitive and specific	- Even dead viruses could reveal positive results
- Relatively fast	- So sensitive that positivity can be of no clinical relevance
- Any virus can be detected depending on the design	- Unable, in principle, to detect viruses not designed to
- Relatively inexpensive	-Could be false negative due to mutations
- Even dead viruses could reveal positive results	

NOTE. IF, immunofluorescence; PCR, polymerase chain reaction.

2.4.1 Virus isolation, antigen detection, and serology

Virus isolation has been the golden standard for many years. Different cell lines susceptible to several viruses are used for inoculation of infected host specimen. Positivity is determined by a certain pattern of swelling or destruction of the cultured cells (cytopathic effect). The pattern is specific for each virus and it is thus only a

trained virologist who can recognize a positive sample. The work is labor-intense and it takes days to weeks before a result can be provided; a sometimes unacceptable turn-around time for the treating physician [64].

Enzyme-linked immunosorbent assay (ELISA) is an antigen detection method that is carried out by first inoculating the patient sample on a surface. Roughly, the further procedure continues by either pre-coating the surface with antibodies that bind the antigen, or the antigen can be directly absorbed to the surface. Then antibodies conjugated with enzymes are added to form an antibody-antigen complex. The enzymes are able to convert the next substance added into a fluorescent signal. This is roughly the procedure of the ELISA. In another antigen detection method, immunofluorescence (IF), the localization of virus proteins to different parts of the cell increases the specificity [65].

Serology has a limited function on the acute phase of a viral infection. Increased levels of IgM suggest acute infection, but normally a substantial increase of IgG is required in order to confirm the infection. The second examine of the serum are preferably made 1-2 weeks after the acute one, which makes the turn-around time too long for many clinical purposes. However, it still plays an important role in some settings. For example in the hematological field, screening of antibodies reveals knowledge about latent viruses, such as herpes viruses, and hepatitis B virus which could reactivate after immunosuppressive treatment. In immunosuppressed patients, however, serology is of limited use as they may have insufficient ability to mount a humoral response and may have received antibodies passively through transfusions. Furthermore, for screening of blood donors and diagnosis of viral hepatitis and HIV, serology is a useful tool [65].

2.4.2 Polymerase chain reaction

Sensitivity in detection of viruses has increased considerably with nucleic acid amplification tests. Less than ten years after Kary Mullis and his colleagues described a specific enzymatic amplification of DNA in vitro [66], he was awarded with the Nobel Prize in Chemistry in 1993 in Stockholm. Ever since, this method have been improved in order to be faster and less labor-intense. Furthermore, it has been improved to be able to estimate the number of viral (genome) copies in the sample. The real-time PCR

described below has of course its base in Mullis' method described in 1986. The major ingredients in a real-time PCR are:

1. The template (in this case the viral genome)
2. DNA building blocks (free nucleotides; dNTPs)
3. A heat stable polymerase (in this thesis, the Taq polymerase) which thrives in 70°C [67]
4. Primers (forward and reverse) complementary to the specific DNA region of interest
5. The probe which is a single stranded oligonucleotide designed to be complementary to a spot between the forward and reverse primers' binding sites). Therefore only specific PCR product can generate fluorescent signal in TaqMan PCR. The probe has a fluorescent reporter dye in its 5' end and a quencher in its 3' end. The quencher inhibits the reporter until the polymerase cleaves the probe.
6. Buffer solutions and magnesium in order to create an optimal environment for the polymerase

The ingredients above are mixed in certain concentrations and then the major steps in the reaction are:

1. Denaturation: Heating the sample/mixture to 95°C in order to denature double stranded DNA (dsDNA) to single stranded DNA (ssDNA). This makes the target region of the viral genome available for the primers and the probe
2. Hybridization: A lowering of the temperature to ~60°C facilitates the primers and probe binding to the viral genome
3. Elongation: Raising of the temperature to 70-80°C, the optimal working temperature for the polymerase. In this step new copies are produced and for each copy, a signal is detected (Figure 2)
4. The steps above are repeated for 40-50 cycles (as the polymerase is heat stable, new enzymes after each cycle are not required).

Not all viruses have a genome consisting of DNA. In fact, most of the respiratory viruses have their genes stored in RNA molecules. As the polymerase mentioned above

only can elongate ssDNA, the RNA has to be converted by a reverse transcriptase (RT) into a complementary DNA (cDNA) before real-time PCR. This is made together with random hexamer oligoprimers, and the viral genome (or actually the complementary sequence) of interest can subsequently be amplified using the PCR scheme above. This method is sometimes called RT-PCR which could be misunderstood as real-time PCR. Numerous different abbreviations are used, but for the experienced reader this is however seldom a problem.

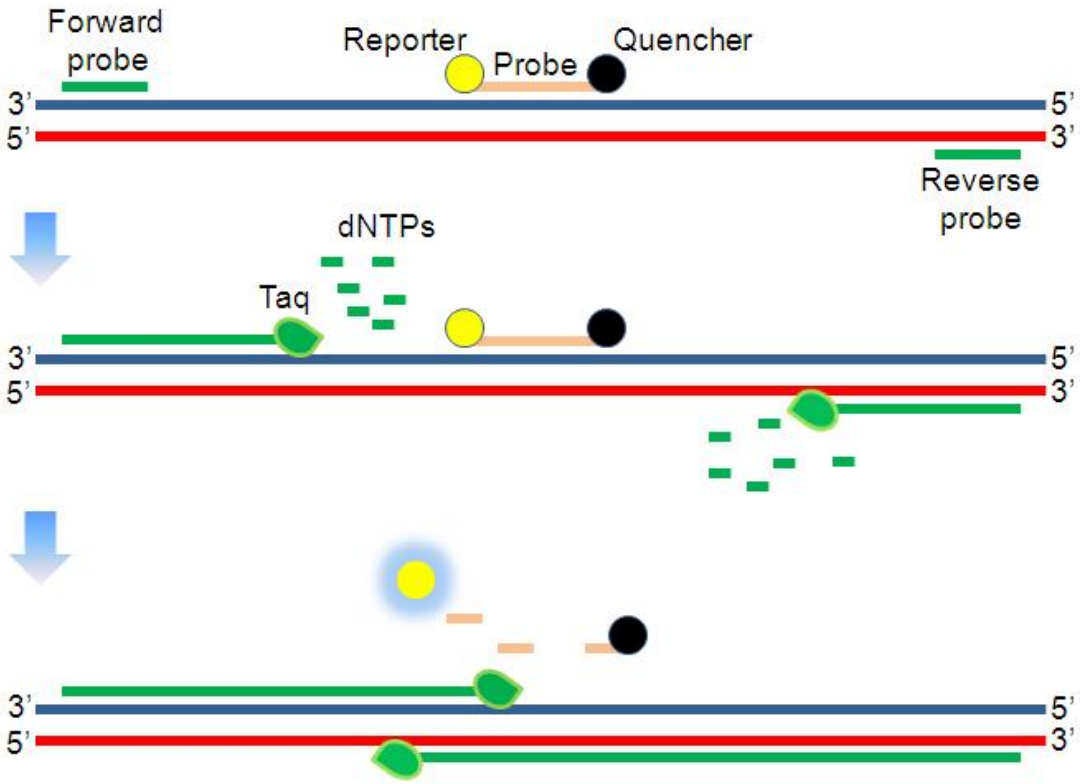

Figure 2. The annealing of primers and probe, elongation by means of polymerase, and the subsequent release of the reporter dye from the quencher as the genome is copied.

2.5 THE VIRUSES

Viruses are found wherever there is life and have probably existed since living cells first evolved [1]. They are the smallest biological units that can infect living organisms. Somewhat unfair, viruses are defined not to be a living form. This is simply due to their lack of own metabolism; they must invade living cells and use their hosts' machinery in order to replicate. Viruses consist of two or three parts: the genetic material (DNA or RNA), a protein coat (capsid) that protects these genes, and in some cases an envelope of a lipid bilayer that surrounds the protein coat when they are outside a cell. However, the shape and size of different viruses vary greatly. An overview of the viruses mentioned in this book is outlined in Figure 3 based on the Baltimore classification. Most of them are normally referred to as respiratory viruses as they cause disease in, or at least are initially transmitted via, the respiratory tract. The viruses' role in both immunocompetent and certain immunocompromised cohorts is discussed below. In particular, their potential as etiological agents to fever is penetrated.

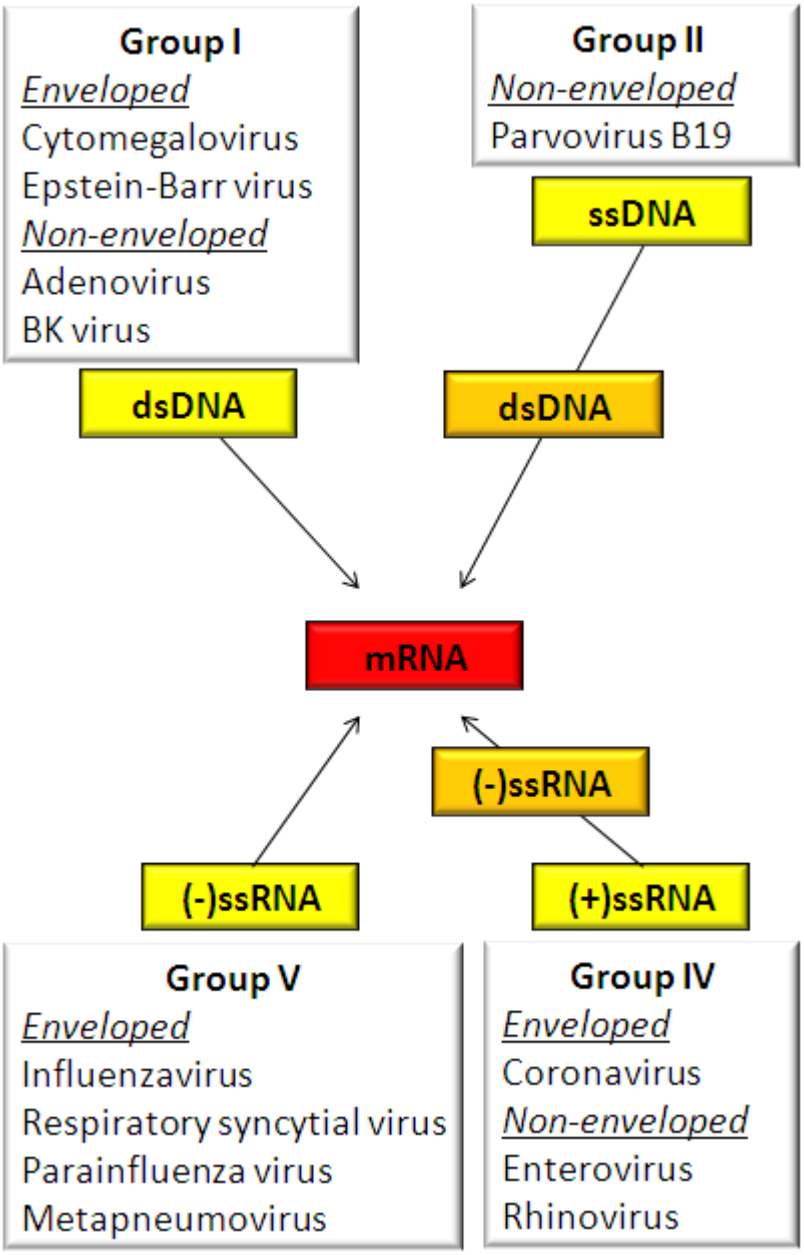

Figure 3. The viruses discussed in this thesis grouped according to the Baltimore classification. The classification is based on the method of viral mRNA synthesis. The viruses presented in group I usually must enter the host nucleus before it is able to replicate. Some of these viruses require host cell polymerases to replicate their genome, while others, such as adenoviruses or herpes viruses, encode their own replication factors. Parvovirus in group II replicates within the nucleus, and form a double stranded DNA intermediate during replication. The DNA viruses are dependent on the cell cycle. The genome of viruses in group IV cannot be directly accessed by host ribosomes to immediately form proteins. Replication in positive-strand RNA viruses is thus via a negative-strand intermediate. The genome of viruses in group V, however, can directly be used by the host cell's machinery in order to replicate. The RNA viruses replicate primarily in the cytoplasm and are not dependent on the cell cycle as the DNA viruses.

2.5.1 Respiratory syncytial virus

Beside influenza virus, the respiratory syncytial virus (RSV) is probably the most well-known virus for parents of small children. This virus is namely the most common cause of lower respiratory tract infections in infants and young children [68, 69], and serological data show that almost all children have been infected before two years of age [70]. Its seasonal variability [71] is reflected by the high pressure on the pediatric health care infrastructure during the winter and early spring! RSV is highly contagious and re-infection can occur at any point later in life. Then it normally causes milder symptoms similar to those caused by "common cold" viruses [70]. However, in adults, foremost elderly, the virus can cause severe disease [72, 73]. RSV is rather pyrogenic but upper respiratory tract infections (URTI) can present with our without fever. Fever is, however, highly associated to lower respiratory tract infections (LRTI) due to RSV [74].

This virus has been shown to cause severe LRTI with a high mortality rate in HSCT recipients [75, 76]. Transplanted patients typically present with fever and upper respiratory tract symptoms (URTS) followed by more severe symptoms as LRTI develops [77, 78]. Studies have shown a cumulative incidence ranging across 0.4-1.5% and 3.5-8.8% in autologous and allogeneic HSCT recipients, respectively [79-81]. In patients with hematological malignancies or HSCT recipients with RSV infection, progression to LRTI was associated with at least two independent risk factors: high age and absence of RSV treatment [82]. This is an interesting finding as reviews of randomized trials have concluded ribavirin not being effective in the treatment of LRTI caused by RSV [83, 84].

2.5.2 Metapneumovirus

The human metapneumovirus (HMPV) is a recently discovered RNA virus [85] that has been shown to cause both URTI and LRTI [86]. The virus is closely related to RSV but have two major genomic differences; the gene order differs and HMPV lack two non-structural genes that are thought to encode proteins with an anti-interferon activity [87]. However, it is unknown how the absence of these proteins affects HMPV pathogenesis. Nearly all children have been infected with HMPV during their first five

years in life [85, 88]. HMPV is thus a major pediatric pathogen and is the second commonest cause of bronchiolitis next to RSV [89, 90]. In temperate climates, the incidence of the virus is increased during the late winter to early spring and is responsible for a significant proportion of URTI and LRTI across all age groups in both healthy and immunocompromised hosts worldwide [91]. HMPV, like RSV, do not appear to cause asymptomatic carriage in the respiratory tract of healthy individuals [85, 89, 92, 93]. URTI due to HMPV can present with or without fever [94] whereas LRTI is recently summarized to be highly associated to fever [74].

URTI with HMPV can progress to severe LRTI and death in both pediatric and adult hematological patients [95-98]. The virus was isolated from bronchoalveolar lavage (BAL) in 26% of symptomatic HSCT recipients and carried a mortality rate of 80% [98]. Beside URTS, the infections were initially characterized by fever before the development of severe LRTI. However, in this patient category, prolonged asymptomatic infection has been described [99]. As for RSV, there is no consensus of the effectiveness of treatment with ribavirin. However, it has been demonstrated to decrease replication of the virus in a mouse model [100] as well as being successful when administrated intravenously in lung transplant recipients [101].

2.5.3 Rhinovirus

The human rhinovirus (HRV) was first described in 1956 [102] and is today known as the major cause of respiratory tract illness [103, 104]. The two species first discovered, A and B, cause rather mild symptoms, the common cold, whereas the recently discovered human rhinovirus C [103, 105, 106] is suggested to cause more severe symptoms [103]. HRV infections occur year round with seasonal peaks of incidence in the early fall and spring [107-110]. Infections with HRV are commonly associated with rhinorrhea, sore throat, nasal congestion, sneezing, cough, and headache [111]. Less often malaise, chills, and low-grade fever occur [104]. The most exciting hypothesis was recently presented, namely that HRV epidemics could interfere with the spread of influenza virus [112]. This is however yet to be confirmed.

HRV have been described as causative pathogens of LRTI in immunocompromised patients [113, 114], either as the sole pathogen or as a co-pathogen with bacteria or

other respiratory viruses. However, two studies that prospectively investigated the incidence of respiratory virus infections in patients with hematological cancer observed no or only a very low number of HRV infections in cases with respiratory symptoms [81, 115]. Results from another study indicated that when detected at high viral load, HRV may cause severe URTI and LRTI, whereas when detected at a medium-low viral load (an event more frequent in immunocompromised subjects), they may represent only bystander viruses [116]. Yet another study found HRV to be the predominant respiratory virus associated with URTI but none of the patients had progression to LRTI, and all patients recovered completely [117]. The disparity of reported incidences could partly be explained by the difficulties of to detect the virus; it is rather hard to culture, and a PCR must be thoroughly designed to cover the great diversity of genotypes.

2.5.4 Enterovirus

In this text human enterovirus (HEV) is represented by the non-polio enteroviruses; coxackievirus, echovirus, and other enteroviruses. Poliovirus is also an enterovirus but is excluded here. All HEV are closely genetically related to HRV and since they share biological features and probably have a similar pathogenetic effect in humans, it has recently been proposed an inclusion of HEV and HRV in the same subset within the Picornaviridae family [118]. The viruses replicate in lymphoid tissue in the pharynx and in the small intestine but in about 5% of cases the virus may spread to other tissues; central nervous system, myo- and pericardium, striated muscles, and skin. The most frequent symptoms are thus fever, sometimes accompanied by a rash or mild URTS. In the cases of viral spread to other organs, more severe syndromes can occur; aseptic meningitis, perimyocarditis, myalgia (Bornholm disease), herpangina and the Hand, Foot and Mouth disease [119]. All these syndromes include fever in the panorama of symptoms.

HEV infections in immunosuppressed individuals are not widely investigated, but some studies are performed on allo-HSCT recipient. Chakrabarti et al reported that 10% of recipients of T-cell depleted grafts developed HEV infections post transplant, but only four episodes were associated with symptomatic illnesses attributable to HEV [120]. Furthermore, there was no mortality directly related to HEV. In contrast, in four HSCT

recipients with acute respiratory illness, HEV was isolated as the sole pathogen in BAL. All infections progressed to severe pneumonia where three were fatal [121]. Yet another group observed HEV infections in three pediatric allo-HSCT patients, who received UCB. Two died from the infection [122]. In a 2-year prospective study on 130 hematological transplanted and non-transplanted patients HEV represented 5% of respiratory viral infections [115]. LRTI was present in one third of the episodes. Unfortunately, none of the reports above have investigated fever associated to HEV infection.

2.5.5 Coronavirus

If excluding the human corona virus (HCoV) that caused the global epidemic of severe acute respiratory syndrome (SARS) in the beginning of this century [123, 124], this group of viruses cause rather harmless URTS [125]. After HRV these viruses play the major role in causing common colds [107], and all HCoV show a seasonal variability in temperate climate countries with frequent transmission and detection during the winter [126, 127]. One of the strains is also associated with croup (acute laryngotracheobronchitis) [128], a disease foremost associated with parainfluenza virus (PIV) type 1 [129]. Two strains of HCoV were first described in the 1960s [130, 131] but as a consequence of an increased interest for this group of viruses after the SARS epidemic, two new viruses, NL63 and HKU1, were recently discovered by groups in the Netherlands and Hong Kong, respectively [132, 133]. Nicely summarized by van der Hoek, fever is reported to be present in 50-70% of the cases of infection with these two new strains [134]. This was not the situation for HCoV-OC45 and HCoV-229E where less than one out of five volunteers inoculated by the virus developed fever [125, 131].

In parallel to other respiratory viruses, HCoV have been subject to investigations of their role as disease-causing pathogens in immunocompromised patients. HCoV have recently been associated with severe LRTI in lung and liver transplant recipients [135] and HCoV-229E has been isolated from HSCT recipients with fever and cough [136]. In another study on five children with ALL and one pediatric renal transplant recipient, HCoV was the sole respiratory pathogen detected. The ALL patients presented with fever alone or together with various URTS.

2.5.6 Influenzavirus

Probably no other respiratory virus is as well known and discussed as the influenzavirus. Every year, the name of the virus is literarily on everybody's lips before the nasopharynx is infected by the actual virus! Although it was not discovered until 1933 [137], epidemics of the virus have been described several times far back in the history. Although influenza type C can be severe and can cause local epidemics, the species is rare compared to types A or B. Only type A and B are thus discussed below. The influenza virus A show great genetic and antigenic variability which arise from two different mechanisms: (1) the *antigenic drift* caused by the lack of proofreading and reparation of the genome during replication; (2) the *antigenic shift* that occurs when two different influenza viruses infect the same cell and assort their segmented RNA. The impact of the changed genome is, as the names of the mechanisms indicate, dependent on a change of the virus' phenotype. Not surprisingly, epidemics and pandemics are more likely to occur after an antigenic shift where a new strain with a new combination of the important proteins hemagglutinin (H) and neuramidase (N) may have been developed. Influenza viruses A and B appear in all age groups but have most impact in the elderly [138]. This is due to a higher risk for them to suffering from a secondary bacterial pneumonia in the convalescent period [139]. Although the influenza virus B cause milder symptoms than does influenza virus A, the disease is rather similar; rapid onset of fever, malaise, muscle pain, and cough.

Several studies have demonstrated infections with either influenza virus A or B in transplanted patients, but no difference in clinical presentation and outcome is determined [140-142]. The mean duration of shedding is longer for allo-HSCT recipients who are not given influenza antiviral treatment; 11 days, which is out of the range for immunocompetent individuals (5-10 days) [143]. A much longer period of shedding (>1 year!) was reported in an immunocompromised patient infected with a multidrug-resistant influenza A virus [144]. Furthermore, with only seven positive cases in allo-HSCT recipients, Peck and colleagues reported afebrile presentation in five of them, and absence of myalgia in all cases [145]. In contrast, in a prospective study on adult leukemia patients undergoing remission-induction chemotherapy, all influenza positive patients presented with fever, rhinorrhea, nasal congestion, headache, and myalgia [146]. Furthermore, in a similar cohort, influenza virus was associated

with fever in 87% of the cases [147]. Severe lymphopenia is identified to be an independent risk factor for progression to LRTI [82].

2.5.7 Parainfluenza virus

These viruses resembled the influenza viruses but were not related to them antigenically; hence *para*influenza virus. There are four subtypes of the virus [148, 149] named PIV 1-4. Although severe infections with PIV 4 have been reported [150, 151], the role of this subtype as a potential pathogen is still unclear [129]. Therefore, PIV refer to PIV 1-3 in the text below. PIV can cause both URTI and LRTI. As mentioned above, the virus is associated with croup in small children but can also cause the same clinical syndrome in adults [129]. PIV have, however, been associated with every kind of upper and lower respiratory tract illness, and there is a strong relationship between PIV and specific clinical syndromes such as bronchiolitis, pneumonia, and tracheobronchitis; all associated with fever. Even though they cause primary infections early in childhood, the immunity generated is not long-lasting and re-infections are therefore common [129]. Epidemiologically, PIV 1 and PIV 2 peak during the fall and winter, whereas PIV 3 peaks during the spring and summer [129, 152].

Immunocompromised children and adults appear to be particularly susceptible to developing severe and fatal LRTI with PIV. Significant disease, including respiratory failure and death, has been reported in solid organ transplant recipients, presenting with cough, dyspnea, and fever [153]. Giant cell pneumonia caused by parainfluenza type 3 was present in a patient with acute myelomonocytic leukemia [154] and all types is a common cause of respiratory illness or even death after HSCT [155-158]. In a Chilean prospective study they found PIV in 13% of the episodes of febrile neutropenia occurring in pediatric cancer patients [159], and in a retrospective study on adult leukemia patients with respiratory tract symptoms, two thirds presented with fever [160]. In a Finnish study on PIV 3 in hematological patients (transplanted and non-transplanted), all positive cases were associated with fever together with cough or rhinorrhea. Infiltration on chest radiograph was a frequent finding [161].

2.5.8 Adenovirus

The great variety of symptoms caused by this DNA virus is reflected by its number of serotypes. Since its discovery in 1953 [162-164], at least 55 different serotypes are described causing a broad range of infections such as respiratory, gastrointestinal, and conjunctival in both the immunocompetent and immunocompromised host [165-168] (Table 2). In contrast to other respiratory viruses, the incubation time for adenovirus (AdV) is rather long, 5-10 days, and its ability to survive outside its host is impressive. This is illustrated by outbreaks of conjunctivitis in swimming pool areas where the transmission of the virus thus are water-borne [169]. AdV is also a common cause of tonsillitis and fever in children [170]. Worldwide, the AdV is a common cause of gastroenteritis with high mortality in children in developing countries [171].

Table 2. Association of adenoviral diseases and principal serotypes in immunocompetent and immunocompromised individuals

Syndrome	Serotypes in species					
	A	B	C	D	E	F
Upper respiratory illness		All	All			
Lower respiratory illness		3, 7, 21			4	
Pertussis syndrome			5			
Acute respiratory disease		7, 14, 21, 55			4	
Conjunctivitis		3, 7, 11	1, 2	8, 19, 37, 53, 54	4	
Gastroenteritis						40, 41
Hemorrhagic cystitis		7, 11, 34, 35				
Hepatitis		3, 7	1, 2, 5			
Myocarditis		7, 21				
Meningoencephalitis		7	2, 5			
Venereal disease			2			
Disseminated disease	31	11, 34, 35	1, 2, 5			40

NOTE. AdV 53 and 54 should be referred to as “types” rather than serotypes. The table is a summary of data derived from three recent publications [165-168].

AdV infections are self-limited in the immunocompetent host. This is not always the case in severely immunocompromised patients such as HSCT recipients, where clinical manifestations including pneumonia, hepatitis, hemorrhagic cystitis, colitis, pancreatitis, meningoencephalitis, and disseminated disease are described [172-179]. The incidence after HCST varies with the patients’ risk factors, but could be dependent

on other factors such as number of body sites investigated and choice of detection method. Most risk factors are in some way associated with the grade and duration of immunosuppression; slow lymphocyte recovery [180, 181], usage of T cell-depleted or CD34+ selected grafts [180-183], grafts from an unrelated donor [184], and GVHD or its therapy [185]. Furthermore, children are at higher risk for acquisition of AdV infection post HSCT [174, 183, 184, 186].

No antiviral drugs are developed specifically against AdV, although drugs as ribavirin and cidofovir have been used [187, 188]. Of these two, cidofovir are the most commonly used but have severe and sometimes unacceptable side-effects; foremost renal [166]. Fortunately, children tolerate the drug better and with preemptive rehydration and a reduced dose, cidofovir have been described to be a safe alternative to withdrawal of the immunosuppressive treatment or expectation [189]. Feuchtinger and colleagues tried the more spectacular option to use virus-specific donor T cells for adoptive transfer of immunity to nine pediatric HSCT recipients with systemic AdV infection [190]. They concluded that the strategy was feasible and effective.

The importance of early detection of AdV infection in the post transplant period has highlighted the difficulties of the development of a proper diagnostic tool. Virus isolation may be too slow for clinical use, whereas PCR are fast and very sensitive. However, the PCR assay is only sensitive if it is designed to cover the specific serotype causing the infection. Many groups have put effort in developing a PCR assay that covers all known AdV serotypes; a demanding but exciting challenge [182, 191-194].

2.5.9 BK virus

This polyomavirus is a small, non-enveloped DNA virus. The name BK virus (BKV) is the initials of the patient from whom it was first isolated [195]. It is genetically closely related to another polyomavirus, JC virus, also discovered in 1971 [196]. Primary infection, typically asymptomatic, occurs during childhood and is followed usually by a lifelong phase of latency in immunocompetent subjects.

Upon immunosuppression, however, the latent infection may be reactivated even when high levels of serum antibodies are present. Its potential to cause harm in the urinary

tract was first reported in 1983 [197] but the emergence of the virus as a disease-causing agent in renal transplant patients began later in the mid-1990s [198]. BKV-associated hemorrhagic cystitis (BKHC) is a potentially serious complication that frequently occurs in recipients of allogeneic HSCT recipients [199, 200]. Clinical manifestations of BKHC can be minor with asymptomatic hematuria, or severe with massive blood loss, pain, urinary obstruction, renal failure and even death [201-203]. The virus' association to fever in this patient category is to my knowledge not investigated. Recently, however, a non-transplanted patient with peripheral T-cell lymphoma, developed acute renal failure which was associated with systemic BKV activation, and the initial symptoms were fever and urinary frequency [204].

2.5.10 Epstein-Barr virus

The Epstein-Barr virus (EBV) is a herpes virus that was first described in 1964 [205]. It infects via saliva, replicates in epithelial cells in the pharynx, and then later primarily in the B cells where lifelong latency is established [206, 207]. In most cases, a primary infection occurs during the first years of life and is asymptomatic or causes symptoms indistinguishable from other viral respiratory infections. However, if it occurs later in life, the immunological response is more powerful and causes a disease with several names; *glandular fever* (enlarged lymph nodes), *mononucleosis* (atypical lymphocytes resembling monocytes seen in the microscope) or *kissing disease* (infection requires exchange of rather high amount of saliva). Whereas the local symptom is tonsillitis, the systemic symptoms are fever, fatigue, lymphadenopathy, and splenomegaly. Liver enzymes are normally elevated and sometimes hepatitis develops. There may be a prolonged convalescence with tiredness and low-grade fever for months. Although the virus is not very contagious and requires close contact in order be transmitted, nearly all adults have been infected with EBV. The virus is associated with several malignancies such as different lymphomas [208].

In the healthy individual, the EBV infection is controlled by humoral and T-cell mediated immune responses. However, reactivation of EBV is frequent in immunocompromised patients [209-211], and EBV-associated lymphoproliferative disease (EBV-LPD) after allogeneic HSCT is a common complication. EBV-LPD is caused by the proliferation of EBV-infected B cells, and once it develops, it rapidly

progresses and is sometimes fatal. It can present with varying clinical signs and symptoms; fever, mononucleosis-like illness with fever, pancytopenia, lymphadenopathy, and even rapidly progressive lymphoma [212, 213].

2.5.11 Cytomegalovirus

The name of cytomegalovirus (CMV) refers to the size of the infected cells, which contain large intranuclear inclusions. Following primary infection the virus establishes latency in lymphocytes and possibly endothelial cells from which it may be reactivated. CMV is a threat even before we are borne. It can cause congenital infection upon primary and reactivated maternal infection. The infant will have a generalized infection at birth, but clinical symptoms are present in only 5-10% of the cases. Although the fetal infection may show minimal manifestations at birth, it can cause severe complications later in life, especially in the central nervous system [214]. CMV can also cause perinatal infection via cervix secretion or via breast milk, but in most cases it is subclinical [215]. Later in life the infection usually is subclinical but can cause a symptom pattern rather similar to mononucleosis. The disease is characterized by protracted fever, malaise, myalgia, and liver function abnormalities.

As for many other herpes viruses, CMV is commonly reactivated upon severe immunosuppression. Despite several new prophylactic options for CMV infection, the virus is still one of the major causes of morbidity and mortality after allo-HSCT where it causes pneumonia, gastroenteritis, and less commonly retinitis and hepatitis [216]. The disease can develop both early and late after the transplantation procedure [217]. Risk factors for CMV infection post HSCT are seropositivity of the recipient [218, 219] and, as for AdV infection, acute GVHD [220, 221]. In some situations, grafts from seronegative donors increased the risk for complications [222]. Usage of a T cell-depleted graft or a graft from a HLA-mismatched donor further increases the risk in seropositive recipients. In addition to direct morbidity and mortality, CMV is associated with opportunistic infection [223-225].

2.5.12 Parvovirus B19

This first member of the parvoviridae family that can cause infection in human was discovered in 1974, and the name refers to its small size and the sample 19 in panel B in which it was found [226]. However, it is a great survivor and is distributed over the globe infecting almost all people at some point during their lives [227]. The incidence of infection shows a seasonal variation in temperate climates, being more common during winter and early spring. Parvovirus B19 (B19) is transmitted through the respiratory route, but can also be transmitted through bone marrow and organ transplantations, and via transfused blood products [228, 229]. The pregnant woman can also infect vertically to the fetus, which is associated with several serious complications to the fetus, including fetal death [230]. One disease associated with the virus is erythema infectiosum (or fifth disease, or slapped cheek syndrome) that mostly occur in children aged 5-15 years. Intranasal inoculation of B19 in healthy volunteers revealed a biphasic clinical course with mild symptoms of fever, malaise, myalgia, and itching in the first peak (8-9 days post inoculation). After 15-17 days, maculopapular rash occurred alone or together with arthralgia [231, 232]. Although B19 viremia is rather infrequent in the healthy population and a large proportion of adults have acquired virus specific antibodies [229, 233-237], asymptomatic infections with B19 are present in 25-50% of cases [238, 239]. In bone marrow, however, B19 DNA is found in 2% of healthy individuals and in up to 15% of children with hematological malignancies without concomitant viremia [240-243]. B19 replicates in erythroid precursors and by that inhibit the erythropoesis [244, 245]. During the time of the first peak of symptoms, the bone marrow has completely lost its erythroid precursors.

The immunocompromised host present a different clinical picture with mild or absent immune mediated symptoms such as rash [246]. Furthermore, persistent B19 infection can cause severe chronic anemia. Treatment with plasma containing specific antibodies against B19 resulted in decreased serum levels of the virus, the appearance of reticulocytes, and interestingly, symptoms of the fifth disease! The infection can also indirectly harm the immunocompromised patient; one recent study showed that B19 in children with ALL is associated with cytopenia resulting in prolonged interruptions of chemotherapy [240]. Here only one out of seven experienced symptoms as the classical rash, whereas six of them were febrile.

2.6 INFECTIONS IN IMMUNOCOMPROMISED PATIENTS

The anticancer treatments can affect the immune system in a variety of ways. The very first line of defense is altered by breakdown of mucocutaneous barriers by for example surgical removal of the primary or metastatic lesions, radiation therapy, GVHD, mucositis, but also the cancer itself by local tumor invasion. Furthermore, indwelling surgical devices such as central venous or urinary catheters increase the risk for infections [247]. Both in a quantitative and qualitative manner, chemotherapy has a negative effect on the next line of the non-specific defense, the phagocytic cells. This dramatically increases the risk of infection which is illustrated by the direct relationship between risk of infection and severity, duration, and rate of decline of neutropenia (most frequently defined as an absolute neutrophil count $<0.5\times10^9$cells/L or $<1.0\times10^9$cells/L and expected to fall) [248]. Neutropenia is therefore the most frequently used marker for immunosuppression in the cohorts discussed below. However, isolated neutropenia is a rare event associated with certain conditions such as Kostmann disease, and thus many of the infections, for example viral, are associated with the parallel decline of lymphocytes. The B and T cells that are responsible for regulating the specific humoral and cell-mediated response, respectively, are also impaired quantitatively and qualitatively by malignancies and its treatment. The altered immunoglobulin production of the B (plasma) cells increases the risk for bacterial, fungal, and viral infections. Furthermore, impaired cellular response is associated with major risk of acquiring viral, fungal, and intracellular bacterial infections [247]. Splenectomy is a part of some treatment strategies and is associated with an increased risk for sepsis with encapsulated bacteria.

2.6.1 Post allogeneic hematopoietic stem cell transplantation

Allo-HSCT recipients are at risk of certain infections in different periods after transplantation. The timing of immune reconstitution determines the timing of these infections. Immune system recovery for HSCT recipients takes place in three phases beginning at the day of transplantation. Before engraftment (<30 days after HSCT) HSCT recipients have two critical risk factors for infection; prolonged neutropenia and altered physical barrier resulting from the conditioning regimen and frequent vascular access required for patient care. During this phase, the infectious complications of

HSCT patients are not very different from those encountered in other profoundly neutropenic patients such as acute leukemia patients. Consequently, the normal oral, gastro-intestinal, and skin flora are sources of infection. Candida, but also, upon prolonged neutropenia, Aspergillus are common infections [249, 250]. Viral infections, especially herpes simplex virus (HSV), are frequent [251], but the infection-related morbidity and mortality at this time is mainly due to severe bacterial sepsis, pneumonia, and fungal infections [39, 252-254]. After engraftment, other herpes viruses and adenovirus are critical pathogens [210, 220, 255-258]. In this period, CMV causes pneumonia, hepatitis, and colitis and potentates super infection with opportunistic pathogens, particularly among patients with active GVHD. Other dominant pathogens during this phase include *Pneumocystis jirovecii* and *Aspergillus species* [259-261]. After 6 months, due to cell-mediated and humoral immunity defects as well as impaired reticuloendothelial system function, allo-HSCT recipients are at risk for infections that include CMV, Varicella-Zoster virus (VZV), EBV, respiratory viruses, and infections with encapsulated bacteria [262-265].

The viral contribution to post HSCT complication is thus substantial. The strategy of monitoring CMV and the use of preemptive treatment in this period is a successful story [220, 266-272] and since AdV have emerged as an important pathogen [179, 273-280] a similar screening for AdV could be of clinical benefit. Our investigation of such a strategy is described in Paper I.

2.6.2 Patients with febrile neutropenia

Chemotherapy-induced neutropenia is one of the major side effects of cancer treatment. The risk of infection is increased by the severity and duration of neutropenia [248, 281]. Due to an impaired inflammatory response, signs of inflammation such as local heat, swelling, exudates, fluctuation and ulceration are often diminished. Fever is thus often the first and sometimes only sign of infection [282, 283]. The approach of empirically administrated parental broad-spectrum antibiotics has substantially decreased the mortality rates due to infections in these patients [284-287], but even though the fever might be the result of bloodstream bacterial infections, 70–90% of the episodes have no causative micro-organisms demonstrated in their blood cultures [288-303]. It can thus be hypothesized that many episodes of febrile neutropenia can be the

result of inflammatory responses to for example transfusions of blood products, malignancy itself, chemotherapeutic drugs, mucosal damage, or – viral infections. The inpatient management using broad-spectrum antibiotics is associated with disruption of family life, nosocomial complications, increased resistance to antibiotics, and high medical costs. As a result, several attempts have been made to identify patients at low risk for bacteremia or serious complications [304-315].

Since research and routine diagnostics have focused primarily on the bacterial contribution to fever, we have a fairly good knowledge about this part of the spectrum. The proportion of Gram-positive and Gram-negative bacteria found, have fluctuated in some parts of the world during the last decades. The main theoretical explanations for this are the use of antibiotic prophylaxis and central venous catheters, as well as emergence of resistant bacteria [316, 317].

As described for each virus in the previous sections, viral infections are common in many settings of immunocompromised patients. However, the viruses' role in febrile neutropenia in hematological patients (not being subjects for allo-HSCT) is not thoroughly explored. Two studies are described in paper II and IV that investigate viruses in the respiratory tract and blood, as potential etiological agents of febrile neutropenia.

3 AIMS

The overall aim of this thesis was to investigate the frequency and clinical impact of a broad range of respiratory viruses in different cohorts of immunocompromised patients. The specific aims of each substudy are described below:

I. To evaluate the clinical benefit of introducing screening for AdV in an already implemented surveillance program for CMV in pediatric and adult allo-HSCT recipients

II. To investigate the prevalence of viruses in pediatric cancer patients with febrile neutropenia using both conventional viral detection methods and real-time PCR

III. To determine the sensitivity of detecting respiratory viruses in adult hematological patients with febrile neutropenia using a flocked nasal swab in the outer part of the nasal cavity compared to nasopharyngeal aspirate

IV. To investigate the prevalence of viruses in adult hematological patients with neutropenic fever, and to compare it to the prevalence of afebrile neutropenic patients.

4 PATIENTS, MATERIALS AND METHODS

All studies on which this thesis is based have ethical permissions from The Ethical Review Board in Stockholm and Human Research Ethics Committee in Westmead (Sydney) where appropriate. Patients were eligible for enrollment after informed consent. Clinical and additional microbiological data were extracted from the patients' medical records.

4.1 PAPER I – ADENOVIRUS IN ALLOGENEIC HSCT RECIPIENTS

4.1.1 The patients

Between March 2006 and September 2007, a total of 20 pediatric and 77 adult recipients of allo-HSCT were recruited from two major Swedish transplantation centers at Karolinska University Hospital and Sahlgrenska University Hospital, respectively. The median age for the adults was 50 years (range 22-69 years) and for the children 12 years (range 1.5-17 years). The predominant underlying diseases for the adults were acute leukemia (48%) and MDS (13%), whereas for the children non-malignancies and MDS were most common (45% and 25%, respectively). A similar proportion of recipients were prepared with MAC (generally cyclophosphamide ± busulfan) and RIC (generally fludarabine ± busulfan or cytarabine), respectively, and for the adults half were T cell depleted *in vivo* with either alemtuzumab or antithymocyte globulin. Fifteen (75%) of the children were T cell-depleted. The grafts used were in half of the cases from a matched unrelated donor (MUD), whereas an HLA-identical sibling could help 43% and 20 % of the adults and children, respectively. UCB was thus infrequently used in adults, but in one fifth of the children this strategy was assumed to be the best choice.

4.1.2 Materials

The recruited recipients were sampled before HSCT and then according to a surveillance scheme routinely used for detection of CMV; weekly during the first nine weeks and then after 3, 6, and 12 months. Peripheral blood was collected for analysis of

AdV in plasma. Peripheral blood mononuclear cells (PBMC) for detection of AdV-specific T cells were collected from twelve randomly selected adult recipients at three time points; 4, 8 and 12 weeks after transplantation.

4.1.3 Methods

The real-time PCR assay used did include primers and probes earlier described [192] with an additional probe 5′FAM-TGCACCAGCCCGGGGCTCAGGTACTCCGA-TAMRA3′ in order to minimize the nucleotide mismatch between the probe and the PCR amplicon derived from the AdV subgroup C [277].

An ELISPOT assay was used for analysis of AdV-specific T cells. In brief, the wells were coated with primary antibodies against human IFN-γ. The PBMC were stimulated by adenolysate, and biotinylated secondary antibodies and streptavidin-bound enzyme conjugate were added to bind to the primary antibody/ IFN-γ complexes. Finally, a colorimetric substrate was added and spots counted in an ELISPOT reader.

Univariate analyses were performed using Fischer's exact test for categorical data. Non-categorical variables were compared with the Mann-Whitney U-test. A multiple logistic regression model was created with Statistica version 8.0 (Windows) for multivariate analysis of risk factors for AdV infection. All tests were two-sided with a p value of <0.05 considered significant.

4.2 PAPER II – VIRAL INFECTIONS IN CHILDREN WITH FEBRILE NEUTROPENIA

4.2.1 The patients

During one year (2007), a total of 90 episodes of febrile neutropenia occurring in 66 children at the pediatric oncology units at the Children's Hospital at Westmead (Sydney, n=43) and Astrid Lindgren Children's Hospital at the Karolinska University Hospital (Stockholm, n=47), respectively, were analyzed with extended viral diagnostics. The median age was 5 years (range 5 months – 18 years) and non-solid

cancer types were slightly overrepresented (69%). In total, more than half of the children suffered from acute leukemia followed by neuroblastomas (10%) and sarcomas (7%).

4.2.2 Materials

Nasopharyngeal aspirate (NPA) and peripheral blood samples were collected in addition to samples for routine laboratory analyses. For detection of CMV, AdV, and EBV and B19, whole blood, plasma, and serum were used, respectively.

4.2.3 Methods

In NPA, conventional viral detection methods were used for detection of PIV, influenza A and B virus, RSV, HSV 1 and 2, VZV, HEV, AdV, and CMV. In addition, NPA was analyzed by real-time PCR for detection of RSV, influenza A and B virus, PIV, HEV, AdV, HRV, human bocavirus (HBoV), HMPV, non-SARS HCoV, and KI/WU polyomavirus [318, 319]. For detection of CMV, AdV, EBV, and B19 in blood, real-time PCR was used [277, 320-322].

Group comparisons were performed by Kruskal-Wallis test using GraphPad Software. All tests were two-sided with p value of <0.05 considered significant.

4.3 PAPER III – NASAL SWAB VERSUS NPA FOR VIRAL DETECTION

4.3.1 The patients

Independently of presence of URTS, a total of 98 episodes of febrile neutropenia occurring in 89 hematological patients were enrolled in this study. The median age was 60 years (range 19-87 years) with acute leukemia and NHL as predominating underlying diseases. The majority suffered from severe neutropenia, <100 neutrophils/mm^3.

4.3.2 Materials

Paired samples of nasal and nasopharyngeal secretion were collected by a flocked nasal swab (fNS) and NPA, respectively.

4.3.3 Methods

First the fNS was inserted at least 20 mm and rotated inside each nostril, and then NPA was obtained via a sterile catheter inserted to the posterior nasopharynx (Figure 4). The fNS was transported without any medium, whereas the NPA was diluted with 2-3 mL of sodium chloride. Within the same day, a 500 µL of medium was added in which the fNS was shaken in order to release the biological material.

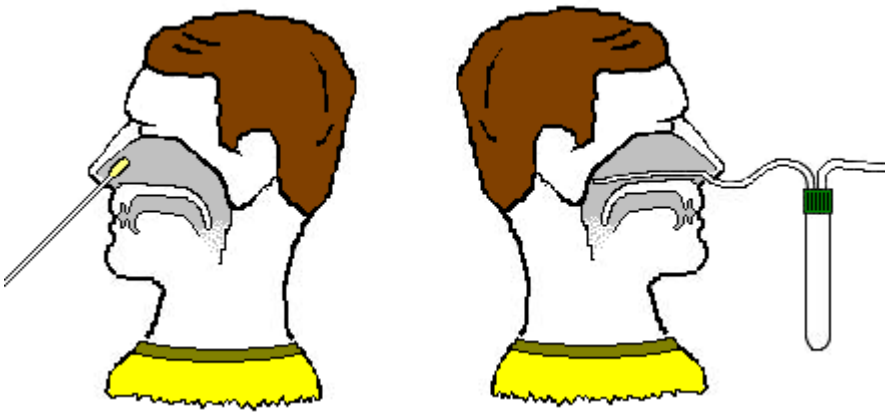

Figure 4. Nasal swab versus nasopharyngeal aspirate for sampling of nasal and nasopharyngeal secretion, respectively.

For both specimens, a real-time PCR assay including a broad panel of respiratory viruses covering AdV, HEV, HBoV, HCoV, HMPV, influenza A and B virus, PIV 1-3, HRV, RSV A and B was used [318].

Cohen's kappa was calculated as a measure of agreement of the results obtained from either method. Wilcoxon signed-rank test and Pearson's correlation coefficient were used when appropriate. All tests were two-sided with p value of <0.05 considered

significant. The statistical software products InStat 3.05 and Prism 5.00 for Windows were used.

4.4 PAPER IV – VIRUSES IN NEUTROPENIC ADULTS WITH AND WITHOUT FEVER

4.4.1 The patients

A proportion of the febrile episodes described in this paper were included in the study where we compared fNS and NPA (paper III). Between January 2008 and February 2010 adult hematological patients presenting at Karolinska University Hospital, Stockholm, with neutropenia were included. In total, 144 of the episodes, occurring in 124 patients, were associated with fever. A vast majority of these were included upon admission due to febrile neutropenia, whereas already hospitalized patients were included as soon as an elevated body temperature was measured. Another 39 patients (47 sampling occasions) were included upon regular clinical check-ups during neutropenia without fever. Patients admitted directly to the Intensive Care Unit where not included. Furthermore, patients having undergone allo-HSCT within the previous two years were excluded. The vast majority of the study subjects suffered from acute leukemia, NHL, multiple myeloma, or MDS.

4.4.2 Materials

At fever onset, the patients were admitted to hospital and sampled according to clinical routine. Bacterial cultures were performed on blood and in some cases from other locals such as urine, central venous catheter, feces, and pharynx. Within 72 hours, NPA and blood was collected for extended viral diagnostics. Whole blood and plasma were used for detection of CMV and AdV, respectively. For detection of EBV, B19, and BKV, serum was used.

4.4.3 Methods

For detection of CMV, EBV, and AdV in blood, real-time PCR assays previously described were used [277, 320-322]. Newly developed primers and probes are described in Table 3, whereas for HRV (A and B) [323], HEV [324], influenza A virus [325], influenza B virus [318], RSV [326], and PIV 1 [327], the design is described elsewhere.

For analysis of lymphocyte counts including subpopulations, peripheral blood was phenotypically characterized in 99 of the patients by staining with two sets of antibodies against the following cell surface markers; Set 1: CD3-FITC, CD8-PE, CD45-PerCP, CD4-APC; Set 2: CD3-FITC, CD16/CD56-PE, CD45-PerCP, CD19-APC. TruCount tubes (BD Biosciences) were used for an absolute count. Thus, cytotoxic T cells, T helper cells, B cells, and NK cells were stained for. The size of monocyte populations was determined using the combination of side scatter and CD45.

Univariate analyses were performed using Fischer's exact test for categorical data. Non-categorical variables were compared with the Mann-Whitney U-test and also by Fischer's exact test after dichotomizing the data above and below the median of both groups. Forward conditional binary logistic regression analyses were performed for multivariate analysis of factors associated with the dependent variable, fever. All tests were two-sided with p value of <0.05 considered significant. Software products used were Prism 5.00 for Windows and PASW Statistics 18.

Table 3. Newly designed primers and probes for different real-time PCR assays used in Study IV.

Virus	Primer/Probe	Sequence (5'-3')
HCoV	FW	YGATAAAGCTGTAGCTCGCAAACT
		ACGCGACCGTGCTGTAGC
		GATCATGAGATATCTGTTCAGAAGAAAATT
		CAGGGAATCATCTGTTCAAAAGAA
	REV	GATTATCCAATTTACGAACCATGCTA
		GCTTGATTATCTAACTTACGCACCATACTA
		ACATATCCAAACGTCTTAACATTCCA
		AAACGTCGGAGCATGCCA
	PRO	VIC-GATAAGAAGAGTAARGTTGTTTC-MGB
		VIC-TGGCTGAACAAGCTG-MGB
HMPV	FW	AAAGCATTAGGCTCATCCTCTACAG
		AAAGCTTTAGGCTCATCTTCAACAG
		AAAGCATTAGGCTCATCATCTACAGG
	REV	ATTGTTAGATGACCTGGCAATGAC
		GTTGGATGACCTGGCAATGAC
		TGTTGGATGATCTGGCAATGAC
		TGTTGTTAGATGATCTGGCAATGAC
		TGTTAGATGACCTGGCGATGAC
	PRO	NED-AGCAAAGCAGAAAGT-MGB
Influenza B virus	PRO[a]	6FAM-CAGATCTGTGCAGTTGAG-MGB
PIV2	FW	TTACCTAAGTGATGGAATCAATCGC
	REV	TCTTTYTCAGAYCTTGTAGCTACATAGCA
	PRO	NED-AAGCTGTTCAGTCACTGC-MGB
PIV3	FW	TCCCCATGGACATTCATYGTT
	REV	TGGCAYAGCAARTTACAATTAGGAA
	PRO	NED-TGCCATGTCCATTTTA-MGB
PIV4	FW	CCAGTCAAATCAAYTGCCTCYG
	REV	GATCTCTRATGCATAGTTCGCAAATT
	PRO	NED-CATGTGGAAGATGTCC-MGB

NOTE. HCoV, human coronavirus; FW, forward; REV, reverse; PRO, probe; HMPV, human metapneumovirus; PIV, parainfluenza virus.

[a] Probe modified, otherwise as described by Tiveljung et al [318]

5 RESULTS AND DISCUSSION

5.1 PAPER I – ADENOVIRUS IN ALLOGENEIC HSCT RECIPIENTS

For all patients, including those who died before the follow-up period ended, a median of 11 samples (range 2–19) was collected weekly for AdV PCR directly after HSCT. A total of 5% (5/97) of the patients, three children and two adults, had at least one sample that tested positive for AdV DNA in plasma, although none of them had clinical evidence of AdV disease. This is in the lower part of the range of reported incidences illustrated in Figure 5 based on a summary made by Marcela Echavarría in 2008 [166]. The overall incidence is however 9% which is just slightly higher than the 5% reported in our study. One important difference however is the fact that not a single patient developed AdV disease, which is a conflicting result from the other studies where the AdV-associated morbidity and mortality of the infected was 10-89%. The disparity between the reports is most likely explained by differences in patient group composition with respect to known factors associated with AdV infection post allo-HSCT (e.g. younger age and T-cell depletion). Moreover, not all relevant publications, such as the classical publication by Lion et al from 2003 [328], were taken into consideration. They found an AdV incidence of 27% in pediatric allo-HSCT recipients and stated that PCR-based monitoring of AdV in peripheral blood permitted early diagnosis of disseminated disease. Except for enteritis in some patients with AdV positivity in stool, detection of the virus at sites other than peripheral blood was not associated with AdV disease. Interesting of this, the same group recently presented data suggesting that detection of AdV in stool permits early detection of impending invasive AdV infection [329]. The incidence of AdV infection in children was 15% in our study. A rather similar overall frequency (19%) has been reported (Figure 6).

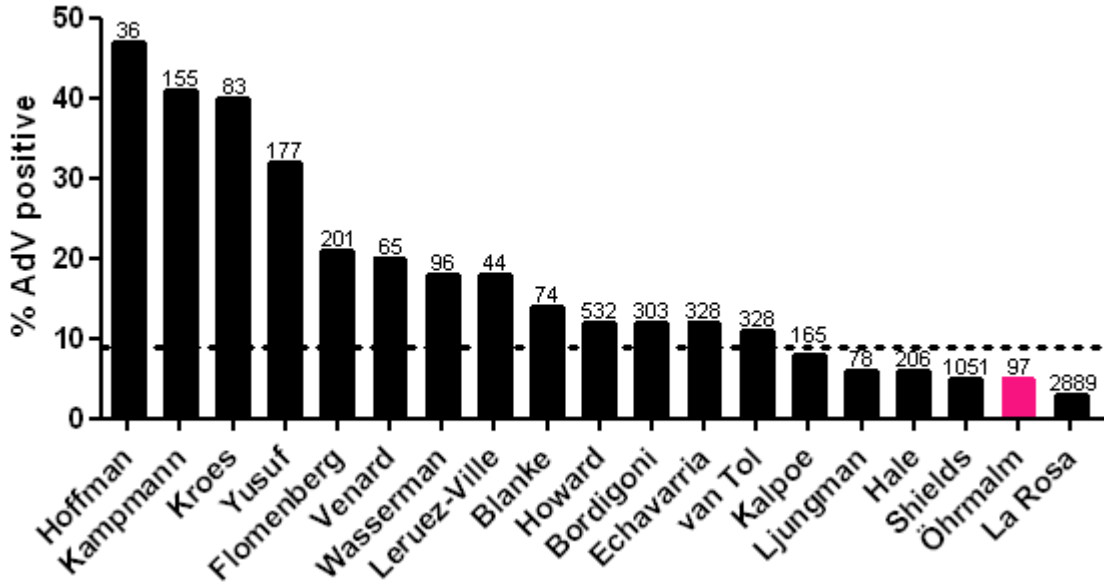

Figure 5. Reported incidences of AdV in HSCT recipients between year 1976 and 2005. The pink column represents the incidence of AdV infection in our study. The dashed line represents the reported overall incidence. The number of included recipients is stated above each column.

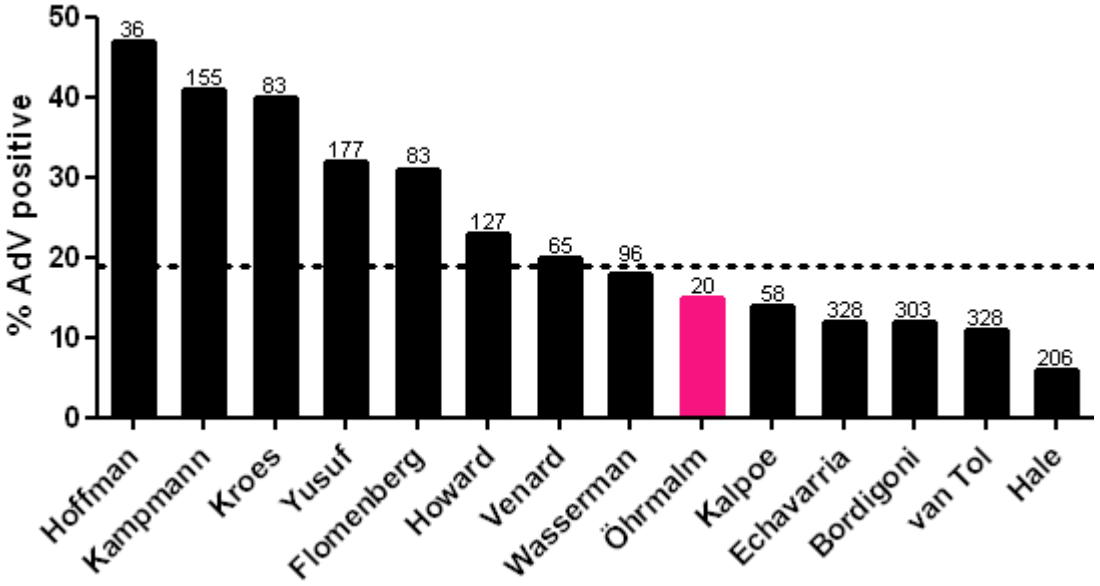

Figure 6. Reported incidences of AdV in pediatric HSCT recipients between year 1979 and 2005. The pink column represents the incidence of AdV infection in children in our study. The dashed line represents the reported overall incidence in children. The number of included recipients is stated above each column.

The AdV titers in Patients no. 1-4 were below 1000 copies/mL plasma, but in the protracted AdV infection in Patient no. 5, the peak was 9000 copies/mL. Determination of serotype was possible in four of our AdV cases; serotype 1, 2, 3, and 31. Serotypes 1 and 2 are very frequently isolated in HSCT recipients, reported in almost all studies where typing was performed. Serotypes 3 and 31 are reported in approximately half of the studies [166].

GVHD or its therapy is a known risk factor for AdV infection in allo-HSCT recipients [178]. In our study, all recipients suffered from GVHD at some time point during the follow-up period, but not necessarily before detection of AdV (Figure 7). GVHD was not found to increase the risk of acquiring AdV infection in our material.

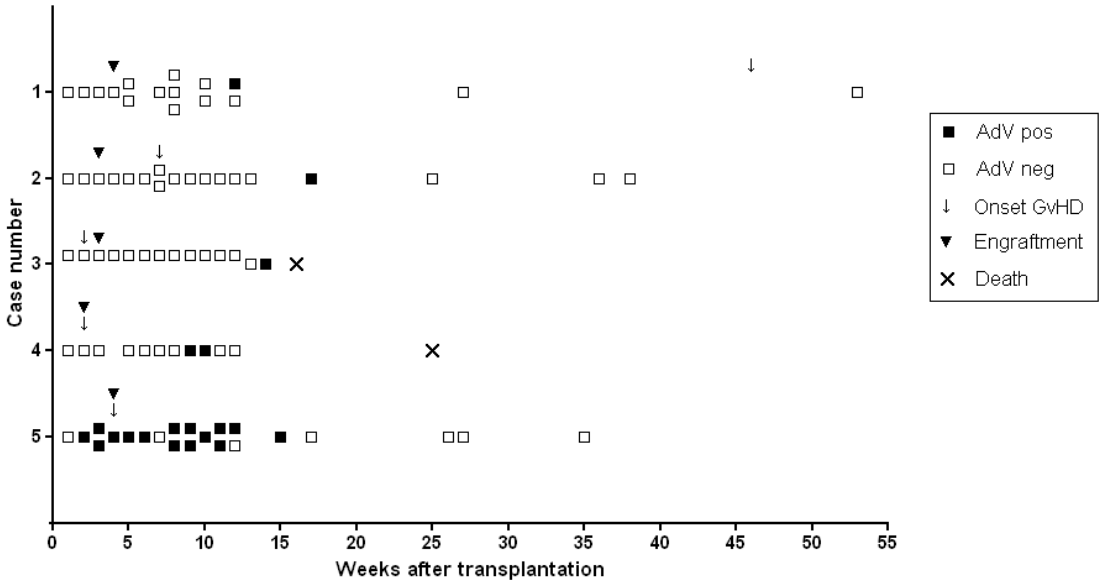

Figure 7. Sampling frequency of the AdV infected patients. AdV was found also in the intestine and kidneys at autopsy in Patient no. 3, and in feces at one occasion for Patient no. 5.

Another known risk factor is younger age [174, 183, 184, 186]. The median age in the AdV positive group was 14 years (range 1.5-42) which was lower than 46 years (range 2-69) in the AdV negative group ($p<0.05$; Figure 8). However, young age did not remain as a risk factor after multivariate analysis including possible confounding factors such as underlying disease, relationship to donor, stem cell source, conditioning regimen, and GVHD.

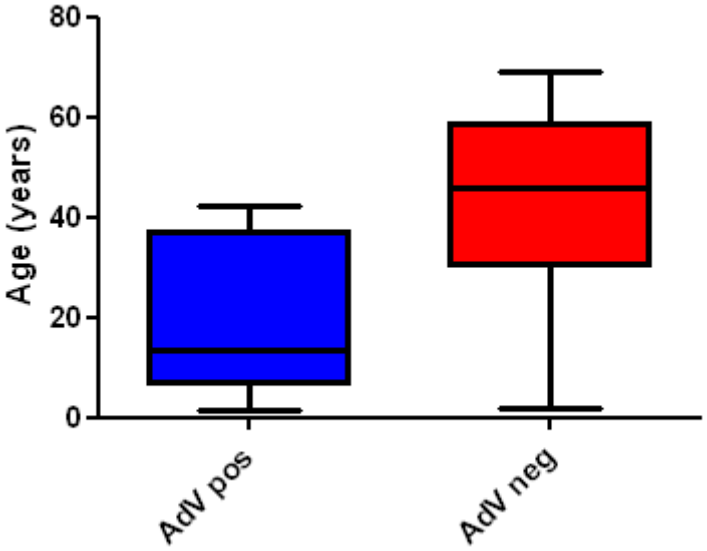

Figure 8. Difference in age between the AdV positive and AdV negative group.

Other known risk factors are MAC, T-cell depletion, and the use of mismatched or unrelated donors [279, 328, 330-332], but those could not be confirmed in our study. However, MDS patients and recipients of bone marrow grafts were overrepresented in the group of AdV positive cases ($p<0.01$ and $p<0.1$, respectively). These associations remained after multivariate analyses with adjusted odds ratios (95% CI) of 56.2 (3.6-876) and 15.7 (1.2-205), respectively. Previously, these factors have not been reported to be associated with higher risk of acquiring AdV infection.

As discussed above, a number of factors influence the incidence of AdV infections in these patients. Two other factors may also contribute to the disparity, namely the number of body sites investigated and the viral detection methods used. However, such relationships could not be seen in a summary of the studies presented above (Figure 9).

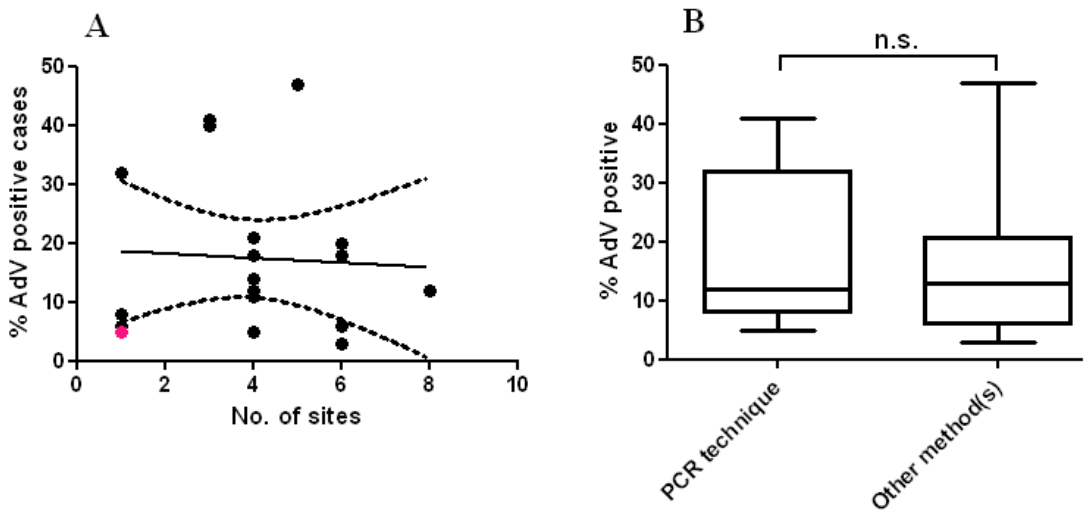

Figure 9. Correlation of reported incidences to A) number of body sites investigated, and to B) viral detection method used. The pink dot in A represents our study. The dashed lines illustrate the 95% CI for the trend line.

Rapid reconstitution of the lymphocyte population and, likely, the presence of AdV-specific T cells of a donor origin, contribute to prevention of AdV infection early after SCT [190, 279, 332]. Our ELISPOT data showed that more than half of randomly selected adults had T-cell immune responses against AdV on at least one time point during the first 3 months. Unfortunately, only a low number of patients were tested, and none of them had AdV infection. Thus, the study could not determine the protective effect of the AdV-specific T cells.

5.2 PAPER II – VIRAL INFECTIONS IN CHILDREN WITH FEBRILE NEUTROPENIA

In paper I the aim was to identify a virus known to cause severe disease in a certain patient category. In paper II the aim is somewhat different. Here we try to identify a broad range of viruses as possible etiological agents of fever, not necessarily disease, in children with febrile neutropenia.

Using conventional viral detection methods on NPA, together with bacterial culture on blood, a total of 26 out of the 90 febrile episodes (29%) revealed a possible etiological agent. Of these, bacteremia and one or more viral pathogens were identified in 21 and

10 episodes, respectively. Thus, with conventional methods, the viral prevalence was 11%. Using the same samples for analysis by real-time PCR with an even broader range of detectable viruses (including CMV, EBV, AdV, and B19 in blood), one or more viral pathogens were detected in 44 of the cases (49%). Together with bacterial culture the total number of episodes with a microbial agent detected was 54 (60%; Table 4). This increase was due to an even more extended panel of viruses investigated, but also the switch to PCR explained additional findings; in a total of 22 samples a virus type that is detectable by conventional methods was detected by PCR. As many as 71% of these were negative when IF and culture were used!

Five out of eight patients with serial samples could demonstrate clearance after a median of 5.5 weeks (range 2.7-14 weeks), but as no sequencing was performed it is possible that the persistent virus actually was a new virus with another genotype. This has been shown to be a common event in a prospective study of infants [333]. Conflicting results are described regarding time of virus shedding in immunocompromised children. Despite no confirming sequencing, prolonged shedding of RSV and HRV was apparent in two studies on children with cancer [334, 335], whereas another study demonstrated clearance in 23 out of 27 cases of viral respiratory tract infection [336]. In the cases with prolonged shedding, HRV was present in three and RSV in one. Whether prolonged shedding occur or not, an important fact is that viral infections can be asymptomatic. Several recent studies have investigated the association between respiratory virus findings and symptoms in children [337-340]. Various results are presented that range from almost no asymptomatic children to an equal distribution of symptomatic and asymptomatic children in virus positive cases. Most common viruses associated with asymptomatic infection were HRV, HEV, and HCoV. Influenzaviruses, RSV, and AdV were rare findings in asymptomatic children.

Table 4. Microbiological findings in 90 episodes of febrile neutropenia revealed by bacterial blood culture and real-time PCR in blood and NPA.

Single virus in NPA	Co-presence of virus in NPA	Virus in blood	Bacteria in blood	Co-presence of virus and bacteria	Episodes with at least one agent detected	Episodes with no agent detected
n=21 (23%)	n = 9 (10%)	n = 4 (4%)	n = 10 (11%)	n = 11 (12%)	n = 54 (60%)	n = 36 (40%)
HRV: 10	HRV, AdV	B19: 2	G^+ : 4	HRV: 5 G^+		
AdV: 3	HRV, HBoV	CMV: 1	G^- : 6	3 G^-		
KIPyV: 3	HRV, OC43	EBV: 1				
HKU1: 2	HRV, WUPyV			PIV3: 2 G^+		
HBoV: 1						
NL63: 1	KIPyV, HEV			KIPyV: 1 G^+		
OC43: 1	KIPyV, HBoV			HBoV		
	RSVB, HKU1					
	RSVB, HKU1					
	HMPV, NL63					

NOTE. AdV, adenovirus; B19, erythrovirus B19; HBoV, bocavirus; CMV, cytomegalovirus; CRP, C-reactive protein; EBV, Epstein-Barr virus; EV, enterovirus; G+, Gram positive bacteria; G−, Gram negative bacteria; HKU1, coronavirus HKU1; HMPV, human metapneumovirus; HRV, human rhinovirus; KIPyV, KI polyomavirus; NL63, coronavirus NL63; NPA, nasopharyngeal aspirate; OC43, coronavirus OC43; PIV3, parainfluenza virus 3; RSV, respiratory syncytial virus; WUPyV, WU polyomavirus

Independently of viral detection method used, the overall yield of potential etiological agents of febrile neutropenia increased significantly with extended viral diagnostics (Figure 10). This finding motivated us to conduct yet another study with a similar aim (Paper IV). However, as discussed in this section, viral findings can be made in asymptomatic individuals, and, as thoroughly penetrated in previous parts of the thesis; viral findings are not always associated with fever. Therefore we included afebrile neutropenic patients as controls in study IV. Moreover, presence of virus was not a useful parameter to predict non-bacterial fevers. In 24% of the cases when a virus was detected there was a concurrent bacterial infection. The corresponding proportion when virus was absent was even less, 22%.

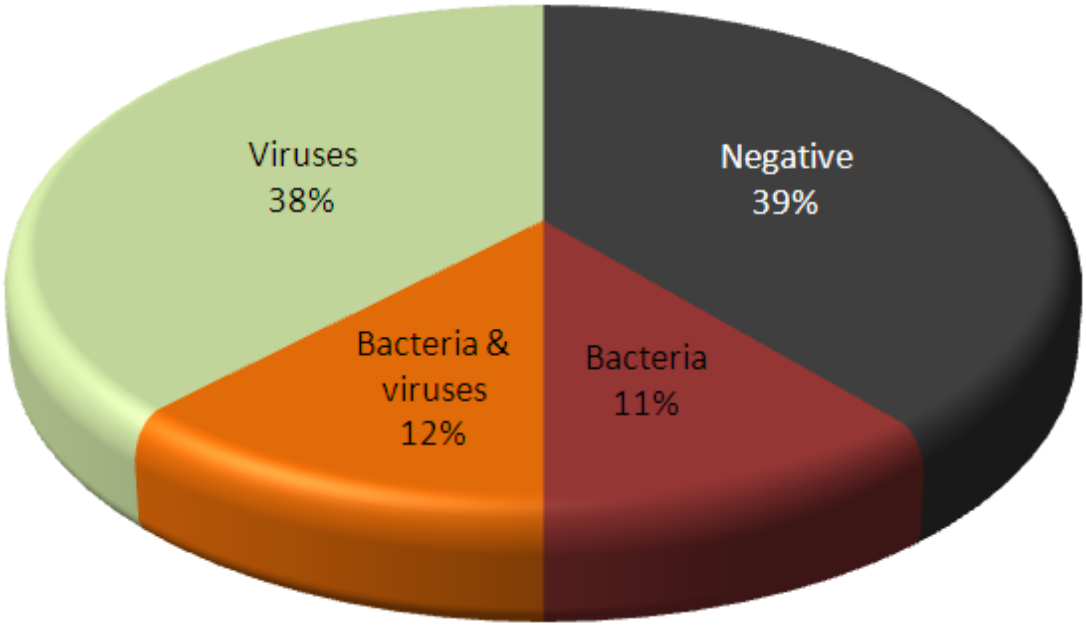

Figure 10. Distribution of viruses and bacteria in the 90 episodes of febrile neutropenia. Viruses detected by either a conventional viral detection method or PCR.

5.3 PAPER III - NASAL SWAB VERSUS NPA FOR VIRAL DETECTION

In the study described in paper II, one of the most frequent explanations for denial to participate was to avoid the use of NPA. The method is, from a strict clinical perspective, harmless and should not be painful. It could, however, be frightening for a child since a long catheter is used and the suction device can be noisy. Furthermore, the technique is somewhat complicated. Using a swab to collect nasal secretion has been shown in many studies to be a rather proper alternative [341-345], but conflicting results exist [346-348]. Most studies are performed on symptomatic children, and the detection methods and viruses sought for differ. Both conventional viral detection methods and PCR assays have been used with foremost RSV and influenzavirus as subjects for investigation. Different depths ranging from 1-4.5 cm have been used, as well as nasopharyngeal swabs (NPS). In addition to the lack of a consensus from the literature, we also suspected that fNS would be insensitive in immunosuppressed individuals due to an altered inflammatory response to infection with subsequent less nasal secretion.

In the first part of the study described in paper IV, we collected nasal secretion with fNS in parallel to NPA. If the fNS showed acceptable sensitivity it was supposed to replace the NPA in the further study. Although several reports have suggested that sensitivity increases when the nasopharynx is sampled instead of the nasal cavity [342, 349], a relationship between reported depth and sensitivity is not evident (Table 5). In fact, a comparison of fNS and NPS showed that the use of nasal swabs was accurate but significantly less painful than nasopharyngeal swabs for virus diagnosis. We thus chose to insert the fNS to a depth of 2-3 cm.

From the first 98 pairs collected, the number of positive samples detected by either method was 20. Only thirteen of these (65%) were detected by the fNS. The number of positive samples is small, but the upper limit in the 95% confidence interval for the overall sensitivity was 85%; an unacceptable low sensitivity for us to replace an established method. Illustrative of this is the fact that we would need to collect 54 additional double-positive pairs, without any additional negative swabs, in order to reach sensitivity above 90%. Thus, we decided to continue using NPA.

Table 5. Summary of studies comparing swabs and nasopharyngeal aspirates.

Study	Depth (cm)	PCR	Viruses	Sensitivity (range)[a]
Sung[b] [345]	1-1.5	No	RSV, Flu A, PIV, AdV	69% overall (PIV 49% –AdV 89%)
Sung[b] [345]	1-1.5	Yes	RSV, Flu A, PIV, AdV	81% overall (RSV 67% –PIV 95%)
Macfarlane [347]	2	No	RSV	66%
Heikkinen [343]	2-3	No	RSV, Flu A+B, PIV, AdV, HRV, HEV	81% overall (RSV 76% –Flu 92%)
Öhrmalm	*2-3*	*Yes*	*RSV, Flu A+B, HRV, HEV, HMPV, HBoV*	*65% overall HRV 78%*
Stensballe [346]	2-3	No	RSV	73%
Abu-Diab [342]	4.5	No	RSV, Flu A, PIV, AdV	98.5% overall (AdV 89% –Flu 100%)
Frayha [350]	NPS	No	RSV, Flu A+B, PIV, AdV	85% overall (RSV 90%)
Ahluwalia [351]	NPS	No	RSV	65%
Cruz [352]	NPS	No	RSV, Flu A+B, PIV, AdV, HRV, HEV	57% overall (HEV 54%, AdV 86%)

NOTE. RSV, respiratory syncytial virus; Flu, influenza virus; PIV, parainfluenza virus; AdV, adenovirus; HRV, human rhinovirus; HEV, human enterovirus; HMPV, human metapneumovirus; HBoV, human bocavirus.

[a] Between the virus with the lowest sensitivity to the virus with the highest sensitivity. If not reported, or too few numbers, single values of sensitivity are presented

[b] Same study using different viral detection methods

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5.4 PAPER IV - VIRUSES IN NEUTROPENIC ADULTS WITH AND WITHOUT FEVER

In study II, we found virus in a rather large proportion of the febrile episodes. However, it is not evident, nor likely, that all viruses found are true etiological agents. To further determine causality, we compared the prevalence of viruses in neutropenic patients with and without fever. The study described in paper IV is similar to study II but differs in some aspects; an adult cohort is investigated instead of a pediatric, the panel of viruses looked for differ slightly and does not utilize conventional methods, and, as mentioned, the study includes afebrile neutropenic patients as controls. Furthermore, additional data on common cell populations in peripheral blood was gathered.

As compared to the 50% in the pediatric study, we found one or more viral pathogens in 42% of the 144 episodes of febrile neutropenia (Figure 11). Half of these pathogens were detected in NPA. Bacterial infection, foremost bacteremia, was present in 35% of the episodes, and in one-third of these cases a viral pathogen was found. Conversely, in one-third of the virus positive cases, a bacterial infection was determined. Thus, viral as well as bacterial infections were more commonly a single infection rather than a co-infection.

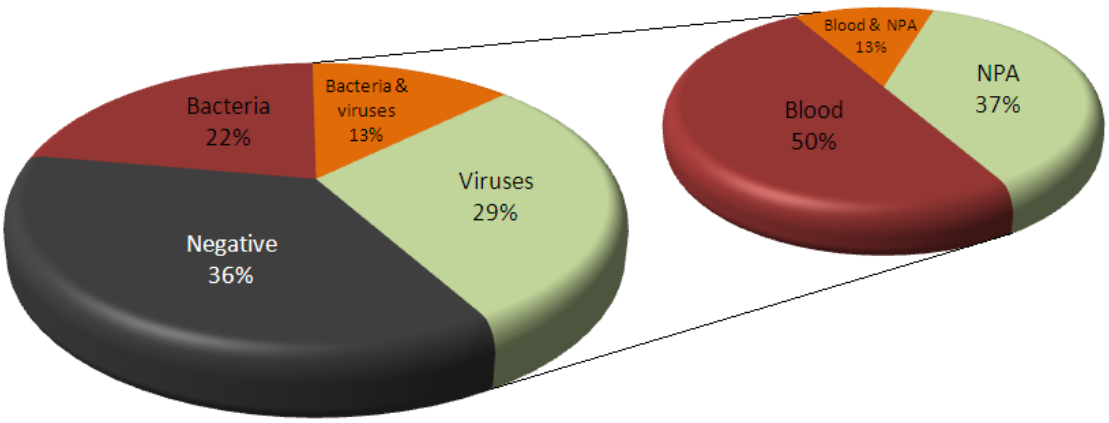

Figure 11. Distribution of viruses and bacteria in the 144 episodes of febrile neutropenia and the proportion of viruses detected in peripheral blood and nasopharyngeal aspirate (NPA), respectively.

Except for the absence of HCoV and PIV, the proportion of detected respiratory viruses in NPA was expected; HRV were present in 13 episodes followed by AdV with 7 episodes. These two viruses were the only represented in the control group. Influenzavirus and RSV were equally represented with 5 episodes each, followed by HMPV with 2 episodes. As described earlier, HRV seems to be less pyrogenic, and it is thus expected to find this virus, if any, in the control group. Rather surprisingly, the predominant virus in blood was BKV which is a common cause of hemorrhagic cystitis in allo-HSCT recipient [199, 200]. In a total of 18 episodes the virus was detected, but with low copy numbers. The second most common virus in blood was CMV, again another virus commonly detected post HCST. No one of the controls had detectable CMV in blood, and only in one afebrile patient BKV was detected. B19 and EBV were also common pathogens in the fever-group, but for EBV the prevalence was equally high in the fever-group as in the control group. The copy numbers were low and it is unlikely that the pathogens found in blood caused disease. However, could they have induced fever?

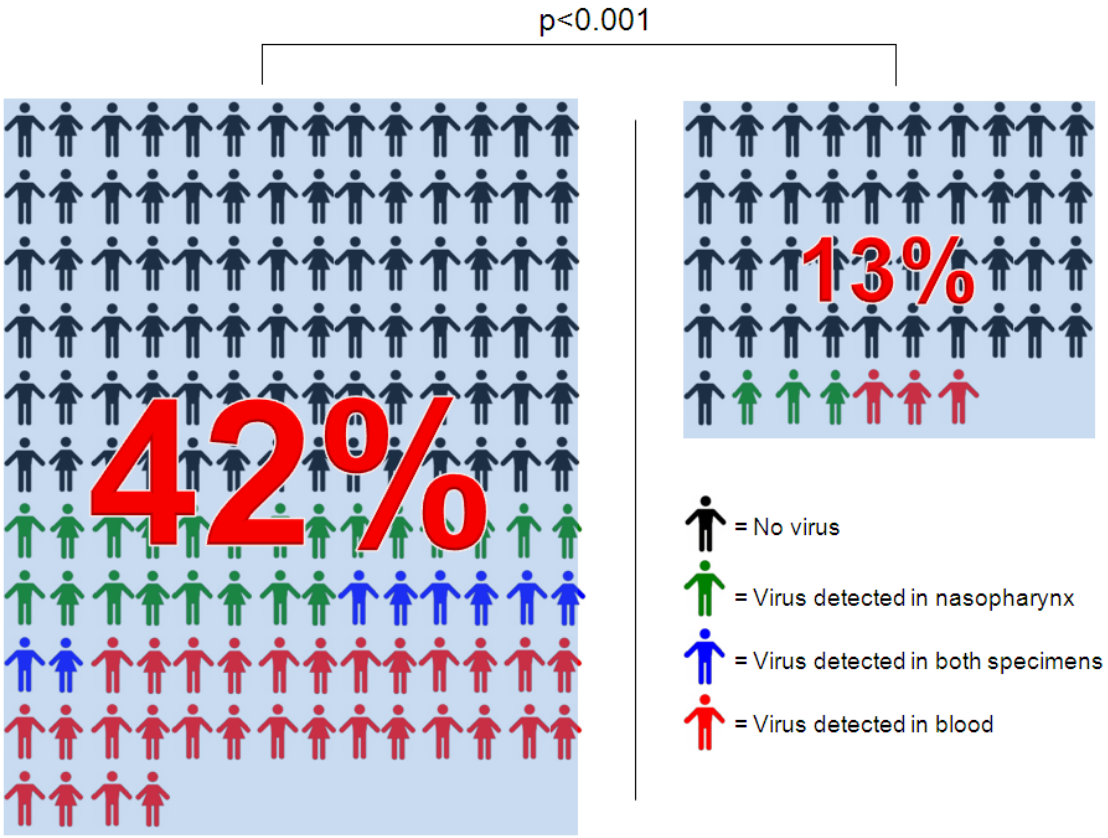

Figure 12. Difference in virus prevalence between the neutropenic patients with and without fever. The p value refers to the univariate analysis.

In summary, a significant difference in virus prevalence was found between the groups (Figure 12). This was true for viruses in NPA and blood separately as well. Unfortunately, the controls were also significantly less immunosuppressed than the febrile patients. However, the difference of the immunological status between the virus-positive and the virus-negative patients was not statistically significant (Figure 13) except for the T helper cells (Figure 14).

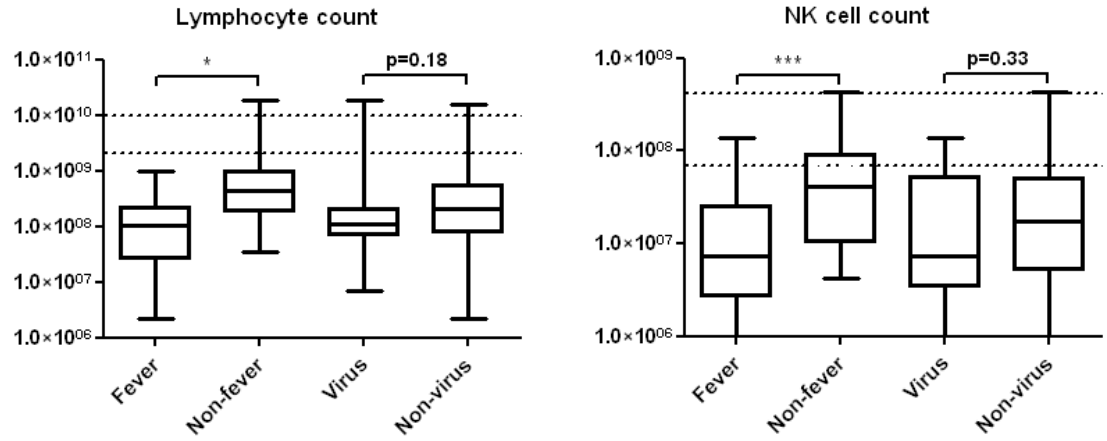

*Figure 13. Comparison of lymphocyte and NK cell count (cells/L) for the febrile and afebrile patients as well as for the virus-infected and virus-free patients. The dashed line represents the reference interval used at the routine laboratory. *, p<0.05; ***, p<0.001.*

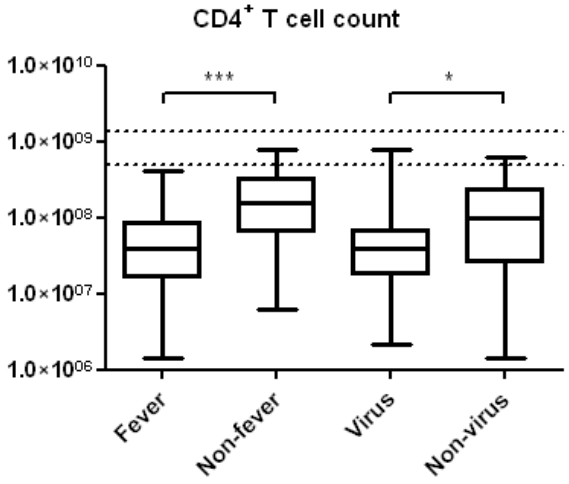

*Figure 14. Comparison of helper T cell count (cells/L) for the febrile and afebrile patients as well as for the virus-infected and virus-free patients. The dashed line represents the reference interval used at the routine laboratory. *, p<0.05; ***, p<0.001.*

In an attempt to rule out possible confounding factors that was detected when comparing the fever-group and the controls regarding several parameters, we created a multivariate regression model. The association remained and no other factor, including immunological status, could thus better explain the association between fever and presence of virus. Furthermore, URTS were by far more frequent in patients with virus detected in NPA (OR=4.4, $p<0.001$).

Nevertheless, there are two major considerations to take into account; (1) the sample size is rather small which could lead to "small sample bias" in a regression model; (2) the most important confounding factor was not measured, namely bacterial infections not detected by conventional bacterial culture. In two recent studies, PCR improved the microbiological documentation of infections in febrile neutropenia [353, 354], and maybe extended bacterial diagnostics could have explained the fever in our study better than the viruses. Not surprisingly, we failed to identify laboratory parameters that could reliably distinguish patients with different infections. Within the fever group there were no differences in CRP according to type of infection. This was also true within the control group. The mean CRP in the fever group was however significantly higher than the mean CRP for the controls. However, this finding is unfortunately of no clinical relevance. As in the pediatric study, a virus finding could not help predicting a non-bacterial fever. Bacteria were detected in 31% of the virus-positive cases, whereas in the virus-negative group, a total of 38% had positive bacterial culture.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

The study described in paper I differs from the studies described in paper II and IV. The attempt to determine the incidence of AdV infections in allo-HSCT recipients was motivated by the knowledge that AdV is associated with morbidity and mortality in this patient category. A high incidence of AdV infection and AdV disease in our study could have supported yet another clinical trial with evaluation of known and newfound risk factors. Preemptive treatment in randomly selected patients with AdV detected in plasma would further have increased our knowledge about the clinical course upon infection. Despite the low number of AdV infections and absence of associated disease in our study, there are good reasons not to leave the matter aside. The incidence could vary with changed transplant regimens; for example usage of T-cell depleted graft or increased proportion of pediatric recipients would motivate reinforced attention. Furthermore, as such a great number of AdV serotypes exist, it is of major importance to have a reliable PCR assay that are proved to cover the whole panel. The assay should be designed to be able to take part in a multiplex assay consisting of other important pathogens post allo-HSCT such as CMV, EBV, and BKV. We have designed and evaluated an in-house made real-time PCR assay that showed to cover all tested serotypes (type 1-50 and 53), and we plan to include it in a fourplex-PCR assay designed for post allo-HSCT surveillance.

The papers II and IV describe another approach to improve the clinical care of immunocompromised patients. We aimed to identify pathogens that not primarily caused severe disease in the patients, but yet were potential etiology to fever. As the regimen of prompt empirical antibiotics is widely used upon febrile neutropenia, our findings suggest that a proportion of the episodes may be associated with overuse of antibiotics. The difference regarding virus prevalence between febrile and non-febrile neutropenic patients in paper IV is a promising result. Rather disappointing, however, was that the presence of virus did not predict bacterium-free fever. Moreover, there are several weaknesses in the study that detract from enthusiasm. Although multivariate regression analyses are developed to adjust for not perfectly matched controls, a much better study design for this matter is a prospective longitudinal study where the patients can be their own controls. Furthermore, results from one homogenous cohort cannot always be translated into another. Still, it is better to have reliable results from a small

cohort possible to interpret, than to have uncertain results from a heterogeneous group such as “hematological patients”. Thus, a smaller but yet more homogenous patient category such as NHL should be investigated one at the time rather than grouped together. For viral detection, we can be sure from study I that PCR is superior to the conventional viral detection methods. In future studies, though, the higher sensitivity of NPA compared to fNS must counterbalance the lower inclusion rate. Finally, all relevant extended diagnostics should be used in parallel rather than one at the time.

The studies on etiology of febrile neutropenia did not reveal absolutely convincing evidence of an association between viral infections and fever. The results are however promising and we therefore plan to conduct a prospective longitudinal surveillance study on a more isolated group of hematological patients in order to further investigate causality. The sampling occasions will include time-points in both the neutropenic phase, with and without fever, and the non-neutropenic phase. In addition to the extended viral diagnostics, we plan to use comprehensive bacterial and fungal diagnostics including PCR technique. Moreover, the patients will be asked for epidemiological information in a higher extent than is done per clinical routine. Finally, as the most clinical relevant question to answer is whether the patients suffer from a bacterial infection or not, we will try to identify reliable predictors thereof. A possible subsequent clinical trial with low-risk patients selected to early discontinuation of the empiric antibiotic treatment could be based on these predictors. In the trial, a continuous improvement of the prediction algorithm could be achieved through a similar extended diagnostic approach. Most importantly, these attempts to advance the clinical care must be made with minimum risk of endanger the health of the patients – step by step.

7 POPULÄRVETENSKAPLIG SAMMANFATTNING

Antalet personer med nedsatt immunförsvar har paradoxalt nog ökat i takt med medicinska landvinningar. Detta beror dels på att patienter med nedsatt immunförsvar som delfenomen i en svår sjukdom övelever längre, dels på att behandling i samband med transplantation och cancer ofta innefattar cellgifter som trycker ner immunsystemets snabbt delande celler. Ett dåligt fungerande immunförsvar innebär en ökad risk att drabbas av infektioner, och dessa patienter kan bli svårt sjuka eller till och med avlida av infektioner som annars vore banala och självläkande hos personer med intakt immunförsvar.

Av denna anledning är resultatet av den ökande mängd genomförda stamcellstransplantationer, då patienternas sjuka benmärg byts ut, till stor del beroende av hur väl komplikationer i form av svåra infektioner kan upptäckas och kontrolleras under det långa efterförlopp som kan vara flera år. Ett belysande exempel på detta är den dramatiska minskningen av dödlighet i komplikationer till infektion med cytomegalovirus (CMV), som har uppnåtts via införandet av rutinmässig övervakning och snabbt insättande av effektiv läkemedelsbehandling mot detta virus. Adenovirus (AdV) är ett annat virus som föreslagits spela stor roll som orsak till sjuklighet och dödlighet hos dessa patienter, och i vissa studier har närmare hälften av alla transplanterade ådragit sig infektion med AdV. Hur vanligt och viktigt detta virus är i dessa sammanhang är dock inte tillräckligt utrett för att rutinmässiga kontroller ska införas på bred front. För att bättre kunna värdera nyttan av ett sådant införande, gjorde vi en studie där vi parallellt med ett befintligt övervakningsprogram för CMV, också tog regelbundna prover avseende AdV i blod hos barn och vuxna som genomgått stamcellstransplantation. Vi fann att 5% (5/97) av patienterna hade AdV i blodet någon gång under uppföljningstiden, och i alla fallen utom ett återfanns ett lågt antal viruspartiklar talande för en låggradig infektion. Ingen av patienterna visade tecken till sjukdom relaterad till AdV. Dessa resultat tyder på en mindre betydelse av AdV-infektion hos denna patientkategori än vad flertalet tidigare rapporter visat. Dock skulle vi med stor sannolikhet påvisat fler AdV-infektioner om vår studie hade inkluderat patienter med fler riskfaktorer för AdV-infektion efter transplantation. Förutom låg ålder, finns andra kända riskfaktorer för AdV-infektion hos transplanterade som de flesta direkt eller indirekt anknyter till graden av nedtryckt immunförsvar. I vår studie kunde vi finna två nya sådana riskfaktorer som inte tidigare rapporterats. Ökad risk sågs för patienter med diagnosen myelodysplatiskt syndrom som anledning till transplantation, samt de som fick benmärg som stamcellskälla i stället för stamceller från perifert blod eller navelsträngsblod. Även om intensiv övervakning och en eventuell antiviral behandling inte hade påverkat det kliniska förloppet hos patienterna i denna studie, kan strategin ändå vara aktuell för andra situationer där patienter med fler riskfaktorer genomgår ett transplantationsförfarande som trycker ned immunförsvaret än mer.

En annan kategori av patienter med kraftigt påverkat immunförsvar är patienter som behandlas med upprepade cellgiftskurer mot cancer. Kort efter behandling sjunker antalet vita blodkroppar drastiskt (neutropeni) och i händelse av feber måste patienterna

åka till sjukhus för inläggning med intravenös behandling med antibiotika, eftersom man vet att bakteriell infektion hos dessa så kallade neutropena feberpatienter kan vara livshotande. Diagnostiken av vad som orsakar febern riktas i praktiken traditionellt mot att leta efter bakterier, och i ungefär en tredjedel av fallen lyckas man med detta, men i de övriga två tredjedelarna förblir orsaken okänd. Det finns alltså anledning att tro att febern inte alltid orsakas av bakterier, och att man i stället skulle kunna fastställa t.ex. virus som orsak om man bara letade efter dessa medelst relevant provtagning. Vinsterna vore stora, eftersom man då skulle kunna skilja ut vilka patienter som är i behov av antibiotika och som måste stanna inneliggande på sjukhus. För de med virusinfektion skulle man kanske i en framtid kunna ändra handläggningsrutinerna och medge hemgång utan antibiotika. Detta skulle betyda minskad tid på sjukhus med ökad livskvalitet för patienterna under denna redan svåra period i deras liv, och därtill minimera risken för ytterligare infektioner med vanliga sjukhussmittor. Minskad användning av antibiotika minskar också risken för resistensutveckling och opportunistiska infektioner, t.ex. med svamp. Totalt skulle detta även reducera sjukvårdskostnaderna, vilket åtminstone kan vara ett krasst sjukvårdspolitiskt argument. Av dessa anledningar har vi därför i två stora studier, undersökt möjligheten att med utökad virusdiagnostik vid neutropen feber åstadkomma ett underlag för en framtida förändring av den kliniska handläggningen av dessa patienter. Både barn och vuxna patienter har ingått i studierna och virusdiagnostiken har riktats mot en bred panel av utvalda virus som vi har goda skäl att anse som de mest potenta att orsaka feber i denna patientgrupp. Det handlar dels om virus som normalt ligger vilande hos friska individer, men som kan blossa upp vid nedsättning av immunförsvaret, dels om virus som är vanligt förekommande i samhället, däribland flera förkylningsvirus och influensa. Vi undersökte förekomsten av dessa virus i blod och i de övre luftvägarna hos patienterna, och kunde samtidigt göra jämförelser mellan olika provtagnings- och påvisningsmetoder. Hos barn fann vi virus i hälften av fallen och de återfanns framförallt i luftvägarna. Totalt, inklusive episoderna där bakterier bedömdes vara orsaken till feber, lämnades endast ca 40% av fallen utan något mikrobiologiskt fynd. Motsvarande siffror hos vuxna patienter var snarlika med virusfynd i drygt 40% av fallen. Här återfanns dock ungefär hälften i luftvägarna och hälften i blod. När vi jämförde med en kontrollgrupp från samma patientkategori, men utan feber, fann vi en markant skillnad i förekomst av virus. Här återfanns ett virus i endast 13% av fallen, jämnt fördelade mellan blod och luftvägar. Den kliniskt viktiga uppgiften att utesluta bakteriell infektion, visade sig dock ej görbart endast genom att påvisa förekomst av virus. Andelen bakteriella infektioner var nämligen ungefär lika stor hos dem som hade respektive inte hade virusinfektion. Den höga andelen virus vid neutropen feber och det faktum att motsvarande feberfria patienter inte hade virus i samma utsträckning, ger dock hopp om att våra fynd kan spela en viktig roll tillsammans med andra variabler i en framtida algoritm för handläggningen av dessa patienter. Detta motiverar därför till fortsatt forskning som nu är under planering i vår grupp.

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