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Febrile Infections in Children with Leukemia

with Special Reference to Respiratory Viral Infections

by

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To Juha, Eemeli, and Kukka

ABSTRACT

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Febrile infections in children with leukemia, with special reference to respiratory viral infections.

From the Department of Pediatrics
Annales Universitatis Turkuensis, Medica-Odontologica, 2008, Turku, Finland
Painosalama Oy, Turku, Finland 2008

Background: Febrile infections are an important cause of morbidity and mortality in children with leukemia. Respiratory viruses occur constantly in the community and are a potential cause of febrile episodes. Only a few studies have been published on respiratory viral infections in children with cancer.

Aims: A 5-year prospective multicenter study was conducted, searching for respiratory viruses in febrile children with leukemia undergoing anticancer treatment. Sixteen viruses were searched for by viral culture, antigen detection, and polymerase chain reaction tests from nasal swab, stool, and blood samples. The expression of MxA protein in blood lymphocytes was analyzed to find out whether it may suggest viral infection in children undergoing anticancer treatment.

Results: During 27743 patient days at risk, the occurrence of febrile episodes was 2.1 per patient years at risk. The study included 138 febrile episodes in 51 children. Evidence of viral infection was obtained in 82 of the 138 (59%) episodes. Dual viral infections were detected in 17 cases (12%). Rhinovirus (22%)**,** respiratory syncytial virus (11%)**,** human herpes virus 6 (7%)**,** human bocavirus (5%), cytomegalovirus (5%), parainfluenza viruses (5%), and influenza A virus (4%) were the most common viral agents. The respiratory viral infections were mainly mild and progressed to pneumonia in only two cases. Blood cultures for bacteria were positive in 19 of the 138 (14%) febrile episodes, and in 11 (58%) of the episodes evidence was found for concomitant viral infection. MxA protein expression occurred in most viral infections.

Conclusions: Respiratory viruses are common causative agents of febrile episodes in children with leukemia. Half of the septicemias were mixed bacterial-viral infections, suggesting that respiratory virus may pave the way to a more severe bacterial disease. After careful microbiological diagnostics, only a few cases remain to be classified as fever of unknown origin. MxA protein may be helpful in the detection of viral infections in children with cancer.

Key words: virus, rhinovirus, respiratory, septicemia, leukemia, children, MxA protein

TIIVISTELMÄ

Minna Koskenvuo

Leukemiaa sairastavien lasten kuumeiset infektiot, erityisesti respiratoriset virusinfektiot.

Lastentenklinikka
Annales Universitatis Turkuensis, Medica-Odontologica, 2008, Turku, Suomi
Painosalama Oy, Turku, Suomi 2008

Tausta: Hengitysteiden virusinfektiot ovat lasten tavallisimpia sairauksia. Infektiota aiheuttava virus voidaan uusilla menetelmillä löytää lähes kaikissa tapauksissa. Leukemiaa sairastavilla lapsilla on perustaudin ja leukemian hoitojen takia tavallista suurempi infektioalttius, ja kuumeilu on tavallista leukemiahoidon aikana. Suurin osa syöpähoidon aikaisten kuumejaksojen syistä jää kuitenkin selvittämättä.

Tavoitteet: Prospektiivisen 5 vuotta kestäneen monikeskustutkimuksen tavoitteena oli etsiä uusimmilla mikrobiologisilla menetelmillä leukemiaa sairastavien lasten kuumeen syy. Tätä varten tutkittiin 16 virusta virusviljelyllä, antigeeniosoituksella ja nukleiinihappo-osoituksella. Näytteitä otettiin nenästä, ulosteesta, virtsasta ja verestä. Lisäksi tutkittiin MxA-proteiinin kykyä osoittaa virusinfektio syöpälapsella.

Tulokset: Tutkimuksen aikana analysoitiin 138 kuumejaksoa 51 leukemialapsella. Kokonaisseuranta-aika oli keskimäärin 1.5 vuotta/lapsi. Kuumejaksojen ilmaantuvuus oli 2.1 jaksoa potilasta kohden suhteutettuna vuoden riskiaikaan. Hengistysteiden virusinfektio voitiin osoittaa 82 kuumejaksossa (59%). Kaksi tai useampi virus löydettiin 12 %:ssa kuumejaksoista. Tavallisimmat virukset olivat rhinovirus (22 %), respiratory syncytial virus eli RS-virus (11 %), human herpes virus 6 (7 %), human bocavirus (5 %), sytomegalovirus (5 %), parainfluenssavirukset (5 %) ja influenssa A -virus (4 %). Kahdelle potilaalle kehittyi pneumonia, muilla oireet olivat lievät. Veriviljely oli positiivinen 19 kuumejaksossa (14 %), ja puolessa tapauksista löydettiin samanaikaisesti respiratorinen virus. MxA proteiini ilmeni veren lymfosyyteissä useimmilla virusinfektioon sairastuneilla syöpälapsilla.

Päätelmät: Kuumeiset respiratoriset virusinfektiot ovat tavallisia leukemiaa sairastavilla lapsilla. Infektion oireet ovat tavallisesti vähäiset, mutta pienelle osalle voi kehittyä veriviljelypositiivinen sepsis tai pneumonia. Kuumeen syy jäi selvittämättä vain harvoissa tapauksissa.

Avainsanat: virus, hengitystieinfektio, leukemia, sepsis, lapsi, MxA proteiini

TABLE OF CONTENTS

ABBREVIATIONS

Ag	Antigen
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
BMT	Bone marrow transplantation
CMV	Cytomegalovirus
CNS	Central nervous system
CRP	C-reactive protein
CSF	Central spinal fluid
CT	Computed tomography
EFS	Event-free-survival
FUO	Fever of unknown origin
GvHD	Graft versus host disease
HBoV	Human bocavirus
HHV-6	Human herpes virus 6
hMPV	Human metapneumovirus
HSCT	Hematopoetic stem cell transplantation
IFN	Interpheron
IL-6	Interleukin-6
MRI	Magnetic resonance imaging
MxA	MxA protein
NOPHO	Nordic Society of Pediatric Hematology and Oncology
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PCT	Procalcitonin
PIN 1, 2, 3	Parainfluenza viruses types 1, 2 and 3
PYR	Patient year at risk
RSV	Respiratory syncytial virus
RT-PCR	Real-time polymerase chain reaction
SD	Standard deviation
WBC	White blood cell count

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following papers, which are referred to in the text by Roman numerals I – IV.

I. Koskenvuo M, Möttönen M, MD, Rahiala J,Pihkala U, Riikonen P, Waris M, Ziegler T, Uhari M, Salmi TT, Ruuskanen O. Respiratory viral infections in children with leukemia. Submitted.

II. Koskenvuo M, Waris M, Allander T, Salmi TT, Ruuskanen O. Human bocavirus in children with acute lymphoblastic leukemia. Eur J Pediatr 2007, in press.

III. Koskenvuo M, Möttönen M, Rahiala J, Pihkala U, Riikonen P, Waris M, Uhari M, Ruuskanen O, Salmi TT. Mixed bacterial-viral infections in septic children with leukemia. Pediatr Infect Dis J 2007, 26(12):1133-6.

IV. Koskenvuo MM, Halminen M, Blomqvist M, Vainionpää R, Ilonen J, Julkunen I, Salmi TT, Mäkelä MJ. Expression of MxA protein in blood lymphocytes of children receiving anticancer chemotherapy. Pediatr Hematol Oncol 2006;23(8):649-60.

1 INTRODUCTION

Malignancies are rare in childhood. They are divided into hematologic malignancies and solid tumors. Hematologic malignancies include acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), as well as some other very rare leukemias. Solid tumors can occur in any solid tissue (Table 1). Leukemia is the most common childhood cancer. It accounts for one third of pediatric malignancies. ALL accounts for 75% of pediatric leukemias and AML for 20% (Pui CH, 1997). The risk of any child developing acute leukemia is about 1 in 2,000, meaning 40-50 new cases per year in Finland (Geaves, 2002). Leukemia was a fatal illness until the early 1970s when effective chemotherapy and irradiation were introduced (Pinkel, 1972). Since then, the outcome of children with leukemia has improved steadily. Overall survival has increased from 25% to 75% with more intensive therapy and treatment adjusted to specific risk groups (Greaves, 2002; Gustafsson et al., 2000; Lie et al., 2005; Ramanujachar et al., 2006). Today, an important goal in improving the outcome of children with leukemia is associated with treatment-related factors. Intensified antileukemia treatment increases susceptibility to infections. Despite effective antimicrobial treatment of infections, they still are the leading cause of treatment-related morbidity and mortality. (Christensen et al., 2005a; Pizzo, 1999; Shaw, 2002; Slats et al., 2005).

The occurrence of febrile episodes during anticancer treatment varies from 2.6 to 4.2 episodes per patient years at risk (Katsimpardi et al., 2006; Lehrnbecher et al., 2004; Lex et al., 2001; Möttönen et al., 1995; Rahiala et al., 1998). The microbiologic cause of infection or the focus of infection in children with cancer is found in 30% to 40% of the cases (Katsimpardi et al., 2006; Lehrnbecher et al., 2004; Lex et al., 2001; Pizzo et al., 1991). The incidence of bacteremia varies from 8.5% to 28% in febrile episodes, depending on the intensity of antileukemia therapy (Paulus et al., 2005; Riikonen et al., 1993; Stabell et al., 2007). Mortality due to invasive infections is low, usually less than 10% but varying from 3% to 17%, depending on the immunologic status of the leukemic patient and on the microbiologic causal agent (Rubnitz et al., 2004; Santolaya et al., 2002; Slats et al., 2005). Most septicemias occur in association with neutropenia and are caused by gram-positive bacteria. A considerable number of febrile episodes have remained classified as fever of unknown origin (FUO).

Respiratory viruses are the most common cause of infections in children, and they are naturally a potential cause of febrile episodes in children with cancer. According to previous reports, respiratory viral infections in children with cancer have not been as common as would have been expected (Arola et al., 1995; Kosmidis et al., 1980; Möttönen et al., 1995; Rahiala et al., 1998). Respiratory syncytial virus (RSV) and parainfluenza viruses have been the most common causative viruses. The most common respiratory virus, rhinovirus, has been searched for only in a few studies (Arola et al., 1995; Christensen et al., 2005b; Long et al., 1987; Möttönen et al., 1995; Wood et al., 1985). Over the past five years, new viruses like human metapneumovirus (hMPV), new coronaviruses (SARS, NL63, HKU1), and human bocavirus (HBoV) have been identified as causes of respiratory disease in humans (Allander et al., 2001; Hon et al., 2003; van den Hoogen et al., 2001). Very recently, a new human polyomavirus, KI virus, was identified from respiratory samples (Allander et al., 2007a; Bialasiewicz et al., 2007). The role of these new viruses in immunocompromised children is not yet clear.

Prompt and reliable etiologic diagnosis of fever is crucial. It determines the patient's treatment and gives information on the clinical course and outcome of the illness. Unnecessary treatments should be avoided. In addition, economic losses, discomfort to the child, and frequent use of broad-spectrum antibiotics are problems. The latter raises antibacterial drug resistance and increases the risk of fungal infections.

A 5-year prospective multicenter survey was conducted, to search for respiratory viruses in febrile children with leukemia. For the purpose, 16 viruses were searched for using virus culture, virus antigen detection, and polymerase chain reaction (PCR) techniques to obtain a maximal yield for virologic diagnosis. In addition, alpha-interferon-induced MxA protein expression in peripheral blood mononuclear cells (PBMC) was analyzed to find out if MxA protein expression is helpful in the detection of viral infections in children with cancer.

Table 1. Occurrence of childhood cancer in the Nordic countries (Denmark, Finland, Iceland, Norway, Sweden) in 1989-2003. No Swedish data included in the occurrence of solid tumors. Data is from register of Nordic Society of Paediatric Haematology and Oncology year 2007.

Diagnostic group	Cases	Incidence*
Leukemias		
Acute lymphoblastic leukemia	2644	4.0
Acute myeloid leukemia	504	0.8
All leukemias	3148	4.7
Solid tumors		
Central nervous system tumors	1900	2.9
Lymphomas	737	1.1
Soft-tissue sarcomas	432	0.7
Sympathetic nervous system tumors	413	0.6
Renal tumors	360	0.5
Malignant bone tumors	252	0.4
Germ-cell, trophoblastic, and other gonadal neoplasms	286	0.4
Retinoblastomas	182	0.3
Carcinoma and other malignant epithelial neoplasms	216	0.3
Hepatic tumors	86	0.1
Other and unspecified malignant neoplasms	72	0.1
All solid tumors	5006	7.5

*) The incidence is shown per 100 000 person years.

2 REVIEW OF THE LITERATURE

2.1 Acute lymphoblastic leukemia

ALL is the most common malignancy in children with 35-45 new cases diagnosed annually in Finland (Gustafsson et al., 2000). The outcome of children with leukemia has improved substantially since the introduction of 'total therapy' by Pinkel et al in 1972 (Pinkel, 1972). Earlier, no remission was achieved in children with hematologic malignancies, and they all died within months. Since early 1980s, the 5-year event-free survival (EFS) of ALL has increased from 57% to 78% in the Nordic countries (Denmark, Finland, Iceland, Norway, Sweden), (Gustafsson et al., 2000). The outcome of children with leukemia is dramatically better than that of adults. While 80% of children are long-term survivors, less than 40% of adults obtain cure with current therapies (Chessells et al., 1998; Freifeld et al., 2006; Gaynon et al., 2000; Schrappe et al., 2000). Adolescents and young adults have shown intermediate outcomes (Nachman et al., 1993; Ramanujachar et al., 2006).

ALL is subclassified according to the morphologic, immunologic, cytogenetic, and molecular genetic features of the leukemia cells. The definite diagnosis is based on examination of bone marrow aspirates. Different chromosomal and gene abnormalities in leukemia define the subclass of this hematologic malignancy, and their prognostic value is variable. Chromosomal abnormalities provide sensitive markers and can be used in monitoring treatment response (Greaves, 2002). The age distribution of ALL patients at diagnosis has been rather constant, with a distinct peak between 2-3 years and with 50% of the patients < 5 years of age (Gustafsson et al., 2000).

The treatment of ALL in the Nordic countries was unified from regional and national protocols to common Nordic treatment protocols for all risk groups, starting in the1980s. Over the next 10 years, the treatment was intensified, based on multidrug chemotherapy avoiding cranial irradiation. With the NOPHO ALL-92 treatment protocol, identical risk-adapted protocols were applied in all the five Nordic countries (Table 2). Children have been assigned to risk-groups on clinical and laboratory criteria at diagnosis. When the NOPHO ALL-92 treatment protocol was adopted, the number of children receiving prophylactic CNS radiation was reduced to less than 10%. The overall 5- year EFS has increased from 57% to 78% since the 1980s, and EFS rates have become almost identical for Denmark, Finland, Iceland, Norway, and Sweden, which emphasizes the advantage of uniform strategies for risk-adapted therapy (Gustafsson et al., 1998; Gustafsson et al., 2000; Saarinen-Pihkala et al., 2004). The results that has been achieved using the NOPHO ALL-92 protocol show that cranial irradiation may be substituted for by high-dose intravenous methotrexate for a vast majority of ALL patients while sustaining a low CNS relapse rate. The cumulative incidence of CNS relapse has been less than 5% since 1992. The first patients treated according to the NOPHO ALL-2000 protocol were diagnosed in February 2001. The probability of total 10-year survival of children who received antileukemia treatment according to NOPHO ALL-92 was 0.84 and the probability of 4-year survival of children who underwent the NOPHO ALL-2000 protocol was 0.89. Figure 1 shows the survival rates for the risk groups, and Figure 2 shows the NOPHO ALL-92 treatment protocol.

Table 2. Risk groups of children with ALL treated according to NOPHO protocols 1984-1998.

Risk group	Years	Age (years)	Criteria
Standard	1984-1998	2- < 10	WBC < 10 x 10^9/l, No high risk criteria
Intermediate		2- <10 or 1- <2 or ≥10	WBC 10- <50 x 10^9/l WBC < 50 x 10^9/l No high risk criteria
High	1984-1991	≥1	and at least one of the following criteria: WBC ≥50 x 10^9/l T cell leukemia Mediastinal mass CNS or testicular involvement t(9;22), t(4;11)
	1992-1998	≥1	and at least one of the following criteria: WBC ≥50 x 10^9/l Mediastinal mass CNS or testicular involvement t(9;22), 22q-, t(4;11) Slow response T cell leukemia
Very high		≥5	ant at least one of the following criteria: Lymphomatous features CNS involvement Slow response T cell leukemia with other HR criteria
Infants	1981-1998	<1	

ALL = acute lymphoblastic leukemia, NOPHO = Nordic Society of Pediatric Hematology and Oncology, WBC = white blood cell count, CNC = central nervous system, HR = high risk

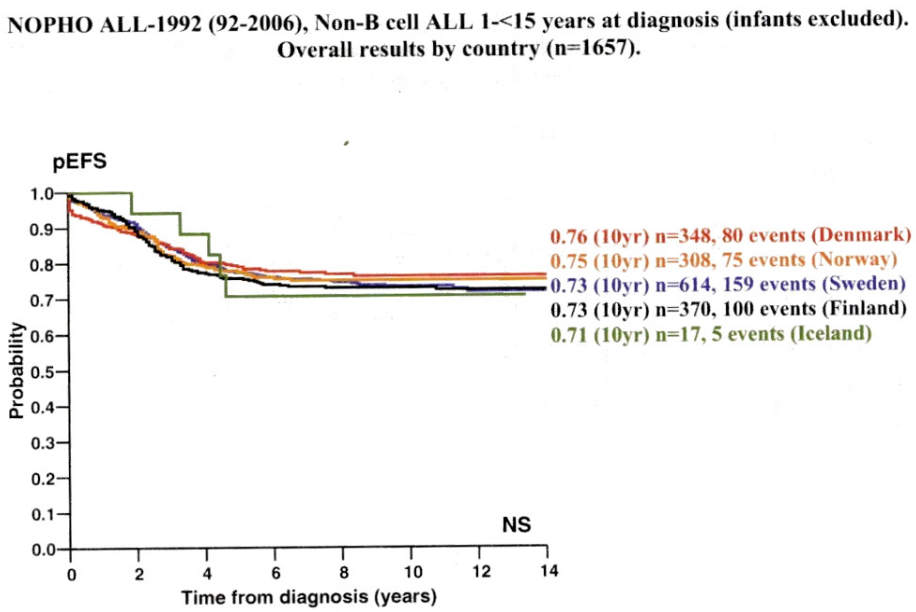

Figure 1. Event-free survival of children with leukemia treated using the NOPHO ALL-1992 protocol (NOPHO register, 2007).

Today, leukemia can be cured in many cases, but treatment-related complications threaten the outcome of these children. Still, 2-5% of the children die of other causes than disease relapse (Christensen et al., 2005a; Rubnitz et al., 2004; Slats et al., 2005). Infections are the main complications associated with treatment. Complications especially occur during intensive chemotherapy. Chemotherapy causes immunosuppression including neutropenia, increasing the risk of severe infections. Invasive procedures and indwelling catheters increase the risk of infections. Repeated admissions and the frequent use of broad-spectrum antibiotics make these immunosuppressed children susceptible to nosocomial infections. Infections are still the main cause of treatment-related death in children with ALL (Christensen et al., 2005a; Hann et al., 1997; Rubnitz et al., 2004; Slats et al., 2005). Children with ALL often suffer from suboptimal nutrition, which may also affect their immune status. ALL itself may lead to hemorrhages, thrombosis and complications of tumor lysis during therapy.

2.2 Acute myeloid leukemia

Acute myeloid leukemia (AML) accounts for 15-20% of childhood leukemias (Rajantie et al., 2003). Down's syndrome and some other genetic conditions predispose to AML. The morphology, immunophenotype, and immunohistochemial features of the blasts distinguish AML from ALL.

The overall survival or the per cent of the children with AML reaching total recovery is 60-65%, and it has been achieved with the intensification of treatment and with improved supportive care methods (Creutzig et al., 2001; Lie et al., 2005; Stevens et al., 1998). According to the best reported results, EFS or the time period without relapses or other complications of the diease or death is up to 50% of children with AML (Lie et al., 1992; Lie et al., 1996; Lie et al., 2003; Lie et al., 2005; Webb et al., 2001; Woods et al., 1996). In the Nordic countries, AML has been treated with shared protocols since 1984. With NOPHO-AML93, stem-cell transplantation was introduced for patients at high risk of relapse. The protocol used currently is NOPHO-AML 2004. Before 1984, very few children with AML were long-term survivors. The 5-year survival rate has increased from 38% to 65% (Lie et al., 2005). Event-free survival after AML treatment has been 48%. The results achieved with the NOPHO-AML protocols are comparable with those of other study groups in Europe and worldwide (Webb et al., 2001; Woods et al., 1996).

Mortality and relapses have decreased significantly with intensified AML treatment, but early and late treatment-related deaths have increased (Lehrnbecher et al., 2004; Riley et al., 1999; Slats et al., 2005). NOPHO-AML93 was a compromise with less toxic treatment and less treatment-related deaths with slightly increased rates of mortality due to the malignancy. The cumulative risk of relapse has not decreased significantly over these years. The main reason for the overall improvement of outcomes in children with AML has been the reduction of therapy-related mortality.

The frequency of early deaths and treatment-related deaths is high among children receiving anticancer treatment for AML. Recently, Riley et al reported a treatment-related mortality rate of 13.8% in British children undergoing therapy for AML (Riley et al., 1999). The result is comparable with those of the German AML-BMF 93 study and reports from the United States (Creutzig et al., 2001). New strategies for supportive has been reported which may help to improve the overall survival of these children

(Creutzig et al., 2001). To reduce the high incidence of early deaths and treatment-related mortality, early diagnosis and adequate treatment of complications are necessary. Prophylactic and therapeutic regimens for better treatment management of bleeding disorders and infectious complications are needed to improve the overall survival of children with AML.

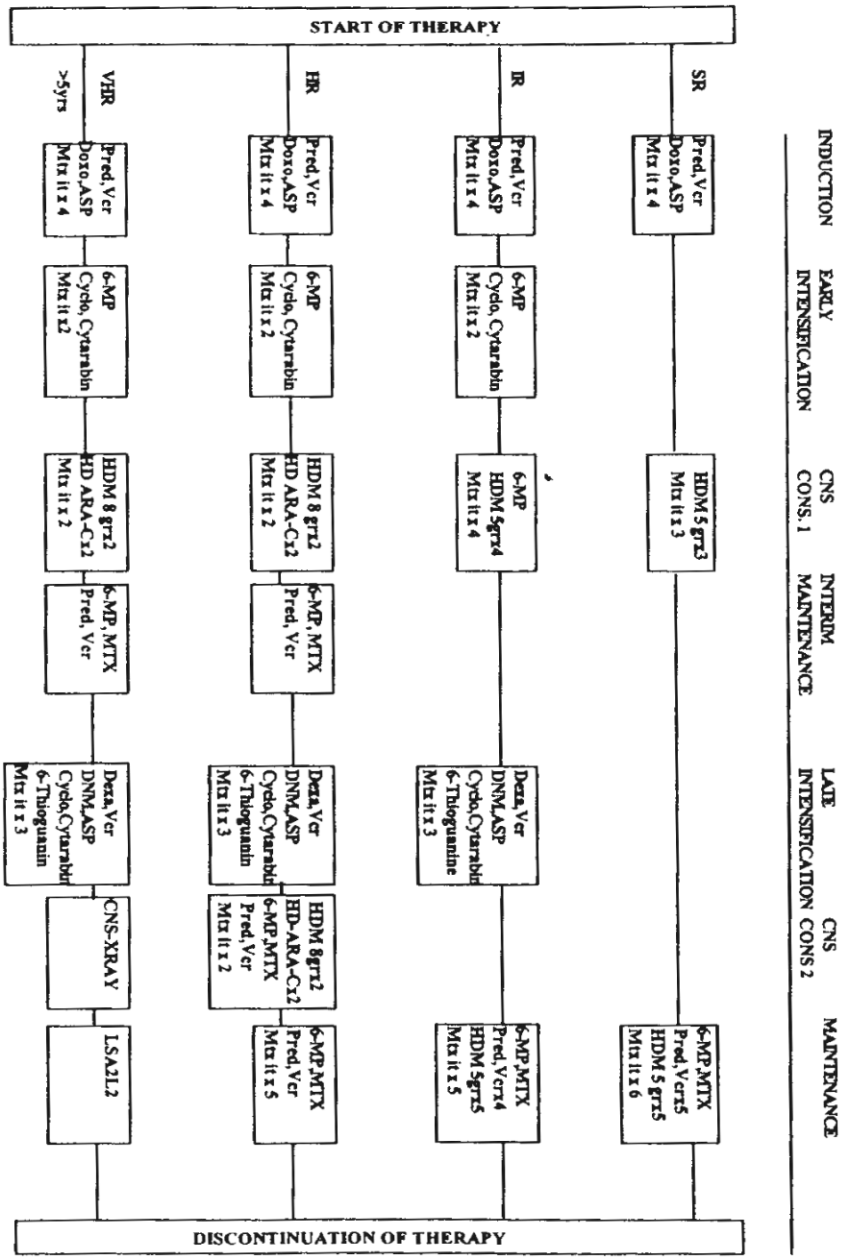

Figure 2. NOPHO ALL-92 treatment protocol.

2.3 Febrile infections in children with leukemia

2.3.1 Fever

Almost all children will experience a febrile reaction at some time. In an immunocompromised child, fever may be the only symptom of infection (Pizzo, 1993; Pizzo, 1999). Fever may also be associated with noninfectious causes, but patients with a severe infection usually have fever despite other predisposing factors. The release of proinflammatory cytokines such as interleukines and tumor necrosis factors from macrophages, lymphocytes, epithelial, or endothelial cells is a consequence of infection or inflammation (Mackowiak et al., 1997). These proinflammatory cytokines and endogenous pyrogenes originating from polymorphonuclear leucocytes are important factors generating fever in the host.

Recent studies of infectious complications in children with leukemia have shown that in about one fourth of chemotherapy cycles fever develops, and 60% of these febrile episodes are associated with clinically or microbiologically documented infections and 40% of the febrile episodes remain classified as fevers of unknown origin (FUO) (Castagnola et al., 2007; Katsimpardi et al., 2006; Lex et al., 2001).

2.3.2 Immunosuppression induced by treatment

The immunocompetent host has a number of complex, non-specific (innate) and specific (adaptive) immune defences against respiratory viruses. Rare defects of the innate immune system are associated with increased susceptibility to viral infections. These include deficiencies of complement, interferon system, NK cells, and phagocytic cells. The impairment of the T lymphocyte system leads to reduced viral clearance, resulting in intensified disease and, possibly, prolonged infection. Poor function of B lymphocytes makes the host susceptible to bacterial infections. Defects of the adaptive immune system can be either primary or secondary, and leukemia is considered to be a secondary cause. Recent studies have concluded that several factors of immunity are associated with increased susceptibility to recurrent infections, and the coexistence of two or more minor immune defects even increases the risk for recurrent febrile infections (Bossuyt et al., 2007; Jonsson et al., 2006). Bossuyt et al studied the immunologic profile of 55 children with recurrent respiratory infections and their 43 controls. They found a partial immune defect or a combination of partial immune defects in 89% of the study patients compared to 37% in controls. Reduced ability to produce specific antibodies to pneumococcal capsular polysaccharide antigens and the IgG2 subclass deficiency were the most common partial immune defects found in the study patients with occurrences of 19% and 20%, respectively. Children with recurrent respiratory infections also had a slightly increased prevalence of C4 deficiency.

The role of factors contributing to immunity in a child with leukemia is complex. The disease itself together with very intensive chemotherapy has an impact on the individual's immunity. The treatment of leukemia is divided roughly into 2 phases: induction and continuation. According to the Nordic protocols, a consolidation phase follows induction before the continuation phase, which again includes an early and a late intensification periods. Neutropenia and lymphopenia can be a consequence of treatment-related toxicity and may therefore vary in time and in degree throughout the course of anticancer treatment. However, febrile neutropenia periods mainly occur

during the induction phase. Children with leukemia are immunocompromised because of the cancer itself or therapy, or both.

Chemotherapy causes immunosuppression, and high-dose steroid treatment also affects immunity. Some cancer patients receive irradiation as part of their treatment protocol, and their immunity is markedly impaired for a time. Increased risk for infection is also associated with damaged skin or mucous membrane, indwelling catheters, and malnutrition. Invasive procedures cause a risk for infections in an immunosuppressed host. Long hospital stays and widespread use of antibiotics also increase the risk for infections.

The intensity of cancer treatment correlates to the severity of immunosuppression in children with leukemia. The immune system is damaged by variety of mechanism during anticancer therapy. The number and function of T and B lymphocytes and neutrophils are decreased as a consequence of anticancer treatment. Methotrexate inhibits DNA synthesis. Alkylating agents such as cyclophosphamide block DNA replication, and 6-mercaptopurine interferes with purine synthesis. All these agents inhibit the inflammatory response to confronted microbes (Caver et al., 1998; Lovat et al., 1993; Stahnke et al., 2001).

The role of neutropenia as increasing the risk of severe infections was already recognized in the 1960s (Wolk et al., 1977). The risk of infection is also affected by the reason of the neutropenia – whether it is caused by bone marrow suppression of by increased consumption of neutrophils because of infection or some other cause. Infections are categorized into neutropenic and non-neutropenic infections, and the treatment of infection is influenced by the neutrophil count. According to the WHO criteria, neutropenia means a neurophil count of $\leq 0.5 \times 10^9$ cells/l. A neutrophil count ≤ 0.5 or even $\leq 0.1 \times 10^9$/l causes a high risk of fever and infections (Cheng et al., 2004). Lymphocyte counts vary according to age but counts $\leq 1.4 \times 10^9$ cells/l are usually defined as lymphopenia (Comans-Bitter et al., 1997).

Recently, programmed cell death because of anticancer treatment has been reported to contribute to the onset of an adaptive immune system either directly or indirectly during infections (Albert et al., 1998; Apetoh et al., 2007; Gorla et al., 1994; Ronchetti et al., 1999; Winau et al., 2006). To current knowledge, apoptosis has been a silent cell death without any effect on the innate or adaptive immune system. Furthermore, some types of tumor cell death can also induce an antitumor immune response, improving the success rates of some anticancer treatment protocols (Casares et al., 2005).

2.3.3 Occurrence

Advances in the intensified treatment of childhood leukemia have been associated with the emergence of infectious complications. The occurrence of febrile episodes has varied from 2.6 to 4.2 episodes per patient year at risk during the anticancer treatment (Katsimpardi et al., 2006; Lehrnbecher et al., 2004; Lex et al., 2001; Möttönen et al., 1995; Rahiala et al., 1998). In general, children with hematological malignancies have more infectious complications than those with solid tumors (Auletta et al., 1999; Madani., 2000). The treatment of solid tumors has also now been intensified. Kocak et al recently reported similar incidences and outcomes of infections in children with solid tumors and those with leukemia (Kocak et al., 2002). In the 1980s, Miser et al reported that the rate of sepsis in children with leukemia and lymphoma was 9.1 per 100 patient years compared to 0.6 episodes per 100 patient years in children with solid tumors

(Miser et al., 1981). The result was explained with a difference in the intensity of treatment of the underlying malignancy. In the same decade, Kosmidis et al conducted a 28-month prospective study of 54 leukemic children to determine the incidence and type of infections associated with febrile episodes during anticancer treatment. They reported that fever was caused by infections in 84 of 119 febrile episodes (71%) (Kosmidis et al., 1980). All severe bacterial infections were found to occur in children with absolute granulocyte counts lower than 0.5 x 10^9/l.

2.3.4 Etiology, clinical profile and outcome

Pizzo et al analyzed 1001 fever episodes in pediatric cancer patients in the early 1980s, and found infectious etiology in 17% of non-neutropenic febrile episodes (Table 2). (Pizzo et al., 1982). In contrast, 52% of febrile episodes of children with neutropenia were associated with a discernible infection. Since then, a focus or microbiological cause of infection of children with cancer has been identified in 30% to 40% of cases.

Rahiala et al analyzed 245 febrile episodes retrospectively in 59 ALL children throughout the course of anticancer treatment. Half of the febrile episodes occurred in the presence of neutropenia (Rahiala et al., 1998). In 33% of cases, the focus of infection or microbiological etiology of infection could be found. The incidence of bacteremia in neutropenic febrile episodes was 28%, and 36% of the episodes remained classified as FUO episodes. Upper respiratory tract infections were the most common causes of fever accounting for 55% of febrile episodes when neutrophil counts were normal. Lex et al studied 313 febrile episodes in 112 children with ALL and T cell lymphoma and reported that 60% of the episodes had clinically or microbiolocically documented etiology (Lex et al., 2001). Gram-positive pathogens showed a higher incidence than gram-negative or fungal ones. The authors concluded that the risk of infection during anticancer treatment may not only be influenced by neutropenia, but drug-specific effects and individual factors may also be important contributors to febrile episodes in these children. Ek et al studied the role of cytarabine as a cause of fever in children with cancer. They reported fever in 67% of chemotherapy cycles including high-dose cytarabine (Ek et al., 2005).

Lehrbecher et al studied 855 infectious complications in 304 children with AML treated using the AML-BFM93 protocol. Of the complications, FUO accounted for 61%, clinically documented infections for 7%, and microbiologically documented infections for 32%, of which 74% were associated with neutropenia. Neutropenia was associated with 74% of these complications. There were 228 (27%) cases of blood culture positive infections. Gram-positive bacteria were the most common causative bacteria. Invasive fungal infections were detected in 15 (5%) patients (Lehrnbecher et al., 2004) .

Infections remain a major cause of therapy-related morbidity and death in children with cancer. Despite more effective supportive care facilities, 2-5% of children with ALL still die of other causes than relapse. In a study conducted in the Nordic countries including 1652 children with ALL under 15 years of age, Christensen et al reported that 56 of the children died, 1% during induction therapy and 2% during remission. Infection was the major cause of death in 38 of 56 cases (68%) (Christensen et al., 2005a). Slats et al analyzed causes of death, other than persistent leukemia or relapsed disease, in 875 children with ALL and 229 children with AML (Slats et al., 2005). The overall mortality rate was 2.6% in children with ALL and 19.2% in children with AML. The main cause of

early death was hemorrhage and infection in remission. Recently, Rubnitz et al analyzed the causes of and risk factors for death unrelated to an underlying malignancy in 1011 children with ALL and 260 children with AML treated between 1983 and 2002 (Rubnitz et al., 2004). They reported an estimated 10-year cumulative incidence of death of 2.9% among patients with ALL. Among children with AML, the estimated 5-year cumulative incidence of death was 7.6%. Infection was the most common cause of death. About 80% of deaths during the anticancer treatment were due to infections. Invasive fungal infections were the most common cause of these deaths during induction therapy and after stem cell transplantation but were rare in the maintenance phase.

Table 3. Risk factors for fever and causes of fever in patients with cancer (Pizzo PA, N Eng J Med 1999) (Pizzo, 1999).

Cancer	Main risk factors	Causes of fever
Low risk	Underlying disease, therapy, neutropenia ≤ 10 days, altered mucosal immunity, indwelling catheter	Fever of unknown origin Bacteria: Gram-positive or gram-negative bacteria Viruses: Respiratory viruses or herpes viruses
High risk	Underlying disease, therapy, neutropenia ≥ 10 days, altered mucosal immunity, defects in humoral or cellular immunity, indwelling catheter	Fever of unknown origin Bacteria: Gram-positive or gram-negative aerobes, anaerobes at sites of mixed infection Viruses: Respiratory syncytial virus, parainfluenza viruses, adenoviruses, herpes simplex virus, cytomegalovirus Fungi: Candida, aspergillus, Cryptococcus, trichosporon, fusarium, phaeohyphomycosis Other: *Pneumocystis carinii,* toxoplasma

2.3.4.1 Respiratory viral infections

Community-acquired respiratory viruses are the main pathogens associated with significant morbidity and mortality among otherwise healthy subjects. In immunocompromised hosts, bacterial and fungal pathogens have traditionally been considered the most important agents often causing lower respiratory tract disease. The primary viruses have included herpesviruses, adenovirus, and, particularly, cytomegalovirus (CMV). Herpesviruses, such as CMV, typically cause a infection during childhood and later become reactivated when host immune defenses are weakened secondary to malignancy or chemotherapy. A group of RNA viruses including rhinovirus, enteroviruses, influenza viruses, respiratory syncytial virus (RSV), and parainfluenza viruses are associated with community-acquired respiratory infections. Table 4 shows the most important respiratory viruses discovered since 1933. Recently, community-acquired respiratory viruses have been recognized as causes of respiratory disease also in immunocompromised patients.

Respiratory infections in children with cancer have accounted for one third of febrile periods during anticancer treatment (Arola et al., 1995; Kosmidis et al., 1980; Möttönen et al., 1995; Rahiala et al., 1998). Only a few studies have been carried out in children with cancer, and most of them have used limited number of detection methods to search for viruses (Table 5). Two Finnish studies from1995 found rhinovirus, RSV, parainfluenza viruses, enteroviruses, adenovirus, and influenza viruses as the most frequent causes of respiratory disease in children with leukemia (other than bone marrow transplant recipients) (Arola et al., 1995; Möttönen et al., 1995). A recent study confirmed the above viral findings but reported lower frequencies, probably as a result of methodologic differences (Christensen et al., 2005a). This Danish study detected viruses in oral washes and found respiratory viruses in 11% of febrile episodes compared to the 37% of the Finnish study, which used nasopharyngeal aspirates for viral studies.

Over the past decade, many studies have reported respiratory viral infections in adult bone marrow transplant (BMT) patients, showing that these patients have the highest risk of developing life-threatening respiratory disease (Hicks et al., 2003; Ison, 2007; Ljungman et al., 1989; Wade., 2006; Whimbey et al., 1996) (See Table 6). In adults, the severity of respiratory viral diseases may vary from mild to life-threatening (Ljungman et al., 1989). Despite advances in microbiologic detection methods, 30-60% of immunosuppressed adult patients with lower respiratory tract disease remain without any definite diagnosis (Couch et al., 1997; Englund, 2001; Whimbey et al., 1997).

Table 4. The most important respiratory viruses.

Virus	Year of discovery
Influenza A virus	1933
Influenza B virus	1940
Influenza C virus	1946
Adenovirus	1953
Parainfluenza types 1, 2, 3	1956
Rhinovirus	1956
Respiratory syncytial virus	1957
Enteroviruses	1958
Parainfluenza type 4	1964
Coronaviruses OC43 and 229E	1965
Human herpes virus 6	1986
Human metapneumovirus	2001
SARS coronavirus	2003
Coronavirus NL63	2004
Human bocavirus	2005
Coronavirus HKU1	2005

Rhinovirus

Human rhinoviruses, members of the *Picornaviridae* family, have more than 100 serotypes. Picornaviruses consist of a single-stranded RNA genome. At least 78 rhinovirus serotypes recognize the intercellular adhesion molecule (ICAM-1) as their

receptor, and the other rhinoviruses, the minor receptor group, recognize the low density lipoprotein receptor (Hayden., 2004). This is important to understand, because one treatment strategy has involved blockage of rhinovirus attachment to ICAM-1 receptor by a soluble ICAM-1. In immunocompetent hosts, rhinovirus clearance is rapid and viral shedding limited to an average of 10 days.

Rhinovirus diagnostics was based on virus culture for 40 years. This was a serious problem, because rhinovirus culturing requires special equipment and expertise and may be time-consuming. This may explain the limited number of associated studies, e.g. of children with cancer. The development PCR techniques in the 1990s has made rhinovirus diagnostics widely available (Bruce et al., 1990; Hyypiä et al., 1989; Vuorinen et al., 2003).

Rhinovirus infections occur throughout the year, but clear fall and spring outbreaks can be indentified (Arruda et al., 1997; Mäkelä et al., 1998). It is well understood that rhinovirus is the most common causative agent of infections worldwide and it is associated with many clinical illnesses (Arruda et al., 1997; Hendley et al., 1969; Mäkelä et al., 1998). Rhinovirus is the most common cause of upper respiratory tract infection, the common cold. Mäkelä et al found viral etiology in 69% of 200 young adults with recent-onset common cold, and rhinovirus was detected in 52% of the patients (Mäkelä et al., 1998). Importantly, during the fall months it caused 80-90% of respiratory infections (Arruda et al., 1997; Papadopoulos, 2004). Acute otitis media and sinusitis are common complications of rhinovirus infections. Recently, Ruohola et al detected rhinovirus in 31% of 79 middle ear fluid samples of children with acute otorrhoea (Ruohola et al., 2006). There is new evidence that rhinoviruses may also cause lower respiratory tract diseases such as bronchiolitis and pneumonia (Fraenkel et al., 1995; Gern et al., 1997; Hayden, 2004; Jartti et al., 2004b; Papadopoulos et al., 1999). Recently, viremia caused by rhinovirus has been reported to occur in normal children in the early course of acute illness (Xatzipsalti et al., 2005). On the hand, it must be stressed that rhinovirus is the most common respiratory virus also found in asymptomatic subjects, especially in young children. The clinical role of asymptomatic rhinovirus carriage is not fully understood (Jartti et al., 2004a; van Gageldonk-Lafeber et al., 2007).

The role of rhinovirus among immunosuppressed patients is not well characterized. It is surprising that Pizzo et al did not include this most common respiratory virus, rhinovirus, in their extensive studies from the 1990s of children with cancer. In 1987, Long et al were the first to report that rhinovirus is the most common cause of respiratory viral disease in children with leukemia (Long et al., 1987) (Table 4). They cultured rhinovirus from 45 of 204 febrile episodes in children with cancer. Arola et al identified rhinovirus infection by culture in 13 immunosuppressed children, who all had an uneventful recovery (Arola et al., 1995). In an extensive study by Bowden et al., rhinovirus accounted for 25% of respiratory infections in adult bone marrow transplant (BMT) recipients and was the third most common viral agent after RSV and parainfluenza viruses (Bowden, 1997). Fatal lower respiratory tract infections have been reported in adult hematologic stem cell transplantation (HSCT) recipients (Gutman et al., 2007). Ghosh et al studied the clinical course and outcome of 22 adult myelosuppressed patients with rhinovirus infection. Most of the illnesses remained as upper respiratory tract infections, but severe pneumonia with a fatal outcome developed in 32% of the cases (Ghosh et al., 1999). In immunosuppressed patients, viral clearance may be delayed and prolonged rhinovirus shedding may occur (Kaiser et al., 2006; Malcolm et al., 2001).

Table 5. Prospective studies on respiratory viral infections in children with cancer.

Author year	Country	Number of children	Mean age Years	Number of febrile episodes	Follow-up time Years	Viral detection methods	Occurrence of respiratory viruses	RSV	Adeno-virus	Influenza A/B	Para influenza 1,2 3	Rhino-virus	Entero viruses	HSV	CMV
Long et al 1987	U.K	200	5.4	204	5	Culture, AG, EM	NG*	25	0	10	15	45	NS	0	0
Wood et al 1985	USA	150	6.4	272	1.4	Culture, AG, serology	29%	3	13	6	10	8	5	24	10
Möttönen et al 1995	Finland	15	7.3	164	2	Culture, AG, serology	26%	1	5	3	16	1	8	7	2
Arola et al 1995	Finland	32	8.1	75	1.4	Culture, AG, serology	37%	6	4	4	5	13	NS	NS	NS
Uys et al 2000	South Africa	14	7.4	22	1	Culture	32%	0	1	0	0	NS	0	4	2
El-Mahal-lawy et al 2005	Egypty	30	8.5	13	1	AG, serology	43%	1	1	10	0	NS	NS	NS	NS
Christensen et al 2005	Denmark	66	NG	206	1	PCR	8% **	4	0	1	1	7	1	2	NS
Täger et al 2006	Chile	25	< 15	44	NG	AG	25% #	3	2	3	2	NS	NS	NS	NS

AG = antigen detection, EM = electron microscopy, RSV = respiratory syncytial virus, HSV = herpes simplex virus, CMV = cytomegalovirus, NS = not studied, NG = not given

Table 6. Studies on respiratory viral infections in adults with stem cell transplantation.

Author year	Country	Number of patients	Number of febrile episodes	Follow-up time Years	Viral detection methods	Occurrence of respiratory viruses	RSV	Adeno-virus	Influ enza A/B	Para influ enza 1,2 3	Rhino-virus	Entero viruses	HSV	CMV
Bowden et al 1997	USA	3680	127#	8 y	Culture, AG	NG	44	NG	14	38	31	NG	NG	NG
Ljungman et al 2001	37 centres in Europe	1973	93#	2.5 y	Culture, AG	2%	42	NG	39	7	2	NG	NG	NG
Chakrabarti et al 2002	UK	83	35	3.2 y	Culture, AG	30%*	13	NG	5	17	NG	NG	NG	NG
Hassan et al 2003	UK	626	29	5 y	Culture, AG	4.3%	8	NG	5	4	11	2	NG	NG
Roghmann et al 2003	USA	62	37#	0.3 y	Culture, AG, PCR	59%	11	NG	4	5	3**	3*	NG	NG
Van Kraaij et al 2005	Netherlands	72	52#	0.5 y	Culture, PCR	63%	3	1	2	2	16	1	NG	NG
Chemaly et al 2006	USA	306	343#	2 y	Culture, AG	NS	107	NG	112	92	32*	32*	NG	NG

# Only respiratory tract infections were included in the study , * Percentage of patients, **The results were given as picornaviruses.
AG = antigen detection, RSV = respiratory syncytial virus, HSV = herpes simplex virus, CMV = cytomegalovirus, NS = not studied, NG = not given

Respiratory syncytial virus

RSV is a single-stranded RNA virus of the *Paramyxoviridae* family. The genome of RSV encodes eight structural and two non-structural proteins. Immunologically important are the F and G proteins inducing protective neutralizing antibodies. However, immune protection after RSV infection is incomplete and early reinfections occur. RSV infections occur worldwide and have an epidemic personality, occuring predominantly during the winter months in temperate climates. (Peret et al., 2000). In Finland, RSV peaks in odd numbered years in double-humped peaks during the fall and spring (Waris, 1991).

RSV infection can be diagnosed by virus culture, by identification of viral antigens, or antibodies. The recent use of RT-PCR for diagnosis of RSV infections has shown high rates of specificity and sensitivity (Freymuth et al., 1995; Jartti et al., 2004b).

RSV has been considered the most important cause of acute respiratory tract viral infections in infants. Primary infections are symptomatic, and all children are infected once until the age of 2 years. Half of them already experience a reinfection (Juven et al., 2000; Openshaw et al., 2005; Virkki et al., 2002). RSV has been reported to cause 50-90% of bronchiolitis cases, 5-40% of pneumonia and acute bronchitis cases, and 10% of croup cases. RSV accounts for half of pneumonias in children < 2 years of age. Worldwide, RSV is estimated to cause 600 000 – 1 million deaths per year among children < 5 years of age (Mäkelä et al., 1994; Openshaw et al., 2005).

Most data on RSV infections in immunosuppressed hosts derive from trials of adult HSCT recipients (Chemaly et al., 2006; Torres et al., 2007; Whimbey et al., 1996). These studies indicate that RSV infection is frequently severe, depending on the degree of the host's immunosuppression, the type of virus, the presence or absence of other infections, and, in HSCT patients, the time of the infection in relation to transplantation. RSV infections may have a mortality rate of 30 to 100% (Whimbey et al., 2000). The timing of the treatment, ribavirin and intravenous immunoglobulin, affects the outcome of the infection in an immunosuppressed host. The surveys by Arola et al and Möttönen et al indicate that RSV is also frequently detected in febrile children with leukemia with no fatal outcomes (Arola et al., 1995; Möttönen et al., 1995)

Adenovirus

Adenovirus is a non-enveloped DNA virus with 51 human serotypes. Adenoviruses have been classified into six species (subgenera) A-F on the basis of antigenicity and other biologic properties. C adenoviruses are endemic and responsible for about 60% of all human adenovirus infections (Echavarria et al., 2006; Larranaga et al., 2000). After primary infection, C viruses may be shed in feces for months or even years. They are able to establish a lifelong, asymptomatic, and persistent infection (Kojaoghlanian et al., 2003). Probable places of persistence are the tonsils and adenoids. Adenovirus infections occur worldwide as epidemic, endemic, and sporadic infections.

Adenoviruses have been cultured from the stools, throat swabs, nasophragyeal aspirates, conjunctival swabs, urine, cerebrospinal fluid, blood, and a variety of biopsy specimens. Adenovirus can also be detected by virus antigen detection, PCR, and serology from paired serum samples. Quantitative RT-PCR used for adenovirus in the

plasma may be a useful tool for identification of immunosuppressed patients at risk for viral invasive disease (Erard et al., 2007).

Primary infection commonly occurs early in life. The usual signs of adenovirus infection are fever, nasal congestion, coryza, pharyngitis, and cough, with or without otitis media. Adenoviral infection can also cause gastroenteritis and epidemic keratoconjunctivitis. In infants and children, adenovirus infections primarily occur between 6 months and 5 years of age. Premature infants are at high risk of developing disseminated neonatal adenovirus infection with pneumonia and high fatality (Larranaga et al., 2000; Larranaga et al., 2007; Lin et al., 2004; Rieger-Fackeldey et al., 2000).

Adenovirus can cause four different syndromes in immunocompromised hosts; gastrointestinal infections with or without hepatitis, urinary tract disease, pulmonary infections, or disseminated infections with multiorgan failure and a potentially fatal outcome (Blanke et al., 1995; Munoz et al., 1998; Shields et al., 1985). The incidence of adenovirus infection in patients receiving BMT varies from 4.9% to 20.9%, and mortality rates may be as high as 60% (Bruno et al., 2003; Chakrabarti., 2007; Hierholzer., 1992; Howard et al., 1999). Higher incidence of severe adenoviral infections have been reported among patients with T cell depleted stem cell transplantations, among patients with graft versus host disease (GvHD), and among patients receiving total body irradiation (Baldwin et al., 2000; Bruno et al., 2003; Erard et al., 2007; Hale et al., 1999). Only a few reports have been published on adenovirus infections among pediatric non-HSCT recipients. Arola et al and Möttönen et al. reported adenovirus as one of the most frequently detected viruses in children with leukemia receiving chemotherapy (Arola et al., 1995; Möttönen et al., 1995). According to these studies, children with leukemia all had a favorable outcome of adenovirus infections.

Parainfluenza viruses

Parainfluenza viruses are single-stranded RNA viruses of the *Paramyxoviridae* family. The two large envelope glykoproteins, fusion protein and hemagglutinin-neuraminidase, are the most important and responsible for antibody neutralization (Hu et al., 1992; Moscona et al., 1991). They have four major serotypes, distinct in their epidemiologic behaviour. Parainfluenza viruses are endemic throughout the year, but outbreaks also occur in winter and spring (Templeton et al., 2004b; Vainionpää et al., 1994).

Parainfluenza viruses can be identified by virus culture and antigen detection. The PCR technique is used to detect viral RNA (Karron et al., 1994). Serology is an indirect method for the detection of parainfluenza virus infection but it is not much used in clinical practice.

Croup or laryngitis is the main clinical manifestation of parainfluenza virus infections in children (Knott et al., 1994; Korppi et al., 1988). Most patients experience cough and rhinitis two-three days before the onset of disease, which rarely manifests as high fever (Hall, 2001; Henrickson et al., 2004). In the community-acquired pneumonias of children, parainfluenza virus type 1, 2 and 3 are common viral pathogens, causing 10% of cases (Farha et al., 2005; Juven et al., 2000; Michelow et al., 2004). Almost all

children face these viruses within their first year of life, but immunity after infection is incomplete and reinfections may occur later in life (Glezen et al., 1984; Knott et al., 1994).

In adult HSCT recipients, parainfluenza viruses, especially type 3, have also been reported to cause serious morbidity and mortality (Nichols et al., 2001). Parainfluenza virus induced pneumonia is associated with a mortality rate of up to 40% in adult HSCT patients (Lewis et al., 1996). Lujan-Zilbermann et al retrospectively analyzed 274 children undergoing HSCT and found parainfluenza type 3 the most frequent cause of respiratory infection after transplantation (Lujan-Zilbermann et al., 2001). Of the 274 children, 12 had a respiratory infection caused by parainfluenza type 3. Three of the 12 children had pneumonia and one died. A 5-year old boy with ALL and HSCT had a persistent parainfluenza type 3 virus infection detected only by RT-PCR both before and after transplantation (Templeton et al., 2004a).

Influenza viruses

Influenza viruses are enveloped RNA viruses of the *Orthomyxoviridae* family. They are classified into types A, B, and C. Influenza A and B viral envelopes contain two glycoproteins, a hemagglutinin (H) and a neuraminidase (N). Influenza viruses show high genetic variability (Ghedin et al., 2005; Johansson et al., 2007). This is particularly evident in influenza A viruses, which have 16 hemagglutinin and 9 neuraminidase subtypes. All these subtypes occur in birds, whereas only some of them occur in humans or other mammals. Epidemics of influenza virus infections occur every year, and worldwide epidemids or pandemics develop occasionally (Taubenberger et al., 2005). Usually, 10-20% of the population is infected by influenza A virus during annual epidemics.

Virus can be cultured from nose, nasopharyngeal and throat secretions within the first days of infection. Rapid diagnosis is made possible by commercial antigen detection tests. Viral RNA can be detected by PCR in clinical specimens (Uryvaev et al., 1990; Zhang et al., 1991). Serology can also be used for diagnostics; antibody titres increase rapidly in the course of infection and decrease to low levels within a couple of weeks.

Many experts consider influenza the most important virus as a cause respiratory disease in humans (Molinari et al., 2007; Monto, 1994; Mäkelä et al., 1998). Early symptoms include fever, chills, headache, sore throat, dry cough, myalgias, anorexia, and malaise. Fever of 38-40^{o}C peaking within 24 hours of onset of disease and lasting for 1-5 days is common. The complications include bacterial sinusitis and otitis media (Heikkinen et al., 2004; Heikkinen, 2006; Peltola et al., 2006c; Ruohola et al., 2006). Lower respiratory tract involvement may also occur, causing exacerbation of asthma, bronchitis, and pneumonia (Hutchinson et al., 2007; Jennings et al., 2007; Joao Silva et al., 2007; Wolf et al., 2006). Pneumonia in patients with influenza virus infection can be primary viral pneumonia or secondary bacterial pneumonia.

Most studies of influenza virus infection in immunosuppressed patients have been conducted in adult HSCT recipients. Elting et al studied the epidemiology of influenza A virus in 294 adult patients with leukemia (Elting et al., 1995). Most influenza infections were found to be nosocomial. The patients presented with symptoms of upper respiratory disease, but development of pneumonia was common with a possible fatal outcome. Data on influenza virus infections of children with cancer are

limited (Chisholm et al., 2001; Kempe et al., 1989). Kempe et al reported that the incidence of influenza virus infection was higher in children with cancer compared to their siblings, but no significant difference in the duration of symptoms was found (Kempe et al., 1989). Children with cancer were hospitalized more often than healthy siblings with influenza infection. According to Kempe et al the clinical complications occurred too infrequently to make any further analyzes about outcome of children with cancer and influenza infection. Complications like upper and lower respiratory tract infections and occasional deaths have been reported in children with cancer and influenza infection, specially in highly immunosuppressed children with bone marrow transplantation (Feldman et al., 1977; Kempe et al., 1989; Potter et al., 1991) . Mortality is particularly high, up to 43% in adult bone marrow transplant recipients with influenza infection (Whimbey et al., 1996; Whimbey et al., 2000) . The frequency of pneumonia has been up to 80% in these adult bone marrow transplant recipients and adults with leukemia (Whimbey et al., 1997; Whimbey et al., 2000) . Chisholm et al found a significant response to immunization also in children with cancer, which supports annual influenza immunization in pediatric patients undergoing treatment for malignancies (Chisholm et al., 2005).

Enteroviruses

The enteroviruses belong to the *Picornaviridae* family. Human enteroviruses include polioviruses, coxackieviruses, echoviruses, and newly discovered enteroviruses designated as “numbered enteroviruses”. Enteroviruses are nonenveloped RNA viruses, and over 70 enterovirus serotypes have been recognized (Bourlet et al., 2003; Caro et al., 2001; Smura et al., 2007). Their normal site of replication lies in the intestinal tract. However, in some cases, the virus spreads to other organs, causing illness. The common role of enteroviruses as a cause of respiratory infections is now better understood (Ruohola A et al, unpublished). Enteroviruses usually occur during summer and fall (Khetsuriani et al., 2006; Weber et al., 1991).

Enteroviruses can be cultured from respiratory samples, blood, cerebrospinal fluid, urine, stools, and solid tissue. Viral culture has been replaced by the sensitive and specific PCR test (Vuorinen et al., 2003). Serological tests are not very useful.

Enteroviruses mainly induce acute respiratory infection, aseptic meningitis, encephalitis, myocarditis, rash, and conjunctivitis. Enterovirus infection can be particularly severe in neonates. Several enteroviruses have been associated with mild upper respiratory tract disease with rhinitis. These serotypes commonly cause upper respiratory disease in young children, but bronchiolitis and pneumonia may also occur. Recently, Rotbart et al reported that 13% of children with respiratory infections caused by enteroviruses presented with pneumonia. (Chang et al., 1999; Jartti et al., 2004b; Rotbart et al., 2001; Yen et al., 2007).

Immunosuppressed patients are particularly susceptible to systemic infections caused by enteroviruses. Especially, coxsackieviruses have been associated with enchephalitis, myocarditis, and disseminated disease in pediatric HSCT recipients (Aquino et al., 1996; Fischmeister et al., 2000; Moschovi et al., 2007). Moschovi et al conducted a 5-year study of enteroviral infections in children with cancer. They reported a fatality rate of 15%. Moreover, 20% of the patients had severe manifestations like enchephalitis and cardiac involvements.

Human metapneumovirus

In 2001, van den Hoogen et al reported a new viral agent causing respiratory disease (van den Hoogen et al., 2001). This new RNA virus, huma metapneumovirus, belongs to the *Paramyxoviridae* family. hMPV occurs worldwide (Ebihara et al., 2003; Wolf et al., 2003; van den Hoogen et al., 2001).

hMPV has been associated with upper and lower respiratory tract disease both in children and in adults (Ebihara et al., 2003; Wolf et al., 2003; van den Hoogen et al., 2001). It may be responsible for 5 to 15% of respiratory infections in children and is second to RSV as a cause of bronchiolitis in early childhood (Williams et al., 2004). hMPV is found less frequently in hospitalized children with respiratory disease than RSV, and the clinical course may be milder. About 55% of children < 2 years of age and nearly 100% of children 5-10 years of age have positive serology for hMPV. hMPV infection can lead to pneumonia, especially in patients with an underlying disease, like asthma, or prematurity (Hamelin et al., 2005; Ulloa-Gutierrez et al., 2004).

The clinical features of hMPV in immunosuppressed hosts are not well characterized (Boeckh et al., 2005; Cane et al., 2003; Debiaggi et al., 2006). hMPV has been reported in children with human immunodeficiency virus infection (Madhi et al., 2003). In a prospective 4-year stydy of adults with hematological malignancies and respiratory infections, 9% were tested positive for hMPV. Most patients were BMT recipients. The most common symptoms were related to upper respiratory tract infections, but mortality with concomitant bacterial infections was also reported (Williams et al., 2005). hMPV is capable of causing fatal disease to immunocompromised hosts. There are four reports of fatal infection in patients with cancer, three in adult bone marrow transplant recipients and one in 17-month-old child with relapsed leukemia (Cane et al., 2003; Englund et al., 2006; Muller et al., 2007; Pelletier et al., 2002) . Further long-term prospective studies are needed to better characterize the severity and outcome of hMPV infection in children and adults with cancer.

Human bocavirus

HBoV is a novel virus of the *Parvoviridae* family. It was first identified in 2005 by Allander et al from the respiratory samples of children with lower respiratory tract disease (Allander et al., 2001). HBoV occurs worldwide and throughout the year, peaking from March to May.

HBoV is detected by the PCR technique, and it can not be cultured. Recently developed serological tests strongly suggest that at least high HBoV copy numbered cases have an acute infection (Kantola K et al., 2008). A Japanese study reported a seroprevalence of 71% in a population aged from 0 month to 41 years (Endo et al., 2007).

The most common symptoms seen in HBoV-positive patients are cough, rhinitis, and fever, but gastrointestinal symptoms and rash have also been reported (Arnold et al., 2006). HBoV has usually been associated with bronchiolitis and pneumonia, but pertussis-like cough and diarrhea have also been reported (Arnold et al., 2006; Lau et al., 2007; Lee et al., 2007). The occurrence of HBoV has been reported to be higher in children than in the adult population, varying from 3.1% to 5.7% in children below 3 years of age. It is the third most common etiologic agent of respiratory disease of children of that age (Arnold et al., 2006; Foulongne et al., 2006). In a large study from

Canada, where respiratory specimens were collected from both children and adults with acute respiratory infection, the incidence of HBoV was 1.5% (Bastien et al., 2006). Weissbrich et al reported a high incidence of 10.3% in hospitalized infants and children with acute respiratory tract disease (Weissbrich et al., 2006). These reports suggest that HBoV may cause respiratory illness more often in infants and children than in adults.

The association of HBoV and respiratory disease has been questioned because of the high rate of coinfections with other respiratory viruses. Weissbrich et al reported a 39% incidence and Sloots et al a 56% incidence of dual infections in pediatric populations (Sloots et al., 2006; Weissbrich et al., 2006). HBoV as sole cause of respiratory illness was reported by Kesebir et al from the USA (Kesebir et al., 2006). Recently, HBoV was found in 19% of 259 children hospitalized for acute expiratory wheezing. In 12 cases (5%), HBoV was the only virus detected, and in 10 of these cases high copy numbers suggested primary infection. HBoV was also detected in the serum of the patients (Allander et al., 2007b). Importantly, HBoV is very rare in asymptomatic subjects (Allander et al., 2007b; Fry et al., 2007; Kesebir et al., 2006).

Recently, HBoV infections in adult and pediatric immunocompromised patients with previous organ transplants or patients with HIV infection have been reported from large respiratory disease surveys, including one disseminated bocavirus infection in an adult HSCT (Arnold et al., 2006; Manning et al., 2006; Schenk et al., 2007; Smuts et al., 2006). One adult cancer patient with severe atypical pneumonia associated with HBoV has been reported (Kupfer et al., 2006). Further studies are needed to determine the clinical profiles and significance of this new respiratory virus in children with cancer.

Coronaviruses

Coronaviruses are enveloped RNA viruses which have the largest genomes of all RNA viruses. Four serogroups have been distinguished: group 1 with serotype 229E and group 2 with serotype OC43. Since 2003, three new coronaviruses have been identified (SARS, NL63, HKU1) (Pyrc et al., 2007; Woo et al., 2005a; van der Hoek et al., 2004). Unlike SARS coronavirus, the other two new coronaviruses have probably been circulating worldwide in humans for a long time.

Non-SARS coronaviruses are difficult to culture. Human coronaviruses can be detected by antigen detection. Currently, diagnosis is mainly based on PCR techniques with nasopharygeal mucus samples and stool samples. IgM antibodies can be found within the first 7 days of infection. IgG seroconversion may be delayed for up to 8 weeks and IgG does not persist (Mo et al., 2005; Woo et al., 2005b).

Human coronaviruses mainly cause colds. The clinical course of disease is indistinguishable from rhinovirus infection. Occasionally, severe lower respiratory tract infections develop (McIntosh et al., 1974; McIntosh et al., 1993; McIntosh et al., 1993).

In November 2002, SARS was reported from China as a cause of contagious, potentially lethal cause of atypical pneumonia. By June 2003, worldwide 8447 cases of this illness from more than 30 countries were recorded with more than 800 deaths (mortality 9.5%). In younger children, SARS often caused a mild respiratory disease. Coronavirus NL63 and coronavirus HKU1 were found in subjects with acute respiratory disease. Coronavirus NL63 has been identified in 1-10% of respiratory tract infections

in children (Pyrc et al., 2007; Woo et al., 2005a; van der Hoek et al., 2004). Coronavirus HKU1 DNA has also been identified in the serum of healthy donors. Estimatedly, human coronaviruses account for up to 30% of respiratory infections in the general population (Hamelin et al., 2005).

Data on coronavirus infections in immunosuppressed patients are scarce and mainly based on anecdotal case reports in both children and adults (Folz et al., 1999; Simon et al., 2007; van Kraaij et al., 2005). Simon et al and Folz et al reported pneumonia associated with coronavirus infection and van Kraaij et al reported a case of lower respiratory tract infection leading to death in adult patient receiving bone marrow transplantation. Further studies are needed to better characterize the severity and outcome of coronavirus infection in children and adults with cancer.

Human herpes virus 6

HHV-6 is the sixth member of the herpes virus family. It is a lymphotrophic DNA virus. HHV-6 has two strain groups, variants A and B. In primary infection variant B is dominant. HHV-6 has infected most people by 2 years of age (Zerr et al., 2005).

HHV-6 causes childhood exanthem roseola infantum, which is sometimes accompanied by neurological illness, such as encephalitis, or severe convulsions with fever (Bertolani et al., 1996; Koskiniemi et al., 1997). The primary infection is followed by persistence and latency in different cells and organs, including monocytes/macrophages, the salivary glands, brain and kidneys. Most adults have detectable viral DNA in the saliva or peripheral blood monocytic cells (Cone et al., 1993).

During immunosuppression, HHV-6 may reactivate and cause a symptomatic disease. A number of studies have clarified the role of HHV-6 in patients receiving HSCT or organ transplantations (Cone et al., 1999; Kadakia et al., 1996; Lehto et al., 2007; Wang et al., 1996). Risk factors for HHV-6 plasma viremia in transplant recipients are low age of the recipient, a sex mismatch between the donor and the recipient, and an underlying disease other than first remission (Zerr et al., 2005). The clinical symptoms associated with HHV-6 viremia in transplant patients are encephalitis, impared memory, and delayed platelet engraftment (Zerr et al., 2001). Adult patients with HHV-6 reactivation have a higher propability to all-cause mortality (hazard ration of 2.9) compared to the patients without HHV-6 reactivation after hematopoietic stem cell transplantation (Zerr et al., 2001). Savolainen et al analyzed 60 pediatric HSCT patients for HHV-6 and HHV-7 and reported that they are commonly detected in blood both pre- and post-transplant (Savolainen et al., 2005). Prolonged reactivation was associated with febrile episodes, rash, and bone marrow suppression in post-stem cell transplant patients, but severe complications were rare (two cases of echephalitis).

Cytomegalovirus

CMV is a DNA virus with structural similarities to herpes simplex virus (Chee et al., 1990). CMV is one of the most succesful human parasites. It infects both vertically and horizontally, and it is responsible for primary infection, reinfection, or reactivation. In populations with a poor socioeconomic background, the vast majority of children have experienced primary CMV infection by puberty. In the developed countries, 40% of adolescents have been infected (Griffiths et al., 1984).

There are two ways to search for CMV: detection of virus or examination of the specific immune response to CMV infection. CMV can be easily cultured or detected by PCR from specimens like urine, saliva, mucus, or blood. A specific IgM test is also used in clinical practice.

CMV disease is mainly restricted to immunocompromised or immunologically immature hosts, like neonates. CMV is the leading cause of congenital infections, with an incidence of 1-2.4% of live births, with possible severe classic "cytomegalovirus inclusion disease" in 10% of them (Ross et al., 2006; Snydman et al., 1995). Congenital CMV infection is the leading infectious cause of brain damage and hearing loss in children and also a relevant health issue in transplant recipients and human immunodeficiency virus (HIV) infected patients (Avetisyan et al., 2007; Ozdemir et al., 2007). Significant progress has been made in the last few years in CMV detection, but the diagnosis of CMV infection can still be problematic in immunocompromised patients.

Furukawa et al followed 68 pediatric patients with solid tumors or leukemia for signs of CMV infection for more than one year (Furukawa et al., 1987). CMV was isolated from 24 of 68 patients at some point of the follow-up, most frequently from the urine. The mean duration of virus shedding was 4.2 months in the primary infection group and 1.7 monts in the reactivation group. The clinical symptoms associated with CMV infection included pneumonia, fever, and hepatitis. Most symptomatic patients had primary infections. No difference was found in the incidence of CMV infection between children with leukemia and with solid tumors. The incidence and outcome of CMV infection is less clearly characterized in patients with hematologic malignancies who receive conventional therapy without the need for transplantation. In adults who receive conventional chemotherapy, CMV associated pneumonia has the mortality ranging from 30% to 57%. An prospective study from University of Maryland Cancer Center reported an incidence of CMV infection in patients with acute leukemia that ranged from 32% to 58% (Wade, 2006) . CMV associated death occurred in 6% of the patients studied (Wade, 2006) . Few studies have examined the role of CMV among non-HSCT children with leukemia. To my knowledge, two case reports of fatal disease and one report of hemolytic uremic syndrome in children with cancer receiving concentional chemotherapy has been published (Adachi et al., 1995; Cavagnaro et al., 2000; Sandoval et al., 2004) . Instead, late CMV infection after stem cell transplant is common (3-17% after allogeneic transplantation) and is associated with 13-fold increase in post-transplant mortality (Boeckh et al., 2003) . These results are from studies made among adult stem cell recipients.

2.3.4.2 Bacterial infections

In leukemic children with fever, the incidence of bacteremia varies from 8.5% to 28%, depending on the intensity of antileukemia therapy (Castagnola et al., 2005; Paulus et al., 2005). Castagnola et al evaluated 153 invasive bacterial infections in 352 children with leukemia receiving anticancer treatment over a 13-year period of time. They reported an incidence rate of 0.092 episodes of invasive bacterial infections per 100 days at risk. Significant changes in the incidence rates were seen, reflecting the intensity of antileukemia treatment. In general, the rate of catheter-related septicemias in children with cancer has been reported to be higher, up to 1.7 episodes per 1000 catheter days (Das et al., 1997).

Septicemia in children with cancer is predominantly thought to be consequence of translocation of microbes from the gut or contamination of a central venous catheter (Santolaya et al., 2002). Myelosuppression, malnutrition and invasive procedures predispose these patients to infections. Relapsed leukemia and recent chemotherapy have been recognized as important risk factors for invasive bacterial infections. Recently, clinical and epidemiological studies found an association of invasive pneumococcal disease with respiratory virus infections (Madhi et al., 2006; Peltola et al., 2006b).

The type of infective agents has changed over time in many centers treating children with cancer. Gram-positive bacteria have become the most common pathogens causing invasive bacterial infections. This phenomenon has been reported from a number of therapeutic trials in the last twenty years (Miser et al., 1981; Paulus et al., 2005; Santolaya et al., 2002). In the Department of Pediatrics, Turku University Hospital, 53 blood culture positive infections were reported between the years 2000 and 2006 among children with cancer. A total of 41 of 53 septicemias were caused by gram-positive bacteria, usually by *Staphylococcus epidermidis*, 12 by gram-negative bacteria, and 3 by *Candida* non-*albicans* (unpublished data). During the 4 year follow-up of children with cancer in 5 Finnish centres, 434 bacterial blood culture positive infections were recorded. Staphylococci (41%) and Streptococci (24%) species were the most common pathogenic (Huovinen P. et al., 2003; Ruuskanen O. et al., 2000). Furthermore, in a large European trial comparing the outcome of febrile infections in children with cancer to that of adults with malignancies, gram-positive bacteria were isolated in 15% of the studied febrile episodes. They were the causes of bacteremia in 60% of the cases, while fungemia occurred in only 2% of all blood culture positive infections (Hann et al., 1997). Generally, in these therapeutic trials, gram-positive and gram-negative bacteria were almost equally represented as causes of blood culture positive infections both in neutropenic and non-neutropenic patients, and fungemia accounted for not more than 10% of cases. Recently, the role of gram-negative bacteria has increased in patients with catheter-related infections (Castagnola et al., 2003; Castagnola et al., 2005; Das et al., 1997; Viscoli et al., 1999). Viscoli et al reported gram-negative bacteria to be related in 48% of catheter-related infections (Viscoli et al., 1999).

The mortality rate of invasive infections in children with leukemia is relatively low, mainly under 10% but varying from 3% to 17%, depending on the immunologic status of the leukemia and on the microbiologic causal agent (Hann et al., 1997; Rahiala et al., 1998; Riikonen et al., 1993; Viscoli et al., 1999).

2.3.4.3 Fungal infections

Severe and protracted neutropenia, intensive phase of cancer treatment, and allogeneic HSCT are the factors most frequently associated with the risk of fungal infection (Abbasi et al., 1999; Hovi et al., 2000; Rosen et al., 2005). Lungs are the most common organ to be affected (Denning, 1998; Viscoli et al., 1999). The most frequently identified yeasts are *Candida* non-*albicans* (Wald et al., 1997).

Clinical and epidemiologic data on fungal infections in children with cancer are limited. Castagnola et al conducted a prospective multicenter 2-year survey of fungal infections in children with cancer (Castagnola et al., 2006). They found 96 episodes of proven, probable, or possible invasive mycosis in as many patients. Of them, 19 were

fungemias and 23 were deep-tissue infections. Most of them (73%) were treated by intensive chemotherapy and 21% by allogeneic stem cell transplantation. The authors found neutropenia in 77% of the episodes. Lymphopenia was present in 75% of patients with normal neutrophil counts. The overall mortality rate was 28% in this study. Earlier, Castagnola et al had reported that the incidence of invasive mycosis in children with leukemia receiving anticancer treatment varied from 0.011 to 0.027 episodes per 100 patient days at risk (Castagnola et al., 2005). They concluded that the correlation between the intensity of anticancer treatment and the rate of infections in children with leukemia was evident. In this study, 13% of the observed invasive bloodstream infections were fungemias. The most frequently found agents were *Aspergillus fumigatus* and *Candida* species. Viscoli et al also conducted a multicenter survey of bloodstream infections in children with cancer and found a total of 191 infections in 156 patients. Fungemia occurred in 9% of the cases, with *Candida* non-*albicans* as the most frequent agent. The mortality rate of fungemias was 22% in this study. Hovi et al studied the occurrence of invasive fungal infections in a Finnish pediatric hematology and oncology unit after improvements in the ventilation system, and after initiating routine azole antifungal prophylaxis (Hovi et al., 2007). They had 98 high-risk subjects who were prospectively surveyed for signs of invasive fungal infections and weekly monitored for serum Aspergillus galactomannan. The incidence of proven invasive fungal infection was 1/31 (3.2%) among children with allogeneic HSCT recipients, 0/26 with autologous HSCT recipients and 1/60 (1.6%) among children undergoing induction treatment. The authors concluded that invasive fungal infections are uncommon in children with high-risk for infections, and regular screening for Aspergillus galactomannan could be useful among pediatric allogeneic HSCT recipients, and two positive samples should prompt further investigations and pre-emptive antifungal therapy.

2.3.4.4 Treatment and prevention

Early empirical broad-spectrum antibiotic therapy has been the cornerstone of the management of febrile neutropenic patients (Pizzo, 1984; Schimpff, 1985). Pizzo reviewed the general principles for the management of fever in patients with neutropenia, emphasizing the importance of immediate admission and patient evaluation with prompt initiation of therapy with broad-spectrum antibiotics. Elevation of axillary temperature once > 38.5°C or repeatedly > 38.0°C should lead to initiation of the antibiotic therapy. According to these principles the antibiotic therapy should be continued if the patient has prolonged (> 1 week) neutropenia, particularly if there is persistent fever. If the patient remains febrile after one week of treatment with broad-spectrum antibiotics, empirical antifungal therapy should be added to the regimen. Furthermore, from 10 to14 days of treatment is adequate for most patients with neutropenia. The principles underlined the significance of washing the hands by all those who cared for these febrile patients with neutropenia (Pizzo., 1993). The goal of the combination of antibiotics is to affect both gram-positive and gram-negative bacteria. Previously, the combination therapy usually included aminoglycosides, which are associated with both nephrotoxicity and ototoxicity and a have very narrow therapeutic index. Since the 1980s, randomized trials comparing third generation cephalosporins alone and in combination therapy have been conducted (Pizzo et al., 1986). In 1997, the Infectious Diseases Society of America published the guidelines for the use of antimicrobial agents in neutropenic patients with fever. According to these

guidelines, the recommendation is either monotherapy with ceftazidime or imipenem or dual therapy with aminoglycoside with antipseudomonal β-lactame. These guidelines are supposed to be followed if vancomycin therapy contraindicated (for severe mucositis, quinolone prophylaxis, colonization with methisilline resistant *Stapylococcus aureus* penisilline-cephalosporine-resistent *Streptococcus pneumoniae*, catheter related infections or hypotension) (Hughes et al., 1997). The use of ceftazidime or imipenem monotherapy has increased resistance problems in many centers (Pizzo et al., 1986; Sanders et al., 1991). The use of vancomycin is also problematic because of the emergence of vancomycin resistant enterococci, which supports the limited use of vancomycin in culture positive resistant staphylococcal infections.

Currently, the combination of antibiotics used in the initiation treatment of febrile neutropenic patients varies between centers. The factors affecting the choice are the local situation of pathogenic agents, the current state of resistance problems, and economic factors. In addition to these general principles, individual factors such as renal function should also be considered.

The current concept is that a treatment of 5 to 7 days is adequate in patients who become afebrile and asymptomatic within one week and whose neutrophils increase over 0.5 x 10^9/l at the same time and whose blood culture remains negative. In patients with blood culture positive infections, antibiotic treatment should be continued for 7 to 14 days. If the patient becomes afebrile but the neutropenia continues, the American guidelines recommend continuation of antibiotic therapy until the neutropenia resolves. In contrast, some centers discontinue antibiotics after 14 days, if the patient with neutropenia is afebrile, the malignancy is in remission, and the bone marrow shows some recovery (Mullen et al., 1990; Nijhuis et al., 2005).

The options for prevention and treatment of respiratory viral infections among children with cancer are immunization, prevention of exposure to infections, and antiviral drug prophylaxis (Sandherr et al., 2006). Antivirals are generally available for influenza A and B viruses and RSV, but only a few studies have been published on the effect of antivirals on the outcome of RSV or influenza infections in immunosuppressed hosts. Table 7 gives a summary of antivirals available and used in immunosuppressed children. Large prospective, randomized trials have shown that oseltamivir is effective for influenza prophylaxis in the general population (Hayden et al., 1999). Chemaly et al have found neuraminidase inhibitors improving outcome of adult patients with leukemia and influenza (Chemaly et al., 2007). Chemaly et al has also reported that oseltamivir as an antiviral treatment may prevent the development of pneumonia associated with influenza infection in adult patients with cancer (Chemaly et al., 2006). They reported that among their 112 adult HSCT patients who developed infections with influenza, 41 were treated with oseltamivir, and only 4 (10%) developed pneumonia compared to untreated 30 (42%) patients, whose infection progressed to pneumonia. Chemaly et al found that fewer adult patients with RSV infection developed pneumonia if they were treated with aerosolized ribavirin (Chemaly et al., 2006). In the treatment of RSV infection in immunocompromised children, inhaled ribavirin has been effective, often combined with intravenous immune globulin or high-titer RSV immunoglobulins (DeVincenzo et al., 2000; Small et al., 2002). Palivizumab (humanized monoclonal anti F protein antibody) has been used for prophylaxis of RSV infection in patients at risk for severe infection (Anonymous , 1998). Parainfluenza virus infections in immunocompromised hosts, mainly transplant recipients, have been treated with

ribavirin with varying degrees of success (Dignan et al., 2006; Glanville et al., 2005; Wright et al., 2005). Acyclovir is a drug of choice for the treatment and prophylaxis of infections caused by herpes simplex virus. Even treatment with low-dose acyclovir has been effective against herpes simplex virus reactivation in children undergoing treatment for cancer (Celkan et al., 2006; Orlowski et al., 2004). Ganciclovir has been used for pre-emptive therapy of cytomegalovirus antigenemia in HSCT recipients mainly (Asano-Mori et al., 2005; Hazar et al., 2004) but only a few reports have been published of successful outcomes in severe CMV and Epstein-Barr virus disease in non-HSCT leukemic children with febrile infections complicated by hemolytic uremic disease and hepatitis (Adams et al., 2006; Cavagnaro et al., 2000). Ganciclovir may also be effective against HHV-6 infections (Wade, 2006). Cidofovir has been administered to pediatric HSCT recipients with severe adenovirus infections but there are no reports on its use in non-HSCT patients. Pleconaril is a drug developed for picornavirus infections. In the study of Hayden et al, early pleconaril treatment was well tolerated and significantly reduced the duration and severity of colds due to picornaviruses in adults (Hayden et al., 2003). There are also a few reports of enteroviral meningitis treated with pleconaril in both adults and children (Abzug et al., 2003; Desmond et al., 2006). Because of its cumulativeness and possible adverse effects, pleconaril is not widely used in clinical practice. To date, only a few case reports have been published on pleconaril therapy in immunosuppressed hosts, and none on children (Rotbart et al., 2001).

Supportive care of infectious children with leukemia include also the use of granulocyte colony-stimulating factor (G-CSF), the use of subcutaneous or intravenous immunoglobulins and the use of hyper-immunoglobulins to prevent varicella zoster infection. Children with ALL treated with CSF have shorter hospitalisations and fewer infections. However, there is no evidence that the duration of neutropenia is shortened or that there is less delays in cancer treatment. Also the data of G-CSF treatment affecting the survival is lacking.The role of CSF regarding febrile neutropenia episodes is still uncertain. During the anticancer treatment, the immunologlobulins has been used for the treatment of persistent viral infections, like parvovirus infection, but no clear benefit has been achieved (Alberti et al., 1999; Flunker et al., 1998; Tang et al., 2007).

Prompt and reliable diagnostics of infections is crucial for identification of patients and hospital staff who may be spreading viruses. Isolation and cohorting of infected patients is part of infection control policy. Restricting visits and prohibiting symptomatic subjects to visit the hospital is important, as well as prohibiting hospital staff with symptoms of respiratory disease to work with immunosuppressed patients. The only vaccine available for respiratory viruses is inactivated influenza vaccine, even if the immune response may be incomplete. The immunization of family members and hospital staff is beneficial. Furthermore, immunocompromised patients are encouraged to receive an annual vaccination against influenza (Whimbey et al., 1997; Whitley et al., 2006). Palivizumab, a specific IgG antibody for RSV may be used for passive immunization, but related study data are very limited (De Vincenzo et al., 1996; Whimbey et al., 2000).

Over the past decade, the risk status of the febrile cancer patient has been evaluated. Attempts to stratify patients into high-risk and low-risk groups as well as different practice guidelines according to risk status have been introduced (Alexander et al.,

2002; Koh et al., 2002; Salzer et al., 2003). Absolute monocyte count < 0.1 x 10^9 /l and fever > 39ºC have been recognized as risk factors for bacterial septicemia (Santolaya et al., 2002; Viscoli et al., 1999). These recent studies suggest that low risk cancer patients may be successfully treated with oral antibiotics. This treatment strategy has many advantages, including improved quality of life, lower costs, and decreased risk of nosocomial infections.

2.3.4.5 Differentiation of viral from bacterial infections in febrile children undergoing chemotherapy for cancer

Clinical findings in febrile children undergoing chemotherapy for cancer often provide inadequate information on the etiology of the infection. CRP is the most widely used inflammatory marker, whereas WBC is not informative enough in these patients. CRP response is reasonably slow, it takes 8 - 24 hours in case of bacterial inflammation (Peltola et al., 2006a). Moreover, CRP values can be influenced by an underlying malignant disease and tissue damage (ref) . More sensitive methods are needed to distinguish bacterial infections from viral infections. For the purpose, a number of macrophage/monocyte-derived cytokines and the precursor protein of calcitonin, procalcitonin, have been studied. Three studies have evaluated IL-6 and PCT in children with cancer (Fleischhack et al., 2000; Kitanovski et al., 2006; Stryjewski et al., 2005). Fleischhack evaluated PCT, CRP, IL-6, IL-8, soluble IL-2 receptor (sIL-2R), and the soluble tumor necrosis factor receptor II (sTNFRII) for their diagnostic relevance in 122 children with febrile neutropenias. A strong correlation to bacterial infections was found for high PCT and IL-8, PCT and CRP, as well as CRP and IL-8.

Human MxA protein is a 78 kDa GTPase, which is granularly distributed in the cytoplasm of lymphocytes (Aebi et al., 1989). Mx (myxovirus resistance) genes are induced almost solely through the production of type I interferons, whereas other cytokines do not seem to enhance MxA protein expression (Rönni et al., 1993; von Wussow et al., 1990). Interferon production is induced by many viral infections, but the detection of interferons in serum is difficult and unreliable, mainly because their half-lives are short. MxA protein is stable and remains elevated for at least one week after viral infection (Halminen et al., 1997). Expression of MxA protein can, therefore, be a useful tool in distinguishing bacterial infections from viral ones (Chieux et al., 1999; Halminen et al., 1997; Nakabayashi et al., 2006).

Table 7. Antiviral agents and treatment and prophylaxis of viral infections.

Virus		Drug	Indication
Herpesviruses			
	Herpes simplex	Acyclovir or valacyclovir	Prophylaxis and treatment
	Varicella zoster	Acyclovir,valacyclovir or famciclovir	Treatment
	Cytomegalovirus	Ganciclovir, valganciclovir, foscarnet, cidofovir	Treatment
	Epstein Barr	Ganciclovir, valganciclovir, foscarnet, cidofovir	
	Human herpes virus 6	Ganciclovir, foscarnet	Treatment
Adenovirus		Cidofovir	Treatment
Respiratory viruses			
	Respiratory syncytial virus	Palivizumab* Ribavirin	Prophylaxis Treatment
	Influenza A/B viruses	Oseltamivir, zanamivir	Treatment
	Rhinovirus	Capsid-binding agents (Pirodavir, Pleconaril)**	Treatment
		Interferon, interferon inducers	Treatment
	Human metapneumovirus	Ribavirin	Treatment
	Parainfluenza viruses	Ribavirin	Treatment

*Humanized monoclonal antibody, **On a compassionate basis only.

3 OBJECTIVES OF THE STUDY

The objective of this thesis was to study febrile episodes in children with leukemia with a focus on the occurrence and clinical characteristics of respiratory viral infections in the children.

The specific study aims were:

1. To search for a new respiratory virus, human bocavirus, in febrile children with leukemia (**I)**

2. To study the occurrence of respiratory viruses in febrile children with leukemia (**II**)

3. To study viral coinfections in leukemic children with blood culture positive bacterial infections (**III**)

4. To detect viral infection by means of MxA protein expression in the blood lymphocytes of febrile children receiving anticancer chemotherapy (**IV**)

4 MATERIALS AND METHODS

4.1 Patients

4.1.1 Multicenter study of febrile episodes in children with leukemia I-III

A five-year prospective multicenter survey searching for viruses, bacteria, and fungi as a cause of fever was carried out in children with ALL and AML during the course of leukemia treatment (Figure 3). The survey was conducted from April 1, 2000 until October 31, 2005 at four Finnish university hospitals (Turku, Oulu, Kuopio, Helsinki). A total of 156 febrile episodes in 51 children with leukemia were studied. The mean follow-up time was 1.5 years per patient (range 0.1 to 2.5 years, SD 0.6), and 33/51 (65%) of the children were followed throughout the course of treatment. The mean age of the children was 5.9 years (range 0.4 to 15.3 years, SD 3.9 years). None of the patients had Down's syndrome. Two children with high-risk AML and five with high-risk ALL (two Philadelphia chromosome positive, two with relapsed leukemia, and one Philadelphia chromosome negative with a highly intensive treatment protocol) finally underwent bone marrow transplantation. The follow-up of these children ended on the day of transplantation. The study was approved by the Ethics Committees of the Medical faculties of Turku, Oulu, Kuopio, and Helsinki Universities. Informed consent was obtained from the patients and their parents.

4.1.2 Study of MxA protein expression in blood lymphocytes of children with cancer IV

Twenty-six children with cancer entered the study for evaluation of MxA protein expression in blood lymphocytes during febrile episodes and during high-dose MTX and Ara-C treatment. The study was conducted at the Department of Pediatrics, Turku University Hospital, from November 1, 1997 until March 31, 1999. Analysis of MxA protein expression was carried out in the Department of Virology, Turku University. Nine children had ALL and one child had AML. Six children had solid tumors (one Wilm's tumor, two neuroblastomas, one teratocarcinoma, one pineal germinoma, and one hepatocellular carcinoma). Three patients had lymphomas (one Hodgkin's disease and two non-Hodgkin's lymphomas). The control group consisted of twenty healthy children without cancer. The mean age of the study patients was 7.2 years (SD 4.7 years) and that of the control patients 5.7 years (SD 3.4 years).

4.2 Study design

4.2.1 Multicenter study of febrile episodes in children with leukemia I-III

Figure 4 shows the study flow chart. According to the NOPHO protocol, children with leukemia are hospitalized whenever fever appears. When the study patient was hospitalized for fever or when fever occurred during hospital stay, viral, bacterial, and fungal specimens were taken to examine the etiology of the febrile episodes. Fever was defined as an axillary temperature ≥ 38.0^{o}C, measured twice within four hours or ≥ 38.5^{o}C measured once. The interval between separate febrile episodes had to be ≥ 7 days from

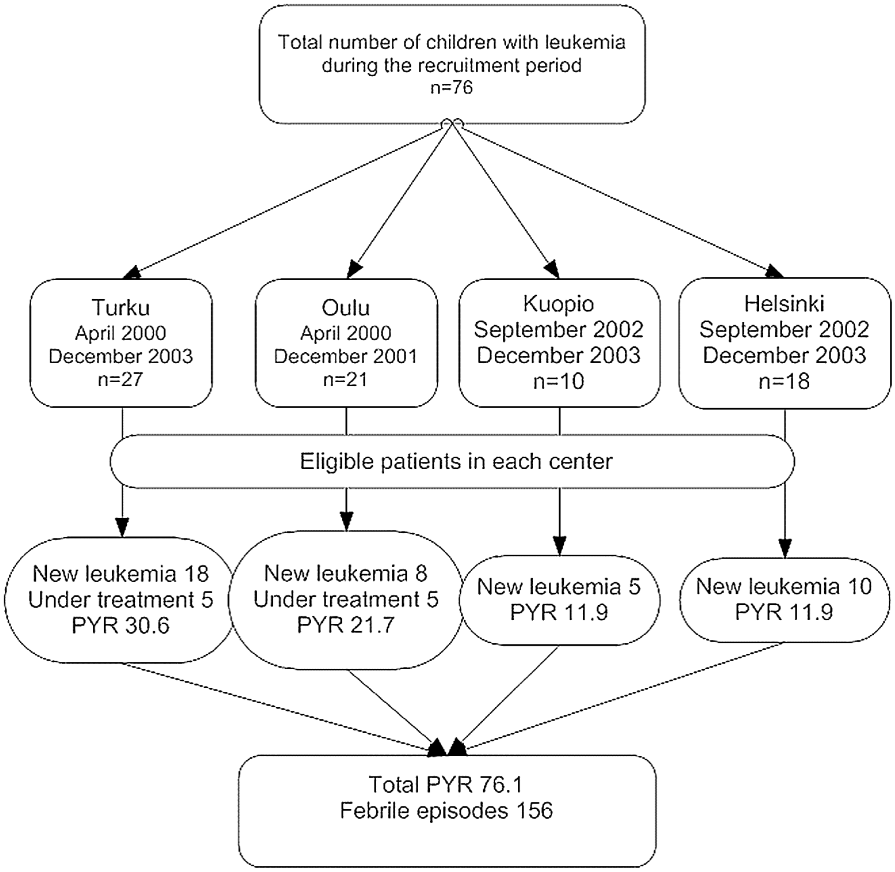

Figure 3. Study population and follow-up times (I-III). PYR = person year at risk.

the day when the fever appeared. Any febrile episodes occurring 48 hours after admission were considered nosocomial and the rest of the febrile episodes community-acquired. All febrile episodes were included and none was excluded because of suspicion of blood transfusion related or cytostatic agent related toxicity. In each episode, the duration of fever, length of hospital stay, and length of antibiotic treatment were analyzed and reported in days. Symptoms like cough, rhinitis, sore throat, eye infection, skin manifestations, vomiting, and diarrhea (≥3 loose stools per day) were also monitored daily.

A blood sample was taken for blood culture. Nasal swab samples were taken to carry out virus culture for 10 viruses, viral antigen detection for 7 viruses, and polymerase chain reaction (PCR) assays for 9 viruses. A stool sample was taken for virus culture and antigen detection for rotavirus and adenovirus. If a child had diarrhea, a stool sample was taken to detect calicivirus and enteroviruses using PCR. Table 5 shows details of the microbiologic examinations. In some patients, follow-up samples for positive viral tests were taken until they turned negative.

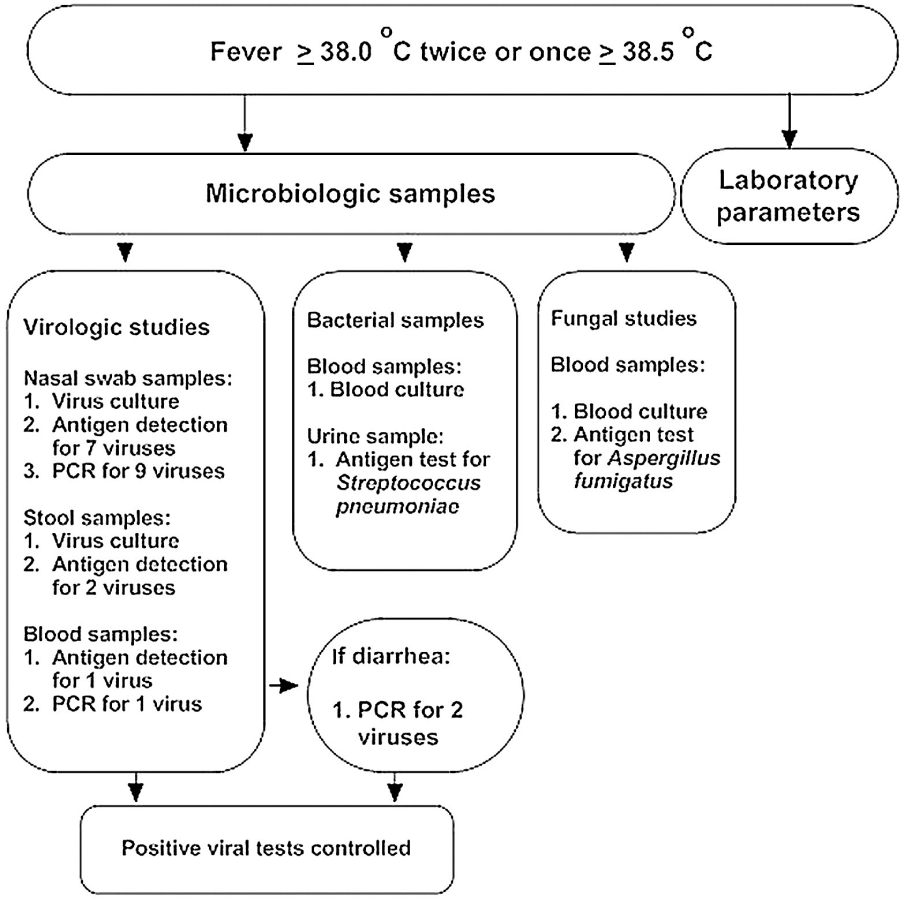

Figure 4. Study flow chart

4.2.2 Study of MxA protein expression in blood lymphocytes of children with cancer IV

The study patients were prospectively divided into two groups - 15 children with acute febrile episodes and 15 afebrile children receiving high-dose MTX or Ara-C treatment. Because of the long period of time, four children participated in both groups so the total number of study patients was 26 children. These study patients receiving MTX or Ara-C had no symptoms of infection one week prior to therapy. Thirty cytostatic chemotherapy periods, 25 with high-dose MTX and 5 with high dose Ara-C, were included in this study. MxA protein expression in blood lymphocytes was evaluated once during the febrile infections and three times during each MTX and Ara-C treatment period; before onset of treatment (day 0), 24 hours after onset of treatment (day 1) and on the day of discharge (days 3 or 4).

4.3 The stage of the immunosuppression (I-IV)

Total neutrophil counts and lymphocyte counts were used as indicative parameters of the stage of immunosuppression. Neutropenia was determined as neurophil count ≤ 0.5 X 10^9cells/l (Cheng et al., 2004). Lymphopenia was defined as lymphocyte count below 1.4 X 10^9 cells/l (Comans-Bitter et al., 1997).

4.4 Microbiologic diagnostics (I – IV)

4.4.1 Viruses

A nasal swab sample was obtained through a nostril by inserting a sterile cotton swab (Applimed SA, Switzerland) to a depth of 2-3 cm and retracting it by rotating movements against nasal mucosa to obtain a maximum yield of mucus. The swab was then inserted into a vial containing viral transport medium (5% tryptose phosphate broth, 0.5% bovine serum albumin and antibiotics in phosphate-buffered saline) (Heikkinen et al., 2002). The specimens were transported to the laboratory during the same day at room temperature for viral analysis. The specimens from other than the Turku University Hospital were mailed to the Department of Virology, Turku University. Table 8 shows the viruses searched for and the methods of identification.

Virus culture was carried out to detect adenovirus, influenza A and B viruses, parainfluenza virus types 1, 2, and 3, RSV, CMV, enteroviruses, and rhinovirus in accordance with routine diagnostic protocols in A549, HeLa, and LLC-MK_2 human foreskin fibroblast cells, and in tertiary monkey kidney cells. The supernatants of cell cultures exhibiting a cytopathogenic effect were further studied by antigen detection for adenovirus, influenza A and B viruses, parainfluenza virus types 1, 2 and 3 and RSV, or by reverse transcription (RT) PCR for enteroviruses and rhinovirus.

Viral antigens were detected by a time-resolved fluoroimmunoassay (Arola et al., 1995). Cell lysate antigens were used to detect influenza A and B viruses and parainfluenza virus types 1, 2 and 3. Hexon antigen was used to detect adenovirus, and semipurified virion antigen to detect RSV. These antigen detection methods have been found to be reliable in the identification of serologically verifiable infection (Hietala et al., 1988; Koskinen et al., 1987; Meurman et al., 1983).

Reverse transcription (RT)-PCR was used to detect rhinovirus, enteroviruses, HHV-6, CMV, coronavirus types OC43 and 229E, hMPV and HBoV, as reported previously (Allander et al., 2007b; Halonen et al., 1995; Kytö et al., 2005; Pitkäranta et al., 1998; Vuorinen et al., 2003; van den Hoogen et al., 2001). RT-PCR used to detect rhinovirus and enteroviruses and HBoV are briefly described here. Nucleic acids for RT-PCR were isolated from nasal swab samples using a commercial kit (High Pure Viral Nucleic Acid Kit, Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Primers amplifying a 120 bp fragment for rhinovirus and enteroviruses were found to be the most sensitive and used for the primary RT-PCR test (Hyypiä et al., 1998). Then, enteroviruses and rhinoviruses were discriminated by a liquid-phase hybridization assay, in which amplicons are identified simultaneously by samarian-labeled rhinovirus and europium-labeled enterovirus probes in a microtiter plate (Lönnrot et al., 1999). PCR positive samples that remained undetectable on liquid hybridization were further tested by another RT-PCR using a primer pair that gives rise

to 530 bp amplification products for rhinoviruses and 650 bp amplicon products for enteroviruses (Arola et al., 1996). HBoV and hMPV were analyzed retrospectively in the nasal swabs stored at -70°C. DNA for HBoV analysis was extracted from a 220 aliquot µl of the specimens using an automated nucleic acid extractor (easyMag, BioMerieux, France). The elution volume was 55 µl. PCR primers BoF (GGAAGAGACACTGGCAGACAA), BoR (GGGTGTTCCTGATGATATGAGC), and probe BoP (Cy5-CTGCGGCTCCTGCTCCTGTGAT-BHQ2) were selected from the NP-1 gene of the HBoV sequence described by Allander et al (Allander et al., 2001). A cloned HBoV plasmid was used as a quantitative standard. PCR reactions were carried out in a 25 µl volume consisting of 5 µl extracted DNA, 1× QuantiTect Probe PCR master mix (Qiagen, Hilden, Germany), 600 nM of each primer, and 100 nM of probe. Amplifications were run on a Rotor-Gene 3000 instrument with the following cycling conditions: 15 min at 95°C; 40 cycles of 15 s at 94°C and 60 s at 60°C. The plasmid standard could be detected diluted to the level of a single copy per reaction.

A commercial antigen test for rotavirus and adenovirus (Diarlex^{R}, Orion Diagnostica, Espoo, Finland), PCR for caliciviruses (Pang et al., 1999) and enteroviruses, and virus culture were carried out in stool samples**.** An antigen test for CMV (Hohenthal et al., 2005) and a PCR assay for HHV-6 (Kytö et al., 2005) were used in blood samples.

In the study reported in paper IV, nasal swab samples for the virus culture and PCR assays of rhinovirus and enteroviruses were studied. HHV-6 and CMV were detected with PCR from PBMC retrospectively from samples restored at -40°C.

4.4.2 Bacteria

Blood culture was carried out according to routine diagnostic protocols, as previously described (Toikka et al., 1999). Bacterial culture was also studied from urine samples. A commercial rapid diagnostic test (Binax NOW®, Binax, Portland, USA) was used to study urinary pneumococcal antigens (Dowell et al., 2001).

4.4.3 Fungus

In papers I-III, fungal culture and antigen test for *Aspergillus fumigatus* were studied from blood samples. In paper IV, only a blood culture was taken.

4.5 Laboratory parameters (I-IV)

Total white blood cell count (WBC), absolute neutrophil count, lymphocyte count, and the first CRP of the febrile episodes were monitored as laboratory parameters.

4.5.1 MxA expression (IV)

Analysis was done as previously described by Halminen et al (Halminen et al., 1997). Briefly, 1-3 ml heparinized venous blood was collected from hospitalized patients during cytostatic treatment. Peripheral blood mononuclear cells (PBMC) were separated on Ficoll-Paque gradient centrifugation (Pharmacia, Uppsala, Sweden). PBMC were fixed with 6% paraformaldehyde solution for 15 min at room temperature and permeabilized for 5 min in 1% Triton X-100 in PBS. Intracellular MxA protein was stained with rabbit anti-MxA antisera (Rönni et al., 1993), followed by secondary

antibody staining with fluorescein-conjugated goat $F(ab')_2$ anti-rabbit IgG (Caltag Laboratories, South San Francisco, CA, USA). Cells were washed with 0.5% BSA in PBS and at a final step with a FACSFlow (Becton Dickinson, Mountain View, CA, USA) solution. Cells were analyzed using a FACScan® cytometer (Becton Dickinson) within 24 hours of staining. Values indicating MxA lprotein expression level are the median channel logarithmic fluorescence counts from the cytometer.

Table 8. Viruses searched for in studies I-III.

Viruses	Sample	Detection method
Rhinovirus	Nasal swab	PCR, culture
Enteroviruses	Nasal swab	PCR, culture
	Stool	PCR, culture
Human bocavirus	Nasal swab	PCR
Human metapneumovirus	Nasal swab	PCR
Coronavirus types OC43 and 229E	Nasal swab	PCR
Cytomegalovirus	Nasal swab	PCR, culture
	Blood	Antigen detection
Human herpes virus 6	Nasal swab	PCR
	Blood	PCR
Adenovirus	Nasal Swab	Antigen detection, culture
	Stool	Antigen detection, culture
Respiratory syncytial virus	Nasal swab	Antigen detection, culture
Parainfluenza viruses types 1, 2 and 3	Nasal swab	Antigen detection, culture
Influenza A virus	Nasal swab	Antigen detection, culture
Influenza B virus	Nasal swab	Antigen detection, culture
Rotavirus	Stool	Antigen detection, culture
Calicivirus	Stool	PCR
Bacteria		
Bacteremia	Blood	Culture
Urinary bacteria	Urine	Culture
		Antigen detection of *Streptococcus pneumoniae*
Fungi		
Fungemia	Blood	Culture
Aspergillus	Blood	Antigen detection

PCR = polymerase chain reaction

4.6 Statistical analysis

4.6.1 Multicenter study of febrile episodes in children with leukemia I-III

The number of febrile episodes was calculated per person year at risk (PYR). The results were given as means ± SD, unless stated otherwise. The significance of the difference in continuous variables between the two groups was tested using Student's t-tests.

4.6.2 Study of MxA protein expression in blood lymphocytes of children with canver IV

Nonparametric Kruskal-Wallis and Mann-Whitney U-tests were used for comparison of MxA protein levels between the groups.

5 RESULTS

5.1 Occurrence of febrile episodes (I – III)

Tables 9 and 10 show the characteristics of the study population and the occurrence of the febrile episodes according to the stage of immunosuppression.

The number of febrile episodes during the survey was 156 in 51 children with leukemia during 27,743 patient days at risk. In 18 episodes, virologic samples were not taken, and 138 febrile episodes were included in the survey (Figure 5). The occurrence of febrile episodes was 2.1/PYR (range 0 to 8.5/PYR). Figure 6 show the distribution of patients according to frequency of febrile episodes. The mean number of febrile episodes was 3.1 (range 0-17) per patient.

Table 9. Characteristics of the study population.

Number of patients	***51***
Type of leukemia	
Acute lymphoblastic leukemia	46
High risk	15
Intermediate risk	12
Standard risk	19
Acute myeloid leukemia	5
Sex (M/F)	22/29
Age (years) at entry	
Mean ± SD	5.9 ± 3.9
Range	0.4 – 15.2
Time (days) spent in the study	
Total	27,743
Mean ± SD	548 ± 219
Range	37 - 913
Duration (days, mean and range) of	
Induction	116 (22 - 145)
Consolidation	109 (17 – 222)
Maintenance	545 (28 – 849)
Number of febrile episodes	
Neutrophil count < 0.5 x 10^9/l	69 (44%)
Neutrophil count < 1.0 x 10^9/l	86 (55%)
Lymphocyte count < 1.4 x 10^9/l	121 (78%)
Total number of febrile episodes	156
Febrile episodes with virologic samples available	138

Table 10. Incidence of neutropenic, non-neutropenic and lymphopenic febrile episodes of 51 children with leukemia. Distribution of hospital days, fever days, antibiotic days during anticancer treatment.

	Induction		***Consolidation***		***Maintenance***		***Whole follow-up***	
	n	/PYR	n	/PYR	n	/PYR	n	/PYR
Hospital days		68		35		7		29
Fever days		17		11		3		5
Antibiotic days		53		27		7		17
Infections with neutrophil count <0.5 X 10^9 /l	40	3	14	1	15	0.3		
Infections with neutrophil count <1.0 X 10^9 /l	44	3	20	2	22	0.4		2
Infections with lymphocyte count <1.4 X 10^9 /l	39	3	36	3	46	1		
Number of infections							156	2

PYR = person year at risk.

The occurrence of febrile episodes with evidence of viral etiology was 1.0/PYR. The occurrence of blood culture positive infections ranged from 1.0/PYR during the induction phase and 0.37/PYR during the consolidation phase to 0.04/PYR during the maintenance phase of leukemia treatment. The occurrence of septicemia with evidence of a viral co-infection ranged from 0.52/PYR during the induction phase and 0.24/PYR during the consolidation phase to 0.04 episodes/PYR during the maintenance phase. The overall occurrence of septicemia with evidence of viral co-infection was 0.14/PYR.

Most of the infections in children with leukemia occurred in the presence of neutropenia. During the whole follow-up, the occurrence of neutropenic infections (neut <1.0 x 10^9 cells/l) was 1.9/PYR. The occurrence of neutropenic infections was higher during the intensive induction therapy, 3.1/PYR, decreased in the consolidation phase to 1.8/PYR and again in the maintenance phase to 0.43/PYR.

Of the febrile episodes, 111 (78%) were community-acquired and 27 (22%) were nosocomial infections.

During the survey, the number of hospital days was 29.3/PYR, days on bacterial antibiotics 17.4/PYR, and fever days 5.1/PYR. Fever days and days on bacterial antibiotics were clustered to the induction period, 45% of the fever days and 53% of the days on antibiotics emerged during the induction phase. Table 10 shows the distributions of hospital days, fever days, and days on bacterial antibiotics.

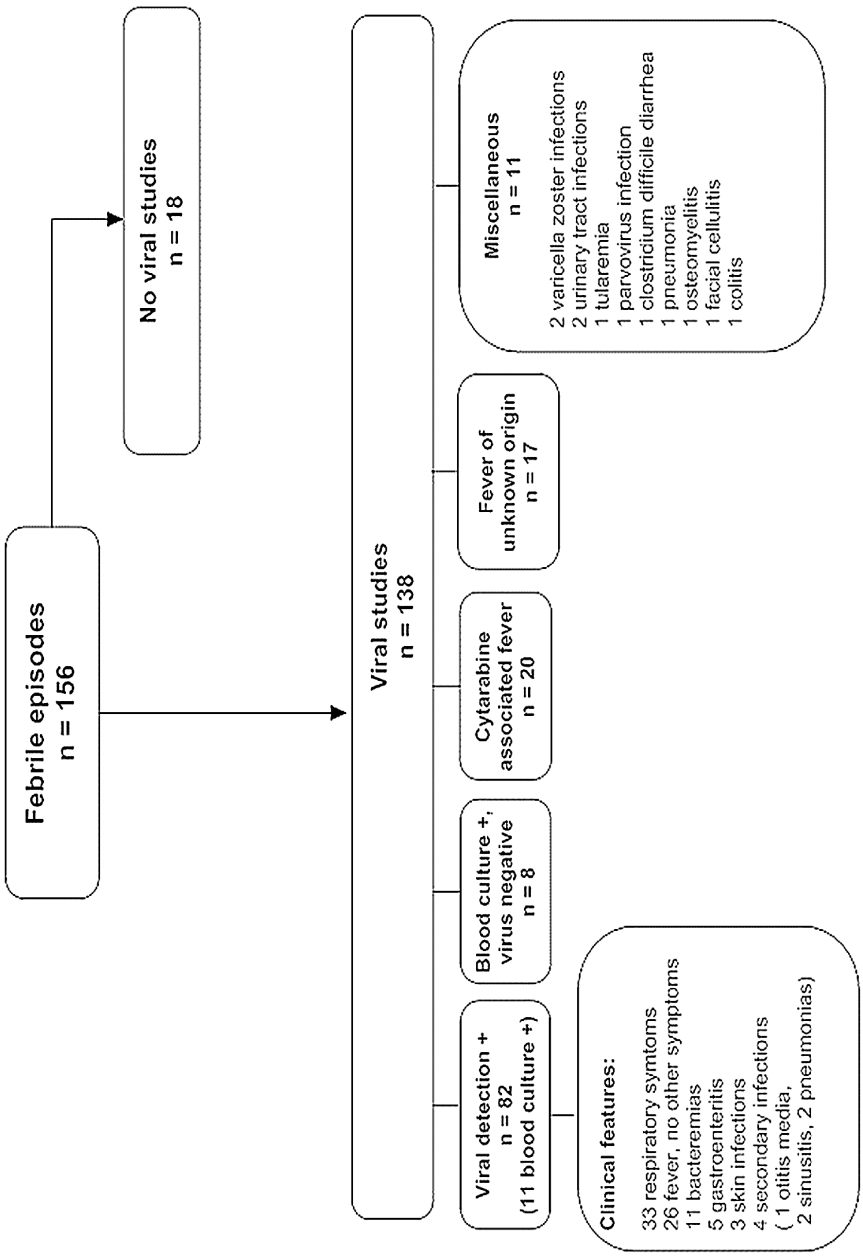

Figure 5. The number of the episodes with virologic studies, and the clinical features of the 138 febrile episodes in 51 children with leukemia .

5.2 Etiology of febrile episodes (I – III)

Table 11 shows the etiology of the febrile episodes. During the survey, the total number of episodes with evidence of viral etiology was 84 of 138 studied episodes (62%) (Figure 3). Bacterial blood cultures were positive in 19 of 156 (11%) febrile episodes in 17 children, and in 11 (58%) of the episodes evidence was found for concomitant viral infection. Evidence of fungal infection was found in 3 cases (2%) of the febrile episodes, but fungemia occurred only in one case. Mixed viral-bacterial infections were found in 14 of 138 (10%) febrile episodes. Mixed bacterial-fungal or viral-fungal infections were found in single cases.

In 37 (27%) of the febrile episodes, all microbiologic tests remained negative, and 20 (14%) of these episodes occurred during Ara-C therapy. None of the febrile episodes were related to blood transfusions. Finally, FUO accounted for 17 (12%) of the febrile episodes.

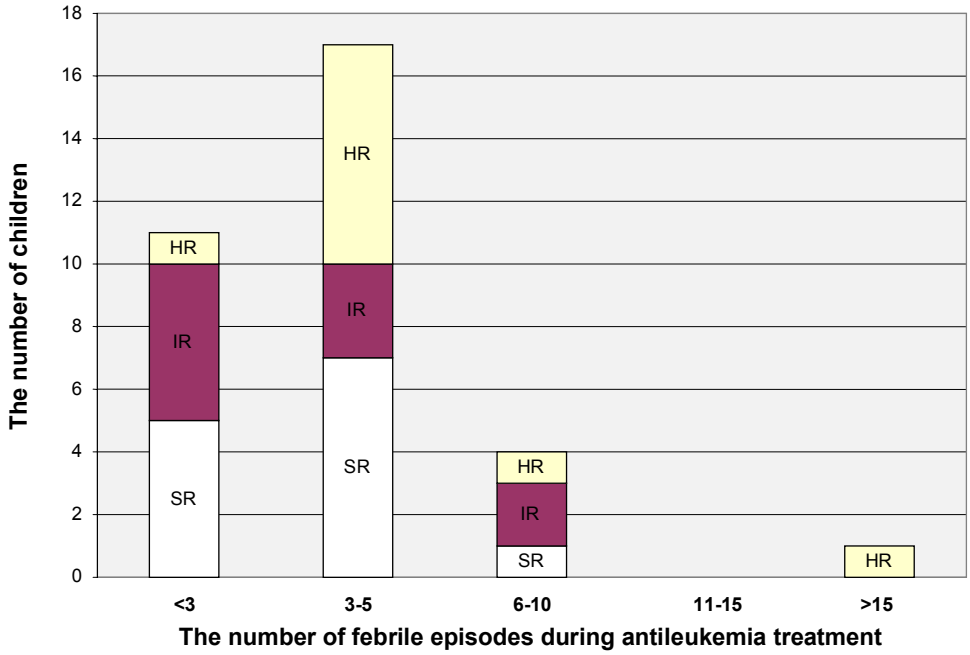

Figure 6. Frequency of febrile episodes in 31 children with leukemia and distribution of patients according to risk-adapted treatment protocols. HR = high risk, IR = intermediate risk, SR = standard risk.

Table 11. Etiology of 138 febrile episodes of 51 children with leukemia.

		Number of episodes	%
Viral infections	Total	84	62
Respiratory infections			
	Rhinovirus	31	22
	Respiratory syncytial virus	15	11
	Human herpes virus 6	10	7
	Human bocavirus	7	5
	Cytomegalovirus	7	5
	Influenza A virus	5	4
	Enteroviruses	4	3
	Parainfluenza type 3 virus	4	3
	Adenovirus	4	3
	Parainfluenza type 2 virus	3	2
	Influenza B virus	2	1
	Coronavirus types OC43 and 229E	1	1
	Herpes simplex virus	1	1
Gastroenteritis			
	Rotavirus	3	2
	Calicivirus	3	2
Other viral infections			
	Varicella zoster virus	1	1
	Parvovirus infections	1	1
Dual viral infections		17	12
Bacterial infections	Total	23	17
Bacteremias		19	12#
Urinary tract infections		2	1
Other bacterial infections			
	Fransicella tularensis	1	1
	Clostridium difficile	1	1
	Streptococcus pneumoniae	6*****	4
Dual bacterial infections		8	6
Fungal infections	Total	3	2
Fungemia	non-albicans *Candida*	1	1
Other fungal infections	*Aspergillus fumigatus*	2	1
Mixed viral-bacterial infections		14	10
Mixed fungal-viral infections		2	1
Mixed fungal-bacterial infections		1	1

* studied in 72 samples, ** 3 children, one with 5 consecutive episodes, *** studied in 85 samples, **** studied in 118 samples, ***** studied 104 samples, # Studied in 156 samples

5.2.1 Viral infections

Respiratory virus was detected in 82 of 138 (59%) febrile episodes. In addition, parvovirus infection was detected in one episode, and in two others varicella zoster infections were detected, but these viruses were not systematically searched for. Rhinovirus (22%), RSV (11%), HHV-6 (7%), HBoV (5%), CMV (5%), and influenza A virus (4%) were the most common viruses found (Table 8). In 12% of the cases, more than one virus was detected, and rhinovirus (n= 14) and RSV (n=7) were the most common viruses in dual viral infections. Dual viral infections are listed as pairs followed by the number of each pair: rhinovirus and RSV 5, rhinovirus and parainfluenza virus type 2 1, rhinovirus and parainfluenza virus type 3 2, rhinovirus and HHV-6 1, rhinovirus and influenza B virus 1, rhinovirus and CMV 2, influenza A and HHV-6 1, CMV and HSV 1, RSV and parainfluenza type 2 virus 1. During the survey, two febrile episodes with more than two viruses were detected (RSV and rhinovirus and enterovirus, rhinovirus and HBoV and CMV).

Most of the viral findings were obtained from nasal swab samples. PCR detected virus in 48 of 68 (71%) virus positive nasal swab samples. It was especially sensitive for picornaviruses (4 cases found by culture compared to 31 by PCR). Throughout the study, the antigen test was more sensitive than virus culture. The antigen test for RSV was positive in 15 febrile episodes compared to 3 episodes positive by virus culture. The antigen test for influenza A virus was also positive in 4 episodes compared to 1 episode positive by virus culture. On the other hand, all viruses detected by virus culture were also positive by the antigen test or PCR assay.

PCR for HBoV was positive in 7 of 138 (5.6%) febrile episodes in 3 children. Two children had a low copy number of HBoV, ≤ 500 copies per nasal swab sample. The tests for other 12 respiratory viruses remained negative in these children. The third child had evidence of possible persistence or reactivation of HBoV infection, with repeated detection of HBoV in five consecutive febrile episodes within 6 months. The number of copies of HBoV were <500, <500, 1400, 81000 and 100000. Concomitant other viruses were detected in only one episode; rhinovirus by PCR from a nasal swab sample and cytomegalovirus by antigen detection from a blood sample.

Stool samples (n = 105) showed adenovirus (n = 2), rotavirus (n = 3), calicivirus (n = 3), enteroviruses (n = 2), and rhinovirus (n = 10). In blood samples (n = 112), cytomegalovirus antigen was positive in 6 cases and HHV-6 PCR in 2 cases.

Rhinoviruses were found throughout the year, but most of them were detected during the fall (September and October) and spring months (from February to June) . RSV occurred in odd numbered years during the spring and fall months. Influenza viruses were found during the winter months in well-detected epidemics. Table 12 shows the seasonality of respiratory viruses.

Follow-up nasal swab samples were taken during 27 febrile episodes within 1-11 days, and in 23 cases the test had changed to virus negative. Three children remained rhinovirus positive and one child RSV positive. One child remained rhinovirus positive in four samples for three weeks. In 9 patients, two consecutive febrile episodes were associated with the same virus in 12 pairs (RSV 6 pairs, rhinovirus 3 pairs, enterovirus 2 pairs, influenza A 1 pair). The intervals between these consecutive episodes varied from 1 week to 3 months (mean 5.8 weeks, SD 5.4). One child was rhinovirus positive in three consecutive febrile episodes over 4 months.

Table 12. Seasonal distribution of respiratory viruses detected during 138 febrile episodes in 51 children with leukemia.

Virus	*Aug*	*Sep*	*Oct*	*Nov*	*Dec*	*Jan*	*Feb*	*Mar*	*Apr*	*May*	*Jun*	*Jul*	*Total*
Rhinovirus	1	3	7	1		1	4	2	2	2	8		31
Respiratory syncytial virus*		1	4	1	1	1	3		1	1	1	1	15
Human herpes virus 6	2	1	1	2	1		1	1				1	10
Enteroviruses*	1			1			1		1				4
Parainfluenza type 2					1				2				3
Parainfluenza type 3				1			1		1	1			4
Influenza A					2		1	1	1				5
Influenza B						2							2
Coronaviruses types OC43 and 229E									1				1
Cytomegalovirus			1				2			4			7
Adenovirus	1	1	1		1								4
Bocavirus		1		1	1		1	2		1			7
Rotavirus#				1				1	1				3
Calicivirus#						1			2				3
Total	5	7	14	8	7	5	14	7	12	9	9	2	99¤

* Epidemics in Finland in odd numbered years

Viruses were found equally in community-acquired and nosocomial febrile episodes (53% vs. 53%, respectively).

The occurrence of the different viruses according to age was not statistically significant (p> 0.05 by the Poisson regression model).The occurrence of positive rhinovirus specimens was more common in the age group of 3 to 6 years, 15.0%, compared to 9.1% in the age group of under 3 years and 9.3% in children older than 7 years of age. The occurrence of RSV infections was 12.1%, 7.5%, and 9.3% in the similar age groups, respectively.

Table 15 shows the influence of neutropenia and lymphopenia on the occurrence of febrile episodes with evidence of respiratory viruses. The occurrence of viral infections was 33% during neutropenic fevers compared to 25% during non-neutropenic fevers (p= 0.47). Furthermore, the occurrence of sole viral infections in the presence of lymphopenia was 32% compared to 14% among non-lymphopenic fever episodes (p=0.13).

5.2.2 Bacterial infections

Bacterial blood cultures were positive in 19 of 156 (11%) febrile episodes in 17 children with leukemia, and in 11 (58%) of the episodes in 11 children evidence was found for concomitant viral infection. Six of them had symptoms before the blood culture was taken. Table 14 shows details of the febrile episodes with blood culture positive infections. Most septicemias (79%) occurred during the induction treatment of leukemia.

Of the septicemias, 79% (15/19) occurred in association with neutropenia and 70% (13/19) in association with lymphopenia.

Bacterial urine culture was positive in 2 cases, both caused by *Enterococcus faecalis*.

The rapid diagnostic test for urinary pneumococcal antigens was positive in 6 febrile episodes. In two cases, it was associated with blood culture positive infections caused by *Streptococcus mitis* with concomitant viruses (rhinovirus and RSV). In one case, it was associated with septicemia caused by *Streptococcus sanguis*. In three other cases, concomitant viruses were detected from a blood sample and from nasal swab samples (CMV from the blood sample, HHV-6 and adenovirus from nasal swab samples).

5.2.3 Fungal infections

Only one child was blood culture positive for non-albicans *Candida* in one febrile episode. Antigen detection for *Aspergillus* was positive in 2 children in 2 febrile episodes, both during the maintenance phase of leukemia treatment. Fungal infections only occurred in association with neutropenia and lymphopenia.

5.3 Clinical profile of febrile episodes (I – III)

5.3.1 Signs and symptoms, serum CRP levels

Table 13 shows the clinical signs and symptoms of viral infections. The respiratory viral infections were mild in most cases and progressed to pneumonia in 2 children, one with RSV and another with rhinovirus infection. Respiratory or gastrointestinal symptoms i.e. cough, rhinitis, sore throat, or diarrhea were recorded in 46/82 (56%) children with virus compared to 30/56 (53%) of those without virus (p=0.38).

Table 13. Signs and symptoms of 85 febrile viral infections in 51 children with leukemia.

Virus	Total number of episodes	Respiratory symptoms (%)*	Skin symptoms (%)	Gastrointestinal symptoms (%)
Rhinovirus	31	18 (58)*	1 (3)	3 (10)
Respiratory syncytial virus	15	7 (47)**		1 (7)
Human herpes virus 6	10	3 (30)		
Human bocavirus	7	3 (43)		1 (14)
Cytomegalovirus	7	3 (43)		2 (29)
Influenza A virus	5	2 (46)		
Enteroviruses	4	1 (25)	2 (50)	1 (25)
Parainfluenza type 3 virus	4	3 (75)		1 (25)
Adenovirus	4	2 (50)		2 (50)
Parainfleunza type 2 virus	3	1 (30)		
Influenza B virus	2	1 (50)		1 (50)
Coronavirus types OC43 and 229E	1	1 (100)		
Herpes simplex virus	1			
Dual viral infections	17	8 (47)		3 (18)
Mixed bacterial-viral infections	11	3 (27)	1 (9)	3 (27)

*13 episodes with cough, 7 with rhinitis,2 with sore throat, 1 with a skin manifestation, 1 with eye symptoms
** 6 episodes with cough, 2 with rhinitis, and 1 with sore throat.

Two patients with a low copy number of HBoV had manifestations of febrile respiratory disease and gastroenteritis. A third patient was positive for HBoV in five consecutive febrile episodes. Fever was the only symptom of infection in three of the five HBoV positive episodes. In two episodes, the patient had rhinitis, stomatitis, and wheezy bronchitis.

Parvovirus infection was detected from the paired serum samples with IgM response, and PCR for parvovirus was also positive from the blood sample. The patient with parvovirus infection had rash and fever for four days. During the follow-up, one varicella zoster infections were seen. That child had a primary infection with VZV antigen positive from a skin lesion.

Among children with blood culture positive infections, 4 of 9 with concomitant respiratory virus (3 rhinovirus positive cases and 1 RSV positive case) had respiratory symptoms, i.e. rhinitis and cough 1-6 days prior to onset of septicemia.

The mean duration of fever was 2.6 (SD 1.7) days among children with respiratory virus infections and 2.1 (SD 1.3**)** days in children with fever of unknown origin (p= 0.44). The mean duration of fever was 3.3 (SD 1.9) days in the children with septicemia, and it was not influenced by a viral co-infection; fever lasted for 3.2 (SD 2.2) days in virus positive cases compared to 3.3 (SD 1.6) days in virus negative cases.

The first serum CRP level in febrile episodes caused by respiratory viral infection (n = 37) was 32 mg/l (SD 27, range 1-195) compared to 67 mg/l (SD 46, range 5-306) in febrile episodes with blood culture positive septicemia (n=19) (p=0.04). The highest CRP level in the group of respiratory viral infections was a mixed viral-fungal infection caused by rhinovirus and Aspergillus flavus detected from sinuses. The mean CRP level in FUO episodes (n = 17) was 30 mg/l (SD 28, range 1-160)

5.3.2 Treatment of febrile episodes

All except three of the febrile episodes were treated with broad-spectrum antibiotics, usually with cloxacillin and ceftazidime. Specific antiviral treatment was given to one child with influenza A infection (zanamivir) and two children with varicella zoster infection (both received acyclovir).

5.3.3 Outcome of febrile episodes

Two children died of infection during follow-up, both without remission in leukemia. The first died of *Streptococcus mitis* septicemia with evidence of concomitant rhinovirus infection. The other died of invasive *Aspergillus fumigatus* infection with preceding rhinovirus involvement in nasal swab samples. Most of the children recovered uneventfully from their febrile episodes during anticancer treatment.

Table 14. Patient characteristics and clinical features of 11 leukemic children with mixed viral-bacterial infections.

No	Virus	Blood culture finding	Virus detection method	Age in years/sex	Underlying disease	Treatment at presentation	Clinical features	Serum CRP level (mg/L)
1	Rhinovirus	*Streptococcus mitis*	RT-PCR	2.2/F	AML, relapse	Induction	Fever for 3 days, vomiting,diarrhea, dying after 3 days	147
2	Rhinovirus	*Staphylococcus epidermidis* and *Bacillus cereus*	RT-PCR	3.8/F	ALL, standard	Consolidation	Fever for 1 day, rhinitis, cough	< 5
3	Rhinovirus	*Streptococcus mitis*	RT-PCR and culture	2.3/F	ALL, standard	Induction	Fever for 2 days, rhinitis, cough	51
4	Rhinovirus	*Escherichia coli*	RT-PCR	3.4/F	ALL, intermediate	Maintenance	Fever for 1 day, rhinitis, cough	12
5	Respiratory syncytial virus	*Staphylococcus hominis* and *Enterococcus faecium*	Antigen detection	3.4/M	ALL, intermediate	Induction	Fever for 3 days, vomiting	49
6	Respiratory syncytial virus	*Bacillus cereus*	Antigen detection	3.6/F	ALL, standard	Induction	Fever for 1 day	< 5
7	Respiratory syncytial virus	*Staphylococcus capitis*	Antigen detection	9.0/F	ALL, intermediate	Consolidation	Fever for 1 day	58
8	Respiratory syncytial virus, Enterovirus Rhinovirus	*Streptococcus mitis*	Antigen detection RT-PCR	3.6/F	ALL, standard	Induction	Fever for 7 days, skin rash	44
9	Calicivirus	*Staphylococcus aureus* and *Klebsiella pneumoniae*	RT-PCR	2.4/F	ALL, standard	Induction	Fever for 2 days	216
10	Calicivirus	*Lactococcus lactis* and *Bacillus cereus*	RT-PCR	2.8/F	ALL, high risk	Induction	Fever for 7 days, diarrhea	123
11	Human herpes virus 6	*Staphylococcus epidermidis*	RT-PCR	4.0/M	AML	Induction	Fever for 7 days	306

ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, CRP = C-reactive protein, RT-PCR = reverse transcriptase PCR, F = female, M = male

Table 15. Occurrence of respiratory viruses in 138 febrile episodes detected by nasal swabs in 51 children with leukemia The proportions of positive viral tests of all detected episodes are shown according to neutrophil and lymphocyte counts.

Virus	Neurophils ≤ 0.5x10^9/l %	Neutrophils ≤ 1.0 x10^9/l %	Neutrophils >1.0 x10^9/l %	Lymphocytes ≤ 1.4x10^9/l %	N
Rhinovirus	32	31	10	20	31
Respiratory syncytial virus	12	12	13	12	15
Human herpes virus 6	11	8	23	11	10
Human bocavirus	0	4	0	3	7
Cytomegalovirus	0	0	4	2	7
Influenza A virus	3	4	2	4	5
Parainfluenza type 3 virus	2	3	4	4	4
Enteroviruses	9	9	0	5	4
Adenovirus	2	1	4	3	4
Parainfluenza type 2 virus	0	0	0	0	3
Influenza B virus	0	1	0	1	2
Coronaviruses (OC43 and 229E)	2	2	0	1	1

* The total occurrence includes the results from blood and stool samples.

5.3.4 MxA protein suggesting viral infection in febrile children with cancer (IV)

MxA protein levels in cancer patients and acute fever were significantly higher than in afebrile cancer patients (median fluorescence intensity 482 vs. 116, p< 0.001) (Table 16). No difference was seen in MxA protein expression between afebrile cancer patients and healthy children (median fluorescence intensity 118 vs. 72, p= 0.21). Patients with laboratory confirmed viral infections had higher MxA protein levels than cancer patients with infections of bacterial or unknown etiology (median fluorescence intensity 717 vs 435, p<0.05). Enterovirus and influenza A virus infections were the most potent MxA protein inducers (intensities varying from 711 to 1669), whereas rhinovirus was the weakest (median intensity of 316) (Figure 7).

A significant increase was seen in MxA protein expression following administration of high-dose MTX and Ara-C. The median fluorescense intensities in febrile children with cancer were higher than in patients receiving cytostatic chemotherapy. The median MxA fluorescense intensity before administration of cytostatic agents was 120. After 24 hours, the value was 187 and it increased up to 268 within 72 hours (both values differing significantly from the baseline, p< 0.005). In only four of these 30 periods of chemotherapy evaluated did the patient have fever. One child was PCR positive for rhinovirus and enteroviruses. One child of 15 was PCR positive for HHV-6. Her MxA protein level was 523. None of the patients were PCR positive for cytomegalovirus.

Table 16. Patients characteristics of children with detected expression of MxA protein

Patient diagnosis	Infection or symptoms	MxA* level	CRP mg/l	WBC x 10^9/l
1. ALL (SR), induction	Enterovirus, *Streptococcus mitis* septicemia	881	70	2,30
1. ALL (SR), induction	Fever and cough, skin infection	562	15	1,70
2. ALL (SR), remission	Staphylococcus aureus abscess	482	42	3,20
3. ALL (SR), remission	Influenza A	723	<1	0,70
4. ALL (HR), remission	Fever of unknown origin	220	41	4,10
5. ALL (HR), remission	Rhinovirus	316	12	1,90
6. AML , remission	Fever of unknown origin	598	61	0,30
6. AML, remission	Fever of unknown origin	685	10	0,20
7. Hodgkin's disease (st IVB)	Enterovirus and rhinovirus	1669	102	1,80
7. Hodgkin's disease(st IVB)	Pneumocystis carinii pneumonia	324	65	1,70
8. Non-Hodgkin lymphoma	Herpes simplex 1	330	29	0,40
9. Lymphoma (mediastinal)	Fever of unknown origin	667	68	0,80
10. Neuroblastoma	Fever and cough	425	14	4,30
11. Neuroblastoma	Fever and mucositis	552	78	0,10
12. Hepatocellular carcinoma	Fever and mucositis	55	98	0,70
13. Germinoma (pineal)	Influenza A virus	711	24	0,50
14. Sacral teratocarcinoma	Adenovirus	403	84	10,90
15. Wilms' tumor	Fever and cough	582	27	1,80
Controls (n = 20)	Healthy	72**		

CRP = C-reactive protein, WBC = white blood cell count, ALL = acute lymphoblastic leukemia, AML = acute myeloid leukemia, SR = standard risk, HR = high risk.

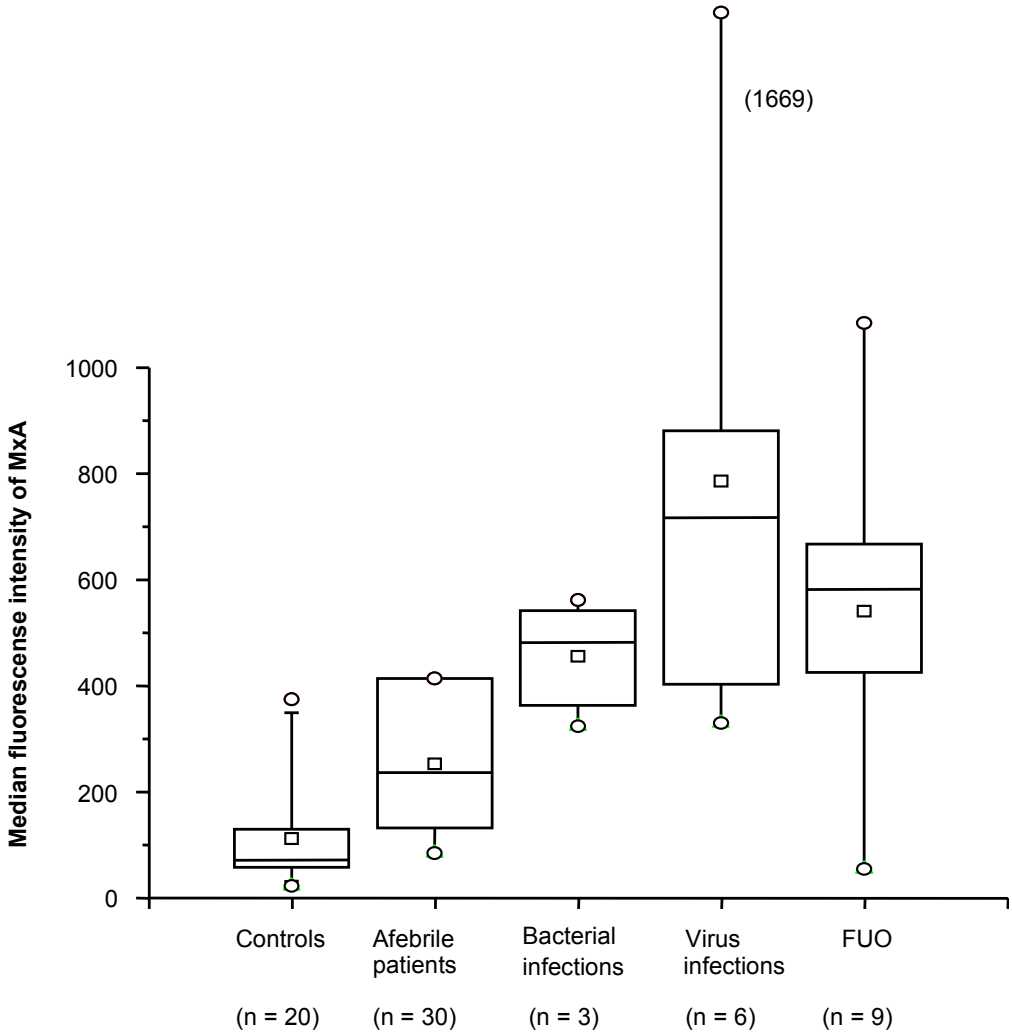

Figure 7. The expression of MxA protein in the different patients groups. The outline of the boxes are the 25% and 75% percentiles where the plots outside the boxes are the maximum and minimum values. The square indicates the mean value.

6 DISCUSSION

6.1 Occurrence of febrile episodes

During 27,743 patient days at risk, the occurrence rate of febrile episodes in leukemic children was 2.1 / PYR. This finding is in agreement with those of earlier studies showing rates of 1.4 – 2.1 / PYR (Lehrnbecher et al., 2004; Mahmood et al., 1996; Möttönen et al., 1995; Rahiala et al., 1998). During the anticancer treatment, febrile episodes with neutropenia or lymphopenia were more common than febrile episodes with normal neutrophil or lymphocyte counts.

The occurrence of febrile episodes with evidence of viral etiology in children with leukemia was 1.0 / PYR. In previous studies, this occurrence has varied from 0.2 / PYR to 0.8 / PYR (Arola et al., 1995; Christensen et al., 2005b; Möttönen et al., 1995). These figures are lower than those for respiratory infections in otherwise healthy children. It should be stressed that the occurrence of respiratory tract disease in healthy children is greatly influenced by age and the form of day care (Griffin et al., 2004; Iwane et al., 2004; Lambert et al., 2007; Louhiala et al., 1995; Monto, 1994; Nokso-Koivisto et al., 2002).In one Finnish questionnaire study, the occurrence of respiratory infections among day care center children $\leq$ 1 year of age was 5.1 / PYR but 1.9 / PYR among children 6-7 years of age in home care (Louhiala et al., 1995). The mean age of leukemic children in this study was 5.9 years, and they were not treated in day care centers. This may partly explain the low occurrence of febrile viral infections. In addition, only febrile respiratory infections were recorded in this study, and they only account for part of all symptomatic respiratory infections.

An interesting finding was the individual variation in the occurrences of febrile episodes (Figure 6). Of 36 children followed throughout the treatment, 5 had recurrent febrile episodes (≥ 6), whereas 11 had only a few (≤ 2) episodes). One child with high-risk leukemia had 17 febrile episodes and another with standard risk leukemia had no febrile episodes during leukemia treatment. This phenomenon is also seen in healthy children. In one Finnish study, 13% of 329 children aged 2-24 months had no respiratory tract infections during the 22-month follow-up, whereas 24% had ≥ 9 episodes. Viral etiology did not correlate to the frequency of respiratory tract infections (Nokso-Koivisto et al., 2002). In addition to differences in the intensity of antileukemia treatment, differences in the immune system may explain differences in susceptibility to infections. Very recent studies have shown several subtle genetic determinants of susceptibility to respiratory infections, including G2m(n) allotypes, FcγRIIa polymorphism, partial C2 and partial C4 deficiency, promoter polymorphism in mannose binding lecting 2, TNF-alpha polymorphism, and IL-6 polymorphisms (Bossuyt et al., 2007; Jonsson et al., 2006; Patel et al., 2007). Coexistence of two or more subtle immune defects may explain the increased the risk for recurrent respiratory infections (Bossuyt et al., 2007; Jonsson et al., 2006). These subtle immunologic defects should also be studied in leukemic children with recurrent febrile episodes.

Most febrile episodes (78%) in the present study were community-acquired. Recently, Urrea et al from Spain reported a high incidence (9.5 /PYR) of nosocomial bacterial infections in children with lymphoblastic leukemia (Urrea et al., 2004). The marked difference to our findings may simply reflect differences in the occurrence of infections between different countries. In Finland, leukemic children are actively discharged

between treatment cycles, making them more susceptible to community-acquired infections.

The occurrence of septicemia ranged from 1.0 / PYR during the induction phase to 0.04/PYR during the maintenance phase, accounting for 14% of febrile episodes. Previously, the occurrence of septicemia has been found to vary from 8.5% to 28%, greatly influenced by the stage of immunosuppression. As expected, neutropenia and lymphopenia also made children susceptible to bacterial septicemia in this study.

6.2 Etiology of febrile episodes

6.2.1 Respiratory viral infections

Evidence of respiratory viral infection was found in 59% of febrile episodes in children with leukemia. Earlier, evidence of respiratory viral infections in children with cancer has been found in 8% to 43% of febrile episodes (Arola et al., 1995; Christensen et al., 2005b; Long et al., 1987; Möttönen et al., 1995; Wood et al., 1985). Rhinovirus, RSV, and HHV-6 were the most frequently detected viruses. FUO accounted for only12% of the febrile episodes. The strength of our study is that it was a prospective survey by 4 centers and lasted for 5 years. The survey accounted for many respiratory virus outbreaks. We searched for 16 respiratory viruses using several different virologic techniques in 36 children during the entire treatment period of 2 years.

Nasal swabs were used as a method for detection of respiratory viruses. Most studies of respiratory viral infections have used nasopharyngeal aspirates (NPA) or nasal washes (NW). NPA gives a mucus sample with good virus recovery rates (Jartti et al., 2004b; Juven et al., 2000; Ruohola et al., 2006). However, NPA retrieval is very unpleasant for the patient and requires a suction device, and, more importantly, it may increase the risk of nasal bleeding in trombocytopenic children with hematologic malignancies. Nasal washes are widely used in the USA. Like NPA, NW requires special devices, and the mucus sample obtained is highly diluted. It has been well demonstrated that nasal swab is a sensitive sampling method for the detection of respiratory viruses in children. Only RSV is an exception. It was detected more often in NPA samples than in nasal swabs when it was searched for by viral culture (sensitivities 97% and 76%, respectively) (Heikkinen et al., 2002) The sensitivity of swab samples rose up to 96% when PCR was used to detect RSV (Waris et al., 2007). In this study, the number of RSV infections could have been higher if PCR had been used for the detection of RSV. However, the high recovery rate (59%) of respiratory viruses favors increased use of nasal swabs for the detection of respiratory viruses in immunosuppressed children. The collection of a nasal swab is simple, safe, painfree, and tolerable for repeated use (Covalciuc et al., 1999; Hall et al., 1975; Heikkinen et al., 2002; McIntosh et al., 1993; Schmid et al., 1998).

The PCR technique was invented by Kary Mullis in1983. The technique has been revolutionary in clinical viral diagnostics. For many respiratory viruses, it is clearly more sensitive than virus culture or virus antigen detection. PCR is especially sensitive for the detection of respiratory picornaviruses (rhinovirus and enteroviruses) (Hyypiä et al., 1998; Vuorinen et al., 2003; van Elden et al., 2002; van Kraaij et al., 2005). Two studies from Turku University have shown that the sensitivity of PCR technique for picornaviruses is 85-97% compared to the 21-25% sensitivity of virus culture (Jartti et

al., 2004b; Vuorinen et al., 2003). In one study of adults with hematologic malignancies, respiratory virus was detected by virus culture in 21% of the cases compared to 63% detected by PCR (van Kraaij et al., 2005). In another retrospective study of 43 patients, virus culture and virus antigen detection of bronchoalveolar lavage showed 9 respiratory viruses. PCR added 8 more respiratory viruses, increasing the recovery rate from 19% to 35% (van Elden et al., 2002). In this study, PCR detected 33 picornavirus positive cases compared to only 4 found by virus culture. It is interesting that with the advances in viral detection methods, the causative viral agent of respiratory infections in children can now be obtained in up to 95% of cases of clinically suspected viral infection (Allander et al., 2007b). Whether there are still unrecognized respiratory viruses or whether the current detection methods are still insensitive remains to be determined.

Rhinovirus was the most frequently detected virus. Eighteen children were rhinovirus positive in 31 of 138 febrile episodes, but the virus mainly caused mild respiratory disease. Earlier on, only 5 studies have addressed the role of rhinovirus in febrile infections in children with cancer (Table 5). Of the 715 febrile episodes of the table, 67 (9%) were positive by virus culture. Only one study has used PCR, and of 206 oral washes (which is not an appropriate sampling method) 7 were rhinovirus positive. The findings of the present study suggest that rhinovirus infection plays a more important role in the etiology of infections in immunocompromised children than has been recognized earlier. Eleven of 73 children with primary immunodeficiency had rhinovirus in their respiratory tract. Four of them cleared the virus successfully, but 7 had severe mixed infections and, finally, 5 died of pneumonitis (Crooks et al., 2000). The clinical significance of rhinovirus infections in leukemic children was low. This finding agrees with those of Arola et al; of their 13 rhinovirus culture positive patients none developed pneumonia and all children recovered uneventfully from the infection (Arola et al., 1995). In adult HSCT patients, rhinovirus infections can readily progress to pneumonia and be ultimately fatal. Ghosh et al followed 22 adult myelosuppressed patients with rhinovirus infection. In 15 of them (68%), the infection remained confined to the upper respiratory tract. Seven patients (32%) developed pneumonia, fatal in all seven (Bowden, 1997; Crooks et al., 2000; Ghosh et al., 1999; Long et al., 1987). Kaiser et al recently reported the first cases of chronic rhinovirus lung infections in lung transplant recipients. Transbronchial biopsy samples showed rhinovirus in the lung parenchyma over a 12-month period. Rhinovirus was identified by PCR in three patients and by culture in two patients. Two of the patients died (Kaiser et al., 2006).

PCR showed HHV-6 in the nasal swabs of 8 patients and in the plasma of two patients. The clinical significance of these findings should be addressed with caution. In HSCT recipients, HHV-6 often leads to viremia, and it may be associated with encephalitis and delayed platelet engraftment. On the other hand, HHV-6 DNA can also be detected in the serum of immunocompetent children without clinical signs of disease. The causality of a detected virus and a clinical disease is sometimes difficult to establish. Causality is supported by a serological response, which can be obtained after positive herpes virus DNAemia. Furthermore, new quantitative PCR techniques may clarify the role of HHV-6 viruses as causative infectious agents (Hermouet et al., 2003; Pitetti et al., 2003). The HHV-6 identified by qualitative PCR may have been a consequence of latent infections without reactivation. Many reports state that herpes simplex virus infection is common in immunosuppressed children. This statement is based on one study carried out 20 years ago (Wood et al., 1985). This study identified

only one herpes simplex virus. This finding agrees with a recent study finding herpex simplex by PCR in the oral swabs of 7 (9%) and blood samples of 2 (3%) of 75 febrile neutropenic children undergoing chemothepapy for cancer (Ramphal et al., 2007).

In this decade, 5 new respiratory viruses have been discovered, i.e., human metapneumovirus, SARS coronaviruses, coronavirus NL63, coronavirus HKU1, and human bocavirus. All these viruses induce respiratory diseases in humans (Allander et al., 2001; Hon et al., 2003; van den Hoogen et al., 2001). In the present study, hMPV and HBoV were searched for. No cases of hMPV were identified. HBoV was discovered in 2001 by Allander et al in a large-scale molecular screening from the respiratory samples of children with lower respiratory tract disease (Allander et al., 2001). Many studies worldwide have since confirmed the common presence of HBoV in children with both upper and lower respiratory disease (Kaplan et al., 2006; Kesebir et al., 2006; Kleines et al., 2007; Kupfer et al., 2006; Maggi et al., 2007; Malcolm et al., 2001). Seroepidemiologic studies suggest that HBoV is a ubiquitous virus acquired early in life (Endo et al., 2007). The association of HBoV and respiratory disease has been questioned because coinfections with other respiratory viruses occur in 33-83% of HBoV positive cases (Allander et al., 2007b; Arnold et al., 2006). Recently, HBoV was identified in 19% of 259 children hospitalized in Turku University Hospital for acute expiratory wheezing. In 12 cases (5%), HBoV was the only virus detected, and in 10 of these cases a high viral load was recorded as well as a serum IgM or IgG response strongly suggesting a primary infection (Kantola K et al., 2008). Furthermore, HBoV DNA was identified in the serum of these patients, suggesting a systemic infection (Allander et al., 2007b). In the present study, the first three cases of HBoV infection in children with cancer were identified. Most of the episodes were associated with HboV in low copy numbers, which we have considered to present asymptomatic shedding in immunocompetent children. Interestingly, one patient with repeated detection of HBoV showed prolonged shedding or reactivation over a 5-month period, supporting the hypothesis that HBoV may persist for a long time after a primary infection. One may speculate that the high viral load detected in two febrile episodes indicates that HBoV could be a causative agent of febrile episodes, while HBoV in low genome copy numbers may represent carriage.

6.2.2 Mixed bacterial-viral infections

Evidence of viral co-infection was found in half of the 19 blood culture positive bacterial infections. This is a novel and preliminary finding in children with cancer. Most children with positive viral tests had a symptomatic respiratory infection or gastroenteritis at the time of septicemia, supporting the view that virus infection had preceded the septicemia. The number of cases in this study was limited but further emphazises the need of larger prospective studies to confirm this phenomenon.

It is well established that viral infections are often complicated by secondary bacterial infections, and concomitant viral-bacterial infections are commonly identified in acute otitis media and pneumonia (Juven et al., 2000; Ruohola et al., 2006). Recently, both virus and bacteria could be identified in 66% of middle ear fluid samples in 79 children with AOM (Ruohola et al., 2006). The classic view is that viruses pave the way for bacterial infections. Epidemiologic studies of influenza and RSV outbreaks have shown a temporal association between viral infections and secondary bacterial infections (Watson et al., 2006). RSV has also been recognized as predisposing factor for

secondary bacterial infections like pneumonia caused by *Streptococcus pneumoniae* in hospitalized children (Korppi et al., 1989). Using serological methods and virus culture, a seasonal association has been found between influenza A infection and meningococcal infections (Cartwright et al., 1991). Recently, Peltola et al found that 78% of their cases with invasive pneumococcal infection had a respiratory viral co-infection, predominantly a rhinovirus or an enterovirus infection (Peltola et al., 2006b). Pneumococcal disease in children has also been associated with influenza or RSV infections (Watson et al., 2006). With another viewpoint, Dunlop et al investigated whether respiratory virus infections predisposes children to severe meningococcal disease (Dunlop et al., 2006). They searched for 12 respiratory viruses in 104 suspected cases and controls, finding evidence that respiratory viral infections contributes to the prodrome of meningococcal disease. Rhinovirus and adenovirus were commonly detected in cases and controls.

The findings of this study suggest that blood culture positive septicemia should no longer be considered simply a sole bacterial infection. Mixed viral-bacterial infections may be more common and clinically more important than earlier understood.

6.3 Clinical profile of febrile episodes

Respiratory viruses induce respiratory infections usually characterized by rhinitis, sore throat, cough, and fever. Some patients develop no respiratory symptoms (Peltola et al., 2006b). In the present study, only half of the febrile children undergoing chemotherapy for leukemia with respiratory virus (rhinovirus or RSV) in their respiratory tract had respiratory symtoms, and the clinical signs and symptoms were mainly mild. The most common symptom of respiratory virus infections was cough alone. This raises two important issues; half of febrile respiratory infections are otherwise asymptomatic, and respiratory symptoms do not develop fully during antileukemia treatment or the respiratory viruses, e.g., rhinovirus or HHV-6, detected are just innocent bystanders without any inflammatory reaction and have nothing to do with the fever recorded. The clinical implication is, however, clear: respiratory viruses should be searched for in febrile leukemic children whether they have respiratory symptoms or not.

Patients with bacterial-viral co-infections have often had more severe clinical profiles and slower responses to antibiotic treatment than those with sole bacterial infections. A plausible explanation is that viruses strengthen the bacteria-induced inflammation either locally in the respiratory tract or generally (Chonmaitree et al., 1996). This phenomenon is well-recognized in the treatment of otitis media and pneumonia (Chonmaitree et al., 1990; Watson et al., 2006). The symptoms of otitis media of a mixed viral-bacterial etiology can persist longer than an otitis with sole bacterial etiology (Ruohola et al., 2006). Dual virus infections may also increase the severity of illness. This phenomenon was reported by Aberle et al from a prospective study of single versus dual respiratory virus infections in 772 hospitalized infants (Aberle et al., 2005). They found that dual infections with RSV involvement were associated with a decreased IFN-gamma response in PBMCs and an increase in disease severity. In our study, CRP levels tended to be higher in septicemia associated with a viral co-infection than in sole bacterial disease and significantly higher than in the comparison group of febrile episodes associated with high-dose cytarabine therapy with no evidence of viral or bacterial etiology. The only child dying of bacterial septicemia had rhinovirus both in her nasal swab and stool samples.

Respiratory infection progressed to pneumonia in only two cases. One was associated with rhinovirus and the other with RSV. In contrast to pediatric patients, myelosuppressed adult patients with respiratory viral infections have been reported to have high rates of fatal pneumonias. Adults have more frequently a specific site of infection, the lower respiratory tract being the most common site. (Ghosh et al., 1999; Ison et al., 2003).

6.4 Treatment, outcome and prevention

All febrile episodes, except three, were treated with intravenous broad-spectrum antibiotics. When most of the episodes were induced by respiratory viruses, a question arises whether antibiotic treatment of all respiratory virus infections is really necessary in leukemic children. First, intravenous antibiotic treatment usually necessitates hospitalization and is unpleasant to the child and the parents. Second, excessive antibiotics (especially third generation cephalosporins) cause antimicrobial resistance problems. Third, the use of broad spectrum antibiotics increases the risk of invasive fungal infections in immunosuppressed children. Fourth, intravenous antibiotic treatment is usually very expensive. (Buxmann et al., 2003; Cotten et al., 2006; Hibbert-Rogers et al., 1995). It is evident that undertreatment of infections in leukemic children is today no problem. On the contrary, it seems that overtreatment may be a problem. We should assess the risk of each febrile episode accurately, and reduce admissions with reliable risk stratification and safely move from intravenous antibiotics to oral antibiotics and treat some cases without any antibiotics at all. A similar risk prediction score system is available for adult cancer patients (Klastersky et al., 2006; Klastersky et al., 2007; Nijhuis et al., 2005). Only a few studies in children with cancer have been conducted for evaluation of risk prediction criteria (Alexander et al., 2002; Mullen et al., 1990; Phillips et al., 2007). Phillips et al evaluated treatment policies in United Kingdom and concluded the following low-risk criteria: The child has to be clinically well, have a negative blood culture (for at least 48 hours), have evidence of neutrophil recovery or neutrophil count > 0.1 x 10^9/l. If these low-risk criteria are met, a child with febrile neutropenia can be discharged with oral antibiotic treatment.

Specific antiviral treatment was given only to two children; one child with influenza A infection (zanamivir) and another with varicella zoster infection (acyclovir). This is understandable because most of the respiratory viral infections were mild. Several antivirals are available for use in immunosuppressed children (Table 5). Point of-care tests can be used for rapid detection of influenza A and B viruses, RSV, and adenovirus. When considered necessary, all these infections may be treated with specific antivirals (Table 7); influenza viruses with oseltamivir, RSV with ribavirin and adenovirus with cidofovir. (Boeckh et al., 2006; Hayden et al., 1999; Johny et al., 2002). Rapid 2-hour PCR techniques are being developed for picornaviruses, and pleconaril should be available for severe enterovirus and rhinovirus infections (Hoffman et al., 2001; Ljungman, 2002; Rotbart et al., 2001). It is evident that new antivirals will be developed in the near future. Pleconaril activates the CYPA3 enzyme system and can therefore not be used in combination with many other drugs. Studies of intranasal pleconaril are being designed. New approaches to antivirals will be developed, e.g., serotonin reuptake inhibitor mirtazapine for the treatment of progressive multifocal leukoencepalopathy induced by JC polyoma virus (Verma et al., 2007).

Two of 51 children died during the present follow-up. One had persistent leukemia, and the other had relapsed disease, and they both finally died of infection. With the advent of aggressive management, the outcome of infections in children with leukemia has improved dramatically. Mortality has dropped from 30% in the 1970s to 1% in the late 1990s (Orudjev et al., 2002). Intensive care is needed in less than 5% of cases (Hann et al., 1997).

It is obvious that infections will remain a significant cause of treatment-related death in children with leukemia.

Intravenous or subcutaneous immunoglobulin therapy offers one approach to the protection of the immunocompromised host against viral infections. Today, prophylactic intravenous immunoglobulin is given to patients after HSCT to protect the immunosuppressed host from infection (Quinti et al., 2007; Winston et al., 2001). Immunoglobulin prophylaxis should also be considered in leukemic subjects who are highly prone to febrile infection, like the child of this study with 17 febrile episodes. Specific passive immunization against RSV with palivizumab (humanized anti-F protein) is available, but it very costly, and together with the already available drug, ribavirin, it lacks extensive studies in immunosuppressed children (Boeckh et al., 2001). In addition to intravenous immunoglobulin, leukemic children with recurrent ferile episodes may benefit from antimicrobial prophylaxis, as shown in children with recurrent acute otitis media or chronic active otitis media (De Diego et al., 2001; van der Veen et al., 2007). The use of G-CSF and other supportive care methods should be evaluated individually and according to severity of the infectious disease.

6.5 Differentiation of viral from bacterial infections in febrile children undergoing chemotherapy for cancer

In this study of children with cancer, elevated MxA protein expression levels were detected after viral infections but not after bacterial infections. Rhinovirus was an exception and did not affect the MxA protein expression during the febrile episodes. This finding is in agreement with earlier ones. Apparently, most rhinovirus infections remain local in the nasopharynx and do not induce any systemic type I interferon response (Mäkelä et al., 1999; Peng et al., 2007). Interestingly, moderately elevated MxA protein levels were recorded following the administration of cytostatic agents in afebrile children with cancer. This may have resulted from a chronic viral infection with reactivation leading to low-level production of type I interferons and, subsequently, elevated MxA protein expression.

Determination of MxA protein expression in patient PBMCs offers a promising tool to be used to distinguish viral infections from bacterial ones. The inadequacy of MxA protein in expressing viral infection in a child with rhinovirus infection is a limitation, because rhinoviruses are the most frequent cause of respiratory virus infections (Arruda et al., 1997; Hayden, 2004; Mäkelä et al., 1998; Winther et al., 2007).

6.6 Limitations of the study

Although many viruses were searched for, they all were not investigated in all episodes included in the study (Figure 3). In addition, the yield of respiratory viruses would probably have increased if more viruses like coronavirus HKU1, coronavirus NL63, parainfluenza virus type 4, and influenza C virus could have been searched for

(Allander et al., 2007b). Furthermore, all respiratory viruses were not searched for by PCR. The occurrence of respiratory viruses in afebrile children with leukemia was not studied. The clinical importance of a positive rhinovirus PCR test can be questioned, since several studies have identified rhinovirus RNA in 12-35% of asymptomatic subjects (Jartti et al., 2004a). Most of the present follow-up samples studied were negative, but the persistence of viruses remained unsolved. As earlier studies have shown, the viral shedding of rhinovirus, RSV, influenza and parainfluenza viruses may be prolonged in immunosuppressed patients compared to immunocompetent hosts, and, on the other hand, some viruses like HHV-6 or parvoviruses may reactivate during immunosuppression (Hicks et al., 2003).

Children with cancer are a highly specific group of patients. These children need to follow certain isolation policies and moreover, they are experiencing multiple procedures like blood withdraws and chemotherapy which induce some pain as well. It is sometimes difficult to follow a study protocol with all needed samples when a child with leukemia is not feeling well. In this study, viral samples could not be taken in 18 of the 156 febrile episodes.

A multicenter study is a challenging project. Quality monitoring is needed to obtain data with a high standard and a minimum amount of gaps. One of the limitations of the present study was a lack of sufficient monitoring, which is mainly explained by our limited financial resources. No telephone follow-up was organized. Febrile episodes were probably not, however, underreported because the parents of Finnish leukemic children very carefully follow the advice to bring the child to the hospital immediately whenever fever occurs.

6.7 Future challenges

The findings of the present studies clearly emphasize the benefits of common use of respiratory viral detection in febrile leukemic children. However, it is important to recognize that leukemic children usually recover well from respiratory viral infections and severe bacterial complications are rare. The necessity of treating viral infections with antibiotics in leukemic children with normal neutrophil counts is an important question. Careful controlled studies of the need of antibiotics used to treat febrile episodes during the maintenance treatment of leukemia are needed. The significance of accurate and rapid viral diagnostics will be emphasized with the advent of new antivirals. The approach to treating viral infections by supporting the immune system of children with leukemia using new immunologic agents is challenging. Improvements in evaluation and prevention of disease transmission are needed for the supportive care of children with cancer. Well-informed focusing of treatment saves costs, reduces admissions, shortens hospital stays and certainly improves the quality of life of the children and their families.

These studies raise a provocative question; should we treat mild febrile respiratory infections of children with cancer with oral antibiotics at home or refrain from the use of antibiotics? Controlled studies are needed to answer the question. Rapid and reliable microbiologic detection methods, including viral diagnostics, are a prerequisite when new treatment strategies are launched for febrile children with cancer. It is necessity for physicians to be thoroughly familiar with the features of febrile diseases of immunosuppressed children to obtain a safe outcome with a good quality of life.

7 SUMMARY AND CONCLUSIONS

The present study showed that respiratory viral infections are common in febrile children with leukemia. Evidence of viral etiology was found in half of the febrile episodes when 16 viruses were searched by PCR, antigen detection, and culture. Rhinovirus, RSV, HHV-6, HBoV, CMV, parainfluenza viruses and influenza viruses were the most frequently detected viruses. Fever of unknown origin finally accounted for only 12% of the febrile episodes.

Three children with leukemia were positive for the new human parvovirus, HBoV, and one of them had five consecutive febrile episodes over half a year. Whether the detection of this new respiratory virus in these children reflects primary infection, reinfection, persistence, or reactivation deserves further studies.

Bacterial septicemia in children with leukemia was associated with virus infection in half of the cases. This novel finding suggests that virus infection may be an important predisposing factor to invasive bacterial infection in children with leukemia.

Determination of MxA protein expression in peripheral blood mononuclear cells may offer a tool for distinguishing viral infections from bacterial infections also in immunosuppressed children with cancer.

In conclusion, our findings suggest that respiratory viruses are the most common etiologic agents of fever in children with leukemia. In one tenth of the cases, respiratory viral infection was associated with septicemia. Most of the viral infections were mild upper respiratory tract infections rarely progressing to pneumonia.

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