Few but Severe Viral Infections in Children With Cancer: A Prospective RT-PCR and PCR-Based 12-Month Study

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INTRODUCTION

During the last decades, survival rates in pediatric cancers have continuously increased and the vast majority of children are cured. One major complication to therapy is still the numerous febrile episodes and in spite of aggressive antibacterial and antifungal treatment some children still die due to infections [1]. Furthermore, a high number of clinical suspected infections remain “fever of unknown origin” (FUO) despite repeated bacterial and fungal cultures. A potential cause of these FUO could be respiratory viral diseases and there is a need of knowing the possible impact of viral respiratory diseases in this population of more reasons: some antiviral drugs are available, for example, for influenza virus; low-risk oral antibiotic treatments are being introduced leaving clinicians with a higher need of knowing the pathogen causing the infection [2,3]; and more centers are loosening the protective isolation allowing the children to go to school earlier in the treatment rendering information on occurrence and morbidity of respiratory viruses in children in anticancer treatment important.

Studies on respiratory viral diseases in non-bone marrow transplantation (BMT) pediatric oncology units are few and most often based on laboratory techniques inferior to the available molecular diagnostics nowadays. RNA viruses account for 30% to 60% of admissions in general pediatric departments [4–6]. Two Finish studies from 1995 [7,8] in non-BMT children in anticancer treatment both confirmed a high occurrence of viral infections. They accounted for 37% of all febrile episodes.

Key words: cancer; febrile neutropenia; PCR; pediatric; immunocompromised hosts; infections; virus

Background. Treatment of low-risk febrile episodes with oral administered antibiotics at home is a new approach in pediatric oncology and protective isolation is loosened in more centers. The impact of viral respiratory infections in febrile diseases in this population is still unclear in terms of occurrence and morbidity. Procedure. A prospective follow-up study of all febrile episodes during 12 months in a pediatric oncology department with a high level of protective isolation was set-up with expanded molecular viral examinations. Reverse transcriptase polymerase chain reaction (RT-PCR) and PCR diagnostics of ten viruses, two atypical bacteria, and one fungus were performed and clinical information on all infections was registered. Results. A total of 250 febrile episodes in 66 patients were registered. In all, 198 respiratory secretions, predominantly oral washes, and 165 anal swabs were analyzed. Twenty-two infections were diagnosed: 7 rhinovirus infections, 4 respiratory syncytial virus (RSV) infections, 2 herpes simplex virus (HSV) infections, 2 varicella-zoster-virus (VZV) infections, 1 influenza B virus infection, 1 parainfluenza virus type 3 infection (PIV3), 1 human metapneumovirus (HMPV) infection, 1 enterovirus infection, 0 adenovirus infections, 0 influenza A virus infections, and 3 non-viral pneumonias: 1 M. Pneumonia, 1 C. Pneumonia, and 1 P. Carinii. The detected pathogens correlated well to the clinical disease. Patients with viral infections were as affected as patients with bacteria in the blood. One of 19 viral infections was lethal, a RSV pneumonia. C-reactive protein concentrations were not able to distinguish viral infections from bacteremias.

Conclusions. The applied sampling method was acceptable and molecular diagnosis of viruses, atypical bacteria and P. Carinii increased the microbiological verification of infections by 35%. Viral infections were few in our protected population but caused severe infectious complications in these patients. Pediatr Blood Cancer 2005;45:945–951.

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in the study of Arola [7] and Mottonen registered more viral infections in leukemic children in maintenance therapy than in matched healthy controls [8]. Other studies agree on the frequency [9], but inconsistency exists concerning morbidity and mortality. Cole [10] described 18 respiratory syncytial virus (RSV) infections in non-BMT children all with benign outcome, whereas Hall [11] reported high mortality of RSV infections. Shaw [12] investigated 2,331 episodes of suspected sepsis in children with cancer in a 5-year period and found 7 of 11 fatal infections to be viral: 4 of viral, 1 of bacterial, and 5 of fungal origin in non-BMT children and 3 of viral and 2 of bacterial origin were registered in BMT patients. Thus in this study, viral infections are the most common cause of lethal infections in children with cancer. The only existing prospective study to our knowledge was a small survey of 22 fever episodes in South Africa. This study also detected more viruses than bacteria but reported no fatal infections [13].

The present study is a prospective, polymerase chain reaction (PCR) and reverse transcriptase polymerase chain reaction (RT-PCR)-based, 1-year examination of ten viruses and three pathogens known to cause atypical pneumonia in a non-BMT setting with protective isolation of all children undergoing immunosuppressive chemotherapy. The purpose is to know the frequency and the morbidity of viral infections in this protected population.

MATERIALS AND METHODS

The study was performed in a university hospital in Denmark on a 15-bed pediatric oncology ward with 99 patients in immunosuppressive treatment from 1 November 2001 to 31 October 2002. The study was approved by the local ethical committee, and informed consent was obtained from 98 patients. One dying patient was excluded. Febrile episodes were registered whenever a patient presented with a body temperature of \( \geq 38.5^\circ C \) or two consecutive measures \( \geq 38.0^\circ C \). Data of clinical information of each infection were prospectively collected: oncological diagnosis and treatment; indwelling catheters; symptoms; neutrophil count at admission (\( \leq 0.5 \times 10^9/L \) is defined as neutropenia); infection treatment; number of days with fever, fever level; CRP-concentrations at admission, the maximal level and the number of days to steady decline were registered. CRP-concentration was measured in nmol/l with lower detection concentration = 48 nmol/l. CRP \( \geq 800 \) nmol/l was considered high and \( \geq 1,800 \) nmol/l very high. A rough clinical estimation of the patient’s clinical state of health during the infection (good, affected, poor) was noted.

Secretions for virological analyses were sampled on admission with fever. (1) Respiratory secretions—children of 3 years or younger: nasopharyngeal aspirate; children of 4 years or older: mouth wash for 60 seconds in 5 ml sterile water; tracheal aspirate or swabs from nose or mouth were made in few cases due to individual obstacles to standard procedure. (2) Anal swab. (3) Swabs from skin ulcers or vesicles if present. Table I describes the study population and Table II the secretions available for analysis. The secretions and swabs kept in 1-ml isotonic NaCl were sent to the laboratory for analyses. Analyses were performed daily in a clinical laboratory with experience in molecular virology. Roche \(^{10}\) viral RNA kits were applied for extraction of RNA and DNA, and magnetic beads were used for extraction of the nucleic acids for parainfluenza virus type 3 infection (PIV3), rhinovirus, and HMPV. Reagents for the Roche Magnapure were applied for extraction with KingFisher \(^{11}\) semiautomatic robot. Qiagen \(^{12}\) one-step RT-PCR kits were used for RNA viruses, and Promega \(^{13}\) kits for DNA amplification were used for DNA-viruses. The gene for \( \beta \)-actin was amplified in all samples in order to control for amplification inhibitors and ensure cellular material in the sample extracted for analysis. Positive and negative controls were run with each sample according to standard set-up. Respiratory secretions were examined fresh by RT-PCR for RSV, influenza A and B, and enterovirus [14], and by PCR for varicella-zoster-virus (VZV), adenovirus, M. Pneumonia, C. Pneumonia, and P. Carinii. PCR was run for adenovirus and RT-PCR for enterovirus in anal swabs and PCR for VZV and herpes simplex virus (HSV) in skin swabs. The samples were stored at \(-80^\circ C\) and respiratory secretions later supplied with analyses in the connected research laboratory for rhinovirus, PIV3 and human metapneumovirus (HMPV) [15–18]. Rhinovirus and HMPV were analyzed with Taqman \(^{19}\) real-time RT-PCR techniques and the other viruses by one-step PCR or RT-PCR with microtiter plate hybridization using biotinylated primers and detection of hybridized material with streptavidin peroxidase [19]. The minimal expected detection level with PCR was as low as 5–50 copies/ml of virus or bacteria depending of the examined pathogen.

Isolation regimens in the ward were unchanged throughout the study period, and the patients were referred to this ward only for all treatments. In-patients with respiratory tract symptoms or diarrhea were isolated from

| TABLE I. Characteristics of the 66 Patients Admitted With Fever |
|---------------------|---------------------|---------------------|
| Patients            | No. of all episodes | No. of individual patients |
| Boys                | 142                | 36                   |
| Girls               | 108                | 30                   |
| Hematological cancer| 156                | 40                   |
| Solid tumors        | 94                 | 26                   |
| Neutropenic         | 148                | —                    |
| \((\leq 0.5 \times 10^9)\) | 102            | —                    |
| Non-neutropenic     | —                 | —                    |
| Weeks of treatment  | —                 | —                    |
| \(\leq 12\)         | 48%                | —                    |
| \(>26\)             | 23%                | —                    |
other patients, and parents wore gowns if leaving the room. Few non-infected visitors were allowed, but public, crowded places (buses, shops, cinemas, etc.) were not advised. School attendance depended on the individual treatment: acute myeloid leukemia (AML) patients and non-Hodgkin lymphoma patients were not allowed to attend day-care or school at any time during therapy whereas children with acute lymphoid leukemia (ALL) could attend school after week 15–46 depending on risk-group. Solid tumor patients were allowed to go to school after 2–3 months. Siblings were not restricted in attending day-care and school, and no recommendations on influenza vaccination were applied for patients, family, or staff. The patients were dismissed to home isolation whenever possible.

Statistics were performed in SPSS using Fisher exact test in parametric data and Kruskal–Wallis test in non-parametric ones. $P < 0.05$ is considered significant. Cases that did not have respiratory secretions analyzed were excluded from the calculations concerning respiratory virus infections. They are included, though, in general considerations of infections in children receiving immunosuppressive chemotherapy.

RESULTS

Table I describes the patients included in this study. Of all 98 patients in the ward, 32 (34%) did not have fever, 28 (29%) had one or two episodes, and 38 (39%) had between three and ten. The median time of admission per infectious episode was 6 days. All 250 febrile episodes were followed clinically and had standard cultures for bacteria performed. A total of 206 infections were investigated by molecular microbiology (Table II), and 19 viral, 2 bacterial, and 1 fungal infections were diagnosed.

Applicability of Molecular Microbiology

Altogether, 2,887 PCR and RT-PCR reactions were run (198 respiratory secretions, 165 anal swabs, 6 skin swabs). False positive results were not an obstacle nor were inhibitors of the reactions: three anal swabs containing inhibitor and one dry swab could not be analyzed. The numbers of positive results from the various specimens are shown in Table II.

Occurrence of Respiratory Viruses and Other Pathogens

PCR and RT-PCR detected 22 infections in 198 respiratory secretions (11%) and 1 in 164 anal swabs. The pathogens detected were: 4 RSV, 1 influenza B virus, 7 rhinovirus, 1 HMPV, 1 PV3, 2 VZV, 2 HSV, 1 enterovirus, 1 $C. Pneumonia$, 1 $M. Pneumonia$, 1 $P. Carini$. One patient had three concomitant viruses: rhinovirus, enterovirus, and VZV and one of the patients with rhinovirus infection had concomitant $H. Influenza$ in sputum. Besides these, no other mixed infections were seen. Fifteen of the viral infections occurred from November to March and the other four from April to October. Paraclinical data of the children with viral diseases reflected infections of considerable impact. Sixteen of 22 infections were detected in neutropenic patients, and 12/22 (55%) were treated with antibiotics for more than 5 days. Cough, fever, and nasal secretion were the most prominent symptoms. CRP-concentrations were considered high in 11/22 patients (50%) and HMPV, influenza virus B, 2 RSV, and 1 HSV and 3 rhinoviruses caused very high CRP-concentrations. Fever was present in at least 3 days in 16 cases (73%) and eight patients had anticancer treatment postponed (36%). One of 19 viral infections was lethal, acute RSV pneumonia.

In all, 104 admissions (42%) had monosymptomatic respiratory disease and 83% of those had respiratory secretions taken for molecular diagnostics. The chosen panel of pathogens diagnosed 12 of these infections (13%) but failed to give information on the remaining 74 cases.
Bacterial and Fungal Findings

Fifty bacteremic episodes were diagnosed from 738 blood cultures (7% of all cultures and 20% of all registered infections). Fifty-two percent were gram negative with predominance of *P. Aeruginosa*. Fourteen percent were polybacterial. Focal bacterial or fungal infections were diagnosed in 6% of the infections. No fungal infections were cultured in blood samples. Most bacteremias were diagnosed in patients with hematological cancers. No lethal blood stream infections occurred.

Microbiological Detection of a Pathogen During Fever

Standard cultures detected 20% bacteremias and 6% focal infections. The applied molecular diagnostics for virus, atypical bacteria, and *P. Carinii* detected a pathogen in another 9% of the infections. It thus increased identification of a pathogen during a fever episode from 26% to 35%, an increase of 35%.

Viral Infection Versus Bacteremia

In order to estimate the morbidity of the 19 viral infections, they were compared with the severity of the 50 blood stream infections, traditionally known as the most severe kind of infection. Patient characteristics, CRP-concentrations, neutrophil count at admission, antibiotic treatment, and influence on anticancer treatment schedule were compared by chi-square test and Fisher exact test. No differences between the two groups were observed but in the three measures below:

(1) Solid tumor patients had significantly more viral infections than patients with hematologic malignancies, \( P = 0.003 \). Solid tumor patients accounted for 53% of all viral infections but only for 37% of the total number of febrile episodes. Children with hematological malignancies accounted for 47% of all viral infections and 63% of all febrile episodes. Solid tumor patients were discharged within 1 week in case of viral infection, whereas six of nine viral infected patients with hematologic malignancies stayed for more than 1 week, \( P = 0.01 \). The difference is reflected in the clinical estimation of the patients condition during the fever episode (good, affected, bad), \( P = 0.01 \). All solid tumor patients with virus were registered in good condition, whereas six of nine patients with hematologic malignancies were affected or bad condition. One AML-patient was admitted for 99 days with RSV pneumonia but exclusion of this extreme case did not alter the significance. Hematological cancer patients and solid tumor patients were admitted for the same number of days in case of bacteremia (\( P = 0.36 \)) and no clinical or paraclinical differences were observed.

(2) Viral infections were mostly present in neutropenic patients, (74%), whereas only 44% of patients with bacteremias were neutropenic, \( P = 0.03 \). Viral infections occurred within the first 5 months of anticancer treatment in 80% of cases.

(3) The number of prescribed shifts in the antibiotic treatment was noted as an indirect measure of how well the patient was responding to treatment. In case of viral infection, treatment was either maintained (67%) or changed up to nine times. In case of bacteremia, 46% was not changed and the rest were changed maximally twice.

CRP-concentrations could not separate viral infections from bacteria in the blood. Dividing the infections into viral and bacteremic cases and comparing CRP-concentrations at admission by chi-square test did not show any difference in levels in the two groups, nor did any comparison of the maximal CRP-concentration during the infection (Fig. 1a,b). The number of days with fever in viral and bacteremic infections was the same but only bacteremias had septic curves with changes from no fever to temperatures \( >39^\circ \)C several times. Anticancer treatment was postponed in about one-third of cases whether the infection was viral or bacterial.

DISCUSSION

Occurrence of Viral Infections

Several confirmative studies have been published about the important impact of respiratory viruses as causative organisms for hospitalization of young children as well as patients with underlying diseases. We improved methods for virus detection by applying modern molecular techniques to respiratory secretions and anal swabs and expected quite a few positive samples. The only other study in children using the PCR and RT-PCR approach for obtaining viral diagnoses in a large population was published by Grondahl and detected 395 of 1,118 samples from general pediatrics in one winter season (35%) [6]. Our laboratory conducted a similar study on clinical samples from general pediatrics in the RSV season in 2002 including a few more viruses. Five-hundred samples with clinically expected RSV were analyzed to detect ampli-cons for RSV, influenza viruses A and B, PIV3, adenovirus, enterovirus, rhinovirus, HMPV, parecho-viruses, *M. Pneumonia*, Chlamydia species, and *B. Pertussis*. Altogether, 352 of 500 samples (70%) were tested positive for one of the pathogens (L.P. Nielsen, MD, personal communication). The detection rate in our population of pediatric oncology patients living in protective isolation was very low. We collected samples not only in the winter season, but also throughout a whole year, and our patients were older than in the above-mentioned studies. Respiratory viruses are more frequent...
in children below 5 years of age [20] as also found in our samples (Table II). However, the occurrence of viruses of 9% presented in this study is very low and calls for considerations on all steps in the investigation: the methods applied, the specimens for examination, and the population studied.

Methodological Considerations

We wanted to establish an acceptable method for the investigations of respiratory pathogens in a clinical pediatric oncology context and ensure specimen from all febrile episodes. Many patients have recurrent episodes and need to do the examination several times. Nasal aspiration is an unwanted method for patients and parents and has caused failure in collecting samples for research in another recent study on the ward. Furthermore, it may not be advisable due to low platelets and mucosal damage. Oral wash was recommended many years ago for examination of P. Carinii by PCR techniques in hematological patients for the same reasons as mentioned above [21]. Oral wash has been validated against bronchial lavage and allows detection of P. Carinii, a lower respiratory tract pathogen, by PCR with sensitivity of 91% and specificity of 94% [22,23]. Also diagnostics of M. pneumonia has changed in most laboratories from serology to PCR detection of a throat swab or oral wash [24]. Viral load in childhood respiratory virus infections is known to be high, and the viruses we detected were high above the detection limit. Our laboratory previously conducted a pilot study on virus detection by PCR in oral washes in leukemic adult patients with pneumonia. The method was acceptable and had reliable results (L.P. Nielsen, MD, personal communication). Sterile water is not a standard fluid for oral wash in the clinic but is well known in the laboratory as a safe liquid to dilute samples or wash pellets for PCR analyzes [25–27]. Intact viral capsids is known to be preserved in sterile water for weeks from former experiences [28]. On the basis of these past studies, oral wash in sterile water was chosen for children of 4 years of age or older and it was well accepted even in children with many febrile episodes. Amplifiable ribonucleic acid was present in all oral washes. Positive controls were tested with the oral washes from patients and no inhibition or false negative controls were detected. We analyzed blinded samples from relatives of the oncological patients, and all samples from related persons were either all negative or detected the same virus (Christensen et al., Common Respiratory Viruses in Patients, Relatives and Staff in Pediatric Oncology, unpublished data). Thus, we found no obvious reason to believe that the low number of viruses detected resulted from methodological problems. However, we do find more viruses in nasal aspirates than in oral washes. It may indicate a higher sensitivity in nasal aspirate or it may be due to the age of the patients.

Population Considerations

The study population complies with a rather restricted isolation regimen. These protected living conditions may reduce the number of respiratory infections. This was confirmed from personal communication with Dr. G.C.F. Chan, Hong Kong, who reports a decrease in the number of respiratory virus infections in general, since SARS was detected compared to the published figures from 2001 [29]. Studies from Belfast on respiratory viruses in medical and pediatric wards had several dual viral infections and repeated viral infections with different pathogens in the same patient possibly reflecting transmission [30,31]. We found only one poly-viral infection and found a low viral load in our ward during these months in another study on virus occurrence in staff and relatives.
Morbidity of Viral Diseases

The seasonal variation of the diagnosed viral infections was as expected, and the clinical manifestations correlated well to the pathogens detected. The morbidity of common respiratory viruses was high. We found a striking similarity of the clinical morbidity of bacteremia and viral diseases in these children. Bacteremia was present in 20% and viral diseases in 9% of the infections. Some of the bacterial blood stream infections might have been contaminations but exclusion of low-pathogenic bacteria did not change the similarity between CRP concentrations, fever days, and admission days in case of viral disease and bacteremia. All these clinical parameters of infectious disease reflect the severity of disease rather than the kind of pathogen. The higher number of shifts in antibacterial antibiotic treatment in case of viral infections supports the finding of a non-bacterial origin of these diseases. It is clear that thorough and sensitive diagnostic methods are necessary together with the clinical examination of the patient to distinguish the type of infection and detect the causative pathogen. With improved antiviral treatment, correct diagnosis becomes of higher clinical relevance in order to ensure rational therapy, to avoid postponing of the anticancer treatment time schedule during courses and during maintenance, and to reduce admission days and expenses [32–39]. The only fatal infection was viral. It is possible that improved antibiotic treatment has succeeded in reducing fatal bacterial infections considerably and death of viral diseases will contribute with a higher share in future infectious deaths in pediatric oncology as seen in Shaw’s large recent study [12].

CRP-concentrations were just as high in viral infections as in case of blood stream bacterial infection (Fig. 1a,b). Some studies find a difference in maximal CRP-concentrations [40,41] but most agree on the considerable overlap between CRP-concentrations in viral and bacterial diseases. This renders clinical use of threshold levels in distinguishing viral and bacterial origin of diseases very difficult [7,42,43]. It is noticed that CRP-concentrations in this population in general were very high. A measure >800 nmol/l is often due to bacteria, and CRP >1,000 nmol/l is considered to be a severe bacterial infection needing antibiotics in general pediatrics. Immunocompetent children rarely have measures above 2,500 nmol/l but we find CRP measured as high as 6,227 nmol/l. The reason for these high levels is unknown and to our knowledge the issue has not been addressed. One might speculate whether the liver is in a state of induction by chemotherapy or produces more C-reactive protein to opsonize microorganisms as a compensatory mechanism in a suppressed immune system.

CONCLUSION

This 12-month prospective study describes 250 febrile episodes in pediatric oncology patients living in protective isolation. The number of nineteen viral infections and three interstitial non-viral pneumonias diagnosed by PCR or RT-PCR was very low, and few mixed infections were found. Protective isolation seemed to be efficient. Oral wash was an acceptable method for collection respiratory secretions for molecular diagnostics. An expanded panel of viruses and three pathogens causing interstitial pneumonia was examined by PCR and RT-PCR increasing the microbiological detection with 35%. There was a fine correlation between the detected pathogens and the clinical diseases of the patients. Morbidity of viral respiratory diseases in this population was high and one RSV infection was fatal. Molecular diagnosis of mouth washings is advisable during the winter season, and early installation of possible antiviral therapy in viral infections should be considered in this population.

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