Viral infections in children receiving anticancer chemotherapy

M C Bouw, *BSc, Drs*

Department of Paediatrics, Wilhelmina Children's Hospital, University Medical Centre, Utrecht, The Netherlands

P B Hesseling, *MB ChB, MMed, MD*
E Nel, *MB ChB, MMed*
G Wessels, *MB ChB, MMed ,MD*

Department of Paediatrics and Child Health, Stellenbosch University and Tygerberg Children's Hospital, W Cape

E Janse van Rensburg, *MB ChB, MMed, PhD, FCPath (SA)*

Quintiles Laboratories, Livingston, West Lothian, Scotland

Objective. To determine the presence of bacterial and viral infectious agents in children with fever during anticancer chemotherapy treatment.

Design. Analysis of data obtained in a prospective cohort study.

Setting. The paediatric oncology unit at Tygerberg Children's Hospital, Stellenbosch University, Parow, Western Cape.

Subjects. All patients up to the age of 15 years who developed fever secondary to anticancer chemotherapy from 9 February 2000 to 9 April 2001.

Outcome measures. Viruses were isolated or antigens detected on venous blood samples, nasopharyngeal aspirates (NPAs), throat swabs, urine and faeces, where possible. Blood for aerobic and anaerobic culture was obtained from an indwelling intravenous catheter and/or a peripheral vein.

Results. Thirty-four patients were analysed for a total of 102 febrile episodes. Evidence of a viral and bacterial infection was found in 31 (30%) and 24 (24%) episodes, respectively. Within these, a combined viral and bacterial infection was demonstrated in 6 (6%) episodes. A total of 35 viral isolates were identified in 31 febrile episodes: herpes simplex virus 1 (HSV-1) (N=14), HSV-2 (N=2), cytomegalovirus (CMV) (N=10), rotavirus (N=5), adenovirus (N=2), para-influenza type 3 (N=1) and hepatitis B (N=1). The blood culture was positive in 24 febrile episodes. The absolute neutrophil count (ANC) on admission was below 0.5×10^9/l in 57 (56%) episodes and thus considered neutropenic. Infectious agents were more frequently identified in neutropenic (54%) than in non-neutropenic (40%) episodes and were more likely to be of bacterial (30%) than viral (15.5%) origin. However, this difference was not significant.

Conclusions. In addition to bacterial infections, viruses are clearly an important cause of fever in children receiving anticancer chemotherapy. Diagnostic tests for viral infections should be used more frequently, and could be of considerable value in evaluating fever and establishing appropriate therapy in these patients.

Fever is a frequent and serious complication in paediatric oncology patients, and often related to infection. The absolute neutrophil and lymphocyte count may be significantly diminished due to cytotoxic chemotherapy and occasionally the neoplastic disease itself, resulting in a host more vulnerable to infections. Symptoms and signs of infection may be minimal or absent in patients with neutropenia as a decrease in the number of neutrophils is associated with a diminished inflammatory response. Arola *et al.* found proof of a respiratory virus infection in 37% of febrile children with cancer while the clinical diagnosis of respiratory tract infection was only 17%.[1] The progression of infection in immunocompromised patients can be rapidly fatal, and patients with early bacterial infections cannot be reliably distinguished from non-bacterially infected patients at presentation.[3,4] Therefore, in these patients, broad-spectrum antibiotic therapy is usually started empirically, even though no bacterial aetiology can be confirmed in the majority of episodes.[1,3] In spite of repeated bacterial cultures, the cause of fever cannot be identified in 60 - 70% of patients and therefore remains 'fever of unknown origin' (FUO).[4-7] This indicates that other infectious agents may play a role. Fungi are recognised as common causes of mainly secondary infection among patients who have received courses of antibiotics, but on occasion these organisms can be the cause of primary infection.[2,4,8] Moreover, as viral infections are the main cause of fever in healthy individuals, it is reasonable to expect the same in febrile oncology patients.[3,9]

Although the incidence of bacterial infections has been studied extensively in adult oncology patients, there are limited data available from paediatric oncology patients and on the occurrence of viral infections.[2,3,5,9] Uys *et al.* performed a prospective study in 1996/1997 in the same paediatric oncology unit as our study. They found proof of

a viral infection in 38% and bacteraemia in 23% of included paediatric oncology patients. However, their study comprised a relatively small number of 22 febrile episodes in 14 patients with neutropenia.[10]

Viral infections could be an important cause of fever and, if diagnosed, enable appropriate treatment in febrile children on anticancer therapy. Therefore, the purpose of this study was to investigate febrile episodes not only for the presence of bacterial infections but also for the frequency, type and outcome of viral infections.

Methods

We analysed data obtained during a prospective study at the paediatric oncology unit of Tygerberg Children's Hospital (Stellenbosch University) from 9 February 2000 to 9 April 2001. This oncology unit mainly treats patients from the Western Cape and Northern Cape up to the age of 15 years. All patients with fever while receiving anticancer chemotherapy in whom specimens could be collected for both viral and bacterial studies were eligible for enrolment.

The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences, Stellenbosch University. Informed written consent was obtained from guardians.

Definitions

Fever was defined as a single axillary temperature ≥38.5°C or as two recordings ≥38°C in a 24-hour period. Patients were considered neutropenic if they had an absolute neutrophil count (ANC) of ≤0.5 x 10^9/l.

Patient evaluation and treatment

All children were treated with accepted international chemotherapy protocols at the time of admission. It was routine practice to insert an indwelling intravenous catheter (Hickman-Broviac type) in patients who required intensive chemotherapy. At presentation with fever, a complete medical history and a thorough physical examination were done, whereafter appropriate specimens were collected for microbiological and virological analysis. All patients were investigated and treated in the study hospital. Clinical signs on admission, outcome of bacterial blood culture, the presence of bacterial DNA, viral isolates, total and differential white blood cell counts and clinical outcome were recorded.

In neutropenic patients, therapy was started with intravenous amikacin and piperacillin. Vancomycin was added if fever persisted beyond 48 hours. Amphotericin was subsequently added in the case of persistent fever for 72 hours. In the event of clinical lesions suggestive of herpes infection or severe mucositis, acyclovir was added. At any stage, treatment was changed when the laboratory identified an organism not sensitive to the drugs administered.

Viral studies

When a child presented with fever, samples were taken from likely sources of infection. The intention was to obtain blood, a nasopharyngeal aspirate (NPA), a throat swab and urine and stool samples from every patient. The samples taken were influenced by the attending physician, the clinical symptoms of the patient, the ability to obtain specimens and time restraints for transport to, and processing of, specimens by the virology laboratory.

For NPAs, a nasogastric tube that had been cut to a length of 7 cm was inserted into the nasopharynx, after which 0.9% saline was instilled. The washings were aspirated and added to an equal volume of viral transport medium. Throat swabs were also transported in viral transport medium. All specimens were processed within 6 hours of collection. Heparinised blood was submitted for rapid detection of cytomegalovirus (CMV)-pp65 antigen.

For para-influenza, influenza, mumps and measles virus isolation, specimens were placed on primary monkey kidney cells and maintained at 33°C. For enterovirus, specimens were maintained on the same cell line at 37°C. Human fibroblast cell cultures were used for the herpesvirus family and HeLa cell cultures for adenovirus. All viral cultures were monitored 3 times a week for cytopathic effect. Once a virus was detected, the cover slip was removed, stained with haematoxylin and eosin, and examined for viral inclusion bodies.

Respiratory syncytial virus enzyme-linked immunosorbent assay (ELISA) (Test Pack, Abbott Laboratories, North Chicago, USA); CMV-pp65 antigen immunofluorescence (Clonab CMV kit, Biotest, Dreieich, Germany); rotavirus-ELISA (Rotaclone, Cambridge Biotech, Worcester, UK) and adenovirus type 40.41-ELISA (Adenoclone, Cambridge Biotech, Worcester, UK) were used for viral antigen detection where appropriate. Herpes simplex virus (HSV) typing was done with monoclonal antibodies (HSV-1 and Bivalent Acculone BioWhittaker, Walkersville, MD, USA). HSV-1 and HSV-2 antibodies were determined using Trinity Biotech USA (HSV COMBINED IgG, IgM, Jamestown, New York).

Bacterial studies

Venous blood samples were collected for aerobic and anaerobic cultures with the Bactec 640 system from both a peripheral vein and the central catheter, if present. Bacterial DNA in serum was measured by amplifying the DNA encoding for the 16S rRNA (861 bp fragment) bacteria. Only a positive blood culture was taken as proof of a bacterial infection. Although urine was collected for bacterial culture and viral studies, the specimens were not midstream specimens and the bacterial cultures indicated contamination, making them unsuitable for analysis of the presence of a bacterial infection.

Statistical analysis

Data are presented as median and range. Statistics were performed in SPSS 14.0 using the Pearson's chi-square test. A p-value of ≤0.05 was considered significant.

Results

Study population

During the 14-month study period, 36 patients with a total of 110 febrile episodes were admitted. Eight episodes (2 patients) were excluded because no viral samples were taken on admission; 102 febrile episodes in 34 patients were evaluated for both a viral and bacterial aetiology. Table I shows the baseline characteristics of this study. The series comprised 11 girls and 23 boys, who were being treated for various malignant diseases (acute lymphoblastic leukemia (N=9), acute myeloid leukemia (N=3), non-Hodgkin's lymphoma (N=10), Hodgkin's lymphoma (N=1), CNS tumour (N=3), Wilms' tumour (N=3), neuroblastoma (N=2), osteosarcoma (N=1), retinoblastoma (N=1) and Langerhans cell histiocytosis

(*N*=1)). The children's age at admission ranged from 8 months to 15 years, with a median of 5.4 years. Patients represented all sectors of the population, 6 being white (18%), 23 coloured (67%) and 5 black (15%). The ANC on admission was below 0.5 x 10⁹/l in 57 (56%) episodes and thus considered neutropenic. For all febrile episodes, the median ANC was 0.32 x 10⁹/l (range 0.00 - 15.0 x 10⁹/l). Respiratory signs (cough, rhinitis), gastro-intestinal symptoms (vomiting) and mucositis were the most prominent infectious symptoms.

TABLE I. BASELINE CHARACTERISTICS

Patients	No. of all episodes (*N*=102)	No. of individual patients (*N*=34)
Boys	66	23
Girls	36	11
Haematological cancer	62	23
Solid cancer	40	11
Median age*	4.8	5.4
Neutropenic†	57	-
Non-neutropenic	45	-

*Median age in years.
†ANC ≤0.5 x 10⁹/l.

Diagnostic test results

Evidence of a viral and bacterial infection was found in 31 (30%) and 24 (24%) of the 102 febrile episodes, respectively. Within these, a combined viral and bacterial infection was present in 6 (6%) episodes. In the remaining 53 episodes (52%), no bacterial or viral infectious agent was identified and they were therefore categorised as FUO. In general, the cause of fever was more often detected in neutropenic (54%) than in non-neutropenic episodes (40%). However, this was not significant (*p*=0.149). Fig. 1 describes the diagnostic test results of all febrile episodes, comparing neutropenic with non-neutropenic episodes.

Viral isolates

Viral isolates are listed in Table II. A total of 35 viral isolates were identified in 31 febrile episodes: HSV-1 (*N*=14), HSV-2 (*N*=2), cytomegalovirus (CMV) (*N*=10), rotavirus (*N*=5), adenovirus (*N*=2), para-influenza type 3 (*N*=1) and (reactivated) hepatitis B virus (*N*=1). In two children, both HSV-1 and HSV-2 antibodies were detected in the blood (it is well known that there is cross-reaction between HSV-1 and HSV-2 antibodies; only typing of viral isolates will be able to distinguish the two). In 1 of these patients, CMV antigen was identified in the blood in addition (this may be cross-reaction within the herpesvirus group). Furthermore, a dual detection of HSV-1 and rhinovirus was identified in another episode. In general, most isolates were detected in the NPAs and throat swabs; none was detected in faeces. In 5 patients, identical viruses were isolated in ensuing episodes, suggesting reactivation or prolonged excretion of the virus. Nineteen (33%) of 31 viral episodes were detected in the neutropenic group, compared with 12 (27%) in the non-neutropenic group (*p*=0.467).
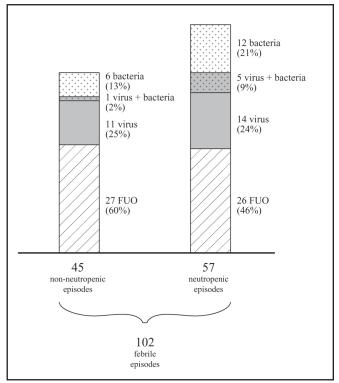

Fig. 1. Diagnostic tests results in 102 febrile episodes. Evidence of a viral and bacterial infection was found in 31 and 24 episodes, respectively. Within these, a dual infection was present in 5 neutropenic and 1 non-neutropenic episode(s).

TABLE II. NUMBER AND TYPE OF VIRAL ISOLATES IN 102 FEBRILE EPISODES

Virus	Total isolates*	In 57 neutropenic episodes*	In 45 non-neutropenic episodes*
HSV-1†	14 (40)†	10 (18)	4 (9)
HSV-2†	2 (6)	1 (2)	1 (2)
CMV†	10 (28)	5 (9)	5 (11)
Rhinovirus†	5 (14)	4 (7)	1 (2)
Adenovirus	2 (6)	1 (2)	1 (2)
Para-influenza type 3	1 (3)	1 (2)	-
Hepatitis B	1 (3)	-	1 (2)
Total positive viral isolates	35	-	-

*Percentages in parentheses.
†Three patients were tested positive for multiple viral isolates: one patient had a dual detection of HSV-1 and HSV-2; another patient for both HSV-1 and rhinovirus; and 1 patient tested positive for HSV-1, HSV-2 and CMV.

Bacterial isolates

The Bactec blood culture was positive in 24 (24%) febrile episodes. The following bacteria were cultured: *Klebsiella pneumoniae* (*N*=4), *Staphylococcus aureus* (*N*=2), *Escherichia coli* (*N*=2), coagulase-negative *Staphylococcus* (*N*=4), *Salmonella* group B (*N*=1), *Streptococcus vestibularis* (*N*=1), *Pseudomonas aeruginosa* (*N*=1), *Propionebacterium avidum* (*N*=1), *Proteus mirabilis* (*N*=1), *Fusobacterium nucleatum* (*N*=1), *Streptococcus pneumoniae* (*N*=1), beta-haemolytic *Streptococcus* group G (*N*=1), *Neisseria* (*N*=1), a dual detection of *Stenothrophomonas maltophilia* and *Acinetobacter baumanii* (*N*=1), and Gram-negative bacils (*N*=1) and Gram-positive bacils (*N*=1), with no further classification into subtypes.

Bacterial DNA was negative in 10 episodes with a positive blood culture, and positive in 13 episodes with a negative blood culture (bacterial DNA was not determined in 1 child with a negative blood culture). Seventeen (30%) of 24 bacteraemias were detected in the neutropenic group, compared with 7 (15.5%) in the non-neutropenic group (i.e. 17/57 v. 7/45; *p*=0.092).

Dual infections

In 6 febrile episodes, evidence of both a viral and bacterial aetiology was found. In 1 of these cases, a bacterial pathogen was demonstrated solely by the presence of a positive bacterial blood culture and in 5 cases by blood culture as well as by DNA PCR. In 5 of these episodes the patients were neutropenic; the 6th patient had an ANC of 0.66×10^9/l.

Clinical outcome

Three patients needed admission to the ICU. One patient with a large-cell anaplastic lymphoma and fever for 15 days without neutropenia (ANC 8.05×10^9/l) was found to have both HSV-1 and HSV-2 antibodies in the blood. An active virus was, however, not isolated from this child, and the antibodies may indicate past infection. Moreover, no bacterial pathogen was identified. Another patient with T-cell lymphoma, fever for 14 days, neutropenia (ANC 0.36×10^9/l) and symptoms of severe mucositis, had HSV-1 in the NPA and throat swab as well as a *S. aureus* identified in the blood. The third patient had acute lymphoblastic leukaemia and severe neutropenia (ANC 0.00×10^9/l) and was found to have a positive blood culture for *K. pneumoniae*. All children made a complete recovery, and no deaths occurred.

Discussion

In the past decades, significant progress has been made in the management of infectious complications during anticancer chemotherapy treatment. The impact and consequences of bacterial infections in this patient group have been extensively studied and described. However, several studies reporting on the incidence of viral infections suggest that viruses are an important overlooked cause of fever and morbidity in this group.[1,3,10-15] One of the first studies on this subject by Craft *et*

> **With improved antiviral drugs, understanding the aetiology of fever has become increasingly important.**

al., showed an incidence of viral infections in 32% of symptomatic children with acute lymphoblastic leukaemia. Rhinovirus was most frequently isolated, followed by herpesvirus and CMV.[14] In a similar prospective study from Chile, Santaloya *et al.* found proof of a viral infection in 26% of 220 neutropenic paediatric patients. However, only children with clinical signs of a viral infection and a CRP level of <40 mg/l were virologically investigated.[15] Moreover, studies in the past are probably based on laboratory techniques inferior to the diagnostics available nowadays. This may have resulted in failure to recognise the true importance of viral infections in patients on anticancer chemotherapy.[2,3,9]

Our study confirms that viruses are found in a significant number of febrile episodes and may be important pathogens in febrile oncology patients. We found proof of a viral infection in 30% and bacteraemia in 24% of febrile episodes. The proportion of viral isolates was comparable between neutropenic and non-neutropenic febrile episodes: 33% and 27%, respectively.

As in several other studies, herpesviruses (herpes simplex virus and CMV) were most frequently isolated.[4,11,12,15] This is meaningful, as it is known that CMV may cause severe and life-threatening infections and necessitate interruption of planned therapy. Identical viruses were isolated in ensuing episodes in 5 patients, suggesting reactivation or prolonged excretion of the virus. It is a well-recognised phenomenon that viruses are isolated from asymptomatic children. Changes in complex immunological defence mechanisms probably account for the increased duration of excretion. In addition, it is known that patients with malignant disease are more vulnerable to multiple infections.[14] We identified multiple infectious agents in the same febrile episode in 6 patients in this study.

Bacterial pathogens found in this study consisted of 14 Gram-negative bacteria (*K. pneumonia* and *E. coli* most frequently identified) and 11 Gram-positive bacteria (6 positive cultures of *Staphylococcus*). Moreover, this study suggests that the cause of fever is more likely to be found and to be of bacterial origin in neutropenic episodes. The occurrence of bacteraemia was 30% in neutropenic compared with 15.5% in non-neutropenic episodes; however, this was not significant.

No aetiology was found in 52% of cases, and the episode was therefore defined as FUO. However, the laboratory techniques used were not designed to identify every possible virus, and the impact of viral infections in this population may therefore be even greater than we recorded. The usefulness of virological diagnoses will be greatly enhanced if rapid, sensitive, diagnostic methods are applied.

Our study shows a similar incidence of viral and bacterial infection as in the study of Uys *et al.*, who found proof of viral infection in 38% and bacteraemia in 23% of febrile neutropenic children. We also found HSV-1 to be the most frequent isolate, with throat swabs and NPAs accounting for the majority of positive isolates. In contrast to their study, where no dual detection of bacteria and viruses was found, we identified a dual infection in 6% of episodes.[10]

Conclusions

With improved antiviral drugs, understanding the aetiology of fever has become increasingly important. It could be of considerable value in adjustment of the antibiotic treatment protocol, reduction of in-hospital admission, and avoiding postponement of crucial chemotherapy. Moreover, even if the viral agent is not the cause of fever, skin or mucous membrane lesions caused by viruses can facilitate bacterial or fungal superinfections and may therefore necessitate antiviral treatment. As it is not possible to definitely exclude a concomitant bacterial infection, antibacterial therapy is still essential in the initial management of these patients.[2,4,6,11] Although frequent and meaningful, viruses may not be as important contributors as bacteria to severe, life-threatening disease. However, this will need further investigation and confirmation in larger studies.

We believe that a search for an aetiology in children who develop fever while receiving anticancer chemotherapy is justifiable. The increasing number of effective antiviral agents as well as the high cost of hospital care and ongoing attempts to identify patients at low risk who may be safely treated at home, further reinforce this.

References

1. Arola M, Ruuskanen O, Ziegler T, Salmi TT. Respiratory virus infections during anticancer treatment in children. *Pediatr Infect Dis J* 1995; 14: 690-694.
2. Hughes WT, Armstrong D, Bodey GP, *et al*. 2002 Guidelines for the use of antimicrobial agents in neutropenic patients with cancer. *Clin Infect Dis* 2002; 34: 730-751.
3. Wood DJ, Corbitt F. Viral infections in childhood leukaemia. *J Infect Dis* 1985; 152: 266-273.
4. Alistair JJ, Wood MD. Management of fever in patients with cancer and treatment-induced neutropenia. *N Engl J Med* 1993; 328: 1323-1332.
5. Persson L, Dahl H, Linde A, Engervall P, Vikerfors T, Tidefelt U. Human cytomegalovirus, human herpesvirus-6 and human herpesvirus-7 in neutropenic patients with fever of unknown origin. *Clin Microbiol Infect* 2003; 9: 640-644.
6. Pizzo PA, Rubin M, Freifeld A, Walsh TJ. The child with cancer and infection. I. Empiric therapy for fever and neutropenia, and preventive strategies. *J Pediatr* 1991; 119: 679-694.
7. Riikonen P, Leinonen M, Jalanko H, Hovi L, Saarinen UM. Fever and neutropenia; bacterial aetiology revealed by serological methods. *Acta Paediatr* 1993; 82: 355-359.
8. Donowitz GR, Maki DG, Crnich CJ, Pappas PG, Rolston KVI. Infections in the neutropenic patient – new views of an old problem. *Hematology Am Soc Hematol Educ Program* 2001; 1: 113-139.
9. Christensen MS, Nielsen LP, Hasle H. Few but severe viral infections in children with cancer: a prospective RT-PCR and PCR-based 12-month study. *Pediatr Blood Cancer* 2005; 45: 945-951.
10. Uys R, Cotton MF, Wessels G, Hesseling PB, Bester R, Janse van Rensburg E. Viral isolates during febrile neutropenia in children with cancer. *J Trop Ped* 2000; 46: 21-24.
11. Pizzo OA, Rubin M, Freifeld A, Walsh TJ. The child with cancer and infection. II. Nonbacterial infections. *J Pediatr* 1991; 119: 845-857.
12. Katsimpardi K, Papadakis V, Pangalis A, *et al*. Infections in a pediatric patient cohort with acute lymphoblastic leukemia during the entire course of treatment. *Support Care Cancer* 2006; 14: 277-284.
13. Kutza AST, Muhl E, Hackstein H, Kirchner H, Bein G. High incidence of active cytomegalovirus infection among septic patients. *Clin Infect Dis* 1998; 26: 1076-1082.
14. Craft AW, Reid MM, Gardner PS, *et al*. Virus infections in children with acute lymphoblastic leukaemia. *Arch Dis Child* 1979; 54: 755-759.
15. Santolaya ME, Villarroel M, Avendano LF, Cofré J. Discontinuation of antimicrobial therapy for febrile, neutropenic children with cancer: a prospective study. *Clin Infect Dis* 1997; 25: 92-97.