

FEBRILE NEUTROPENIA IN PAEDIATRIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION, *IN-VITRO* SENSITIVITY DATA AND CLINICAL RESPONSE TO EMPIRICAL ANTIBIOTIC THERAPY

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ABSTRACT

Objective: To find the in-vitro sensitivity data and clinical response in order to determine the changes required in empiric antibiotic therapy for management of febrile neutropenia in paediatric patients undergoing peripheral blood stem cell transplantation.

Design: A descriptive study.

Place and Duration of Study: Paediatric bone marrow transplant unit at Bismillah Taqee Institute of Health Sciences and Blood Disease Center from September 1999 to May 2004.

Patients and Methods: All patients were treated according to institutional protocol for febrile neutropenia. Empirical antibiotics include Ceftriaxone and Amikacin. In non-responders, changes made included Imipenem and Amikacin, Piperacillin Tazobactam/Ticoplanin or Vancomycin/Cloxacilin/Ceftazidime. In non-responders, amphotericin was added until recovery.

Results: Out of 52 patients, 5 did not develop any fever; in the remaining 47 patients there were 57 episodes of febrile neutropenia. The mean days of febrile episodes were 4.71 (range 3-8). Fever of unknown origin (FUO) occurred in 31 (54.3%) episodes. Microbiologically documented infection (MDI) occurred in 17 (29.8%) episodes of fever. Clinically documented infection (CDI) occurred in 9 (15.7%) episodes. Gram-negative organisms were isolated in 10 while gram-positive organisms in 7. *Klebsiella*, *S. aureus* were the most common isolates. Empirical therapy was effective in 12 of the 33 (36%) episodes. Out of 28, 26 (92%) responded to Imipenem/Amikacin as second line therapy while those who received any other second line combination, only 11 out of 22 (50%) showed response. Systemic Amphotericin was used in 4 patients, 2 responded. Infection related mortality rate was 4%.

Conclusion: Gram-negative infections predominated, Imipenem/ Amikacin found to be most effective therapy while a low mortality rate is recorded in our setting suggesting good infection control.

KEY WORDS: *Febrile neutropenia. Paediatric stem cell transplantation. Antibiotic sensitivity.*

INTRODUCTION

Paediatric bone marrow and peripheral blood stem cell transplantation procedures are increasingly performed for malignant and non-malignant haematological disorders. Congenital disorders like beta-thalassaemia major and Fanconi's anemia in children are important indications in developing world, where consanguineous marriages result in an increasing proportion of these disorders. Bacteria and fungi cause 95% of infections in allogeneic stem cell transplant setting.^{1,2} Life threatening complications due to bacterial infections have been reported in 10-20% of febrile episodes in neutropenic patients.³ The clinical hallmark of bacteremia in the febrile neutropenic host has been the development of fever. Empirical treatment, i.e. broad-spectrum antimicrobial therapy without waiting for microbiological and/or clinical documentation of an infection, is justified in patients with fever and neutropenia (febrile neutropenia, FN). There is high

frequency of severe infection that is associated with a low granulocyte count. Gram-negative infections can cause morbidity and mortality; particularly, if they are unrecognized or inappropriately treated. Any delay in instituting effective gram-negative antibiotic coverage in FN patients can result in high mortality. However, there is more time available to make treatment modification when dealing with gram-positive infections.³ There has been a trend towards more gram positive infections reported in this group of patients.⁴ In contrast, few reports from the developing world still show predominant gram-negative infections.^{5,6} In pediatric stem cells transplant patients, FN may differ from adults in presentation, antibiotic choice, response and outcome. This is the data of febrile neutropenic episodes in pediatric patients received peripheral blood stem cell transplantation for various malignant and non-malignant disorders, institutional antibiotic policy was revised according to in-vitro sensitivity data and clinical response.

PATIENTS AND METHODS

It was a descriptive study conducted at Paediatric Bone Marrow Transplant Unit at Bismillah Taqee Institute of Health Sciences and Blood Disease Center from September 1999 to

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May 2004. All patients were treated according to institutional protocol for conditioning, graft versus host disease (GvHD) prophylaxis and antimicrobial therapy for FN. Fiftyone allogeneic and one autologous transplant were performed for various haematological conditions; including aplastic anemia, Fanconi's anemia, b-thalassemia major, acute leukemia, lymphoma, and neuroblastoma.

Conditioning regimen for b-thalassemia major consisted of Cyclophosphamide 50mg/kg/day for 4 days, Busulphan 4 mg/kg/day for 4 days (Bu/Cy). In aplastic anemia, Cyclophosphamide 50mg/kg/day for 4 days and ATG 5 mcg/kg/day for 3 days in heavily pre-transfused patients. For acute lymphoblastic leukemia and acute myeloid leukemia, Busulphan 4mg/kg for 4 days and Cyclophosphamide 50mg/kg/day for 4 days followed by stem cells transfusion after one day rest. CD-20 antibodies purging was done in CD 20+ pre-B-cell leukemia. One autologous stem cells transplantation (SCT) was performed who received (BEAM) B.C.N.U, Etoposide, ARA-C, and Melphalan as conditioning regimen. Graft-verses-host diseases prophylaxis was cyclosporine 5 mg/kg/day, methotrexate 5 mg/m² on day 1, 3, 6, 11 and then weekly. Anti-fungal prophylaxis was given with Itraconazole 3 mg/kg/day. Granulocyte colony stimulating factor was started on fourth day posttransplant (3-5 mcg/kg/day) till Absolute Neutrophil Count (ANC) reached more than 2000 cells/mm.³

All patients aged less than 20 years admitted in BMT ward from September 1999 to May 2004 for stem cells transplantation were included. Neutropenia was defined as an ANC of less than 500 cells/mm.³ Patients who had more than one episodes of neutropenic fever were enrolled more than once. Fever was defined as single oral temperature of 38.3°C (101°F) or a temperature of 38.0°C (100.4°F) for more than one hour. Each episode of fever was defined as the period from the first day of fever till the day when maximum temperature was less than 37.5°C (100°F) and patient remained afebrile for 48 hours. Patients with history of allergic reaction to any of the antibiotics used in the study, or age more than 20 years were excluded.

After a detailed history and thorough physical examination during the neutropenic episodes, complete blood count, electrolytes, urinalysis, liver, renal function test and blood cultures from peripheral line and central venous catheter (CVC) were checked. Chest X-rays were performed only when clinically indicated. Stool of patients, who had diarrhea, was sent for microscopy. For sore throat and cough, throat swabs and sputum was collected for culture and sensitivity. Catheter-related infections were identified by local signs of infection at the site of catheter or by CVC blood cultures with a greater colony count than in peripheral blood cultures when taken at the same time. All patients were started with first line antibiotic regimen according to protocol, Ceftriaxone 80 mg/kg/ day in two divided doses along with Amikacin 15 mg/kg/day in two divided doses. Antibiotic response was observed during next 72 hours, whether patient had organism isolated from culture or any site of infection identified like pneumonia, whether the fever has resolved, and whether patient's condition has deteriorated. Second line therapy was started in those who did not respond to first line that included Inj. Imipenem + Amikacin. After 5-7 days, if fever did not settle then either Vancomycin

was added or if there was suspicion of fungal infection, amphotericin-B was chosen.

Treatment outcome was classified as a success without modification when patient recovered from fever and neutropenia on initial empirical therapy. Success with modification involved ultimate recovery from fever and neutropenia but requiring alteration of different antibiotic, antifungal or antiviral agent. The treatment was considered as failure if fever persisted for longer than 7 days without any response leading to patient's death or the patient showed clinical deterioration with or without persistence of primary isolated microorganism or detection of a new organism.

Febrile episodes were classified according to the kind of infection as (i) fever of unknown origin (FUO); (ii) microbiologically documented infection (MDI); (iii) clinically documented infection (CDI); and according to the suspected source or site of infection (unknown, bacteraemia, fungaemia, viraemia, lower respiratory tract infection, upper respiratory tract infection, gastrointestinal tract infection, soft tissue infection and line related infection).⁶ FUO was defined as both the absence of any clinical or radiological sign of infection other than fever and no isolation of causative organism.

The diagnosis of microbiologically documented infections was based on both isolation of causative organism from body fluids and accompanied by clinical symptoms adopted from the case definitions of the Centers for Disease Control (CDC) surveillance system for nosocomial infection.⁷ Bacteraemia or fungaemia (MDI) was defined as fever with positive blood cultures for bacteria or fungi with or without septic symptoms or signs of localized infection. Fever arising from a clinically evident source of infection including radiological findings without detection of any pathogen was classified as clinically documented infection (CDI).^{7,8} Bacterial isolates were identified and tested according to standard identification techniques and antibiotic susceptibility tests as suggested in NCCLS document M-100-S58.⁹

RESULTS

From November 1999 to May 2004, a total of 57 episodes of febrile episodes were documented in 52 pediatric transplants who received PBSC for allogeneic (MRD) (n=51) or autologous (n=1) transplantation. There were 38 males and 14 females whose age ranged from 2 to 20 years (mean age 10.37 years). Clinical characteristics of these patients are given in Table I.

The incidence of neutropenic fever was 91% (52/57). Mean days of febrile episodes were 4.71(3-8) days. *E. coli*, *S. aureus*, *Klebsiella*, and *P. aeruginosa*, were most commonly encountered organisms (Table II). FUO occurred in 31 (54.3%) episodes, MDI occurred in 17 (29.8%) episodes of fever and CDI occurred in 9 (15.7%) episodes. Fungal and viral episodes were not documented microbiologically, but there was clinical response to anti-fungal therapy and anti-viral therapy in 2 and 1 episode respectively. Twelve of the 33 (36%) episodes responded to empirical therapy. Twenty-six out of 28 (92%) episodes responded to Imipenem alone or with combination, as a second line therapy while those who received any other second line combination, only 11 out of 22 (50%) episodes, the fever effervesced. All 7 episodes of gram-positive sepsis responded to Vancomycin or Cloxacillin. Four episodes required systemic Amphotericin, 2 responded. Infection related mortality rate was 4%. Response to antibiotic regimen

is shown in Table III.

A total of 120 samples were sent for culture during 57 febrile neutropenic episodes; 17 cultures yielded an organism. Three most common gram negative organisms isolated from specimens were *E. coli*, *Klebsiella*, and *P. aeruginosa*. Four drugs consistently showing sensitivity against these organisms were Imipenem, Cefepime, and Amikacin. While 3rd generation

cephalosporins, and quinolones were highly resistant.

DISCUSSION

High-dose chemotherapy with stem cell or bone marrow transplantation (BMT) is currently used in the treatment of solid tumors^{9,10} hematological malignancies, acquired and congenital hematological diseases.¹¹ For both autologous and allogeneic transplantation, bacterial and fungal infections are the important source of early morbidity and mortality.^{12,13} Recently, peripheral blood stem cells (PBSC) have been increasingly used as a source of stem cells and carry the advantage of faster neutrophil and platelets recovery, whereas there is increased risk of chronic graft versus host disease. (GVHD).^{14,15} Febrile neutropenia is mostly seen during the first week of transplantation.

Traditionally, broad-spectrum antibiotic combination is used as empirical therapy in febrile neutropenia, 44% febrile episodes require a modification. Modification of antibacterial therapy is a reality in clinical practice in the treatment of febrile neutropenia. Several studies have reported various response rates to empirical therapy from 50-70%.^{16,17} This study has included a large group of profoundly neutropenic pediatric patients for stem cells transplantation (absolute neutrophil count of <100/cmm).

A study performed in Italy showed 156 episodes of fever occurred in 102 children during first 100 days post bone marrow transplant (BMT). Fever of unknown origin (FUO) was found in most cases (40%) followed by other infections (33.4%), Pneumonia (19.2%) and Septicemia (7.1%). The overall incidence of mortality was 22.6% and has mortality due to infection 17.4%.¹⁸

A retrospective review of 75-haemopoietic stem cell transplantation showed fever in 74 patients (98%). FUO occurred in 43%, Bacteremia without focus occurred in 29%, whereas 17% neutropenic fever was CVC associated. The median duration of fever was 12.5 days and time of engraftment was 14 days.¹⁹

In this study, 57 episodes of febrile neutropenia occurred in 50 children who received PBSC for malignant and non malignant hematological conditions, the incidence of neutropenic fever was 91%, the overall incidence of mortality was 4%, which is compatible with the data from developed countries.¹⁹ The median duration of fever in our group of patients was 4.7 days and median time of engraftment was 11 days.

In this cohort patients, FUO, MDI and CDI, were seen in 31 (54.3%), 17 (29.8%) and 9 (15.7%) episodes respectively, which is in accordance with the results reported in literature.^{18,19,22,24} Gram-negative bacilli, especially, *P. aeruginosa*, *Escherichia coli* and *Klebsiella* species, remain prominent causes of infection in neutropenic patients.²¹ The most commonly encountered organisms were similar in our group.

In the selection of the initial antibiotic regimen, one should consider the type, frequency of occurrence, and antibiotic susceptibility of bacterial isolates recovered from other patients at the same hospital. The use of certain antibiotics may be limited by special circumstances, such as drug allergy or organ (e.g., renal or hepatic) dysfunction. The rate of gram-negative infections is increasing in most of the centers and

Table I: Characteristics of pediatric patients, who underwent peripheral blood stem cells transplantation from 1999-2004.

Diagnosis episode	Number of patients (%)	Febrile neutropenic	
AML	3 (5.7)	4	
ALL	2 (3.8)	2	
AA	24 (46)	31	
β-Thalassaemia major	15 (28)	14	
Fanconi's anaemia	2 (3.8)	2	
PNH	1 (1.9)	1	
HD	1 (1.9)	1	
CML	1 (1.9)	1	
DBA	1 (1.9)	1	
MDS	1 (1.9)	1	
NB	1 (1.9)	1	
Total transplants	52		
Allogeneic (MRD)	51		
Autologous	1		

ALL; acute lymphoblastic leukemia, AML; Acute myeloid leukemia, AA; aplastic anaemia, HD; Hodgkin's disease, CML; chronic myeloid leukemia, MRD; matched related donor.

Table II: Documentation of infection, and antibiotic sensitivity in pediatric patients who underwent peripheral blood stem cells transplantation from 1999-2004.

Organisms	Number (%)	Antibiotic sensitivity
Total sepsis	57	-
No. of blood samples cultured	120	-
No. of organism isolated	17 (14%)	-
Gram negative Isolates	10 (58.8%)	-
<i>E. coli</i>	2	Imipenem, Cefepime, Amikacin
<i>Pseudomonas</i> spp.	2	Amikacin, Imipenem, Ceftazidime
<i>Klebsiella</i> spp.	4	Imipenem, Cefepime, Amikacin
<i>Xanthomanas multophilia</i>	1	Imipenem,
<i>Bacillus</i>	1	Ceftriaxone, Imipenem, Cefepime
Gram positive isolates	7 (41.2%)	
<i>S. aureus</i>	6	Ceftriaxone, Amoxicillin-Clavulanate
MRSA	1	Vancomycin,
Mixed infection	1	

Table III: Pattern of response to antibiotic regimen use in pediatric patients who underwent peripheral blood stem cells transplantation from 1999-2004.

Ceftriaxone/Amikacin	Piperacillin/Amikacin	Imipenem/Amikacin	Vancomycin
12/33 (36%)	2/5 (40%)	21/23 (91%)	3/3 (100%)
Cefepime/Amikacin	Teicoplanin/Piperacillin	Imipenem/Cloxacillin	Imipenem/Piperacillin
6/10 (60%)	1/2 (50%)	1/1 (100%)	1/1 (100%)
Imipenem	Cefepime/Gentamycin	Ceftazidime	Amphotericin-B Anti viral
3/3 (100%)	1/1 (100%)	1/4 (25%)	2/4 (50%) 1/1 (100%)

double gram-negative coverage is recommended by most of the guidelines. As gram-negative sepsis is a life-threatening condition, aminoglycoside plus Ceftriaxone was used as first line empirical therapy in the present cohort, but it worked in only 12 of the 33 (36%) episodes. Imipenem has excellent activity against gram-negative organisms, Viridians streptococci and pneumococci used as second line empirical therapy in our group of patients. Response to second line therapy was 26 out of 28 (92%) episodes. Similar results have been observed in other studies in febrile neutropenic patients.^{21,23} While those who received any other combination, only 11 out of 22 (50%) episodes, the fever effervesced. All 7 episodes of gram-positive sepsis responded to Vancomycin or Cloxacillin. Four episodes required systemic Amphotericin in which 2 responded.

Institutional antibiotic policy was revised in 2002 which showed 50% response to first line therapy which included, Ceftriaxone and Amikacin, 5.5% response to second line, Piperacillin-Tazobactam plus Amikacin, third line antibiotics included Ceftazidime work in 7% cases. Imipenem and Amikacin responded in 30/40 patients. Ten patients had fungal infection.⁵ According to new antibiotic policy, we continue to use Ceftriaxone plus Amikacin at first position and Imipenem and Amikacin started as second line therapy. This present study showed 36% sensitivity to first line therapy, which was 50% in the previous study whereas sensitivity to imipenem was 92%, which is still satisfactory. Failure or low response to Ceftriaxone could be due to production of extended spectrum β -lactamases (ESBL). β -lactamase is an enzyme that hydrolyzes the beta lactam ring of beta-lactam antibiotics. The extended spectrum cephalosporin such as third generation was widely used as a β -lactamase inhibitor especially for enteric bacilli such as *E. coli* and *Klebsiella*. Later on it was found that new type of beta lactamase is produced by *Klebsiella* spp. and *E. coli* that hydrolyze the beta-lactam ring of cephalosporins also. These new β -lactamases are collectively known as ESBLs.²⁰ Although ESBLs was not detected by our lab, however, antibiogram is suggestive of ESBL producer organisms. Based on the above evidence, it is suggested that the institutional antibiotic policy for pediatric PBSCT patients should be revised periodically in order to decrease posttransplant morbidity and mortality.

CONCLUSION

Pediatric poststem cell transplant, febrile neutropenia could be managed in a developing country setting. The morbidity and mortality remain comparable to more advanced countries. Gram-negative infections are predominating infection in this group of patients. These results suggest a high resistance to third generation cephalosporins. Institutional antibiotic policy should be revised at regular interval in order to decrease post transplant morbidity and mortality.

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