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Post-transplant infections: single center experience from the developing world

Khalil Ullah [a,*], Shahid Raza [a], Parvez Ahmed [a], Qamar-un-Nisa Chaudhry [a], Tariq Mahmood Satti [a], Suhaib Ahmed [b], Sajjad Hussain Mirza [b], Fahim Akhtar [b], Khalid Kamal [a], Farrukh Mahmood Akhtar [a]

[a] *Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan*
[b] *Armed Forces Institute of Pathology, Rawalpindi, Pakistan*

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Summary
Objective: To describe our experience of post-transplant infections in allogeneic stem cell transplants at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan.
Methods: From July 2001 to September 2006, patients with malignant and non-malignant hematological disorders having human leukocyte antigen (HLA)-matched sibling donors were selected for transplant. Pre-transplant infection surveillance was carried out, and strict prophylaxis against infection was observed. After admission to the hospital, patients were kept in protective isolation rooms, equipped with a HEPA filter positive-pressure laminar airflow ventilation system. Bone marrow and/or peripheral blood stem cells were used as the stem cell source. Cyclosporin and prednisolone were used as prophylaxis against graft-versus-host disease (GVHD). The engraftment was monitored with cytogenetic/molecular analysis and change of blood group. Survival was calculated from the date of transplant to death or last follow-up.
Results: One hundred and fifty-four patients received allogeneic stem cell transplants from HLA-matched siblings for various hematological disorders at the Armed Forces Bone Marrow Transplant Centre, Rawalpindi, Pakistan between July 2001 and September 2006. Indications for transplant included aplastic anemia ($n = 66$), β-thalassemia major ($n = 40$), chronic myeloid leukemia ($n = 33$), acute leukemia ($n = 8$), and miscellaneous disorders ($n = 7$). One hundred and twenty patients were male and 34 were female. The median age of the patient cohort was 14 years (range $1^1/_4 - 54$ years). One hundred and thirty-six patients and 135 donors were cytomegalovirus (CMV) IgG-positive. One hundred and forty patients (90.9%) developed febrile episodes in different phases of post-transplant recovery. Infective organisms were isolated in 150 microbiological culture specimens out of 651 specimens from different sites of infections (23.0% culture positivity). Post-transplant infections were confirmed in 120 patients (77.9%) on the basis of clinical assessment and microbiological, virological, and histopathological examination. Mortality related to infections was 13.0%. Fatal infections included CMV disease (100% mortality, 6/6),

* Corresponding author. Tel.: +92 51 561 31287; fax: +92 51 9271860.
E-mail address: dr_khalil58@yahoo.com (K. Ullah).

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disseminated aspergillosis (66.7% mortality, 4/6), pseudomonas septicemia (42.9% mortality, 9/21), and tuberculosis (25% mortality, 1/4).
Conclusions: More than 90% of our patients developed febrile episodes with relatively low culture yield. The majority of infections were treated effectively, however CMV, aspergillosis, and pseudomonas infections remained problematic with high mortality.

Introduction

Stem cell transplantation (SCT) is the most effective treatment for hematological disorders. Opportunistic infections are one of the main causes of morbidity and mortality after SCT and the successful outcome of SCT is largely determined by infectious complications. According to International Bone Marrow Transplant Registry (IBMTR) data for the period 1996—2000, infections contributed to 17% of deaths in allogeneic SCT and 21% in autologous SCT.[1]

The epidemiology of these infections depends on the degree, type, and duration of immune suppression, the use of prophylactic antibiotics, surveillance for organisms associated with nosocomial infections, the emergence of drug-resistant organisms, and the use of isolation precautions.[2] The relative frequencies of opportunistic pathogens vary at different periods post-SCT. Therefore it is important to define the pattern of infectious complications at different times after SCT.[3] Factors that influence the risk of infections include mucosal damage, presence of a right atrial catheter, and prolonged neutropenia prior to SCT. The role of graft-versus-host disease (GVHD) and its treatment in the pathogenesis of these infections has been well documented.[4]

Bloodstream infections in patients with profound neutropenia are associated with prolonged hospitalization and poor short-term survival. Systemic fungal infections are serious complications as they carry higher morbidity and mortality compared with frequently isolated nosocomial blood-borne pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, etc.[5] Systemic candidiasis is a serious complication associated with high morbidity and mortality. Antifungal prophylaxis with fluconazole during the early post-transplant period has been shown to significantly reduce the incidence of candidemia and improve short-term survival. Nosocomial fungemia due to *Candida* species has steadily increased over the past four decades in SCT recipients.[6]

For decades, various approaches have been undertaken in an attempt to reduce the risk of translocated oral and bowel flora, which, along with central venous catheters are the source of serious infections in SCT patients through the years. Numerous regimens have been tried, including neomycin and polymyxin, trimethoprim—sulfamethoxazole (TMP—SMX), and most recently, the oral quinolones, particularly ciprofloxacin. Although the practice of oral prophylaxis is routine in many SCT centers, the current problems with drug resistance may force a careful reconsideration of this still unproven approach.[7,8]

Cytomegalovirus (CMV) infection remains one of the most important complications of allogeneic SCT, although the impact on morbidity and mortality has been reduced during the last decade by improvements in management. The different preventive strategies for CMV disease include use of the appropriate blood products, immunoglobulin, and use of antiviral agents either as chemoprophylaxis or pre-emptive therapy. The currently available antiviral agents for the prevention of CMV infection and to treat disease are acyclovir, valacyclovir, ganciclovir, valganciclovir, foscarnet, and cidofovir.[9—11]

The Armed Forces Bone Marrow Transplant Centre has provided bone marrow transplant facilities in Pakistan since 2001. The bone marrow transplant program is in the evolutionary stages in this country and no consolidated data have yet been published. With this background we describe herein our initial experiences of post-transplant infectious complications in various malignant and non-malignant hematological disorders for the period from July 2001 to September 2006.

Patients and methods

Patients with malignant and non-malignant hematological disorders having human leukocyte antigen (HLA)-matched sibling donors were selected for transplant. The age limit for β-thalassemia major, Gaucher's disease, and Fanconi's anemia was 14 years. For aplastic anemia, the age limit was 40 years, and for myelodysplastic syndrome (MDS), acute leukemias, chronic myeloid leukemia (CML), and lymphomas, the age limit was 55 years. These patients were stratified into different risk groups according to well-established criteria. Patients with aplastic anemia were categorized into severe and very severe aplastic anemia on the basis of degree of pancytopenia and marrow cellularity according to Camitta criteria. Patients with β-thalassemia major were categorized into risk classes I, II, and III on the basis of presence or absence of hepatomegaly, degree of fibrosis on liver biopsy, and adequacy of iron chelation according to Lucarelli's Pesaro group risk classification. Patients with CML were classified into standard and high risk on the basis of duration of disease, phase of disease, response to therapy, age, and patient/donor sex combination according to European Group for Blood and Marrow Transplantation (EBMT) risk stratification criteria.

Pre-transplant infection surveillance

As per our transplant protocol, after HLA-typing all patients and sibling donors underwent pre-transplant infection surveillance. Prospective surveillance was carried out to detect the spectrum of microbial pathogens in our patients and to treat them with appropriate antibiotics. Surveillance cultures were taken from the nose, throat, stool, and urine for the pattern of organisms involved, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus spp*, *Salmonella spp*, *Shigella spp*, and enteropathogenic

Escherichia coli. All stool samples were also examined microscopically for intestinal parasites including *Entamoeba histolytica*, *Giardia lamblia*, intestinal flagellates, helminths, and Cryptosporidium.

Peripheral blood films were screened for malarial parasites by conventional microscopy after preparing thick and thin Giemsa-stained peripheral blood smears. All patients and donors were screened for tuberculosis by Mantoux test and chest X-ray, while sputum for acid-fast bacilli (AFB) examination and PCR for *Mycobacterium tuberculosis* were done in suspicious cases. As per our anti-tuberculosis treatment policy, patients and/or donors with a positive Mantoux test or with active tuberculosis received four-drug anti-tuberculosis therapy (rifampin, isoniazid, pyrazinamide, and ethambutol) for at least two months. After re-evaluation these patients were subjected to allogeneic SCT, and anti-tuberculosis therapy was continued for another 6 months post-transplant.

Virological screening for hepatitis B, hepatitis C, HIV, CMV, and Epstein—Barr virus (EBV) was carried out by ELISA and molecular analysis (PCR) where indicated. All patients with positive hepatitis B, hepatitis C, and HIV results were considered unfit for transplant. Virological screening for hepatitis B virus (HBV) was done by testing for hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antigen (anti-HBc) using the MONOLISA plus enzyme immunoassay kit (Bio-Rad). All HBsAg- and anti-HBc-negative patients were subjected to transplant, while HBsAg- and anti-HBc-positive patients were further tested for HBV-DNA by real-time PCR (Sacace Biotechnologies, Italy). These patients, however, were considered unfit for transplant. Hepatitis C virus (HCV) screening was done by testing for anti-HCV antibodies by ELISA (Innotest HCV Ab IV, Italy; fourth generation enzyme immunoassay kit). HCV-RNA was tested for by PCR using QIAamp viral RNA kit (Qiagen, Germany). Anti-HCV-positive patients with normal ALT and negative PCR were subjected to transplant, while HCV PCR-positive patients were considered unfit for transplant. CMV antigenemia testing was done by CMV Brite Kit (IQ Corporation B.V., The Netherlands) to detect CMV antigen PP65 in peripheral blood polymorphonuclear (PMN) cells, and CMV PCR was performed using CMV Real-TM Quant RG real-time kit for quantitative CMV PCR (Corbett Research and Sacace Biotechnologies, Italy). Test results of 20 000 copies/ml were considered positive. CMV antigenemia was monitored weekly for the first 100 days by ELISA and CMV molecular analysis and thereafter monthly for one year. Pre-transplant screening for Varicella zoster virus (VZV) was carried out by indigenously manufactured complement fixation test (CFT). These tests were performed in pre-transplant assessment only to detect high-risk patients. VZV-positive patients were given acyclovir prophylaxis in high doses for longer duration (9 months). Pre-transplant screening for EBV was carried out by EBV viral capsid antigen (VCA) IgM enzyme immunoassay by using DIA PRO diagnostic bioprobes (Srl, Italy).

After admission to the hospital, patients were kept in protective isolation rooms, equipped with a HEPA filter positive-pressure laminar airflow ventilation system. Clinical examination of patients was performed twice a day as well as when required. All patients were provided with a bacteria-reduced diet. Leuko-depleted and irradiated blood products were used during the post-transplant period.

Antimicrobial protocol

Antimicrobial prophylaxis consisted of ciprofloxacin 250—500 mg twice daily (10—30 mg/kg daily in two divided doses) from the start of conditioning for gut decontamination. Empiric broad-spectrum anti-pseudomonal penicillin in combination with aminoglycoside was started once neutropenic patients developed febrile spikes as defined. These consisted of piperacillin—tazobactam 4.5 g IV 6-hourly (adults and children >12 years) or 90 mg/kg 6-hourly (children <50 kg) along with amikacin 15 mg/kg/day. Blood cultures were taken at the start of fever and whenever antibiotics were changed. Results of bacteriological cultures were usually available by the third day. In culture-negative patients who had persisting symptoms, antibiotics were changed on the fifth day.

Fluconazole 50—100 mg daily (3—6 mg/kg daily) and acyclovir 250—500 mg three time a day (5 mg/kg three times daily) were used as antifungal and antiviral prophylaxis, respectively, starting at day −2, continuing until day +180. Patients who required antifungals in therapeutic doses were given amphotericin 1.0—1.5 mg/kg/day. Patients with a confirmed fungal infection responding poorly to amphotericin or developing deranged renal functions were given either voriconazole or caspofungin depending on availability. Acyclovir was switched to therapeutic doses upon confirmation/strong suspicion of susceptible viral infection. Prophylaxis for CMV infection consisted of acyclovir at a dose of 10 mg/kg three times daily from day −2 to day +180. Once the antigenemia or rising copies of CMV PCR was confirmed, pre-emptive antiviral therapy with ganciclovir was started at 5 mg/kg every 12 hours for 14 days or until 7 days after the clearance of CMV antigen from blood, whichever was later, and then ganciclovir (5 mg/kg/day) continued for the next 14 days. CMV disease was treated with ganciclovir (5 mg/kg every 12 hours) for three weeks followed by 5 mg/kg/day for the next 4 weeks.

Pentamidine sulfate (300 mg) nebulization monthly was used as prophylaxis for *Pneumocystis jiroveci* infection at the time of conditioning. TMP—SMX combination was used as prophylaxis after hematological recovery and continued for 9 months post-transplant. Chloroquine sulfate 250—500 mg (10—15 mg/kg) weekly was used as prophylaxis against malaria and continued for 6 months post-transplant. Amoebic dysentery, giardiasis, and helminthic infestations are quite prevalent in our country, so all patients also received metronidazole 200—400 mg three times daily (7.5 mg/kg three times a day) for 7 days and albendazole (200—400 mg) as a single dose before transplant.

Documentation of infections and definitions

Neutropenia was defined as an absolute neutrophil count (ANC) $<0.5 \times 10^9$/l and was considered to have ended at an ANC $>1.0 \times 10^9$/l. An infection was defined as nosocomial when there was no evidence of infection at the time of admission to the hospital. A febrile temperature spike of >38 °C on two occasions 30 minutes apart or >39 °C on one occasion in neutropenic patients was considered as febrile neutropenia. A blood stream infection (BSI) was defined when the pathogen was isolated from blood culture and was not related to infection at another site. Blood culture samples

were drawn from the catheter lumens as well as from a peripheral vein. Single blood culture isolates were sufficient to classify a febrile episode as bacteremia. Catheter-associated sepsis was defined as primary sepsis if there were signs of infection at the insertion site of an intravascular catheter and one of the following: (1) fever $>38\,^{\circ}C$, (2) chills, (3) hypotension. Pneumonia was defined as fever $>38\,^{\circ}C$, production of sputum, cough, dyspnea, ronchi/rales, or pleural rub with radiological evidence/organism isolated from bronchoalveolar lavage (BAL) or cultured from blood. A soft tissue infection was defined when there was localized pain, redness, swelling, or purulent discharge with positive culture from the site involved. Disseminated aspergillosis was defined when Aspergillus infection was established at more than one site. *Pneumocystis jiroveci* (*carinii*) pneumonitis (PCP) was defined when radiological evidence of pneumonitis was combined with isolation of *P. jiroveci* from induced sputum or BAL fluid. We designed consensus definitions for CMV antigenemia, CMV infection, CMV disease, and CMV syndrome. CMV antigenemia was defined as detection of CMV antigen in blood. CMV infection was defined as detection of CMV antigen in blood with signs of respiratory or gastrointestinal system symptoms (pneumonia/diarrhea) without detection of CMV antigen from the appropriate tissue specimen. CMV disease was defined as patients having detectable CMV antigenemia along with appropriate tissue diagnosis. CMV syndrome was defined by fever, pancytopenia, and CMV antigenemia. CMV pneumonia was defined by the presence of cough, fever, and dyspnea combined with radiological evidence of lung infiltrates and detection of CMV in BAL. CMV enteritis was defined by the presence of diarrhea and detection of CMV in involved gut biopsy. Infection-related mortality was defined as the death rate associated with disseminated bacterial, viral, and fungal infections.

Transplant procedures

Patients with aplastic anemia received lymphoglobulin (SANG Stat, Lyon, France) 15 mg/kg daily for 3 days (total dose 45 mg/kg) and cyclophosphamide 50 mg IV daily for four days (total dose 200 mg/kg). Patients with thalassemia (class I and class II) and Gaucher's disease received conditioning with busulfan 3.5 mg/kg PO in divided doses daily for four days (total dose 14 mg/kg) followed by cyclophosphamide 50 mg/kg once daily IV for four days (total dose 200 mg/kg). Class III thalassemic patients received long-duration conditioning with hydroxyurea 30 mg/kg daily and azathioprine 3 mg/kg daily (from day -45 to day -11), fludarabine 20 mg/m^2 daily (from day -17 to day -11), busulfan 3.5 mg/kg PO in divided doses daily for four days (total dose 14 mg/kg), followed by cyclophosphamide 40 mg/kg once daily IV for four days (total dose 160 mg/kg). Patients with acute leukemias and CML received busulfan 4 mg/kg daily for four days (total dose 16 mg/kg), followed by cyclophosphamide either 40 mg/kg daily for four days (total dose 200 mg/kg) or 60 mg/kg daily for two days in high-risk CML patients (total dose 120 mg/kg). Patients with Fanconi's anemia received lymphoglobulin 15 mg/kg daily for three days (total dose 45 mg/kg), fludarabine 30 mg/m^2 daily for 5 days (total dose 150 mg/m^2), and cyclophosphamide 5 mg/kg daily for four days (total dose 20 mg/kg). Patients with non-Hodgkin's lymphoma (NHL) received Campath-1H 20 mg daily for five days, fludarabine 20 mg/m^2 for four days, and melphalan 140 mg/m^2 for one day only.

Stem cell source

The stem cell source for thalassemic patients was primarily bone marrow, whereas peripheral blood stem cells (PBSC) were the main stem cell source for CML patients. However in aplastic anemia, bone marrow and PBSC were both used as the stem cell source. Bone marrow was harvested from the sibling donor on day 0 under general anesthesia by multiple punctures in both iliac crests. Granulocyte-colony stimulating factor (G-CSF) mobilized PBSC were harvested from sibling donors. All donors received G-CSF (Filgen) 10 μg/kg/day for 5 days prior to PBSC harvest. PBSC were harvested on day -2 and day -1 to achieve a standard dose of mononuclear cells $>4.0 \times 10^8$/kg body weight of patient by using a COBE Spectra cell separator. The median apheresis time was 245 min (range 220—270 min). Bone marrow/PBSC harvests were infused into the patient on day 0 under cover of steroids and antihistamines.

GVHD prophylaxis

As prophylaxis against GVHD, cyclosporin (5 mg/kg/day IV in two divided doses) and prednisolone (0.5 mg/kg/day) were used from day -2 onwards. The IV dose of cyclosporin was switched over to oral cyclosporin at the time of discharge from hospital. The oral dose of the cyclosporin at the time of switchover from IV dose was doubled and continued for 9 months. Thereafter cyclosporin was gradually tapered off over the next three months (total duration one year). Cyclosporin dose was adjusted according to blood levels as well as according to the renal status of the patient. Trough levels of cyclosporin were maintained between 200 and 300 ng/ml. Prednisolone was gradually tapered off until day +90 post-SCT. Intravenous methotrexate (10 mg/m^2) was given on days +1, +3, +6, and +11 along with folinic acid rescue therapy. Thalassemia patients also received IV immunoglobulin 500 mg/kg on day -1 and then 250 mg/kg on days +8 and +22.

GVHD was diagnosed and graded both clinically and histologically.[12] GVHD was managed with escalating doses of cyclosporin and steroids. In steroid-resistant GVHD, interleukin-2 receptor antibodies and anti-thymocyte globulin (ATG) were used. Follow-up bone marrow examinations were done on days +30, +100, +180, and +360. The engraftment was monitored with cytogenetic/molecular analysis and change of blood group.

Statistical methods

Survival was calculated from the date of transplant to death or last follow-up according to Kaplan—Meier and Cox (proportional hazard) regression analysis methods. The analysis was performed with Stats Direct software and MS excel software.

Results

From July 2001 to September 2006, a total of 154 patients received allogeneic SCT from HLA-matched sibling donors at the Armed Forces Bone Marrow Transplant Centre,

Rawalpindi for various hematological disorders. Diseases included were aplastic anemia (66), β-thalassemia (40), CML (33), acute leukemia (8), and miscellaneous disorders, which included Fanconi's anemia (3), MDS (2), Gaucher's disease (1), and NHL (1).

One hundred and twenty patients were male and 34 were female. The median age of the patients was 14 years (range $1^{1}/_{4} - 54$ years). Eighty-eight donors were male and 66 were female. Sixty-six patients were transplanted across gender. Forty-nine patients had ABO mismatch transplants with major ABO mismatch in 30 patients and minor ABO mismatch in 19 patients. In 125 transplants both the patient and the donor were CMV-positive, in 11 transplants the patient was positive while the donor was negative, in 10 transplants the patient was negative and the donor was positive, while in eight transplants both the patient and donor were CMV-negative. Forty-seven patients received bone marrow, 53 received PBSC, and 54 received both bone marrow and PBSC harvest. Patient/donor characteristics and transplant procedures are shown in Table 1.

Out of 154 patients who underwent SCT, 140 patients had febrile episodes either alone or in association with other signs and symptoms during different post-transplant recovery phases. Relevant cultures and samples were taken in all febrile patients to ascertain causative organisms. Out of 651 cultures from different sites, 150 culture specimens (23.0%) were positive. Post-transplant infection was confirmed in 120 patients (77.9%) on the basis of clinical assessment and microbiological, virological, and histopathological analysis.

Bacterial infections were seen in 79/154 (51.3%) patients. The majority of bacterial pathogens were Gram-negative organisms (60.8%) compared with Gram-positive organisms (39.2%). Infective organisms isolated in our patients in order of frequency were coagulase-negative Staphylococcus (CoNS) in 23 patients (14.9%), followed by *Pseudomonas spp* in 21 patients (13.6%), *Klebsiella spp* in nine patients (5.8%), MRSA in eight patients (5.2%), *Acinetobacter spp* in eight patients (5.2%), *E. coli* in seven patients (4.5%), and *Enterobacter spp* in three (1.9%) patients. *Mycobacterium tuberculosis* was seen in four patients (2.6%). The majority of bacterial pathogens were isolated from either blood drawn from a peripheral vein or fluid collected from the central venous line (heparinized saline used to lock the line when not in use).

Fungal infections were seen in 23 patients (14.9%). Among fungal infections, *Candida albicans* was isolated from blood, central venous line fluid, and sputum in 16 patients (10.4%). *Aspergillus spp* was isolated from blood, tissues, sputum, BAL, and pus in six patients (3.9%). *Pneumocystis jiroveci* was isolated from BAL in one patient (0.6%).

Plasmodium falciparum was isolated from peripheral blood in one patient (0.6%). CMV infection was documented in 28 patients (18.2%). CMV disease (two enterocolitis, four pneumonia) was seen in six patients (3.9%). Vesicular eruptions in dermatomal pattern suggestive of herpes zoster infection were clinically seen in six patients (3.9%). The frequencies of infections seen in our patients are shown in Table 2.

Infection-related morality was observed in 20 patients (13.0%). The various infective causes of mortality were Pseudomonas septicemia, disseminated aspergillosis, CMV disease, and disseminated tuberculosis. Fatal Pseudomonas septicemia was observed in the early recovery phase whereas fatal fungal and CMV infections were observed in the mid and late recovery phases. Mortality related to tuberculosis was observed in the mid recovery phase. Out of 21 patients who developed Pseudomonas infection, nine died of septicemia during the first 30 days post-SCT.

Four patients out of six who developed Aspergillus infection died of disseminated disease involving the lungs, liver, and subcutaneous tissues. Two patients developed Aspergillus infection during the mid recovery phase. Of these, one patient developed bilateral pneumonitis and nodular ulcerating lesions in the lower abdomen and right thigh on day +45; the tissue biopsy and culture from the site revealed the growth of *Aspergillus fumigatus*. The other patient developed grade III acute GVHD, which was complicated by pulmonary and hepatic aspergillosis at day +74; tissue biopsy and culture confirmed the growth of *Aspergillus fumigatus*. The remaining two patients developed disseminated aspergillosis during the late recovery phase at day +123 and day +187. Both patients had chronic extensive GVHD, which was complicated by jaundice, hepatomegaly, and subcutaneous nodular swellings involving the thighs. Tissue biopsy specimens confirmed the growth of *Aspergillus fumigatus*. All patients received amphotericin B initially against Aspergillus infection. Two patients were treated with amphotericin B combined with itraconazole, while one patient was treated with voriconazole and the other received caspofungin. None of these patients responded to antifungal therapy and they died on days +59, +72, +135, and +201 post-SCT.

Out of six patients with CMV disease, two developed enterocolitis during the mid recovery phase at days +42 and +59. CMV pneumonitis was observed in four patients during the late recovery phase at days +98, +116, +127, and +139 post-SCT. All patients were treated with ganciclovir, however none responded to antiviral therapy and they died at days +54, +67, +110, +123, +133, and +155 post-SCT.

A total of four patients had tuberculous infection at days +21, +30, +92, and +241 post-SCT. Out of these, one patient developed tuberculous lymphadenopathy with superior mediastinal widening at day +92. Mediastinoscopic tissue biopsy and cultures were positive for *M. tuberculosis* with caseating granulomatas. The patient did not respond to treatment and died on day +124 post-transplant. The frequency of infections in the early, mid, and late recovery phases, and the sites of infection are shown in Tables 3 and 4.

Aspergillus infection was observed in aplastic anemia and CML only, whereas Candida infection was more common among thalassemic patients. CMV disease was more frequent in thalassemia and CML patients. Apart from these, the other infections were evenly distributed in all the hematological disorders as shown in Table 5.

In our study, at the end of 5 years, the overall survival and disease-free survival were 72.7% and 70.7%, respectively, with a median follow-up of 56.2 ± 3.1 months. Disease-free survival for the different hematological disorders in our patients as compared to those reported by the Center for International Blood and Marrow Transplant Research (CIBMTR) and EBMT are shown in Figure 1. Overall mortality was observed in 27.3% (42/154). Mortality related to infections was 13.0% (20/154). Post-transplant fatal infections in different hematological disorders are shown in Table 6.

Table 1 Patient/donor characteristics and transplant procedures

	All disorders (n = 154)		Thalassemia (n = 40)		Aplastic anemia (n = 66)		CML (n = 33)		Acute leukemia (n = 8)		Misc. (n = 7)[a]	
	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor
Sex												
Male	120	88	28	16	52	37	30	25	6	5	4	5
Female	34	66	12	24	14	29	3	8	2	3	3	2
M/F ratio	3.5:1	1.3:1	2.3:1	0.6:1	3.7:1	1.3:1	10:1	3.1:1	3:1	1.6:1	1.3:1	2.5:1
Age												
Median age (years)	14	17	4	5.5	17	18	25	27	21	15	10	14
Range (years)	1¼–54	1–54	1¼–14	1–18	5–38	3–45	7–54	7–54	5–36	5–36	2–40	8–35
CMV status												
Patient (+): donor (+)	125		25		54		33		6		7	
Patient (+): donor (−)	11		5		6		-		-		-	
Patient (−): donor (+)	10		6		2		-		2		-	
Patient (−): donor (−)	8		4		4		-		-		-	
Risk stratification			Pesaro risk class		Camitta classification		Risk group		Risk group		Risk group	
			Class I	25	VSAA	52	Standard risk	23	Relapse disease (8); high risk (0)		FA (3); MDS (2); Gaucher's disease (1); NHL (1)	
			Class II	10	SAA	14	High risk	10				
			Class III	5								
ABO incompatibility												
Major	30		6		14		8		1		1	
Minor	19		10		5		2		0		2	
Conditioning regimen			Class I and II: Bu 14/Cy 200; class III: hydroxyurea, azathioprine, Flu, Bu 14/Cy 160		Cy 200 mg/kg		Bu 16/Cy 200		Bu 16/Cy 200		FA: Flu, ATG, Cy; Gaucher's disease: Bu 14/Cy 200; MDS: Bu 16/Cy 200; NHL: Campath, Flu, Mel	
					ATG 45 mg/kg		Bu 16/Cy 120					
Source of stem cell												
BM	47		35		6		1		-		5	
PBSC	53		5		8		30		8		2	
PBSC + BM	54		-		52		2		-			
Transplant												
First	154		40		66		33		8		7	
Second	6		3		3		-		-			

Table 1 (*Continued*)

	All disorders (n = 154)		Thalassemia (n = 40)		Aplastic anemia (n = 66)		CML (n = 33)		Acute leukemia (n = 8)		Misc. (n = 7)[a]	
	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor	Patient	Donor
Donor/recipient gender value												
M/M	70		10		31		22		5		2	
F/F	18		6		8		-		2		2	
M/F	17		6		6		3		-		2	
F/M	49		18		21		8		1		1	

CML, chronic myeloid leukemia; FA, Fanconi's anemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; Bu, busulfan; Cy, cyclophosphamide; Flu, fludarabine; ATG, antithymocyte globulin; Mel, melphalan; BM, bone marrow; PBSC, peripheral blood stem cells.
[a] Fanconi's anemia (3), MDS (2), Gaucher's disease (1), and NHL (1).

Table 2 Frequency of infections, number of febrile episodes, and microbiological culture yield

	Patients (%)
Infections	
Bacterial infections[a]	79/154 (51.3)
Fungal infection[b]	23/154 (14.9)
Cytomegalovirus disease	6/154 (3.9)
Herpes infections	6/154 (3.9)
Tuberculosis	4/154 (2.6)
Pneumocystis jiroveci	1/154 (0.6)
Malaria	1/154 (0.6)
Total	120/154 (77.9)
Febrile episodes	140/154 (90.9)
Microbiological culture yield	150/651 (23.0)

[a] Coagulase-negative Staphylococcus (23), *Pseudomonas aeruginosa* (21), *Klebsiella spp* (9), methicillin-resistant *Staphylococcus aureus* (8), *Acinetobacter spp* (8), *Escherichia coli* (7), *Enterobacter spp* (3).
[b] *Candida albicans* (16), *Aspergillus spp* (6), *Trichosporon beigelii* (1).

Discussion

Opportunistic infections of varying severity with bacterial, viral, and fungal organisms occur in >90% of patients after allogeneic SCT and contribute significantly to morbidity and mortality after engraftment.[2] Fatal opportunistic infections have been reported in 4—15% of related transplant recipients and 12—28% of unrelated transplant recipients.[13] Data from 18 different transplant centers in the UK for 1825 transplant patients showed a 6.2% incidence of severe infections requiring readmission to hospital less than 100 days following bone marrow transplant (BMT). The majority of infections occurred in recipients of allogeneic SCT (58%) and proved fatal in 3.7% of all patients. Common organisms encountered were CMV, *P. jiroveci*, *Streptococcus pneumoniae*, *Pseudomonas spp*, and *Aspergillus spp* with mortalities of 84%, 67%, 33%, 85%, and 87%, respectively.[14]

Bacterial infections are frequently seen during the post-transplant neutropenia phase. During the early post-transplant period, damage to the mucosal surfaces and neutropenia allow commensal bacteria easy entry into the systemic circulation. Routine frequent use of indwelling central venous catheters in transplant patients results in local infection and sepsis. The most common pathogens are Gram-positive bacteria, especially CoNS. Besides these, Gram-negative organisms including *E. coli*, *Klebsiella spp*, and *P. aeruginosa* are the most common bacterial pathogens during the early post-transplant neutropenic phase.[15] Usually antibiotic treatment alone is adequate, but occasionally removal of the catheter is necessary if the infection is not responsive. The classic approach for the management of these infections has been a semi-synthetic penicillin/extended-spectrum cephalosporin with an aminoglycoside. These antibiotics are used empirically at first indication of fever in neutropenic patients.[16]

Over the past two decades the percentage of infections caused by Gram-negative organisms has steadily decreased from 70% to 30%, and Gram-positive bacteria now account for

Table 3 Post-transplant opportunistic bacterial, fungal, viral, and parasitic infections by recovery phase

Organisms	Early recovery phase (pre-engraftment) (<30 days)	Mid recovery phase (post-engraftment) (30–100 days)	Late recovery phase (>100 days)	Total
CoNS	13	5	5	23
MRSA	5	2	1	8
Pseudomonas aeruginosa	14	4	3	21
Klebsiella pneumoniae	5	3	1	9
Escherichia coli	4	2	1	7
Acinetobacter spp	3	3	2	8
Enterobacter spp	-	3	-	3
Mycobacterium tuberculosis	2	1	1	4
Pneumocystis jiroveci	1	-	-	1
Candida albicans	11	3	2	16
Aspergillus spp	-	3	3	6
Trichosporon beigelii	1	-	-	1
Cytomegalovirus	-	3	3	6
Varicella zoster virus	-	-	6	6
Plasmodium falciparum	1	-	-	1
Total number of infections	60	32	28	120

CoNS, coagulase-negative Staphylococcus; MRSA, methicillin-resistant *Staphylococcus aureus*.

70% of bacterial infections compared with 30%, 25 years ago.[17,18] Data from developing countries, though limited, show that 40–50% of patients develop bacterial infections; Gram-negative bacteria are still the major organisms, although incidence of Gram-positive organisms seems to be on the increase, consistent with Western data.[19,20] George et al.[21] from India reported 36.9% bacterial infections, 45.7% viral infections, and 11.1% fungal infections in transplant patients. The major bacterial pathogens were Gram-negative organisms (72.7%) compared with Gram-positive organisms (27.3%). Common Gram-negative organisms were Pseudomonas, *E. coli*, and Klebsiella, while CoNS were the main Gram-positive organisms.

In our series, bacterial infections were observed in 51.3% of patients during the early post-transplant period (Gram-negative infections 60.8% and Gram-positive infections 39.2%). *Pseudomonas aeruginosa* and CoNS were major bacterial pathogens.

Tuberculosis has been demonstrated to be a significant problem following allogeneic SCT in endemic countries, with an incidence of 0.1–5.5%.[22] The incidence of active tuberculosis after allogeneic SCT has been reported in 3.1% of

Table 4 Post-transplant infections by site of infection

Organisms	Blood	CV line fluid	Nasal swab	Throat swab	Sputum	BAL	Pleural fluid	Pus	Tissue	Clinical diagnosis	Total
CoNS	13	10	-	-	-	-	-	-	-		23
MRSA	3	3	-	-	2	-	-	-	-		8
Pseudomonas aeruginosa	8	8	1	-	1	-	-	1	2		21
Klebsiella pneumoniae	2	2	-	-	4	-	-	-	-		8
ESBL-producing Klebsiella	1	-	-	-	-	-	-	-	-		1
Escherichia coli	4	-	-	3	-	-	-	-	-		7
Acinetobacter spp	-	4	2	-	-	-	-	-	2		8
Enterobacter spp	-	2	-	1	-	-	-	-	-		3
Mycobacterium tuberculosis	-	-	-	-	-	1	2	-	1		4
Pneumocytis jiroveci	-	-	-	-	-	1	-	-	-		1
Candida albicans	6	6	-	-	4	-	-	-	-		16
Aspergillus spp	2	-	-	-	1	1	-	1	1		6
Trichosporon beigelii	-	-	-	-	-	1	-	-	-		1
Cytomegalovirus	-	-	-	-	-	-	-	-	6		6
Varicella zoster virus	-	-	-	-	-	-	-	-	-	6	6
Plasmodium falciparum	1	-	-	-	-	-	-	-	-		1
Total	40	35	3	4	12	4	2	2	12	6	120

CV line, central venous line; BAL, bronchoalveolar lavage; CoNS, coagulase-negative Staphylococcus; MRSA, methicillin-resistant *Staphylococcus aureus*; ESBL, extended-spectrum beta-lactamase.

Table 5 Post-transplant opportunistic infections by disease

Organisms	Aplastic anemia	β-Thalassemia	CML	Acute leukemia	Misc.	Total
CoNS	11	5	4	2	1	23
MRSA	2	3	2	1	0	8
Pseudomonas aeruginosa	10	2	4	3	2	21
Klebsiella pneumoniae	4	2	2	0	1	9
Escherichia coli	1	2	2	1	1	7
Acinetobacter johnsonii	3	3	1	1	0	8
Enterobacter spp	2	1	0	0	0	3
Mycobacterium tuberculosis	1	1	2	0	0	4
Pneumocystis jiroveci	0	0	0	1	0	1
Candida albicans	1	9	3	1	2	16
Aspergillus spp	3	0	3	0	0	6
Trichosporon beigelii	0	0	0	1	0	1
Cytomegalovirus	1	3	2	0	0	6
Varicella zoster virus	2	3	1	0	0	6
Plasmodium falciparum	1	0	0	0	0	1
Total	42	34	26	11	7	120

CML, chronic myeloid leukemia; CoNS, coagulase-negative Staphylococcus; MRSA, methicillin-resistant *Staphylococcus aureus*.

patients in Korea.[23] Similarly in Turkey, where tuberculosis is endemic, a 30—40 times higher incidence has been reported in BMT patients compared to the general Turkish population.[24] A study from Taiwan shows a trend towards increased risk of having pulmonary tuberculosis in allogeneic SCT as compared to autologous SCT ($4.8 \pm 1.8\%$ vs. 0).[25] In 2001, George et al.[26] and Chandy et al.[27] from Vellore, India reported 1.38% and 2.2% incidence of tuberculosis in transplant recipients, respectively. However, updated data from the same center in 2006 show 1.7% tuberculosis in transplant patients.[21] In our series, four (2.6%) patients developed tuberculosis. Of these, two patients developed the disease in the early post-transplant period whereas the other two developed tuberculosis in the mid and late recovery phases. These infections were most probably due to reactivation of previous tuberculosis infection.

PCP accounts for fewer than 10% of the cases of interstitial pneumonia in patients with allogeneic SCT.[28] One of our patients developed PCP pneumonia during the first post-transplant month. Because of early diagnosis and initiation of treatment this patient made a complete recovery.

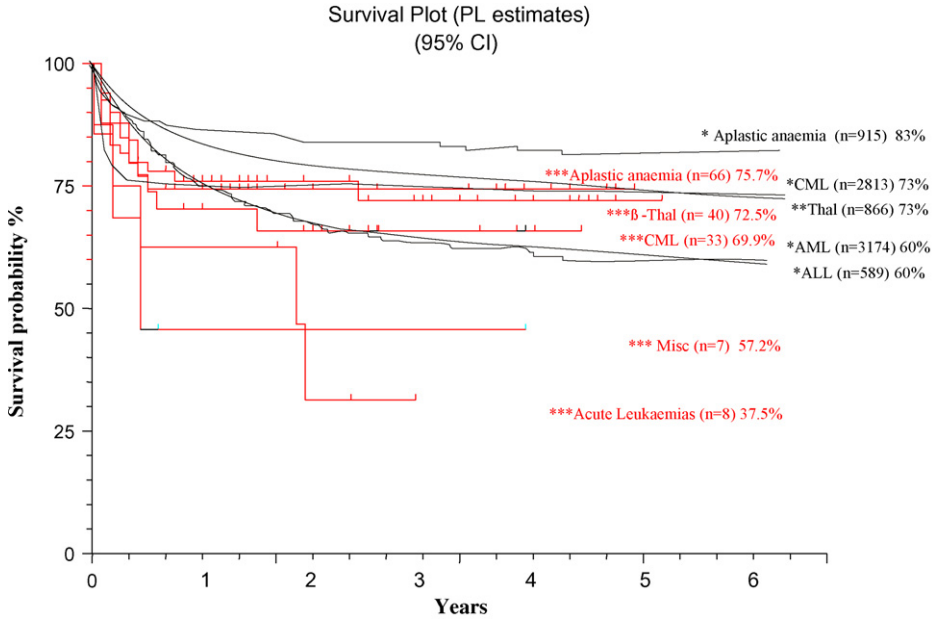

Figure 1 HLA-identical sibling hematopoietic stem cell transplantation disease-free survival (DFS) by disease. *CIBMTR post-transplant (DFS) data, 1998—2004; Marcelo et al., Report on state of the art in blood and marrow transplantation. *CIBMTR Newsletter* 2006;**12**:5—10. **EBMT Working Party on Pediatric Diseases, Pesaro group post-transplant (DFS) data, 1982—2001; Lucarelli et al., The cure of thalassemia by bone marrow transplantation. *Bone Marrow Transplant* 2001;**28**(Suppl 1):S11—3. ***Armed Forces Bone Marrow Transplant Centre Pakistan, post-transplant (DFS) data, 2001—2006.

Table 6 Causes of death by disease

Disease	Relapse	aGVHD grade IV	cGVHD	VOD	ICH	ARF	Septicemia	Fungal infection	CMV	TB	Total
Aplastic anemia (n = 66)	-	1	2	2	-	4	4	2	1	-	16
β-Thalassemia (n = 40)	-	1	-	1	-	-	2	-	3	1	8
CML (n = 33)	-	1	1	2	-	-	2	2	2	-	10
Acute leukemia (n = 8)	5	-	-	-	-	-	-	-	-	-	5
NHL (n = 1)	-	-	-	-	-	-	-	-	-	-	-
MDS (n = 2)	-	-	-	1	-	-	-	-	-	-	1
Fanconi's anemia (n = 3)	-	-	-	-	1	-	-	-	-	-	1
Gaucher's disease (n = 1)	-	-	-	-	-	-	1	-	-	-	1
Total (n = 154)	5	3	3	6	1	4	9	4	6	1	42

Overall mortality 27.3% (42/154); infection-related mortality 13.0% (20/154); non-infection-related mortality 14.3% (22/154).
aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; VOD, veno-occlusive disease; ICH, intracranial hemorrhage; ARF, acute renal failure; CMV, cytomegalovirus; TB, tuberculosis; CML, chronic myeloid leukemia; NHL, non-Hodgkin's lymphoma; MDS, myelodysplastic syndrome.

Nosocomial fungemia due to *Candida spp* and systemic candidiasis are serious post-transplant complications associated with high morbidity and mortality. Antifungal prophylaxis with fluconazole during the early post-transplant period has been proved beneficial.[6] From January 1997 to December 1999, out of 204 allogeneic marrow transplants performed at the University of South Carolina, USA, 14 patients developed nosocomial candidemia while receiving fluconazole (100—200 mg daily) prophylactically, and the incidence of hematogenous candidiasis was only 6.8%.[29]

Invasive fungal infections (IFI) with *Aspergillus spp* are a significant cause of morbidity and mortality after SCT.[30,31] More than half of transplant patients affected by aspergillosis die from their fungal infections. Reactivation in transplant patients has been estimated to be 30—50% and is associated with a poor prognosis.[32,33] Retrospective analysis of data from 48 transplant patients shows a 33% overall risk of Aspergillus relapse with 88% mortality.[34] Between April 1980 and December 2002, a total of 1453 patients were transplanted at HMR Quebec, Canada. Five hundred and twenty-five patients had 939 episodes of blood stream infections. Out of these, aspergillemia was observed 23 times in 21 patients.[35] Voriconazole has been used as first line treatment of aspergillosis and has been shown to be more effective and better tolerated than amphotericin B. Despite a substantial number of side effects, voriconazole is widely used after SCT.[36—38] Studies from Asian countries like Israel and India have observed an increasing incidence of fungal infections from BMT units.[39,40] In our series, fungal infections were observed in 14.9% of patients. *Candida spp* was the major fungal pathogen (69.5%) as compared to *Aspergillus spp* (26%). The overall incidence of fungal infections in our patients is similar to that found in other Asian countries like India; however, aspergillosis was a more common infection in transplant recipients in Vellore, India, whereas candidiasis was the more common infection in our patients. We used fluconazole prophylaxis routinely at a dose of 50—100 mg daily. Sixteen patients (10.4%) developed *Candida spp* infection during the early post-transplant period. All patients responded to amphotericin B and no fatality was seen. Six of our patients developed disseminated aspergillosis in the mid and late recovery phases and this proved fatal in four patients. All patients were initially treated with amphotericin B and itraconazole. Patients who did not respond also received voriconazole—caspofungin.

CMV infection is the most common fatal infection following a BMT, accounting for 15—20% mortality. The incidence of CMV infection in 785 BMT patients in one institution has been reported to be 49% in autologous transplants and 46% in allogeneic transplants.[41] CMV interstitial pneumonitis is the most common infectious complication.[15] Ganciclovir alone or in combination with immunoglobulins has been shown in several studies to result in resolution of pneumonitis in 50—70% of patients.[42,43] George et al.[21] reported 21.3% CMV infection and 20.5% CMV disease in transplant patients, with 90% mortality. In our series, CMV infection was documented in 28/154 (18.2%) patients and CMV disease was seen in 3.9% of patients with 100% mortality. One patient developed disease during the early recovery phase while two developed disease during the mid recovery phase and three patients developed disease during the late recovery phase. One hundred and thirty-six patients and 135 donors were CMV IgG-positive during pre-transplant workup. Five out of six patients also had extensive chronic GVHD and were on heavy immunosuppression. Four patients were treated with ganciclovir, while in two patients diagnosis was confirmed after death. Unfortunately none of these patients survived.

Varicella zoster virus (VZV) infection is most problematic in sero-positive patients during the mid and late recovery phases, usually due to reactivation of the virus. Its usual manifestation is dermatomal vesicular eruptions at a median of 5 months after BMT and occurs in 25—40% of patients.[44] Four of our patients developed herpes zoster infection during the late recovery phase, between 1 and 2 years post-SCT, and were successfully treated with acyclovir.

In summary, infections were documented in 77.9% of our patients with 13.0% mortality. The major causes of mortality were CMV enterocolitis/pneumonia, disseminated aspergillosis, pseudomonas septicemia, and disseminated tuberculosis with mortalities of 100% (6/6), 66.7% (4/6), 42.9% (9/21), and 25% (1/4), respectively.

The high rate of mortality from CMV and Aspergillus infections in our series was probably due to disseminated infections as a result of the heavy immunosuppression that

these patients were receiving due to GVHD. None of our patients developed protozoal or helminthic infections apart from a single case of malaria. This could be due to our effective prophylaxis.

Pakistan is a developing country in Southeast Asia where tuberculosis and malaria are prevalent and the majority of the population is sero-positive for CMV. We were apprehensive about an increased incidence of tuberculosis, malaria, and CMV disease in our patients when we started the bone marrow transplant program in our country. We screened all patients and donors for these diseases.

One of our major concerns is the limited availability of newer, less toxic antifungal and antiviral drugs. Another major concern is poor patient compliance with treatment not uncommon in developing countries such as Pakistan. There is a need to improve social services in our country as well as to educate the patients and their families to comply with treatment during the post-transplant period.

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