INFECTIONS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION WITH A NONMYELOABLATIVE CONDITIONING REGIMEN

Running title: Infections in nonmyeloablative HCT

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Abstract

Hematopoietic cell transplantation (HCT) following nonmyeloablative conditioning (NMSCT) could be associated with a reduced risk of infection compared to standard allogeneic HCT. We retrospectively analyzed incidence and risk factors of infection in 62 patients undergoing NMSCT with low-dose TBI +/- fludarabine and postgrafting CsA and MMF. The proportion of patients with any infection was 77%, but the majority of infectious events occurred beyond day 30. Donor other than sibling, older age, early disease and male gender were significant risk factors. The incidence of bacteraemia was 55% at 1 yr and the number of bacteraemic episodes was 0.9 per patient (0.08 before day 30). The risk of bacteraemia increased with older age and the use of a donor other than an HLA-identical sibling, but not with neutropenia. The incidence of infections other than bacteraemia correlated with the use of corticosteroids. The risk of CMV infection increased with high-risk CMV serology, and risk of CMV disease with high-risk CMV serology, older age, first transplantation and a diagnosis of lymphoma. In conclusion, after NMSCT, infections are not frequent in the first 30 days post-transplant but careful long-term monitoring is necessary thereafter.

Key words

Hematopoietic stem cell transplantation, allogeneic transplantation, nonmyeloablative conditioning, infections.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HCT) with a myeloablative conditioning remains the only curative treatment for several haematological malignancies[1]. However, because of its toxicity, this approach is restricted to younger and fitter patients. This observation led several groups to set up transplant protocols with a nonmyeloablative conditioning (NMSCT for "nonmyeloablative stem cell transplantation") in which elimination of malignant cells is shifted from the preparative regimen to the GVL effect[2, 3]. The Seattle team has proposed an original approach to NMSCT with a conditioning regimen based on single dose (2 Gy) TBI +/- fludarabine (90 mg/m^2), followed by post-transplant immunosuppression with cyclosporine A (CsA) and mycophenolate mofetil (MMF) that permitted to perform the transplant in an ambulatory care setting[4-6]. The authors observed a low transplant-related mortality (even in older patients), which was most often attributed to graft-versus-host disease (GVHD) and/or infections[5, 6]. Thus, one major objective to improve outcome following NMSCT is to achieve rapid immune reconstitution (to permit the occurrence of the GVL effect[7] and avoid opportunistic infections), without severe GVHD.

Infections represent up to 63% of causes of death in standard allogeneic transplant recipients at the time of autopsy[8]. Theoretically, NMSCT could be associated with a reduced risk of infection because they cause less disruption of the gastrointestinal mucosa [9-11] and less severe neutropenia[9, 12, 13]. In addition, the prolonged presence of host immunity could provide some protection against infectious events[14]. However, it is not yet clear whether NMSCT would indeed result in a reduced risk of infection compared to standard myeloablative allogeneic transplantation. Available data have sometimes indicated a reduced incidence of infection after NMSCT[9, 10] but other studies have observed similar rates of infection after the two forms of allogeneic transplantation[11, 12, 15-18]. We therefore conducted a retrospective study to describe the incidence and types of infection in 62 recipients of a NMSCT in our institution.

Patients and methods

Patients

Patients' characteristics are described in Table 1. The study population included 62 consecutive patients, 16 females and 46 males, aged 54±11 yrs (M±SD), who had undergone NMSCT between March 2000 and May 2003. NMSCT were performed in patients deemed unfit for HCT with myeloablative conditioning because of age (> 55 yrs for sibling or > 50 yrs for UD transplants), comorbidity (poor organ function or active infection) or relapse after a conventional transplant. Among them, 55 had haematological malignancies and 7 had renal cell carcinoma, one-third in early disease (untreated or 1st partial (PR) or complete (CR) remission) and two-thirds in more advanced stages. The donor was an HLA-identical sibling in about 50% of the cases and an alternative donor in the other 50%. All patients on study were followed up for a minimum of 6 months or until death. Patients and donors gave written informed consent to the transplant procedure, as well as to the collection and analysis of their clinical data and this was approved by the Ethics Committee of the University of Liège.

Clinical management

In accordance with the regimen developed by the Seattle team[19], patients received low-dose TBI (2Gy) either alone (n=19) or with fludarabine (90mg/m^2) (n=33),. Cyclophosphamide (3 g/m^2) was substituted for TBI in case of previous irradiation precluding TBI (n=10). PBSC were mobilized with G-CSF 10 µg/kg/d. Graft manipulations consisted in CD8 depletion (n=28) or CD34 selection (n=7). An average dose of 5.7±2.9 x 10^6 CD34+ cells/kg was infused. Post-transplant immunosuppressive therapy was carried out with Cyclosporine A (CsA, from day –1 to day 180 or longer in case of alternative donor or chronic GVHD) and mycophenolate mofetil (MMF, 15 mg/kg b.i.d. from day -1 to day 28 or day 42 in case of alternative donor) as previously described[14, 20]. The diagnosis and grading of acute GVHD was established as previously reported[21]. First-line treatment of GVHD consisted in corticosteroids (2 mg/kg). G-CSF (5 µg/kg/d) was administered only when the

granulocyte count was below 1.0 x 10^9/L. Disease evaluation was routinely carried out on days 40, 100, 180 and 365. All patients had a central venous catheter device. DLI were given either prophylactically after CD34 selection or in case of poor chimerism or relapse .

Infection prophylaxis and treatment

Patients also received acyclovir (400 mg t.i.d. orally), oral antifungal prophylaxis with itraconazole solution (200 mg b.i.d.) and aerosolized pentamidine, but no antibacterial prophylaxis, until the end of immunosuppressive therapy. In case of fever, above 38.3°C once or above 38°C on three consecutive measurements, empirical antibiotherapy was started with a combination of cefepime + amikacin. Before the initiation of antibiotherapy, blood cultures were drawn through each lumen of the central catheter and a peripheral vein. Additional blood cultures were obtained when clinically indicated. Patients were weekly screened for CMV by antigenemia and PCR in blood. Pre-emptive therapy with ganciclovir was initiated after a positive antigenemia or PCR and discontinued after day 100 and at least three consecutive negative results.

Definitions

According to the definitions of the Infectious Diseases Working Party of the EBMT, bacteraemia or fungemia were defined by the isolation of bacteria or fungi from any blood culture in the context of fever or other clinical signs consistent with infection[22]. For coagulase-negative staphylococci, at least two blood cultures with the same antimicrobial susceptibilities were required to be positive. Varicella-zoster virus (VZV) infections were defined as typical cutaneous vesicular lesions or atypical lesions associated with the detection of VZV by culture or IF. Any positive result in CMV screening tests was considered as CMV infection whereas CMV disease was defined by the evidence of CMV infection at one body site (PCR or histopathologic finding) in association with clinical signs or symptoms. Invasive fungal infections were defined according to the EORTC criteria[23]. Fever of unknown origin (FUO) was defined as fever alone without any clinical sign or bacteriological documentation.

Statistical analysis

Patients' characteristics (Table 1) and proportions of patients with a given number of infections (Table 2) were analyzed by appropriate contingency tables. Times to infectious events were studied by Kaplan-Meier analyses. Univariate and multivariate Cox regression models were used to analyze the influence of selected variables on the risk of infection. Times to events were the outcomes, with censoring at time of subsequent transplant, death or last follow-up. Variables were selected with a forward stepwise procedure (criterion $p<0.05$) and P values were calculated with the likelihood ratio test. Statistical analyses were done using Microsoft Excel (Microsoft Corp., Redmond, WA), GraphPad Prism (GraphPad Software, San Diego, CA) and SAS (SAS Institute, Cary, NC) softwares.

Results

Types of infection

No patient experienced toxic mucositis or significant gastrointestinal toxicity. The mean neutrophil nadir was 580/µL (range 0-3000). It 35 patients, the neutrophil count did not decrease below 500/µL. Patients received G-CSF for a mean of 5.5 (0-17) days. The median time to 1,000 PMN/µL was 8.5 (1-22) days. There were 25 deaths (40%). Sixty percent of the deaths were due to relapse/progression of the underlying disease. Infection was the primary cause of death in 10% of the patients but infections contributed to death in 24% of them. The proportion of patients presenting any infection during the course of their follow-up was 77% (Table 2) but the majority of these first infections occurred well beyond day 30 post-transplantation (figure 1A).

The actuarial incidence of bacteraemia at one year was 55% (Figure 1B). Overall, there were 0.9 episode of bacteraemia/patient (Table 3). However, these bacteraemias were seldom (0.08 per patient at risk) observed in the first 30 days post-transplant. The largest number of bacteraemias was due to coagulase-negative staphylococci (41%). Gram-negative bacteria accounted for 25% of events.

Other agents causing bacteraemias included streptococci (9%), miscellaneous other bacteria (21%) and fungi (4%). The actuarial incidence at one year of infections other than bacteraemia was 72%, but again these infections were significantly delayed (Figure 1C). The major types of such infections were pneumonias and upper respiratory tract (URT) infections (Table 3).

Infectious agents

Before day 30, the main agents responsible for infections were bacteria (N=18 (82%)) and that dominance persisted throughout the observation period (Table 3). Again there was a low incidence of bacterial infections (n=17, 0.27/patient at risk) in the first 30 days following transplantation. Considering all infectious episodes, Gram-positive bacteria were the agents most frequently responsible for infection: Coagulase-negative staphylococci were involved in 24/132 events and streptococci in 10/132 events. Gram-negative bacteria were involved in 28/132 cases and miscellaneous other bacteria in 15/132 cases.

Fungal infections represented an incidence of 0.03 event/patient at risk before day 30, but their incidence tended to increase thereafter (0.10/patient at risk between days 100 and 365) (Table 3). Candidas were identified in 5% of infectious episodes, while aspergillus was documented in 7% of all infections. However, there was a further 3% incidence of possible fungal infections.

The actuarial incidence of VZV infection was 10% at one year. The actuarial incidences of CMV infection and disease were 52% and 18%, respectively (Figure 2). No case of CMV reactivation or disease occurred among the low risk patients (donor and recipient serologically negative). After excluding low risk patients, rates of CMV infection and disease were 68% and 23%, respectively., i.e. 36% and 9% in the intermediate risk group (donor seropositive and recipient seronegative), and 80% and 26%, in the high risk category (recipient seropositive) (Table 4).

Risk factors for infection

We then examined, in univariate and multivariate analysis, risk factors for infection, separately analyzing all infections, bacteraemias, infections other than bacteraemia, CMV infection and CMV

disease, interstitial pneumonia and VZV infection. These factors included data concerning the donor (relationship to patient, age, sex, ABO compatibility, CMV status), patient (age, sex, diagnosis, pre-transplant disease status, CMV status), transplant procedure (conditioning regimen, consecutive number of the transplant, graft manipulation, use of DLI) and complications (neutropenia, aGVHD, cGVHD, use of corticosteroids).

When looking at the overall risk of infection, we identified in univariate analysis donor other than HLA-identical sibling (p=0.0077) and absence of DLI as significant risk factors (p=0.0132). In multivariate analysis, only having a donor other than a HLA-identical sibling (p=0.0010) was a significant risk factor.

For bacteraemia, in univariate analysis only donor source reached statistical significance, with a one-year 43% incidence of bacteraemia with a sibling donor and 66% with an alternative donor (p=0.0061). This result was confirmed in multivariate analysis (p=0.0015), where the risk of bacteraemia increased also with the age of the recipient (p=0.0064) .

For infections other than bacteraemia, in univariate analysis only administration of DLI was associated with an increased risk (p=0.00440), whereas in multivariate analysis only the use of corticosteroids increased the risk (p=0.0066).

In univariate analysis, only high-risk pretransplant CMV status (R+/D-, R+/D+) (p<0.0001) influenced the risk of CMV infection, which nevertheless tended to be higher in older patients (p=0.0721) and patients with donor other than HLA-identical sibling (p=0.0679). In multivariate analysis, the risk of CMV infection increased only with a high-risk CMV status (p<0.0001) and, among these patients, no additional factor was identified.

Similarly, in univariate analysis, the risk of CMV disease increased in patients with high-risk pretransplant CMV status (p=0.0054). Among these patients, in multivariate analysis, the risk of CMV disease increased with the age of the patient (p=0.0137), a first transplantation (p=0.0057) and a diagnosis of lymphoma (p=0.0010).

Discussion

NMSCT are usually reserved for patients unable to undergo myeloablative conditioning because of older age, poor clinical condition or previous transplantation with a high-dose regimen. It is therefore impossible to select, for the purpose of comparison, an appropriately matched group of patients undergoing transplantation after a standard myeloablative conditioning. Compared to myeloablative regimens, nonmyeloablative conditioning is associated with less myelotoxicity and produces fewer extra-haematological toxicities, in particular less damage to the mucosal barriers of the gastrointestinal tract[9-11, 24]. However, the relative intensity of these treatments ranges from the reduced-intensity conditioning (RIC) regimens that still induce severe neutropenia and are carried out in inpatient protected units[10, 15, 25, 26], to the truly nonmyeloablative regimen developed by the Seattle team that causes little neutropenia, can be performed as outpatient and cause much less morbidity[9, 12, 13, 24]. Using the Seattle regimen, we indeed encountered virtually no toxic mucositis and no gastrointestinal toxicity. In addition, we have previously shown that this regimen was associated with little myelosuppression[20] and with prolonged persistence of host immune cells[14]. However, it was not clear whether these factors had the potential of providing some protection against infections.

In our population of 62 NMSCT recipients, the proportion of patients presenting any infection during follow-up was 77%. This overall proportion is quite similar to the one found after standard allogeneic transplantation[20, 27], but the majority of these first infections occurred well beyond day 30 post-transplantation (Figure 1A). Although earlier papers in small number of patients were widely discrepant in that respect[11, 18], this reduced rate of early infection[10] and this delayed time to first infection[9, 10] in NMSCT recipients have also been described by others. Therefore, the absence of mucosal damage and of neutropenia in many NMSCT patients possibly did confer some protection against early infections in our patients. This is consistent with a previous study showing that neutropenia at initiation of a RIC is already a risk factor for subsequent infection in the early post-transplant period[15]. However, in our hands, among NMSCT recipients, those with early neutropenia did not have a higher rate of early infection.

Bacteria were evidently the leading agents causing infection. This was particularly true in the early post-transplant phase, but remained so thereafter as well, albeit to a lesser extend. Among them, Gram-positive bacteria were the agents most frequently responsible for infection, in agreement with data in standard allogeneic transplantation[27-30] and probably related to the fact that all patients carried a central venous catheter device. Similarly, the pattern of fungal infections was consistent with previous studies[9, 10] that reported incidences of proven or probable fungal infection in NMSCT recipients[12, 16, 17] comparable to those observed in standard transplants after a myeloablative conditioning[31, 32]. Some[9, 12, 16] but not all[17] previous studies have shown that advanced disease, age above 40, GVHD, corticosteroid use, CMV infection or relapse were predictive of fungal infections in NMSCT patients, but the number of patients with such infections in our series was too low to analyze these factors.

After excluding seronegative patients with a seronegative donor, the actuarial incidences of CMV infection and CMV disease were 68% and 23%, respectively. In some previous studies, CMV infection and disease have been delayed after NMSCT[13, 33]. It is hypothesized that residual host T lymphocytes may contribute to resistance to CMV disease and that, as the level of these cells decline over time, the risk of infection increases[34]. However, similarly to some other studies[26], we did not observe any such delay in NMSCT recipients. On the other hand, the incidence of CMV infection and disease was clearly dependent on recipient and donor serostatus[13, 17, 26, 33]. In addition, older age and an alternative donor, but not the use of corticosteroids[17], acute GVHD[26], graft manipulation[26] or use of fludarabine for conditioning[35], tended to be risk factors for CMV infection. However, it cannot be excluded that the fact that CMV disease was more frequent after a first transplant may in fact in part be related to the use of fludarabine, as the vast majority of patients not receiving this drug were second transplants. Although this was not found for CMV infection, older age was indeed associated with CMV disease in multivariate analysis. It has also been suggested that the use of ATG or Campath-1H in the conditioning regimen[25] or the use of MMF post-transplant[36] could enhance the risk of CMV infection and disease, but this is not evaluable in our series. Chronic GVHD has been recognized as a significant risk factor after conventional allogeneic transplantation[37], but not after NMSCT.

Bacteraemias occur in about 20% to 60% of the cases after allogeneic HCT[28]. In our study, the incidence of bacteraemia at one year was 55%. In addition, occurrence of bacteraemia was again delayed beyond the first 30 days. Contrarily to the findings of others using a similar conditioning regimen[9], neutropenia was not a risk factor for bacteraemia. In agreement with others[17], the agents responsible for bacteraemias were mainly coagulase-negative staphylococci, and in general, Gram-positive bacteria were the agents most often responsible for infection. This result is totally in agreement with the literature[17].

A number of risk factors were consistently identified by univariate and multivariate analysis. Age above 60 years and use of an alternative donor were often associated with an increased risk of overall infection, bacteraemia, CMV infection and CMV disease. This has not been reported before in NMSCT patients, except for CMV infection, and could be related to poorer immune reconstitution or increased incidence of GVHD in those patients, although GVHD per se was not associated with an increased risk in our patients. In univariate analysis, absence of DLI was associated with an increased overall risk of infection. This probably reflects the fact that DLI were mostly given in case of transplantation with an HLA-identical sibling, but also that DLI may enhance immune recovery. For infections other than bacteraemia, only the use of corticosteroids significantly increased the risk. The influence of corticosteroids on the infectious risk is well described in the literature[9, 11, 12, 16, 17, 35]. The initial disease category gave inconsistent information, with lymphoma patients having more CMV disease. The use of TBI or graft manipulation were not linked to an increased risk of infection in our population.

In conclusion, NMSCT recipients are at risk for the same type of infections with the same kind of pathogens than myeloablative transplant recipients. However, infections are less frequent in the first 30 days post-transplant. Methods of prophylaxis, empirical antibiotic therapy and pre-emptive treatments should be the same as for conventional allografts. Careful long-term monitoring of these patients is necessary.

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Table 1. Patients' characteristics.

Number	62
Age (yrs) (M±SD)	54±11
Sex	
Female	16 (26%)
Male	46 (74%)
Diagnosis	
Leukaemia	23 (37%)
Lymphoma/myeloma	32 (52%)
Solid tumour	7 (11%)
Status at transplant	
Untreated	9 (14%)
CR	17 (28%)
PR	7 (11%)
Relapse/Resistant	29 (47%)
Donor type	
Sibling	30 (49%)
Other family	4 (6%)
Unrelated	28 (45%)
VZV status (Recipient)	
Positive	52 (84%)
Negative	10 (16%)
CMV status (Recipient/Donor)	
-/-	15 (24%)
-/+	11 (18%)
+/-	20 (32%)
+/+	16 (26%)
Transplant number	
First	30 (49%)
Second	25 (40%)
Third	7 (11%)
Graft manipulation	
No	27 (44%)
CD34	7 (11%)
CD8	28 (45%)
DLI	
No	29 (47%)
Yes	33 (53%)
Acute GVHD	
0-1	42 (68%)
2	17 (27%)
3-4	3 (5%)
Chronic GVHD	
None	42 (68%)
Limited	11 (18%)
Extensive	9 (14%)
Corticosteroids	
No	26 (42%)
Yes	36 (58%)

Table 2. Absolute numbers and proportions (% of patients at risk) of patients with infectious complications after transplantation. Only infections before disease progression were considered.

All infections	
≥ 1 infection	48 (77%)
≥ 2 infections	31 (50%)
≥ 3 infections	18 (29%)
≥ 4 infections	11 (18%)
≥ 5 infections	8 (13%)
Infections before day 30	
≥ 1 infection	21 (34%)
≥ 2 infections	6 (10%)
≥ 3 infections	0 (0%)
Infections day 31-day 100	
≥ 1 infection	27 (48%)
≥ 2 infections	10 (18%)
≥ 3 infections	5 (9%)
Infections day 101-day 365	
≥ 1 infection	30 (59%)
≥ 2 infections	12 (23%)
≥ 3 infections	6 (11%)

Table 3. Number of infections (N per patient at risk) according to time after transplantation. Only infections before disease progression were considered.

	Days 0-30	**Days 31-100**	**Days 101-365**	**Days > 365**
N patients at risk	**62**	**56**	**51**	**20**
All infections	21 (0.34)	38 (0.68)	41 (0.80)	10 (0.50)
Infections by agent				
Viral	1 (0.02)	1 (0.02)	5 (0.10)	4 (0.20)
Fungal	2 (0.03)	3 (0.05)	5 (0.10)	1 (0.05)
Bacterial	17 (0.27)	33 (0.59)	28 (0.55)	5 (0.25)
Parasitic	0 (0.00)	0 (0.00)	1 (0.02)	0 (0.00)
Unknown	1 (0.02)	1 (0.02)	2 (0.04)	0 (0.00)
Total	**21**	**38**	**41**	**10**
Infections by type				
Bacteraemia	5 (0.08)	16 (0.29)	17 (0.33)	1 (0.05)
IP	1 (0.02)	1 (0.02)	3 (0.06)	0 (0.00)
Other pneumonia	4 (0.06)	9 (0.16)	8 (0.16)	5 (0.25)
Upper resp. tract	0 (0.00)	4 (0.07)	2 (0.04)	2 (0.10)
Abdominal	2 (0.03)	2 (0.04)	2 (0.04)	1 (0.05)
Genito-urinary	1 (0.01)	1 (0.02)	1 (0.02)	0 (0.00)
Zoster/varicella	0 (0.00)	0 (0.00)	2 (0.04)	1 (0.05)
Skin (other)	0 (0.00)	1 (0.02)	2 (0.04)	0 (0.00)
CNS	0 (0.00)	1 (0.02)	1 (0.02)	0 (0.00)
Miscellaneous	0 (0.00)	0 (0.00)	1 (0.02)	0 (0.00)
FUO	8 (0.13)	3 (0.05)	2 (0.04)	0 (0.00)
Total	**21**	**38**	**41**	**10**

Table 4. CMV reactivation and CMV disease.

CMV status		**N**	**CMV**	**CMV**
Recipient	**Donor**		**infection**	**disease**
Negative	Negative	15	0 (0%)	0 (0%)
Negative	Positive	11	4 (36%)	1 (9%)
Positive	Negative	20	14 (70%)	5 (25%)
Positive	Positive	16	14 (88%)	5 (31%)
Total		**62**	**32 (52%)**	**11 (18%)**

Legends to the figures

Figure 1. Actuarial incidence of infections (A), bacteraemias (B) and infections other than bacteraemias (C).

Figure 2. Actuarial incidence of CMV infection (A) and CMV disease (B).

Figure 1A

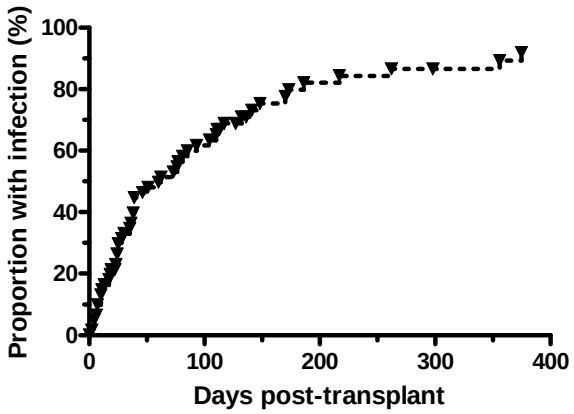


Figure 1B

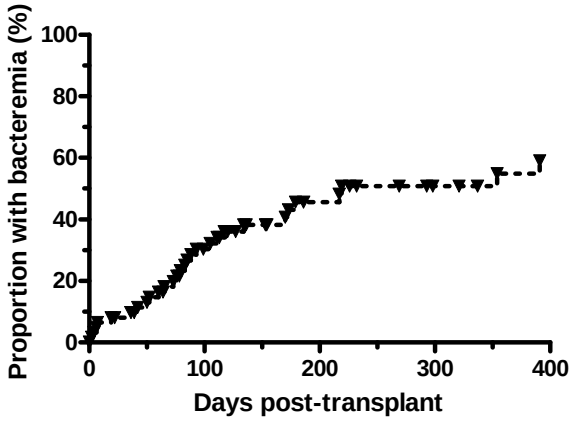


Figure 1C

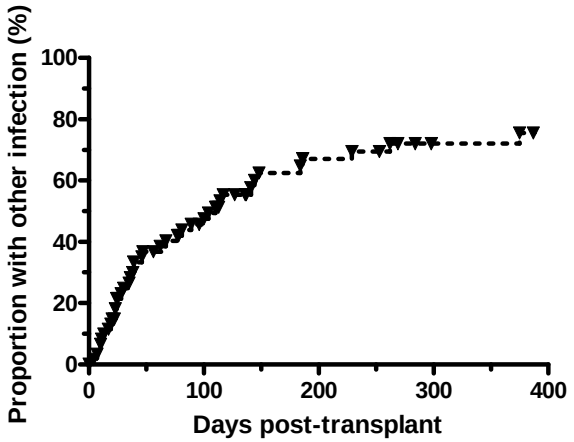
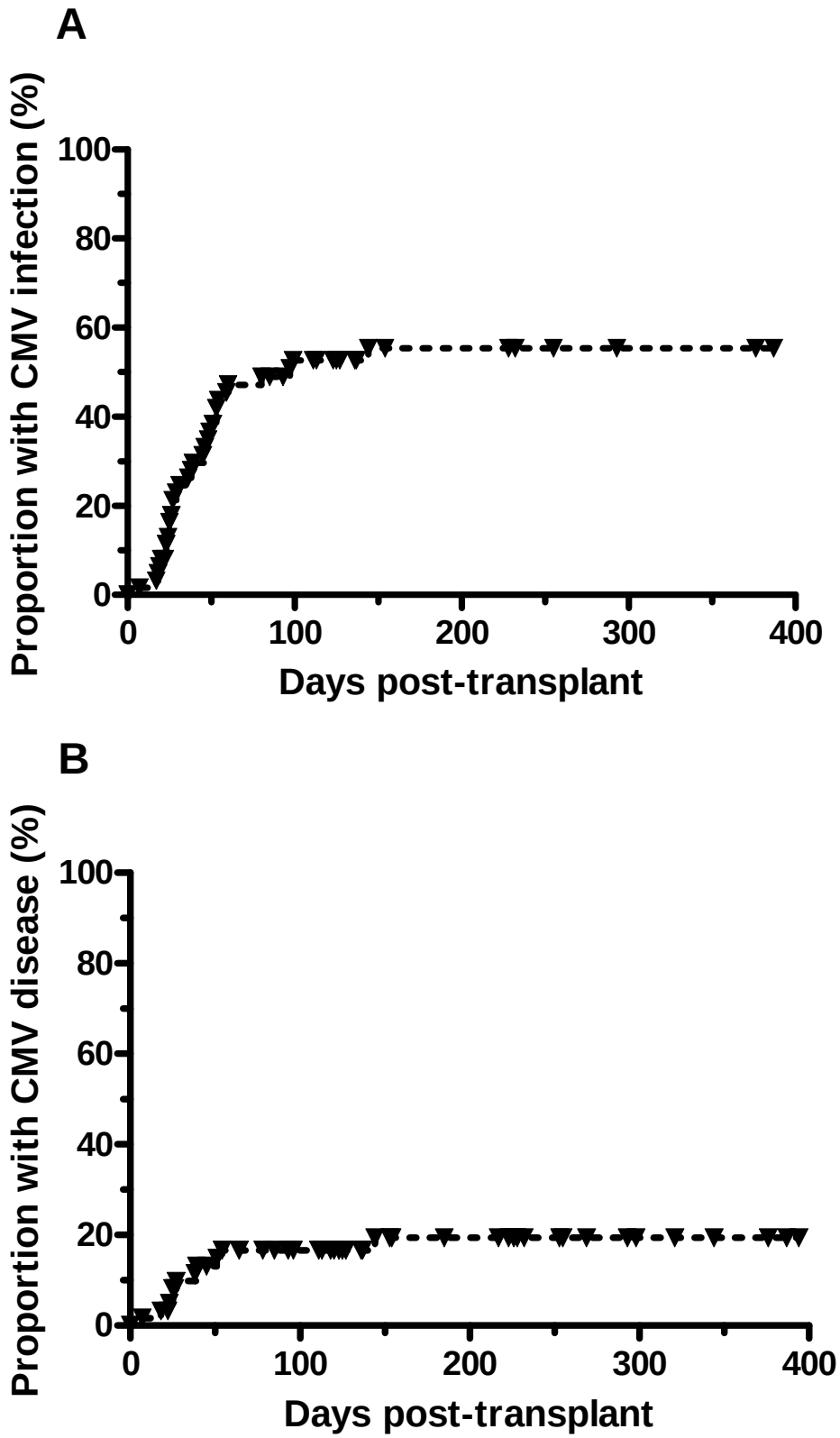


Figure 2


A
Proportion with CMV infection (%)
100
80
60
40
20
0
0
100
200
300
400
Days post-transplant
B
Proportion with CMV disease (%)
100
80
60
40
20
0
0
100
200
300
400
Days post-transplant