Cytopenias after day 28 in allogeneic hematopoietic cell transplantation: impact of recipient/donor factors, transplant conditions and myelotoxic drugs

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ABSTRACT

Background
Secondary cytopenias are serious complications following hematopoietic cell transplantation. Etiologies include myelotoxic agents, viral infections, and possibly transplant-related factors such as the intensity of the conditioning regimen and the source of stem cells.

Design and Methods
We retrospectively analyzed data from 2162 hematopoietic cell transplant recipients to examine the effect of these factors on overall cytopenias occurring after 28 days in hematopoietic cell transplantation.

Results
Advanced age of the patient, recipient cytomegalovirus seropositivity, unrelated donor status, human leukocyte antigen mismatch and lower doses of transplanted $CD34^+$ cells ($\leq 6.4\times10^6$/kg) significantly increased the risk of cytopenias after day 28. Non-myeloablative hematopoietic cell transplantation had protective effects on anemia and thrombocytopenia after day 28 (adjusted odds ratio 0.76, probability value of 0.05 and adjusted odds ratio 0.31, probability value of <0.0001, respectively) but not on overall or ganciclovir-related neutropenia. This lack of protection appeared to be due to the use of mycophenolate mofetil in the majority of recipients of non-myeloablative hematopoietic cell transplants. Peripheral blood stem cells did not confer protection from cytopenias when compared to bone marrow.

Conclusions
Elderly patients appear to be more prone to cumulative toxicities of post-transplant drug regimens, but non-myeloablative conditioning, optimized human leukocyte antigen matching, and higher doses of $CD34^+$ cell infusions may reduce the risk of cytopenia after day 28.

Key words: non-myeloablative allogeneic hematopoietic stem cell transplantation, ganciclovir-related neutropenia, cytopenias after day 28.

Citation: Nakamae H, Storer B, Sandmaier BM, Maloney DG, Davis C, Corey L, Storb R, and Boeckh M. Cytopenias after day 28 in allogeneic hematopoietic cell transplantation: impact of recipient/donor factors, transplant conditions and myelotoxic drugs. Haematologica 2011;96(12):1838-1845. doi:10.3324/haematol.2011.044966

Acknowledgments: we thank Daniel Stachel, MD, for reviewing charts, and Gary Schoch and Craig Silva for database services.

Funding: the authors are grateful for research funding from the National Institutes of Health, Bethesda, MD grants P01CA018029, P30CA015704, P01HL036444, P01CA078902, and K24HL093294. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health nor its subsidiary Institutes and Centers. Hirohisa Nakamae was funded by the Graduate School of Medicine, Osaka City University, Osaka, Japan.

Manuscript received on April 5, 2011. Revised version arrived on August 18, 2011. Manuscript accepted on August 19, 2011.

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Introduction

Secondary cytopenia is a common complication after hematopoietic cell transplantation (HCT). Causes include viral infection, septicemia, graft-*versus*-host disease (GVHD), and myelotoxic drugs.[1-5] Of the commonly used drugs with myelotoxic potential, ganciclovir is particularly prone to cause neutropenia, which occurs in up to 40% of allograft recipients and may increase the risk of invasive bacterial and fungal infections.[1,6] The underlying mechanism of ganciclovir-related neutropenia is a dose-dependent inhibition of DNA-polymerase in hematopoietic progenitor cells.[7] We previously reported that ganciclovir-related neutropenia is associated with low marrow cellularity, hyperbilirubinemia, and elevated serum creatinine levels after myeloablative conditioning (M-HCT).[1] However, it is not known how non-myeloablative conditioning (NM-HCT) influences the incidences of secondary cytopenias in general and ganciclovir-related neutropenia in particular.

Less toxic non-myeloablative conditioning regimens that can be successfully used in elderly patients and/or patients with comorbidities have been developed.[8-13] Non-myeloablative conditioning does not eradicate host hematopoiesis and allows relatively prompt hematopoietic recovery within 28 days after transplantation.[14,15] NM-HCT may, therefore, be associated with a lower incidence of cytopenias, including ganciclovir-related neutropenia. In addition, the increased use of hematopoietic growth factors for secondary neutropenia at moderate levels in recent years may also be associated with a lower risk of profound levels of neutropenia.

The purpose of this study was to examine risk factors for the occurrence of cytopenias 28 days after HCT as a surrogate for secondary neutropenia overall, and ganciclovir-related neutropenia in particular.

Design and Methods

Study population

This retrospective study population consisted of 2162 consecutive patients who underwent HCT between 1998 and 2006 at the Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, WA, USA). The retrospective analysis was approved by the Institutional Review Board of the FHCRC. Informed consent was obtained from all the patients before HCT. We compared events between 534 patients undergoing NM-HCT and 1628 contemporaneous patients undergoing M-HCT who served as a comparison group (Table 1). Clinical and laboratory data were extracted from the computerized database and from patients' charts.

The most common regimens for NM-HCT were fludarabine (30 mg/m^2/day for 3 consecutive days) together with low-dose total body irradiation (2 Gy, day 0), or low-dose total body irradiation (2 Gy, day 0) alone. In contrast, many different types of conditioning regimens were used for M-HCT. The most common regimen for M-HCT consisted of cyclophosphamide (60 mg/kg/day for 2 consecutive days) followed by total body irradiation (12 Gy or 13.2 Gy) or busulfan (4 mg/kg/day for 4 consecutive days) followed by cyclophosphamide (60 mg/kg/day for 2 consecutive days). The NM-HCT group included more elderly patients, almost exclusive use of peripheral blood stem cells as the source of stem cells, and higher doses of transplanted CD34$^+$ cells than those in the M-HCT group (Table 1).

In terms of GVHD prophylaxis, M-HCT patients most commonly received a combination of a calcineurin inhibitor (either cyclosporine or tacrolimus) and short-term methotrexate (15 mg/m^2 intravenously on day 1, and 10 mg/m^2 on days 3, 6, and 11). All NM-HCT patients received post-grafting immunosuppressants including mycophenolate mofetil (MMF) and a calcineurin inhibitor, cyclosporine or tacrolimus (Table 1)

MMF was administered at a dose of 15 mg/kg orally twice a

Table 1. Characteristics of the patients for the neutropenia/transfusion analysis (allogeneic transplant from 1998 to 2006, hematologic malignancy n=2162).

	Myeloablative (n=1628)	Non-myeloablative (n=534)
Patient's age		
≤ 40 years	770 (47%)	95 (18%)
> 40 years	858 (53%)	439 (82%)
Donor's age[a]		
≤ 40 years	788 (56%)	196 (42%)
> 40 years	618 (44%)	273 (58%)
Patient self-reported race[b]		
Caucasian	1297 (80%)	475 (90%)
Non-Caucasian	315 (20%)	52 (10%)
Donor self-reported race[c]		
Caucasian	826 (80%)	274 (88%)
Non-Caucasian	211 (20%)	39 (12%)
Patients' CMV status[d]		
Negative	799 (49%)	212 (40%)
Positive	827 (51%)	322 (60%)
Donors' CMV status[e]		
Negative	972 (60%)	305 (57%)
Positive	654 (40%)	229 (43%)
Patients' gender		
Male	918 (56%)	333 (62%)
Female	710 (44%)	201 (38%)
Donors' gender		
Male	865 (53%)	285 (53%)
Female	763 (47%)	249 (47%)
Sex mismatch		
Other	1224 (75%)	383 (72%)
Female into male	404 (25%)	151 (28%)
Donor		
Related	777 (48%)	283 (53%)
Unrelated	851 (52%)	251 (47%)
HLA mismatch		
No	1331 (82%)	485 (91%)
Yes	297 (18%)	49 (9%)
Stem cell source		
Peripheral blood stem cells	929 (57%)	491 (92%)
Bone marrow	699 (43%)	43 (8%)
Use of mycophenolate mofetil		
No	1490 (92%)	0
Yes	138 (8%)	534 (100%)
CD34 cell dose[f]		
PBSC (median, range×10^6)	7.47 (0.02-57.9)	7.99 (0.76-42.6)
Marrow (median, range×10^6)	3.20 (0.02-35.6)	4.83 (0.71-22.3)
> 6.4×10^6	569 (42%)	333 (64%)
≤ 6.4×10^6	774 (58%)	186 (36%)
ABO mismatch[g]		
No	810 (50%)	307 (57%)
Yes	816 (50%)	227 (43%)
Major	337 (21%)	93 (17%)
Minor	365 (22%)	110 (21%)
Bi-directional	114 (7%)	24 (5%)

[a]unknown for 287; [b]unknown for 23; [c]unknown for 812; [d]unknown for 2; [e]unknown for 2; [f]unknown for 300; [g]unknown for 2; PBSC: peripheral blood stem cells.

day from day 0 to day 27 and discontinued for the human leukocyte antigen (HLA) matched-related NM-HCT patients; while for the unrelated NM-HCT patients MMF was given at a dose of 15 mg/kg orally two or three times a day from day 0 to day 40, with tapering to day 96. For the single HLA-antigen and combined HLA-antigen and allele-mismatched NM-HCT patients, 15 mg/kg MMF was given three times a day and then tapered at day 100 over 2 months.[8,9,12,16,17]

Transfusion requirements

Red blood cell transfusions

Red blood cells were routinely transfused when the hematocrit fell below 26%. In patients with severe uremia, other causes of platelet dysfunction, active bleeding, or thrombocytopenia refractory to platelet transfusion, the hematocrit was maintained at 30% or above. A hematocrit of at least 30% was also maintained in patients with a history of cardiac or peripheral vascular disease, and in patients over 65 years old.

Platelet transfusions

A platelet threshold for transfusion of 1.0×10^{10}/L was used for clinically stable, afebrile patients without evidence of hemorrhage, infection, or uncontrolled GVHD. Transfusions at higher platelet levels were given to patients receiving anti-coagulant medications and patients with abnormal coagulation times, platelet dysfunction such as uremia, or other bleeding diatheses. Invasive procedures, anticoagulation management, prevention of blood clots and management of central venous catheter-associated thrombosis required maintenance of higher platelet levels.

Infection surveillance, prophylaxis and pre-emptive therapy against cytomegalovirus

Cytomegalovirus (CMV) surveillance with polymerase chain reaction (PCR) analysis or the pp65 antigenemia assay was performed on a weekly basis until day 100 as previously described.[18,19] After day 100, surveillance and pre-emptive therapy were recommended for CMV intermediate- and high-risk patients on a weekly or biweekly basis until day 365.

Pre-emptive ganciclovir treatment was started when CMV pp65 antigenemia/PCR became positive during the first 100 days after HCT. Ganciclovir (5 mg/kg IV twice daily) for 7 to 14 days was administered as induction therapy followed by a half-dose of ganciclovir (5 mg/kg IV daily) or valganciclovir 900 mg once a day orally as maintenance therapy until negative surveillance or day 100.[20] All doses were adjusted based on the patients' renal function according to the manufacturers' recommendation. After day 100, pre-emptive therapy was recommended for patients with CMV pp65 antigenemia or more than 1000 copies/mL (assessed by PCR) as previously described.[18,19]

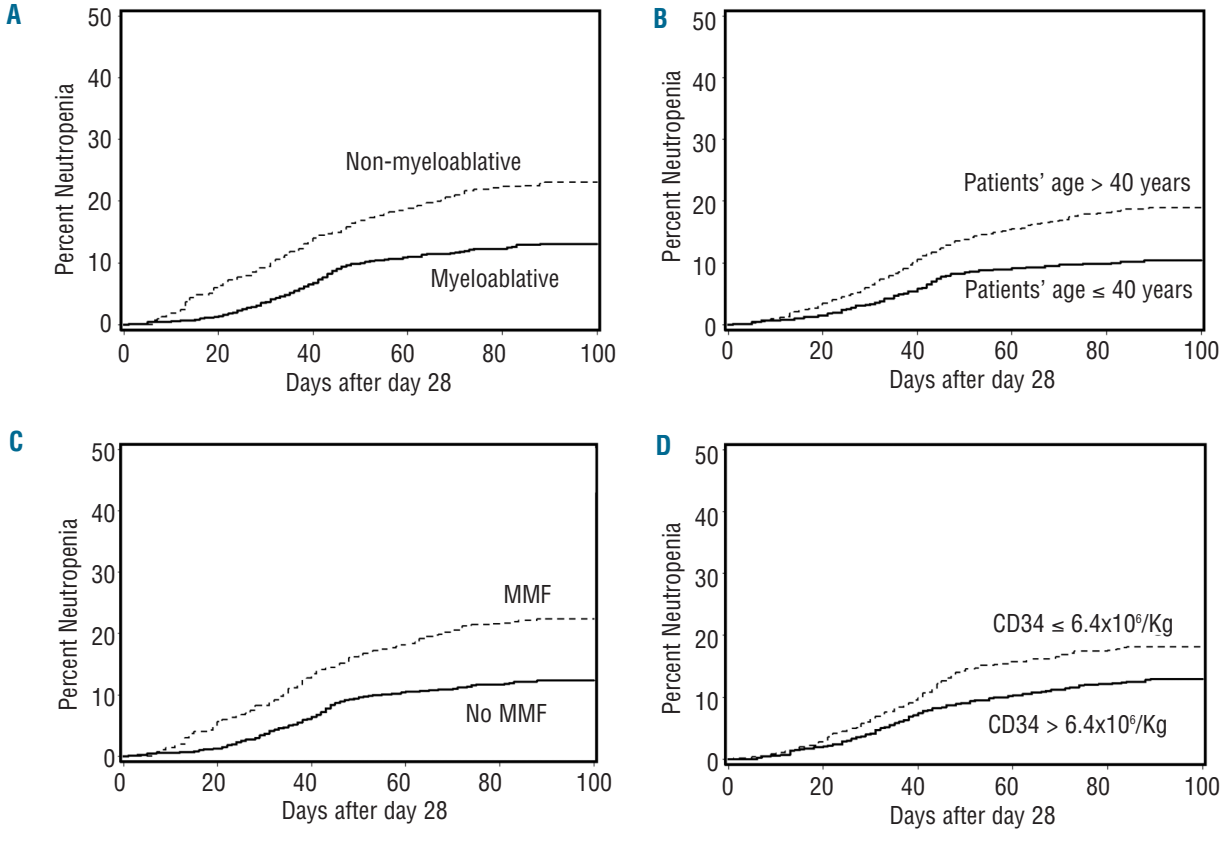

Figure 1. Cumulative incidence of neutropenia after day 28. The probabilities of neutropenia after day 28 (absolute neutrophil count < 500/L) according to (A) non-myeloablative (NM-HCT) *vs.* myeloablative hematopoietic stem cell transplantation (M-HCT), (B) patients' age (≤ 40 *vs.* > 40 years), (C) MMF use (yes *vs.* no) and (D) $CD34^+$ cell dose (≤ 6.4×10^6/kg *vs.* > 6.4×10^6/kg) are illustrated. The cumulative incidence of development of neutropenia was higher for NM-HCT than it was for M-HCT. However, in a multivariate analysis NM-HCT was not a significant risk factor for development of neutropenia.

Pneumocystis jirovecii prophylaxis consisted of trimethoprim sulfamethoxazole as the primary agent and dapsone as the secondary agent. Identical doses of both drugs were used for all patients, regardless of conditioning regimen.[21] However, recipients of non-myeloablative conditioning regimens started prophylaxis at day 28 after HCT while recipients of myeloablative transplantation received pretransplant dosing which was then resumed after neutrophil engraftment.

Definitions of cytopenias after day 28

We evaluated neutropenia, anemia and thrombocytopenia after day 28, and ganciclovir-related neutropenia. Ganciclovir-related neutropenia was defined as non-relapse–related neutropenia (absolute neutrophil count < 500/μL and < 200/μL) after the start of pre-emptive therapy for pp65 antigenemia /PCR positivity in patients with an absolute neutrophil count greater than 1000/μL at the time of CMV infection. Neutropenia after day 28 was defined as absolute neutrophil counts less than 500/μL and less than 200/μL occurring any time between day 28 post-HCT and day 120 among relapse-free patients. We used transfusion support after day 28 as a surrogate marker of anemia and thrombocytopenia. Significant anemia and thrombocytopenia beyond day 28 were both defined as the upper 25th percentile of transfusion support after day 28, up to the first of day 80, death or relapse. Specifically, we defined patients who received more than 0.8 units of red blood cell transfusions per week as cases with anemia after day 28; similarly, we defined patients who were given more than 1.6 units of platelets per week up to day 80 as cases with thrombocytopenia.

Statistical analysis

The characteristics of NM-HCT and M-HCT patients were summarized using frequency counts and percentages for categorical variables and medians and ranges for continuous variables. The cumulative incidence of neutropenia after day 28 was estimated by previously described methods, with death or relapse treated as a competing risk. Univariate and multivariate Cox regression models were used to estimate hazard ratios and 95% confidence intervals (95% CI) for risk factors associated with neutropenia after day 28 and ganciclovir-related neutropenia as defined above. Univariate and multivariate logistic regression models were used to estimate odds ratios for risk factors associated with anemia and thrombocytopenia. Cox regression was used to perform a landmark analysis among patients alive and disease-free at day 100, to evaluate the impact of prior cytopenias and other risk factors on subsequent non-relapse mortality, defined as any death without prior relapse. Covariates included recipient/donor age and sex, recipient/donor race, donor CMV serostatus, sex mismatch, HLA disparity, donor relationship, intensity of conditioning, stem cell source, T-cell-depleted conditioning, year of transplantation, disease risk, GVHD prophylaxis, acute GVHD and chronic GVHD. Acute and chronic GVHD and other post-transplant factors were analyzed as time-dependent variables. Variables with a significance level of less than 0.05 in the univariate models were candidates for the multivariate models. All *P* values are two-sided and unadjusted for multiple comparisons.

Results

Risk factors for cytopenias after day 28

Among the 1818 patients with neutrophil engraftment at day 28, 711 (39%) had at least one form of cytopenia after day 28: 103 (6%) had neutropenia only, 128 (7%) had anemia only, 102 (6%) had thrombocytopenia only and 123 (7%) had all three cytopenias. Neutropenia after day 28 was significantly more frequent in NM-HCT than in M-HCT (23% and 13%, respectively) (Figure 1A).

In univariate analysis, the risk factors for neutropenia (< 500/μL) after day 28 included the patients' age (> 40 years), recipient CMV seropositivity, patients at higher risk of CMV, unrelated donor status, receipt of NM-HCT, use of MMF, lower CD34$^+$ cell dose (≤ 6.4×10^6/kg) in the graft, chronic GVHD, high bilirubin level (> 6 mg/dL), and elevated creatinine (> 2 mg/dL) (Table 2). In a multivariate model, we identified patients' age, CMV seropositivity, unrelated donor status, HLA mismatched donor, MMF use and lower CD34$^+$ cell dose (≤ 6.4×10^6/kg) as significant risk factors for neutropenia after day 28 in both HCT with bone marrow and peripheral blood stem cells (Table 3) (Figure 1B-D). Analysis of a lower threshold for defining neutropenia (< 200/μL) did not reveal additional risk factors (*data not shown*).

Results of univariate analyses for anemia and thrombocytopenia after day 28 are presented in Table 4. ABO-mismatched donors, patients' age (> 40 years), female donor, patients at higher risk of CMV, unrelated donor, HLA-mismatched donor, bone marrow as the stem cell source and lower CD34$^+$ cell dose (≤ 6.4×10^6/kg) were risk factors for anemia after day 28. Risk factors for thrombocytopenia after day 28 included ABO-mismatched donor, unrelated donor, HLA-mismatched donor, bone marrow as the stem cell source and CD34$^+$ cell dose (≤ 6.4×10^6/kg) (Table 4). In a multivariate model, ABO-mismatched donor, patients' age (> 40 years), CMV infection, unrelated donor, HLA-mismatched donor and lower CD34$^+$ cell dose (≤ 6.4×10^6/kg) were identified as common risk factors for anemia and thrombocytopenia after day 28 in both HCT with bone marrow and peripheral blood stem cells (Table 5). CMV serostatus was no longer significant when active post-transplant CMV infection was included in the model.

NM-HCT was significantly associated with a lower incidence of anemia and thrombocytopenia after day 28 in both univariate and multivariate models (Tables 4 and 5).

Risk factor for ganciclovir-related neutropenia

The cumulative incidence of ganciclovir-related neutropenia was similar for NM-HCT and M-HCT recipients (26% and 22%, respectively). A univariate model for ganciclovir-related neutropenia is shown in Table 2. In the univariate model, we found patients' age (> 40 years), unrelated donor status, MMF use, lower CD34$^+$ cell dose (≤ 6.4×10^6/kg) and high bilirubin level (> 6 mg/dL) to be significant risk factors for ganciclovir-related neutropenia (Table 2). All factors except MMF use and high bilirubin levels remained statistically significant risk factors for ganciclovir-related neutropenia (Table 3). Analysis of a lower threshold for neutropenia (< 200/μL) did not reveal additional risk factors (*data not shown*).

The impact of cytopenias after day 28 on non-relapse mortality

In a multivariate analysis of non-relapse mortality, we included acute GVHD, age of the patients and donors, sex of the patients and donors, patients' CMV status, donor relation, HLA disparity, stem cell source, MMF use and the intensity of the conditioning regimen as covariates. After adjustment for these factors, neutropenia, anemia and thrombocytopenia after day 28 were all significant inde-

Table 2. Univariate risk factors for neutropenia (absolute neutrophil count <500/μL).

	Ganciclovir-induced[1]			Neutropenia after day 28[2]		
	N	HR (95% CI)	*P* value	N	HR (95% CI)	*P* value
Patients' age						
≤ 40 years	225	1.0		718	1.0	
> 40 years	468	1.90 (1.3-2.8)	0.0004	1100	1.96 (1.5-2.6)	<0.0001
Donors' age						
≤ 40 years	320	1.0		819	1.0	
> 40 years	284	1.35 (1.0-1.9)	0.08	771	1.28 (1.0-1.6)	0.06
Patients' sex						
Male	350	1.0		1046	1.0	
Female	343	0.78 (0.6-1.1)	0.12	772	0.85 (0.7-1.1)	0.17
Donors' sex						
Male	376	1.0		976	1.0	
Female	317	0.91 (0.7-1.2)	0.56	842	1.04 (0.8-1.3)	0.73
Sex mismatch						
Others	540	1.0		1356	1.0	
F into M	153	1.13 (0.8-1.6)	0.53	462	1.28 (1.0-1.6)	0.06
Patients' race						
Caucasian	521	1.0		1474	1.0	
Others	160	0.75 (0.5-1.1)	0.15	323	0.94 (0.7-1.3)	0.69
Donors' race						
Caucasian	325	1.0		917	1.0	
Others	109	0.75 (0.5-1.2)	0.25	219	0.81 (0.5-1.2)	0.31
Patients' CMV status						
Negative	64	1.0		848	1.0	
Positive	628	1.22 (0.7-2.2)	0.48	968	1.98 (1.5-2.5)	<0.0001
Donors' CMV status						
Negative	313	1.0		1053	1.0	
Positive	379	0.74 (0.5-1.0)	0.06	764	1.09 (0.9-1.4)	0.46
CMV risk group						
D-/R-	13	1.0		602	1.0	
D+/R-	51	0.80 (0.2-2.9)		246	1.39 (0.9-2.1)	
R+	628	1.02 (0.3-3.2)	0.73	968	2.21 (1.6-3.0)	<0.0001
Donor relation						
Related	324	1.0		907	1.0	
Unrelated	369	1.56 (1.1-2.1)	0.007	911	1.42 (1.1-1.8)	0.004
HLA mismatch						
No	572	1.0		1523	1.0	
Yes	121	1.22 (0.8-1.8)	0.33	295	1.31 (1.0-1.8)	0.08
Stem cell source						
Peripheral blood	463	1.0		1266	1.0	
Bone marrow	230	1.14 (0.8-1.6)	0.44	552	0.95 (0.7-1.2)	0.68
Conditioning						
Myeloablative	522	1.0		1361	1.0	
Non-myeloablative	171	1.25 (0.9-1.8)	0.22	457	1.85 (1.5-2.4)	<0.0001
MMF use						
No	468	1.0		1243	1.0	
Yes	225	1.41 (1.0-1.9)	0.04	575	1.93 (1.5-2.4)	<0.0001
CD34⁺cell dose (10^6)						
> 6.4/kg	290	1.0		807	1.0	
≤ 6.4/kg (PB)	161	1.72 (1.2-2.5)	0.004	407	1.59 (1.2-2.1)	0.0008
≤ 6.4/kg (BM)	153	1.36 (0.9-2.0)	0.13	370	1.20 (0.9-1.6)	0.24
Time-dependent associations						
Acute GVHD						
0–I	125	1.0		408	1.0	
II–IV	568	1.38 (0.9-2.1)	0.12	1410	0.90 (0.7-1.2)	0.42
Chronic GVHD						
No	287	1.0		790	1.0	
Yes	406	0.41 (0.1-1.2)	0.06	1028	0.07 (0.0-0.1)	<0.0001
Bilirubin						
≤ 6 mg/dL	571	1.0		1522	1.0	
> 6 mg/dL	122	1.86 (1.3-2.7)	0.003	296	2.01 (1.5-2.7)	<0.0001
Creatinine						
≤ 2 mg/dL	479	1.0		1311	1.0	
> 2 mg/dL	214	1.45 (1.0-2.1)	0.05	507	1.40 (1.1-1.8)	0.01
Antigenemia/PCR						
≤ 5 and ≤ 1000	408	1.0				
6–10 or 1001–10^4	107	0.90 (0.5-1.5)				
> 10 or > 10^4	178	1.53 (1.1-2.2)	0.05			

continued in next column

[1]Based on 693 patients positive for CMV by antigenemia or PCR, without prior relapse, and absolute neutrophil count (ANC) > 1000/μL; [2]based on 1818 patients relapse-free at day 28, and with ANC > 1000/μL.

Table 3. Multivariate risk factors for neutropenia (absolute neutrophil count < 500/μL).

	Ganciclovir-induced[1]			Neutropenia after day 28[2]		
	N	HR (95% CI)	*P* value	N	HR (95% CI)	*P* value
Patients' age						
≤ 40 years	225	1.0		718	1.0	
> 40 years	468	1.72 (1.2-2.6)	0.008	1098	1.66 (1.3-2.2)	0.0004
Donors' age						
≤ 40 years	320	1.0				
> 40 years	284	1.53 (1.0-2.3)	0.03			
(missing)	89	0.96 (0.6-1.6)	0.87			
Patients' CMV status						
Negative				848	1.0	
Positive				968	1.84 (1.4-2.4)	<0.0001
Donor relation						
Related	324	1.0		906	1.0	
Unrelated	369	2.03 (1.4-3.0)	0.0004	910	1.45 (1.1-1.9)	0.003
HLA mismatch						
No				1522	1.0	
Yes				294	1.44 (1.1-2.0)	0.02
MMF use						
No	468	1.0		1241	1.0	
Yes	225	1.39 (1.0-2.0)	0.07	575	2.02 (1.5-2.6)	<0.0001
CD34⁺cell dose (10^6)						
> 6.4/kg	290	1.0		807	1.0	
≤ 6.4/kg (PB)	161	1.79 (1.2-2.7)	0.004	407	1.57 (1.2-2.1)	0.003
≤ 6.4/kg (BM)	153	1,61 (1.0-2.6)	0.04	370	1.74 (1.2-2.5)	0.002
(missing)	89	1.51 (0.9-2.6)	0.13	234	1.74 (1.2-2.6)	0.006

[1]Based on 693 patients positive for CMV by antigenemia or PCR, without prior relapse, and with an absolute neuthophil count (ANC) > 1000/μL; [2]based on 1816 patients relapse-free at day 28, and ANC > 1000/μL; PB: peripheral blood; BM: bone marrow.

pendent risk factors for non-relapse mortality after HCT (neutropenia: HR=1.81, 95% CI 1.4–2.4, *P*<0.0001; anemia: HR=1.56, 95% CI 1.2–2.1, *P*=0.002; thrombocytopenia: HR=2.35, 95% CI 1.7–3.2, *P*<0.0001) (Table 6).

Discussion

This study provides novel and somewhat unexpected results on the risk of cytopenias after HCT. Older recipient age, low CD34⁺ cell dose, an unrelated donor, and HLA mismatch were risk factors for cytopenias after transplantation. Non-myeloablative conditioning was associated with significantly reduced incidences of anemia and thrombocytopenia after day 28, but not of neutropenia.

Table 4. Univariate risk factors for significant anemia and thrombocytopenia after day 28.

	Anemia[1]			Thrombocytopenia[2]		
	N	OR (95% CI)	*P* value	N	OR (95% CI)	*P* value
ABO mismatch						
No	1068	1.0		1068	1.0	
Yes	973	1.78 (1.5-2.2)	<0.0001	973	1.51 (1.2-1.8)	<0.0001
Patients' age						
≤ 40 years	827	1.0		827	1.0	
> 40 years	1216	1.25 (1.0-1.5)	0.03	1216	1.01 (0.8-1.2)	0.92
Donors' age						
≤ 40 years	931	1.0		931	1.0	
> 40 years	841	0.88 (0.7-1.1)	0.24	841	0.99 (0.8-1.2)	0.94
Patients' sex						
Male	1179	1.0		1179	1.0	
Female	864	1.16 (1.0-1.4)	0.13	864	0.91 (0.7-1.1)	0.34
Donors' sex						
Male	1090	1.0		1090	1.0	
Female	953	1.23 (1.0-1.5)	0.03	953	0.96 (0.8-1.2)	0.71
Sex mismatch						
Others	1524	1.0		1524	1.0	
F into M	519	1.11 (0.9-1.4)	0.34	519	1.08 (0.9-1.4)	0.49
Patients' race						
Caucasian	1666	1.0		1666	1.0	
Others	355	0.92 (0.7-1.2)	0.54	355	0.98 (0.8-1.3)	0.90
Donors' race						
Caucasian	1038	1.0		1038	1.0	
Others	241	0.96 (0.7-1.3)	0.82	241	1.04 (0.7-1.4)	0.83
Recipient CMV serostatus						
Negative	960	1.0		960	1.0	
Positive	1081	1.28 (1.1-1.6)	0.01	1081	1.14 (0.9-1.4)	0.19
Donor CMV serostatus						
Negative	1205	1.0		1205	1.0	
Positive	837	0.97 (0.8-1.2)	0.79	837	0.87 (0.7-1.1)	0.19
CMV serostatus risk group						
D-/R-	692	1.0		692	1.0	
D+/R-	268	1.18 (0.9-1.6)		268	1.03 (0.7-1.4)	
R+	1081	1.35 (1.1-1.7)	0.03	1081	1.15 (0.9-1.4)	0.42
Donor relation						
Related	1010	1.0		1010	1.0	
Unrelated	1033	1.58 (1.3-1.9)	<0.0001	1033	2.15 (1.7-2.6)	<0.0001
HLA Mismatch						
No	1716	1.0		1716	1.0	
Yes	327	1.60 (1.2-2.1)	0.0003	327	2.03 (1.6-2.6)	<0.0001
Stem cell source						
Peripheral blood	1348	1.0		1348	1.0	
Bone marrow	695	1.45 (1.2-1.8)	0.0003	695	1.82 (1.5-2.2)	<0.0001
Conditioning						
MA	1544	1.0		1544	1.0	
NMA	499	0.71 (0.6-0.9)	0.004	499	0.30 (0.2-0.4)	<0.0001
MMF use						
No	1420	1.0		1420	1.0	
Yes	623	0.77 (0.6-1.0)	0.02	623	0.44 (0.3-0.6)	<0.0001
$CD34^+$cell dose (10^6)						
> 6.4/kg	855	1.0		855	1.0	
≤ 6.4/kg (PB)	447	1.43 (1.1-1.8)	0.004	447	1.31 (1.0-1.7)	0.04
≤ 6.4/kg (BM)	462	1.81 (1.4-2.3)	<0.0001	462	1.93 (1.5-2.4)	<0.0001

[1]Based on 2043 patients relapse-free at day 28; significant anemia post-day 28 is defined as red blood cell transfusions > 0.8 units per week from day 28 to the first of day 80, death or relapse. [2]Based on 2043 patients relapse-free at day 28; significant thrombocytopenia post-day 28 is defined as platelet transfusions > 1.6 units per week from day 28 to the first of day 80, death or relapse. PB: peripheral blood; BM: bone marrow; MA: myeloablative; NMA: non-myeloablative.

Table 5. Multivariate risk factors for significant anemia and thrombocytopenia after day 28.

	Anemia[1]			Thrombocytopenia[2]		
	N	OR (95% CI)	*P* value	N	OR (95% CI)	*P* value
ABO mismatch						
No	1066	1.0		1066	1.0	
Yes	973	1.67 (1.4-2.0)	<0.0001	973	1.27 (1.0-1.6)	0.03
Patients' age						
≤ 40 years	825	1.0		825	1.0	
> 40 years	1214	1.43 (1.1-1.8)	0.001	1214	1.36 (1.1-1.7)	0.007
CMV infection[3]						
Negative	1301	1.0		1301	1.0	
Positive	738	1.39 (1.1-1.7)	0.002	738	1.36 (1.1-1.7)	0.005
Donor relation						
Related	1008	1.0		1008	1.0	
Unrelated	1031	1.30 (1.1-1.6)	0.02	1031	1.86 (1.5-2.3)	<0.0001
HLA Mismatch						
No	1715	1.0		1715	1.0	
Yes	324	1.33 (1.0-1.7)	0.04	324	1.46 (1.1-1.9)	0.007
Conditioning						
MA	1540	1.0		1540	1.0	
NMA	499	0.78 (0.6-1.0)	0.06	499	0.32 (0.2-0.4)	<0.0001
$CD34^+$cell dose (10^6)						
> 6.4/kg	855	1.0		855	1.0	
≤ 6.4/kg (PB)	446	1.54 (1.2-2.0)	0.002	446	1.52 (1.1-2.0)	0.004
≤ 6.4/kg (BM)	462	1.79 (1.4-2.3)	<0.0001	462	1.57 (1.2-2.1)	0.002
(missing)	276	1.45 (1.1-2.0)	0.02	276	1.43 (1.0-2.0)	0.03

[1]Based on 2039 patients relapse-free at day 28; significant anemia post-day 28 is defined as red blood cell transfusions > 0.8 units per week from day 28 to the first of day 80, death or relapse. [2]Based on 2039 patients relapse-free at day 28; significant thrombocytopenia post-day 28 is defined as platelet transfusions > 1.6 units per week from day 28 to the first of day 80, death, or relapse. [3]CMV infection defined as any active CMV infection before day 100; CMV serostatus; a separate multivariate model that included CMV recipient serostatus instead showed a significant association of CMV seropositivity with anemia (adjusted OR 1.33, 95% CI 1.1-1.6, P=0.005) and thrombocytopenia (adjusted OR 1.25, 95% CI 1.0-1.5, P=0.04). PB: peripheral blood; BM: bone marrow; MA: myeloablative; NMA: non-myeloablative.

We hypothesized that non-myeloablative conditioning is associated with less neutropenia after day 28. Surprisingly, in this study we did not find a significant reduction of neutropenia either overall or in the context of ganciclovir use. Overall, neutropenia after day 28 occurred in 13% of patients. The exact contribution of MMF to the relatively high rates of neutropenia in NM-HCT recipients cannot be determined since MMF was given to all patients receiving non-myeloblative conditioning. MMF was significantly associated with neutropenia even after controlling for donor relatedness (which determined the duration of drug use). However, neutropenia is an important adverse effect of MMF and cumulative toxicity with ganciclovir is plausible and has been described.[22] Our study also identified other factors that might explain the high rate of neutropenia in NM-HCT. We found older recipient age to be a risk factor for both neutropenia after day 28 and ganciclovir-related neutropenia. NM-HCT is more commonly done in older patients. The effect of older recipient age may be mediated by subclinical renal dysfunction (especially tubular function[23]), which may lead to inadvertent overdosing of myelotoxic drugs that are eliminated through the kidneys and whose doses are adjusted only by creatinine clearance (which does not measure tubular function). Such an effect would be consistent with the pharmacokinetic properties and the toxicity profile of ganciclovir, which

Table 6. Multivariate risk factors for non-relapse mortality. Landmark analysis at day 100, n=1489, 331 events.

	HR (95% CI)	*P* value
Neutropenia[1]		
No	1.0	
Yes	1.81 (1.4-2.4)	<0.0001
Anemia[2]		
No	1.0	
Yes	1.56 (1.2-2.1)	0.002
Thrombocytopenia[3]		
No	1.0	
Yes	2.35 (1.7-3.2)	<0.0001
GVHD		
0-I	1.0	
II-IV	1.37 (1.0-1.8)	0.03
Patients' age		
≤40 years	1.0	
>40 years	1.51 (1.2-2.0)	0.002
Donors' age		
≤40 years	1.0	
>40 years	1.12 (0.9-1.5)	0.42
(missing)	1.03 (0.7-1.5)	0.87
Donor/patient sex		
Other	1.0	
Female/male	1.25 (1.0-1.6)	0.07
Patient CMV status		
Negative	1.0	
Positive	1.05 (0.8-1.4)	0.72
Donor relation		
Related	1.0	
Unrelated	1.08 (0.8-1.4)	0.59
HLA mismatch		
No	1.0	
Yes	1.62 (1.2-2.2)	0.001
Stem cell source		
Peripheral blood	1.0	
Bone marrow	0.87 (0.7-1.1)	0.31
MMF use		
No	1.0	
Yes	2.12 (1.4-3.3)	0.0006
Conditioning		
Myeloablative	1.0	
Non-myeloablative	0.84 (0.5-1.3)	0.44

[1]Neutropenia was defined as an absolute neutrophil count (ANC) < 500/μL occurring any time between day 28 post-HCT and day 120 among patients, relapse-free at day 28, and ANC > 1000/μL. [2]Significant anemia post-day 28 is defined as red blood cell transfusions > 0.8 units per week from day 28 [3]Significant thrombocytopenia post-day 28 is defined as platelet transfusions > 1.6 units per week from day 28 to the first of day 80, death, or relapse.

includes predominantly neutropenia but not thrombocytopenia and anemia.

There are limited data on cytopenia after day 28 relative to the intensity of the conditioning regimen. Severe GVHD, myelotoxicity associated with drugs such as ganciclovir, trimethoprim sulfamethoxazole or MMF, as well as viral and severe fungal or bacterial infections have all been associated with an increased risk of neutropenia after day 28.[1,2,4,22] Furthermore, Bruno *et al.* previously reported unrelated donor, grade II-IV acute GVHD, impaired renal function, the combination of busulfan and cyclophosphamide, total body irradiation, stem cell dose and infections as risk factors for secondary failure of platelet recovery among M-HCT.[5] The present study extended our previous findings that NM-HCT may also have protective effects against thrombocytopenia and anemia.[24] We identified HLA mismatch, CMV serostatus and the CD34$^+$ cell count as additional risk factors for both outcomes in multivariable models. We speculate that the higher doses of CD34$^+$ cells and the reduced intensity of the conditioning regimen used in NM-HCT contributed to the lower rates of anemia and thrombocytopenia.

The CD34$^+$ cell dose rather than the stem cell source *per se* was an important risk factor for all cytopenias examined in this study. When the cell dose was included in the multivariable models the stem cell source was no longer significant, suggesting that the protective effect of peripheral blood stem cells for anemia and thrombocytopenia seen in the univariate analyses was mediated by the higher dose of CD34$^+$ cells (Tables 3 and 5).

CMV serostatus of the recipient was a risk factor for both anemia and thrombocytopenia requiring blood products (Table 5), a finding not previously appreciated in HCT recipients.[25] When CMV serostatus and active CMV infection were included in a multivariable analysis, active CMV infection remained significant while CMV serostatus was no longer significant, suggesting that active CMV infection or pre-emptive therapy was responsible for the effect. The relative contribution of CMV infection compared to that of its treatment cannot be determined from this study. Ganciclovir has not been associated with thrombocytopenia or anemia or an increased use of blood products in several placebo-controlled randomized trials in HCT recipients.[6,26,27] A previous risk factor analysis in myeloablative HCT recipients between 1990 and 1997 did not identify CMV serostatus as a risk factor for thrombocytopenia, but the use of platelet products was not analyzed in that study.[5] Based on the lack of association with anemia and thrombocytopenia in randomized trials of ganciclovir, we speculate that CMV infection itself might be responsible for the effect.[4,28]

Our study has several limitations, including the retrospective nature of the analysis and that the analysis of concomitant medications was performed by protocol only. With regard to the non-myeloablative conditioning, the results can probably not be extrapolated to other types of reduced-intensity conditioning regimens. However, the strength of the analyses lies in the large sample size, the number of clinically important factors analyzed, a homogeneous transplant protocol, and highly standardized supportive care strategies.

In conclusion, the study provides a comprehensive analysis of factors associated with cytopenias after day 28 in HCT recipients. Unexpectedly, NM-HCT did not reduce the risk of neutropenia after day 28 overall or in the context of ganciclovir treatment. The high rates of neutropenia appear to be linked to the use of MMF and ganciclovir, emphasizing the need for less toxic immunosuppressive and anti-CMV drugs or strategies. In contrast, NM-HCT showed a protective effect against anemia and thrombocytopenia after day 28, probably through less toxic conditioning and higher doses of CD34$^+$ stem cells or almost exclusive use of peripheral blood stem cells. Finally, the study identified potentially modifiable factors that could be used before transplantation to minimize the risk of post-transplant cytopenias, including non-myeloablative conditioning, optimized HLA matching, and higher doses of CD34$^+$ cell infusions.

Authorship and Disclosures

The information provided by the authors about contributions from persons listed as authors and in acknowledgments is available with the full text of this paper at www.haematologica.org.

Financial and other disclosures provided by the authors using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are also available at www.haematologica.org.

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