

ORIGINAL ARTICLE

Short- and long-term impact of neutropenia within the first year after kidney transplantation

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SUMMARY

The aim of this retrospective single-center study was to investigate the short- and long-term impact of neutropenia occurring within the first year after kidney transplantation, with a special emphasis on different neutropenia grades. In this unselected cohort, 225/721 patients (31%) developed 357 neutropenic episodes within the first year post-transplant. Based on the nadir neutrophil count, patients were grouped as neutropenia grade 2 ($<1.5\text{--}1.0 \times 10^9/\text{l}$; $n = 105$), grade 3 ($<1.0\text{--}0.5 \times 10^9/\text{l}$; $n = 65$), and grade 4 ($<0.5 \times 10^9/\text{l}$; $n = 55$). Most neutropenia episodes were presumably drug-related (71%) and managed by reduction/discontinuation of potentially responsible drugs (mycophenolic acid [MPA] 51%, valganciclovir 25%, trimethoprim/sulfamethoxazole 19%). Steroids were added/increased as replacement for reduced/discontinued MPA. Granulocyte colony-stimulating factor was only used in 2/357 neutropenia episodes (0.6%). One-year incidence of (sub)clinical rejection, one-year mortality, and long-term patient and graft survival were not different among patients without neutropenia and neutropenia grade 2/3/4. However, the incidence of infections was about 3-times higher during neutropenia grade 3 and 4, but not increased during grade 2. In conclusion, neutropenia within the first year after kidney transplantation represents no increased risk for rejection and has no negative impact on long-term patient and graft survival. Adding/increasing steroids as replacement for reduced/discontinued MPA might supplement management of neutropenia.

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Key words

drug-related side effects, infection, kidney transplantation, neutropenia, rejection

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Introduction

Although neutropenia is a very common problem within the first year after kidney transplantation, only a few studies investigated its clinical consequences and management

[1,2]. Hartmann et al. found no impact of leucopenia/neutropenia on the one-year incidence of rejection and infections in 102 kidney and/or pancreas transplantations receiving tacrolimus (Tac)—mycophenolic acid (MPA)-based immunosuppression [1]. By contrast, Zafrani et al.

reported a higher risk of rejection in 395 kidney transplant recipients, if MPA was discontinued for more than six days in response to neutropenia [2]. Furthermore, they found a higher risk of infections in patients with neutrophil counts below 1000 cells/mm³ ($=1.0 \times 10^9$ cells/l). Both studies reported no increased risk of neutropenia for patient death and allograft failure at one-year post-transplant. Notably, a detailed analysis with respect to different grades of neutropenia was not performed, and long-term outcomes beyond the first year post-transplant were not assessed [1,2].

Current recommended therapeutic management for neutropenia consists of reduction/discontinuation of the causative drugs (mostly MPA, valganciclovir, or trimethoprim/sulfamethoxazole) [3–5]. Unfortunately, reduction/discontinuation of MPA can increase the risk of allograft rejection, if no replacement is given [6]. Several studies suggested that administration of granulocyte colony-stimulating factor (G-CSF) can accelerate recovery from neutropenia, which might shorten the time without adequate MPA exposure and might also reduce neutropenia-associated infection mortality [1,2,7,8]. However, G-CSF is expensive (~350 \$US per dose of 300 µg) and a few studies raised concerns regarding an increased risk of rejection or allograft dysfunction [8–11]. Therefore, a general management strategy for neutropenia after kidney transplantation has not been established. The policy at our institution is to increase or add steroids, if MPA is reduced/discontinued in the context of neutropenia, unless the risk of rejection was considered as very low or a severe concomitant infection existed.

The aim of this retrospective single-center study was to investigate the short- and long-term impact of neutropenia occurring within the first year after kidney transplantation, with a special emphasis on different neutropenia grades. In addition, we evaluated the concept of adding or increasing steroids as replacement for reduced/discontinued MPA.

Materials and methods

Patient population

The ethics committee of Northwestern and Central Switzerland approved this retrospective study (www.knz.ch; project ID 2021-00304). We assess all kidney transplantations performed at the University Hospital Basel from February 1, 2008, until January 31, 2019, for eligibility ($n = 773$). We excluded 52 transplantations (6.7%) for the following reasons: no baseline blood

count data ($n = 14$), missing follow-up data ($n = 9$), less than 25 blood count evaluations because of early referral ($n = 19$), death within the first month post-transplant ($n = 3$), and graft loss within the first month post-transplant ($n = 7$). The final population for analysis consisted of 721 transplantations having 30'019 blood counts performed within the first year post-transplant (median 39 per transplant [IQR 34–46]).

Immunosuppression

Initial immunosuppression was selected based on the presence/absence of donor-specific HLA-antibodies (HLA-DSA), ABO-blood group compatibility and HLA-matching as previously reported [12–15]. Recipients of an allograft with ≥ 1 HLA-mismatch but no HLA-DSA (i.e., standard risk patient) received an induction therapy with basiliximab 20 mg on day 0 and 4, and a triple therapy with tacrolimus (Tac), MPA and prednisone (P) or a steroid-free regimen consisting of Tac-MPA and a mTOR-inhibitor. In case of a rejection-free clinical course, immunosuppression was modified and reduced within the first six months to establish a dual Tac-MPA therapy on the long-term. Target trough levels of tacrolimus were 10–12 ng/ml for the first month, 8–10 ng/ml for months two to three, 6–8 ng/ml for months four to six, and 4–8 ng/ml thereafter.

Recipients of an allograft with HLA-DSA (i.e., high-risk patient) received an induction therapy consisting of a polyclonal anti-T-lymphocyte globulin (ATG-Fresenius or Thymoglobulin) as well as intravenous immunoglobulins (IvIg). Maintenance immunosuppression consisted of Tac-MPA-P. Target tacrolimus trough levels were 10–12 ng/ml for the first month, 8–10 ng/ml for months two to three, 6–8 ng/ml for months four to six, and 4–8 ng/ml thereafter. Steroids were tapered to 0.1 mg/kg body weight by month three post-transplant and maintained at this level. All ABO-blood group incompatible transplant recipients received one single dose of rituximab four weeks prior to transplantation and immunoadsorption depending on the anti-blood group titers.

MPA trough levels were measured at all outpatient visits (21545 measurements in 720 transplantations; median 29 per transplant [IQR 25–34]).

Medication as infection prophylaxis

All patients received prophylaxis with trimethoprim/sulfamethoxazole (160 mg/800 mg three times per week) against pneumocystis jirovecii infection for six months.

The CMV prevention strategy at our center has been described previously [16]. Briefly, high-risk patients (D+/R-) received prophylaxis with oral valganciclovir (Valcyte, Roche) 450 mg twice daily adjusted for renal function. Intermediate-risk patients (R+) received prophylaxis with valganciclovir if they had an induction therapy with ATG or were ABO-incompatible. All other intermediate-risk patients were managed by regular monitoring and deferred therapy. Low-risk patients (D-/R-) received no prophylaxis and had no regular screening. Prophylaxis was given for a minimum of 3 months and prolonged if immunosuppression was still considered as high (e.g., recent rejection therapy).

Assessment of allograft rejection

Patients were monitored by surveillance biopsies at 3 and 6 months post-transplant. Clinically indicated allograft biopsies were performed when serum creatinine increased by >20% from baseline. Findings were graded according to the Banff 2015 classification [17]. Clinical and subclinical rejection episodes were treated according to the phenotype and severity. Clinical T-cell-mediated rejection (TCMR) were mainly treated with i.v. steroid pulses (3–5*500 mg methylprednisolone) and a steroid taper. Subclinical TCMR were mainly treated with p.o. steroids (3*200 mg prednisone) and a steroid taper. Patients with clinical antibody-mediated rejection (ABMR) and mixed rejection episodes received ATG ± IvIg. Subclinical ABMR or mixed rejection was treated with i.v. or p.o. steroid pulses and a steroid taper. Borderline changes, which are by far the most frequent rejection phenotype in the current era of immunosuppression, were regarded and treated as TCMR [18].

Blood count and definition/classification of neutropenia

Hemoglobin is given as g/l, the other blood counts are reported as 10^9 *cells/l, which corresponds to 10^3 *cells/mm³ (i.e., $1.5*10^9$ *cells/l = 1500 cells/mm³). Neutropenia episodes were graded according to the nadir neutrophil count using the definitions of the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 (<https://ctep.cancer.gov>) as follows: neutropenia grade 2 (<1.5–1.0* 10^9 *cells/l), neutropenia grade 3 (<1.0–0.5* 10^9 *cells/l), and neutropenia grade 4 (<0.5* 10^9 *cells/l). The cause of neutropenia was determined retrospectively after careful patient chart review

including all available diagnostic tests and response to therapeutic interventions.

Management of neutropenia

Depending on the severity and dynamics of neutropenia, potentially offending drugs were reduced/discontinued stepwise or simultaneously (MPA, valganciclovir, trimethoprim/sulfamethoxazole). If MPA was reduced/discontinued, steroids were either added (e.g., 5–15 mg/day) or increased (e.g., doubling of the existing dose), unless the risk of rejection was considered as very low (e.g., HLA-identical donor) or a severe concomitant infection existed. All interventions were at the discretion of the attending transplant physician. Usual viral infections were investigated if suspicion was high or neutropenia persisting.

Statistical analysis

We used JMP software (SAS Institute Inc., Cary, NC) for statistical analysis. Categorical data are presented as counts and/or percentages and were analyzed by chi-square test or Fisher's exact test as appropriate. Continuous data are shown as median and interquartile ranges [IQR] and compared by Wilcoxon rank-sum tests. For all tests, a (two-tailed) p-value <0.05 was considered to indicate statistical significance. Time-to-event analyses were performed by the Kaplan–Meier method and compared by the log-rank test.

Results

Baseline characteristics

Two hundred and twenty-five of 721 patients (31%) developed at least one neutropenic episode within the first year post-transplant. According to the nadir neutrophil count, they were further divided into three subgroups as follows: neutropenia grade 2 ($n = 105$), neutropenia grade 3 ($n = 65$), and neutropenia grade 4 ($n = 55$). Baseline characteristics of the four groups are summarized in Table 1. Patients with neutropenia had a significantly higher proportion of CMV-risk constellations and received more often CMV-prophylaxis with valganciclovir (both $P < 0.0001$). Other patient and donor characteristics as well as initial immunosuppression was similar. Interestingly, patients with neutropenia had significantly lower neutrophil counts immediately prior to transplantation compared to patients without neutropenia ($P = 0.0002$), while hemoglobin, platelet counts, and lymphocyte counts were similar (Table 1).

Table 1. Baseline characteristics of patients grouped according to the occurrence and grade of neutropenia within the first year after transplantation.

Parameter	No neutropenia (n = 496)	Neutropenia grade 2 (n = 105)	Neutropenia grade 3 (n = 65)	Neutropenia grade 4 (n = 55)	P-value
Recipient sex, n (%) females	175 (35%)	30 (29%)	24 (37%)	19 (35%)	0.58
Recipient age at transplant	56 (45–63)	55 (44–64)	55 (41–62)	56 (41–65)	0.85
Renal disease					0.51
ADPKD	90 (18%)	22 (21%)	13 (20%)	11 (20%)	
Diabetic nephropathy	38 (8%)	12 (11%)	5 (8%)	5 (9%)	
Glomerulonephritis	181 (36%)	29 (28%)	17 (26%)	15 (28%)	
Interstitial nephropathy	17 (3%)	6 (5%)	3 (5%)	6 (11%)	
Vascular nephropathy	50 (10%)	9 (9%)	10 (15%)	4 (7%)	
Other nephropathies	62 (13%)	19 (18%)	10 (15%)	8 (14%)	
Unknown nephropathy	58 (12%)	8 (8%)	7 (11%)	6 (11%)	
Donor type, n (%) living donors	227 (46%)	42 (40%)	25 (38%)	21 (38%)	0.41
Donor age	55 (46–64)	54 (47–65)	54 (41–64)	57 (44–64)	0.85
≥second kidney transplantation	75 (15%)	21 (20%)	10 (15%)	7 (13%)	0.60
HLA A/B/DRB1/DQB1 mismatches	5 (3–6)	5 (3–6)	5 (3–6)	5 (3–6)	0.92
Pretransplant HLA-DSA	93 (19%)	18 (17%)	6 (9%)	3 (5%)	0.01
ABO incompatible	46 (9%)	9 (9%)	7 (11%)	6 (11%)	0.95
CMV constellation					
High risk (D+/R–)	64 (13%)	25 (24%)	28 (43%)	25 (47%)	<0.0001
Intermediate risk (R+)	290 (59%)	64 (61%)	26 (40%)	19 (36%)	
Low risk (D-/R-)	141 (28%)	16 (15%)	11 (17%)	9 (17%)	
Prophylaxis with valganciclovir	151 (30%)	44 (42%)	38 (58%)	29 (53%)	<0.0001
Induction therapy					0.34
Anti IL2-receptor antibody	363 (73%)	75 (71%)	53 (81%)	46 (84%)	
ATG ± Ivlg	109 (22%)	24 (23%)	9 (14%)	6 (11%)	
None	24 (5%)	6 (6%)	3 (5%)	3 (5%)	
Initial immunosuppression					0.64
Tac-MPA-P	466 (94%)	97 (92%)	60 (92%)	52 (94%)	
Tac-MPA-mTOR	28 (6%)	8 (8%)	5 (8%)	2 (4%)	
Other	2	–	–	1 (2%)	
Blood count before transplant					
Hemoglobin [g/l]	117 (107–126)	116 (104–126)	117 (106–125)	120 (104–126)	0.63
Platelets [$\times 10^9/l$]	215 (175–265)	211 (154–256)	222 (174–259)	12 (169–245)	0.35
Leucocyte count [$\times 10^9/l$]	11.2 (7.2–18.4)	9.4 (5.8–15.7)	9.1 (6.2–13.2)	9.1 (6.2–17.1)	0.01
Neutrophil count [$\times 10^9/l$]	5.8 (4.2–8.0)	4.7 (3.3–8.3)	4.7 (3.8–6.0)	4.8 (3.5–6.9)	0.0002
Lymphocyte count [$\times 10^9/l$]	1.1 (0.8–1.5)	1.0 (0.7–1.4)	1.1 (0.9–1.5)	1.0 (0.8–1.4)	0.14
Days of neutropenia in first year					
Total days	n.a.	14 (7–21)	20 (10–33)	21 (14–44)	<0.0001
Days $<1.5 \times 10^9/l$	n.a.	14 (7–21)	9 (2–25)	5 (0–17)	
Days $<1.0 \times 10^9/l$	n.a.	n.a.	7 (4–14)	7 (0–14)	
Days $<0.5 \times 10^9/l$	n.a.	n.a.	n.a.	7 (7–12)	

ADPKD, autosomal polycystic kidney disease; HLA-DSA, donor-specific HLA-antibodies; ATG, anti T-cell globulin; Ivlg, intravenous immunoglobulins; Tac, tacrolimus; MPA, mycophenolic acid; mTOR, mTOR inhibitors; P, prednisone.

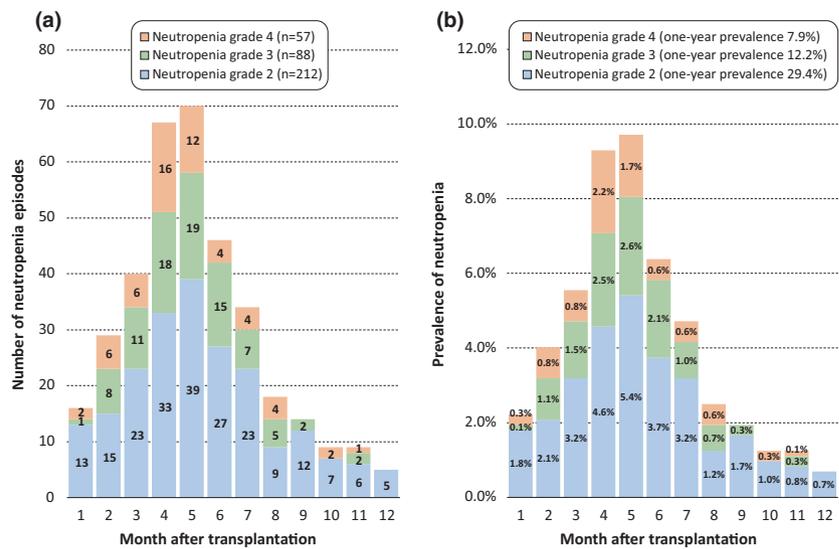
Neutropenia episodes

Overall, the 225 patients experienced 357 episodes of neutropenia. Sixty-four percent of patients had only one episode, 23% had two episodes, 7% had three episodes, and 6% more than three. Two hundred and twelve of 357 neutropenia episodes (59%) were grade 2, 88/357

episodes (25%) were grade 3, and 57/357 episodes (16%) were grade 4.

Neutropenia episodes were rarely observed within the first month after transplantation (4.5% of all episodes), they peaked between months 4 and 6 (51.3% of all episodes) and were rare again beyond month 9 (6.4% of all episodes) (Fig. 1a). The monthly prevalence of

Figure 1 Temporal distribution of 357 neutropenia episodes within the first year after transplantation, stratified by the grade of neutropenia. (a) Number of neutropenia episodes per month. (b) Monthly prevalence of neutropenia in the whole population of 721 patients.



neutropenia ranged from 0.7% (month 12) to 9.7% (month 5) (Fig. 1b). We observed no correlation between the neutropenia grade and the time post-transplant ($P = 0.18$). The median duration of the neutropenic episodes was 12 days (IQR 7–18 days). Higher grades of neutropenia lasted significantly longer: grade 2 (median 10 days; IQR 6–15), grade 3 (median 14 days; IQR 7–20), and grade 4 (median 14 days; IQR 11–28) ($P < 0.0001$).

Median neutrophil count at the nadir was $1.29 \times 10^9/l$ (grade 2), $0.80 \times 10^9/l$ (grade 3), and $0.28 \times 10^9/l$ (grade 4). At the time point of the neutrophil nadir,

hemoglobin, platelet count, and lymphocyte count were not different among the three groups ($P = 0.41$, $P = 0.10$, and $P = 0.36$) and mostly in the normal range, indicating isolated neutropenia in the vast majority of cases (Table 2).

Drug exposure at the start of neutropenia is summarized in Table 3. MPA dose per day and the mean MPA trough level in the preceding month were significantly higher in the grade 4 neutropenia group compared with grade 2 and 3. No other significant correlations between the grade of neutropenia and the drug exposure were found.

Table 2. Blood count at the nadir of neutropenia episodes.

Parameter	Neutropenia grade 2 ($n = 212$)	Neutropenia grade 3 ($n = 88$)	Neutropenia grade 4 ($n = 57$)	P -value
Hemoglobin				
Median (IQR) [g/l]	117 (104–127)	115 (108–127)	120 (106–132)	0.41
<100 g/l, n (%)	39 (18%)	11 (13%)	6 (11%)	0.23
Platelets				
Median (IQR) [$\times 10^9/l$]	204 (157–256)	211 (173–258)	219 (175–291)	0.10
< $75 \times 10^9/l$, n (%)	1 (0.5%)	1 (1%)	1 (2%)	0.60
Lymphocyte count				
Median (IQR) [$\times 10^9/l$]	0.94 (0.61–1.44)	0.85 (0.58–1.26)	0.92 (0.48–1.28)	0.36
Normal range [†] [$\geq 0.9 \times 10^9/l$], n (%)	111 (53%)	43 (50%)	30 (53%)	0.80
<0.9 to $\geq 0.8 \times 10^9/l$, n (%)	21 (10%)	8 (9%)	3 (5%)	
<0.8 to $\geq 0.5 \times 10^9/l$, n (%)	41 (19%)	18 (21%)	9 (16%)	
<0.5 to $\geq 0.2 \times 10^9/l$, n (%)	32 (15%)	16 (18%)	11 (19%)	
< $0.2 \times 10^9/l$, n (%)	7 (3%)	2 (2%)	4 (7%)	
Leucocyte count				
Median (IQR) [$\times 10^9/l$]	2.69 (2.29–3.31)	2.15 (1.70–2.57)	1.57 (1.11–2.16)	<0.0001
< $2.0 \times 10^9/l$, n (%)	22 (10%)	34 (39%)	38 (67%)	<0.0001
Neutrophil count [$\times 10^9/l$]				
	1.29 (1.16–1.38)	0.80 (0.68–0.92)	0.28 (0.15–0.39)	<0.0001

[†]Normal range according to the used instrument and in-house reference

Table 3. Drug exposure at start of neutropenia episodes.

Parameter	Grade 2 (n = 212)	Grade 3 (n = 88)	Grade 4 (n = 57)	P-value
Tacrolimus	209 (99%)	87 (99%)	56 (98%)	0.95
mTOR	9 (4%)	4 (5%)	1 (2%)	0.60
MPA				
On MPA	176 (83%)	77 (88%)	51 (89%)	0.36
Mycophenolate-mofetil	109 (51%)	51 (58%)	37 (65%)	0.16
Mycophenolate-sodium	67 (32%)	26 (30%)	14 (25%)	0.58
Equivalent MPA dose (mg/day)*	1000 (750–1500)	1000 (1000–1500)	1500 (1000–2000) [†]	0.003
MPA trough level	2.0 (1.1–3.3)	1.7 (1.0–2.7)	1.7 (0.6–2.9)	0.22
mean MPA trough level preceding month	2.4 (1.4–3.2)	2.4 (1.4–3.6)	3.0 (1.8–4.4) [‡]	0.008
Azathioprine	8 (4%)	–	1 (2%)	0.05
Prednisone				
On prednisone	157 (74%)	68 (77%)	50 (88%)	0.07
Dose (mg/Tag)	7.5 (7.5–15)	7.5 (7.5–12.5)	7.5 (7.5–12.5)	0.76
Trimethoprim/Sulfamethoxazole	151 (71%)	68 (77%)	45 (79%)	0.34
Valganciclovir				
On valganciclovir	77 (36%)	45 (51%)	23 (40%)	0.06
Dose (mg/Tag)	450 (450–450)	450 (450–900)	450 (225–450)	0.58

MPA, mycophenolic acid; mTOR, mTOR inhibitors.

*720 mg mycophenolate-sodium equals 1000 mg mycophenolate-mofetil.

[†]Grade 4 vs grade 2 ($P = 0.0007$), grade 4 vs grade 3 ($P = 0.01$), grade 3 vs grade 2 ($P = 0.54$).

[‡]Grade 4 vs grade 2 ($P = 0.003$), grade 4 vs grade 3 ($P = 0.01$), grade 3 vs grade 2 ($P = 0.74$).

Therapeutic interventions and presumed causes of neutropenia

Overall, interventions were made in 253/357 neutropenia episodes (71%). In those 253 episodes, 383 interventions were recorded (=1.5 interventions per episode). The most frequent intervention was a modification in MPA dosage (51%), followed by valganciclovir (25%) and trimethoprim/sulfamethoxazole (19%). Other interventions were rare (5%) and included discontinuation of azathioprine ($n = 5$), reduction/discontinuation of mTOR inhibitors ($n = 5$), and discontinuation of proton pump inhibitors ($n = 10$). G-CSF was only used on two occasions (0.6%). Depending on the grade of neutropenia, more interventions were made, including a higher frequency of MPA discontinuation and substitution with prednisone, by either adding it or increasing its dose (Table 4).

The cause of neutropenia was determined retrospectively and was inferred by the evolution of neutrophil counts after interventions. Overall, single drugs were regarded as the cause of neutropenia in 17%, a combination of drugs in 54%. Infections alone were rarely assigned as the causative event (1.4%). A combination of infection and drugs was assumed in 11%, and in 17% of all neutropenia episodes the cause remained unknown. The causes of

neutropenia were significantly different among the three grades ($P = 0.001$; Fig. 2).

After resolution of neutropenia, 275/357 cases (77%) were on MPA at a median dose of 1000 mg/day (IQR 500–1000), and 279/357 cases (78%) were on prednisone.

No pneumocystis jirovecii infection was observed following 71 interventions regarding trimethoprim/sulfamethoxazole. Valganciclovir was altered in 96 neutropenia episodes. Subsequent CMV infections occurred in 13 patients (14%): CMV tissue-invasive disease ($n = 2$), CMV syndrome ($n = 1$), asymptomatic CMV viremia ($n = 10$). Hospitalization was required in 1/13 patients. Eleven of 13 patients (85%) with subsequent CMV infections had high-risk constellations (D+/R-).

Neutropenia and rejection

We performed two analyses to investigate the impact of neutropenia and the subsequent interventions on the development of allograft rejection. First, on the level of neutropenia episodes we compared the frequency of rejection observed in the month preceding neutropenia ($n = 69$ biopsies) with the frequency of rejection observed since the onset of neutropenia until one month after its resolution ($n = 96$ biopsies). The rejection frequency was not different within the two time

Table 4. Therapeutic interventions.

Parameter	Grade 2 (n = 212)	Grade 3 (n = 88)	Grade 4 (n = 57)	P-value
Any intervention	130 (61%)	75 (85%)	48 (84%)	<0.0001
Number of interventions, 1/2/3	89/36/5	40/28/7	14/24/10	<0.0001
MPA	<u>n = 176</u>	<u>n = 77</u>	<u>n = 51</u>	
No change	86 (49%)	15 (19%)	8 (16%)	<0.0001
Reduced	43 (24%)	7 (9%)	4 (8%)	
Discontinued	45 (26%)	52 (68%)	39 (76%)	
Stopped	2 (1%)	3 (4%)	–	
Valganciclovir	<u>n = 77</u>	<u>n = 45</u>	<u>n = 23</u>	
No change	30 (39%)	15 (33%)	4 (17%)	0.18
Reduced	6 (8%)	2 (4%)	–	
Discontinued	34 (44%)	24 (54%)	17 (74%)	
Stopped	7 (9%)	4 (9%)	2 (9%)	
Trimethoprim/Sulfamethoxazole	<u>n = 151</u>	<u>n = 68</u>	<u>n = 45</u>	
No change	125 (83%)	47 (69%)	21 (47%)	0.0002
Discontinued	22 (15%)	17 (25%)	20 (44%)	
Stopped	4 (2%)	4 (6%)	4 (9%)	
On prednisone	<u>n = 157</u>	<u>n = 68</u>	<u>n = 50</u>	
Increased	34 (22%)	32 (47%)	31 (62%)	<0.0001
From mg/day	7.5 (5–10)	7.5 (7.5–10)	7.5 (5–10)	0.40
To mg/day	15 (15–22.5)	15 (15–20)	20 (15–20)	0.25
No prednisone	<u>n = 55</u>	<u>n = 20</u>	<u>n = 7</u>	
Prednisone added	12 (22%)	15 (75%)	7 (100%)	<0.0001

The underlined number indicate the number of cases/patients evaluated.

MPA, mycophenolic acid.

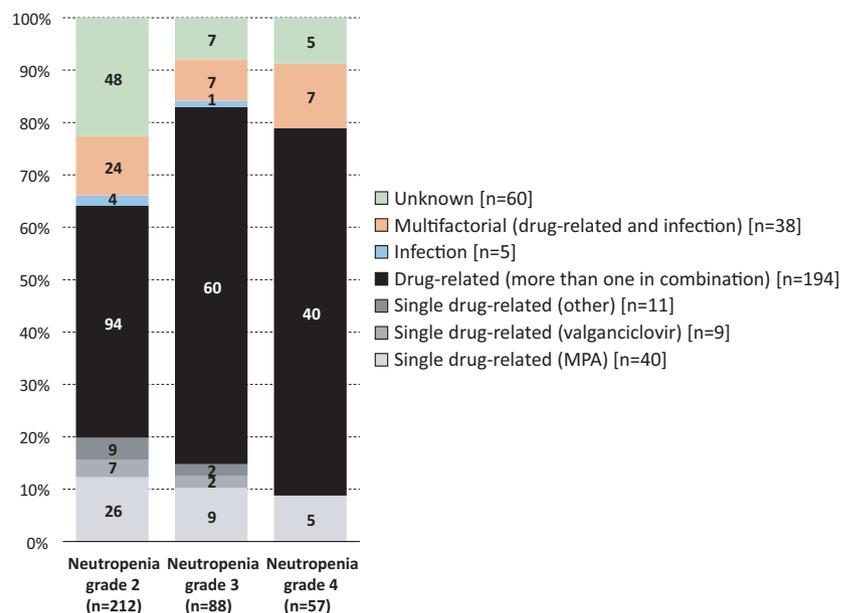


Figure 2 Assigned causes of 357 neutropenia episodes, stratified by the grade of neutropenia.

frames (36/69 [52%] vs 46/96 [48%]; $P = 0.59$). Among the 82 rejection episodes, clinical rejection was more frequent in the month preceding neutropenia than in the later time frame (16/36 [44%] vs 8/46 [17%]; $P = 0.01$).

Next, we investigated the overall one-year incidence of rejection between patients with and without neutropenia. No differences were observed regarding the incidence of clinical rejection ($P = 0.62$), (sub)clinical rejection ($P = 0.58$), (sub)clinical TCMR ($P = 0.67$), or

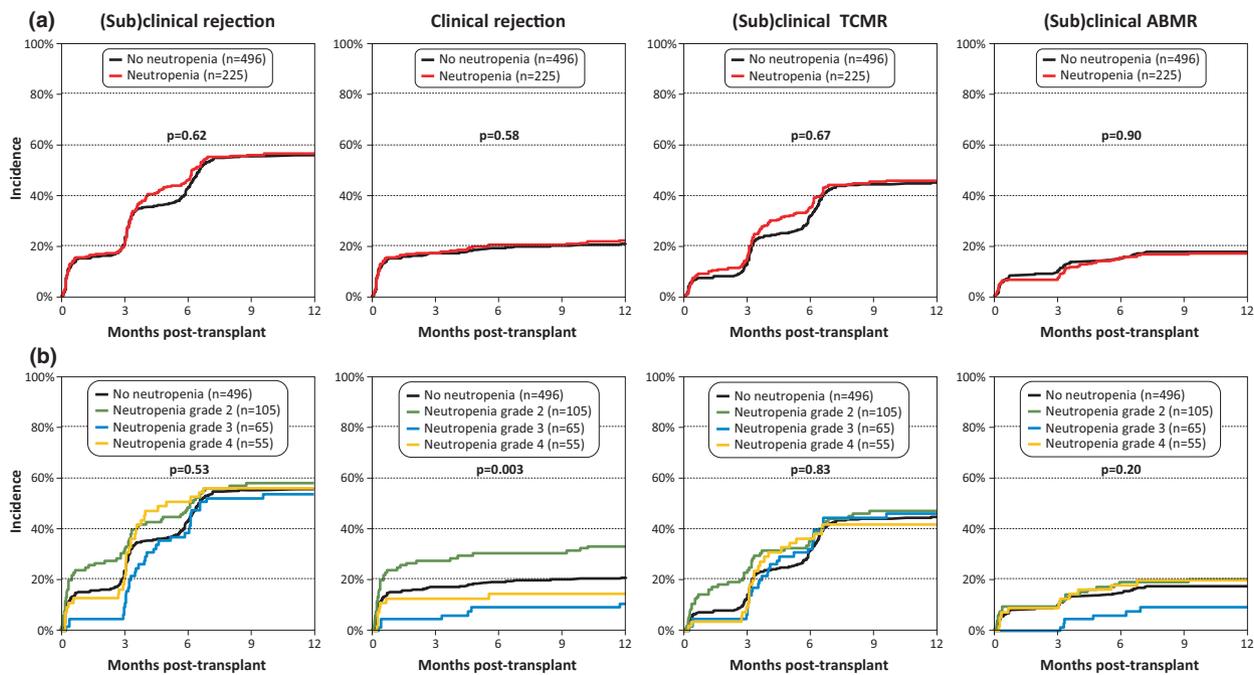


Figure 3 Incidence of rejection within the first year after transplantation. (a) Comparison of patients with and without neutropenia. (b) Comparison of patients without neutropenia and the three groups defined by the grade of neutropenia.

(sub)clinical ABMR ($P = 0.90$) (Fig. 3). In a sensitivity analysis comparing neutropenia grades, we found significant differences regarding the incidence of clinical rejection ($P = 0.003$), but not (sub)clinical rejection ($P = 0.53$), (sub)clinical TCMR ($P = 0.83$), or (sub)clinical ABMR ($P = 0.20$) (Fig. 3). The difference in clinical rejection was driven by a higher incidence of rejection within the first month post-transplant in patients with neutropenia grade 2 compared with the other three groups.

Risk of infection during neutropenia

To assess the risk of infection associated with neutropenia, we compared the frequency of infections during neutropenia with its frequency outside of neutropenic phases on an individual patient level. Therefore, each patient served as his/her own control reducing biases because of patients-specific confounders. Within the first year after transplantation, we observed infections in 174/225 patients (77%) with neutropenia. They had in total 463 infections; 57 occurred during neutropenia (12%) and 406 outside of neutropenic phases (88%). The incidence of infections during neutropenia was significantly higher than outside of neutropenic phases in grade 3 neutropenia (6.0 per year vs 1.8 per year; paired T-test $P = 0.02$) and grade 4 neutropenia (6.3 per year vs 2.5 per year; paired T-test $P = 0.01$), but similar in

grade 2 neutropenia (2.0 per year vs 1.9 per year; paired T-test $P = 0.85$). The incidence of infections outside of neutropenic phases was not different among the three groups (1.9 vs 1.8 vs 2.5 per year; one-way ANOVA $P = 0.23$). The most common infectious diseases during neutropenia were CMV ($n = 14$), urinary tract infections ($n = 14$), upper airway infections ($n = 8$), and neutropenic fever ($n = 6$).

One-year outcomes

Three hundred thirty-three of 357 neutropenia episodes (93%) occurred in the outpatient setting, requiring hospitalization in 12/333 cases (3.6%). The main reason for hospitalization was infectious diseases. The hospitalization rate correlated with the grade of neutropenia (grade 2: 3/196 [1.5%]; grade 3: 3/83 [3.6%]; grade 4: 6/53 [11.3%]; $P = 0.003$). Twenty-four of 357 neutropenia episodes (7%) occurred during hospitalization for other reasons.

The incidence of death within the first year after transplantation was similar between patients with and without neutropenia (5/225 [2.2%] vs 5/496 [1.0%]; $P = 0.30$). Two of 5 patients died during or within 10 days after resolution of neutropenia. Both died unexpectedly at home likely because of an acute cardiac event. The other 3/5 patients died more than 100 days after neutrophil counts normalized. Graft loss was

significantly more frequent in patients with neutropenia (6/225 [2.7%] vs 2/496 [0.4%]; $P = 0.01$). However, 5/6 graft losses were unrelated to neutropenia or its treatment (i.e., therapy-resistant early onset ABMR [$n = 2$], very marginal donor organ [$n = 2$], hyperfiltration injury in a kidney from a pediatric donor [$n = 1$]).

Median eGFR at one-year post-transplant, calculated according to the CKD EPI formula [19], was similar between patients with and without neutropenia (51 ml/min [39–67] vs 53 ml/min [41–68]; $P = 0.37$), and among the three neutropenia grades ($P = 0.43$).

Long-term outcomes

For this analysis, all cases with functioning allograft at one-year post-transplant ($n = 703$) were included, stratified by the occurrence and grade of neutropenia within

the first year after transplantation. Graft-, patient-, and death-censored graft survival were not different between patients with and without neutropenia ($P = 0.77$, $P = 0.79$, and $P = 0.23$). Furthermore, no differences were observed among the three grades of neutropenia (Fig. 4).

Discussion

The key observation in this study is that different grades of neutropenia within the first year after kidney transplantation do not represent an increased risk for rejection and have no impact on long-term patient and graft survival. However, neutropenia grade ≥ 3 (i.e., neutrophil count $<1.0 \times 10^9/l$) is associated with a higher risk of infectious diseases and a higher rate of hospitalization.

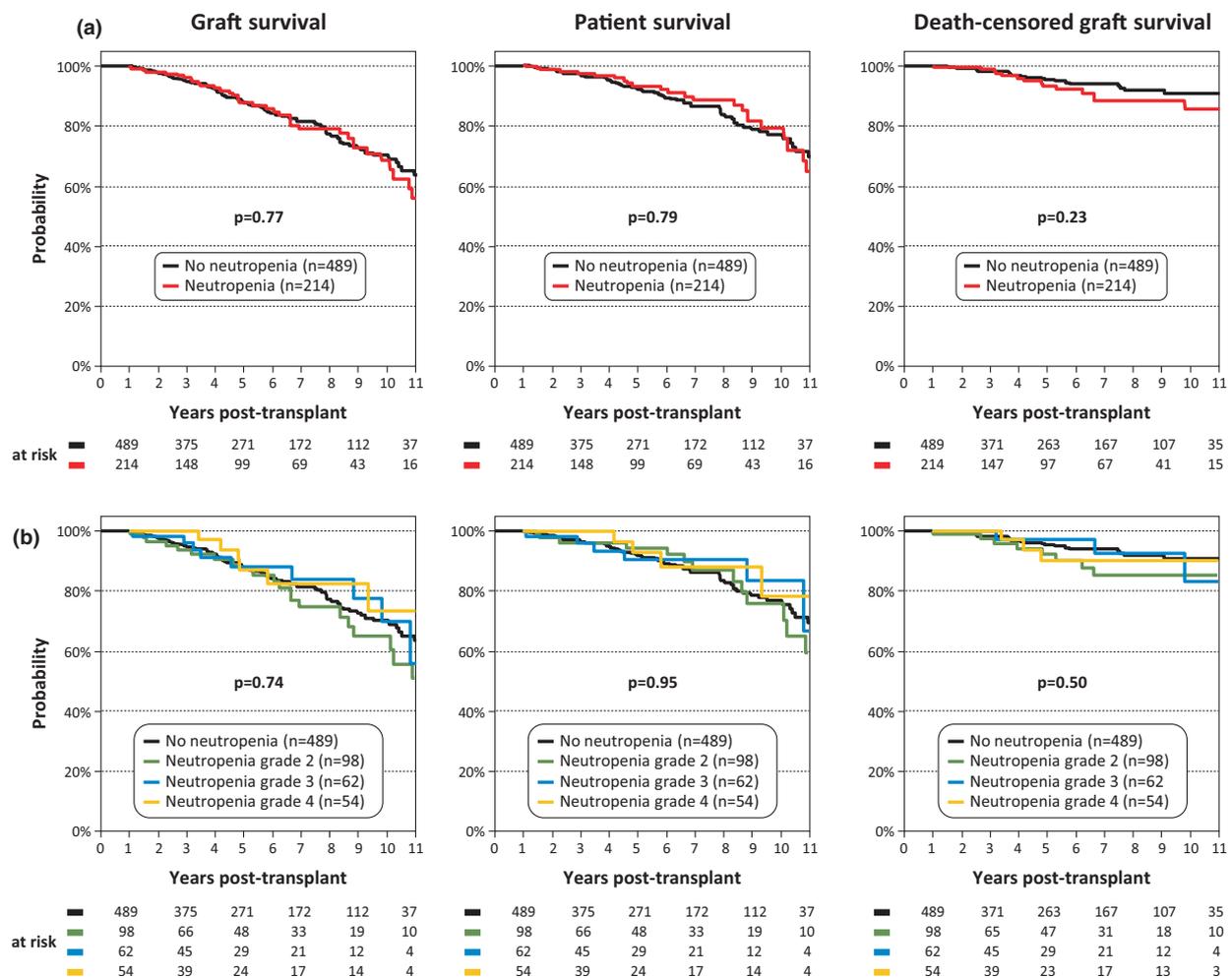


Figure 4 Graft-, patient-, and death-censored graft survival beyond the first year after transplantation. In this analysis, only patients with a functioning allograft at one-year post-transplant are included ($n = 703$), stratified by the occurrence and grade of neutropenia within the first year after transplantation. (a) Comparison of patients with and without neutropenia. (b) Comparison of patients without neutropenia and the three groups defined by the grade of neutropenia.

To the best of our knowledge, this is the largest study ($n = 721$) investigating short- and long-term clinical impact of different neutropenia grades after kidney transplantation. Consistent with previous publications, we did not observe a higher one-year mortality or graft failure rate between patient with and without neutropenia [1,2]. Our study extends these data also to long-term outcomes.

Zafrani et al. reported in a cohort of 395 patients (220 on Tac-MPA) a higher risk of clinical rejection, if MPA was discontinued for more than 6 days during management of neutropenia [2]. Despite investigating the association of neutropenia grades and occurrence of rejection with two different approaches and various rejection definitions, we did not observe a higher risk. This absent association might be related to the management policy at our institution, which includes a replacement of reduced/discontinued MPA with steroids. Clearly, this must be regarded as an observation in a retrospective study, which would ideally be clarified in a prospective interventional study. Nevertheless, we believe that replacing MPA with some steroids (i.e., doubling the existing dose or adding 5–15 mg/day) is an attractive concept to prevent rejection. On the other hand, the added steroids might increase the risk for infectious diseases, which has to be balanced against the benefit to prevent rejection. Interestingly, steroids might even have beneficial effect during systemic inflammation processes and have emerged as an ancillary part of treatment for many infectious diseases [20–22].

The precise determination of the cause of neutropenia is very challenging and often impossible because of the multifactorial etiology and concurrent therapeutic interventions. Our study confirms that neutropenia within the first year post-transplant is mostly drug-related and has a peak prevalence around month 3 to 6 [2,7,10]. We could add more granular data to the temporal prevalence and the most likely cause of neutropenia. Indeed, our data suggest a very vulnerable time frame between months 4 and 6 because of cumulative toxic effects of MPA and valganciclovir while having minimal or no steroids. Concurrent viral infections, such as CMV, could induce a further hit on granulopoiesis. Furthermore, patients with a lower neutrophil production capacity might be more susceptible to develop neutropenia. This is supported by the intriguing observation that patients developing neutropenia had lower neutrophil counts immediately before the transplantation.

In our study, the majority of neutropenia episodes did not go along with a reduction in other blood cell counts (i.e., platelets and hemoglobin). In addition,

although lymphopenia was present in almost 50% of neutropenia episodes, we found no correlation with neutropenia grades, suggesting that neutropenia was in most cases a neutrophil-restricted disease process.

Some observations in this study can be used for clinical management of neutropenia episodes. First, neutropenia grade 2 is often self-limiting and requires only mild interventions. Second, trimethoprim/sulfamethoxazole alone is rarely responsible for neutropenia but can be temporarily discontinued without a detectable increased risk for pneumocystis jirovecii infection. Third, valganciclovir can be safely discontinued in most cases, but it requires a cautious follow-up especially in CMV high-risk patients, because most subsequent CMV infections occurred in this group. And fourth, MPA seems to be the main causative drug for neutropenia and can be safely reduced/discontinued without having an increased risk for rejection, if substituted with steroids.

G-CSF was only used in 2/225 patients (0.9%) or 2/357 neutropenia episodes (0.6%). Both patients had neutropenia grade 4 requiring hospitalization and empiric antibiotic therapy because of neutropenic fever. G-CSF was given much more frequently in the study by Hartmann et al. (21/59 patients [36%]) and Zafrani et al. (15/112 patients [13%]), but short-term outcomes are very similar to our data [1,2]. Ultimately, only a prospective randomized study can clarify, if and for which grades of neutropenia G-CSF is beneficial. An individualized approach for using G-CSF might be a good option, depending also on the center's experience in daily practice.

The advantages of this study are the large contemporary and unselected cohort, the availability of surveillance biopsies, the detailed temporal blood count data, and the evaluation of different neutropenia grades regarding short- and long-term outcomes. However, it has also certain limitations. Management of neutropenia was not fully standardized as in all other studies published so far. Therefore, personal decisions on an individual patient level can introduce a bias, which we regard as small. Furthermore, we describe the outcomes for the management concept of adding/increasing steroids as replacement for reduced/discontinued MPS, but a side-by-side comparison with a control group is lacking. Clearly, a prospective randomized trial could resolve this limitation but is unlikely to ever be performed. In addition, we acknowledge all limitations inherent to retrospective single-center studies such as restricted applicability to different patient populations or healthcare systems.

In conclusion, our study indicates that neutropenia within the first year after kidney transplantation is not

associated with an increased risk for rejection and has no negative impact on long-term patient and graft survival. Adding/increasing steroids as replacement for reduced/discontinued MPA might help to prevent rejection and supplement management of neutropenia.

Authorship

LI, JH, and SS participated in research design. All authors participated in the writing/revising of the paper. LI, PA, CW, PHM, and SS participated in the performance of the research. LI and SS participated in data analysis.

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Conflict of interest

None to declare.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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