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Pre-transplant testosterone and outcome of men after allogeneic stem cell transplantation

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ABSTRACT

Testosterone is an important determinant of endothelial function and vascular health in men. As both factors play a role in mortality after allogeneic stem cell transplantation (alloSCT), we retrospectively evaluated the impact of pre-transplant testosterone levels on outcome in male patients undergoing alloSCT. In the discovery cohort (n=346), an impact on outcome was observed only in the subgroup of patients allografted for acute myeloid leukemia (AML) (n=176, hereafter termed 'training cohort'). In the training cohort, lower pre-transplant testosterone levels were significantly associated with shorter overall survival (OS) [hazard ratio (HR) for a decrease of 100 ng/dL: 1.11, P=0.045]. This was based on a higher hazard of non-relapse mortality (NRM) (cause-specific HR: 1.25, P=0.013), but not relapse (cause-specific HR: 1.06, P=0.277) in the multivariable models. These findings were replicated in a confirmation cohort of 168 male patients allografted for AML in a different center (OS, HR: 1.15, P=0.012 and NRM, cause-specific HR: 1.23; P=0.008). Next, an optimized cut-off point for pre-transplant testosterone was derived from the training set and evaluated in the confirmation cohort. In multivariable models, low pre-transplant testosterone status (<250 ng/dL) was associated with worse OS (hazard ratio 1.95, P=0.021) and increased NRM (cause-specific HR 2.68, P=0.011) but not with relapse (cause-specific HR: 1.28, P=0.551). Our findings may provide a rationale for prospective studies on testosterone/androgen assessment and supplementation in male patients undergoing alloSCT for AML.

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Introduction

Allogeneic stem cell transplantation (alloSCT) is an effective therapy for many hematologic malignancies, but is still hampered by substantial procedure-related mortality and morbidity. Today, there is growing recognition that endothelial dysfunction is implicated in the pathogenesis of a variety of potentially fatal early and late complications of alloSCT, such as transplant-associated thrombotic microangiopathy, cardiovascular disorders, and graft-*versus*-host disease (GvHD).[1,2]

In particular, therapy refractory GvHD is a substantial determinant of non-relapse mortality (NRM) after alloSCT, and "endothelial vulnerability" was proposed as a hypothesis to explain why some patients with acute GvHD fail to respond to escalating immunosuppressive therapy and ultimately succumb to GvHD and/or treatment related complications.[3-6]

In male individuals, low serum testosterone has been linked to endothelial dysfunction and all-cause and cardiovascular disease-related mortality in various non-transplant settings.[7-9] This is of particular importance as alloSCT is increasingly used in elderly populations characterized by declining sex hormone activity. Notably, with regard to hematologic malignancies, testosterone and androgens have also been used as adjunct in the treatment of acute myeloid leukemia (AML).[10-12]

Based on these considerations, we sought to evaluate the impact of pre-transplant testosterone levels on outcome in male patients undergoing alloSCT.

Methods

Patients

Patients were eligible for this study if they were allografted for a hematologic malignancy between 2002 and 2017 at the University Hospital Heidelberg, Germany, and had serum samples available for measurement of pre-transplant testosterone levels. Pre-transplant testosterone levels were assessed in all male patients meeting these criteria (discovery cohort, n=346). The independent confirmation cohort consisted of male patients diagnosed with AML who had undergone allografting at the University Hospital Essen between 2009 and 2013 and had serum samples available for measurement of testosterone levels (n=168). In addition, pre-transplant testosterone levels were also measured in a small pilot cohort of female patients allografted for AML in Heidelberg (n=32).

Written informed consent for sample and data collection according to the Declaration of Helsinki was obtained for all patients, and the local ethics committees approved the study. Patient data were obtained from medical records and chart review. Disease status prior to alloSCT was assessed applying published criteria.[13] Further details regarding transplant procedure are provided in the *Online Supplementary Appendix.*

Assessment of pre-transplant testosterone serum levels

Serum samples were collected between 0 and 2 weeks before alloSCT and cryopreserved at −80°C. Serum levels of total testosterone were assessed retrospectively in the last serum sample before start of the conditioning treatment. The measurements were carried out using accredited laboratory methods. A detailed description of the methodology is provided in the *Online Supplementary Appendix*. Serum concentrations of total testosterone were expressed in ng/dL (for conversion of ng/dL into nM divide by a factor of 28.8). None of the patients had received sex hormone therapy previous to, or at the time of, sample collection.

Statistical analysis

Overall survival (OS), progression-free survival (PFS) (time to relapse or death from any cause), time to relapse, and non-relapse mortality (NRM) (time to death in absence of prior relapse) were calculated from the date of alloSCT to the appropriate end point. NRM and recurrence of the underlying malignancy were considered as competing events. Since acute GvHD and its treatment are major contributors to post-transplant mortality, OS, PFS, incidence of NRM and relapse were also assessed after acute GvHD (i.e. in patients who developed acute GvHD, from the date of its onset).

Since the normal physiological range of serum testosterone has not been well defined, pre-transplant testosterone was first analyzed as a continuous variable in the uni- and multivariable models. Cox proportional hazards regression analysis was applied for OS, PFS, and OS and PFS after acute GvHD. Relapse and NRM were analyzed by cause-specific Cox models. Prognostic impact of pre-transplant testosterone on OS, PFS, OS and PFS after acute GvHD was assessed by hazard ratios (HR) and, in case of time to relapse and NRM, by cause-specific hazard ratios (CHR) from corresponding (cause-specific) Cox models. Multivariable (cause-specific) Cox regression models were used to adjust for additional co-variates. All statistical tests were two-sided. For further details on the statistical methods used for analysis see the *Online Supplementary Appendix.*

Results

Discovery cohort

Patients', disease and transplant characteristics of the male patients of the discovery cohort (n=346) are summarized in *Online Supplementary Table S1*. Median follow up of survivors was 65 months (95%CI: 57-73). Median pre-transplant total testosterone serum level was 400 ng/dL [interquartile range (IQR) 269-584].

In univariable analysis, lower pre-transplant testosterone as continuous variable was significantly associated with shorter OS and PFS (HR for a decrease of 100 ng/dL, 1.11, 95%CI: 1.03-1.20, P=0.005 and HR 1.12, 95%CI: 1.05-1.20, P=0.001, respectively). This was due to a significantly higher risk of both NRM and relapse (CHR 1.15, 95%CI: 1.02-1.28, P=0.018 and CHR 1.11, 95%CI: 1.01-1.20, P=0.023, respectively). However, in both the "slim" and "full" multivariable models, significant associations of pre-transplant testosterone with any end point could not be confirmed. This was based on a statistically significant interaction between pre-transplant testosterone and the diagnosis AML for the end point NRM (*Online Supplementary Table S2*).

Accordingly, lower levels of pre-transplant testosterone were significantly associated with shorter OS and PFS, and higher hazard of NRM and relapse only in the subgroup of patients allografted for AML (n=176) but not for other diagnoses (n=170) as revealed by the univariable models (*Online Supplementary Table S3*). Patients' and transplant characteristics of AML *versus* non-AML patients are also provided in *Online Supplementary Table S1*. Consequently, further analyses were restricted to male patients with AML, henceforth referred to as the 'training cohort' (n=176).

Pre-transplant testosterone and post-transplant outcome in the training cohort

Patients', disease and transplant characteristics of the training cohort are summarized in Table 1. Median pre-transplant total testosterone serum level was 423 ng/dL (IQR 256-611; for distribution, see histogram in *Online Supplementary Figure S1A*) and the estimated median follow up of survivors was 36 months (95%CI: 32-47). The cumulative incidence of acute GvHD grade 3-4 on day +100 post-transplant was 5.1% (95%CI: 1.7-8.4). Pre-transplant testosterone had no impact on the hazard of acute GvHD grade 3-4 (HR 0.92 95%CI: 0.74-1.15, P=0.448).

Since testosterone might also reflect the individual's health and nutritional status prior to alloSCT, pre-transplant testosterone levels were correlated to additional patients' characteristics: body-mass index (BMI), levels of C-reactive protein (CRP), performance status and comorbidities prior to alloSCT. These data were only available for the training cohort and are summarized in *Online Supplementary Table S4*. Pre-transplant testosterone levels were weakly negatively correlated to pre-transplant CRP levels (Spearman's rho: -0.17, P=0.025) and were not correlated to the BMI (Spearman's rho: -0.12, P=0.107). However, pre-transplant testosterone levels were lower in obese patients of the training cohort (BMI ≥30 kg/m², P=0.028) (*Online Supplementary Figure S2A*). The median pre-transplant testosterone levels were similar between patients with elevated (>5 mg/L) and non-elevated (≤5

Table 1. Patients' disease and transplant characteristics of male acute myeloid leukemia (AML) patients of the training and confirmation cohorts.

Parameter	Training cohort n=176	Confirmation cohort n=168	P
Age [years] at alloSCT (median, IQR)	59 (50-64)	56 (45-63)	0.818
Disease stage before alloSCT[a], n (%)			0.022
Early	74 (42)	78 (47)	
Intermediate	32 (18)	45 (27)	
Late	69 (39)	44 (26)	
NA	1	1	
Conditioning[b], n (%)			0.003
RIC	114 (65)	132 (80)	
MAC	62 (35)	34 (20)	
NA	0	2	
Stem cell source, n (%)			0.632
Peripheral blood	168 (96)	158 (94)	
Bone marrow	8 (4)	10 (6)	
Donor, n (%)			0.182
Related	41 (23)	29 (17)	
Unrelated	135 (77)	139 (83)	
Recipient – donor sex match, n (%)			0.078
Matched	127 (72)	135 (80)	
Male – female	49 (28)	33 (20)	
Pre-transplant testosterone, ng/dL (median, IQR)	423 (256-611)	469 (309-580)	0.043

alloSCT: allogeneic stem cell transplantation; AML: acute myeloid leukemia; CI: confidence interval; IQR: interquartile range; MAC: myeloablative conditioning; NA: not available or not assessable; RIC: reduced intensity conditioning. [a]According to Gratwohl *et al.*[13] [b]According to Bacigalupo *et al.*[37] and Bornhäuser *et al.*[38]

mg/L) CRP levels prior to alloSCT (*P*=0.127) (*Online Supplementary Figure S2B*). There was a trend towards lower pre-transplant testosterone levels in patients with lower Karnofsky performance status (KPS ≤80%), whereas testosterone levels were similar between the low hematopoietic cell transplantation-specific comorbidity index[14] group (HCT-CI 0), intermediate (HCT-CI 1 to 2), and high risk (HCT-CI 3 or more) HCT-CI groups (*Online Supplementary Figure S2C and D*; for further details see the *Online Supplementary Appendix*).

In univariable analysis, lower pre-transplant testosterone was correlated with shorter post-transplant OS and PFS, due to higher hazards of both relapse and NRM. Low pre-transplant testosterone was also associated with non-relapse and overall mortality after onset of acute GvHD. The results of the univariable analyses of the training cohort are given in Table 2.

In the multivariable models, lower levels of pre-transplant testosterone were significantly associated with worse OS (HR for a decrease of 100 ng/dL, 1.11, *P*=0.045) and PFS (HR 1.11, *P*=0.022) ("full" models). Other factors with a statistically significant impact on OS and PFS were patient age and advanced disease stage (Table 3). Notably, lower pre-transplant testosterone was associated with a higher hazard of NRM (CHR 1.25, *P*=0.013) rather than relapse (CHR 1.06, *P*=0.277) in patients allografted for AML as revealed by the "slim" models (Table 3).

In contrast to the univariable models (Table 2), no significant association of testosterone with survival after onset of acute GvHD could be observed in multivariable analy-

sis (Table 3). Consequently, and due to the relatively low number of events, no multivariable models were fitted for NRM/relapse following acute GvHD. When pre-transplant testosterone as continuous variable was analyzed in multivariable models including age, CRP, BMI, KPS and comorbidities as co-variates, the associations of lower testosterone with worse OS and shorter PFS remained significant (*Online Supplementary Table S5*).

Finally, pre-transplant testosterone was assessed and evaluated in a small pilot cohort of female patients allografted for AML in the Heidelberg center (n=32) (*Online Supplementary Table S6*). As expected, median pre-transplant total testosterone serum levels were substantially (20-fold) lower than in males. Importantly, no association of testosterone (per decrease of 10 ng/dL) with any end point was observed in the univariable models (*Online Supplementary Table S7*).

Pre-transplant testosterone and post-transplant outcome in the confirmation cohort

Patients', disease and transplant characteristics of the confirmation cohort are summarized in Table 1. As compared to the training cohort, significantly fewer patients were transplanted for late-stage disease and were allografted after myeloablative conditioning (Table 1). In the confirmation cohort, median pre-transplant total testosterone serum level was 469 ng/dL (IQR 309-580) (*Online Supplementary Figure S1B*). The estimated median follow up of survivors was 47 months (95%CI: 39-53). The cumulative incidence of acute GvHD grade 3-4 on day

Table 2. Univariable analysis of pre-transplant testosterone in the training (n=176) and in the confirmation (n=168) cohorts.

	Training cohort (n=176) Testosterone per 100 ng/dL decrease		Confirmation cohort (n=168) Testosterone per 100 ng/dL decrease	
	HR 95%CI	P	HR 95%CI	P
End point				
OS	1.16 (1.05-1.28)	0.002	1.12 (1.03-1.25)	0.027
PFS	1.18 (1.08-1.28)	0.0005	1.09 (0.99-1.20)	0.088
OS after acute GvHD	1.19 (1.01-1.41)	0.040	1.15 (1.03-1.30)	0.012
PFS after acute GvHD	1.22 (1.04-1.43)	0.015	1.12 (1.01-1.23)	0.037
	CHR 95%CI	**P**	**CHR 95%CI**	**P**
NRM	1.28 (1.09-1.52)	0.003	1.19 (1.03-1.30)	0.019
Relapse	1.12 (1.01-1.25)	0.033	1.01 (0.88-1.15)	0.916
NRM after acute GvHD	1.45 (1.11-1.89)	0.005	1.19 (1.03-1.39)	0.022
Relapse after acute GvHD	1.06 (0.86-1.32)	0.552	1.05 (0.91-1.22)	0.490
	HR 95%CI	**P**	**HR 95%CI**	**P**
Acute GvHD grade 3-4	0.92 (0.74-1.15)	0.448	1.23 (0.99-1.54)	0.055

AML: acute myeloid leukemia; CHR: cause-specific hazard ratio; CI: confidence interval; GvHD: graft-*versus*-host disease; HR: hazard ratio; OS: overall survival; PFS: progression-free survival; NRM: non-relapse mortality.

+100 post-transplant was 11.9% (95%CI: 7.0-16.8) and pre-transplant testosterone had no significant impact on risk of acute GvHD grade 3-4 (HR 1.23 95%CI: 0.99-1.54, *P*=0.055).

In univariable analysis, similar to the training cohort, lower pre-transplant testosterone (as continuous variable) showed significant associations with shorter OS, and worse OS and PFS after onset of acute GvHD. In this cohort of AML patients, this was due in both instances to a higher hazard of NRM rather than relapse (Table 2). These findings were further substantiated in the corresponding multivariable models of the confirmation cohort (Table 4).

Optimized pre-transplant testosterone cut-off value and illustration of outcome correlations

Continuous effects, in general, are less instructive and often hard to interpret, particularly with regard to interventions in a possible future clinical trial setting. Therefore, in order to facilitate further evaluation of pre-transplant testosterone status, an optimal cut-off determination with regard to post-transplant OS was conducted in the training cohort. The analysis revealed multiple cut-off points (*Online Supplementary Figure S3*). The value of 250 ng/dL (corresponding to 8.7 nM, significance level of *P*=0.018) was used as optimal cut-off point, since it agrees with the reports in the literature on testosterone and mortality[15,16] and exactly reflects the lower level of our center's reference range (250-1000 ng/dL).

When the optimized cut-off of 250 ng/dL was analyzed in multivariable models including age, CRP, BMI, KPS and comorbidities as confounding variables, the associations of lower testosterone status (<250 ng/dL) with worse OS and shorter PFS remained significant (HR 2.0 for both OS and PFS) in the training cohort (*Online Supplementary Table S8*).

The optimized cut-off of 250 ng/dL was next evaluated in the multivariable models of the confirmation cohort, showing that low pre-transplant testosterone status (<250 ng/dL) was correlated with worse survival both post transplant (HR approx. 2) and after onset of acute GvHD (HR approx. 2.3) (Table 5). Again, the association of low pre-transplant status with OS after alloSCT was mainly driven

by a nearly 2.7-fold increased hazard of NRM (Table 5).

For illustration purpose, patients of both cohorts were stratified according to high (≥250 ng/dL) and low (<250 ng/dL) pre-transplant testosterone status; the corresponding plots for the end points post-transplant and after onset of acute GvHD are given in Figure 1 and Figure 2, respectively.

Non-relapse causes of death

To further explore the association of pre-transplant testosterone with NRM, we made a detailed analysis of non-relapse causes of death in both cohorts. In the training cohort, a total of 35 non-relapse deaths occurred. These were caused by severe infections (including sepsis) in 20 (57%), acute GvHD (i.e. lethal complications of acute GvHD and/or its treatment) in 13 (37%), and cardiovascular events in two (6%) patients. In the confirmation cohort, a total of 47 non-relapse deaths occurred. Again, the most common non-relapse cause of death was infection/sepsis in 32 (68%) followed by lethal acute GvHD in nine (19%), and cardiovascular events in six (13%) patients, respectively. Notably, as compared to patients not succumbing to NRM, in both cohorts, serum levels of pre-transplant testosterone tended to be lower in patients who died of lethal complications of acute GvHD (Figure 3).

Correlation of pre-transplant testosterone with pre-transplant serum levels of suppressor of tumorigenicity-2

When both cohorts were combined, pre-transplant serum levels of soluble suppressor of tumorigenicity-2 (ST2) were available for a total of 218 patients. Pre-transplant testosterone levels were weakly negatively correlated to pre-transplant soluble ST2 levels (Spearman's rho: -0.13, *P*=0.048). However, when regarding the pre-transplant testosterone cut-off value of 250 ng/dL, median pre-transplant ST2 was significantly higher in patients with low pre-transplant testosterone status (<250 ng/dL) as compared to patients of the high (≥250 ng/dL) pre-transplant testosterone group (573 pg/mL, IQR 255-2082 *vs.* 350 pg/mL, IQR 212-587, *P*=0.005, respectively) (*Online Supplementary Figure S4*).

Discussion

The present study is, to the best of our knowledge, the first to evaluate testosterone status in the context of outcome and mortality after alloSCT. So far, studies in the alloSCT setting have focused on the impact of chronic GvHD and its treatment on the androgen status,[17] or have investigated the relationship between sex hormone levels and overall gonadal function, mainly in the context of late complications after alloSCT.[18,19] Evaluation of testosterone status in the context of outcome and mortality after alloSCT, therefore, appears to meet an unmet need, given that sex hormones are involved in the regulation of a wide range of physiological processes that affect metabolism, tissue and cardiovascular homeostasis, inflammatory and immune responses,[20] and thus may interfere with alloSCT outcome.
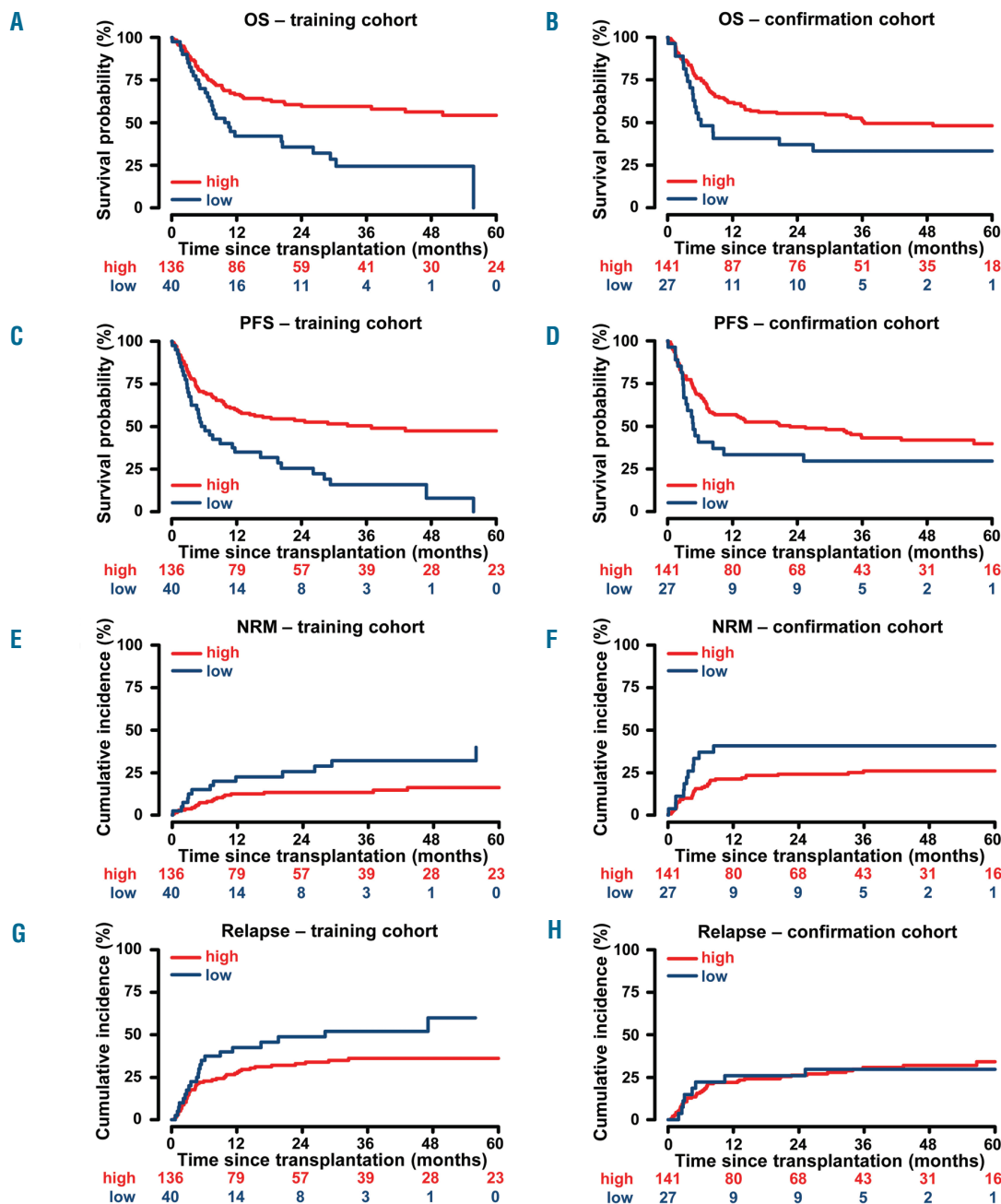

Figure 1. Impact of pre-transplant testosterone status on outcome measures after allogeneic stem cell transplantation (alloSCT) in the training and in the confirmation cohorts. The cut-off point of 250 ng/dL was derived from the Heidelberg training cohort of men allografted for acute myeloid leukemia (AML) (n=176). It was used to stratify patients in low (<250 ng/dL) and high (≥250 ng/dL) pre-transplant testosterone groups, and then applied to an independent cohort of male AML patients who underwent alloSCT in the Essen center (confirmation cohort, n=168) (see *Online Supplementary Figure S1*). (A and C) Distribution of overall survival (OS) and progression-free survival (PFS) since transplant in the training cohort. (B and D) Distribution of OS and PFS since transplant in the confirmation cohort. (E and G) Incidence curves of non-relapse mortality (NRM) and relapse after alloSCT in the training cohort. (F and H) Incidence curves of NRM and relapse after alloSCT in the confirmation cohort. Curves of patients with low (<250 ng/dL) and high (≥250 ng/dL) pre-transplant testosterone status are shown in blue and in red, respectively.

In our study, impact of pre-transplant testosterone on outcome was observed only in the subgroup of male patients allografted for AML. Lower levels of pre-transplant testosterone were associated with worse OS and PFS post-transplant, largely due to a significantly increased risk of NRM. The reason for this disease-specific association is unclear.

In general, testicular damage and dysfunction, which result in low testosterone status, appear to be rather long-term sequelae of cytotoxic chemotherapy, and the clinically most significant toxicities are observed after regimens employing higher doses of alkylating agents.[21] In contrast, adult AML patients considered eligible for alloSCT are usually treated by combination therapy employing anthracyclines and antimetabolites in both first-line and salvage treatment approaches. As patients in advanced disease stage are likely to have received more aggressive therapy prior to transplant and to account for the more deleterious effect of treatment intensity, we have considered disease stage prior to alloSCT as confounding variable in all multivariable models. In both cohorts, the associations of pre-transplant testosterone with worse outcome were not confounded by disease stage prior to transplant.

One possible explanation for the observed disease-specific association may be related to the immunoregulatory cytokine network in human AML, including leukemia cell-derived angiopoietins and the interleukin-33/ST2 axis, and the likely cross-talk between leukemic and endothelial cells.[22-26] Notably, soluble ST2 and angiopoietin-2 were known to be associated with endothelial dysfunction and cardiovascular risk,[27,28] and in the context of alloSCT, both were highly correlated with therapy-refractoriness of acute GvHD and high overall mortality.[3,29] Given the inverse correlation of pre-transplant serum levels of testosterone with soluble ST2 observed in our patients, some speculation about endothelial involvement in the present study may appear justified.

Analysis of non-relapse causes of death revealed that pre-transplant testosterone levels tended to be lower in patients that later succumbed to acute GvHD and/or its treatment, and lower pre-transplant testosterone levels were also associated with shorter survival after onset of GvHD. In this regard, our findings fit into the concept of "endothelial vulnerability".[3,4] In the setting of alloSCT, this concept describes a condition/predisposition that manifests itself particularly after a severe challenge, such as conditioning therapy or GvHD. Since this "vulnerability" is, at least in part, a characteristic of the recipient's endothelial cell system, a corresponding increased risk of mortality may already be identified prior to transplantation by assessing endothelial biomarkers. Accordingly, low(er) pre-transplant testosterone, which represents a known determinant of endothelial dysfunction in men,[7] may therefore promote and/or enhance this "vulnerability", resulting in the observed increase in treatment-related mortality.

However, it should be noted that our study design is not able to definitely support (or refute) the hypothesis of endothelial involvement. Although the associations of lower pre-transplant testosterone levels with worse outcome in the training cohort continued to hold true when additional confounding variables that reflect comorbidities and the patients' nutritional and overall status were included in the multivariable models, the possibility that lower pre-transplant testosterone is primarily an expres-
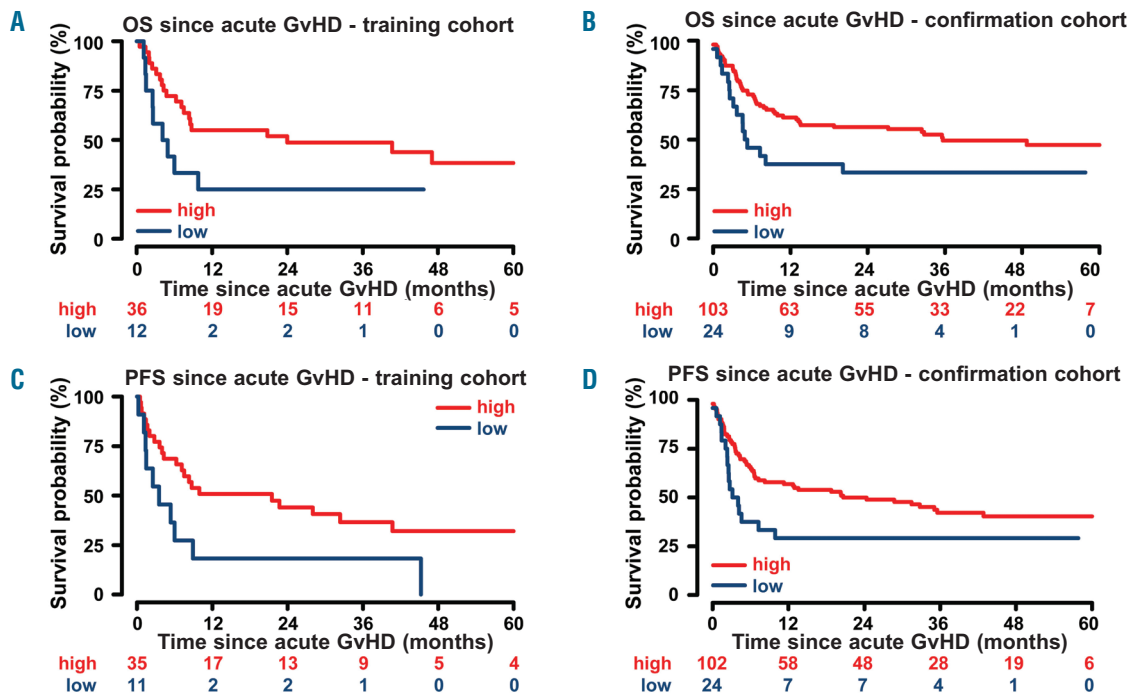
Figure 2. Impact of pre-transplant testosterone status on outcome measures after onset of acute *graft-versus*-host disease (GvHD) in the training and in the confirmation cohorts. (A and C) Distribution of overall survival (OS) and progression-free survival (PFS) since onset of acute GvHD in the training cohort. (B and D) Distribution of OS and PFS since onset of acute GvHD in the confirmation cohort. Curves of patients with low (<250 ng/dL) and high (≥250 ng/dL) pre-transplant testosterone status are shown in blue and in red, respectively.

sion of patients' general health status prior to alloSCT cannot be ruled out.

Currently, there is an ongoing "testosterone debate" in the field of cardiovascular medicine. Although the association between testosterone levels and cardiovascular mortality seems to be conclusive, prospective data on testosterone treatment are scarce, and so far no prospective controlled study has been able to show that treatment with testosterone or normalization of testosterone levels can reduce cardiovascular events.[30] On the contrary, results of a recently published prospective controlled study, which is one of seven co-ordinated National Institutes of Health (NIH)-supported trials of testosterone treatment in elderly men ("T Trials"),[31] indicate that testosterone may even increase cardiovascular risk, as reflected by increasing coronary artery plaque volume following testosterone treatment.[32] Thus, although of potential clinical value, any long-term health benefits of testosterone supplementation remain to be established.

As regards myeloid malignancies, testosterone among other sex steroids has been shown to exert cytostatic and cytotoxic effects on several myeloid leukemia cell lines *in vitro*.[33] However, although an early pilot study on AML patients using androgens as an adjunct in different first-line and maintenance treatment approaches showed an unexpectedly high rate of long-term survivors in a group of patients who achieved complete remission,[10] larger randomized studies failed to show beneficial effects on overall and disease-free survival.[11] Only recently, a prospective controlled trial in elderly patients with AML suggested that maintenance therapy with low-dose oral norethandrolone, a synthetic androgen with similar anabolic activity to testosterone, significantly improves overall, disease- and event-free survival,[12] and this has renewed interest in this treatment approach. However, in this study, the beneficial effects of androgen maintenance therapy were observed only in patients with low disease burden at diagnosis and only in the absence of relapse during the first year of treatment.[12] This might imply that androgen treatment has had some impact on disease-independent mortality. Interestingly, in the aforementioned trial, female patients were also treated, and the multivariable

Table 3. Multivariable analysis of the training cohort with the end points overall survival (OS), progression-free survival (PFS), non-relapse mortality (NRM), and relapse following allogeneic stem cell transplantation and OS and PFS after onset of acute graft-*versus*-host disease (GvHD) (complete case analysis).

	OS (n=175)		PFS (n=175)		NRM* (n=175)		Relapse* (n=175)		OS after acute GvHD* (n=48)		PFS after acute GvHD* (n=45)	
	HR 95% CI	*P*	HR 95% CI	*P*	CHR 95% CI	*P*	CHR 95% CI	*P*	HR 95% CI	*P*	HR 95% CI	*P*
Covariate												
Testosterone	1.11		1.11		1.25		1.06		1.14		1.12	
(per 100 ng/dL decrease)	(1.00-1.22)	0.045	(1.02-1.22)	0.022	(1.05-1.47)	0.013	(0.95-1.19)	0.277	(0.95-1.35)	0.152	(0.95-1.33)	0.171
Disease stage[†]												
Early	Ref		Ref		Ref		Ref		Ref		Ref	
Intermediate	0.92		0.94		0.62		1.14		3.65		3.35	
	(0.46-1.86)	0.822	(0.50-1.78)	0.857	(0.19-1.95)	0.411	(0.55-2.36)	0.720	(1.26-10.61)	0.017	(1.13-9.88)	0.029
Late	1.87		1.74		2.11		2.17		2.98		4.59	
	(1.03-3.36)	0.038	(1.04-3.08)	0.036	(1.00-4.45)	0.050	(1.25-3.76)	0.006	(1.16-7.64)	0.023	(1.80-11.68)	0.001
Age (per 10-year increase)	1.26		1.28		1.13		1.28		1.11		1.13	
	(1.02-1.54)	0.029	(1.06-1.55)	0.010	(0.84-1.52)	0.415	(1.01-1.61)	0.040	(0.78-1.59)	0.564	(0.80-1.59)	0.498
Conditioning												
MAC	Ref		Ref		–	–	–	–	–	–	–	–
RIC	0.70 (0.41-1.21)	0.203	0.69 (0.41-1.15)	0.152								
Donor												
Related donor	Ref		Ref		–	–	–	–	–	–	–	–
Unrelated Donor	1.36		1.35									
	(0.82-2.26)	0.233	(0.84-2.15)	0.214								
Recipient - donor sex match												
Matched	Ref		Ref		–	–	–	–	–	–	–	–
Male recipient /	0.77		0.89									
female donor	(0.46-1.31)	0.341	(0.55-1.43)	0.630								
Donor source												
PB	Ref		Ref		–	–	–	–	–	–	–	–
BM	1.60		1.24									
	(0.62-4.13)	0.333	(0.49-3.15)	0.652								

Number of events: OS, n=87; PFS, n=102; NRM, n=35; relapse, n=67; OS after acute GvHD, n=30; PFS after acute GvHD, n=32. *Slim model. [†]According to Gratwohl *et al.*[13] BM: bone marrow; CHR: cause-specific hazard ratio; CI: confidence interval; HR: hazard ratio; MAC: myeloablative conditioning; PB: peripheral blood; RIC: reduced intensity conditioning.

models revealed no impact of gender on survival. In our cohorts of male patients allografted for AML, pre-transplant testosterone did not correlate with post-transplant relapse risk, suggesting an only limited, if any, impact on disease control. Conversely, and similar to the androgen maintenance trial,[12] in our patients, the associations of testosterone with post-transplant survival were largely driven by increased NRM.

Besides its retrospective nature, several potential limita-

tions of our study need to be addressed. First, and as already stated above, we cannot definitely exclude the possibility that testosterone solely reflects the individual's health and nutritional status prior to alloSCT, and thus unknown confounders cannot be ruled out. Second, due to its observational character, our study does not provide evidence for causality between testosterone and post-transplant outcome, and therefore our results here need to be interpreted with caution. Further, measurements of
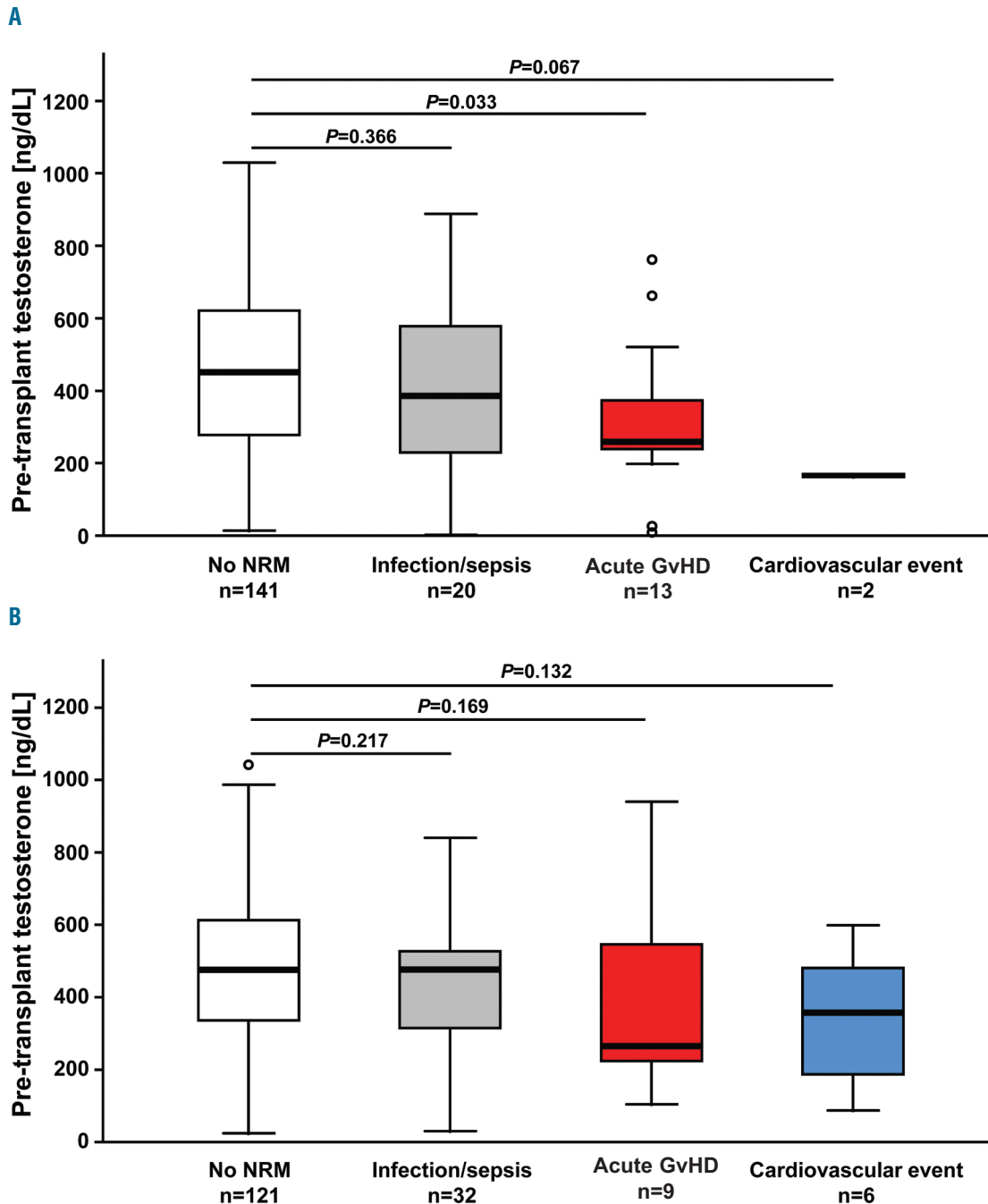

Figure 3. Comparison of pre-transplant testosterone serum levels according to different non-relapse causes of death in the training and in the confirmation cohorts. Non-relapse causes of death were grouped into three categories: severe infection/sepsis, death due to acute graft-*versus*-host disease (GvHD) (i.e. lethal complications of acute GvHD and/or its treatment), and cardiovascular events. In both the training (A) and the confirmation (B) cohorts, as compared to patients not succumbing to non-relapse mortality (NRM), serum levels of pre-transplant testosterone tended to be lower in patients who died of lethal complications of acute GvHD. Box plots are depicted. Number of patients/events for each group is indicated. *P*-values by Mann-Whitney U test.

Table 4. Multivariable analysis of the confirmation cohort with the end points overall survival (OS), progression-free survival (PFS), non-relapse mortality (NRM), and relapse following allogeneic stem cell transplantation and OS and PFS after onset of acute graft-*versus*-host disease (GvHD) (complete case analysis).

	OS (n=165)		PFS (n=165)		NRM* (n=165)		Relapse* (n=165)		OS after acute GvHD* (n=127)		PFS after acute GvHD* (n=126)	
	HR 95% CI	*P*	HR 95% CI	*P*	CHR 95% CI	*P*	CHR 95% CI	*P*	HR 95% CI	*P*	HR 95% CI	*P*
Co-variate												
Testosterone	1.15		1.10		1.23		1.02		1.19		1.15	
(per 100 ng/dL decrease)	(1.03-1.28)	0.012	(0.99-1.22)	0.062	(1.05-1.43)	0.008	(0.88-1.16)	0.818	(1.05-1.33)	0.004	(1.03-1.28)	0.011
Disease stage†												
Early	Ref		Ref		Ref		Ref		Ref		Ref	
Intermediate	1.62		1.55		1.88		1.22		2.01		1.75	
	(0.93-2.83)	0.088	(0.92-2.62)	0.101	(0.87-4.09)	0.109	(0.61-2.45)	0.579	(1.09-3.74)	0.026	(0.98-3.10)	0.057
Late	2.48		2.43		3.30		1.98		3.21		2.95	
	(1.48-4.15)	<0.001	(1.48-3.97)	<0.001	(1.65-6.62)	<0.001	(1.03-3.78)	0.039	(1.78-5.80)	<0.001	(1.70-5.12)	<0.001
Age (per 10-year increase)	1.12		1.09		1.32		1.02		1.22		1.16	
	(0.90-1.37)	0.307	(0.89-1.33)	0.424	(1.03-1.71)	0.031	(0.82-1.26)	0.893	(0.99-1.50)	0.068	(0.96-1.40)	0.127
Conditioning												
MAC	Ref		Ref		–	–	–	–	–	–	–	–
RIC	1.81		1.65									
	(0.88-3.71)	0.10	(0.85-3.21)	0.137								
Donor												
Related donor	Ref		Ref		–	–	–	–	–	–	–	–
Unrelated Donor	1.22		0.93									
	(0.65-2.29)	0.528	(0.52-1.65)	0.806								
Recipient - donor sex match												
Matched	Ref		Ref		–	–	–	–	–	–	–	–
Male recipient /	1.63		1.15									
female donor	(0.95-2.81)	0.075	(0.68-1.97)	0.604								
Donor source												
PB	Ref		Ref		–	–	–	–	–	–	–	–
BM	1.29		1.75									
	(0.49-3.39)	0.605	(0.77-3.97)	0.182								

Number of events: OS, n=87; PFS, n=97; NRM, n=46; relapse, n=51; OS after acute GvHD, n=67; PFS after acute GvHD, n=75. *Slim model. †According to Gratwohl *et al.*[13] BM: bone marrow; CHR: cause-specific hazard ratio; CI: confidence interval; HR: hazard ratio; MAC: myeloablative conditioning; PB: peripheral blood; RIC: reduced intensity conditioning.

total testosterone levels were based on a single serum sample. However, it should be noted that single-point measurements of total testosterone and androgens were demonstrated to be a reliable indication of the long-term hormonal status of men.[34] Certainly, further prospective, and preferably interventional, studies are needed to confirm our findings.

As regards intervention, the "optimal" cut-off value for definition of "low testosterone" needs to be discussed. Most studies on cardiovascular health and cardiovascular risk assessment applied values between 100-350 ng/dL (corresponding to 3.5-12.1 nM), as indicated by a recent systematic review.[35] In the prospective "T trials", testosterone levels <275 ng/dL (approx. 9.5 nM) were used as indications for intervention.[31] However, men undergoing alloSCT represent a distinct patient cohort. And although the available evidence indicates that testosterone replacement therapy is largely considered to be safe in most men, some controversies remain, and the inherent risk of adverse effects, particularly in selected high-risk popula-

tions, should not be ignored.[36] In our study, median pre-transplant testosterone levels in all male cohorts were above the aforementioned range. It should further be noted that, since data on clinical signs of hypogonadism cannot be ascertained retrospectively, patients of the low pre-transplant testosterone group (<250 ng/dL) in our study may not necessary be androgen deficient. In the present study, an optimized cut-off point of 250 ng/dL was derived and was shown to be associated with a 2-fold risk of death post transplant and after onset of acute GvHD in men allografted for AML. This cut-off value of 250 ng/dL is consistent with one definition of "low testosterone"[35] and was shown to be associated with mortality also in non-transplant settings,[15,16] and may thus be applied in a pilot clinical trial. Certainly, any future prospective study should also assess clinical signs of androgen deficiency, including additional biochemical testing.

In summary, our study suggests that pre-transplant testosterone has an impact on NRM and thus may be a determinant of outcome in male patients allografted for

Table 5. Multivariable analysis of the confirmation cohort with optimized pre-transplant testosterone cut-off value (complete case analysis).

Co-variate	OS (n=165)		PFS (n=165)		NRM (n=165)		Relapse (n=165)		OS after acute GvHD* (n=127)		PFS after acute GvHD* (n=126)	
	HR 95% CI	P	HR 95% CI	P	CHR 95% CI	P	CHR 95% CI	P	HR 95% CI	P	HR 95% CI	P
Testosterone												
≥250 ng/dL	Ref		Ref		Ref		Ref		Ref		Ref	
<250 ng/dL	1.95		1.81		2.68		1.28		2.29		2.29	
	(1.11-3.43)	0.021	(1.05-3.12)	0.033	(1.25-5.74)	0.011	(0.57-2.84)	0.551	(1.27-4.12)	0.006	(1.29-4.10)	0.005
Disease stage[†]												
Early	Ref		Ref		Ref		Ref		Ref		Ref	
Intermediate	1.67		1.62		2.04		1.35		2.12		1.90	
	(0.96-2.93)	0.072	(0.95-2.75)	0.076	(0.92-4.51)	0.079	(0.66-2.77)	0.417	(1.13-3.96)	0.019	(1.05-3.42)	0.033
Late	2.53		2.46		3.09		2.04		3.27		3.09	
	(1.51-4.23)	<0.001	(1.51-4.00)	<0.001	(1.51-6.33)	0.002	(1.03-4.05)	0.042	(1.80-5.93)	<0.001	(1.76-5.40)	<0.001
Age (per 10-year increase)	1.12		1.10		1.28		0.98		1.23		1.17	
	(0.91-1.39)	0.280	(0.90-1.35)	0.353	(0.93-1.64)	0.121	(0.68-1.28)	0.873	(0.99-1.53)	0.065	(0.96-1.43)	0.117
Conditioning												
MAC	Ref		Ref		Ref		Ref		–		–	
RIC	1.79		1.63		1.62		1.69					
	(0.88-3.67)	0.110	(0.84-3.15)	0.146	(0.56-4.64)	0.370	(0.71-4.01)	0.233				
Donor												
Related donor	Ref		Ref		Ref		Ref		–		–	
Unrelated Donor	1.19		0.91		1.25		0.72					
	(0.64-2.22)	0.579	(0.52-1.61)	0.753	(0.50-3.13)	0.634	(0.35-1.49)	0.375				
Recipient - donor sex match												
Matched	Ref		Ref		Ref		Ref		–		–	
Male recipient / female donor	1.57		1.13		1.45		0.93					
	(0.93-2.65)	0.094	(0.67-1.91)	0.643	(0.70-3.00)	0.316	(0.44-1.98)	0.854				
Donor source												
PB	Ref		Ref		Ref		Ref		–		–	
BM	1.42		1.92		1.59		2.01					
	(0.53-3.78)	0.485	(0.83-4.42)	0.125	(0.35-7.30)	0.551	(0.74-5.49)	0.173				

Number of events: OS, n=87; PFS, n=97; NRM, n=46, relapse, n=51; OS after acute GvHD, n=67; PFS after acute GvHD, n=75. *Slim model. [†]According to Gratwohl *et al.*[13] Number of events: OS, n=87; PFS, n=97; NRM, n=46; relapse, n=51; OS after acute GvHD, n=67; PFS after acute GvHD, n=75. *Slim model. [†]According to Gratwohl *et al.*[13] PFS: progression-free survival; BM: bone marrow; CHR: cause-specific hazard ratio; CI: confidence interval; GvHD: graft-*versus*-host disease; HR: hazard ratio; MAC: myeloablative conditioning; PB: peripheral blood; RIC: reduced intensity conditioning; OS: overall survival; NRM: non-relapse mortality.

AML. Considering recent successful post-remission androgen maintenance treatment approaches in AML,[12] and the fact that an individual's testosterone status is modifiable, our results may provide a rationale for the design of interventional clinical studies evaluating testosterone/androgen status and supplementation in patients undergoing alloSCT.

References

1. Tichelli A, Bucher C, Rovó A, et al. Premature cardiovascular disease after allogeneic hematopoietic stem-cell transplantation. Blood. 2007;110(9):3463-3671.

2. Cooke KR, Jannin A, Ho V. The contribution of endothelial activation and injury to end-organ toxicity following allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2008;14(1 Suppl 1):23-32.

3. Luft T, Dietrich S, Falk C, et al. Steroid-refractory GvHD: T-cell attack within a vulnerable endothelial system. Blood. 2011;118(6):1685-1692.

4. Radujkovic A, Dai H, Kordelas L, et al.

Asymmetric dimethylarginine serum levels are associated with early mortality after allogeneic stem cell transplantation. Haematologica. 2019;104(4):827-834.

5. Lindås R, Tvedt TH, Hatfield KJ, Reikvam H, Bruserud O. Preconditioning serum levels of endothelial cell-derived molecules and the risk of posttransplant complications in patients treated with allogeneic stem cell transplantation. J Transplant. 2014;2014:404096.

6. Tatekawa S, Kohno A, Ozeki K, et al. A Novel Diagnostic and Prognostic Biomarker Panel for Endothelial Cell Damage-Related Complications in Allogeneic Transplantation. Biol Blood Marrow Transplant. 2016;22(9):1573-1581.

7. Akishita M, Hashimoto M, Ohike Y, et al. Low testosterone level is an independent determinant of endothelial dysfunction in men. Hypertens Res. 2007;30(11):1029-1034.

8. Empen K, Lorbeer R, Dörr M, et al. Association of testosterone levels with endothelial function in men: results from a population-based study. Arterioscler Thromb Vasc Biol. 2012;32(2):481-486.

9. Araujo AB, Dixon JM, Suarez EA, Murad MH, Guey LT, Wittert GA. Clinical review: Endogenous testosterone and mortality in men: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2011;96(10):3007-3019.

10. Hollard D, Sotto JJ, Berthier R, Leger J, Michallet M. High rate of long-term survivals in AML treated by chemotherapy and androgenotherapy: a pilot study. Cancer. 1980;45(7):1540-1548.

11. Hayat M, Jehn U, Willemze R, et al. A randomized comparison of maintenance treatment with androgens, immunotherapy, and chemotherapy in adult acute myelogenous leukemia. A Leukemia-Lymphoma Group Trial of the EORTC. Cancer. 1986;58(3):617-623.

12. Pigneux A, Béné MC, Guardiola P, et al. Addition of Androgens Improves Survival in Elderly Patients With Acute Myeloid Leukemia: A GOELAMS Study. J Clin Oncol. 2017;35(4):387-393.

13. Gratwohl A, Stern M, Brand R, et al. Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. Cancer. 2009;115(20):4715-4726.

14. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106(8):2912-2919.

15. Shores MM, Matsumoto AM, Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. Arch Intern Med. 2006;166(15):1660-1605.

16. Haring R, Völzke H, Steveling A, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J. 2010;31(12):1494-1501.

17. Björk Y, Smith Knutsson E, Ankarberg-Lindgren C, et al. Androgens in women after allogeneic hematopoietic cell transplantation: impact of chronic GvHD and glucocorticoid therapy. Bone Marrow Transplant. 2017;52(3):431-437.

18. Grigg AP, McLachlan R, Zaja J, Szer J. Reproductive status in long-term bone marrow transplant survivors receiving busulfan-cyclophosphamide (120 mg/kg). Bone Marrow Transplant. 2000;26(10):1089-1095.

19. Tauchmanovà L, Selleri C, De Rosa G, et al. Gonadal status in reproductive age women after haematopoietic stem cell transplantation for haematological malignancies. Hum Reprod. 2003;18(7):1410-1416.

20. dos Santos RL, da Silva FB, Ribeiro RF Jr, Stefanon I. Sex hormones in the cardiovascular system. Horm Mol Biol Clin Investig. 2014;18(2):89-103.

21. Howell SJ, Shalet SM. Testicular function following chemotherapy. Hum Reprod Update. 2001;7(4):363-369.

22. Zhang J, Ye J, Ma D, et al. Cross-talk between leukemic and endothelial cells promotes angiogenesis by VEGF activation of the Notch/Dll4 pathway. Carcinogenesis. 2013;34(3):667-677.

23. Cogle CR, Goldman DC, Madlambayan GJ, et al. Functional integration of acute myeloid leukemia into the vascular niche. Leukemia. 2014;28(10):1978-1987.

24. Hatfield K, Øyan AM, Ersvaer E, et al. Primary human acute myeloid leukaemia cells increase the proliferation of microvascular endothelial cells through the release of soluble mediators. Br J Haematol. 2009; 144(1):53-68.

25. Reikvam H, Hatfield KJ, Fredly H, Nepstad I, Mosevoll KA, Bruserud Ø. The angioregulatory cytokine network in human acute myeloid leukemia - from leukemogenesis via remission induction to stem cell transplantation. Eur Cytokine Netw. 2012; 23(4):140-153.

26. Afferni C, Buccione C, Andreone S, et al. The Pleiotropic Immunomodulatory Functions of IL-33 and Its Implications in Tumor Immunity. Front Immunol. 2018; 9:2601.

27. Andersson C, Enserro D, Sullivan L, et al. Relations of circulating GDF-15, soluble ST2, and troponin-I concentrations with vascular function in the community: The Framingham Heart Study. Atherosclerosis. 2016;248:245-251.

28. Patel JV, Lim HS, Varughese GI, Hughes EA, Lip GY. Angiopoietin-2 levels as a biomarker of cardiovascular risk in patients with hypertension. Ann Med. 2008;40(3):215-222.

29. Vander Lugt MT, Braun TM, Hanash S, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. N Engl J Med. 2013;369(6):529-539.

30. Gencer B, Mach F. Testosterone: a hormone preventing cardiovascular disease or a therapy increasing cardiovascular events? Eur Heart J. 2016;37(48):3569-3575.

31. Snyder PJ, Ellenberg SS, Cunningham GR, et al. The Testosterone Trials: Seven coordinated trials of testosterone treatment in elderly men. Clin Trials. 2014;11(3):362-375.

32. Budoff MJ, Ellenberg SS, Lewis CE, et al. Testosterone Treatment and Coronary Artery Plaque Volume in Older Men With Low Testosterone. JAMA. 2017;317(7):708-716.

33. Blagosklonny MV, Neckers LM. Cytostatic and cytotoxic activity of sex steroids against human leukemia cell lines. Cancer Lett. 1994;76(2-3):81-86.

34. Vermeulen A, Verdonck G. Representativeness of a single point plasma testosterone level for the long term hormonal milieu in men. J Clin Endocrinol Metab. 1992;74(4):939-942.

35. Huo S, Scialli AR, McGarvey S, et al. Treatment of Men for "Low Testosterone": A Systematic Review. PLoS One. 2016;11(9):e0162480.

36. Grech A, Breck J, Heidelbaugh J. Adverse effects of testosterone replacement therapy: an update on the evidence and controversy. Ther Adv Drug Saf. 2014;5(5):190-200.

37. Bacigalupo A, Ballen K, Rizzo D, et al. Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant. 2009;15(12):1628-1633.

38. Bornhäuser M, Kienast J, Trenschel R, et al. Reduced-intensity conditioning versus standard conditioning before allogeneic haemopoietic cell transplantation in patients with acute myeloid leukaemia in first complete remission: a prospective, open-label randomised phase 3 trial. Lancet Oncol. 2012;13(10):1035-1044.