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The Prognostic Value of YKL-40 Concentrations in Nonmyeloablative Conditioning Allogeneic Hematopoietic Cell Transplantation

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Increased plasma concentrations of YKL-40, also called chitinase-3-like-1 protein (CHI3L1), have been correlated with disease severity in a variety of malignant and inflammatory diseases. The objective of the current study was to assess pretransplant recipient and donor *CHI3L1* polymorphisms and plasma YKL-40 concentrations as prognostic biomarkers in a cohort of 149 patients treated with hematopoietic cell transplantation (HCT) after nonmyeloablative conditioning for hematologic malignancies. Recipients with pretransplant YKL-40 concentrations above the age-adjusted 95th percentile (high) had higher relapse-related mortality (33% versus 18%, $P = .04$; hazard ratio (HR) = 4.41, $P = .01$), lower progression-free survival (38% versus 64%, $P < .01$; HR = 2.84, $P = .01$), and overall survival (42% versus 69%, $P = .01$; HR = 3.09, $P = .01$). Recipients transplanted with donors with high YKL-40 concentrations had an increased probability and risk of grade 2-4 acute graft-versus-host disease (aGVHD) (93% versus 62%, $P < .01$; HR = 2.25, $P = .02$). *CHI3L1* polymorphisms were associated with plasma YKL-40 concentrations, but not with clinical outcomes. In conclusion, our study suggests that plasma YKL-40 could function as a biomarker for relapse risk and treatment-related toxicity, and possibly as a tool complementing clinical risk scores such as the HCT comorbidity index.

Biol Blood Marrow Transplant 17: 1299-1307 (2011)

KEY WORDS: YKL-40, Ch13L1, Hematopoietic cell transplantation, Nonmyeloblative conditioning, Graft-versus-host disease, Prognosis

INTRODUCTION

With the introduction of reduced-intensity conditioning (RIC), allogeneic hematopoietic cell transplantation (HCT) has become a curative treatment option for a variety of malignant hematologic diseases in older and medically infirm patients. However, complications such as graft-versus-host disease (GVHD), infections, and relapse are still major causes of morbidity and mortality. In the older and medically infirm patient population that is only eligible for RIC, prognostic factors such as the Kahl score [1] or the HCT comorbidity index [2,3] have proven useful in predicting the risk of relapse and treatment-related mortality (TRM). However, to appropriately balance the likelihood of disease control against the risk of debilitating complications, still more accurate pretransplant tools are needed to predict outcome. Inflammatory biomarkers serve as prognostic tools in a variety of disease settings, and in allogeneic HCT it has been shown that rising or increased concentrations of markers such as C reactive protein (CRP), interleukin (IL)-6, and tumor necrosis factor (TNF)-α in the posttransplantation period are predictive of treatment-related toxicity [4-8]. The predictive value of inflammatory biomarkers in the pretransplant period is less well investigated. However, increased pretransplant concentrations of CRP have been shown to associate with increased incidence of infectious complications, acute GVHD (aGVHD), and TRM [9,10].

YKL-40, also called chitinase-3-like-1 protein (CHI3L1), is a phylogenetically conserved heparin-, chitin-, and collagen-binding member of the family of mammalian chitinase-like proteins. It is regarded

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Financial disclosure: See Acknowledgments on page 1306.

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Received September 10, 2010; accepted January 4, 2011

1083-8791/$36.00
doi:10.1016/j.bbmt.2011.01.008

as an acute-phase reactant, and increased concentrations of YKL-40 have been observed in a variety of inflammatory diseases of infectious and noninfectious etiology (reviewed by Johansen et al. [11]). In cancer, elevated YKL-40 concentrations have been associated with advanced disease [12], and in a large prospective study of a general Danish population cohort, high YKL-40 concentrations were predictive of the development of gastrointestinal cancer in subjects without known cancer [13]. YKL-40 is secreted by cancer cells [14], vascular smooth muscle cells [15], connective tissue cells [16,17], neutrophile granulocytes, and macrophages [18-20], and although its exact biological function is unknown, it is involved in of cell proliferation and differentiation [21], angiogenesis [22], matrix remodeling, and inflammation [14]. Secretion of YKL-40 is induced by interferon (INF)-γ [23] and IL-6 (J.S.J., unpublished results), and it has been suggested that it is involved, as an opsonin, in activating innate immune responses [14,24]. In a murine knockout model, the YKL-40 analog Brp-39 has been shown to be of importance in establishing Th2 polarized immune responses and augmenting the accumulation of macrophages, dendritic cells, and T cells by inhibiting apoptosis [25]. Compared to another acute-phase reactant such as CRP, YKL-40 has a different temporospatial secretion profile and therefore only a weak correlation to CRP [26-29]. Furthermore, YKL-40 may reflect disease activity more accurately, as it originates from cancer cells and inflammatory cells directly involved in the pathological process, whereas CRP is secreted by hepatocytes as a response to IL-6 [14].

Because of the ability of YKL-40 to predict survival and disease severity across a large variety of cancers and inflammatory disorders, and because of its involvement in processes central to oncogenesis and inflammation, we hypothesized that pretransplant YKL-40 concentration or *CHI3L1* genotype could be a valuable prognostic marker in the setting of nonmyeloablative conditioned allogeneic HCT, and that both could capture the inherent risk of relapse associated with cancer and comorbid conditions.

METHODS

Study Cohorts

The study cohort consisted of 149 consecutive recipients treated with allogeneic HCT following nonmyeloablative conditioning for hematologic malignancies (acute myeloid leukemia/myelodysplastic syndrome [AML/MDS], n = 54 [36%]; chronic myeloid leukemia [CML], n = 3 [2%]; chronic lymphocytic leukemia (CLL), n = 21 [14%]; non-Hodgkin lymphoma [NHL], n = 16 [11%]; and multiple myeloma [MM], n = 16 [11%]) between March 2000 and July 2007, at the bone marrow transplantation unit at Rigshospitalet, Copenhagen, Denmark. Sixty-seven (45%) of the recipients were in complete remission (CR), and 82 (55%) were not in complete remission. When stratified according to risk of relapse after allogeneic HCT [1], 32 (21%) had low risk and 72 (48%) and 45 (30%) had standard and high risk, respectively. For related donors (n = 86 [58%]), donor selection was based on serologic typing for HLA-A and -B and on molecular typing for HLA-C, -DRB1, and DQB1. For unrelated donors (n = 63 [42%]), donor selection was based on molecular typing for HLA-A, -B, -C, -DRB1, and -DQB1. When available, HLA-identical siblings were preferred to matched unrelated donors (in 11 unrelated donors, a single allele mismatch was present), and cytomegalovirus serostatus (cytomegalovirus [CMV]-negative recipient and donor, n = 31 [21%]; other combinations, n = 118 [79%]) and gender mismatch (male recipient/female donor, n = 34 [23%]); other combinations n = 115 (77%) were taken into account when possible. All recipients received transplants of peripheral blood stem cells (PBSC) after conditioning with fludarabine 30 mg/m^2 for 3 days and 2 Gy of total-body irradiation (TBI), except for 2 recipients who were conditioned with 2-Gy TBI only. Donor treatment, conditioning regimen, and supportive care have been described previously [30]. Acute GVHD and chronic GVHD (cGVHD) were diagnosed according to standard criteria [31].

Blood samples for plasma measurement and DNA extraction were obtained from recipients during pretransplantation workup, which was scheduled approximately 2 to 3 weeks prior to conditioning. Recipients were not treated with chemotherapy in the time period between pretransplant workup and conditioning. However, some recipients could be recovering from chemotherapy-induced nadirs. From related donors and domestic unrelated donors, samples were obtained during preleukapheresis workup. Plasma samples were obtained before treatment with granulocyte colony-stimulating factor (G-CSF) was begun. For international donors, plasma samples were not available. DNA was available from all recipients and donors, and plasma was available from 112 (75%) recipients and 92 (62%) donors.

For the purpose of comparing the distribution of genotypes in the recipient and donor cohorts to the general population, a cohort of 100 Danish Caucasian healthy blood donors served as controls. DNA and plasma samples were available from all control subjects.

Informed consent was obtained from all recipients, donors, and controls, and the local ethics committee approved the study.

Genotyping

DNA from samples was extracted using the Promega Maxwell 16 blood DNA kit (Promega Corporation, Madison, WI). The control cohort was genotyped

for SNP1 (rs2153101), SNP2 (rs946263), SNP3 (rs4950929), and SNP4 (rs4950928), whereas recipient and donor cohorts were genotyped only for SNP1, -2, and -4. Polymerase chain reaction (PCR) amplification was performed as previously described [32] in reaction volumes of 12 μL or 24 μL, prior to genotyping by direct Sanger sequencing or pyrosequencing, respectively. Pyrosequencing was performed, according to the manufacturer's protocol, on a PSQ 96A pyrosequencing platform (Biotage AB, Uppsala, Sweden) following Streptavidin Sepharose High-Performance Beads (GE Healthcare Bio-Sciences Corp., Piscaway, NJ) and Vacuum Prep Tool (Biotage AB) purification. Direct Sanger sequencing was performed using ABI BigDye Terminator v1.1 and v3.0 Cycle Sequencing Kit on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Bedford, MA) using forward PCR primers and purification by EtOH precipitation as previously described [32]. All primers for PCR and pyrosequencing were produced by DNA Technology A/S, Risskov, Denmark, and are shown in Supplementary Table S1.

YKL-40 Plasma Analyses

Plasma was prepared from EDTA-anticoagulated blood within 8 hours of sampling and stored at −80°C until YKL-40 analysis. YKL-40 concentrations are stable at room temperature in EDTA-anticoagulated blood for up to 8 hours [33]. Plasma concentrations of YKL-40 were determined in duplicates using a commercial sandwich-type enzyme-linked immunosorbent assay (Quidel, Santa Clara, CA). The detection limit was 10 μg/L. The intra-assay coefficients of variations were 5% (at 40 μg/L), 4% (at 104 μg/L), and 4% (at 155 μg/L). The interassay coefficient of variation was <6%. Plasma YKL-40 was determined by ELISA in a large cohort of 8899 subjects, aged 20-95 years, from the Danish general population [13]. In this cohort, 3130 subjects (1837 women, 1293 men, aged 21-84 years) had no known disease at time of blood sampling in 1991-1994 and remained healthy and alive during the 16-year follow-up period. There was no sex difference in plasma YKL-40 levels. Because plasma YKL-40 increased exponentially in these healthy subjects with increasing age, the 95th percentile of plasma YKL-40 by age was determined. This age correction of plasma YKL-40 is important to account for, when plasma YKL-40 is evaluated in patients. The present study cohorts were therefore divided into subjects with normal plasma YKL-40 concentrations, that is, below the age-adjusted 95th percentile, and into subjects with high plasma YKL-40 concentrations, that is, equal or above the age-adjusted 95th percentile.

Statistics

The inferred haplotypes and linkage disequilibrium, expressed as the squared correlation coefficient (R^2) quantified between all pairs of biallelic loci, were estimated using SNPAlyze version 4.0 (Dynacom, Yokohoama, Japan). The Hardy-Weinberg equilibrium was analyzed using gene frequencies obtained by simple gene counting and the chi-square test with Yates' correction. Inferred haplotypes, linkage disequilibrium, and Hardy-Weinberg equilibrium were analyzed separately for controls, recipients, and donors. Fisher's exact test and the Kruskal-Wallis test were used where appropriate. Correlation analyses were performed using Spearman's rank correlation test. Overall survival (OS) was measured from the time of transplantation until death from any cause. Recipients still alive at the time of analysis were censored at last follow-up date. Progression-free survival (PFS) was calculated from date of transplantation to date of first relapse or death. Recipients who were alive and in remission were censored at date of last follow-up. TRM was defined as death in complete remission or death related to transplantation where it was not possible to assess disease status before death. Relapse-related mortality (RRM) was defined as death during relapsed or progressive disease. Probability of OS and PFS were estimated by the Kaplan-Meier method, and comparisons were made with the log-rank test. In the calculation of cumulative incidences for RRM, TRM, and GVHD, death before relapse, death without relapse, death without GVHD, and retransplantation were handled as competing events, where appropriate in these analyses. Comparisons of cumulative incidences were performed using Gray's k-sample test. For the purpose of Cox regression, competing events were censored. All significant associations between outcome measures and CHI3L1 genotype and age-adjusted plasma YKL-40 concentrations were investigated in multivariate Cox regression models. The multivariate models were restricted to include the covariate of interest, recipient age, donor type (related donor versus unrelated), Kahl score [1] and GVHD, because of a relatively low number of events. The presence of grade 2-4 acute and extensive cGVHD were considered as 1 time varying covariate. All P values were 2-tailed, and $P < .05$ was considered significant. All statistical analyses were performed using the R project for statistical computing (http://www.r-project.org/), except for receiver-operating characteristic (ROC) analyses (Prism 5 for Windows, La Jolla, CA).

RESULTS

Plasma YKL-40 Concentrations and Transplantation Outcome

The 3 cohorts differed significantly with regard to median age (control: 31 years [range: 19-63]; recipients: 52 years [range: 19-69]; donors: 44 years [range:

19-68]; $P < .001$) and median plasma YKL-40 concentrations, with the highest concentrations in recipients (mean 102.4 ng/mL; standard deviation [SD] = 128.9; range 9-967), intermediate in donors (mean 57.0 ng/mL; SD = 42.4; range: 14-240), and lowest in controls (mean 37.3 ng/mL; SD = 23.3; range: 20-145). The number of subjects with plasma YKL-40 concentrations above the age-adjusted 95th percentile was significantly higher in the recipient cohort (32 out of 112 [29%]) compared to the control (7 out of 100 [7%]) and donor cohorts (14 out of 92 [15%]) (recipient cohort versus control cohort: $P < .001$; recipient cohort versus donor cohort: $P = .03$), whereas no significant difference was observed between the control and donor cohorts ($P = .10$).

The median follow-up time in the transplantation cohort was 879 days (range: 30-3229) with 5 years OS, PFS, 1-year cumulative incidence of grade 2-4 aGVHD, and 3 years cumulative incidence of extensive cGVHD of 53%, 46%, 66%, and 45%, respectively. The median time from last course of chemotherapy to conditioning was 173 days (range: 19-3528 days).

No significant differences in number of recipients with pretransplant YKL-40 concentrations above the age-adjusted 95th percentile were observed between disease groups (data not shown). The fraction of recipients not in remission at the time of transplantation with age-adjusted plasma YKL-40 levels above the 95th percentile was nonsignificantly higher than in recipients in complete remission (25% versus 33%, $P = .41$). A similar nonsignificant trend was observed for the Kahl score, which is a prognostic tool that stratifies recipients into low, intermediate, and high risk of relapse according to the malignant disease [1]. The fraction of recipients with high age-adjusted plasma YKL-40 concentrations approximately doubled when moving from low-risk disease (16%) to intermediate (31%) and high risk (34%) ($P = .27$).

When stratified according to the age-adjusted plasma YKL-40 concentrations, recipients with concentrations equal to or above the 95th percentile had a significantly higher RRM (33% versus 18% at 3 years, $P = .04$), which translated into both a significantly lower PFS (38% versus 64% at 3 years, $P < .01$) and OS (42% versus 69% at 3 years, $P < .01$) (Figure 1A-1C). The impact of the age adjusted YKL-40 concentration was most pronounced within the first year posttransplant. No association between recipient pretransplant age adjusted YKL-40 concentrations and TRM (25% versus 13% at 3 years, $P = .12$) or GVHD (grade 2-4 aGVHD at 1 year: 66% versus 66%, $P = .99$; grade 3-4 aGVHD at 1 year: 16% versus 18%; extensive cGVHD at 2 years: 28% versus 37%) was observed. In Cox regression analyses, pretransplant recipient plasma YKL-40 concentrations above or equal to the 95th percentile were also significant risk factors for RRM, PFS, and OS in both the univariate model and the multivariate model adjusted for donor pretransplant age-adjusted YKL-40 concentration, recipient age, donor type, relapse risk (Kahl score), and presence of GVHD as a time-dependent variable (Tables 1,2).

Donor plasma YKL-40 concentrations were also associated with transplantation outcome. Grafts from donors with YKL-40 concentrations equal to or above the 95th percentile were associated with a significantly higher cumulative incidence of grade 2-4 aGVHD at 1 year (93% versus 62%, $P < .01$) and lower probability of OS (48% versus 64% at end of follow-up years, $P = .05$). In both the univariate and multivariate Cox regression analyses, elevated donor plasma YKL-40 concentrations were a significant risk factor for developing grade 2-4 aGVHD, whereas only a trend toward increased risk of lower OS was observed (Tables 1,2). No significant associations between plasma YKL-40 concentrations and any other outcome measures were observed (PFS 48% versus 68%, $P = .19$; RRM 21% versus 17%, $P = .39$; TRM 30% versus 14%, $P = .11$; grade 3-4 aGVHD 29% versus 17%, $P = .32$; extensive cGVHD 44% versus 33%, $P = .93$).

As an alternative to the age-adjusted 95th percentile, ROC curve analysis was also performed to determine

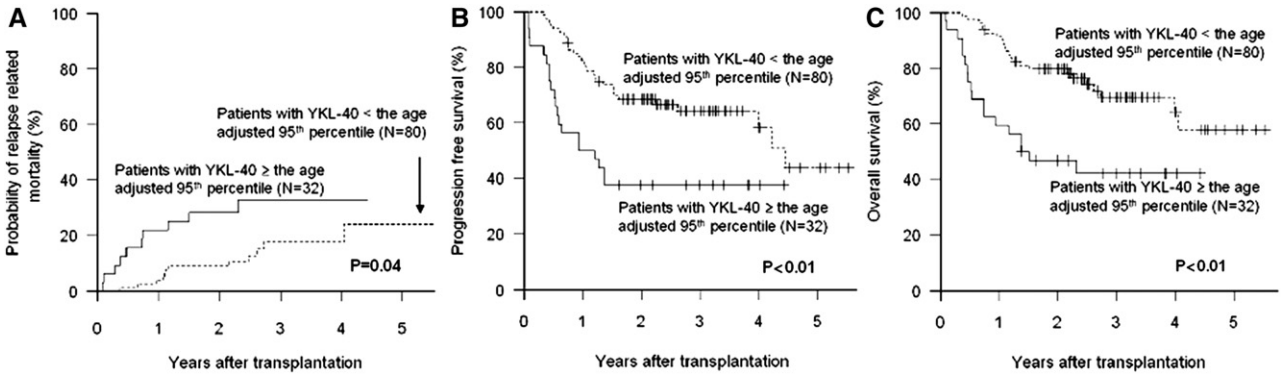

Figure 1. Transplantation outcome stratified according to pretransplantation age-adjusted 95th percentile recipient plasma YKL-40 concentrations. Cumulative incidence of relapse-related mortality (A) and Kaplan-Meier estimates of progression-free survival (B) and overall survival (C).

Table 1. Univariate Cox Regression Analyses According to the Age-Adjusted Plasma YKL-40 Concentrations

Covariate	Outcome	HR	95% CI	*P*
Recipient pretransplant YKL-40 concentrations	Relapse related mortality	2.95	1.27-6.88	.01*
	Progression free survival	2.54	1.43-4.50	<.01*
	Overall survival	2.79	1.50-5.19	<.01*
Donor YKL-40 concentrations	Grade 2-4 acute GVHD	2.35	1.25-4.38	.01*
	Overall survival	2.35	0.99-5.53	.05

HR indicates hazard ratio; CI, confidence interval.
HR was analyzed with recipient or donor plasma YKL-40 concentrations < the age-adjusted 95th percentile as reference group (n=80; donor n=78) compared to recipients or donors with plasma YKL-40 concentrations ≥ the age-adjusted 95th percentile (recipient n=32; donor n=14).
*$P < .05$.

a clinically relevant YKL-40 cutoff concentration. For RRM, PFS, and OS, the recipient pretransplant YKL-40 concentration ROC *P* values were .16, .28, and .03, with area under the curves (AUC) of 0.60, 0.56, and 0.62, respectively. For grade 2-4 aGVHD and donor YKL-40 concentrations, the ROC *P* value was .03, with an AUC of 0.64. Although 2 of the ROC analyses yielded significant ($P < .05$) results, all the AUCs where only slightly more than 0.5. The cutoff concentrations were between 81 and 83 ng/mL (except for grade 2-4 aGVHD, where it was 50 ng/mL) yielding specificities between 44% and 49% and sensitivities between 70% and 74%. No further effort was put into pursuing these cutoff concentrations, as their clinical relevancy and usefulness would be minimal.

Genetic Variation in *CHI3L1*

All polymorphisms adhered to the Hardy-Weinberg equilibrium ($P > .05$). Moderate to strong linkage disequilibrium was observed, with squared correlation coefficients between 0.81-0.94) for SNP1, SNP2, and SNP4 in all 3 cohorts. Because of perfect linkage disequilibrium between SNP1 and SNP3 in the control cohort, recipients and donors were not genotyped for SNP3. Haplotypes were only inferred for subjects where all SNPs had been successfully genotyped (control cohort, n = 100; recipients, n = 143; donors, n = 142) and the same 3 most common haplotypes, called H1-H3 (H1: T [SNP1], A [SNP2], T [SNP3], C [SNP4]; H2: H1: A [SNP1], G [SNP2], G [SNP3], G [SNP4]; and H3: H1: A [SNP1], A [SNP2], G [SNP3], G [SNP4]) accounted for more than 98% of the investigated chromosomes (control cohort: H1 = 85.5%, H2 = 14.5%, H3 = 2.0%; recipients: H1 = 77.3%, H2 = 30.3%, H3 = 1.1%; donors: H1 = 78.5%, H2 = 18.3%, H3 = 2.1%). There were no significant differences in the distribution of genotypes or haplotypes between the 3 cohorts ($P > 0.05$) (Supplementary Table S2).

Association between the *CHI3L1* Genotype and YKL-40 Concentration

YKL-40 concentrations were not compared between controls, recipients, and donors because of differences in demographics. However, within each study cohort, all the investigated polymorphisms displayed similar patterns of gene-dose–dependent associations with the plasma concentration of YKL-40 (Table 3). Subjects homozygous for the major alleles had the highest concentrations, whereas subjects heterozygous or homozygous for the minor alleles had either intermediate or low plasma YKL-40 (Table 3). The same relationship was evident between the inferred haplotypes and plasma YKL-40 concentrations,

Table 2. Multivariate Cox Regression Analyses

	Outcome											
	Relapse-Related Mortality			Progression-Free Survival			Overall Survival			Grade 2-4 Acute Graft-versus-Host Disease		
Covariate	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Recipient pretransplant YKL-40 concentrations, low vs high[$]	4.41	1.37-14.14	.01*	2.84	1.38-5.87	.01*	3.09	1.39-6.87	.01*	1.51	0.83-2.75	.18
Donor pretransplant YKL-40 concentrations, low vs high[$]	4.40	0.93-20.68	.06	2.07	0.83-4.19	.12	2.61	1.00-6.85	.05	2.25	1.16-4.37	.02*
Recipient age	1.00	0.96-1.05	.77	0.99	0.96-1.01	.31	1.00	0.97-1.04	.97	0.87	0.60-1.27	.34
Donor type, related vs unrelated	0.43	0.14-1.35	.15	1.09	0.55-2.18	.80	1.25	0.57-2.76	.57	1.26	0.73-2.15	.59
Kahl score [1]	2.70	1.04-7.04	.04*	1.12	0.70-1.78	.64	1.07	0.63-1.81	.80	0.98	0.96-1.01	.68
Graft-versus-host disease, absence vs presence[#]	0.18	0.05-0.70	.01*	0.44	0.20-0.99	.05	0.54	0.22-1.36	.19	NA	NA	NA

HR indicates hazard ratio; CI, confidence interval; NA, not applicable.
[$]Low is defined as YKL-40 plasma concentrations below the age-adjusted 95th percentile and high as YKL-40 plasma concentrations equal to or above the age-adjusted 95th percentile. [#] To adjust the analyses for the absence or presence of acute or chronic graft-versus-host disease, both grade 2-4 acute and extensive chronic graft-versus-host disease were entered as 1 time-dependent covariate.
*$P < .05$.

where subjects homozygous for the major (haplotype H1) or minor alleles (haplotype H2), had the highest or lowest concentrations, respectively, whereas subjects carrying any other combinations of minor and major alleles had intermediate concentrations. The apparent association between gene-dose and YKL-40 plasma concentrations, despite no adjustment for age having been performed, can be explained by the variation in age of the subjects within each cohort being small enough to negate the age effect on plasma concentrations.

Association between *CHI3L1* Genotype and Transplantation Outcome

Apart from SNP1 in the recipient cohort, all recipients homozygous or transplanted with donors homozygous for the minor alleles of SNP1, SNP2, SNP4, and the H2 haplotype, which were associated with low YKL-40 plasma concentrations, had OS and PFS of zero (Supplementary Table S3). These observations were significant for PFS in the recipients only (Table 4). No obvious convergence between recipient and donor genotypes or haplotypes was observed. Of the 6 recipients who were homozygous for the H2 haplotype, only 2 were transplanted with donors that also were H2 homozygous. There was no association between recipient or donor genotypes and RRM, TRM, aGVHD, or extensive cGVHD (Supplementary Table S3).

DISCUSSION

As it has become feasible to treat older and more medically infirm patients, because of the introduction of reduced-intensity regimens and better supportive care, there has been increased interest in developing tools that can predict outcome pretransplant. Although prognostic tools based on pretransplant demographics, such as the HCT comorbidity index [2,3], have proven very successful, less attention has been focused on the value of pretransplant analyses of inflammatory biomarkers. In the pretransplant setting, CRP has been associated with treatment-related toxicity [9,10], whereas no association has been observed for preconditioning concentrations of TNF-α receptor 1 [8], which is a surrogate marker for TNF-α. In this context, we would like to propose the novel inflammatory biomarker YKL-40. YKL-40 has previously been associated with outcome in a variety of different malignant and inflammatory diseases [11]. Because of its close correlation with age, it has been suggested that plasma YKL-40 concentrations should be defined as either low or high in relation to age-matched healthy controls [12]. Two findings in the current study could encourage using this approach. First, the correlation with age was evident as the median YKL-40 concentration was significantly higher in the older donor cohort compared to the control, with the high YKL-40 concentrations in the recipients probably being a consequence of contributions from both age and the malignant disease [11,12]. Second, to establish clinically relevant YKL-40 cutoff concentrations for different outcome measures by using ROC curves failed, yielding very low sensitivities with no clinical value. Thus, to avoid the introduction of bias to our analyses because of a large age span in both the donor and recipient cohorts, high YKL-40 concentrations were defined as being above the 95th percentile of a large healthy Danish cohort, as previously described (J.S.J., unpublished results).

In our study, we observed that recipients with pretransplant plasma YKL-40 concentrations above the age-adjusted 95th percentile had a significantly poorer

Table 3. Plasma YKL-40 Concentrations in Controls, Recipients, and Donors Stratified According to Genotype

Genotype		Controls			Recipients			Donors		
		n	Mean ± SD, ng/ml (range)	*P*	n	Mean ± SD, ng/ml (range)	*P*	n	Mean ± SD, ng/ml (range)	*P*
		100	37 ± 23 (20-145)		112	102 ± 129 (9-967)		92	57 ± 42 (14-240)	
SNP1	T/T	70	40 ± 23 (20-138)		70	118 ± 149 (21-967)		59	65 ± 48 (23-240)	
	A/T	26	33 ± 25 (20-145)		37	85 ± 82 (18-448)		25	43 ± 21 (20-81)	
	A/A	4	20 ± 0 (20-20)	<.001*	4	15 ± 4 (9-17)	<.001*	4	22 ± 6 (14-27)	.002*
SNP2	A/A	74	40 ± 22 (20-138)		73	118 ± 146 (21-967)		61	64 ± 48 (23-240)	
	A/G	23	31 ± 26 (20-145)		35	80 ± 84 (17-448)		23	44 ± 21 (20-81)	
	G/G	3	20 ± 0 (20-20)	<.001*	3	14 ± 4 (9-16)	<.001*	4	22 ± 6 (14-27)	.006*
SNP3	T/T	70	40 ± 23 (20-138)			NA			NA	
	G/T	26	33 ± 25 (20-145)			NA			NA	
	G/G	4	20 ± 0 (20-20)	<.001*		NA	NA		NA	NA
SNP4	C/C	70	40 ± 23 (20-138)		71	119 ± 148 (21-967)		57	67 ± 48 (23-240)	
	C/G	26	32 ± 25 (20-145)		36	82 ± 83 (18-448)		27	42 ± 20 (20-81)	
	G/G	4	20 ± 0 (20-20)	<.001*	4	15 ± 4 (9-17)	<.001*	4	22 ± 6 (14-27)	.001*
Haplotype	H1/H1	69	40 ± 23 (20-138)		69	119 ± 150 (21-967)		57	67 ± 48 (23-240)	
	HX/HX	28	32 ± 24 (20-145)		39	83 ± 81 (17-448)		27	42 ± 20 (20-81)	
	H2/H2	3	20 ± 0 (20-20)	<.001*	3	14 ± 4 (9-16)	.002*	4	22 ± 6 (14-27)	<.001*

SD indicates standard deviation; NA, not applicable; H1/H1, homozygous for haplotype H1; HX/HX, any other haplotype combination than H1/H1 or H2/H2; H2/H2, homozygous for haplotype H2.
*$P < .05$.

Table 4. Univariate 5-Year Kaplan-Meier Estimates of Overall Survival and Progression-Free Survival Stratified According to Recipient or Donor CHI3LI Genotype or Haplotype

		Recipient genotype					Donor genotype				
			OS		PFS			OS		PFS	
CHI3LI genotype		N	(%)	*P*	(%)	*P*	N	(%)	*P*	(%)	*P*
SNP1	T/T	89	49	.40	51	.02*	91	51	.17	44	.38
	A/T	48	60		51		47	59		50	
	A/A	8	30		0		6	0		0	
SNP2	A/A	93	49	.08	50	.02*	97	53	.19	47	.29
	A/G	47	64		45		41	56		45	
	G/G	6	0		0		6	0		0	
SNP4	C/C	88	48	.26	50	.01*	88	53	.25	45	.36
	C/G	48	60		45		48	60		50	
	G/G	7	0		0		6	0		0	
Haplotype	H1/H1	86	47	.17	50	.02*	87	52	.14	44	.33
	HX/HX	51	61		44		49	61		52	
	H2/H2	6	0		0		6	0		0	

OS indicates overall survival; PFS, progression-free survival; H1/H1, homozygous for haplotype H1; HX/HX, any other haplotype combination than H1/H1 or H2/H2; H2/H2, homozygous for haplotype H2.
*$P < .05$.

prognosis because of increased RRM, which translated into both poorer PFS and OS. The impact of pretransplant YKL-40 concentration was most pronounced within the first year posttransplant, where after survival curves tended to become parallel. In multivariate analyses, the relationship between YKL-40 and RRM was independent of type of hematologic cancer and of other known risk factors for relapse, such as recipient age, donor type, presence of GVHD, remission status at the time of transplantation, and relapse risk of the malignant disease (Kahl score [1]). Although no significant association between disease severity and YKL-40 concentrations was observed, the number of recipients with YKL-40 concentrations above the 95th percentile was higher in the subset not in remission at the time of transplantation and almost doubled when moving from recipients with low risk of relapse to intermediate or high risk [1]. Our observation of an association between relapse-related outcome measures and YKL-40 is therefore probably not a measure of the efficacy of the transplantation procedure, but more likely a reflection of the severity of the underlying disease. Several lines of evidence have recently linked YKL-40 to oncogenesis. In gene microarray analyses, increased expression of YKL-40 mRNA in different carcinoma tissues have been observed [34-36], and in in vitro tumor models YKL-40 has been shown to promote tumor progression and blood vessel formation through the mitogen-activated protein kinase, which is an important pathway in mitogenesis and cell survival [12,37,38]. In addition to YKL-40's role in angiogenesis, its proinflammatory properties may also play a part in oncogenesis, as the inflammation observed around malignancies regulates important aspects of cancer proliferation, differentiation, and invasion [39].

In the current study, an association between donor plasma YKL-40 concentrations above the age-adjusted 95th percentile and grade 2-4 aGVHD was observed. As YKL-40 has been implicated in activation of dendritic cells and inhibition of macrophage and T cell apoptosis [23,25], it is not implausible that increased YKL-40 production adopted from the donor could augment inflammatory reactions leading to GVHD. The finding is not supported by an increase in TRM, and no association between *CHI3L1* genotype and outcome was observed. Thus, even though the observation of the concentration of an inflammatory marker in the donor weeks before transplantation being predictive of outcome is intriguing, there is a risk of type I error, and further investigations are needed before it can be ascribed any clinical significance.

The populations were genotyped for 4 polymorphisms previously observed to be associated with plasma YKL-40 concentrations [40-42]. All polymorphisms adhered to the Hardy-Weinberg equilibrium in all 3 cohorts, and genotype frequencies were comparable to those listed in dbSNP [43]. No differences in genotype or haplotype distribution were observed between the recipient cohort and control and donor cohorts, indicating that the polymorphisms as such do not play a role in disease susceptibility. In agreement with previously published data, apparent gene-dose–dependent relationships between all the investigated polymorphisms and plasma YKL-40 concentrations were observed [40-42]. The lack of correlation between genotypes associated with high YKL-40 concentrations and outcome is in contrast to our observation of a relationship between high YKL-40 concentrations and outcome. However, as a total of 37 genetic variants have been identified throughout the entire *CHI3L1* gene locus [43], and with the numerous regulatory elements that control gene transcription and translation, it is unlikely that the expressional control of *CHI3L1* is limited to the polymorphisms investigated in the current study. An

unexpected finding in our study was that recipients, who either themselves were homozygous for any of the polymorphism minor alleles or were transplanted with donors that were homozygous, all died within the time of follow-up. As the minor alleles have been shown to be associated with lower YKL-40 concentrations, this finding is in contrast to the common consensus of low plasma YKL-40 concentrations being associated with superior disease outcomes [12,40]. Considering that only 6 to 8 recipients and donors were homozygous for the minor alleles, the association between genotype and PFS warrants further investigation in a larger cohort, as a possible type I error cannot be ruled out.

The biggest limitation of our study is its generalizability. Although, there is no biological reason for the YKL-40 effect to be limited to nonmyeloablative conditioning HCT only, other factors related to high-dose conditioning, T cell depletion, and the demographics of the study cohort, may impact the predictive power of YKL-40. Another limitation of the study is the size and heterogenic composition of the cohort, which limits the statistical power of subset analyses and increases the risk of false positive results.

In conclusion, this study is the first to investigate the association between plasma YKL-40 concentrations and *CHI3L1* genotype in allogeneic HCT. Although the current study is purely descriptive, our findings suggest a role for recipient pretransplant plasma YKL-40 concentrations as an independent risk factor associated with relapse-related outcome measures. The exact biological functions of YKL-40 are poorly understood, and whether the associations between plasma YKL-40 concentrations and disease outcome are causal or just bystander effects is still unknown. However, an inflammatory biomarker that defines relapse risk and possibly treatment-related toxicity could be a valuable tool complementing clinical risk scores such as the HCT comorbidity index. Further studies, both clinical, in independent cohorts, and experimental with analyses of the functional relevance of YKL-40, are needed to confirm these findings, explain their molecular background, and possibly establish YKL-40 as an independent prognostic factor.

ACKNOWLEDGMENTS

The authors thank Anne Bjørlig, Tonni Løve Hansen, and Dorthe Mogensen for excellent technical assistance, and the staff and recipients at the Department of Hematology at Rigshospitalet for invaluable help and support.

Financial disclosure: The work was supported by grants from the Danish Medical Research Council, the Novo Nordisk Research Foundation, the Benzon Foundation, the Danish Rheumatism Association, the Danish Cancer Society, Rigshospitalet, and the Lundbeck Foundation.

AUTHORSHIP STATEMENT

Contribution: A.M.M. performed experiments and drafted the manuscript. B.K. designed the research, analyzed results and drafted the manuscript. J.S.J. performed experiments and was involved in manuscript revision. T.N.M. collected data and was involved in manuscript revision. H.O.M was involved in design and practical aspects of the experimental work and manuscript revision. L.V. was involved in data analyses and manuscript revision. P.G. designed the research, analyzed results, and revised the manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at 10.1016/j.bbmt.2011.01.008

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