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Pentraxin-3 levels in graft-versus-host disease during allogeneic hematopoietic stem cell transplantation

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Acute and chronic graft-versus-host-diseases (aGVHD and cGVHD, respectively) are serious complications after hematopoietic stem cell transplantation (HSCT), impairing survival and quality of life. Because the underlying pathomechanism of GVHD is still poorly understood, we investigated the novel inflammatory marker Pentraxin-3 (PTX3) for its potential role in acute and chronic GVHD compared with autologous HSCT and healthy individuals. We collected plasma samples from patients undergoing autologous ($n = 12$) and allogeneic ($n = 28$) HSCT and from healthy individuals ($n = 15$) throughout 7 days before and up to 1 year after HSCT. PTX3 levels in patients with aGVHD were significantly higher (36.4 ± 23.6 ng/mL) than in allogeneic patients without aGVHD (10.4 ± 4.4 ng/mL, $p = 0.0001$), autologous controls (11.4 ± 6.7 ng/mL, $p = 0.001$), or healthy individuals (1.9 ± 0.6 ng/mL, $p < 0.001$). PTX3 levels in patients with cGVHD (13.6 ± 6.3 ng/mL) were significantly lower than in allogeneic patients without cGVHD (25.1 ± 13.8 ng/mL, $p = 0.04$) and higher than in autologous controls (8.9 ± 7.8 ng/mL, $p = 0.07$) and healthy individuals (1.9 ± 0.6 ng/mL, $p < 0.001$). Severity of aGVHD and cGVHD correlated with PTX3 levels. Rising PTX3 levels after HSCT indicated unfavorable outcome. We show that PTX3 levels correlate with the severity of aGVHD, cGVHD, and—with reservations—survival in patients undergoing allogeneic HSCT.

Acute and chronic graft-versus-host diseases (aGVHD and cGVHD, respectively) are serious complications after allogeneic hematopoietic stem cell transplantation (HSCT) [1,2]. Despite considerable progress in research, morbidity and mortality of GVHD remain high and the underlying pathophysiology is still not fully understood [3,4]. During aGVHD, a broad array of immune cells and cytokines such as tumor necrosis factor-α (TNFα) and interleukin-1β (IL-1β) are released [5]. This contributes to the impairment of organ functions (e.g. skin, liver, and gut) and injury mimicking autoimmune diseases [6]. Numerous currently utilized therapies in GVHD are likely to harbor a negative impact on immune reconstitution as well as on a desirable graft-versus-leukemia effect [7]. We set out to investigate a novel marker of inflammation for its potential role in aGVHD and cGVHD in individuals undergoing HSCT and focused on pentraxin-3 (PTX3) [8]. PTX3 is an acute-phase protein that belongs to the pentraxin superfamily. The widely used C-reactive protein (CRP) is the prototypic member of the short pentraxin subfamily and PTX3 is the prototypic member of the long pentraxin subfamily [9]. Whereas CRP increases slowly after infection, PTX3 levels increase within hours, making it a useful marker for the diagnosis of sepsis.

Recently, it has been shown that PTX3 is rapidly induced and secreted by diverse cell types in response to inflammatory cytokines and plays a critical role in both autoimmune and infectious diseases [9,10]. PTX3 was first found to be released by human mononuclear phagocytes [11], but it is also produced by dendritic cells, fibroblasts, renal epithelial cells, and endothelial cells in response to Toll-like receptor agonists, TNFα, IL-1β, and serum-amyloid A [12]. PTX3 regulates the clearance of apoptotic cells by binding to apoptotic membranes, regulating the maturation of dendritic cells and the secretion of soluble factors and thereby contributing to tissue

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damage after inflammation [13]. PTX3 was also found to interfere with the activation of the classical complement pathway by binding to C1q [14]. Studies in healthy individuals show that circulating PTX3 levels are low [15] but rapidly increase in patients depending on the severity of infection [16]. Moreover, PTX3 plasma levels positively correlate with primary graft dysfunction after lung transplantation and acute renal allograft rejection [17,18]. Furthermore, PTX3 was described as an early predictor of complications in hematologic patients with neutropenic fever [19].

Interestingly, no data on PTX3 in either aGVHD or cGVHD are available, although the aforementioned studies suggest the possibility that it might be involved in the pathophysiology of GVHD. We hypothesized that PTX3 may play a role in GVHD based on what is known of the role of PTX3 in inflammatory regulation and diseases and on previous studies published on the regulation of PTX3 release. For example, Holler et al. showed that increased TNFα serum levels can predict the course of aGVHD [20]. Many subsequent studies underlined the role of TNFα in aGVHD [21], leading eventually to clinical trials with TNFα antagonist. Barak et al. found that TNFα and IL-1β levels correlated with severity of established cGVHD [22]. Both TNFα and IL-1β are known to be strong inducers of PTX3 [23]. This certainly does not prove a biological role of PTX3 in GVHD, but taking into account that PTX3 mediates apoptotic cell clearance [24] and also suppresses P-selectin-mediated recruitment of leukocytes [25], this indicates that PTX might have a role in GVHD. PTX3 plasma levels also positively correlate with primary graft dysfunction after lung transplantation and acute renal allograft rejection and therefore can be of biological relevance in the pathophysiology of GVHD as well [17,18].

Because there is a critical need for a deeper understanding of the pathophysiologic mechanisms behind either form of GVHD, we performed a prospective pilot study on a small cohort of patients at a specialized tertiary care center with the aim of determining whether PTX3 levels are associated with GVHD status and possibly patient survival. This article provides the first description to date of PTX3 levels in both types of GVHD in patients undergoing HSCT.

Methods

The study was designed as a prospective cohort study including 40 patients who were followed longitudinally. A total of 41 consecutive patients were eligible for the study. Because one patient died 2 days after enrollment, she was excluded from further analysis, leaving 40 patients for further analysis. We collected plasma samples prospectively from these patients undergoing autologous ($n = 12$) and allogeneic ($n = 28$) HSCT, as well as from healthy individuals ($n = 15$). Inclusion criteria included age between 18 and 75 years, hematologic disease in remission, and scheduled autologous or allogeneic HSCT. Samples were obtained at the following time points: at admission (7 days before HSCT; T–1); on the day of HSCT (T0); during aplasia (absolute neutrophil count [ANC] $<$ 0.5 G/L; T+1); on the day of engraftment (ANC $>$ 0.5 G/L; T+2); and 1 month (T+3), 3–6 months (T+4), and 6–12 months after HSCT (T+5). PTX3 was analyzed by enzyme-linked immunosorbent assay (Human Pentraxin-3/TSG-14 Quantikine ELISA Kit, R&D Systems). All values were measured at the time point closest to maximum GVHD activity. Time-matched samples were used for comparison of GVHD and no-GVHD patients. Nonrelapse mortality (NRM) was defined as any death not related to the underlying malignancy. Relapse was defined as recurrence of malignancy after achievement of complete remission with NRM as a competing risk. Overall survival (OS) was calculated from day 0 of HSCT to the day of death from any cause or last follow-up. Patients were censored at the date of last contact.

The diagnosis and the severity of aGVHD and cGVHD were determined based on the modified Glucksberg and National Institutes of Health classification [26–28]. All patients received antiinfective prophylaxis as described previously [29]. The immunosuppressive therapy for aGVHD included steroids (2 mg/kg/day), calcineurin inhibitors, and, for salvage, extracorporeal photopheresis (ECP) in the majority of patients. Similarly, steroids in combination with calcineurin inhibitors and ECP were given for the treatment of cGVHD. The study was approved by the institutional review board of the Medical University of Vienna. All patients and all healthy volunteers declared written informed consent in accordance with the Declaration of Helsinki.

Statistical analysis

Statistical comparisons of plasma levels with patient cohorts were made using unpaired Student's t test. Fisher's exact test was used to examine the significance of the association between two variables. Statistical comparisons of plasma levels were done using one-way ANOVA for detection of overall differences, followed by post hoc analysis with correction for multiple testing. The log–rank test was used to compare survival curves. $p < 0.05$ was considered significant (Prism 6.0, GraphPad Software, USA). Univariate analysis was used for testing the influence of an independent variable on PTX3 levels. Multiple regression analysis was used to test the influence of several independent variables on PTX3 levels. Cumulative incidence of NRM was estimated using the Kaplan–Meier method with adjusting for relapse as a competing risk event and for relapse with NRM as a competing risk. Cumulative incidences of aGVHD and cGVHD were estimated considering relapse/progression and death as competing events and the data are presented as mean and standard deviation and median and interquartile range, respectively.

Results

Detailed patient characteristics are depicted in Table 1. PTX3 did not show any significantly different curve kinetic from admission until 12 months after HSCT between the allogeneic and the autologous groups. Maximum PTX3 levels were reached at T0 in the allogeneic group (34.2 ± 25.2 ng/mL) and at T+1 in the autologous group (32.3 ± 28.1 ng/mL). PTX3 levels were not significantly different between women and men (11.1 ± 9.3 ng/mL vs. 13.8 ± 13.5 ng/mL). No patient characteristic or clinical parameter (age, gender, body mass index [BMI], donor