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Predictive Value of Clinical Findings and Plasma Biomarkers after Fourteen Days of Prednisone Treatment for Acute Graft-versus-host Disease

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A B S T R A C T

We examined the hypothesis that plasma biomarkers and concomitant clinical findings after initial glucocorticoid therapy can accurately predict failure of graft-versus-host-disease (GVHD) treatment and mortality. We analyzed plasma samples and clinical data in 165 patients after 14 days of glucocorticoid therapy and used logistic regression and areas under receiver-operating characteristic curves (AUC) to evaluate associations with treatment failure and nonrelapse mortality (NRM). Initial treatment of GVHD was unsuccessful in 49 patients (30%). For predicting GVHD treatment failure, the best clinical combination (total serum bilirubin and skin GVHD stage: AUC, .70) was competitive with the best biomarker combination (T cell immunoglobulin and mucin domain 3 [TIM3] and [interleukin 1 receptor family encoded by the IL1RL1 gene, ST2]: AUC, .73). The combination of clinical features and biomarker results offered only a slight improvement (AUC, .75). For predicting NRM at 1 year, the best clinical predictor (total serum bilirubin: AUC, .81) was competitive with the best biomarker combination (TIM3 and soluble tumor necrosis factor receptor-1 [sTNFR1]: AUC, .85). The combination offered no improvement (AUC, .85). Infection was the proximate cause of death in virtually all patients. We conclude that after 14 days of glucocorticoid therapy, clinical findings (serum bilirubin, skin GVHD) and plasma biomarkers (TIM3, ST2, sTNFR1) can predict failure of GVHD treatment and NRM. These biomarkers reflect counter-regulatory mechanisms and provide insight into the pathophysiology of GVHD reactions after glucocorticoid treatment. The best predictive models, however, exhibit inadequate positive predictive values for identifying high-risk GVHD cohorts for investigational trials, as only a minority of patients with high-risk GVHD would be identified and most patients would be falsely predicted to have adverse outcomes.

INTRODUCTION

The frequency of acute graft-versus-host-disease (GVHD) after allogeneic hematopoietic cell transplantation is in the 50% to 70% range, with gastrointestinal symptoms the most common presentation [1,2]. The overall severity of GVHD has recently shifted toward less severe organ involvement and improved survival [1,3]. Newer methods of GVHD prophylaxis, such as post-transplantation cyclophosphamide, depletion of naïve T cells from the graft, and better HLA matching of unrelated donors and recipients, have improved outcomes [4-10]. Nevertheless, GVHD remains a formidable problem because of incomplete treatment responses, need for prolonged immune suppression, treatment-related toxicity, and fatal infections.

The backbone of GVHD therapy is prednisone or methylprednisolone [2,11]. Ideally, GVHD management could be improved by adopting a personalized treatment strategy where patients predicted to have treatment-responsive GVHD could be effectively treated with lower doses of prednisone or shorter-duration systemic therapy in conjunction with oral topical glucocorticoid [12-14]. This approach to treatment is inappropriate for patients who have findings at disease onset that are associated with more severe GVHD and a greater risk of nonrelapse mortality (NRM) [2,12]. Several studies have tested the hypothesis that plasma biomarkers measured at GVHD onset or at a set time after transplantation can predict whether GVHD is likely to respond to initial treatment [3,15-19]. We recently reported a study of plasma biomarkers measured

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before the clinical onset of acute GVHD [20]. A panel of IL6, T cell immunoglobulin and mucin domain 3 (TIM3), and soluble tumor necrosis factor receptor-1 (sTNFR1) was associated with subsequent peak grade 3 and 4 GVHD and a panel of interleukin 1 receptor family encoded by the IL1RL1 gene (ST2) and sTNFR1 was associated with NRM at 1 year. The positive predictive value (PPV) of all biomarker panels published to date, including ours, is suboptimal. When the probability of poor outcomes is low, the sensitivity and specificity of predictive tests must be very high to achieve clinically useful positive and negative predictive values at any biomarker cut point. This degree of biomarker predictive accuracy has not yet been met with regard to outcomes in patients with acute GVHD.

In the current study, we examined the hypothesis that biomarkers in blood samples drawn 2 weeks after the start of glucocorticoid therapy for GVHD, with or without concomitant clinical findings, can accurately predict failure of GVHD treatment and NRM. The overall aim was to identify patients at high risk of treatment failure and NRM as accurately as possible, so that early interventions could be tested for efficacy in preventing these outcomes.

METHODS

Allogeneic Hematopoietic Cell Transplantation

Pretransplantation conditioning regimens generally contained high-dose cyclophosphamide with busulfan or 12 to 13.2 Gy total body irradiation or fludarabine with 2 to 4 Gy total body irradiation. Recipients were usually given a calcineurin inhibitor plus methotrexate or mycophenolate to prevent GVHD. Prophylactic medications included antimicrobial drugs for infection and ursodiol for cholestasis.

Study Design

Patients who consented to research blood collection and analysis of clinical data under protocols approved by the Fred Hutchinson Cancer Research Center institutional review board and who developed acute GVHD comprised the study cohort. Blood samples for biomarker analysis were collected prospectively under the auspices of 2 protocols, 1 an observational study and the other a GVHD prophylaxis protocol (NCT00489203). Clinical and laboratory manifestations of GVHD and outcomes were reviewed in retrospect. After 14 ± 7 days of glucocorticoid treatment for GVHD, we collected a blood sample and concomitant clinical data related to GVHD, including gut GVHD stage (diarrhea, upper gut symptoms), skin GVHD stage, prednisone dose, laboratory tests (serum albumin, total serum bilirubin, calculated glomerular filtration rate), and a subjective global assessment of the course of GVHD from baseline (improved, worsened, or unchanged, based on notes from the attending physician and patient care team). Demographic factors previously noted to have an impact on GVHD treatment outcomes (time from transplantation to start of glucocorticoid treatment, HLA mismatch, and GVHD prophylaxis) were also analyzed as potential predictors of outcome [21]. In the blood samples, we measured the levels of 8 analytes, 6 that had been shown to be most predictive of the severity of GVHD and NRM in a previous study of blood samples drawn before the clinical onset of GVHD [20], and 2 recently described analytes associated with GVHD, angiopoietin-2 and vascular endothelial growth factor [18,22].

Outcomes of interest were NRM within 1 year after the blood sample was drawn and failure of initial glucocorticoid treatment for GVHD. *NRM* was defined as a death during the year after GVHD treatment day 14 ± 7 without prior recurrent or progressive hematologic malignancy. Failure of initial treatment of GVHD was defined during a 56-day observation period after the start of therapy as either initiation of secondary systemic therapy for GVHD, death, the onset of chronic GVHD by National Institutes of Health criteria, or an average prednisone dose ≥.5 mg/kg/day during days 55 and 56 after initiation of treatment. This threshold prednisone dose for defining GVHD treatment failure was determined by comparing the average prednisone dose during treatment days 29 to 56, 50 to 56, and 55 to 56 as related to NRM within 1 year after treatment day 14 (Supplement Table S1, Supplement Figure S1). The highest area under a receiver-operating characteristic curve was found with average prednisone dose on treatment days 55 to 56.

Collection, Processing, and Analysis of Blood Samples

Blood samples were collected as previously described [20,23]. The Luminex microbead method (Luminex, Austin TX) was used for measurement of hepatocyte growth factor, IL6, sTNFR1, TIM3, TNFα, and ST2 [20,23]. For the analyte angiopoietin 2, capture (MAB098) and detection (BAM0981) antibodies were obtained from R&D Systems (Minneapolis MN); the lower limit of detection was 30 pg/mL. For vascular endothelial growth factor, capture (MAB293) and detection (BAF293) antibodies were obtained from R&D Systems; the lower limit of detection was 8.0 pg/mL.

Statistical Methods

All biomarker values were log transformed before analysis. Values at the lower limit of detection were assigned that value. Logistic regression was used to evaluate the association of plasma biomarkers or clinical factors with outcome, including the calculation of area under the receiver operating characteristic curve. We have chosen binary endpoints because these are directly amenable to calculation of predictive parameters such as sensitivity and PPV, which are critical for evaluating potential clinical applications. The best models using biomarkers alone or using clinical and laboratory factors alone were determined by forward selection at the .05 level of significance. Backward selection yielded the same model in each case. The plasma biomarker and clinical factors from the best models were then combined in a single model.

RESULTS

Demographics of Patients

The study cohort of 165 patients (Table 1) included 81 who had provided samples before the onset of GVHD in a prior study [20] and 84 who provided samples solely for this study. Blood samples were drawn at a median of 14 days (interquartile range, 13 to 17 days) after starting glucocorticoid therapy, which was at a median of 44 days after transplantation (interquartile range, 35 to 58).

Stages and grade of acute GVHD at baseline and at the time of the 14-day evaluation after initiation of prednisone therapy are given in Table 2.

Table 1
Characteristics of 165 Patients Treated for Acute GVHD

Age in years, median (range)	45 (8-71)
Diagnosis	
Acute leukemia	81 (49)
Myelodysplastic syndrome	37 (22)
Lymphoma	23 (14)
Chronic myeloid leukemia	15 (9)
Other diagnosis	9 (6)
Donor type	
Related	59 (36)
Unrelated	106 (64)
HLA match	
Matched	132 (80)
Mismatched	33 (20)
Graft type	
Marrow	30 (18)
Peripheral blood	135 (82)
Sex match	
Female donor to male recipient	46 (28)
Other	119 (72)
Conditioning regimens	
Myeloablative*	134 (81)
Reduced intensity nonmyeloablative	31 (19)
CMV serostatus before transplantation (donor/recipient)	
+/+	47 (29)
−/+	37 (22)
+/−	25 (15)
−/−	56 (34)
GVHD prophylaxis	
Calcineurin inhibitor + methotrexate	118 (72)
Calcineurin inhibitor + methotrexate + mycophenolate mofetil	4 (2)
Calcineurin inhibitor + mycophenolate mofetil	35 (21)
Other regimens	8 (5)

Data presented are n (%) unless otherwise indicated.
CMV indicates cytomegalovirus.
*Five patients received antithymocyte globulin as part of their conditioning therapy.