BRIEF ARTICLE

Prediction of Veno-Occlusive Disease Using Biomarkers of Endothelial Injury

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Predicting the development of veno-occlusive disease (VOD) of the liver remains challenging. We hypothesized that biomarkers of endothelial injury in myeloablative allogeneic transplantation recipients could predict VOD occurrence. We evaluated 4 biomarkers—von Willebrand Factor (vWF), thrombomodulin, E-selectin, and soluble intercellular adhesion molecule-1 (sICAM-1)—weekly in the peritransplantation period in an attempt to predict VOD. In the patients who received sirolimus, vWF, thrombomodulin, and sICAM-1 levels were significantly elevated in patients with VOD compared with those without VOD on day −1 ($P \leq .035$), day +7 ($P \leq .0001$), and day +14 ($P \leq .004$). E-selectin was predictive on day +7 ($P = .007$). Levels of vWF ≥1400 IU/mL and thrombomodulin ≥100 ng/mL on day +7 were both 100% sensitive and 100% specific in predicting VOD. These biomarkers were informative when adjusted for other risk factors for VOD in regression analysis. Among patients not receiving sirolimus, biomarkers of endothelial injury were not informative. We conclude that vWF, thrombomodulin, and sICAM-1 elevations before and early after transplantation may be useful in predicting VOD in patients receiving sirolimus.

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INTRODUCTION

Veno-occlusive disease (VOD, also referred to as sinusoidal obstruction syndrome) of the liver occurs in 5%-15% of patients after myeloablative allogeneic hematopoietic stem cell transplantation (HSCT). VOD is thought to result from conditioning-related injury to hepatic sinusoidal endothelium and hepatocytes, compounded by cytokine-mediated effects related to allogenicity [1]. Although clinical risk factors for VOD are well established, precise prediction of VOD in individuals remains elusive.

In previous work, we demonstrated an increased frequency of VOD after sirolimus-based graft-versus-host disease (GVHD) prophylaxis (relative risk [RR], 1.55, $P = .33$ without concomitant methotrexate [MTX]; RR, 2.35, $P = .005$ with concomitant MTX) [2]. Sirolimus may act as an endothelial toxin or may prevent endothelial repair after conditioning-related or mechanical injury. It is commonly used to coat endovascular stents to prevent restenosis [3] and has been associated with another endothelial injury syndrome, thrombotic microangiopathy, after transplantation [4].

We hypothesized that the occurrence of VOD can be predicted by measuring biomarkers of endothelial injury, particularly in patients receiving sirolimus therapy.

METHODS

We performed a retrospective analysis of biomarkers of endothelial injury using banked plasma and serum samples collected weekly in the peritransplantation period, with clinical VOD as the outcome of interest. We selected 4 biomarkers—von Willebrand factor (vWF), thrombomodulin, soluble intracellular adhesion molecule-1 (sICAM-1), and E-selectin—based on

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their association with VOD, known endothelial expression pattern, and ability to be measured in stored plasma or serum. The biomarkers were measured using commercially available ELISA kits (vWF: American Diagnostica, Greenwich, CT; thrombomodulin: Diagnostica Stago, Parsippany, NJ; sICAM-1 and E-selectin: R&D Systems, Minneapolis, MN). vWF and thrombomodulin were assayed in plasma, and sICAM-1 and E-selectin were assayed in serum.

All patients in the study group underwent myeloablative HSCT using cyclophosphamide and total body irradiation, as described previously [5]. In brief, cyclophosphamide (1800 mg/m^2 on days −5 and −4) was administered, followed by total body irradiation at 14.0 Gy, delivered in 7 fractions at a dose rate of 10 cGy/min. Lead blocks were used to compensate for lung absorption. Tacrolimus was administered at 0.02 mg/kg/day i.v. by continuous infusion beginning on day −3, with a target serum concentration of 5-10 ng/mL. Sirolimus was administered as a 12-mg oral loading dose on day -3, followed by a 4-mg/day single dose, with a target serum concentration of 3-12 ng/mL as assessed by high-performance liquid chromatography. Recipients of matched related and matched unrelated grafts were included.

Patients were selected to represent 2 GVHD prophylaxis groups: sirolimus/tacrolimus (SIR$^+$) and tacrolimus/MTX (SIR$^-$) with or without VOD (VOD$^+$/VOD$^-$). A sufficient number of patients were randomly selected from our database to ensure the availability of least 10 samples for assay at each of 3 time points (days -1, +7, and +14); however, not all patients in groups other than the SIR$^+$VOD$^+$ reference group had serum and plasma measurements at each time point. Assays were performed before the clinical development of VOD. VOD was diagnosed based on the Baltimore criteria [6], with diagnosis confirmed by Doppler ultrasonography and/or liver biopsy with wedged hepatic venous pressure gradient measurement.

Statistical Analysis

All assays were performed in duplicate, and the results presented here are the mean of 2 assays. The 2-sided exact Wilcoxon rank-sum test was used for comparison of continuous variables, and the 2-sided Fisher exact test was used for comparison of categorical variables. All biomarkers were first evaluated at each time point. To establish a cutoff value for predictive biomarkers, analysis of the receiver operator characteristic (ROC) curve was performed at each time point. To assess whether the cutoff value determined in the ROC analysis predicted the occurrence of VOD in the presence of other prognostic factors, exact logistic regression analysis was performed at each time point, adjusting for age, recipient–donor sex mismatch, and donor type. In addition, to test for a group difference (ie, VOD$^+$ vs VOD$^-$) over time, a mixed model for repeated measures was explored for each biomarker using PROC MIXED in SAS version 9.2 (SAS Institute, Cary, NC). The level of each biomarker was log-transformed for modeling. All tests were 2-sided. Testing for multiple biomarkers was not adjusted for in the level of significance.

RESULTS

Table 1 summarizes characteristics of the study patients. Significant intragroup differences in baseline characteristics can be seen, with SIR$^+$ patients engrafting earlier than SIR$^-$ patients (14 days vs 16 days; $P < .01$) and SIR$^+$VOD$^+$ patients being more likely to receive an unrelated donor graft and to experience delayed platelet recovery. Only 2 patients (1 SIR$^+$VOD$^+$ and 1 SIR$^+$VOD$^-$) were exposed to gentuzumab ozogomycin before HSCT. A total of 61 patients were needed for the analysis to ensure the availability of 10 samples at each of the 3 analysis time points; however, in the SIR$^-$VOD$^+$ group, only 9 patients with banked samples were ultimately identified. In the SIR$^+$VOD$^+$ group, all 10 patients had samples at all time points. The median time to development of VOD was 17 days (range, 11-28 days) in the SIR$^+$ group and 21 days (range, 10-40 days) in the SIR$^-$ group ($P = .35$).

Comparison of Biomarkers

Significant differences in biomarker levels were detected between SIR$^+$ patients with VOD and those without VOD. vWF, thrombomodulin, and sICAM-1 levels were significantly elevated in VOD$^+$ patients compared with VOD$^-$ patients on days -1, +7, and +14 (Table 2 and Figure 1). E-selectin level was significantly elevated only on day +7 ($P = .007$). A repeated-measures analysis performed for each predictive biomarker found that each was significantly associated with the occurrence of VOD when measured over time (vWF, $P = .003$ mU/mL; thrombomodulin, $P = .002$ ng/mL; sICAM1, $P = .004$ ng/mL).

In contrast, biomarker levels did not differ significantly between the SIR$^-$VOD$^+$ and SIR$^-$VOD$^-$ groups at any of the time points tested (Figure 1), except for thrombomodulin level on day +7 (median, 46 vs 16; $P = .0003$) and day +14 (median, 43 vs 21.5; $P = .02$). This difference was not seen on the repeated-measures analysis, at least in partly because of the small sample size. Even though the significant differences in thrombomodulin level were seen between the SIR$^-$VOD$^+$ and SIR$^-$VOD$^-$ groups, the thrombomodulin level in the SIR$^-$VOD$^+$ group was much lower than that in the SIR$^+$VOD$^+$ group (median, 227.5; $P < .001$).