

Review

Predicting Immuno-Metabolic Complications After Allogeneic Hematopoietic Cell Transplant with the Cytokine Interleukin-33 (IL-33) and its Receptor Serum-Stimulation 2 (ST2)

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ABSTRACT

Patients undergoing allogeneic hematopoietic cell transplantation (HCT) are at risk for numerous acute and long-term complications from this procedure. Post-transplant diabetes mellitus (PTDM) is a common but under-recognized problem. Similar to graft-versus-host disease (GVHD), new-onset diabetes is characterized by immune dysregulation that can negatively impact transplant outcomes. This review will discuss the biology of IL-33/ST2 in acute GVHD and PTDM development, and how this cytokine axis could be leveraged for predicting and treating immuno-metabolic complications after transplant.

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1. INTRODUCTION

Allogeneic hematopoietic cell transplant (HCT) is an effective therapy for high-risk hematologic malignancies. It leverages the powerful graft-versus-leukemia (GVL) effect, in which alloreactive donor T lymphocytes recognize and eliminate malignant cells. Conversely, the major complication of HCT is graft-versus-host disease (GVHD), a process in which donor-derived T cells recognize normal organs as foreign, and initiate a complex cascade of humoral and cellular immune events, resulting in widespread recipient tissue damage [1]. Acute GVHD is observed in 30–60% of HCT recipients [2]. Patients with acute GVHD experience a significantly higher mortality rate compared to those without GVHD [3]. In the post-HCT period, previously euglycemic patients can also develop hyperglycemia related to insulin resistance and insulin insufficiency. Metabolic complications after HCT are common, with new-onset post-transplant diabetes mellitus (PTDM) developing in about 20–50% of patients, and generally preceding the diagnosis of acute GVHD. Similar to acute GVHD, PTDM is also associated with inferior overall survival (OS) following transplant [4,5].

GVHD and insulin resistance are both conditions that involve the accumulation of pro-inflammatory, type 1 T helper (Th1)

cells that overwhelm the anti-inflammatory capacities of regulatory T cells (Tregs) and type 2 T helper (Th2) cells [6,7]. The cytokine interleukin-33 (IL-33), an alarmin, and its receptor serum-stimulation 2 (ST2) are involved in many inflammatory and autoimmune diseases, including allergic and respiratory disorders, inflammatory bowel disease, and rheumatoid arthritis. In HCT, soluble ST2 (sST2), a decoy receptor for IL-33, is a highly validated biomarker of steroid-refractory acute GVHD [8–11]. Diabetes and obesity are associated with low-level inflammation of adipose tissue. Interestingly, animal models of metabolic diseases demonstrate decreased levels of Tregs in the visceral adipose tissue (VAT), and the administration of IL-33 expands VAT Tregs and improves insulin sensitivity in these models [12,13]. This paper will review acute GVHD and PTDM in the context of immune dysregulation, the biology of the IL-33/ST2 axis as it relates to these disorders, and the role of plasma sST2 as a predictor of immuno-metabolic complications after HCT.

2. ACUTE GVHD

Acute GVHD usually occurs in the first 100 days after transplant, and is classically divided into three phases: recipient conditioning, donor T cell activation, and inflammatory effector [14]. First, pre-HCT conditioning regimens—incorporating radiation, chemotherapy, and depleting antibodies—damage and activate host tissues. Within this pro-inflammatory milieu, antigen presenting cells (APCs) upregulate MHC expression and lead to the next stage of acute GVHD. The second phase involves donor T-cell activation

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Table 1 | Biomarkers for immuno-metabolic complications after allogeneic hematopoietic cell transplant

Outcome	Biomarker	N	Date analyzed	Direction of response	References
Treatment-resistant GVHD, NRM	sST2	20 + 381 + 673 + 75	Therapy initiation, D+14	Increased	Vander Lugt et al. [8]
Treatment-resistant GVHD, NRM	sST2, REG3 α , TNFR1	328 + 164 + 300	GVHD onset	Increased	Levine et al. [10]
NRM	sST2, TNFR1	74 + 76	GVHD onset	Increased	McDonald et al. [67]
GVHD, NRM in CBT	sST2	113	D+28	Increased	Ponce et al. [9]
GVHD, NRM	sST2, REG3 α	620 + 309 + 358	D+7	Increased	Hartwell et al. [11]
GVHD, NRM	sST2, REG3 α	248 + 367	Therapy initiation, 28 days after GVHD treatment	Increased	Srinagesh et al. [68]
NRM	sST2, REG3 α , TNFR1	170 + 245	Pre-HCT, D+7, D+14, D+21	Increased	Rowan et al. [69]
Malignancy relapse, NRM	sST2,REG3 α	702 + 902	D+28	Relapse decreased, NRM increased	Aziz et al. [70]
PTDM, NRM	sST2	36 + 26	Engraftment, D+30	Increased	Johnpulle et al. [60]
PTDM, all-cause mortality	sST2	55	D+14	Increased	Rowan et al. [61]

GVHD, graft-versus-host disease; sST2, soluble ST2; REG3 α , regenerating islet-derived protein 3- α ; TNFR1, tumor necrosis factor receptor 1; CBT, cord blood transplant; HCT, hematopoietic cell transplant; PTDM, post-transplant diabetes mellitus.

authors concluded that biomarker-based scores can be used to guide risk-adapted therapy at the onset of acute GVHD, and suggested that high-risk patients with an Ann Arbor score of 3 are candidates for intensive primary therapy or novel agents, while low-risk patients with a score of 1 are candidates for rapid taper of systemic steroids [10].

Given the success of biomarker panels in risk-stratifying acute GVHD at symptom or treatment onset, Hartwell and colleagues sought to determine whether a biomarker signature before acute GVHD onset could be predictive of treatment response and NRM [11]. Blood samples on day +7 after HCT were obtained from a multicenter set of 1,287 patients, and four GVHD biomarkers, sST2, REG3 α , TNFR1, and IL-2R α , were measured to model 6-month NRM. The most accurate model included concentrations of sST2 and REG3 α and identified high-risk and low-risk groups, with NRM of 28% and 7%, respectively ($p < 0.001$). The simplified two-protein biomarker set also correlated with the previously described Ann Arbor GVHD score. Independent of important clinical risk factors, such as the degree of HLA mismatch, the genetic relationship between donor and recipient, the intensity of conditioning regimen, and the age of the recipient, the biomarker algorithm was able to stratify patients into high or low risk for NRM [11]. While HLA mismatch and donor-recipient relationship were significantly associated with NRM in univariate analyses, their incorporation into the algorithm did not significantly improve its performance over the sST2-REG3 α -biomarker panel alone. High-risk patients had a three-fold higher NRM risk than low-risk patients (NRM 19% versus 6%, respectively; $p < 0.001$) and experienced greater than twice the steroid-refractory GVHD as did low-risk patients (treatment refractory 35%, versus 15%; $p < 0.001$). Furthermore, high-risk patients experienced twice as much severe gastrointestinal GVHD as did low-risk patients [11]. The increase in REG3 α and sST2 prior to onset of GVHD symptoms suggests the biomarkers can identify subclinical gastrointestinal pathology. These observations have led to biomarkers and the Ann Arbor scoring system being incorporated into clinical practice. Clinical trials using biomarker risk stratification at the time of GVHD onset followed by treatment with the novel agents itacitinib (JAK1 inhibitor) or natalizumab (α 4 integrin

blocker) for Ann Arbor scores 1 (low-risk) or 3 (high-risk), are currently underway (NCT03846479 and NCT02133924, respectively).

6. PTDM AFFECTS HCT OUTCOMES

As HCT survival increases, long-term care needs to focus on preventing or minimizing chronic treatment-related complications. Along with GVHD, the study of the metabolic complications after HCT is important due to their negative impact on cardiovascular health and survival. Compared with recipients of autologous HCT, recipients of allogeneic HCT are at increased risk for metabolic syndrome and PTDM. Approximately 20 to 50% of HCT recipients develop new-onset PTDM, and mortality for those with PTDM is three-fold higher than those without hyperglycemia [4,5]. Clinical risk factors for PTDM include TBI, increased body mass index, unrelated donor source, ablative conditioning, older age, and higher doses of corticosteroids [5,55]. However, the mechanistic causes for PTDM have not been well-described.

Griffith *et al.* conducted a prospective study of patients undergoing HCT with calcineurin-inhibitor-based GVHD prophylaxis to evaluate PTDM risk factors and to describe the incidence of new-onset PTDM [5]. Adult patients, without a prior diagnosis of type-2 diabetes mellitus and who were scheduled for their first HCT, were included. Baseline laboratory values, including fasting glucose, fasting lipid profile, C-peptide, and insulin, along with height, weight, and vital signs, were obtained one day prior to conditioning. The authors prospectively defined PTDM as a fasting blood glucose ≥ 126 mg/dL or a random blood glucose ≥ 200 mg/dL [5]. Weight and fasting glucose were measured weekly until either PTDM diagnosis or day +100 post-HCT.

Patients underwent either ablative or reduced-intensity (RIC) conditioning regimens. The GVHD prophylaxis incorporated calcineurin inhibitors and methotrexate (in ablative) or mycophenolate mofetil (in RIC). Acute GVHD was treated according to standardized institutional guidelines, with grade II-IV aGVHD treated with 1–2 mg/kg/day prednisone equivalent for 10–14 days, followed by a 10% weekly taper. The primary endpoint was PTDM

incidence by day 100. Assessments of PTDM risk factors and survival were the secondary aims.

Eighty-four patients completed the study. The demographic data, BMI, blood pressure, category of malignancy did not differ between patients who did and did not develop PTDM. Fifty (60%) patients developed PTDM. The cumulative incidence of PTDM was 50% by day 41 and 59% by day 98. The median time from transplant to PTDM diagnosis was 23 days, and generally preceded GVHD diagnosis or corticosteroid treatment. Univariate risk-factor analysis showed an increased risk of PTDM in patients who received ablative conditioning, peak systemic steroids ≥ 1 mg/kg/day, unrelated donor HCT, or TBI. Pre-HCT fasting C-peptide levels were higher in patients with PTDM (4.45 ng/mL *versus* 2.6 ng/mL; $p = 0.025$) [5]. The incidence of PTDM was 72.5% in patients with a fasting, pre-transplant C-peptide above the cohort median of 3.6 ng/mL as compared with 50% of patients with C-peptide ≤ 3.6 ng/mL. Furthermore, in a multivariate logistic regression model, pre-HCT fasting C-peptide level >3.6 ng/mL (OR 5.9; 95% CI 1.77–20.44; $p = 0.004$), unrelated donor HCT (OR 4.3; 95% CI, 1.34–14.2; $p = 0.014$), and peak steroid use >1 mg/kg/day (OR 5.09; 95% CI, 1.19–23.2, $p = 0.035$) were independent predictors of PTDM development [5]. The mean survival of patients with PTDM was 2.26 years compared to 2.7 years for those without PTDM ($p = 0.021$). Analysis of PTDM as a time-dependent variable showed its significant impact on OS (HR 3.27; 95% CI, 1.3–8.2; $p = 0.01$). Not only did pretransplant, fasting C-peptide greater than the cohort median (>3.6 ng/mL) predict PTDM diagnosis, it was also associated with inferior OS (1.7 years *versus* 2.9 years, $p = 0.012$). Thus, a strong relationship between elevated fasting C-peptide levels and both PTDM and mortality was identified. In this study, the elevation of fasting C-peptide levels was thought to represent normal/physiologic β -cell compensation related to established insulin resistance. Taken together, these data suggest that insulin resistance precedes PTDM, and that factors associated with insulin resistance lead to inferior HCT outcomes. The fact that the elevations in C-peptide were noted before transplant means that these findings cannot be due to immunosuppression or alloreactivity.

Based on the C-peptide data, and to determine if PTDM was due to preexisting insulin resistance, Engelhardt and colleagues prospectively followed 20 patients, without diabetes, and who underwent related HLA-identical, peripheral blood HCT with either ablative or RIC conditioning, for the development of new-onset PTDM [56]. To measure whole-body, peripheral, and hepatic insulin sensitivity, the authors performed oral glucose tolerance testing (OGTT) and the gold-standard euglycemic hyperinsulinemic clamp on patients both before and 90 days after HCT [56]. In the first 100 days post-HCT, 55% of recipients developed PTDM (at a median of 22 days post-HCT), and PTDM diagnosis preceded both corticosteroid use and the development of grades II–IV acute GVHD. No patients were receiving corticosteroids at the time of PTDM diagnosis. During pre-HCT OGTT, elevated fasting blood glucose (87 mg/dL *versus* 101 mg/dL; $p = 0.005$), but not 2-hour postprandial glucose level, predicted PTDM diagnosis [56]. When compared to patients who maintained euglycemia, HCT recipients who developed PTDM exhibited lower pre-HCT whole-body insulin sensitivity, as well as lower skeletal muscle glucose uptake post-HCT. There were no significant differences in hepatic glucose production or hepatic insulin sensitivity among patients with PTDM

and euglycemic HCT recipients. The estimated two-year OS was decreased in patients developing PTDM compared to those who maintained euglycemia (55% *versus* 100%; $p = 0.039$) [56]. These data suggest that the risk of progression to PTDM depends on pre-existing, subtle whole-body insulin resistance before HCT, which develops into more robust peripheral/skeletal muscle insulin resistance after HCT. These findings also firmly repudiate the misconception that PTDM is an iatrogenic phenomenon secondary to hyperglycemia-inducing immunosuppressive medications. Targeting insulin resistance or the immuno-metabolic consequences of pre-diabetes could be a rational approach for decreasing complications and improving HCT outcomes.

7. THE IL-33/ST2 AXIS IN OBESITY AND TYPE-2 DIABETES MELLITUS

The accumulation of VAT in obesity leads to chronic inflammation, insulin resistance, and the development of type-2 diabetes mellitus [57]. Immunologically, the progression of insulin resistance to diabetes mellitus is characterized by the accumulation of Th1 cells and depletion of immunosuppressive Tregs in adipose tissue, leading to low-grade systemic and localized inflammation [7,12]. The expansion of VAT Tregs through the administration of recombinant IL-33 led to lower adiposity, reduced fasting glucose, and improved glucose tolerance and insulin sensitivity in genetically obese mice [13]. IL-33 also induced the accumulation of Th2 cells in adipose tissue and polarization of adipose tissue macrophages toward an M2 alternatively activated phenotype, which is associated with protection against obesity-related metabolic events [58]. Conversely, ST2^{-/-} mice not susceptible to the effects of IL-33 had increased body weight, abnormal glucose regulation, and impaired insulin secretion when compared to wild-type control animals fed a high-fat diet [58]. Taken together, the balance between adipose tissue inflammation *versus* homeostasis and the ratio Th1 cells to ST2+ VAT Tregs are critical determinants of metabolic regulation. In turn, the IL-33/ST2 signaling axis appears to be a major factor driving this equilibrium, which could be disturbed after HCT.

Corticosteroids are commonly used during HCT, and glucocorticoid-induced hyperglycemia is well known. However, there is a paucity of data describing the direct effects of glucocorticoids on the IL-33/ST2 signaling axis. In a mouse model of allergic airway disease, treatment with intranasal budesonide reduced inflammation but bronchoalveolar IL-33 levels remained unchanged [59]. More research will be needed to describe the effects of glucocorticoids on the IL-33/ST2 axis during HCT.

8. sST2 AS A BIOMARKER FOR PTDM

Evidence suggests that IL-33 regulates insulin sensitivity, established insulin resistance precedes PTDM, and PTDM leads to decreased survival after HCT. As discussed, sST2 is a validated biomarker for steroid-refractory acute GVHD and mortality after transplant. Therefore, Johnpulle and colleagues hypothesized that PTDM was related to dysregulated IL-33/ST2 signaling, and that sST2 would predict PTDM diagnosis [60]. Serum sST2 was measured at neutrophil engraftment and at day +30 in 36 euglycemic HCT patients, who were followed prospectively as above for the development of PTDM. An independent validation cohort of

26 patients without pre-HCT diabetes was analyzed retrospectively for PTDM diagnosis. Compared to patients without PTDM, patients with PTDM had higher levels of sST2 at engraftment ($p = 0.02$) and at D+30 ($p < 0.01$). Elevated sST2 levels among PTDM patients were confirmed in the second cohort at engraftment ($p = 0.01$) (Table 1). Multivariate analysis revealed that high engraftment sST2 levels continued to predict PTDM and NRM, independent of conditioning and GVHD. These data indicate a relationship between glucose homeostasis and the IL-33/ST2 axis after adult transplantation [60].

Pediatric HCT recipients also demonstrate a significant association between PTDM and sST2 [61]. At a different transplant center, 55 pediatric HCT patients without preexisting diabetes were followed for the first 100 days post-HCT. The authors monitored fasting and random blood glucose levels on a weekly basis and obtained plasma sST2 levels at day +14. PTDM was diagnosed in 31% of patients. The median time to PTDM diagnosis was day +19 (range 3 to 44 days). Between patients with and without PTDM, gender, age, malignancy, matched or related donor status, disease risk index, use of high-dose corticosteroids (≥ 1 mg/kg methylprednisolone equivalent for at least 72 hours), and BMI did not differ significantly. Patients who received umbilical CBT were more likely to develop PTDM ($p = 0.0363$). Patients with PTDM had worse HCT outcomes, including increased likelihood of admission to the intensive care unit (71% PTDM versus 8% without PTDM; $p = 0.007$) and greater risk for mechanical ventilation (53% PTDM versus 5% without PTDM), as well as decreased OS at one year (50% PTDM versus 90% without PTDM; $p = 0.0239$). Patients with PTDM also had higher mean sST2 levels at day +14 compared to patients without PTDM (sST2 95.1 ± 90.0 ng/mL versus 28.2 ± 28.7 ng/mL; $p = 0.0002$).

In a subsequent analysis, sST2 levels were categorized as either “high” or “low” using the median sST2 level of the cohort as the threshold [61]. Patients with a high sST2 level had increased risk of developing PTDM (HR 3.92; 95% CI 1.42–10.84; $p = 0.0085$). Interestingly, analysis of pre-HCT sST2 levels did not show significant differences between patients who developed PTDM and those who did not, although this comparison was limited by an incomplete dataset. Collectively, two HCT studies from different institutions involving adult or pediatric cohorts have now shown that PTDM is associated with decreased survival, and that high sST2 levels in the first 2–3 weeks of transplant predict PTDM development.

Although sST2 has been shown to be a validated predictor of glucose dysregulation, it remains unclear if this protein is the best biomarker for PTDM. Similarly to the identification of biomarkers of acute GVHD, proteomic and transcriptomic approaches will need to be applied to the study of metabolic complications to identify the best and most precise biomarkers for PTDM. Furthermore, additional studies in metabolic HCT outcomes are needed to determine the effects of corticosteroids and the IL-33/ST2 signaling axis. However, in the studies reviewed here, sST2 levels were typically analyzed before the development of PTDM and corticosteroid use and, so, steroid administration should not affect the initial values.

9. CONCLUSIONS/FUTURE DIRECTIONS

GVHD and PTDM are common complications after HCT, which are characterized by immune dysregulation and inferior survival.

The pleiomorphic cytokine IL-33, its receptor ST2, and sST2 are intricately linked to both conditions. In HCT, depending on the inflammatory milieu, IL-33 can have differing effects. During baseline homeostasis, the pre-HCT administration of IL-33 seems to expand ST2+ Tregs and dampen GVHD severity. However, in the setting of tissue damage, post-HCT administration of IL-33 can activate IFN γ -producing T cells that exacerbate GVHD [23,45]. Similar to alloreactivity, the development of insulin resistance and type-2 diabetes mellitus is associated with chronic inflammation and immune activation. Animal models of insulin resistance have demonstrated an accumulation of Th1 cells and depletion of ST2+ Tregs in adipose tissue [12,13,58]. Within these preclinical models, IL-33 administration results in VAT ST2+ Treg expansion and improvement in insulin sensitivity [13].

Given the role of IL-33/ST2 in regulating cellular immunity and insulin sensitivity, it is not surprising that the decoy receptor sST2 is a validated biomarker for acute GVHD, PTDM, and transplant mortality [8–11,60,61]. In addition, cardiovascular diseases such as coronary artery disease, heart failure, and stroke have also been linked to IL-33/ST2 signaling, and these complications are significant causes of morbidity and mortality for long-term survivors of HCT [62–66]. Thus, monitoring sST2 may have implications beyond just glucose metabolism and GVHD.

sST2 as well as other GVHD biomarkers are now being incorporated into therapeutic clinical trials. As our understanding of IL-33/ST2 increases, this cytokine axis could be leveraged for treating immuno-metabolic complications after transplant. For example, Zhang and colleagues demonstrated that by blocking sST2 with a monoclonal antibody, IL-33 levels can be increased in the peritransplant period, leading to less severe GVHD and improved HCT outcomes in an animal model [43]. A similar strategy could be used to mitigate metabolic inflammation and insulin resistance. In conclusion, modulation of IL-33 signaling, heightened awareness of metabolic complications after HCT, and optimized treatment strategies for insulin resistance could all potentially improve transplant outcomes. This intriguing therapeutic possibility warrants the development of experimental animal models of PTDM, as well as randomized clinical trials for the treatment and prevention of metabolic complications after HCT.

CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

AUTHORS' CONTRIBUTION

UKR and BGE both had equal contribution to the conception of the work, drafting the work, revising it and approval of the final manuscript.

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