

platelet activation tests use aggregation, release of dense granules (serotonin), or release of alpha granules (surface exposure of P-selectin from the alpha granule membrane). Kanack and colleagues studied the release of the alpha granule protein thrombospondin-1 (TSP-1), as assayed by a commercially available ELISA. The authors contend, with a strong rationale, that such an ELISA is more readily performed in general hospital laboratories.

How close is this work to achieving this vision? The authors used a limited number of known patient samples, already identified as positive or negative in the conventional assays, that is, SRA and PEA. Clinical information on the patients providing the samples was very limited. They show that, for these patient serum samples, the SRA and PEA are concordant. After presenting initial trial experiments, the authors optimized a method for cryopreservation, thawing and TSP-1 ELISA. In particular, whether their TSP release assay (TRA) is conducted by provision of exogenous PF4 (PF4-TRA) or exogenous unfractionated heparin (hep-TRA), they find that the ratio of sample TSP-1 to that induced by a concurrent normal control serum is diagnostic. If the ratio exceeds 2, it is HIT. If the ratio is close to 1, it is not HIT. Importantly, the ratio should be high with therapeutic heparin (on order 0.5 U/ml) and low at high heparin (on order 100 U/ml).

What is needed next for the vision of the authors to become reality? First, the interindividual variability in healthy donor platelet responses must be resolved. The authors suggest the use of pooled platelets or pedigreed donors in the future. Whichever is chosen needs validation. Second, the control serum should be better described; for example, how many donors provide it, and is it heat inactivated or frozen? Third, this ELISA requires that the laboratory coat the plates overnight before the assay the next day. How will that influence laboratory practice and throughput? Fourth, recombinant human PF4 at a high concentration in PF4-TRA can be expensive; for HIT testing, hep-TRA seems more promising. Finally, HIT platelet activation assays, even in the best of hands, continue to have standardization concerns.<sup>5</sup> True validated standardization will require that coded

patient samples be distributed among independent reference laboratories, which then use identical protocols and reagents to make the call of the assay results. Breaking the code, scoring the results, and linking to the clinical course will begin to make it clear if these new approaches can be used to improve care of patients and to help providers.

*Conflict-of-interest disclosure:* S.E.M. has an intellectual property interest and is a member of the Scientific Advisory Board of Verolox Therapeutics. ■

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## TRANSFUSION MEDICINE

Comment on *Hod et al*, page 2730

# To Fe, or not to Fe, that is the question

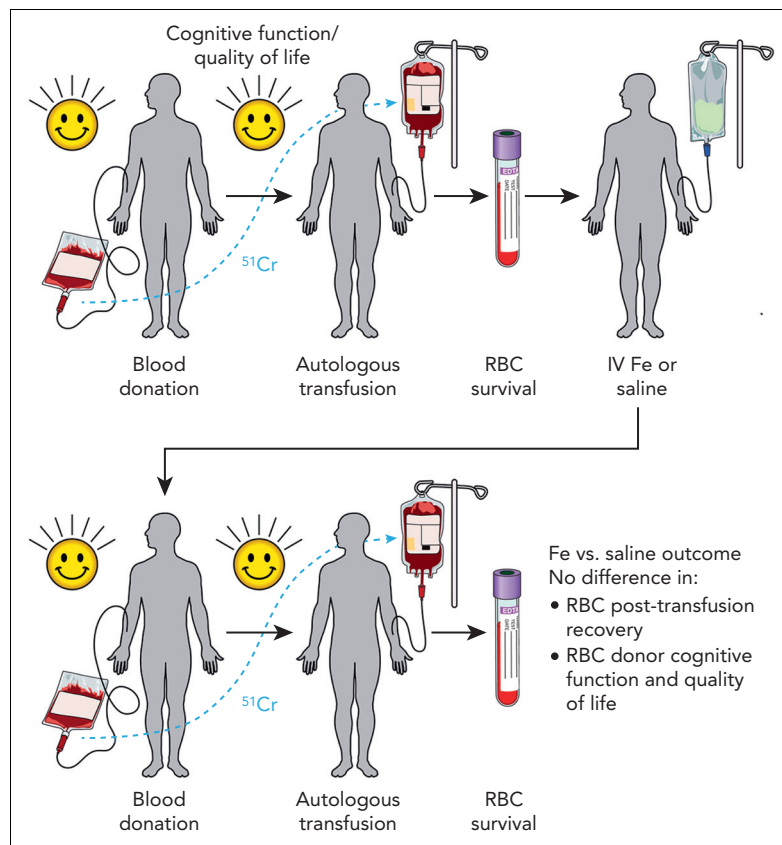
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**In this issue of *Blood*, Hod et al<sup>1</sup> present results of the Donor Iron Deficiency Study (DIDS), a randomized controlled trial that demonstrates that iron repletion in iron-deficient blood donors fails to improve posttransfusion recovery of a subsequently donated red blood cell (RBC) unit or the quality of life and cognitive performance of the blood donor.**

RBC transfusion represents the most common therapeutic intervention in hospitalized patients. However, unlike the vast majority of patient interventions, transfused RBCs are a biologic directly harvested from altruistic donors. The majority of blood donor eligibility considerations, including questionnaires and infectious disease testing, are designed to ensure the safety of the transfused recipient. However, in addition to the time and resources required of blood donors, blood donation itself is not without risk. Most complications are transient (eg, vasovagal reactions) and have little, if any, long-term sequelae. However, a substantial percentage of repeat blood donors develop iron deficiency. Despite this, the effects of donor iron deficiency on the quality of

the donated unit and the overall health of the donor remain incompletely understood.

First described more than 80 years ago,<sup>2,3</sup> the development of iron deficiency among blood donors results from differences between rates of iron loss from blood donation and iron repletion from a regular postdonation diet. Donation of a single whole blood unit results in the loss of 200 to 250 mg of iron. Without supplemental iron, dietary iron intake typically requires >170 days to replace iron lost during donation,<sup>4</sup> significantly longer than the minimum 56 days between whole blood donations mandated in the United States. In addition, other causes of chronic blood loss (eg, menstruation),



Study schema of the DIDS. Frequent blood donors who were iron (Fe) deficient (defined as ferritin <15 µg/L and zinc protoporphyrin >60 µmol/mol heme) but eligible for blood donation were recruited. Each participant donated an RBC unit, which was stored for 40 to 42 days, labeled with chromium-51 (<sup>51</sup>Cr), autologously transfused, and then assessed for 24-hour posttransfusion recovery. These individuals were then randomized to receive intravenous (IV) saline or iron. Approximately 145 days (129-162 days, with some pandemic-related exceptions) after randomization, participants donated a second RBC unit, which was likewise stored for 40 to 42 days, labeled with <sup>51</sup>Cr, autologously transfused, and assessed for 24-hour posttransfusion recovery. Assessment of quality of life (via the RAND Health Survey) and cognitive function (via the Cognition Fluid Composite Score) was done before each blood donation and measurement of 24-hour posttransfusion recovery. Although iron repletion successfully corrected iron deficiency, on average, RBC units donated from iron-repleted individuals failed to display any difference in 24-hour posttransfusion recovery when compared with saline-treated controls. Secondary analyses, including quality of life and cognitive function, likewise found no difference between iron-repleted and saline-treated blood donor participants.

reduced intake of bioavailable iron (eg, dietary choices), or reduced iron absorption (eg, use of acid blocking agents) can further increase iron deficits in blood donors.<sup>5</sup> As a result, substantial effort has been taken to define the scope and clinical implications of iron deficiency in blood donors.<sup>5</sup>

Although iron-deficient individuals with hematocrit levels below the requirements for donation are deferred, up to 35% of individuals with acceptable hematocrit levels for donation may have ferritin levels indicative of iron deficiency. Given the nonanemic sequelae of iron deficiency, most notably impaired neurological function,<sup>6</sup> expert opinion-based policy has recommended that donation facilities “monitor, limit, or

prevent iron deficiency in blood donors.”<sup>7</sup> In this context, the Hemoglobin and Iron Recovery Study demonstrated that iron supplementation can increase the rate of hemoglobin and ferritin recovery after donation of a single whole blood unit.<sup>4</sup> However, a recent placebo-controlled trial in iron-deficient blood donors showed that although iron repletion increased hemoglobin, no changes in self-reported fatigue or general well-being were noted,<sup>8</sup> suggesting that the benefits of iron supplementation on overall donor health may be minimal.

Before the DIDS, 2 key questions regarding blood donor iron deficiency remained: (1) Does iron repletion of iron-deficient blood donors improve the

quality of the subsequently donated RBC product as measured by posttransfusion recovery? (2) Does iron repletion of iron-deficient blood donors improve donor cognitive performance? To address these questions, Hod et al enrolled 79 frequent blood donors who were iron deficient and eligible to donate blood (see figure). Among these 79 study participants, 54 were women (68%) and 25 were men (32%), consistent with the sex-biased prevalence of iron deficiency among blood donors.<sup>1</sup>

The study used a trial design that enabled within-subject measurements before and after intravenous iron or saline. Study subjects first received an autologous transfusion of chromium-51 radiolabeled 42-day-old RBCs, followed by quantitation of radiolabeled cells in circulation 24 hours posttransfusion; according to US Food and Drug Administration regulations, >75% of radiolabeled RBCs must be detectable 24 hours posttransfusion. Within 30 days of this first measurement of posttransfusion RBC recovery, subjects were randomized to receive 1 g intravenous iron or saline. Approximately 5 months after randomization, a second autologous collection and transfusion of radiolabeled RBCs was performed, followed by a second measurement of 24-hour posttransfusion RBC recovery. Within-subject change in posttransfusion RBC recovery between the first and second transfusions was the study’s primary outcome. In addition, laboratory markers of iron status and standardized assessments of cognitive function and quality of life were performed.

Blood donors in the iron repletion group showed increased hemoglobin, ferritin, and hepcidin levels and decreased zinc protoporphyrin over time. The hemoglobin concentration in RBC units collected from donors in the iron repletion group was also higher compared with donors in the saline group. Comparing the first and second autologous transfusions among all study subjects, there was no significant difference in 24-hour posttransfusion RBC recovery between the iron repletion and saline groups. Assessment of quality of life and cognitive function also did not reveal any significant differences between donors in the iron and saline groups.

Altogether, the DIDS randomized controlled trial suggests that, on average, correction of iron deficiency in frequent

blood donors does not significantly affect posttransfusion RBC recovery or donor cognitive function. Nonetheless, it remains unclear if there are specific subsets of donors for whom iron repletion might improve blood product quality or cognition. Indeed, female subjects, but not male subjects, who received iron repletion showed a statistically significant increase in posttransfusion RBC recovery. Moreover, the study excluded individuals 16 to 18 years old, who comprise >10% of donors in the United States and are at higher risk of developing iron deficiency and related complications.<sup>9,10</sup> Such individuals may be particularly vulnerable to iron deficiency due to ongoing neurological development where the consequences of iron deficiency may not be acutely manifest, but instead become apparent at much later time points postdonation. Identification of individuals at highest risk of morbidity due to iron deficiency could help guide donor-specific recommendations for iron repletion, changes in the eligible age range, and alterations in donation frequency. Although these additional questions remain, the present results provide important guidance on the impact of iron replacement for iron-deficient blood donors and suggest that the overall quality of the subsequently donated unit and the cognitive performance of the donor remain largely unaffected by iron replacement.

*Conflict-of-interest disclosure:* S.R.S. is a consultant for Alexion, Novartis, Cellics, and Argenx, and receives honoraria for speaking engagements for Grifols. R.B. declares no competing financial interests. ■

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## TRANSPLANTATION

Comment on Kong et al, page 2740

# Chronic GVHD on the move

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**In this issue of *Blood*, Kong et al present an additional pathophysiologic link between autoimmune diseases and chronic graft-versus-host disease (GVHD).<sup>1</sup> They demonstrate that peripheral T helper (T<sub>h</sub>) cells and tissue-resident T helper (T<sub>rh</sub>) cells not only are clonally related but also are able to traffic between peripheral blood and target organs of chronic GVHD.**

Recent progress has been made in treating chronic GVHD, and understanding of its highly complex pathophysiology is increasing. Despite these advances, this autoimmune-like clinical condition, with its diverse manifestations, remains an obstacle to successful allogeneic hematopoietic cell transplantation (allo-HCT), due to its impact on quality of life, morbidity, and mortality. In fact, the incidence of chronic GVHD has increased, owing to the increasing age of the patient population, the increased use of unrelated donors, and a lower level of treatment-related mortality.<sup>2</sup> In approximately half the patients with chronic GVHD, only 1 or 2 organs are involved, but many patients have multiorgan involvement, which can occur simultaneously or as sequential manifestations over the years. The particular organs that become involved, and whether flares of chronic GVHD occur, are largely unexplained and cannot be predicted clinically.

One critical insight is that chronic GVHD is not a single entity caused by a distinct immunopathologic mechanism; rather, its manifestations are mediated by several

acute and chronic inflammatory pathways, as well as by dysregulated immunity leading to aberrant tissue repair, fibrosis, and immune dysfunction.<sup>3,4</sup> In contrast to acute GVHD, which is typically caused by postthymic donor T cells, chronic GVHD cannot be attributed to just one cell population. Even nascent, stem cell-derived T cells that have undergone selection in the host's thymus may cause auto-reactivity, as thymic and lymphoid tissues can be damaged by transplant conditioning and/or acute GVHD. Similarly, B cells and plasma cells can produce autoantibodies, resulting in chronic GVHD that clinically resembles antibody-mediated autoimmune diseases. The modeling and study of such highly complex, pathologic events, either in preclinical mouse models or by use of primary human samples, remain challenging, and only a few have achieved success in this endeavor.

Defu Zeng and his group are among the researchers who have made significant contributions to a better understanding of the pathophysiology of GVHD. They recently reported that following allo-HCT,