BLOOD DONATION AND DONOR RECRUITMENT

Brief report

Thrombophilia screening of related hematopoietic stem cell donors before collection: results of a single-center activity

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INTRODUCTION

Allogeneic stem cell transplantation (ASCT) is a life-saving therapeutic option for specific onco-hematological diseases that is made possible through the donation of hematopoietic stem cells (HSCs) from a healthy related or unrelated donor. With the exception of umbilical cord blood, donation occurs through collection of HSCs by two modalities: bone marrow harvest from the iliac crests and leukapheresis after mobilization with granulocyte colony-stimulating factor (G-CSF) and eventually Plerixafor. This latter modality is currently the most common due to its greater convenience, as it does not require general anesthesia or hospitalization and is associated with a more rapid donor recovery.

Ensuring donor safety is an utmost responsibility of the medical staff at each donation center, which carefully evaluates all potential donors and provides the final clearance; the absence of any contraindication to donate is documented, based on medical history and health status at the time of assessments. Although the administration of G-CSF has been proven to be safe over 25 years of clinical use, initial concerns were raised about potential secondary effects such as an increased risk of cancer, autoimmune diseases, and arterial or venous thromboembolic events. Regarding this latter, in vitro studies on HSC donors[1,2] demonstrated an increased hypercoagulable state associated with G-CSF, emphasizing the importance of assessing each donor's coagulation profile to manage potential thrombotic predisposition. However, clinical experience accumulated over the years has ruled out an increased incidence of thrombotic events following HSC donation, regardless of the modality of donation[3,4].

Importantly, eligibility criteria of unrelated adult donor are usually more stringent than those of related donors; in particular, a high variability of thrombophilia screening before collection of HSCs has been documented among the donation centers[5,6]. Given the observed increased use of related donors due to the recent expansion of haploidentical ASCT[7], it is therefore crucial to define the optimal approach for related donors as well.

The present study is an attempt to contribute to this issue by describing the local policy on thrombophilia screening and the management of prothrombotic risk at the donation center of the ASST Grande Ospedale Metropolitano Niguarda, reinforcing the principle of protecting healthy donors from adverse events. The study includes related donors (HLA-identical sibling and haploidentical) who donated HSCs via leukapheresis over the past six years.

Arrived: 06 February 2025
Revision accepted: 22 May 2025
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MATERIALS AND METHODS

Thrombophilia screening

After referral by the physicians who are treating the ASCT recipient, each potential HSC donor undergoes a thorough medical history evaluation[8], physical examination and a work-up including instrumental and laboratory tests at the donation center. According to our local policy, laboratory testing includes the "routine" thrombophilia screening by the plasma levels of protein C, free protein S, antithrombin (AT), PT/PTT, lupus anticoagulant (LAC), and homocysteinemia for all potential donors; factor V Leiden and the G20210A mutation of prothrombin (FII) are tested only in donors with a first-degree relative with a history of venous thromboembolism (VTE) while potential donors with a personal history of prior venous or arterial thromboembolism are excluded. This approach aligns with the evaluation criteria for adults unrelated donors in our country[9].

Hematopoietic stem cell mobilization

Mobilization, i.e., the "transfer" of HSCs from the bone marrow to peripheral blood for subsequent collection via apheresis, is achieved by administering G-CSF starting 4-5 days before the scheduled donation date, upon informed consent obtained for both mobilization and donation. The most commonly used agents are lenograstim and filgrastim, including biosimilars. At our center, both are used at a dose of 10 μg/kg/day, administered in two injections every 12 hours, beginning on the evening of the fifth day before donation. A second mobilizing agent, plerixafor, is administered to donors in addition to G-CSF when their blood CD34+ count is not adequate for an optimal donation ("poor-mobilizers")[10]. The trigger for Plerixafor administration is a CD34+ count on peripheral blood <20/μL on the fifth day of mobilization with G-CSF[11].

Leukapheresis

The stem cell collection is performed through leukapheresis at the donation center, by processing up to a maximum of three total blood volumes according to the levels of HSCs in peripheral blood and the target of CD34+ cells as requested by the transplant center. The final actual processed volume depends on predicted yield[12] and procedure is performed using peripheral venous access unless derogation due to lack of suitable peripheral access of a donor being the only eligible one. The procedure can be repeated the following day if the target is not achieved on the first collection day.

Post-donation follow-up

Donors are followed up by the staff of the same donation center through clinical and laboratory evaluations at 24 or 48 hours, one week, one month, six months, and annually for ten years post-donation or whenever deemed to be necessary. The follow-up aims at monitoring potential adverse events associated to donation in the short, medium, and long term.

RESULTS

A total of 99 donations obtained via leukapheresis from an equal number of adult related donors at the donation center of the ASST Grande Ospedale Metropolitano Niguarda were included in the study. Collections occurred between September 2018 and September 2024 and related donors were HLA-identical siblings or haploidentical donors, these latter being parents, siblings or offspring in most cases. The male-to-female ratio was 57/42, with a median age of 44 years (range: 19-70). All donors except five underwent the leukapheresis procedure using peripheral venous access and 82 out of 99 collected the requested HSCs with one procedure. None of the donors had a history of VTE. Three donors had a first-degree relative with VTE and were therefore tested for Factor V Leiden and the G20210A mutation of FII in addition to standard thrombophilia screening: all mutation tests were negative. Results are summarized in **Table I**.

Protein S, protein C and AT values were found out of range in four (4%), two (2%) and three (3%) donors, respectively. However, only one donor required enoxaparin prophylaxis due to clinically significant protein S deficiency (plasma levels of 39%), confirmed on a repeated testing. Enoxaparin was administered subcutaneously at a dose of 6.000 IU/die beginning the first day of G-CSF up to the day following leukapheresis. Tolerance was optimal with no adverse events and the CD34+ target was achieved after a single leukapheresis. All the remaining donors with any protein C, protein S or AT value out of range had plasma levels above 50%, that had been deemed non clinically significant by our hemostasis center and therefore not deserving prophylaxis. Thirteen donors with hyperhomocysteinemia received folic acid supplementation at 5 mg/day from work-up until donation. PT/PTT were within the normal range in all donors, and no one tested positive for LAC.

No arterial or venous thromboembolic events were observed during mobilization, donation, or follow-up.

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Blood Transfus. 2025; 23(5): 389-392 doi: 10.2450/BloodTransfus.1021

Table I - *Coagulation tests among the 99 donors*

Test	Median (range)	Normal range	No. out of range
Protein C	104 (64-150)%	70-140%	5
Free protein S	92 (39-150)%	60-139%	6
AT	100 (51-132)%	83-118%	6
LAC ratio	1.05 (0,83-1,25)	≤1,25	0
Homocysteinemia	13,2 (9,7-61,8) µmol/L	3-15 µmol/L	12

Out-of-range values are both below or above the normal range; No. is the number of donors with any value out of range.

As of December 15, 2024, the median post-donation follow-up duration is 47 months (range: 2-74), with a total observation period of 352 person-years.

DISCUSSION

The present results confirm the safety of stem cell donation in terms of thromboembolic risk (no events occurred after a follow-up of 352 person-years) and describe our local approach of thrombophilia screening among related donors, mirroring that of the adult unrelated setting. Among the 99 donors included in the present study, only one had a clinically significant protein S deficiency requiring prophylactic anticoagulation, which showed to be safe and did not interfere with mobilization and donation. To our knowledge only one similar study[13] has been published to date, reporting comparable laboratory findings, although all 72 donors in that study received prophylactic low-molecular-weight heparin as part of that center's policy.

Similarly, the absence of VTE among our donors supports the policy of administering genetic testing of Factor V Leiden and G20210A FII mutation only to donors with a family history of VTE and not to all donors, enhancing cost-effectiveness. If we had extended those genetic testing to all donors, it is likely that some of these mutations might have been detected, due to their prevalence to the general population of 0.7 to 7%[14]; however, the absence of VTE in our cohort supports the current targeted screening strategy, because hypothetical undetected Factor V Leiden or G20210A FII mutations would have been non clinically significant in terms of thromboembolic risk associated with the donation. Theoretically, combined genetic alterations conferring higher thrombotic risk would be undetected by the current targeted strategy although this eventuality is very rare. Reassuringly, a recent study on over 21.000 unrelated donors[4] reported thrombotic event rates of 132 and 131 per 100.000 person-years among leukapheresis and bone marrow harvest donors, respectively, aligning with general population rates[15] and reinforcing the safety of donation. Importantly, we believe that careful evaluation is essential for related as well as unrelated donors, upon the shared principle of donor safety as a priority. From November 2022, exclusion of related donors with high-risk thrombophilia among all the Italian donation centers is applied, even in the absence of prior VTE[16]. An updated multicenter evaluation among donation centers would be of great interest, after the paper by Coluccia et al. published over 10 years ago and containing little information about thrombophilic screening (only No.=4 donation centers)[5].

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CONCLUSIONS

In conclusion, the present study describes a targeted strategy of thrombophilia screening among related HSC donors, confirming the safety of donation in terms of thrombotic risk, consistently with the main current literature. Among 99 donors managed by the same team of healthcare workers over the six last years, no VTE events occurred at a median follow-up of four years and a total observation period of 352 person-years, with only one requiring anticoagulant prophylaxis.

While meticulous evaluation remains essential to prioritize donor safety, our findings do not currently indicate the need for additional screening measures. Further multicenter studies could provide additional insights to potentially harmonize screening practices among the donation centers.

ACKNOWLEDGMENTS

We thank all the healthcare workers of the Immunohematology and Transfusion Medicine Service at the ASST Grande Ospedale Metropolitano Niguarda for their dedication to donors' care and safety.

AUTHORSHIP CONTRIBUTIONS

RC conceived the study, evaluated the donors, performed leukapheresis and wrote the manuscript; LB, CG, CL, LR, AMM, AS, MP evaluated the donors and performed leukapheresis; PC and EV supervised the manuscript; FD helped in data collection and analysis.

The Authors declare no conflicts of interest.

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