

Letter to the Editor

Circulating endothelial cells as markers of endothelial dysfunction during hematopoietic stem cell transplantation for pediatric primary immunodeficiency

To the Editor:

Endothelial damage is the pathological hallmark of numerous acute complications occurring during hematopoietic stem cell transplantation (HSCT): sinusoidal obstruction syndrome (SOS), transplantation-associated thrombotic microangiopathy (TA-TMA), capillary leak syndrome (CLS), and pulmonary arterial hypertension (PAH).¹ These disorders remain an important cause of morbidity and mortality, despite improvement in their prevention and their therapy. Many factors are thought to induce endothelial dysfunction: the underlying disease, the conditioning regimen, the immunosuppressive therapy, the intercurrent infections, and graft-versus-host disease (GVHD).¹⁻³ Circulating endothelial cells (CECs) are specific and sensitive markers of endothelial dysfunction. They have been used in a variety of pathological conditions to monitor the occurrence of vascular damage.⁴ Because vascular complications impact the outcome of HSCT for several types of primary immunodeficiency (PID),

we decided to monitor endothelial dysfunction during HSCT for PID through the measurement of CECs.

Thirty-four pediatric patients treated in the department of Pediatric Immuno-Hematology at the Necker-Enfants Malades Hospital who received allogeneic HSCT after full-intensity conditioning regimen for PID were enrolled between April 2011 and December 2013. Informed consent for participation in the study was obtained from the children's parents in accordance with French regulatory requirements and the Declaration of Helsinki.

CECs were monitored on fresh total blood samples within 6 hours after the samples were taken. The quantification of CECs was performed according to the consensus isolation and enumeration protocol.⁵ The monitoring was performed at the time of transplant (day 0), day 15, day 30, and eventually day 60 post-transplant if the patient was still hospitalized. Blood samples were not collected within 5 days of a severe sepsis to avoid any confounding factor.⁶

Conditioning regimens were performed according to the guidelines of the European Group for Blood and Marrow Transplantation for primary immunodeficiency and metabolic disease. GVHD prophylaxis consisted of *in vitro* T-cell depletion in case of haploidentical or mismatched unrelated donor, or a combination

TABLE I. Hematopoietic transplantation characteristics

	No vascular event (n = 23)	Vascular event (n = 11)	Total patients (n = 34)
Sex ratio ♀/♂	9/14	3/8	12/22
Age: median (range)	3 y (0.5-14 y)	0.9 y (0.25-9 y)	3 and 5 y (0.25-14 y)
Diagnosis			
SCID	6	2	8
CID	8	5	13
HLH	5	3	8
Neutrophil disorders	3	1	5
Donor			
Matched sibling donor	7	0	7
MUD	7	5	12
MMUD	4	2	6
Haploidentical donor	5	4	9
Defibrotide conditioning regimen	8	9	17
Bu/Flu/ATG	16	6	22
Bu/Flu/ThioTEPA/ATG	6	5	11
Mel/Flu/Alemtuzumab	1	0	1
Graft characteristics			
<i>In vitro</i> T-depletion	5	6	11
No <i>in vitro</i> T-depletion	18	5	23
CD34 cell dose (10 ⁶ /kg)	8.2 (1.4-24.2)	13 (5-24.2)	10.5 (1.4-24.2)
Engraftment day	19 (9-49)	18.5 (13-31)	19 (9-49)
Rejection	2	1	3
GVHD	8	3	11
Grade 1-2	6	1	7
Grade 3-4	2	2	4
Time onset (days)	16.5 (2-60)	27 (10-47)	17 (2-60)
CMV systemic replication	4	3	7
Severe sepsis	2	1	3
Duration of hospitalization post-HSCT (days)	78 (38-212)	92 (56-155)	83 (38-212)
Death	3	5	8

For CD34 number, engraftment, time of onset of GVHD, and duration of post-HSCT hospitalization, median time is indicated with range in brackets.

ATG, Antithymocyte globulin; Bu, busulfan; CID, combined immunodeficiency; CMV, cytomegalovirus; Flu, fludarabine; HLH, hemophagocytic lymphohistiocytosis; Mel, Melphalan; MMUD, mismatched unrelated donor; MUD, matched unrelated donor; SCID, severe combined immunodeficiency.

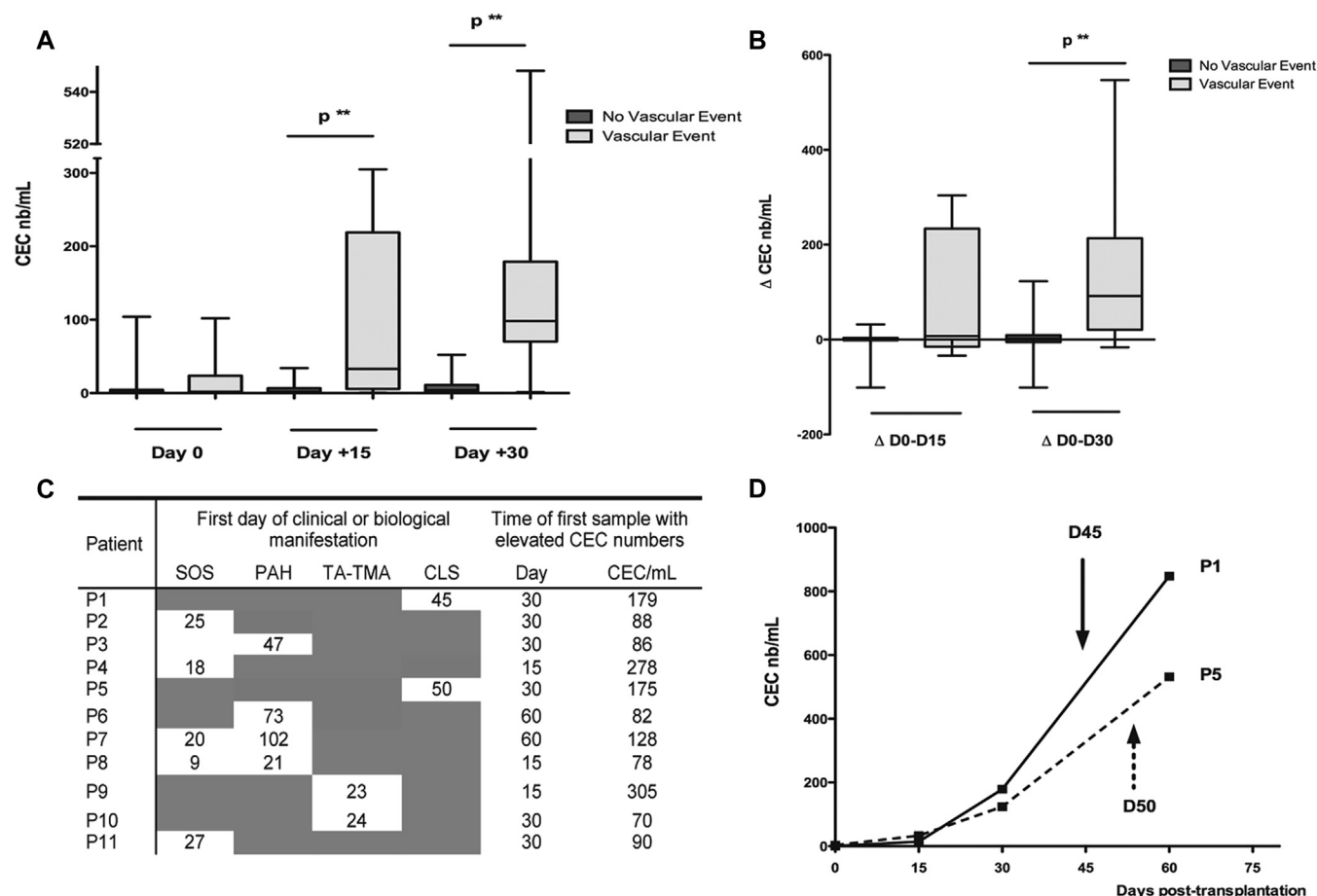


FIG 1. Comparison of CEC counts (**A**) between patients presenting with or without VE at day 0, 15, and 30 post-transplant. CEC counts increase (**B**) significantly during the first month ($\Delta D0-D30$) post-transplant in patients with VE as compared with patients without VE. Statistical analyses were performed with Mann-Whitney *U* test. VE characteristics (**C**). CEC count kinetics in patients with CLS (**D**). Vertical arrows symbolize onset of clinical symptoms. $**P = .001-.01$.

of cyclosporine with mycophenolate mofetil in case of matched sibling or matched unrelated donor. All patients received ursodeoxycholic acid as SOS prophylaxis from the start of the conditioning regimen until day 60. Young patients (aged <1 year) and patients with an underlying disease at high risk for SOS received prophylactic defibrotide treatment from day 0 to day 28. Engraftment was defined as the first of 3 consecutive days of an absolute neutrophil count of 0.5 g/L. Assessment and grading of acute GVHD were done using established standardized criteria. Diagnosis of SOS was made according to the Baltimore criteria. PAH was screened by Doppler echocardiography and confirmed by right-sided cardiac catheterization. TA-TMA was defined as evidence of hemolysis with presence of schizocytes >2/hpf, negative Coombs test, and elevated blood pressure. CLS was defined as a clinico-biological state of hypotension, hypovolemia, generalized edema, and hypoalbuminemia. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software, Inc, La Jolla, Calif) with Mann-Whitney *U* test and Fisher exact test.

The median age at transplant was 3.5 years (the range was 3 months to 14 years), with a large male predominance. Indication for HSCT included severe, combined immunodeficiencies (8 patients), combined immunodeficiencies (13 patients), hemophagocytic lymphohistiocytosis (8 patients), and neutrophil disorders (5 patients). The patients' characteristics are

summarized in Table I. There was no significant difference between the patients with or without vascular events (VE) on univariate analysis, except for the younger age of the population presenting vascular complication ($P = .04$).

Vascular complications occurred in 11 patients (32%) and are detailed in Fig 1, C. Of note, all patients who developed SOS were under defibrotide prophylaxis. Two patients developed CLS in association with grade IV GVHD. TA-TMA occurred in 2 patients and resolved in both after cyclosporine withdrawal. Patients with VE had significantly higher CEC counts (median = 33/mL; range: 5.5-305) as compared to patients without VE (median = 2/mL; range: 0-34) at day 15 ($P = .005$). The CEC count was persistently higher at day 30 in patients with VE (median = 98/mL; range: 1-548) as compared to patients without VE (median = 4/mL; range: 2-52; $P = .002$) (Fig 1, A). Moreover, the increase of CEC counts was significantly higher in patients with VE during the first month post-transplant (median $\Delta D0-D30 = 92$; range: 16.5-547; $P = .007$) as compared to patients without (Fig 1, B). Among the patients without VE, all but 2 patients had a CEC count lower than 15/mL at day 15 (sensitivity: 55%, specificity: 91%). None of the patients without VE had a CEC level above 60/mL during the entire follow-up, while all patients who developed endothelial complication had at least 1 blood sample with CEC count above this threshold (sensitivity

and specificity: 100%). Only 2 patients with VE had low CEC counts at day 30; they developed PAH at day 73 and 102 and had abnormal CEC counts measured at day 60 (77/mL and 125/mL). In our study, CEC counts failed to efficiently predict the onset of SOS. However, high CEC counts were detected before the onset of other vascular complications in 7 patients out of 8 (Fig 1, C). Of note, no correlations on univariate analysis were found between CEC counts and the following variables: underlying disease, intensity of conditioning regimen,⁷ occurrence of cytomegalovirus reactivation, defibrotide prophylaxis, GVHD, and type of complication or death.

In summary, we show that high CEC counts are associated with the occurrence of endothelial complications in children undergoing HSCT for PID. These encouraging preliminary results warrant the initiation of larger studies with tighter monitoring schedules to validate the extensive use of CEC enumeration as a predictive tool of vascular complication in high-risk patients.

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This work was supported by the Assistance Publique-Hôpitaux de Paris.

Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2014.05.039>