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Response

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To the Editor:

We are glad to see interest in our findings regarding the possibility that some of the hematopoietic stem cell transplantation (HSCT) thrombotic microangiopathies (TMAs) might be triggered by mutations in complement regulatory genes acquired from HSCT donors. Here we try to clarify the points raised by Gavriilaki et al. [1] in their letter to the editor.

First, we agree that our patients' disease might be viewed as atypical hemolytic uremic syndrome (aHUS), but patients were not selected: We have screened all consecutive patients referred to our center by experienced hematologists involved in HSCT because of reported post-HSCT TMA. The diagnostic criteria are those currently in use. In particular, proteinuria and impaired renal function are present in the criteria of Jodele et al. [2] as well as in those of the Bone Marrow Transplant Clinical Trials Network [3]. Although minor differences among the published diagnostic criteria exist [4], we clearly stated that all our HSCT patients showed evidence of platelet consumption, nonimmune-mediated hemolysis, and signs of renal damage [5], in line with a diagnosis of TMA; thus, a bias of selection can be ruled out.

Second, unfortunately, no method has been described that reliably detects complement activation in TMA. For example, patients with primary aHUS, which is clearly related to complement activation, show C3 consumption only in half of cases [6]. Complement activation products such as C5a and the terminal complement complex C5b-9 are often high in aHUS, but many patients cannot be accurately classified because of significant value overlaps [7]. The modified Ham test, proposed by Gavriilaki et al. [8], is an elegant and very promising method but needs to be validated in larger series of patients and control subjects. In our experience complement activation markers are clinically poorly informative even if they are frequently elevated in TMA patients. Nevertheless, in response to Gavriilaki et al. [1], we had the measured plasma levels of C5b9 in 4 of 6 patients with complement mutations (354 ± 64 ng/mL) and in 5 of 10 patients without mutation (332 ± 29 ng/mL), showing a significant increase compared with normal control subjects (167 ± 71 ng/mL; $P = .01$) but no statistical difference between the 2 group.

Third, donor DNAs were obtained from HSCT recipient peripheral blood after the documented achievement of full bone marrow donor chimerism. At the same time, recipient DNAs were obtained from buccal brushing. Contamination of DNA buccal brushing with donor DNA was excluded by means of quantitative fluorescent PCR. We assessed donor–recipient chimerism on unfractionated bone marrow samples with a commercial assay based on quantitative PCR detection of insertion/deletion polymorphisms, all outside the HLA complex (AlleleSEQR Chimerism Assay; Celera Genomics, Rockville, MD). The method for chimerism evaluation was not reported in our article because of space constrain, but the different genetic patterns obtained further support the full bone marrow donor chimerism. As far as the statement that "patients' complement proteins…should be…universally replaced by the donor's proteins," this raises issues regarding the very unclear mechanism by which mutations predispose to the disease. The vast majority of the mutations are in heterozygous state, raising the possibility of interactions between mutated and nonmutated proteins [9]. We simply have no data on

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mechanisms to discuss linking the detected mutations and the TMA.

Finally, our article was not meant to describe in detail each case but rather to share with the scientific community the observation that regulatory complement gene variants can be acquired via HSCT (in 37% of cases in our unselected series) and may express itself in the recipient. We agree that large prospective studies are necessary to better elucidate the still unclear pathogenesis of this severe and life-threatening condition. Our data indicate that in addition to the mutations of complement genes observed pretransplant in patients [10], the mutations acquired post-transplant from the donor should also be taken into account. We underline that 6 of 16 patients (37.5%) screened is not a trivial proportion and cannot be considered a casual finding given the incidence of these variants in the general population. Ours was not a planned study; therefore, a control group without TMA was not originally scheduled.

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