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Contribution: D.C.R. designed the study, analyzed the data and wrote the manuscript. C.L. and E.C. performed G6PD assays and commented on the manuscript. J.B. collected data and commented on the manuscript. D.G. and C.D. performed TCD measurements and commented on the manuscript. S.L.T. helped design the study and contributed to the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Response

G6PD deficiency and cerebrovascular disease in sickle cell anemia?

We would like to thank Dr Rees and colleagues for their interest in our paper. Their own work on the King's College sickle cell anemia (SCA) cohort does not confirm our Centre Hospitalier Intercommunal Créteil (CHIC) study showing that G6PD deficiency is an independent significant risk factor for abnormal high arterial velocities on transcranial Doppler (TCD).

Measuring G6PD activity in SCA patients is difficult because of the high level of reticulocytes, which may result in an overestimation of the activity. In addition, the dosage has to be performed 3 months away from transfusions. Rees et al, however, found the same incidence of G6PD deficiency in their cohort as we did in ours (11% vs 11.1%); therefore, it seems unlikely that the observed discrepancy between our 2 studies could be explained by differences in G6PD activity measurements.

In contrast, we believe that major differences in the number of TCD evaluations, the length of study follow-up, and the definition of abnormal TCD could explain, in large part, the divergent results between our studies.

The CHIC experience of exploration by TCD is longer. Systematic TCD screening for CHIC began in 1992, whereas that of the King's College began in 2004.[1]

The number of patients with available G6PD status and TCD was 155 in the King's College cohort, but 325 in the CHIC cohort.

Furthermore, only 10/155 (6.5%) patients had TAMMX greater than or equal to 200 cm/second in the King's College cohort versus 55/325 (16.9%) in the CHIC cohort. This can be due to 2 factors:

· ***A shorter follow-up.*** As we demonstrated in our newborn cohort,[2] velocities greater than or equal to 200 cm/second occur before the age of 9 years; thus, the study by Rees et al could have missed this occurrence.

· ***Differences in the definition of abnormal velocities.*** Rees et al have defined TAMMX greater than 169 cm/second as abnormal, whereas we have considered as abnormal TAMMX greater than or equal to 200 cm/second (as in the Stroke Prevention Trial in Sickle Cell Anemia [STOP] study) and only initiated transfusion programs in these patients. They justify the cutoff by stating that they used TCD imaging (also used in our cohort), which tends to give lower values than TCD nonimaging. It is unlikely that the difference is as large that TAMMX greater than or equal to 169 cm/second could be considered abnormal. Moreover, no significant difference was found in a recent report comparing both methods.[3] Thus, the study by Rees et al may have included patients with only marginally abnormal velocities. In addition, if these patients were transfused early, it could have influenced the occurrence of vasculopathy.

Our findings on the role of G6PD as a risk factor for high velocities was strengthened by our study in 280 SCA patients from the CHIC cohort using magnetic resonance angiography/magnetic resonance imaging (MRA/MRI), (follow-up of 2139 patient-years), which showed that G6PD deficiency ($P = .008$) was a highly significant risk factor for stenoses.[4]

As Rees et al did not find an association between G6PD deficiency and cerebrovasculopathy in their cohort, we believe that additional studies in different cohorts will be necessary to definitively confirm the influence of G6PD deficiency on cerebral vasculopathy.

Nevertheless, our findings of an association between G6PD deficiency and abnormal TCD and stenoses seem relevant because

of accumulating data now supporting a link between oxidative enzymopathies and vascular disease.[5] In G6PD-deficient endothelial cells, there is increased oxidative stress and decreased NO levels.[6] This "enzymatic vasculopathy" hypothesis could be particularly relevant in SCA, a monogenic disease with highly variable clinical profile, probably due to polymorphism in other factors, including those influencing vascular biology.

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Conflict-of-interest disclosure: The authors declare no competing financial interests.

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To the editor:

Proportions of immature CD19$^+$CD21$^-$ B lymphocytes predict the response to extracorporeal photopheresis in patients with chronic graft-versus-host disease

B lymphocytes are increasingly assigned an important role in the pathogenesis of auto-and alloimmune diseases including chronic graft-versus-host disease (cGVHD)[1-4] where abnormalities of B-cell homeostasis in patients with active cGVHD have been observed.[1,4] Extracorporeal photopheresis (ECP) achieves high response rates in steroid-refractory cGVHD.[5] At present, no reliable biomarkers are available for prediction of response to ECP and monitoring of patient outcome. We thus investigated the distribution of immature CD19$^+$CD21$^-$ B cells and memory CD19$^+$CD27$^+$ B cells in peripheral blood (PB)[1] before ECP (n = 19), 6 (n = 28), 12 (n = 34), and 21 months (n = 33) after start of ECP in 49 patients (28 male, 21 female) with moderate (n = 25) or severe (n = 24) cGVHD[6] and correlated results with clinical response.[7] Informed consent was obtained in accordance with the Declaration of Helsinki. Eighteen patients were included in a prior report.[1]

Complete (CR) and partial response (PR) to ECP was observed in 25 of 34 patients (74%) after 12 months. ECP nonresponders at 6 months had a significantly higher ($P = .02$) percentage of immature CD19$^+$CD21$^-$ B lymphocytes (mean 22%, range 4%-52%) in PB before start of ECP compared with CR (mean 8%; range 1.3%-29%) and PR (mean 16%; range 1.6%-55%) patients (Figure 1A). Percentages of memory CD19$^+$CD27$^+$ B cells before first ECP were not significantly different ($P = .12$) between the groups. The CD21$^-$/CD27$^+$ ratio was significantly higher ($P = .03$) in ECP nonresponders (mean 17.4; range 0.5-50) compared with CR patients (mean 1.6; range 0.3-5).

Percentages of immature CD19$^+$CD21$^-$ B lymphocytes were significantly ($P < .001$) lower in CR patients compared with ECP nonresponders 6 (mean 5% vs 25%; range 1%-15% vs 14%-43%), 12 (mean 6% vs 24%, range 1.2%-16% vs 1.5%-58%), and 21 (mean 6% vs 26%; range 1%-21% vs 2%-60%) months after start of ECP (Figure 1B). In addition, in ECP responders compared with ECP nonresponders the CD21$^-$/CD27$^+$ ratio was significantly lower 6 months (mean 2.2 vs 9.6; range 0.1-6.5 vs 1.2-20; $P = .001$), 12 (mean 2.7 vs 6; range 0.2-15 vs 0.6-16; $P = .006$), and 21 months (mean 2 vs 17; range 0.2-9 vs 0.6-76; $P = .001$) after start of ECP.

To our knowledge, relative amounts of immature CD19$^+$CD21$^-$ B lymphocytes represent the first reported cellular biomarker able to predict response to ECP. Whether our findings are ECP-specific has to remain speculative because no other treatment cohorts were analyzed and the mechanisms of action of ECP are still subject to further research.[5] CD19$^+$CD21$^-$ B lymphocytes could also serve as a novel biomarker for measuring disease activity of cGVHD quantitatively and objectively, which would be highly desirable.[8] Of note, CD21$^-$ B lymphocytes are increased in proportion in autoimmune diseases such as systemic lupus erythematosus and active cGVHD.[1,10] Increased proportions of CD21$^-$ B lymphocytes could be part of the autoimmune pathogenesis compatible with inefficient censoring of autoreactive B cells in cGVHD.[9] Disrupted B-cell homeostasis and relative rather than absolute preponderance of an activated B-cell pool has recently been reported in cGVHD.[4] Our findings warrant larger prospective studies analyzing the predictive value of B-cell subsets for successful ECP treatment of cGVHD.

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