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REFERENCES

1. U.S. Food & Drug Administration. Information about immune globulin (human) product shortage. 2018. https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/information-about-immune-globulin-human-product-shortage. Accessed November 21, 2019.
2. Ciszewski TS, Ralston S, Acteson D, Wasi S, Strong SJ. Protein levels and plasmapheresis intensity. *Transfus Med*. 1993;3:59-65.
3. Schulzki T, Seidel K, Storch H, et al. A prospective multicentre study on the safety of long-term intensive plasmapheresis in donors (SIPLA). *Vox Sang*. 2006;91:162-173.
4. Food and Drug Administration HHS. Volume Limits - Automated Collection of Source Plasma. https://www.fda.gov/media/70951/download. Accessed July 22, 2019.
5. Gustafson M. Source Plasma Donor Hemovigilance Activities and Results. 2017. https://www.pptaglobal.org/images/presentations/2017/Gustafson_PlasmaVigilance100817.pdf. Accessed July 22, 2019.
6. Buffaloe GW, Heineken FG. Plasma volume nomograms for use in therapeutic plasma exchange. *Transfusion*. 1983;23:355-357.
7. Lemmens HJ, Bernstein DP, Brodsky JB. Estimating blood volume in obese and morbidly obese patients. *Obes Surg*. 2006;16:773-776.

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White matter volume changes in adult beta-thalassemia: Negligible and unrelated to anemia and cognitive performances

To the Editor:

We read with great interest the study by Choi on brain involvement in anemic patients, showing white matter volume reduction according to anemia and cognitive impairment severity.[1] The findings that they reported with a quantitative analysis and showing the interplay among anemia, white matter volume changes and cognition, were both fascinating and reasonable. However, the miscellaneous recruitment of anemic patients did not help understand whether the phenomenon of white matter volume decrease was effectively a biomarker of any type of anemia. Indeed, white matter involvement has been widely shown in sickle cell disease,[2] while brain involvement is still uncertain or not fully studied in other forms of anemia. For example, recent studies with control groups have failed to detect significantly increased vascular-like white matter changes in beta-thalassemia patients.[3,4] Even very sensitive advanced MRI analyses (ie, magnetization transfer and diffusion tensor based techniques) failed to reveal structural white matter changes in beta thalassemia patients.[5] On these premises, the finding of a significant supratentorial white matter volume decrease in anemic patients in the paper by Choi appears rather in conflict with the increasing evidence of no white matter involvement in beta-thalassemia (both at conventional and advanced MR imaging investigations), at least among patients treated according to current treatment guidelines. Even though beta-thalassemia patients were only a subgroup with a limited sample size in the paper by Choi, they still represented the majority of non-sickle anemia patients (ie, 14/26 patients). For these reasons, white matter structural and volumetric changes were investigated in our large samples of adult beta-thalassemia patients (48 transfusion dependent patients, age = 38 ± 10 years, and 23 non transfusion dependent patients, age = 30 ± 11.5 years) and controls (56 healthy subjects, age = 34 ± 11 years) using a high-resolution 3DT1 MPRAGE sequence that was part of our MRI study protocol for all study participants (the same 3 T scanner and the same sequence was used, see Russo et al[5] for reference). Notably, most patients and controls had undergone a comprehensive cognitive test battery (WAIS-IV), and data on hemoglobin levels in beta-thalassemia patients were available thus allowing for a full comparison among cognitive, laboratory and white matter volume findings.

First of all, using the most validated analysis of white matter volume quantification (voxel based morphometry, VBM, see Supplementary material) we detected only a small cluster close to the ventricular wall in the left temporal lobe (coordinate: x = −30 y = −27 z = −8) of significant volume increase in TDT patients vs healthy controls ($P < .05$ cluster level corrected, cluster size = 277 voxels; Figure 1). No other subgroup comparison showed significant differences (whole group of patients vs controls, NTDT vs TDT, NTDT vs controls). In addition, no correlations were found in the patients' group between the mean white matter volumes (extracted from the above mentioned cluster), and both the hemoglobin values and the full scale Intelligence Quotient scores, that were significantly worse in beta thalassemia patients (see Tartaglione et al.[6]).

Considering the striking contrast between the present study and the study by Choi, we also applied to our sample a tensor-based morphometry analysis (TBM; see Supplementary material), i.e. the less common white matter analysis performed by Choi. By means of TBM, we failed to find any significant difference by comparing either patients subgroups or healthy controls and

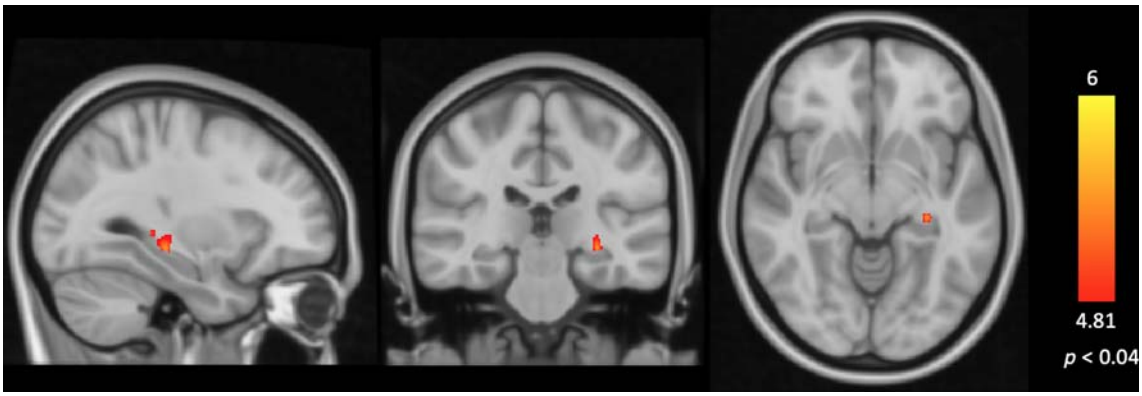

FIGURE 1 Contrast t-map representing the comparison between HC and TD from the VBM analysis in the WM

beta-thalassemia patients (as a whole group or according to disease severity).

Our analysis, performed on a large sample of beta-thalassemia patients with two different methods to study the morphometry of the white matter, revealed no structural changes comparing patients (whole group) and healthy controls or comparing beta-thalassemia patients subgroups. More precisely, just the VBM analysis revealed a small alteration in the white matter volume in the TDT subgroup (compared to controls) but, even in this small cluster, we were not able to detect any correlation between the white matter changes and anemia severity or cognitive performances. Whether discrepancies between the present study and the study by Choi were due to differences in sample size, recruitment criteria, treatments or environmental/genetic factors remains unknown. In any case, our experience on a large sample of patients does not confirm the relationship among anemia, cognition and white matter changes at least among beta-thalassemia patients, thus challenging the statement by Choi and colleagues at the hemoglobin levels maintained in our patients (93.7 ± 9.7 g/L). In addition, adding this piece of evidence, our findings further strengthen the suspicion of a limited value of MRI brain monitoring in neurologically asymptomatic beta thalassemia patients, at least when the current treatments guidelines are observed.

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REFERENCES

1. Choi S, O'Neil SH, Joshi AA, et al. Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome. *Am J Hematol*. 2019;94(10):1055-1065.
2. Manara R, Talenti G, Rampazzo P, et al. Longitudinal evaluation of cerebral white matter hyperintensities lesion volume in children with sickle cell disease. *Br J Haematol*. 2017;176(3):485-487.
3. Tartaglione I, Russo C, Elefante A, et al. No evidence of increased cerebrovascular involvement in adult neurologically-asymptomatic β-Thalassaemia. A multicentre multimodal magnetic resonance study. *Br J Haematol*. 2019;185(4):733-742.
4. Premawardhena A, Ranawaka U, Pilapitiya T, et al. Headache: an important symptom possibly linked to white matter lesions in thalassaemia. *Br J Haematol*. 2019;185(3):541-548.
5. Russo AG, Ponticorvo S, Tartaglione I, et al. No increased cerebrovascular involvement in adult beta-thalassemia by advanced MRI analyses. *Blood Cells Mol Dis*. 2019;78:9-13.

6. Tartaglione I, Manara R, Caiazza M, et al. Brain functional impairment in beta-thalassaemia: the cognitive profile in Italian neurologically asymptomatic adult patients in comparison to the reported literature. *Br J Haematol*. 2019;186(4):592-607.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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Lower white matter volume in beta-thalassemia associated with anemia and cognitive performance

To the Editor:

We would like to thank Manara et al for their interest in our manuscript, "Anemia predicts lower white matter volume and cognitive performance in sickle and non-sickle cell anemia syndrome," that investigates the relationship between anemia, white matter volume and cognitive performance in chronically anemic and non-anemic subjects.[1] Manara et al raised concerns about whether the relationship between anemia and white matter volume could be generalized outside of sickle cell disease to beta-thalassemia, as their own previous MR imaging studies failed to reveal abnormalities in patients with beta-thalassemia.[2–4] They performed group comparisons between patients with beta-thalassemia and controls but showed minimal to no differences in T1-voxel intensity (white matter density; VBM) or Deformation Indices (white matter volume/Jacobian determinants; TBM).

The discrepancies between our two analyses can be attributed to possible differences in study populations or in specific analytic methodologies. To test these possibilities, we first chose to restrict our previous analysis to patients with thalassemia and control subjects. This sub-cohort consisted of 16 thalassemia patients (age = 26.8 ± 8.2; F = 10, M = 6; hemoglobin = 10.2 ± 0.9 g/dL) and 40 controls (age = 27.7 ± 11.3; F = 28, M = 12; hemoglobin = 13.4 ± 1.3 g/dL). A detailed table of each patient's characteristics is provided in Table S1. Age and sex were not statistically different but hemoglobin count was statistically lower in our patient group ($P < .001$).

We performed TBM analysis to search for group differences between beta-thalassemia patients and controls using Deformation Index (DI) maps as described in our manuscript (section 2.5). A group of 10 000 random permutations were run to determine a null distribution of t-statistics at each voxel. Note, 50 473 voxels showed a statistically significant difference at a False Discovery Rate of $q = 0.10$ using Benjamini and Hochberg's method (Figure 1A). While fewer voxels were found significant in this more restricted population, they were spatially consistent with the results in our manuscript. The largest clusters were observed in the prefrontal cortex where beta-thalassemia patients had lower DI than controls. Thalassemia patients had larger DI than controls in the clusters found in the occipital lobe. This is also consistent with the clusters found in our previous study where the severity of anemia correlated with higher DI (opposite from most other voxels where anemia correlated with lower DI). We additionally ran correlations between hemoglobin and DI using the same procedures as our previous study, but only including thalassemia patients and controls (Figure 1B). We found 2 804 significant voxels where most clusters overlapped with clusters found in the group comparison. Fewer significant voxels were observed in comparison to our findings in our manuscript but this was expected due to the limited patient sample size and lower range of hemoglobin values.

Hemoglobin showed a significant positive correlation with mean DI across all significant voxels identified through both group testing ($P < .001$; $r^2 = 0.21$) and correlation testing ($P < .001$; $r^2 = 0.40$). Consistent with our previous study, females (n = 38) did not show significant relationships between mean DI and cognitive measures. In males (n = 18), mean DI from group testing was significantly correlated with Perceptual Reasoning Index ($P = .037$; $r^2 = 0.24$), driven by Block Design ($P = .045$; $r^2 = 0.23$), and Digit Span ($P = .044$; $r^2 = 0.23$). Matrix Reasoning showed a trend towards significance ($P = .094$; $r^2 = 0.16$). Mean DI obtained from correlation testing showed a significant positive association with Digit Span in males only ($P = .106$; $r^2 = 0.34$).

-Our observations here were largely consistent with the findings in our previous study. However, we did identify some key methodologic differences between the two studies that could contribute to the disparity with Manara's findings. We used non-parametric permutation testing that is robust to the underlying distribution while the parametric statistical method used by Manara et al assumed that calculated t-statistics followed a normal distribution. We used Benjamini and Hochberg False Discovery Rate (FDR-BH) for multiple comparison correction, instead of family-wise error rate (FWER) correction. The FWER is a more conservative approach than FDR which means that less voxels will survive correction. The FWER can be advantageous because it limits the risk of including false positives, but disadvantageous due to its lower power and sensitivity compared to FDR. We note that performing the same statistical analysis as Manara et al, 2044 significant voxels survived in our group comparisons (Figure 2). Lastly, we manually inspected and carefully edited masks at boundaries of the brain because we noted that imperfect skull stripping adversely impacted brain registration that is central for Deformation Index calculation; we cannot know if similar attention to detail was applied in the Manara analyses.

We cannot exclude that physiological differences between the two study populations might also have contributed to the disparity. Our study population was younger and much more ethnically diverse, with more than half common from the Far East. To improve genotypic homogeneity, we excluded patients with alpha thalassemia syndromes from our sub analysis, but retained three patients with transfused hemoglobin E-Beta thalassemia. Since few of our patients have been followed at Children's Hospital Los Angeles for their entire lives, we did not always know how well their hemoglobin levels were maintained in early childhood. For example, subject 15 (who had 38 discrete white matter hyperintensities)