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Pathologically stiff erythrocytes impede contraction of blood clots: Reply to comment

Dear Editor,

We thank Ilich et al.[1] for their comments on our paper[2] and their accurate summary emphasizing the importance of red blood cell (RBC) properties in thrombosis associated with sickle cell disease (SCD). The authors refer to their publication on this topic[3] and find that our results are consistent with their findings on the role of RBC rigidity in blood clot contraction and structure. The fact that related results were obtained in the two studies using different approaches is reassuring. Both research projects, which evolved over many years,[4-9] showed the effect of stiffer RBCs from SCD patients on clot contraction and the resulting clot structure, showing lesser compression of RBCs, and the effects of chemical stiffening of RBCs. While these two publications mutually reinforce the findings, these complementary but independent studies take different approaches conceptually and methodologically.

Faes et al. focused on exploring SCD in more depth, and carried out informative and important results with both human patient samples and in vivo murine models, and specifically assessed the role of RBC sickling in the retention of those cells within the clot volume, leading to a larger final clot size following contraction in their murine SCD studies. They discuss the influence of this effect on the pathogenesis of thrombosis, and show a reversal of this effect with RBC exchange. Importantly, they show that sickled RBCs increase resistance to fibrinolysis of contracted clots, pointing to another aspect of the clinical importance of exploring these mechanisms of thrombosis.

Using an alternative approach, we focused on the general mechanistic role of RBC mechanical properties on the impairment of clot contraction. Our study quantified in detail the effects of RBC stiffness on clot contraction, through in vitro studies of SCD blood, and other stiff RBCs, using chemical treatment, llama ovalocytes, and stiffening with the Wright b antibody. We determined the extent of contraction, kinetics of the three phases, work done by platelets, and percentages of biconcave RBCs, polyhedrocytes and intermediate forms. Previously, we quantified the effects of different factors on clot contraction, including variations in platelets, fibrin and RBCs, and used experiments with blood from SCD patients to show a clinically relevant example of the effect of stiffness of RBCs reducing the extent of clot contraction (fig. 5FG in[9]). However, the mechanism underlying the reduction in contraction remained unexplored. Our continuing studies further explored the role of RBC rigidity in reducing the extent of clot contraction and formation of polyhedrocytes. We carried out extensive studies on clots from SCD patients as an example of a condition with increased RBC rigidity, but also used other methods to stiffen RBCs, to ensure that the effects were not influenced by other aspects of SCD. Moreover, we looked at RBC from SCD patients in the absence of sickling to focus on the effect of this disease on the RBC membrane.

While we recognized the importance of the study by Faes et al. and cite it in our paper, we acknowledge that a detailed discussion in the context of our findings would have been appropriate. We think highly of this quite significant contribution from such an eminent group of scientists.

SCD is an important, clinically relevant condition associated with an increased risk of thrombosis that has received too little attention, so it is beneficial that multiple groups are studying the pathogenesis of thrombosis in SCD. Through these two papers, we now have a better understanding of the effects of RBC stiffness on clot contraction.

KEYWORDS

blood clotting, clot contraction, erythrocytes, sickle cell disease, thrombosis

CONFLICT OF INTEREST

None of the authors has any relevant conflict to declare.

AUTHOR CONTRIBUTIONS

All authors contributed to writing and editing this letter.

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