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Circulating microparticles in children with sickle cell anemia: a heterogeneous procoagulant storm directed by hemolysis and fetal hemoglobin

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Chronic hemolytic anemias are made up of sickle cell anemia (SCA), beta (β)-thalassemia, paroxysmal nocturnal hemoglobinuria, autoimmune hemolytic anemia, and unstable hemoglobinopathies. They are associated with a high thrombotic risk. In SCA patients, a high rate of both venous and arterial thrombosis (deep vein thrombosis, pulmonary embolism, stroke, pregnancy-related venous thromboembolism) has been reported.[1] Interestingly, these subjects commonly present with laboratory features of a subclinical hypercoagulable state,[2] charac-

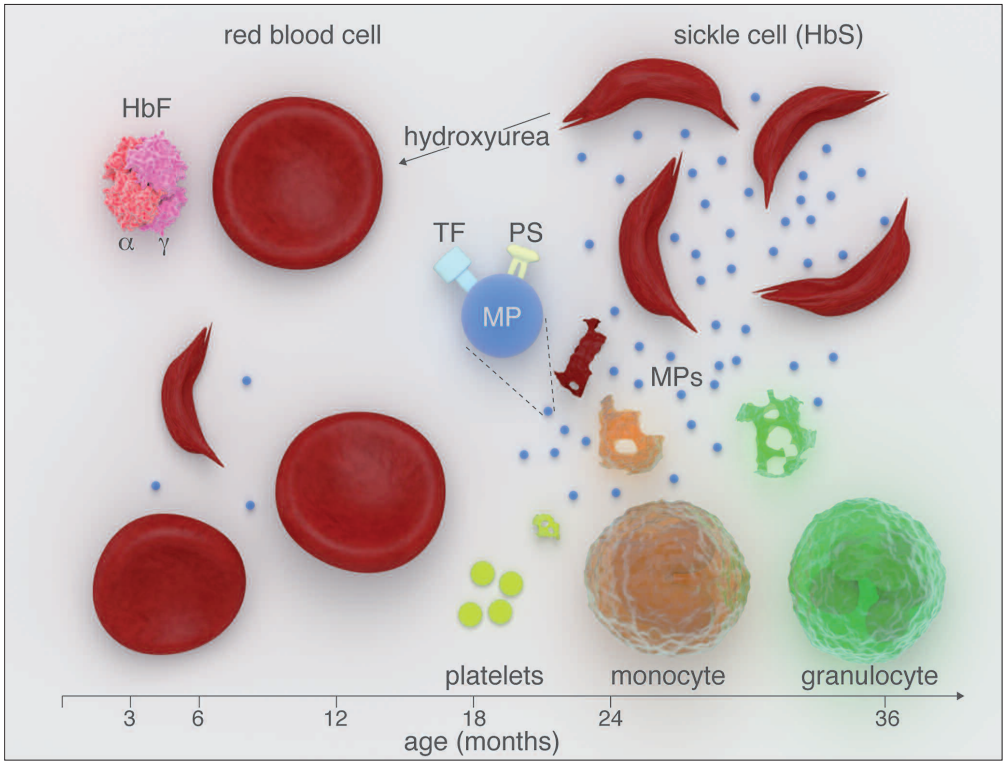

Figure 1. Mixed microparticles in SCA children act at the interface between hemolysis and clotting activation. Microparticles (MPs) of different cellular origin (e.g. red blood cells, platelets, monocytes, granulocytes) are released upon hemolysis, blood cell apoptosis, and activation. Hemolysis increases when the physiological switch from fetal (HbF) to adult hemoglobin occurs and sickle cells (characterized by HbS) are produced. *Vice versa*, hemolysis decreases when hydroxyurea therapy is started, with subsequent upregulation of HbF ($\alpha 2\gamma 2$) levels. On their surface MPs expose procoagulant phospholipids (i.e. phosphatidylserine, PS) and proteins (i.e. Tissue Factor, TF), and activate other blood cells (e.g. platelets, monocytes) and hemostasis. HbF levels inversely correlate to circulating MPs and regulate specific MP patterns, particularly those platelet-derived.

terized by increased plasma levels of markers of thrombin generation, e.g. D-Dimer, thrombin-antithrombin complex (TAT), prothrombin Fragment 1+2 (F1+2), during the non-crisis 'steady state' and, in particular, during acute pain episodes. In fact, hemolysis is a known procoagulant condition[3] due to the release of cell-free plasma hemoglobin and the depletion of nitric oxide.[4] Additional biological mechanisms of hemolysis-associated hypercoagulation include: red blood cell membrane abnormalities leading to exposure of anionic procoagulant phospholipids (i.e. phosphatidylserine), endothelial dysfunctions with overexpression of cell adhesion molecules, platelet activation, thrombocytosis following functional hyposplenism or surgical splenectomy.[2,4-6]

Importantly in this scenario is the occurrence in SCA patients of increased levels of circulating microparticles (MPs).[7] MPs are vesicles of less than 1 μm resulting from the shedding of activated or apoptotic blood and vascular cell membranes. Among their various functions, MPs exert procoagulant actions through the expression on their surface of procoagulant phospholipids (i.e. phosphatidylserine) and proteins (i.e. Tissue Factor).[8] Elevated circulating MPs are found in different clinical conditions at high thrombotic risk, e.g. diabetes mellitus, atherosclerosis, acute coronary syndrome and myocardial infarction, sepsis, antiphospholipid syndrome, malignancy.[9-12] Specifically, in SCA, MPs of erythrocyte origin produced during hemolysis carry negative niches that activate the intrinsic phase of blood coagulation (tenase and prothrombinase) leading to thrombin generation.[13] A significant correlation between the total number of MPs and the levels of markers of hypercoagulability (i.e. D-dimer, TAT, and F1+2) has been repeatedly demonstrated in SCA patients.[7,13-15] In this condition, MPs likely represent the interface between hemolysis and blood clotting activation. However, as shown for the first time by the study of Nébor *et al.* published in this Journal,[16] in SCA children there is a variety of MPs which originate not only from erythrocyte, but from virtually all blood cells, mainly platelets. In contrast to the situation for adult patients,[13-15] only limited data are available regarding MP characterization in SCA children.[5] Nébor *et al.* found that, although platelet-derived and erythrocyte-derived MPs were the most common type in this condition, all other cell origins, e.g. monocyte-, granulocyte-, and endothelial cell-derived MPs, were represented. Interestingly, the age-related reduction in HbF levels during childhood was associated with an increase in MP levels, particularly those from platelets and monocytes, and to a lesser extent those from erythrocytes. While confirming the already known inverse relation between HbF concentration and MPs formation[5] and thrombin generation,[13] these data show for the first time the specific cellular patterns involved in the process. In the same way, in this population the reactivation of fetal hemoglobin (HbF) synthesis (which impairs HbS polymerization) induced by hydroxyurea, the current standard therapy option in SCA, correlated with the reduction in plasma levels of MPs, particularly those of platelet and erythrocyte origin. Attempts to standardize the methodology for the isolation, analysis and count of MPs have been shown to have limitations and these tests can be influenced by many different factors, from blood collection up to gate analysis.[17] However, the data published here open up new perspectives on how all blood cellular compartments are involved in the clotting activation associated to SCA and, possibly, to all hemolytic anemias. In these circumstances, different subtypes of MPs act as messengers between hemolysis and

the hemostatic system activation. This also expands our vision of the possible mechanism(s) involved in the hemolytic crisis brought on by other comorbid conditions, such as sepsis. Along the same lines, there is evidence that the HbF levels, an important regulatory mechanism of SCA severity and hemolysis, govern MP concentration by acting on specific MP subtypes.

We can imagine that, in SCA children, a storm of various (mainly platelet-derived) procoagulant MPs takes place with chronic hemolysis and is driven by HbF levels (Figure 1).

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Are ongoing trials on hematologic malignancies still excluding older subjects?

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Hematologic malignancies are diseases that mainly affect older subjects. Multiple myeloma,[1] myelodysplastic syndromes[2] and chronic myeloid leukemia[3] are common in advanced age. Nevertheless, there is evidence that older patients with hematologic malignancies have often been excluded from clinical trials (CTs).[4,5] Their exclusion prevents clinicians from obtaining information concerning the efficacy and safety of treatments in older patients and might represent an important barrier to the treatment of these patients.[6] Published literature reflects trials performed some years before their publication. It is not known whether older individuals are gradually being included in more trials as a consequence of the aging of the population and of the recommendation provided by Regulatory Agencies, e.g. FDA and ICH, to include older individuals in CTs.[7,8] The aims of this study were to assess the presence and the extent of underrepresentation of older individuals in ongoing CTs on hematologic malignancies registered in an online open-access CT registry maintained by the World Health Organization (WHO), and to evaluate