HEME- AND IRON-TRIGGERED INFLAMMATION AND VASCULOTOXICITY CONTRIBUTES TO THE PATHOPHYSIOLOGY OF HEMOLYTIC ANEMIAS

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INTRODUCTION & AIMS

Increasing evidence from animal studies suggests that free heme and iron exert vasculo-toxic and proinflammatory effects due to their ability to induce endothelial and immune cell activation. A role for heme and iron in triggering inflammation and vasculopathy and promoting the pathogenesis of atherosclerosis has been postulated. In this study, we were interested to elucidate whether heme- and iron-driven vascular and inflammatory abnormalities manifest in patients with hemolytic diseases.

METHODS

To this purpose, we analyzed systemic markers of vascular damage, oxidative stress and inflammation and their correlation with heme and iron levels in serum samples from cohorts of transfusion-dependent patients with β-thalassemia major and intermedia (β-thal major and int.), sickle cell disease (SCD) and spherocytosis (SPH). One cohort of SCD patients received simple transfusions (SCD1) and the second cohort exchange transfusions (SCD2).

SYSTEMIC HEME & IRON OVERLOAD IN THAL, SCD AND SPH

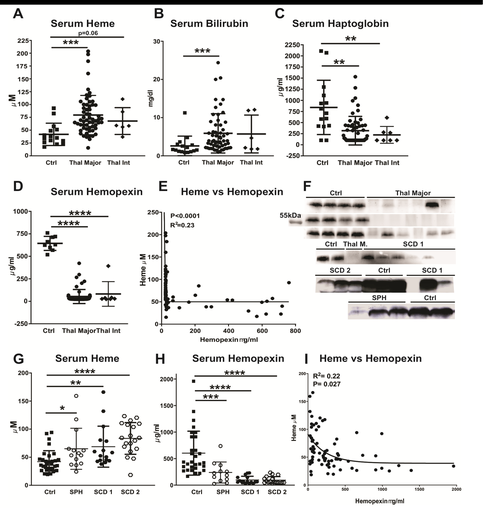


Figure 1. Patients with hemolytic diseases are hallmarked by hemolysis and heme scavenger depletion.
(A-E) Measurement of total heme, bilirubin, haptoglobin and hemopexin in sera of healthy subjects (Ctrl), and patients with β-thal major and int; (F) Representative western blot for hemopexin in sera of healthy subjects (Ctrl) and patients with β-thal major, SCD (SCD1; SCD2) and SPH;
(G-I) Measurement of total heme and hemopexin in sera of Ctrls and patients with SCD (SCD1; SCD 2) and SPH. Values represent mean ± standard deviation (SD). *P<0,05; **P<0,01; ***P<0,001; ****P<0,0001.

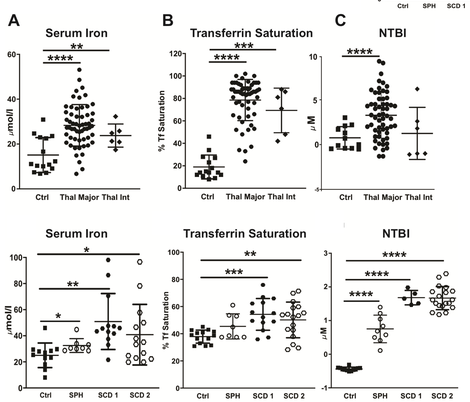


Figure 2. Patients with hemolytic diseases are hallmarked by elevated Tf saturation and NTBI formation.
(A-C) Measurement of iron, transferrin saturation and non-transferrin-bound iron (NTBI) in sera of healthy subjects (Ctrl) and patients with β-thal major and int (upper panel); Ctrls and patients with SCD (SCD1; SCD2) and SPH (lower panel). Values represent mean ± standard deviation (SD). *P<0,05; **P<0,01; ***P<0,001; ****P<0,0001.

INAPPROPRIATE HEPCIDIN LEVELS IN HEMOLYTIC PATIENTS

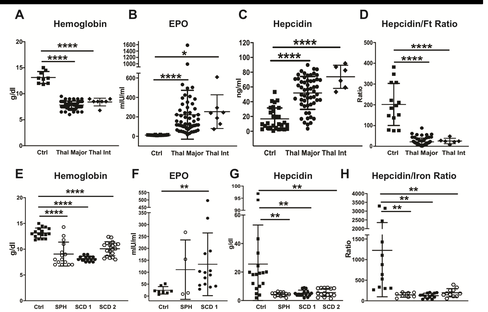


Figure 3. Iron overload in patients with hemolytic diseases is associated with inappropriate hepcidin levels.
(A-D) Measurement of hemoglobin, erythropoietin (EPO), hepcidin and hepcidin/serum ferritin (Ft) ratio in sera of (A-D) healthy subjects (Ctrl), and patients with β-thal major and int; (E-H) Ctrls and patients with SCD (SCD 1; SCD 2) and SPH. Values represent mean ± standard deviation (SD). *P<0,05; ***P<0,001; ****P<0,0001.

HEME & IRON-DRIVEN VASCULAR DYSFUNCTION AND OXIDATIVE STRESS IN HEMOLYTIC PATIENTS

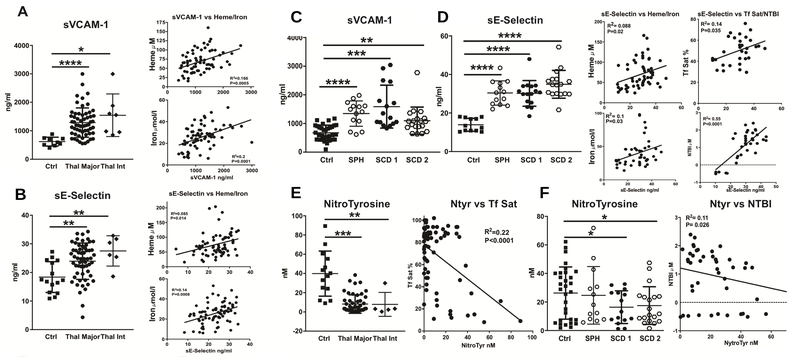


Figure 4. Patients with hemolytic diseases are characterized by altered biomarkers of vascular dysfunctions. (A-D) Measurement of the soluble adhesion molecules sVCAM-1 and sE-selectin (A-D), and nitrotyrosine (E-F) in sera of (A,B,E) healthy subjects (Ctrl) and patients with β-thalassemia major and int; (C,D,F) Ctrls and patients with SCD (SCD 1; SCD 2) and SPH. Values represent mean ± standard deviation (SD). *P<0,05; **P<0,01; ***P<0,001; ****P<0,0001.

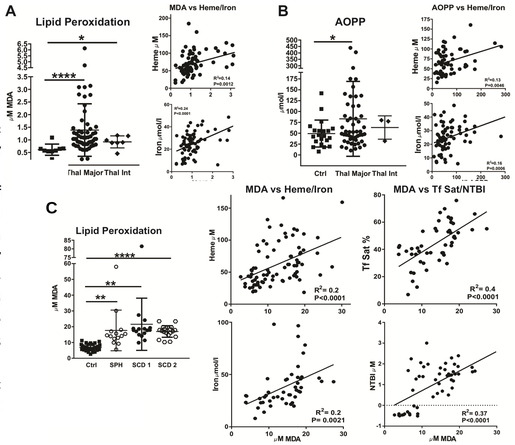


Figure 5. Patients with hemolytic diseases are hallmarked by oxidative stress.
(A,B) Measurement of malondialdehyde (MDA) and advanced oxidized protein products (AOPP) in sera of healthy subjects (Ctrl) and patients with β-thalassemia major and int; (C) Measurement of malondialdehyde (MDA) in sera of Ctrls and patients with SCD (SCD 1; SCD 2) and SPH. Values represent mean ± standard deviation (SD). *P<0,05; **P<0,01; ****P<0,0001.

INFLAMMATION IN HEMOLYTIC PATIENTS

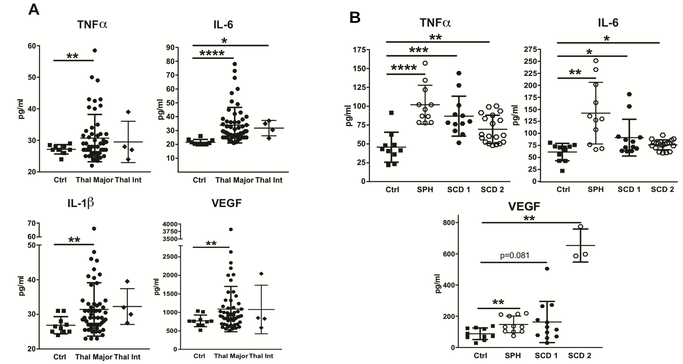
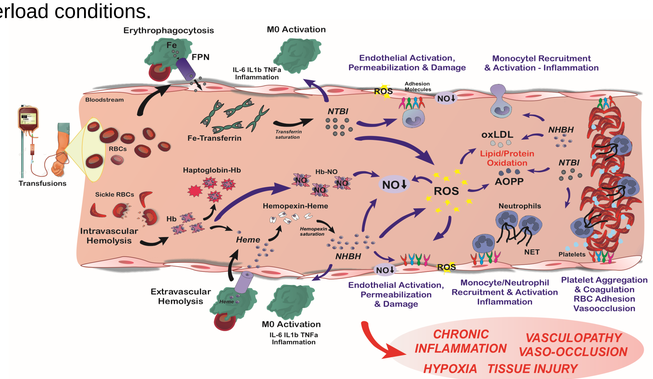


Figure 6. Patients with hemolytic diseases are hallmarked by elevated circulating inflammatory cytokines. (A) Measurement of TNFα, IL-6, IL-1β and VEGF in sera of healthy subjects (Ctrl) and patients with β-thal major and int; (B) Measurement of TNFα, IL-6 and VEGF in sera of Ctrls, and patients with SCD (SCD 1; SCD 2) and SPH. Values represent mean ± standard deviation (SD). *P<0,05; **P<0,01; ***P<0,001; ****P<0,0001.

CONCLUSIONS

These results support the involvement of serum hemoglobin, heme and iron in the pathogenesis of vascular dysfunction in hemolytic diseases and suggest a vasculotoxic action for these molecules. These findings are relevant for disorders hallmarked by vasculopathy, such as sickle cell disease and β-thalassemia, as well as for cardiovascular disease. Our data further highlight the key protective role of heme/iron scavengers and support the potential therapeutic benefit of iron chelation therapy or hemopexin treatment to counteract heme-/iron-driven vascular toxicity in hemolytic and iron-overload conditions.



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