Platelet subpopulations in decompensated cirrhotic children

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INTRODUCTION

Pediatric patients with decompensated cirrhosis present alterations in both pro-and antihemostatic drivers leading to so-called rebalanced hemostasis[1]. Little is known about platelet function and activation in this patient group. It has been shown that after maximal platelet stimulation two distinct platelet subpopulations are present: procoagulant and proaggregant platelets[1]. Procoagulant platelets function as a scaffold to support the coagulation cascade by exposing a negative phospholipid, phosphatidylserine, on their surface. Proaggregant platelets bind fibrinogen via the activated GPIIbIIIa to support platelet aggregation.

AIM

Decompensated cirrhotic pediatric patients were compared to age-matched healthy volunteers to study exposure of phospatidylserine and activation of GPIIbIIIa.

METHODS

Phosphatidylserine exposure was measured in both patients and healthy volunteers with flow cytometry using Annexin V (binds phosphatidylserine), CD41 (binds GPIIbIIIa) and PAC-1 (binds activated GPIIbIIIa) **(Fig 1)**. In brief, peripheral blood was collected in a citrate tube (Monovette plastic 3.0 mL, 3.2% citrate, 106 mM (Sarstedt, Numbrecht, Germany); 9:1 blood:citrate ratio) after drawing a discard tube. Whole blood was diluted in a mix of HEPES-buffer, Ca+, fluorochrome coupled antibodies and agonists. Platelets were maximally stimulated with cross-linked collagen related peptide (CRP-XL; 5 µg/ml) and thrombin (5 IU/mL). GPRP was added to the mix to avoid clot formation. Samples were incubated for 10 minutes at room temperature and incubation was stopped by adding 1 mL of HEPES-Ca2+ buffer and analysed within 1 hour. Informed consent was obtained for every patient. The study was approved by the local ethics committee.

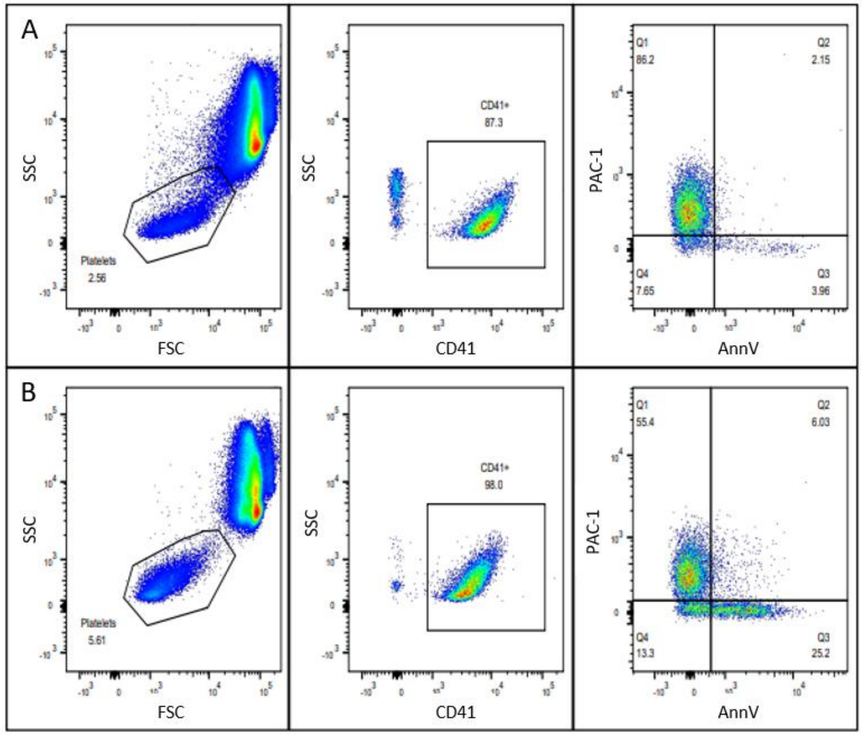

Figure 1. Example of platelet flow cytometry in a cirrhotic patient (A) and in a healthy volunteer (B). Platelets were first gated on forward-side scatter proporties and CD41. PAC-1 and Annexin V were used for activated GPIIbIIIa and phophadidylserine respectively.

RESULTS

Fifteen pediatric patients with cirrhosis and eight pediatric healthy volunteers were included. Etiologies of cirrhosis were biliary atresia (n = 9), PFIC (n = 4), alpha-1-antitrypsine deficiency (n = 1) and unknown (n = 1). Results are shown in Figure 2. Mean age of patients was 33 months compared to 38 months in healthy volunteers. Procoagulant and proaggregant platelets were mutually exclusive (figure 1). Percentage procoagulant platelets was lower in patients (15,0%) compared to healthy volunteers (35,3%) with resulting higher proaggregant platelets in patients (70,3%) compared to healthy volunteers (45,4%).

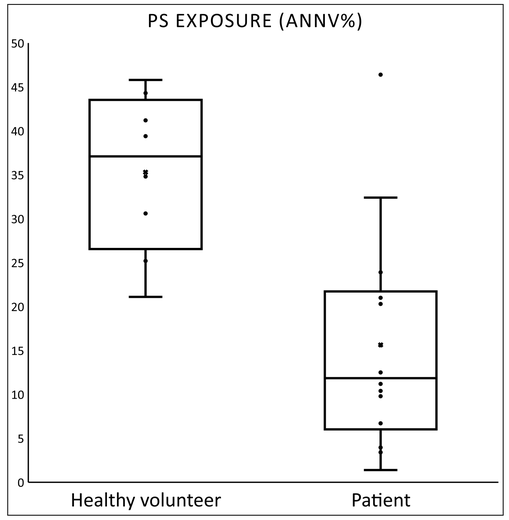

Figure 2: Phosphatidyl serine (PS; AnnexinV%) exposure in patients and healthy volunteers

CONCLUSIONS

Platelet subpopulations seem to be different in decompensated cirrhotic children compared to age-matched healthy volunteers after maximal platelet stimulation: a lower phosphatidylserine exposure is seen in decompensated cirrhotic children compared to healthy controls.

REFERENCES

[1]Tripodi et al. N Engl J Med 2011; 365:147-156

[2]Sodergren et al. Platelet subpopulations remain despite strong dual agonist stimulation and can be characterised using a novel six-colour flow cytometry protocol. Sci Rep 2018, 8, 1441,

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