Circulating microparticle tissue factor activity Is increased in patients with cirrhosis

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Footnote Page

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List of Abbreviations (in the order of their mention)

F, factor; TF, tissue factor; MP, microparticle; TAT, thrombin-antithrombin complexes; MELD, model for end-stage liver disease.

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Cirrhosis is associated with a procoagulant imbalance.[1] Reduced expression of the anticoagulants protein C and antithrombin and increased expression of the procoagulants factor VIII and von Willebrand factor may increase coagulation in these patients.[1] Levels of procoagulant microparticles (MPs) and particularly MPs carrying the highly procoagulant protein tissue factor (TF) have not been investigated in cirrhosis patients.

To determine if MP TF activity is increased in patients with cirrhosis, we analyzed 33 patients with alcoholic and/or hepatitis C virus related cirrhosis (10 Child-Pugh A, 9 Child-Pugh B and 14 Child-Pugh C) using a previously reported assay.[2] None used drugs known to interfere with coagulation, had hepatocellular carcinoma, infection or transfusion within two weeks before blood draw. We also used 9 healthy volunteers as controls. All patients and controls gave their informed consent to participate to the study, which was approved by the Institutional Review Board. Levels of markers of activation of coagulation and fibrinolysis increased with cirrhosis severity (Fig. 1A, B). As expected, plasma from healthy individuals contained very low levels of MP TF activity.[2] Importantly, MP TF activity increased with cirrhosis severity and was 17 fold higher in Child-Pugh C patients compared with controls (Fig. 1C). MP TF activity correlated with MELD score (Spearman's correlation: $r = 0.523$; $P = 0.002$). Similarly, when restricting the analyses to the 22 patients with a relatively stable condition (outpatients or planned hospitalization), we observed higher MP TF activity in patients with cirrhosis than in controls ($p = 0.007$). A previous study reported that MP TF activity was increased 38-fold in acute liver injury/acute liver failure patients.[3] This study used platelet poor plasma while we used platelet free plasma.[4]

We also assessed levels of total MPs, but did not observe any difference between patients and controls either using flow cytometry (annexin V binding; Fig. 1D) or an activity assay (Zymuphen MP Activity, Hyphen BioMed, Fig. 1E). This lack of difference in levels of phosphatidylserine positive MPs may be because the majority of these MPs are derived from platelets and we have found that platelet-derived MPs are not increased in cirrhosis.[4]

In conclusion, patients with cirrhosis have increased levels of circulating MP TF activity that may contribute to the activation of coagulation and thrombosis in these patients.

References

1. Tripodi A, Primignani M, Lemma L, Chantarangkul V, Mannucci PM. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. J Hepatol Aug 2013;59:265-270.
2. Owens AP, 3rd, Passam FH, Antoniak S, Marshall SM, McDaniel AL, Rudel L, et al. Monocyte tissue factor-dependent activation of coagulation in hypercholesterolemic mice and monkeys is inhibited by simvastatin. J Clin Invest Feb 1 2012;122:558-568.
3. Stravitz RT, Bowling R, Bradford RL, Key NS, Glover S, Thacker LR, et al. Role of procoagulant microparticles in mediating complications and outcome of acute liver injury/acute liver failure. Hepatology Jul 2013;58:304-313.
4. Rautou PE, Bresson J, Sainte-Marie Y, Vion AC, Paradis V, Renard JM, et al. Abnormal plasma microparticles impair vasoconstrictor responses in patients with cirrhosis. Gastroenterology Jul 2012;143:166-176 e166.

Figures Legend

Figure 1. MP Procoagulant Activity In Patients With Cirrhosis. Blood was obtained from healthy controls (n=9), and from Child-Pugh A (n=10), B (n=9) and C (n=14) patients with cirrhosis. Plasma D-dimer (A), thrombin-antithrombin complexes (TAT) (B), microparticle (MP) tissue factor (TF) activity (C), Annexin V positive MPs (D) and MP phosphatidylserine (PS) procoagulant activity (E) levels were measured. Data are given as median (horizontal bar), 25th and 75th percentile (boxes), and 10^{th} and 90^{th} percentile (error bar).

*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. Only significant differences are shown.

Other Manuscript Elements

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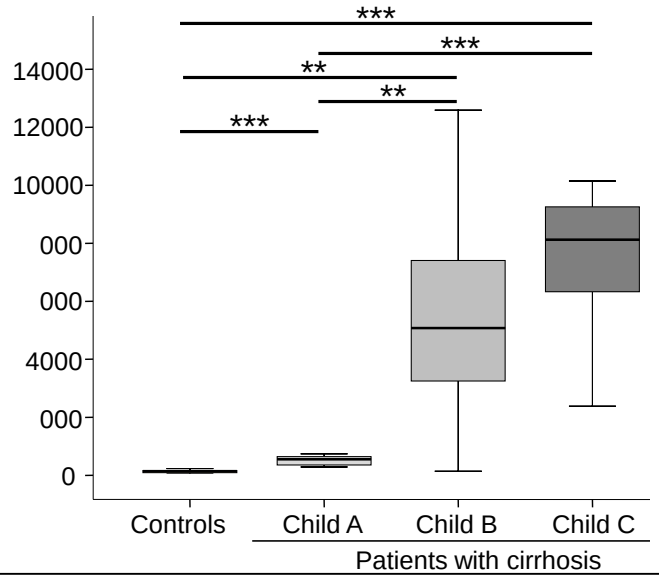
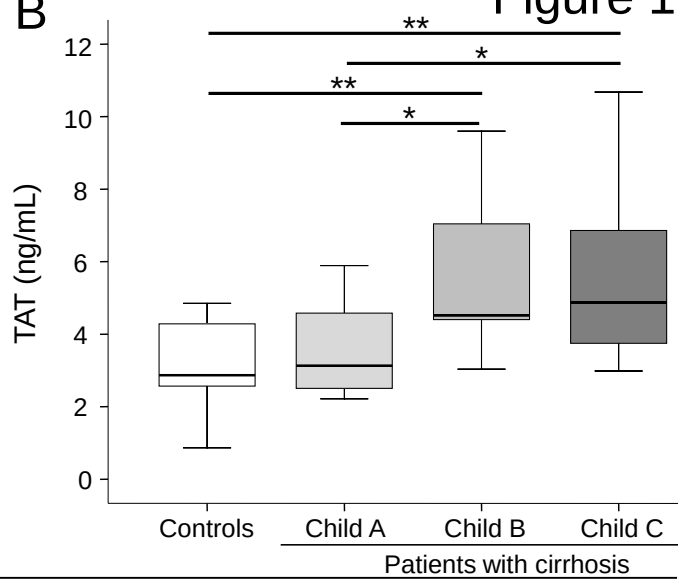
Permissions.

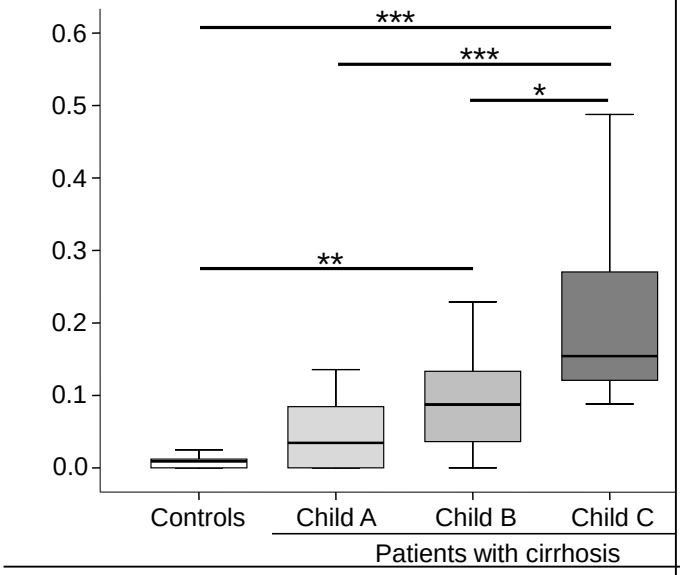
Not applicable.

Figure 1

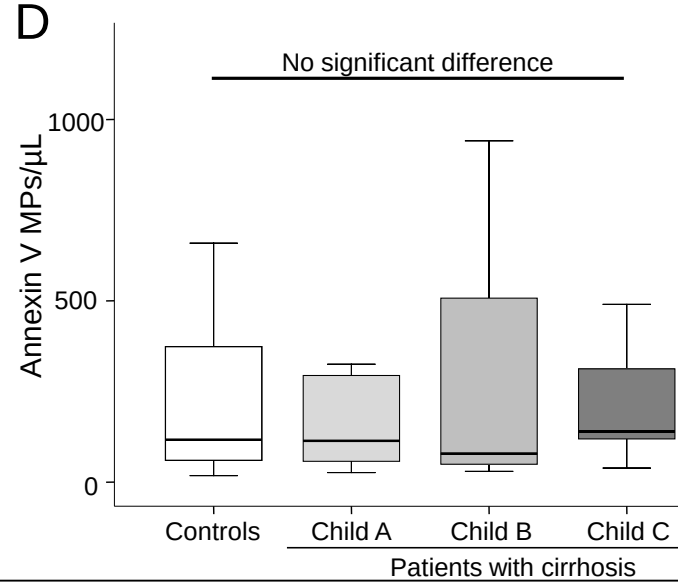

***
***
**
**
***
14000
12000
10000
000
000
4000
000
0
Controls
Child A
Child B
Child C
Patients with cirrhosis

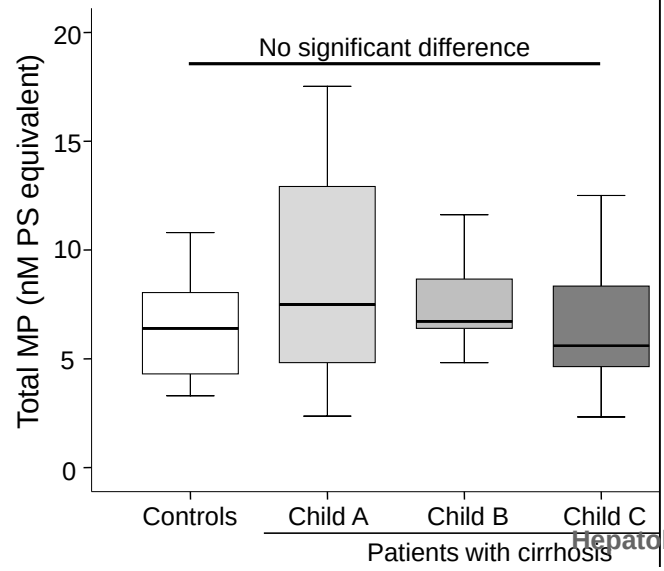
B


**
*
**
*
TAT (ng/mL)
12
10
8
6
4
2
0
Controls
Child A
Child B
Child C
Patients with cirrhosis


***
***
*
**
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Controls
Child A
Child B
Child C
Patients with cirrhosis

D


No significant difference
Annexin V MPs/µL
1000
500
0
Controls
Child A
Child B
Child C
Patients with cirrhosis


No significant difference
Total MP (nM PS equivalent)
20
15
10
5
0
Controls
Child A
Child B
Child C
Patients with cirrhosis