References

[1] Violi F, Basili S, Raparelli V, Chowdary P, Gatt A, Burroughs AK. Patients with liver cirrhosis suffer from primary haemostatic defects? Fact or fiction J Hepatol 2011;55:1415–1427.
[2] Lisman T, Adelmeijer J, de Groot PG, Janssen HL, Leebeek FW. No evidence for an intrinsic platelet defect in patients with liver cirrhosis – studies under flow conditions. J Thromb Haemost 2006;9:2070–2072.
[3] Lisman T, Porte RJ. Rebalanced hemostasis in patients with liver disease: evidence and clinical consequences. Blood 2010;6:878–885.
[4] Patel MD, Joshi SD. Abnormal preprocedural international normalized ratio and platelet counts are not associated with increased bleeding complications after ultrasound-guided thoracentesis. AJR Am J Roentgenol 2011;1:W164–W168.
[5] Pereboom IT, Adelmeijer J, van Leeuwen Y, Hendriks HG, Porte RJ, Lisman T. No evidence for systemic platelet activation during or after orthotopic liver transplantation. Liver Transpl 2009;8:956–962.
[6] Pereboom IT, Adelmeijer J, van Leeuwen Y, Hendriks HG, Porte RJ, Lisman T. Development of a severe von Willebrand factor/ADAMTS13 dysbalance during orthotopic liver transplantation. Am J Transplant 2009;5:1189–1196.
[7] Pereboom IT, Lisman T, Porte RJ. Platelets in liver transplantation: friend or foe? Liver Transpl 2008;7:923–931.
[8] Zimmerman GA, Weyrich AS. Signal-dependent protein synthesis by activated platelets: new pathways to altered phenotype and function. Arterioscler Thromb Vasc Biol 2008;3:s17–24.
[9] Laffi G, Marra F, Gresele P, Romagnoli P, Palermo A, Bartolini O, et al. Evidence for a storage pool defect in platelets from cirrhotic patients with defective aggregation. Gastroenterology 1992;2:641–646.
[10] Lesurtel M, Graf R, Aleil B, Walther DJ, Tian Y, Jochum W, et al. Platelet-derived serotonin mediates liver regeneration. Science 2006;5770:104–107.

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Reply to: Platelet function in patients with cirrhosis

To the Editor:

We thank Drs. Lisman and Porte for their very interesting comments concerning our review on the presence or absence of platelet dysfunction in liver cirrhosis. We agree that extrinsic and intrinsic factors may contribute to maintain a normal primary hemostasis in cirrhosis, despite potential defects due to thrombocytopenia and anemia. Among the extrinsic factors, we agree that the enhanced ratio von Willebrand factor/ADAMTS 13 may have a role. However, we have recently demonstrated the existence of a novel intrinsic factor that could contribute to maintain a normal hemostasis. We demonstrated that, upon stimulation, platelets produce physiologic amounts of isoprostanes [1], which are chemically stable eicosanoids derived from arachidonic acid interaction with reactive oxidant species (ROS) [2]. In virtue of these chemical characteristics, isoprostanes are useful in the late phase of platelet activation, where they serve to propagate platelet aggregation via activation of the glycoprotein IIb/IIIa [1]. We have recently discovered that the enzyme NADPH oxidase, one of the most important cellular producers of ROS, has a key role in the formation of platelet isoprostanes. Thus, in patients with hereditary deficiency of the enzyme, platelet formation of isoprostanes is down-regulated. In particular, platelets from these patients have impaired platelet activation, reinforcing the formation of isoprostanes as a relevant intracellular pathway for platelet function [1]. On the basis of these findings, we analyzed the behavior of platelet isoprostanes and NADPH oxidase activation in a population affected by cirrhosis. We found that platelet production of isoprostanes was increased coincidentally with an up-regulation of platelet NADPH oxidase, suggesting a cause-effect relationship between them [3]. These changes were more evident in patients with Child-Pugh's classes B and C, indicating the platelet over-expression of this pro-aggregating molecule typically occurs in patients with severe liver failure. This finding was associated with systemic signs of platelet activation such as enhanced soluble CD40L, corroborating the concept that in liver cirrhosis platelets are more prone to be activated.

The fact that platelets possess all the armamentarium to activate NADPH oxidase [4,5] is of relevance, not only for the hemostatic system, but also for the extra-hemostatic effects potentially played by platelets. The important issue raised by Drs. Lisman and Porte is still to be adequately addressed, even if there is evidence of a role of platelets in tumor metastasis, wound healing and, overall, inflammation. Our data contribute to further expand the knowledge on the pro-inflammatory role of platelets in as much as NADPH oxidase is an enzyme of the innate immune system with a crucial role for the bacterial killing via the production of ROS [6]. In this regard, platelets may represent another important component of the innate immune system, potentially involved in the atherosclerotic process [7,8]. Thus, ROS generation by NADPH oxidase is used by platelets to oxidize LDL which are ultimately taken up by macrophages via scavenger receptors [9]. Such an extra-hemostatic effect may implicate a key role for platelets in the inflammatory process that initiates atherosclerotic disease. The impact of this phenomenon in liver cirrhosis remains, however, to be established.

How the complexity of these platelet functions relates to the progression of liver cirrhosis, including bleeding and thrombosis, is still a matter of debate and needs further investigation. For this reason, we fully agree with Drs. Lisman and Porte that, currently, the use of routine *in vivo* and *ex vivo* tests to explore global platelet function in cirrhosis is not useful for clinicians. However, future research may identify new platelet biomarkers that will help better understand the role of platelets in the bleeding and thrombotic events that complicate the clinical course of cirrhosis.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

References

[1] Pignatelli P, Carnevale R, Di Santo S, Bartimoccia S, Sanguigni V, Lenti L, et al. Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction. Arterioscler Thromb Vasc Biol 2011;31:423–434.
[2] Morrow JD, Hill KE, Burk RF, Nammour TM, Badr KF, Roberts II LJ. A series of prostaglandin F2-like compounds are produced in vivo in humans by a non-cyclooxygenase, free radical-catalyzed mechanism. Proc Natl Acad Sci U S A 1990;87:9383–9387.
[3] Basili S, Raparelli V, Riggio O, Merli M, Carnevale R, Angelico F, et al. NADPH oxidase-mediated platelet isoprostane over-production in cirrhotic patients: implication for platelet activation. Liver Int. 2011;31:1533–1540.
[4] Pignatelli P, Sanguigni V, Lenti L, Ferro D, Finocchi A, Rossi P, et al. gp91phox-dependent expression of platelet CD40 ligand. Circulation 2004; 110:1326–1329.
[5] Seno T, Inoue N, Gao D, Okuda M, Sumi Y, Matsui K, et al. Involvement of NADH/NADPH oxidase in human platelet ROS production. Thromb Res 2001;103:399–409.
[6] Cox D, Kerrigan SW, Watson SP. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. J Thromb Haemost 2011;9:1097–1107.
[7] Li Z, Yang F, Dunn S, Gross AK, Smyth SS. Platelets as immune mediators: their role in host defense responses and sepsis. Thromb Res 2011;127:184–188.
[8] Hansson GK, Hermansson A. The immune system in atherosclerosis. Nat Immunol 2011;12:204–212.
[9] Carnevale R, Pignatelli P, Lenti L, Buchetti B, Sanguigni V, Di Santo S, et al. LDL are oxidatively modified by platelets via GP91(phox) and accumulate in human monocytes. FASEB J 2007;21:927–934.

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The use of acoustic radiation force-based shear stiffness in non-alcoholic fatty liver disease

To the Editor:

Palmeri and colleagues are to be congratulated on their study assessing the utility of acoustic radiation force image impulse (ARFI) to assess the severity of liver fibrosis in patients with non-alcoholic fatty liver disease (NAFLD) [1]. However, there are several aspects from the study that need to be addressed. Firstly, the number of unsuccessful stiffness reconstructions is significant at 37/172 (21.5%). This would represent a major drawback for any screening test used to gauge the severity of underlying liver fibrosis. In addition, the area under the curve (AUC) of 0.9 for the threshold given ($\geqslant$4.24 kPa) should be re-calculated considering the frequency of the fibrosis stages and the distribution of non-advanced and advanced liver fibrosis (DANA) in the study population using the method described by Poynard and colleagues [2]. In common with all studies evaluating the accuracy of non-invasive markers using liver biopsy as the gold standard, the authors have omitted failed examinations from the final analysis. This is misleading as the accuracy and reproducibility of ARFI is then confined to successful readings and not the study population as a whole. I think all such studies should have a statistical test similar to "an intention-to-treat" analysis in clinical drug trials so that the accuracy of the study test is applied in the context of an entire study population and not only those in whom a result is achieved. Furthermore, as can be seen from Fig. 2, the shear modulus across the liver fibrosis stages is very narrow from stage 0 to stage 4. Once again, this may make interpretation of results difficult, particularly for the lesser stages i.e. stages F0–2, when clinicians may wish to identify early disease to act early to prevent disease progression. This group of patients is still likely to require a liver biopsy to differentiate non-alcoholic steatohepatitis from NAFLD, negating the prime benefit of a non-invasive test. It would be useful to assess the diagnostic accuracy of the test between different stages of fibrosis from F0 *vs.* F1–4 to F0123 *vs.* F4. One could then derive meaningful cut-offs to help guide clinical decisions based on the sensitivity, specificity, negative and positive predictive values as well as positive and negative likelihood ratios, of a respective test.

Conflict of interest

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References

[1] Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, et al. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with non-alcoholic fatty liver disease. J Hepatol 2011;55:666–672.
[2] Poynard T, Halfon P, Castera L, Munteanu M, Imbert-Bismut F, Ratziu V. Standardization of ROC curve areas for diagnostic evaluation of liver fibrosis markers based on prevalences of fibrosis stages. Clin Chem 2007;53:1615–1622.

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