# Letter in response to 'Coagulation and fibrosis in chronic liver disease'

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## LETTERS

### The contribution of megakaryocytes to liver fibrosis

In a recent paper in *Gut*, Calvaruso *et al*[1] give a succinct summary of the coagulation changes which predispose to fibrosis in chronic liver disease. However, they did not mention the important contribution of the megakaryocyte, in addition to thrombin, to liver fibrosis. This is exemplified by the observation of frequently fatal, liver fibrosis which can occur in acute megakaryoblastic leukaemia (AMKL) and transient myeloproliferative disorder (a precursor of AMKL) in children with Down syndrome.[2] These children demonstrate sinusoidal fibrosis and extramedullary haematopoiesis in the liver, raising the possibility that the fibrosis is caused by cytokines elaborated by the liver megakaryocytes.[3] Abnormal megakaryocytic progenitor cells which proliferate in the foetal liver, release cytokines, including platelet derived and transforming growth factors, potent stimulators of growth and collagen synthesis in fibroblasts, leading to liver fibrosis.[3] Although in this context, a case against the presence of megakaryocytes in the adult liver may be made, it is important to note that extramedullary haematopoiesis has been demonstrated in the liver in many hematological disorders including haemoglobinopathies and storage disorders like Gaucher's disease. In the non-haematological conditions, however, the elaboration of the fibrogenic cytokines from the bone marrow megakaryocytes can occur in the presence of thrombin.

Thrombin, the key molecule in the coagulation system, can stimulate megakaryocytes to release fibrogenic mediators including basic fibroblast, and vascular endothelial growth factors (VEGFs).[4] Both these growth factors are released physiologically to help in sustaining haematopoietic colony growth in the presence of other cytokines.[4] In instances of vascular injury, VEGF, in particular, can help in endothelial proliferation and angiogenesis as a part of wound healing, an exaggerated form of which leads to fibrosis.[4] Functional thrombin receptors are also shown to be expressed on cells of the megakaryocytic lineage with studies demonstrating direct effects of thrombin on megakaryocytic morphology, release reaction and proplatelet formation.[5]

In the article, the authors explain thrombin-induced activation of platelets as an important role player in the events leading to fibrosis. However, decreased platelet production from the reduced number of megakaryocytes rather than their increased destruction is the main mechanism for thrombocytopenia associated with chronic hepatitis C virus (HCV) infection.[6] Direct infection of the megakaryocytes by HCV in this situation can cause the release of the fibrogenic factors stored in their granules. Also, megakaryocyte development, maturation and differentiation is regulated by thrombopoietin, the levels of which correlate inversely with the development of liver fibrosis with HCV infection.[6] These observations would suggest a predominant role for the platelet precursor rather than the platelets themselves in the development of hepatic fibrosis.

The interaction between the bone marrow precursor cells and a critical molecule in haemostasis can be considered beneficial in maintaining a regulated release of platelets and other key cytokines in cases of endothelial injury, where excess bleeding need to be controlled and wound healing needs to commence as soon as possible. Uncontrolled thrombin generation as such can lead to exaggerated wound healing, or fibrosis, through many mechanisms including stimulation of the megakaryocytes. This important role of megakaryocyte should be explored in conjunction with the other coagulation factors to further improve our understanding of liver fibrosis.

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### Letter in response to 'Coagulation and fibrosis in chronic liver disease'

We read with interest the paper by Calvaruso and colleagues discussing the role of coagulation in fibrosis in chronic liver disease.[1] Fibrotic diseases, and especially liver fibrosis, are a major public health issue, and remain too often refractory to therapy. Novel treatment options are therefore eagerly awaited. Overall, we agree with the author's conclusions that compelling evidence supports a close relationship between thrombin and hepatic fibrogenesis and that targeting the coagulation cascade might be an attractive therapeutic avenue for the management of liver fibrosis. This notion is underscored by the fact that anticoagulant therapy already showed promise in idiopathic pulmonary fibrosis where it improved overall survival.[2]

Although we would like to commend the authors on writing a valuable review on this highly relevant topic, we do feel they might have focused too much on the role of thrombin. The realisation that both coagulation and PAR-2 are instrumental in fibrogenesis boosted research on factor (F)Xa–PAR-2 driven signal transduction in pathophysiology. These recent experiments challenged the concept that FXa-induced signal transduction is simply reminiscent of thrombin-induced signal transduction.[3]

Calvaruso and colleagues briefly mention FXa by stating that similarly to thrombin, FXa acts as a potent mitogen on fibroblasts. Rather surprisingly, they state that the mitogenic effect might be mediated by the effector cell protease receptor-1 (EPR-1). This is surprising because the existence of EPR-1 is highly controversial.[4] Indeed, the cDNA encoding an inhibitor of apoptosis, survivin, is reportedly identical to that of EPR-1 except for a few nucleotide differences and with its orientation opposite to EPR-1. The published EPR-1 cDNA sequence is consequently believed to be derived from survivin mRNA. Most probably, therefore, FXa exerts its effects in fibrogenesis via PAR-2 activation.[5]

Interestingly, the authors of the recent review[1] do suggest that (next to thrombin–PAR-1) PAR-2 activation might also contribute to liver fibrosis. This notion is based on the facts that PAR-2 expression is upregulated in livers of bile duct-ligated rats, that hepatic stellate cells (HSCs) express PAR-2 and that PAR-2 activation induced myofibroblast differentiation, cell proliferation and collagen synthesis by HSCs. It is hypothesised that PAR-2 activation is elicited by mast cell tryptase. Although this is a likely candidate to activate PAR-2, we feel the potential role of FXa should not have been overlooked. The mitogenic effects of FXa in fibroblasts and smooth muscle cells of different tissues are well known.[3] In addition, we recently showed that FXa induces fibroblast migration, extracellular matrix synthesis, transforming growth factor β release and fibroblast differentiation into myofibroblasts. All these fibroproliferative responses were mediated by PAR-2 activation, thereby at least suggesting that the FXa–PAR-2 axis acts as an important modifier in fibrotic disease.[5]

Overall the role of thrombin in liver fibrosis is indeed compelling, but the potential importance of FXa, which has already

been shown to be important in animal models of glomerulonephritis, restenosis, asthma and fibrosarcoma,[3] might be as important. From a clinical perspective, one might even argue that FXa is a more attractive therapeutic target for liver fibrosis than thrombin. This notion is not only based on the fact that FXa inhibitors are more effective at lower doses than thrombin inhibitors but also on the fact that FXa inhibitors block both FXa-dependent profibrotic signalling and thrombin generation.

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## Applicability of BARD score to Japanese patients with NAFLD

We read the article by Harrison *et al*[1] with great interest. The authors proposed an easily calculated composite score for predicting the risk of advanced fibrosis in patients with non-alcoholic fatty liver disease (NAFLD), called the BARD score: the weighted sum of the three variables (body mass index (BMI) ⩾28 = 1 point, aspartate aminotransferase/alanine aminotransferase ratio (AAR) ⩾0.8 = 2 points, diabetes = 1 point). When a BARD score of 2–4 was used, the area under the receiver operating characteristic curve (AUROC) was found to be 0.81 with an odds ratio (OR) of 17 (95% CI 9.2 to 31.9) for detecting advanced fibrosis. The positive predictive value (PPV) and negative predictive value (NPV) were 43% and 96%, respectively. We studied the reliability of the BARD score for identifying the risk of advanced fibrosis in Japanese patients with NAFLD.

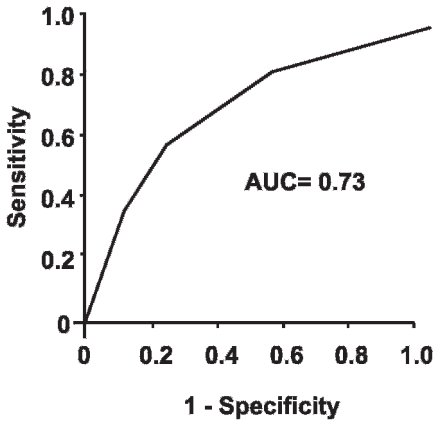


**Figure 1** Simple steatosis plus non-alcoholic steatohepatitis (NASH) with fibrosis stages 0–2 vs NASH with fibrosis stages 3–4. AUC, area under the curve.

A total of 122 patients (61% female; median age, 59 years) with NAFLD who underwent liver biopsy at our hospital were studied. Median BMI was 26 kg/m$^2$ (range, 18–45); 33% of patients had a normal BMI of <25, whereas 46% were overweight (BMI 25–29), 21% were obese (BMI ⩾30) and 1% were extremely obese (BMI ⩾40). Common co-morbidities included hypertension (30%), diabetes (37%) and hyperlipidaemia (59%). Liver biopsy showed that 9 (7%) had simple steatosis, and 113 (93%) had non-alcoholic steatohepatitis, including 67 (55%) with mild fibrosis (stage 0–2) and 46 (38%) with advanced fibrosis (stage 3–4). When a BARD score of 2–4 was used, the AUROC was 0.73 (fig 1) with an OR of 4.9 (95% CI 2.2 to 10.8) for detecting advanced fibrosis. The PPV and NPV were 59% and 77%, respectively.

The BARD score was less predictive of advanced fibrosis in Japanese patients than in the study by Harrison *et al*, probably because of two major reasons. First, their subjects were predominantly Caucasians; only 2% were Asian Pacific Islanders. Although mean BMI is lower in Asian populations than in non-Asian populations, Asians have a higher percentage of body fat for a given BMI than non-Asians.[2] In a study conducted in Japan,[3] nearly half of the subjects with NAFLD were not overweight or obese, suggesting that different genetic and environmental factors are related to susceptibility to hepatic steatosis in the Japanese population. In our cohort, BMI was similar in patients with and without advanced fibrosis, as shown in table 1. Our results are consistent with those of Hashimoto *et al*,[4] who found that older age, the presence of diabetes and elevated AAR were significantly associated with more advanced fibrosis in Japanese patients, whereas higher BMI was not. Secondly, Harrison *et al* assessed only AAR, glycated haemoglobin (HbA1c) and the quantitative assessment check index score among laboratory variables as potential risk factors for advanced fibrosis. Since decreased platelet count and decreased albumin concentration were significantly associated with more advanced fibrosis in our cohort (table 1), we assessed the value of the NAFLD fibrosis score,[5] which includes these two variables, for the detection of advanced fibrosis. The AUROC for the NAFLD fibrosis score was 0.84, with a PPV and NPV of 59% and 89%, respectively (data not shown). Albeit slightly more complex, the NAFLD fibrosis score more accurately detected advanced fibrosis in our patients than the BARD score.

In summary, the BARD score can be easily derived from readily available clinical data, but may be less reliable for excluding the presence of advanced fibrosis in Japanese patients with NAFLD than in the study by Harrison *et al*.

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**Table 1** Risk assessments of clinical parameters for advanced fibrosis

| Characteristics | Simple steatosis and NASH fibrosis 0–2 (n = 76) n (%) | NASH fibrosis 3–4 (n = 46) n (%) | p Value | OR (95% CI) |
|---|---|---|---|---|
| Age ⩾50 years | 45 (59) | 37 (80) | 0.016 | 2.8 (1.2 to 6.7) |
| Female gender | 42 (55) | 33 (72) | 0.070 | 2.1 (0.9 to 4.5) |
| Body mass index ⩾28 kg/m$^2$ | 24 (32) | 15 (33) | 0.906 | 1.0 (0.5 to 2.3) |
| Hypertension | 18 (24) | 18 (39) | 0.070 | 2.1 (0.9 to 4.6) |
| Diabetes | 21 (28) | 24 (52) | 0.007 | 2.9 (1.3 to 6.1) |
| Hyperlipidaemia | 52 (68) | 20 (43) | 0.007 | 0.4 (0.2 to 0.8) |
| AST/ALT ⩾0.8 | 16 (21) | 27 (59) | <0.0001 | 5.3 (2.4 to 11.9) |
| Platelets <200×10$^9$ cells/l | 25 (33) | 36 (78) | <0.0001 | 7.3 (3.1 to 17.1) |
| Albumin <4.1 g/dl | 24 (32) | 32 (70) | <0.0001 | 5.0 (2.2 to 10.9) |

ALT, alanine aminotransferase; AST, asparate aminotransferase; NASH, non-alcoholic steatohepatitis.

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