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Endothelial *JAK2*^{V617F} does not enhance liver lesions in mice with Budd-Chiari syndrome

To the Editor:

Budd-Chiari syndrome is defined as hepatic venous outflow obstruction in the absence of congestive or restrictive heart disease. Myeloproliferative neoplasms are the leading cause of Budd-Chiari syndrome, diagnosed in 25–50% of such patients.[1,2] In most patients with Budd-Chiari syndrome and myeloproliferative neoplasms, Janus kinase 2 gene (*JAK2*) V617F mutation is found in myeloid cells. *JAK2*^{V617F} has also been detected in liver endothelial cells of patients with Budd-Chiari syndrome, attributed to a common cell of origin for myeloid and endothelial cells, called hemangioblast.[3–5] In Budd-Chiari syndrome, *JAK2*^{V617F} is associated with poorer prognostic features at presentation and earlier need for hepatic decompression procedures.[1] This observation leads to the hypothesis that *JAK2*^{V617F} enhances liver injury and fibrosis induced by hepatic venous outflow obstruction, thus worsening Budd-Chiari syndrome.

In order to test this hypothesis, we applied a recently described surgical model of Budd-Chiari syndrome to mice expressing *JAK2*^{V617F}.[6] *JAK2*^{V617F} expression in myeloid cells promotes major vasodilation and hemostasis impairment, making surgery extremely challenging in these animals.[7] Accordingly, we analyzed the endothelial component using mice expressing *JAK2*^{V617F} specifically in endothelial cells. We generated these transgenic mice by crossing conditional *Jak2*^{V617F} knock-in mice with inducible *Cadherin5*$^{Cre\text{-}ERT2}$ mice. Recombination was induced in *Jak2*^{V617F} *knock-in – Cadherin5*$^{Cre\text{-}ERT2}$ (hereafter referred to as *JAK*^{V617F}) mice by tamoxifen injection (1 mg/day/mice intraperitoneously for five consecutive days, two consecutive weeks) at the age of five weeks. Littermate controls (hereafter referred to as *JAK2*^{WT}) received the same treatment. Partial inferior vena cava ligation (pIVCL), or sham surgeries were performed at the age of 12 weeks and mice were sacrificed six weeks postoperatively (Fig. 1).[6] Based on previous experiments using this surgical model, we included 8 to 10 mice per group.[6] All experiments were performed in accordance with the European Community guidelines for the care and use of laboratory animals (N°07,430) and were approved by our institutional ethical committee (17-053).

As expected, *JAK2*^{WT} mice undergoing pIVCL had higher portal pressure, liver expression of profibrogenic genes and liver

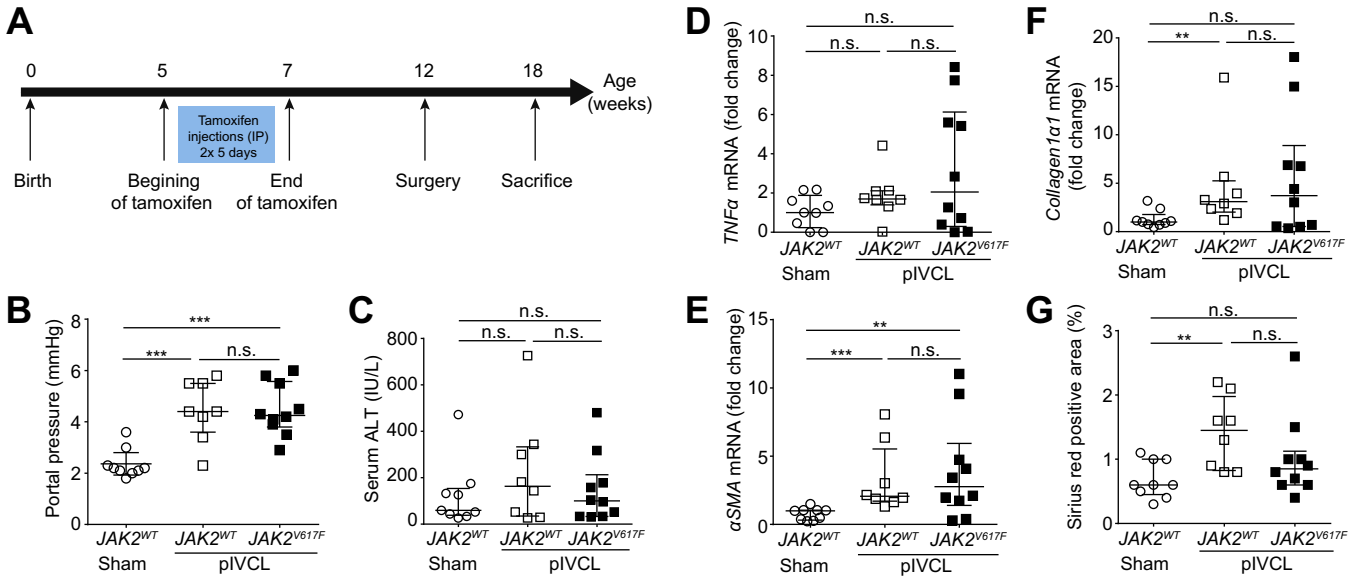

Fig. 1. Endothelial *JAK2*^{V617F} does not enhance liver injury in mice after partial inferior vena cava ligation. Partial inferior vena cava ligation was performed in 12-week aged male and female *Jak2*^{V617F} knock-in - *Cadherin5*$^{Cre\text{-}ERT2}$ (*JAK*^{V617F}) mice and in littermate controls (*JAK2*^{WT}). Sham surgery was also performed. All mice were on a C57BL/6 background. (A) Mice were sacrificed 6 weeks postoperatively. (B) Portal pressure, (C) serum ALT level, (D) liver *TNFα*, (E) *αSMA* and (F) *collagen1α1* gene expressions were determined (supplementary CTAT Table) and (G) liver fibrosis was quantified (Sirius red positive areas). Data are given as median (horizontal bar) and interquartile range (error bar). Comparisons between groups of mice were performed using the Mann–Whitney U test. $^{*}p$ <0.05; $^{**}p$ <0.01; and $^{***}p$ <0.001. ALT, alanine aminotransferase; *JAK2*, Janus kinase 2; pIVCL, partial inferior vena cava ligation; n.s., no significant difference.

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fibrosis than sham mice, while showing no change in serum aminotransferases or in liver expression of proinflammatory genes (Fig. 1).[6] However, as shown (Fig. 1), the expression of *JAK*^{V617F} in liver endothelial cells did not affect any of these parameters.

In conclusion, we found no evidence in an animal model that endothelial *JAK2*^{V617F} can explain the more severe presentation of patients with Budd-Chiari syndrome and *JAK2*^{V617F}. The explanation for increased severity of these patients should therefore be sought mostly in myeloid *JAK2*^{V617F}. Thus, future therapeutic strategies to improve the management of patients with Budd-Chiari syndrome and myeloproliferative neoplasms might focus on myeloid cells rather than on endothelial cells. Beside the cytoreductive agent hydroxyurea, treatments for myeloproliferative neoplasms now also include the JAK2/1 inhibitor ruxolitinib. One phase II trial recently reported that ruxolitinib is safe in patients with splanchnic vein thrombosis.[8] Whether ruxolitinib is useful in this setting to improve patient outcomes should be evaluated in larger studies.

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Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

J.P. and M.B.H contributed equally to the work. M.B.H. and D.A.S. performed mouse surgeries. J.P., A.H. and M.T. analyzed liver samples. J-L.V. generated *Jak2*^{V617F} *knock-in* mice. J.P. and P-E. R. wrote the manuscript. C.M.B, D.V. and V.H.S discussed and analyzed the results. All authors critically revised the manuscript.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jhep.2018.01.010.

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