

# JAK2 and Endothelial Function: New Options for Anti-Thrombotic Therapies

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Thromb Haemost 2018;118:1512–1514.

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The activation of Janus kinases (JAKs) is a crucial enzymatic step in the signal transduction of many growth factors and cytokines. Four members, JAK1, JAK2, JAK3 and tyrosine kinase 2 (TYK2) have been identified and they all are well-known due to their function in the haematopoietic and immune system. Their profound involvement in the regulation of the immune response made JAKs to be attractive drug targets for inflammatory disorders such as rheumatoid arthritis<sup>1</sup> and cancers of the immune system such as multiple myeloma.<sup>2</sup> Genetic alterations of JAKs were described for all four JAKs and associated with human diseases. While inherited loss of function mutations in JAK3 and TYK2 were found in severe combined immune deficiency and atopic dermatitis, respectively, somatic gain of function mutations in JAK1, JAK2 and JAK3 resulted in myeloproliferative neoplasms (MPNs) and leukaemia/lymphomas. For example, generation of fusion proteins consisting of JAK2 and TEL, breakpoint cluster region (BCR) or pericentriolar material-1 were described in chronic myeloid leukaemia, acute myeloid leukaemia or acute lymphoblastic leukaemia.<sup>3,4</sup> In addition, the point mutation JAK2V617F gained fame after it was identified as the major driving force in non-BCR-ABL1 MPNs in particular in polycythaemia vera, essential thrombocythaemia and primary myelofibrosis.<sup>5–7</sup> The JAK2V617F mutation can also be found, but much less frequent, in the hypereosinophilic syndrome, chronic or juvenile myelomonocytic leukaemia, acute myeloid leukaemia and refractory anaemia with ringed sideroblasts (for review see Haan et al).<sup>8</sup>

So far, it was common belief that the acquired somatic JAK2V617F mutation affects mainly haematopoietic stem cells, multi-potent progenitor cells,<sup>9,10</sup> cells from the lymphoid lineage<sup>11,12</sup> and some differentiated cells like granulocytes.<sup>7</sup> However, several recent studies reported occurrence of the JAK2V617F mutation in endothelial cells (ECs) of patients with MPN and Budd–Chiari syndrome.<sup>13–15</sup> In fact, thromboembolic events represent a major cause of morbidity and mortality during the chronic phase of MPN

patients, before the onset of the accelerated phase with evolution to myelofibrosis or acute leukaemia. Thrombotic manifestations in MPN patients are affecting unusual sites like the portal vein or splanchnic veins. Indeed, splanchnic vein thrombosis affects 0.9 to 5% of the patients with polycythaemia vera and 3 to 10% of the patients with essential thrombocythaemia.<sup>16</sup> Vice versa, approximately 40 and 30% of Budd–Chiari syndrome or portal vein thrombosis patients, respectively, have underlying MPNs.<sup>17</sup>

Although the thrombotic risk in the MPN patients can be associated with an increased haematocrit, leucocytosis or platelet dysfunction, it is so far unknown what makes MPN patients to be prone to thromboembolic events. Since ECs are critically involved in the regulation of vascular structure, cellular adhesion, vascular tone and thromboresistance, it may be very well possible that the JAK2V617F mutation in ECs of an MPN patient pre-disposes him to thrombosis.

Indeed, this problem was tackled by Guadall et al and the outcome of their studies is described in an article of the current issue of *Thrombosis and Haemostasis*.<sup>18</sup> To address this problem, the authors used isogenic JAK2V617F and JAK2 wild-type (WT) induced pluripotent stem (iPS) cells from an MPN patient and redirected these iPS cells towards the endothelial lineage. Tube formation assays in matrigel and nitric oxide formation as characteristic parameters of ECs first revealed that the iPS cells can be differentiated towards the endothelial lineage and that no principal difference between the cells from the two genotypes was detectable. However, the authors detected a gain of function in JAK2V617F cells when compared with the WT cells; this became visible by increased levels of phosphorylated JAK2 and signal transducer and activator of transcription 3 (STAT3). These findings resemble the enhanced intra-cellular JAK2/STAT3 signalling as observed in JAK2V617F haematopoietic cells. Moreover, the authors observed an increased proliferation in the cells with the JAK2V617F mutation when compared with WT ECs.

received  
July 31, 2018  
accepted  
July 31, 2018

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Stuttgart · New York

DOI <https://doi.org/10.1055/s-0038-1668586>.  
ISSN 0340-6245.

Next, the authors were able to link the observed findings to an activated status of ECs that is prone to thrombotic events by looking at the formation of Weibel–Palade bodies and expression of von Willebrand factor (vWF) and P-selectin (CD62P). All these parameters, numbers and fluorescence intensity of Weibel–Palade bodies as well as expression of vWF and P-selectin were significantly higher and also accompanied by accumulation of P-selectin at the cell surface in JAK2V617F cells when compared with WT cells.

To characterize the consequences of JAK2V617F expression in the ECs further, the authors obtained the respective transcriptomic profile of these cells. The authors found 428 genes being differentially expressed (259 up- and 169 down-regulated) in JAK2V617F versus WT cells. Importantly, a gene set enrichment analysis showed that various over-expressed genes were highly related to pro-inflammatory and pro-adhesive properties, to extracellular matrix regulation, and to generation of glycoproteins, processes that are also involved in venous stenosis and thrombosis. In particular, the induction of a pro-thrombotic phenotype of the JAK2V617F cells was underlined by the over-expression of the interleukin (IL)-33 receptor; IL-33 is known to induce tissue factor expression in ECs.<sup>19</sup> This was further supported by the dysregulation of *SULF1* expression, which is involved in desulphation of cellular heparan sulphate proteoglycans, and of the sphingosine-1-phosphate receptor 3 that contributes to vWF release from Weibel–Palade bodies as well as to P-selectin surface exposure.<sup>20,21</sup>

Next, the authors functionally substantiated the pro-thrombotic expression profile and examined whether the interactions between white blood cells (WBCs) and the JAK2V617F ECs or WT ECs is showing a difference. To this end, the authors established cell adhesion assays using WBC from healthy donors or from MPN patients. The authors found that JAK2V617F cells bound stronger to WBC from healthy donors than their WT counterparts. This difference was even more exaggerated upon usage of WBCs from a JAK2V617F MPN patient. Together, these assays confirmed that the JAK2V617F mutation provides a pro-adherent phenotype to ECs.

Altogether, the novelty aspect in the study of Guadall et al consists in the overarching finding that differentiation of pluripotent stem cells from MPN patients harbouring the JAK2V617F mutation could give rise to ECs which display pro-adherent and pro-thrombotic features, which can contribute to the thrombotic events seen in MPN patients. Moreover, the findings also support the view that JAK2V617F ECs present in the bone marrow niche could contribute to the development of myelofibrosis.

Overall, and with respect to thrombosis, this needs to be seen together with the other cells critically involved in blood clotting and thrombus formation such as platelets. Apart from the erythropoiesis, the JAK2 V617F point mutation in the normal haematopoietic progenitor cells causes also the growth of megakaryocytes, which results in higher production of platelets. Although platelets lack the nucleus to direct ribonucleic acid transcription, they and even more their megakaryocytic progenitors react very well to stimulation by JAK2 activating factors such as thrombopoietin, or

IL-3.<sup>22,23</sup> A study investigating platelet haemostatic and adhesive molecules found that platelets from essential thrombocythaemia JAK2V617F patients expressed higher membrane tissue factor and P-selectin.<sup>24</sup> In addition, a recent report indicated that JAK2 can be involved in collagen-induced platelet activation.<sup>25</sup> Hence, presence of the gain of function JAK2V617F mutation in megakaryocytes and platelets and as shown here for ECs explains, at least in part, the high frequency of major arterial and venous thrombotic complications as well as the micro-vascular, platelet-mediated disturbances in MPN patients. Consequently, this raises the thought about the druggability of the thrombotic status by JAK2 inhibitors. In fact, only one inhibitor, ruxolitinib, targeting JAK2/JAK1 has been approved for treatment of hydroxyurea-intolerant polycythaemia vera, and intermediate- or high-risk myelofibrosis but not essential thrombocythaemia.<sup>26</sup> Another inhibitor, tofacitinib, targeting preferentially JAK1 and JAK3 has been approved for methotrexate-resistant rheumatoid arthritis.<sup>27</sup> Ruxolitinib is a well-tolerated drug, which decreases inflammatory cytokines, leukocytosis and thrombocytosis and thereby improves the clinical status as well as prolongs survival of patients with myelofibrosis.<sup>26,28,29</sup> Although it is common believe that the ruxolitinib effects are mainly caused by the inhibition of cytokine action, it will be interesting to see its effects in other MPNs such as essential thrombocythaemia where it is in late-phase clinical trials. Further, since JAK2 activation is mainly triggered by IL-3, IL-5, granulocyte-macrophage colony-stimulating factor, interferon- $\gamma$ , erythropoietin, thrombopoietin, growth hormone and prolactin,<sup>30</sup> one may, in addition to MPNs and leukaemia, think about mountain sickness, acromegaly, prolactinomas or inoperable hypophyseal tumours as treatment entities. Given the relative safety of ruxolitinib, and in light of the findings with ECs from the paper of Guadall et al, it is provoking to speculate that JAK2 inhibition, either with ruxolitinib or other more specific new inhibitors such as pacritinib or NS-018, could represent an avenue for more wider therapeutic options associated with EC proliferation, for example, in atherosclerosis or myocardial infarction. This would even not require a JAK2 gain of function mutation since ruxolitinib inhibits both, mutated and WT JAK2. Overall, more research, more testing and more clinical trials are needed to improve the current picture.

#### Conflict of Interest

None.

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