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Bone morrow microvascular dencity in chronic mieloproliferative neoplasms in patients with and without *jak2* (v617f) mutation

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The incidence of *JAK2* (V617F) mutation in myeloproliferative neoplasms (MPNs): polycythemia vera (PV), essential thrombocythemia (ET) and chronic idiopathic myelofibrosis (MF) is very high, which leads to constitution activation of *JAK2* and an independent growth of the haemopoetic cell lines.

Angiogenesis in MPNs was studied by an endothelial marker (CD34) and bone marrow microvessel density (MVD) compared in patients with and without *JAK2* (V617F) mutation.

MVD in *JAK2* mutation was 16.48±8.77 (per high-power microscopy field – HPF), 12.51±5.59, 20.51±7.77 for PV, ET and MF, respectively. Patients without mutation had MDV 11.32±5.74, 12.80±5.40 and 15.00.±7.57 for PV, ET and MF, respectively. There was no difference ($p>0.05$) in mean MVD in ET, while MVD was higher in PV and MF patients with *JAK2* mutation ($p< 0.01$).

These results show pronounced angiogenesis in the bone marrow of MF and PV patients with *JAK2* mutation. Probably it is induced by the activation of the *JAK2* signaling pathway.

Key words: bone marrow microvessel density, polycythemia vera, essential thrombocythemia, chronic idiopathic myelofibrosis, *JAK2* (V617F) mutation

Neoangiogenesis is an integral part in the progression of solid tumors and the microvascular density in tumor tissue correlates well with its growth and its capacity to metastasize (10). Increased number of bone morrow blood vessels is established in various hematologic disorders, such as acute lymphoid or myeloid leukemia, myelodysplastic syndrome, chronic myeloid leukemia and plasma cell proliferative disorders (3, 9). In normal conditions, human bone marrow is supplied by a relatively smaller number of blood vessels.

Marked neoangiogenesis is a characteristic feature of myeloproliferative neoplasms (MPNs): polycythemia vera (PV), essential thrombocythemia (ET), and chronic idiopathic myelofibrosis (MF) (3, 10). These Philadelphia-negative (Ph_) chronic MPNs display a high frequency of *JAK2* (V617F) mutation. It is an acquired somatic mutation in the *JAK2* gene resulting in a valine to phenylalanine substitution at position 617 (JAK2V617F) (7).

Currently available data indicate that *JAK2* (V617F) participates in the pathogenesis of these neoplasms, because the mutation leads to constitution activation of *JAK2* and thus haemopoetic cell lines acquire a potential for independent growth (10).

That is why we studied angiogenesis in MPNs and compared bone marrow microvessel density (MVD) in patients with and without *JAK2* (V617F) mutation.

Materials and Methods

Forty three patiens with MPNs were investigated (21 men and 22 women, 23 to 77 years of age).

Tissue specimens

Patients were selected based on the availability of well-preserved bone marrow biopsy specimens, suitable for additional stainings. Paraffin sections were stained with HE and Gomori. A histopathological diagnosis of the MPNs was made in accordance to WHO (9). Paraffin section (5 μm thick) was processed by peroxidase-antiperoxidase technique. Monoclonal antibody Mo a Hu CD34 class II clone QBEnd (Dako) was used for detection of microvessels. We counted the number of vessels per (HPF – 10 x 40) in the areas of most dense vascularization. Five areas were evaluated from each patient. MDV was determined as a median value of all measurings. The data are presented as the mean and standard deviation. Statistical comparisons using analysis of variance and Student's t-test was performed. $P<0.05$ was considered statistically significant.

JAK2 (V617F) mutation was determinate by reverse-transcribed polymerase chain reaction (RT-PCR method).

Results

Histology

Eighteen of the patients were with PV. In bone morrow there were an increased number of the erythroid precursors and the myeloid-erythroid ratio was usually decreased. The megakaryocytes were pleomorphic, grouped together and had deeply lobulated nuclei without atypical and dysplastic features.

ET was established in twelve patients. The bone morrow in ET was usually normocellular for age or moderately hypercellular with an increased number of either large or giant megakaryocytes. They showed hyperlobulated nuclei and/or appeared in clusters or were diffusely dispersed. The megakaryocytic clusters were found around the sinusoids or close to the bone trabeculae. Reticulin fibrosis was minimal or lacking.

MF was found in thirteen patients. Nine of them were in the cellular phase and four in fibrotic phase. In the cellular phase, the bone morrow was hypercellular and displayed panmyelosis. Megakaryocytes were atypical and often appeared in clusters around the sinusoids and/or bone trabeculae. Abnormal nuclear lobulation, naked nuclei, and large bizarre forms were frequently observed. Micromegakaryocytes were often present. MF patients in the fibrotic phase revealed various degrees of fibrosis and marked hypocellularity. Megakaryocytes had deeply lobulated and hyperlobulated nuclei, abundant mature cytoplasm, and smooth nuclear contours.

Bone morrow microvascular dencity

MVD in *JAK2* (V617F) mutation was 16.48±8.77 (10 x 40), 12.51±5.59, 20.51±7.77, respectively for the patients with PV, ET and MF (Fig. 1). Patients without mutation had MDV 11.32±5.74, 12.80±5.40 and 15.00.±7.57 for PV, ET and MF, respectively (Fig.2). There was no significant difference in mean MVD in ET between the groups

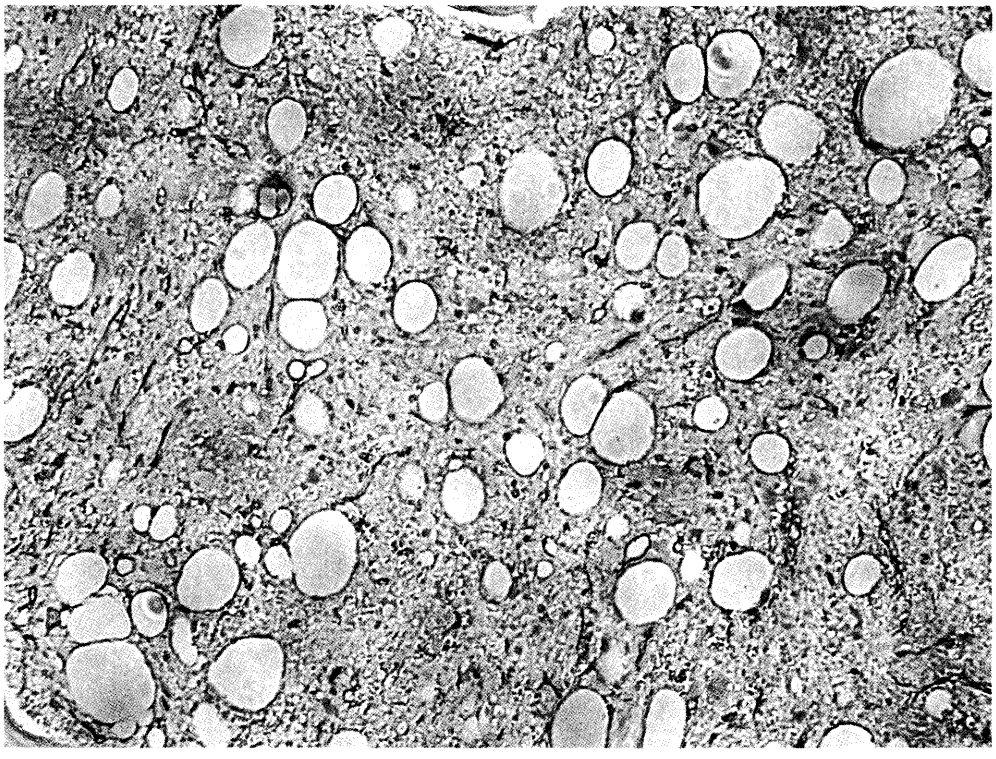

Fig 1. MPN in *JAK2* positive patient with PV.

($p>0.05$). MVD was higher in both PV and MF with *JAK2* (V617F) mutation as compared to *JAK2* (V617F) negative patients ($p<0.01$). The microvessels had moderate to high expression of CD34 in the cytoplasm of the endothelial cells. Scattered CD34 positive cells were also found, but the positive reaction was only in the nuclei.

Disscusion

An increased MVD in MPNs compared to controls has been already established (3, 4). Patients with MF had the highest MVD, followed by PV and ET. In addition, an altered vascular architecture has been established in MPNs (3). By confocal microscopy, tortuous and branched microvessels were observed in both PV and MF (3).

Our quantitative results considerably extend the results of previous studies and show a correlation between angiogenesis in bone morrow and *JAK2* mutation for patients with MF and PV. MF and PV patients with *JAK2* mutation had higher MVD than *JAK2* negative patients. This finding in patients is confirmed by in vitro studies, which show that angiogenesis is induced by activation of the *JAK2* signaling pathway, while vascular sprouting is inhibited by *JAK2* blockade (11).

JAK2 gene was mapped on the short arm of chromosome p24 in 1992 by Pritchard and his colleagues (5). It has 140 kb spanning 25 exons to form 1132 aminoacid JAK2 protein (7). *JAK2* mutation is implicated with mobilization of CD34-positive cells and

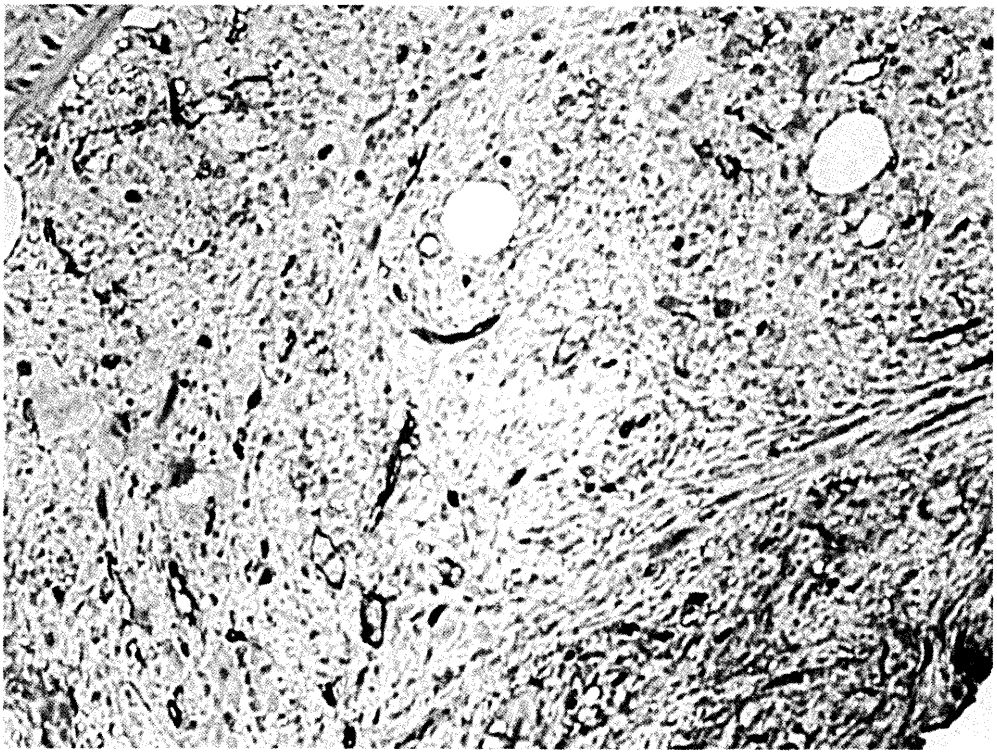

Fig 2. MPN in *JAK2* negative patient with PV.

MPNs progression (2). In this study endothelial cells were marked with CD34, but a set of haematopoietic cells also were CD34 positive. MPNs are thought to originate at the level of a primitive haematopoietic progenitor or stem cells (8). During embryogenesis endothelial cells and haematopoietic cells share a common cell origin (8). *JAK2* mutation is found in both endothelial hepatic venule cells and haematopoietic cells in patients with PV (8). The increased MVD in patients with *JAK2* mutation indicate that the endothelial cells are a part of the malignant transformations in MPNs.

We found that CD34 antibody visualized endothelial cytoplasm of microvessels. The scattered CD34 positive cells showed nuclear expression. CD34 expression was present only in the nuclei of haemopoietic stem cells with *JAK2*V617F mutation, while in their differentiated progeny, it remains mostly in the cytoplasm (6). The signals that are required for the translocation of normal and mutated *JAK2* to the nucleus remain unknown. It has been suggested that the activation of the kinase, coding from *JAK2* by phosphorylation may be the first step of several modifications which operate the nuclear translocation and when the cell undergoes differentiation these modifications are shut off, and mutated JAK2 protein remains predominantly in the cytoplasm. On the basis of these data we consider that the endothelial cells at least in part are differentiated cells.

We were unable to find a change in MVD between *JAK2* positive and *JAK2* negative patients with ET. Our results differ from other studies, which report an increased bone morrow angiogenesis in ET (4). According to Medinger et al. (2009) CD105 is

more reliable marker for newly formed vessels than CD34. On the other side, this probably indicates that angiogenesis is not involved, at least during the early stages of ET.

In conclusion, the formation of blood vessels is pronounced in MF and PV in bone marrow of patiens with *JAK2* mutation. Whether this is a major oncogenic event or a consequence of clonal haematopoietic cell proliferation remains to be clarified.

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