Angiogenesis in pulmonary hypertension with myelofibrosis

In patients with pulmonary arterial hypertension (PAH), myelofibrosis (MF) is common and contributes to impaired hematopoiesis. In most cases MF is not a clonal disease and it has been suggested that the same mechanisms that trigger the vascular injury resulting in PAH also contribute to the development of MF.[1] PAH is characterized by disorganized cellular proliferation in plexiform lesions, which morphologically resemble the abnormal vessels seen in primary MF (PMF) or secondary to a myeloproliferative disorder (post polycythemic myelofibrosis, PPMF, or post essential thrombocytosis, PTMF). However, there are differences as well: patients with MF and PAH have a higher level of serum VEGF and lower levels of circulating endothelial progenitor cells (EPC) than myeloproliferative MF patients.[2] Distinctive features of MF associated with PAH include normal or low circulating CD34 cell count, polyclonal platelets and granulocytes, absence of peripheral blood dacrocytes and the JAK2 1849G>T(V617F) mutation.[3,4]

We have recently shown that there is an overexpression of pericytes around the wide and tortuous capillaries in the bone marrow of patients with PMF, PPMF and PTMF,[5] i.e. the proportion of pericyte covered vessels was significantly higher in these patients as compared to controls. To further characterize the mechanisms of MF in a non-malignant setting, we wanted to investigate if patients with PAH and MF also have signs of enhanced angiogenesis and an overexpression of pericytes in their bone marrows or if the overexpression of pericytes is specific for PMF.

In this study, 3 patients, all females, 32, 34 and 48 years old with a diagnosis of PAH were evaluated with bone marrow biopsies because of suspicion of MF which was confirmed by routine bone marrow pathological examination. These patients have previously been described.[3] Bone marrow biopsies from 19 patients with PMF or MF secondary to a myeloproliferative disorder (10 men and 9 women; age range, 50-84 years; mean age, 66 years) and biopsies from 9 healthy individuals (9 men and one woman, 48-85 years; mean age 66 years) were used as control. These subjects have also been described previously in detail.[5] None of the patients with MF secondary to a myeloproliferative disorder underwent heart catheterization on suspicion of PAH, thus, there were no clinical signs suggesting that PAH was the underlying cause of the patients' MF. The PAH/MF patients were diagnosed and followed up at Baylor College of Medicine and the Methodist Hospital Houston, Texas, USA and the PMF, PPMF, PTMF and healthy controls at the Department of Hematology, Karolinska University Hospital Huddinge, Stockholm or Odense University Hospital, Denmark.

Sections from the bone marrow biopsies were double stained for CD34 (an endothelial cell marker) and SMA-α to identify pericytes. We chose CD34 as our endothelial cell marker to avoid misinterpreting megakaryocyte extensions and platelets as vessels, since megakaryocytes are abundant in PMF bone marrows.[6] As some pericytes will not express SMA-α, depending on localization and maturity, we also stained for desmin and PDGFR-β, as previously described.[5] Each section was evaluated for microvascular density (MVD) and vessel perimeters were measured using specialized software. Sections were also analyzed for percentage of pericyte positive vessels, as previously described.[5] Results are given as means and standard deviations, but also as median and range (5th-95th percentile). It was not possible to compare medians in a non-parametric test because of the low number of PAH patients.

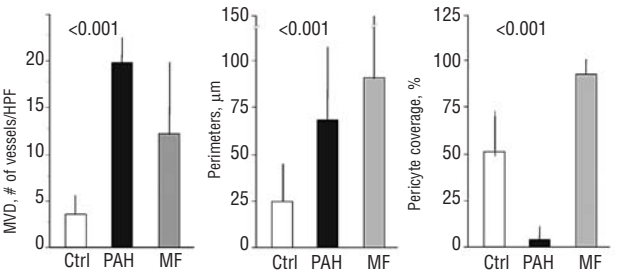

Figure 1. Microvascular density (MVD; left panel), capillary perimeters (middle panel) and percentage of vessels covered by pericytes (right panel) in controls (open bars), patients with PAH and MF (black bars) and patients with PMF, PPMF and PEMF (grey bars). Mean and SD values.

All 3 patients with PAH and MF had a markedly elevated MVD as compared to healthy controls (mean 19.7±5.0 *vs.* 3.66±2.3, $p<0.001$; median 21.0 (13-25) *vs.* 3.25 (03-8.4)) (Figure 1, left panel), but similar to patients with PMF or MF secondary to myeloproliferative disorder ($p=0.16$). Patients with PAH and MF had vessels that morphologically resembled those in patients with myeloproliferative MF in that they showed large distended lumina with irregular branching.[5] Vessels in the bone marrow of patients with PAH and MF had significantly increased perimeters as compared to controls (mean 70.3±46 *vs.* 19.2±16; $p<0.001$; median 60.0 (23-150) (Figure 1, middle panel). Compared with patients with myeloproliferative MF, perimeters were a little less, but this did not reach statistical significance ($p=0.3$)

Surprisingly, when staining for pericytes with SMA-α we found that patients with PAH were almost totally lacking pericytes in their bone marrow. Only 3% of the vessels were covered with pericytes (individual values were 0%, 0% and 10%) as compared with 91.6% of vessels in patients with myeloproliferative MF ($p<0.001$) and 51.1% of vessels in healthy controls ($p<0.001$) (Figure 1, right panel). Simultaneously stained slides from appropriate controls showed expected pericyte appearance, ruling out technical factors as the cause for the absence of pericytes in the PAH patients. Stainings with desmin and PDGFR-β were negative in PAH/MF patients and controls, but occasional PDGFR-β positive pericytes were seen in patients with myeloproliferative MF.[5]

PAH and myeloproliferative MF are both characterized by pathological angiogenesis and it has been suggested that the processes that initiate PAH could also result in myelofibrosis.[1] Here, we present data that clearly show that the MF in PAH has distinct phenotype with regard to angiogenesis which differs from clonal myeloprolifer-

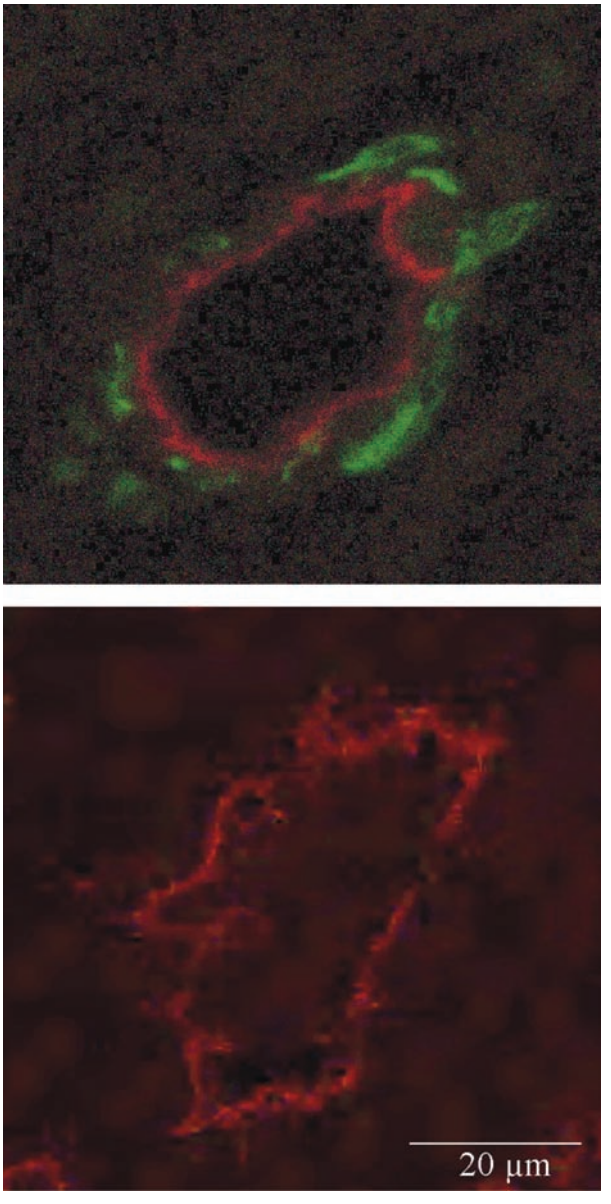

Figure 2. **Micrographs of normal (left) and PAH (right) bone marrow capillaries stained for CD34 (red, for endothelial cells) and SMA-α (green, for pericytes).**

ative MF. As expected, patients with PAH had increased MVD and morphologically aberrant vessels in their bone marrows. However, in this limited sample, instead of an increase in pericyte coating that characterizes vessels from patients with a myeloproliferative disorder, vessels from patients with PAH showed a striking loss of pericytes. This finding suggests that involvement of pericyte precursors (e.g. mesenchymal cells) or recruitment of pericytes might be specific for myelofibrosis secondary to a myeloproliferative disorder.

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