Myeloid-derived suppressor cells (MDSCs) are immature myeloid cells that accumulate in patients with malignancies, sepsis or chronic inflammation. Features of MDSCs are: myeloid origin, immature state, strong ability to reduce cytotoxic functions of T/NK cells, potential to differentiate into endothelial cells favoring neoangiogenesis. MDSCs are conventionally divided into polymorphonuclear (PMN)- and monocytic (M)-MDSCs. PMN-MDSCs are characterized by the production of reactive oxygen species and arginase-1 (Arg-1), that directly inhibits T-cell proliferation and activation, while M-MDSCs predominantly express inducible nitric oxide synthase and produce nitric oxide.

Myelofibrosis (MF) is characterized by clonal expansion of a hematopoietic stem cell, extramedullary hematopoiesis, bone marrow (BM) fibrosis, splenomegaly, BM and splenic neoangiogenesis and an acquired mutation of JAK2, CALR or MPL gene. Inflammation plays a relevant role in MF pathogenesis, as proven by high levels of inflammatory cytokines with prognostic significance and by a state of chronic oxidative stress.

Aims: To characterize circulating MDSCs and assess their localization and quantification in spleen samples from MF patients.

Methods:
Peripheral blood mononuclear cells (PBMCs) from MF patients (n=15) and healthy subjects (HDs; n=10) were stained with surface antibodies (CD11b, CD14, HLA-DR, CD15, CD33) and intracellular Arg-1 and analysed by flow cytometry. Formalin-fixed paraffin-embedded samples of spleen specimens (MF n=41; HDs n=20) were immunostained with anti-Arg-1 antibody using the automated platform Dako Omnis Envision Flex with the EnVision FLEX, High pH, HRP Rabbit/Mouse High pH [GV800] revelation kit.

Results:
In PBMCs, the percentage of PMN-MDSCs (identified as CD11b+HLA-DRlow/-CD33dimCD14+CD15+) was higher (p=0) in patients (median 9.2, range 1-59) than HDs (median 1.7, range 0.1-4.5) and directly correlated with the allelic burden (R=0.86, p=0.006) in JAK2-mutated patients. Patients in pre-fibrotic phase (n=7) had levels of PMN-MDSCs (median 9.2, range 1-31.4) comparable to HDs; on the contrary, the percentage of PMN-MDSCs in patients with BM fibrosis (n=8; median 13.3, range 3.5-59) was higher (p=0) than in HDs. All PMN-MDSCs were Arg-1+, both in MF and in HDs. Gene expression studies are ongoing. The expression of the CXCR4 receptor on PMN-MDSCs was lower, although not statistically significant, in MF (median 1.1, range 0-9.3) than in HDs (median 2.5,
PMN-MDSCs in MF inversely correlated ($R=0.89$, $p=0.007$) with CXCR4 expressed on CD34+ cells. The percentage of M-MDSCs (identified as CD11b+HLA-DRlow/CD33hiCD14+CD15-) was higher ($p=0.015$) in HDs (median 0.49, range 0.25-1.5) than in MF (median 0.29, range 0.02-0.76).

Immunohistochemical staining of spleen sections showed a higher ($p=0$) percentage of Arg-1+ cells (calculated on total spleen cells) in MF (median 20, range 5-60) than in HDs (median 7, range 2-20); Arg-1+ cells were present in all patients and predominantly located in the red pulp in MF patients, while in HDs were detected in the white pulp.

**Summary/Conclusion:**

The identification of MDSCs and their relation with the mutational status suggest a new mechanism of MF pathogenesis, either related to the chronic inflammatory status of patients or as players of the neoangiogenesis that characterizes the disease. Moreover, the low expression of CXCR4 on PMN-MDSCs could be, on one hand, related to the low expression of this receptor on CD34+ cells or suggest a MF-specific recruitment mechanism, other than that observed in solid tumors.