Philadelphia-negative myeloproliferative neoplasms display alterations in monocyte subpopulations frequency and immunophenotype

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Abstract
Philadelphia-negative myeloproliferative neoplasms (MPN) are clonal hematological diseases associated with driver mutations in JAK2, CALR, and MPL genes. Moreover, several evidence suggests that chronic inflammation and alterations in stromal and immune cells may contribute to MPN’s pathophysiology. We evaluated the frequency and the immunophenotype of peripheral blood monocyte subpopulations in patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (MF). Peripheral blood monocytes from PV (n = 16), ET (n = 16), and MF (n = 15) patients and healthy donors (n = 10) were isolated and submitted to immunophenotyping to determine the frequency of monocyte subpopulations and surface markers expression density. Plasma samples were used to measure the levels of soluble CD163, a biomarker of monocyte activity. PV, ET, and MF patients presented increased frequency of intermediate and non-classical monocytes and reduced frequency of classical monocytes compared to controls. Positivity for JAK2 mutation was significantly associated with the percentage of intermediate monocytes. PV, ET, and MF patients presented high-activated monocytes, evidenced by higher HLA-DR expression and increased soluble CD163 levels. The three MPN categories presented increased frequency of CD56+ aberrant monocytes, and PV and ET patients presented reduced frequency of CD80/86+ monocytes. Therefore, alterations in monocyte subpopulations frequency and surface markers expression pattern may contribute to oncoinflammation and may be associated with the pathophysiology of MPN.

Keywords Myeloproliferative neoplasms · Monocytes · Immune cells · Inflammation · Flow cytometry · Pathophysiology

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Introduction

Philadelphia-negative myeloproliferative neoplasms (MPN) are clonal hematological diseases characterized by expansion of precursor and mature myeloid cells in bone marrow and peripheral blood. The most frequent are polycythemia vera (PV), essential thrombocytosis (ET), and primary myelofibrosis (MF) [1, 2]. Erythrocyte hyperproliferation and high hemoglobin and hematocrit levels characterize PV, which may be accompanied by increased granulocytes count. ET and MF patients display atypical megakaryocyte proliferation: ET is characterized by increased peripheral platelet count, while MF is associated with inefficient erythropoiesis and deposition of collagen and reticular fibers in bone marrow [3, 4].

MPN pathophysiology is associated with genetic driver mutations in the Janus Kinase 2 (JAK2), calreticulin (CALR), and thrombopoietin receptor (MPL) genes, which are gain-of-function mutations that trigger autonomous and constitutive activation of the JAK-STAT pathway. The JAK2V617F mutation is the most frequent genetic alteration in MPN. It is present in more than 95% of PV patients, while 50–70% of ET and 50–60% of MF patients harbor the JAK2 mutation. In addition, 20–30% of ET and MF patients may harbor CALR mutation and 5–10% patients may present MPL mutation [1, 2, 5].

Beyond the genetic alterations, MPN are considered as oncoinflammatory diseases due to the exacerbated inflammatory status [6–8]. Recent studies have reported increased levels of pro-inflammatory cytokines, chemokines, and growth factors in bone marrow niche and peripheral blood from PV, ET, and MF patients [6, 9, 10] and associated them with patients’ clinic-laboratory parameters [9, 10]. Therefore, the pro-inflammatory profile seems to contribute to genetic and epigenetic instability, predisposition to cardiovascular events, fibrotic progression, and leukemic transformation in MPN [5, 6, 11, 12].

MPN patients may present alterations in immune cell compartments, such as decreased frequency and effector activities of natural killer cells [13], dysfunctions in lymphocyte subpopulations [14, 15], and immune response failure against pathogens [16–18]. Neutrophils, monocytes, and platelets activities have been associated with oncoinflammation and predisposition to cardiovascular events [19, 20].

Classical monocytes (CD14++CD16−) correspond to 80–95% of peripheral blood monocytes in normal subjects and react against pathogens through phagocytosis, expression of scavenger receptors, high peroxidase activity, and antimicrobial mechanisms. Intermediate monocytes (CD14++CD16+) corresponding to 2–10% of peripheral blood monocytes are associated with inflammation potentiation, neoangiogenesis, lymphocytes chemoattraction, and secretion of interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNF-α). Non-classical monocytes (CD14+CD16++) correspond to 2–10% of peripheral blood monocytes and are related to vascular patrolling, immune surveillance, antiviral response, natural killer, and T lymphocytes modulation, and angiogenesis process [21–24].

In MPN, monocytosis has been associated with reduced life expectancy in older patients, worse prognosis in younger MF patients and the occurrence of cytogenetic and epigenetic abnormalities in PV patients [6, 25, 26]. Considering that immune cells play major roles in the modulation of inflammatory response, which is a key component in MPN pathophysiology, the present work investigated the frequency and immunophenotype of monocyte subpopulations in PV, ET, and MF patients.

Subjects, material, and methods

Demographic and clinic-laboratory features of patients and control subjects

Twenty milliliters of peripheral blood samples were collected from 16 PV patients (8 women, 8 men, median age = 62 years), 16 ET patients (8 women, 8 men, median age = 66.5 years), and 15 MF patients (5 women, 10 men, median age = 63 years) previously diagnosed according to the 2016 World Health Organization criteria. The control group consisted of 10 healthy individuals (6 women, 4 men, median age = 49 years old) from the university community.

In the PV group, 13 patients were JAK2V617F positive and three had undetermined mutational status; in the ET group, 12 patients were JAK2V617F positive, two were CALR positive, and two were triple negative; in the MF group, nine patients were JAK2V617F positive, two were CALR positive, one was triple negative, and three patients had undetermined mutational status. At the time of blood collection, eight PV patients, 11 ET patients, and nine MF patients were undergoing treatment with hydroxycarbamamide (Table 1). None of the MPN patients evaluated in this study was in use of JAK inhibitors or interferon-alpha at the time of blood collection.

The Ethics Committee for Human Research from the School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo (FCFRP-USP) and from the University Hospital of the Ribeirão Preto Medical School (HCFCRP-USP), University of São Paulo (USP), Ribeirão Preto, Brazil approved the study protocols (#24508619.1.0000.5403 and 84215.1.0000.5403).
All the participants signed the informed consent form.

### Plasma samples

Plasma samples were obtained after centrifuging peripheral blood collected in EDTA tubes (Vacutainer®; Becton, Dickinson and Company) at 400 × g for 10 min at 22 °C (Eppendorf 5810R Centrifuge) and stored at −80 °C in a freezer for further quantification of soluble CD163 (sCD163) biomarker using ELISA.

### Isolation of peripheral blood monocytes

Blood samples were diluted in phosphate buffered saline (PBS) and submitted to Ficoll®-Paque (Sigma-Aldrich, San Luis, MO, USA) density gradient centrifugation protocol at 400 × g for 35 min at 22 °C (Eppendorf 5810R Centrifuge) to obtain peripheral blood mononuclear cells. The cells were counted in a hemocytometer and the cell concentration was adjusted to 1 × 10⁷ cells per ml for immunophenotyping.

### Immunophenotyping of monocyte subpopulations

For monocyte immunophenotyping, 500 µl of mononuclear cells suspension in PBS were incubated with 5 µl of combined antibodies as follows: (i) an unlabeled tube for negative cells acquisition; (ii) a tube with anti-CD45 APC-H7, anti-CD14 APC, anti-CD16 PECy5, and anti-CD80/86 PE antibodies; (iii) a tube with anti-CD45 APC-H7, anti-CD14 APC, anti-CD16 PECy5 and anti-HLA-DR FITC antibodies; and (iv) a tube with anti-CD45 APC-H7, anti-CD14 APC, anti-CD16 PECy5, and anti-CD56 FITC antibodies (Table 2).

After antibody labeling, cells were washed once with PBS, centrifuged at 400 × g for 5 min, filtered, and suspended in 1 mL of PBS and analyzed in a BD FACSAria™ Fusion flow cytometer, with 10,000 events acquired.

The monocyte population was gated by forward scatter area (FSC-A) and side scatter area (SSC-A) using pseudocolor dot plots (Supplementary Fig. 1-I). Doublets were excluded applying FSC-A versus FSC-height (FSC-H) gate (Supplementary Fig. 1-II). CD14+ cells—total monocyte population—were selected (Supplementary Fig. 1-III). Monocyte subpopulations were defined applying CD45 versus CD16 dot plots (Supplementary Fig. 1-IV). CD16 heatmap gradient expression and CD16 median of fluorescence intensity (MFI) were used to delimit the monocyte subpopulations in classical (CD16−), intermediate (CD16+), and non-classical (CD16+++) (Supplementary Fig. 1-V). The frequency of each monocyte subpopulations were analyzed by dot plots and expressed by percent of labeled cells.

The CD64, CD80/86, HLA-DR, and CD56 expression in monocytes were evaluated by histograms in each monocyte subpopulations gates and the results were expressed in median of fluorescence intensity (MFI). The data were analyzed using BDFACS™ Diva software in the flow cytometer and FlowJo™ v10 software.

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**Table 1** Demographic and clinic-laboratory data from patients and control subjects

| Age (years) Median, range | CTRL | PV | ET | MF |
|---------------------------|------|----|----|----|
| 62.5 (36–89)              |      |    |    |    |
| Gender (n)                | n = 10 | n = 16 | n = 16 | n = 15 |
| Male                      | 4     | 8  | 8  | 10 |
| Female                    | 6     | 8  | 8  | 5  |

**Mutation status**

| JAK2V617F+ | 13 | 12 | 9 |
| CALR+      | –  | 2  | 2 |
| Triple negative | – | 2 | 1 |
| Undetermined | – | 3 | 3 |

**Treatment**

| Hydroxycarbamide | –  | 8  | 11 | 9 |

CTRL Controls; ET Essential thrombocythemia; MF Primary myelofibrosis; PV Polycythemia vera

**Table 2** Features of the antibodies used for immunophenotyping

| Antibody | Clone | Fluorochrome | Volume | Source          |
|----------|-------|--------------|--------|-----------------|
| CD14     | M5E2  | APC          | 5µL    | BD Pharmingen   |
| CD16     | 3G8   | PECy5        | 5µL    | BD Pharmingen   |
| CD45     | 2D1   | APC-H7       | 5µL    | BD Pharmingen   |
| CD56     | B159  | FITC         | 5µL    | BD Pharmingen   |
| CD64     | 10.1  | FITC         | 5µL    | BD Pharmingen   |
| CD80     | 2D10.4| PE           | 5µL    | BD Pharmingen   |
| CD86     | IT2.2 | PE           | 5µL    | BD Pharmingen   |
| HLA-DR   | G46-6 | FITC         | 5µL    | BD Pharmingen   |

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#24508619.1.3001.5440). All the participants signed the informed consent form.
Quantification of soluble CD163 in plasma

Plasma concentration of the monocyte biomarker sCD163 was quantified using the DuoSet ELISA kit (R&D Systems, Minneapolis, Minn, USA), according to the manufacturer’s instructions. The concentration of sCD163 was determined by linear regression and the results were expressed in pg/mL.

Statistical analyses

The Mann–Whitney and ANOVA non-parametric statistical tests were used to compare monocyte subpopulations frequency and surface markers MFI among PV, ET, and MF patients and the control group. Statistical differences were considered significant when the p value was < 0.05. The results were expressed as mean and standard deviation. Statistical analyses were performed using the GraphPad Prism 5.0 software (Graph-Pad Software, San Diego, CA, USA).

Results

Frequency of monocyte subpopulations in MPN

Compared with the control group, (i) PV (35.68 ± 24.42, p < 0.0001), ET (39.56 ± 25.32, p = 0.0002) and MF (32.34 ± 26.02, p < 0.0001) patients presented decreased frequency of classical monocytes; (ii) PV and MF patients presented increased frequency of intermediate (PV = 39.00 ± 20.03, p = 0.0002; MF = 31.66 ± 15.67, p = 0.0002) and non-classical monocytes (PV = 27.35 ± 26.56, p = 0.0072; MF = 33.53 ± 26.90, p = 0.0043); and (iii) ET patients presented increased frequency of intermediate monocytes (38.53 ± 20.84, p = 0.0012). PV, ET, and MF patients presented similar frequencies of monocyte subpopulations (Fig. 1).

Frequency of intermediate monocytes is associated with JAK2 mutation in MPN

The frequencies of classical, intermediate, and non-classical monocytes from PV, ET, and MF patients were analyzed according to JAK2 mutation status. JAK2V617F+ MPN patients presented higher frequency of intermediate monocytes than JAK2V617F− patients (50.60 ± 22.82, p = 0.0090) (Fig. 2). ET and MF patients positive for JAK2V617F or CALR mutations had similar frequencies of monocyte subpopulations (data not shown).

Fig. 1 Percentage of monocyte subpopulations in myeloproliferative neoplasms (MPN). Polycythemia vera (PV), essential thrombocytemia (ET), and primary myelofibrosis (MF) patients presented lower frequency of classical monocytes (p < 0.0001, p = 0.0002, p < 0.0001, respectively); PV, ET, and MF presented increased intermediate monocytes frequency (p = 0.0002, p = 0.0012, p = 0.0002, respectively). PV and MF patients presented increased frequency of non-classical monocytes compared to the controls (p = 0.0072, p = 0.0043, respectively). Statistical differences were calculated using ANOVA non-parametric test and considered significant when p < 0.05. The results were expressed in mean and standard deviation.
Surface markers distribution in monocyte subpopulations from MPN patients

The frequency of CD64+ monocyte subpopulations did not differ in PV, ET, and MF patients (data not shown).

In PV, there is higher frequency of CD80/86+ non-classical than CD80/86+ classical monocytes (classical = 18.81 ± 22.54, non-classical = 37.77 ± 25.60, p = 0.0428) (Fig. 3A). In ET, there is more CD80/86+ non-classical monocytes than classical and intermediate monocytes (classical = 33.45 ± 37.25, intermediate = 35.64 ± 30.86, non-classical = 60.03 ± 25.82, p = 0.0203, and p = 0.0229, respectively) (Fig. 3B). There were no differences in MF patients (Fig. 3C).

PV patients presented higher frequency of HLA-DR+ classical monocytes than HLA-DR+ non-classical monocytes (classical = 90.06 ± 18.64, non-classical = 63.86 ± 35.13, p = 0.0104) (Fig. 3D). The frequency of HLA-DR+ intermediate monocytes is also higher than the frequency of HLA-DR+ non-classical monocytes (intermediate monocytes = 84.05 ± 23.04, non-classical = 63.86 ± 35.13, p = 0.0545) (Fig. 3D). ET and MF patients did not present significant differences (Fig. 3E–F).

CD56+ non-classical monocytes frequency is higher than CD56+ classical monocytes in PV (classical = 30.23 ± 18.28, non-classical = 51.74 ± 26.25, p = 0.0414) (Fig. 3G). CD56+ non-classical monocytes frequency is higher than CD56+ classical and intermediate monocytes in ET patients (classical = 33.68 ± 26.85, intermediate = 46.84 ± 24.92, non-classical = 69.71 ± 24.50, p = 0.0056, and p = 0.0240) (Fig. 3H). No differences were seen in MF patients (Fig. 3I).

MPN patients and the control group had similar frequencies of CD64+ monocytes. Compared with the control group, PV (30.08 ± 24.59, p < 0.0001) and ET (43.14 ± 33.50, p = 0.0016) patients exhibited reduced frequency of CD80/86+ monocytes, but the CD80/86 positivity in total monocytes population was unaltered in MF patients (Fig. 4A).

Compared with the control group, PV (18.81 ± 22.54, p = 0.0013, and 26.76 ± 20.22, p = 0.005, respectively) and ET (33.45 ± 37.25, p = 0.0406, and 35.64 ± 30.86, p = 0.0128, respectively) patients exhibited lower frequencies of CD80/86+ classical and intermediate monocytes. MF patients presented higher frequency of CD80/86+ intermediate monocytes than PV (43.74 ± 36.58, p = 0.0007) and ET patients (60.03 ± 25.82, p = 0.0229). PV patients had lower frequency of CD80/86+ non-classical monocytes than the control group (37.77 ± 25.60, p = 0.0194) and ET patients (60.03 ± 25.82, p = 0.0440) (Fig. 4B).

PV (77.66 ± 31.65), ET (73.17 ± 30.14), and MF (82.51 ± 31.28) patients displayed increased frequency of HLA-DR+ monocytes when compared with controls (p < 0.0001) (Fig. 4A). Classical monocytes from PV (90.06 ± 18.64, p = 0.0046), ET (83.63 ± 19.17, p = 0.0251), and MF (83.22 ± 30.48, p = 0.0229) patients had higher HLA-DR positivity than controls. Intermediate monocytes from PV (84.05 ± 23.04, p = 0.0026), ET (72.79 ± 29.13, p = 0.0185), and MF (80.62 ± 32.34, p = 0.0111) presented the same profile as classical monocytes. PV (63.86 ± 18.43, p = 0.0312) and MF (84.01 ± 35.67, p = 0.0262) patients exhibited higher frequency of HLA-DR+ non-classical monocytes.
monocytes than controls. MF patients had higher frequency of non-classical HLA-DR⁺ monocytes than PV patients (p = 0.0384) (Fig. 4B).

Total monocytes from PV (40.77 ± 21.09, p = 0.132), ET (50.08 ± 29.00, p = 0.0026), and MF (59.58 ± 27.07, p < 0.0001) patients displayed higher CD56 positivity than controls. MF patients had more CD56⁺ monocytes than PV patients (p = 0.0038; Fig. 4A). PV (30.23 ± 18.28, p = 0.0060), ET (33.68 ± 26.85, p = 0.0174), and MF (50.58 ± 25.26, p = 0.0005) patients presented higher frequency of CD56⁺ classical monocytes than controls. MF patients exhibited higher frequency of CD56⁺ intermediate monocytes (62.70 ± 29.55, p = 0.0185); ET (69.71 ± 24.50, p = 0.0140) and MF (66.27 ± 26.19, p = 0.0462) patients presented higher frequency of CD56⁺ non-classical monocytes (Fig. 4B).

Expression density of CD64, CD80/86, HLA-DR and CD56 in MPN monocytes

MPN patients and control subjects did not significantly differ in monocytes CD64 expression. Compared with controls, PV patients expressed lower CD80/86 levels (329.81 ± 440.44, p = 0.0275), while MF patients expressed similar CD80/86 levels in the total monocyte population (Fig. 5A).

PV (2900.03 ± 2527.01, p = 0.0025), ET (1420.83 ± 1255.32), and MF (9355.63 ± 6921.76, p = 0.0033) patients expressed more HLA-DR in the
total monocytes population than control subjects (775.86 ± 616.83). MF patients expressed more HLA-DR in the total monocytes population than PV (p = 0.0066) and ET (p = 0.0040) patients (Fig. 5A). PV (2759.00 ± 2533.28, p = 0.0172) and ET (1085.36 ± 912.21, p = 0.0109) patient’s classical monocytes and MF patient’s intermediate monocytes (9840.06 ± 7074.64, p = 0.0250) presented increased HLA-DR expression than controls (Fig. 5B).

PV (208.61 ± 554.73; p = 0.0132) and ET (505.77 ± 672.22; p = 0.0026) patients’ total monocytes population expressed lower CD56 levels than control subjects (CTRL).
controls (798.76 ± 554.73). MF patients’ monocytes presented higher CD56 expression than PV and ET (1004.62 ± 1432.14, p = 0.02247, and p < 0.0001), while ET patients’ monocytes presented higher CD56 expression than PV (p = 0.0033) (Fig. 5A). Compared with the control group, monocyte subpopulations from ET and MF patients expressed similar levels of CD56, while classical, intermediate, and non-classical monocytes from PV patients expressed lower CD56 levels (189.36 ± 74.22, p = 0.0463; 206.55 ± 100.38, p = 0.0038; and 229.91 ± 86.61, p = 0.0006, respectively) (Fig. 5B).

**sCD163 concentration in MPN patients’ plasma**

Plasma sCD163 concentration was quantified in PV (n = 13), ET (n = 13), MF (n = 14) patients and control subjects (n = 11). PV (11,454.52 ± 4939.96, p = 0.0499), ET (11,675.92 ± 4652.72, p = 0.0281), and MF (16,247.93 ± 9556.81, p = 0.0281) patients presented higher sCD163 concentration than the control group (Fig. 6). The three groups of MPN patients did not differ with respect to the sCD163 plasma concentration.

**Discussion**

The pro-inflammatory status seem to contribute to MPN pathophysiology [4, 6, 8], once the isolated presence of driver mutations are insufficient to fully explain the mechanisms underlying the MPN pathogenesis, progression, and phenotypes [27]. Many authors have reported the association of oncoinflammation [6–8, 27] with genetic instability, symptomatology, endothelial dysfunction, and pro-coagulant state in MPN [9, 10, 18].

PV, ET, and MF patients, in the present study, exhibited increased frequency of intermediate and non-classical monocytes in peripheral blood. Intermediate and non-classical monocytes are cells that present mature phenotypes
Monocyte subpopulations contribute to the pathogenesis and progression of cardiovascular diseases like thrombosis—a frequent comorbidity of MPN patients, reflecting the pro-inflammatory status [31, 32].

Expansion of intermediate and non-classical monocytes occurs in bone marrow and peripheral blood from PV patients, in which high plasma levels of the pro-inflammatory cytokines IL-6, IL-8, MIP-1α, and TNF-α secreted by monocytes correlate with disease severity and risk to leukemic transformation [33].

In our study, higher frequency of intermediate monocytes in JAK2+ patients strengthens the hypothesis that exacerbated inflammation linked to JAK-STAT pathway activation may contribute to pro-inflammatory monocytes differentiation [33].

The higher frequency of non-classical monocytes in MF patients when compared with PV and ET patients and the control group was a relevant finding, as it suggested that these cells participate in the mechanisms of secretion of factors associated with fibrosis. The significant increased frequency of non-classical monocytes in PV patients’ peripheral blood when compared with the control group suggests the potential participation of these cells in the risk for thromboembolic events. The literature has reported the participation of non-classical monocytes in intravascular immune responses, wound healing, and regulation of vascular homeostasis [21, 34].

Here we described the altered expression of monocytes markers in PV, ET, and MF patients. PV and ET patients presented lower frequency of CD80/86-expressing monocytes than controls and MF patients. Low CD80 expression is associated with weak induction of immune surveillance and facilitation of necrotic cells spread in patients with colorectal cancer [35]. This profile was also reported in other neoplasms, like multiple myeloma, leukemia and carcinomas, and represents one of the mechanisms of immune response evasion that leads to insufficient activation of T lymphocytes [36].

The lower frequency of CD80/86+ monocytes in PV and ET patients compared with healthy individuals and MF patients suggested that circulating monocytes, in these patients, present less costimulatory capacity. On the other hand, CD80/86 expression levels in MF patients’ monocytes were similar to those detected in controls, pointing to differences in costimulation profile between the three diseases.

The frequency of HLA-DR+ monocytes was higher in PV, ET, and MF than in controls. High HLA-DR expression is related to monocytes activation and pro-inflammatory cytokines secretion [37]. In inflammatory diseases like rheumatoid arthritis and sepsis, high HLA-DR expression and low CD80/86 expression are associated with increased activation of circulating monocytes and reduced costimulatory activity, respectively [37]. PV and ET patients from the

Compared with classical monocytes and play complex activities, including modulation of effector functions of helper lymphocytes and natural killer cells, neutrophil recruitment, intravascular homeostasis, and immune surveillance. These monocyte subpopulations are able to induce potent inflammatory responses that give support to the chronic-inflammatory milieu in neoplastic diseases [24].

Murine B-cell acute lymphoblastic leukemia model present increased frequency of non-classical and reduced frequency of classical monocytes in peripheral blood and spleen [28]. The frequency of intermediate and non-classical monocytes is augmented in acute myeloid leukemia and associated with genetic instability and worse prognosis. The increased frequency of these cells in acute myeloid leukemia is inversely correlated with TCD4+ lymphocytes and positively correlated with granulocytes count. This data emphasizes that CD16+ monocytes may modulate the effector functions of other immune cells [29].

Tie-2-expressing monocytes, with pro-angiogenic ability, and monocytes with T lymphocytes-suppressive capacity were described in chronic lymphocytic leukemia [30]. The gene signature in chronic lymphocytic leukemia is associated with increased CD16 expression and corresponds to the expansion of intermediate and non-classical monocytes. In addition, monocytes’ genomic abnormalities, immunosuppressive characteristics and dysfunctions in phagocytic and costimulatory activity were associated with the leukemic cells pro-tumorigenic microenvironment [30], emphasizing the influence of the microenvironment in the phenotype and effector functions of monocytes.

Fig. 6 Quantification of soluble CD163 (sCD163) in myeloproliferative neoplasms (MPN). The plasma concentration of the monocyte activity biomarker sCD163 in patients with polycythemia vera (PV, n = 11), primary myelofibrosis (MF, n = 14), and control subjects (CTRL, n = 11) was plotted in the scatter plot. The MPN patients presented higher plasma concentration of sCD163 than CTRL group (PV: p < 0.0499; ET: p < 0.0273; MF: p < 0.0079). Statistical differences were determined using ANOVA non-parametric test and considered significant when p < 0.05.
present study exhibited this profile, which is also associated with tumor cells resistance to apoptosis in other neoplasms, such as in colon cancer [38, 39].

The fact that PV, ET, and MF patients exhibited high sCD163 plasma levels, which is a specific biomarker of monocyte activation, suggested the presence of high-activated monocytes in peripheral blood of these patients. Plasma CD163 concentration correlates to the exacerbated activity of monocytes and macrophages in bacterial sepsis, hepatitis, rheumatoid arthritis, Crohn’s disease, ulcerative colitis, atherosclerotic events, and type 2 diabetes [40].

Newly diagnosed patients with multiple myeloma present increased number of extracellular vesicles carrying sCD163 in plasma and increased CD163 antigenic expression on intermediate monocytes seems to be implicated in malignant transformation of plasma cells [41]. sCD163 levels have an important prognostic value in solid and hematological neoplasms, as they correlate with patients’ worse overall survival and lower progression-free survival [42].

PV, ET, and MF patients presented high frequency of CD56+ aberrant monocytes in peripheral blood. CD56+ monocytes expand during chronic-inflammatory and neoplastic conditions, but their effector functions were not fully elucidated [43]. CD56+ monocyte counts are increased in peripheral blood from patients with endocrine neoplasms in thyroid and adrenal glands, gastrointestinal neoplasms, leukemias, and lymphomas [44]. This subpopulation is known as “tumor-lysing monocytes”, express natural killer cell markers and induce apoptosis of tumor cells when stimulated in vitro by IFN-α. It highlights their possible contribution to immune surveillance as lysis-promoting cytotoxic cells when stimulated [44].

CD56+ monocytes are expanded in patients with moderate and severe COVID-19 as compared with mild-asymptomatic patients. These cells are associated with IFN-γ and granzyme B secretion and high-activated profile; their number increase in the first 5 days of infection and they remain proliferative at the 16th day after the PCR test confirmation. CD56+ monocytes probably participate in the exacerbated immune response that lead to tissue and organ damage and dysfunction in COVID-19 and the frequency of these cells positively correlate with hypertension in these patients [45].

The frequency of CD56+ monocytes in acute myeloid leukemia correlates with worse prognosis and low patients’ overall survival [46]. In MPN, CD56+ monocytes were recently reported in ET patients and associated with CXCL1 secretion, a chemokine that have been considered as a potential biomarker of ET evolution to MF [47].

The present work reported higher CD56 expression in MF monocytes, which indicates a possible association between CD56 expression and MPN severity, once MF is the disease category with worse prognosis.

Taken together, the results point to alterations in monocyte subpopulations frequency in MPN patients’ peripheral blood, suggesting that exacerbated oncoinflammatory activity affects the monocyte compartments. JAK2V617F mutation is probably linked to the intermediate monocytes shifting. Surface markers pattern alterations and increased sCD163 levels indicates hyperactivation and possible perturbations in monocytes’ effector activities. Therefore, monocytes may contribute to oncoinflammation in MPN.

Further investigations are necessary to better understand the immune response alterations in MPN in order to determine the immune profiles of PV, ET, and MF patients, helping to develop new therapeutic interventions that restore the immune cell antitumor activity and dampen the exacerbated oncoinflammation in MPN.

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Author contributions VLB collected the samples, performed monocytes isolation and immunophenotyping, analyzed data, and wrote the paper. GDB, FCA, LSB, and JPL helped to collect samples, isolate monocytes, and select patients. PVBP performed samples acquisition for the immunophenotyping analysis. CF and RCC performed the ELISA assay. VLB, LFP, FGF, and FAC designed the study, discussed the results, provided the research funding, and revised the manuscript.

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Declarations

Conflict of interest The authors have no conflict of interest to declare.

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