



Cytokines frequently implicated in myeloproliferative neoplasms

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ABSTRACT

Classical myeloproliferative neoplasms (MPN) include polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). MPN has been defined as a chronic inflammation-driven tumor model. It is clear that there is a close link between chronic inflammation and MPN pathogenesis. Several studies have demonstrated cytokine profiles in MPN patients. Other studies have used cell lines or animal models aiming to clarify the underlying mechanism of cytokines in the pathogenesis of MPN. However, important questions remain: (1) among all these cytokines, which are more predictive? and (2) which are more critical? In this review, we summarize cytokines that have been investigated in MPN and highlight several cytokines that may be more significant in MPN. We suggest that cytokines are more critical in PMF than PV or ET. These cytokines include IL-1 β , TNF- α , IL-6, IL-8, VEGF, PDGF, IFNs and TGF- β , all of which should be more closely investigated in MPN. Based on our extensive literature search, several key factors have emerged in our understanding of MPN: first, TNF- α could correlate with MPN progression including PMF, PV and ET. IL-1 β plays a role in PMF progression, while it showed no relation with PV or ET. Second, IL-8 could be a prognostic factor for PMF, and IL-6 could be important for MPN progression. Third, VEGF and PDGF play an indirect role in MPN development and their inhibitors could be effective. Fourth, different subtypes of IFNs could have different effects in MPN. Finally, TGF- β is closely linked to MF, although the data are inconsistent. Agents that have targeted these cytokines described above are already in clinical trials, and some of them have even been used to treat MPN patients. Taken together, it will be critical to continue to investigate the precise role of these cytokines in the pathogenesis and progression of MPN.

1. Introduction

The term “myeloproliferative disorders” was first proposed by William Dameshek in 1951. With the discovery of the Philadelphia chromosome, myeloproliferative disorders were classified into two groups: BCR-ABL positive chronic myeloid leukemia (CML) and BCR-ABL negative chronic myeloproliferative disorders (CMD) [1]. The latter was replaced by the name myeloproliferative neoplasms (MPN), which is more representative of the clonal features for this disease group. It includes several subtypes: PV, ET, PMF, chronic neutrophilic leukemia (CNL), unclassified myeloproliferative neoplasms (MPN-U) and chronic eosinophilic leukemia, not otherwise specified (CEL-NOS) [2]. The first three entities (PV, ET and PMF) represent classical MPN. Epidemiology of MPN in Europe and USA shows the incidence of MPN is about 2–3/100,000, and an increased tendency has been observed in the last 10 years [3,4]. Many advances have been made in MPN pathogenesis and treatment since the discovery of the JAK2V617F mutation as an MPN disease marker in 2005 [5,6]. Soon after, other gene mutations were identified in MPN including mutations in the

myeloproliferative leukemia protein (MPL) and calreticulin (CALR) [7–10]. Emerging results from clinical surveys of gene mutations in MPN patients and transgenic animal model studies revealed that these gene mutations could be driven factors for the clonal evolution in developing MPN [11,12]. Ruxolitinib, a JAK-inhibitor, has been used for PMF patients as a standard treatment, and it has been investigated in PV or ET patients with hydroxycarbamide (HC) failure/intolerance as a second line treatment [13]. However, other factors could be involved in classical MPN pathogenesis. Hasselbalch proposed the idea that chronic inflammation plays a critical role in MPN development [14]. This was later confirmed by many studies that demonstrated the critical role of inflammation in the initiation and progression of MPN; it has been proposed that MPN could be an inflammation-driven tumor model [15–19]. Clinical surveys have focused on inflammatory mediators such as cytokines, and many cytokines have been evaluated in blood serum or bone marrow plasma from MPN patients by different MPN centers [20,21]. Furthermore, IFN- α has been used in treating MPN effectively for more than fifty years and has a stable therapeutic status in MPN management even with the common usage of Ruxolitinib [22].

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Table 1 Cytokines evaluated in the literature. Several cytokines have been investigated in MPN patients using different techniques from 1997 to present. Detailed information about each study will be presented in this review.

Populations	Sample source	Cytokines	Technology	Authors	Years
ET = 8, PV = 6, CML = 25, MF = 1	PB	sIL-2R	ELISA	Kawatani T	1997
ET = 20, PV = 10, CML = 15, MF = 10	PB	TNF- α , IL-1 α , IL-1 β , IL-2, sIL-2R, IL-6, IL-10	ELISA	Bourantani KL	1999
ET = 31, PV = 22, CML = 17	PB and BM	TPO, IL-6, sIL-6R, IL-11, SCF, IL-3, IL-8	ELISA	Hui-chi HSU	1999
ET = 40, PV = 8, MF = 25, CML = 10, Control = 27	PB	IL-1 α , IL-1 β , IL-2, sIL-2Ra, IL-6, TPO	ELISA	Panteli KE	2005
PV = 18	PB and BM	IL-11, IL-8	ELISA	Hermouet S	2002
PV = 10, SE = 6	PB	EPO, VEGF	ELISA	Murphy P	2002
ET = 40, PV = 8, MF = 25, CML = 10, Control = 27	PB	VEGF	ELISA	Panteli KE	2007
ET = 15, PV = 58, SE = 37	PB and BM	IL-6, IL-8, IL-11, Leptin, HGF, MCP-1, TIMP-1, SCF	ELISA	Boissinot M	2011
ET = 28, PV = 20	PB	HGF, IL-6, IL-11, C-MET	mRNA		
ET = 5, PV = 5, MF = 10	PB	IL-10, IL-22, IL-23	ELISA	Gangemi S	2012
PV = 65, MF = 127, Control = 35	PB	79 cytokines	Human cytokine array	HO CL	2007
PV = 65, MF = 127, Control = 35	PB	IL-1 β , IL-1RA, IL-2, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, EGF, etoxin, FGF-b, GM-CSF, HGF,	Multiplex cytokine assay	Vaidya R	2012
PV = 17, ET = 21	PB	IFN- α , IFN- γ , IP-10, MCP-1, MIG, MIP-1 α , MIP-1 β , RANTES, TNF- α , VEGF	Multiplex cytokine assay	Tefferi A	2011
ET = 5, PV = 15, MF = 41, Control = 18	PB	IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-12, GM-CSF, IFN- γ , MCP-1, PDGF-BB, TNF- α , VEGF, HGF	Multiplex cytokine assay	Pourelot E	2014
ET = 11, PV = 20, MF = 16, Control = 34	PB	sIL-2Ra	ELISA	Wang JC	2014
	PB	GM-CSF, IFN- α 2, IFN- γ , IP-10, MCP-1, MIP-1 α , MIP-1 β , RANTES, TNF- α , IL-1 β , IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17A	Multiplex cytokine assay	Cacemiro MDC	2018

EPO, erythropoietin; FGF-b, fibroblast growth factor-basic; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; IFN, interferon; IL, interleukin; IP-10, IFN- γ -inducible protein 10; MCP-1, monocyte chemoattractant protein-1; MIP, macrophage inflammatory protein; PDGF, platelet-derived growth factor; RANTES, regulated on activation normally T-cell expressed and secreted; SCF, stem cell factor; VEGF, vascular endothelial growth factor; TPO, thrombopoietin; TNF- α , tumor necrosis factor α .

Altogether, these studies suggest that there is a close relationship between MPN and inflammation, though it is still not clear whether inflammation or gene mutation is the trigger for MPN. Tumor development is always a multi-step process that involves cooperating effects of both intrinsic and extrinsic factors. We, therefore, suggest that both gene mutation and chronic inflammation are critical in the development of MPN. Immunotherapy is very popular, especially chimeric antigen receptors T (CART) cell therapy, which has revolutionized tumor treatment not only in hematology malignancies but also in solid tumors [23]. For MPN, it is possible to use gene-modified T cells to kill the MPN clone, which would exert an immunomodulating role by balancing inflammatory cytokine levels. The latter could control the disease. At the very least, cytokine-directed immunotherapy for MPN could be a new treatment strategy in the future. In this review, we summarize experimental results of cytokine profiles in MPN and potential clinical applications of cytokines in treating MPN.

2. Chronic inflammation state and MPN: Results from epidemiology

Phenomena of autoimmune myelofibrosis or PMF presenting autoimmune phenomena have been reported [24–26]. A large population-based study including 11039 MPN patients and 43550 matched controls showed that 2.6% MPN patients had a previous history of autoimmune problems such as immune thrombocytopenic purpura (ITP), Crohn's disease (CD), polymyalgia rheumatica (PMR), giant cell arteritis, Reiter's syndrome and aplastic anemia. Patients with a history of these autoimmune problems presented an increased risk of developing MPN [27]. More recently in 2011, results from a systematic review of a literature search indicated that autoimmune conditions are associated with the development of myeloid neoplasms including MPNs. In particular, CD was found to be associated with the occurrence of MPN, and JAK2 mutations were also identified in CD patients [28]. One recent case study with 323 MPN patients and 333 chronic lymphocytic leukemia (CLL) patients demonstrated an increased risk of MPN in populations with a history of autoimmune disease. Interestingly, this study showed JAK2-positive MPN patients presented a higher incidence of prior ischemic disease and thromboembolic events compared to JAK2-negative MPN patients [29]. It has been established that autoimmune disease is essentially chronic inflammation disease and that vascular problems are due to persistent chronic inflammation; we, therefore, suggest that there is a link between MPN and chronic inflammation. Furthermore, we speculate that persistent chronic inflammation could more likely be due to gene mutation, which results in JAK2-positive MPN.

3. Cytokine profile in MPN: Results from experimental investigation

Cytokines are small molecules constituted by proteins or glycoproteins, which regulate the immune cell function and the immune system. They are secreted mainly by immune cells but also by other cells such as epithelial cells in response to all kinds of stimulation such as injury, infection and stress. They are considered mediators of inflammation and play critical roles in acute and chronic inflammation. In addition to the classically defined cytokines such as interleukins (ILs) and interferons (IFNs), there are a variety of other factors including tumor necrosis factors (TNFs), chemokines, colony stimulating factors (CSF), cell growth factors (TGF, HGF, PDGF and FGF), and angiogenic factors (such as VEGF) [30,31]. The major immunomodulatory role of cytokines is the recruitment of immune effector cells to eradicate invasive stimuli. This process comprises transmission and feedback of information between the immune cell producing center and exertion of local activities that include angiogenesis, cell proliferation and cell migration. A balance in the cytokine network between pro-inflammatory and anti-inflammatory effects is required for effectively controlling normal

function. Otherwise, its dysregulation could generate organ disorders or even diseases. Dysregulation of cytokine networks has been reported in MPN [21]. Cytokines evaluated in 15 MPN studies (literature from 1997 to 2018) are summarized in Table 1.

Cytokine evaluation in MPN dates back to 1997. Early studies have evaluated limited subtypes of cytokines because of technology limitations or kit restrictions. Now with the application of multiplex cytokine assays, a panel of cytokines can be measured per sample at the same time and can provide more complete information of the cytokine profile in MPN patients. As indicated in Table 1, about 79 kinds of cytokines have been evaluated at least once [32]. Due to limited sample numbers, it is difficult to make a concise conclusion for all the cytokines. However, several cytokines among these have been frequently evaluated in different studies, and further correlative analysis indicates that 12 of the 79 cytokines could be prognostic factors for MPN patients. The 12 cytokines are IL-1 α , IL-1 β , IL-2Ra, IL-6, IL-8, IL-11, IFN, TNF- α , TGF- β , VEGF, PDGF and MIP-1. Therefore, we focus on these 12 cytokines in this review.

3.1. IL-1

The IL-1 family consists of seven cytokines with antagonistic activity (IL-1 α , IL-1 β , IL-18, IL-33, IL-36 α , IL-36 β and IL-36 γ), three receptors antagonists (IL-1Ra, IL-36Ra and IL38) and an anti-inflammatory cytokine (IL-37) [30]. IL-1 α is constitutively expressed in several types of cells at a steady state, especially in epithelial cells, whereas its expression is increased in response to pro-inflammatory and stress-associated stimuli. Its major functions are inducing sterile and pathogen-induced inflammation as well as T helper cell 17 (Th17) responses [33]. IL-1 β is produced by a limited number of cells such as monocytes, macrophages and dendritic cells. It mediates inflammation not only at the tissue level but also systemically. It can propagate inflammation by inducing other pro-inflammatory cytokines such as IL-6 and TNF- α , and it bridges innate and adaptive immunity by activation of Th1 and Th17 cells [34]. Furthermore, IL-1 β can impair anti-tumor immunity by promoting the accumulation of myeloid-derived suppressor cells (MDSCs). IL-1 β is crucial for maintaining homeostasis as well as for emergency hematopoiesis. It induces the production and secretion of IL-17 and subsequently promotes the production of granulocyte colony-stimulating factor (G-CSF), which drives proliferation of hematopoietic stem cells and myeloid cells [35]. IL-1 has been well-studied in several hematological malignancies such as acute myeloid leukemia (AML), myelodysplastic syndrome (MDS) and multiple myeloma (MM) [36]. Early experimental studies in 1999 in 55 myeloproliferative disease (MPD) patients (ET = 20, PV = 10, CML = 15, MF = 10) demonstrated no detectable IL-1 α and IL-1 β in serum by ELISA [37]. Later, Panteli et al. [38] reported that IL-1 α and IL-1 β were detectable in MPD patients (ET = 40, PV = 8, MF = 25, CML = 10, Control = 27) by ELISA in 2005, however, it had no difference with that observed in the control group with a very low level. Tefferi et al. [39,40] showed a higher level of IL-1 β and IL-1RA was observed in PV (n = 65) and PMF (n = 127) patients compared to the control group (n = 35) with a multiplex cytokine assay. For IL-1 β , the difference for the PV group was not significant, but the difference for the PMF group was significant. However, the difference for IL-1RA was significant for both PV and PMF groups compared to the control group. A recent French study using a multiplex cytokine assay to evaluate the cytokine profile in PV and ET patients showed both PV and ET patients have lower levels of IL-1 β , which is less than 2.19 pg/ml; however, there was no control group in this study [41]. One recent Spanish study using a multiplex cytokine assay evaluated 16 cytokines in 11 ET patients, 16 PMF patients, 20 PV patients and 34 healthy controls, and it showed these MPN patients presented a significantly higher level of IL-1 β compared to the healthy controls [42]. These results described above indicate that a serum IL-1 β level could be higher in MPN patients compared to control groups, though at a very low level, and this

difference could be more obvious in PMF patients. A recent Italian study confirms this phenomenon: an elevated level of serum IL-1 β was observed in 36 MF patients compared to 10 controls [43]. Furthermore, increased proliferation of CD34+ cells was observed from MF patients when using culture medium containing IL-1 β . This suggests that IL-1 β is strongly correlated with MF progression [43]. Up to now, there are three IL-1 blockers that have been approved by the Food and Drug Administration (FDA) in the USA: anakinra, rilonacept and canakinumab. These IL-1 blockers have been widely tested in several clinical trials in diabetes, rheumatic disease, and liver disease [35,44]. A phase II clinical trial with anakinra in treating 47 early stage MM patients revealed its efficacy in decreasing the C-reactive protein and prolonging progression-free survival (PFS) and overall survival time (OS) [45]. A monoclonal antibody (MABp1) directed against IL-1R and neutralizing anti-IL-1 α has been approved and is in clinical trial for treating colorectal cancer, metastatic cancer, diabetes, among others. A phase III trial of MABp1 in patients with advanced colorectal cancer has revealed promising results [46]. PMF patients would benefit from IL-1 inhibitor treatment and this requires investigation in clinical trials.

3.2. Soluble IL-2 receptor (sIL-2R)

Interleukin 2 (IL-2) was discovered in 1976 and has been well-characterized. It was initially called T cell growth factor for its immune stimulation effects downstream of the lymphocyte activating factor [47]. The IL-2 receptor has three subunits: α , β and γ . While the β and γ subunits are constitutively active, the α unit is expressed only after cell activation. sIL-2R is a 40–45 kDa truncated protein that is cleaved from the IL-2R protein once T cells are activated; hence, it is a surrogate marker for T cell activation. sIL-2R is also produced by dendritic cells, activated B cells, monocytes and malignant cells [48]. Since its discovery in 1985, elevated sIL-2R has been described in a variety of diseases including hematological malignancies, particularly non-Hodgkin lymphoma (NHL), viral infections such as EBV infections and autoimmune disease. Moreover, it has been used as an early predictor of disease and as a potential treatment for diseases including graft versus host disease (GVHD) after hematopoietic stem cell transplantation and hemophagocytic syndrome [49–51]. The first experimental study in 1997 using ELISA to test sIL-2R in MPD patients (ET = 8, PV = 6, CML = 25 [CML-Chronic Phase (CP) = 18, CML-Accelerated Phase (AP) or -Blast Phase (BP) = 7], MF = 1) revealed a higher level of sIL-2R in all MPD patients compared to the control group (CML in AP or BP: 2693 \pm 694 U/ml, $p < 0.0001$; CML in CP: 792 \pm 63 U/ml, $p < 0.0001$; PV: 553 \pm 89 U/ml, $p < 0.05$; ET: 449 \pm 56 U/ml; PMF: 628 U/ml vs. controls: 395 \pm 25 U/ml) [52]. Other studies also reported an elevated level of IL-2 and sIL-2R in MPN patients compared to the control group [37,38]. Results from a study with a larger sample size showed that an elevated IL-2 level was observed both in the PV group with a median of 9.2 pg/ml (range: 0–29 pg/ml) and in the PMF group with a median of 5.1 pg/ml (range: 0–1562 pg/ml) compared to the control group with a median of 6.1 pg/ml (range: 0–28 pg/ml) [39,40]. However, these differences were not significant. For sIL-2R, an elevated serum level was also observed for the PV and PMF groups compared to the control, but the difference for the PV was not significant, while the difference for the PMF was significant [39,40]. Another study confirmed that there was a significant elevation of serum sIL-2R in PMF patients compared to the control group [53]. In short, these results indicate that elevated sIL-2R is associated with PMF but is not linked to ET or PV. There are five drugs targeting the IL-2 axis: recombinant IL-2, anti-CD25 antibodies (basiliximab and daclizumab), anti-CD25 radio-immunoconjugate, anti-CD25 immunotoxin and anti-CD25 antibodies drug conjugate. Among these five drugs, recombinant IL-2 has been widely used in treating solid tumors like melanoma and glioma. Daclizumab has been used in multiple sclerosis and GVHD with success [54]. Drugs targeting IL-2 axis may be useful in controlling PMF or slowing down its progression towards an advanced stage.

3.3. IL-6

Interleukin 6 was discovered in 1986 and was initially called BSF-2. It is produced not only by T cells but also by macrophages, fibroblasts, synovial cells, endothelial cells, glial cells and keratinocytes [55]. Its expression can be induced by a variety of stimuli, including cytokines such as IL-1, TNF and PDGF. It is important for the immune response by promoting B and T cell differentiation [56]. It can interact with two molecules: IL-6R and gp130. IL-6 signal transmission induces activation of JAK/STAT, extracellular regulated protein kinases (ERK) and the phosphoinositide 3-kinase (PI3k) signal pathway [57,58]. Experimental results have demonstrated that there is a close link between IL-6 and acute microbe infection. It has also been reported to play a critical role in autoimmune disease and even in tumor development such as Castleman disease, MM and lymphoma [59,60]. The first experimental study of IL-6 in MPN dates back to 1991 when the serum IL-6 level in reactive thrombocytosis (RT) patients was evaluated with an indirect proliferation assay by using patient serum to culture IL-6 dependent B9 cell lines. Results showed there was a significantly elevated level of IL-6 (38.1 ± 94.6 U/ml) compared to the normal control (2.91 ± 1.08 U/ml, $p < 0.001$) [61]. Later, in 1999, serum IL-6 level evaluation in MPD patients using ELISA showed no difference between the MPD group and control group [37]. Another study measuring IL-6 and sIL-6R in clonal thrombocytosis (CT) and RT patients showed a median value of IL-6 at 40 pg/ml (range: 4–633 pg/ml) for RT and 5 pg/ml (range: 1–124 pg/ml) for CT, and this difference was significant. Results obtained with sIL-6R also revealed a significantly higher level for both RT and CT compared to that of the control group [62]. However, one study in 2005 showed an elevated IL-6 level for myeloid metaplasia with myelofibrosis (MMM) and CML patient but not for PV or ET patients compared to that of the control group [38]. Results from Tefferi et al. showed an elevated level of IL-6 for PMF with a median of 6.2 pg/ml (range: 0–186 pg/ml) and PV with a median of 7.2 pg/ml (range: 0–39 pg/ml) compared to a healthy control group with a median of 0.6 pg/ml (range: 0–9). The difference between PMF and healthy control was not significant, while the difference between PV and healthy control was significant [39,40]. Results from a French group demonstrated a median level of IL-6 was 11.8 pg/ml (range: 5.12–62.1 pg/ml) and 10.8 pg/ml (range: 6.04–39.5 pg/ml) for PV and ET, respectively. The difference between these two groups was not significant [41]. Results from a Spanish group showed PV, ET and PMF patients presented significantly higher levels of IL-6 compared to healthy controls [42]. Taken together, these studies indicate that IL-6 could be important for MPN progression.

Additional experiments in MPN cell lines or MPN mouse models would be useful to confirm the role of IL-6 in MPN progression. Since the 1990s, an IL-6/IL-6R blockade drug has been developed by Kishimoto et al. [59]. Now a variety of drugs are available such as tocilizumab, siltuximab, clazakizumab, sarilumab and soluble gp130Fc [63]. Tocilizumab has been widely used to treat some intractable inflammatory diseases such as Castleman's disease and rheumatoid arthritis (RA) [64]. Results from siltuximab clinical trials in lymphoma, MM and some advanced stage solid tumors have revealed its clinical values as a novel treatment [65]. A huge number of clinical trials concerning this axis are ongoing and aim to generalize its clinical use. A phase I/II clinical trial of tocilizumab addition to standard prophylaxis for GVHD after hematopoietic stem cell transplantation showed a lowering incidence of acute GVHD [66]. These effective clinical trial results suggest it could be an alternative treatment for those MPN patients who have a particularly high level of IL-6.

3.4. IL-8

Interleukin-8, also known as CXCL8, belongs to the elastin-like recombinamer CXC chemokine family. It is produced by macrophages, epithelial cells, airway smooth muscle cells and endothelial cells [67]. It

has two receptors: IL-8RA (CXCR1) and IL-8RB (CXCR2). These two receptors are members of the G protein-coupled receptors (GPCR) family, which contains seven transmembrane domains, and are expressed on granulocytes, monocytes, mast cells and some natural killer (NK) cells [68]. Its major function is the recruitment of immune cells, particularly neutrophils and NK cells, to the sites of infection or injury. The CXCL8-CXCR1/2 axis has been widely investigated in solid tumors such as breast cancer, prostate cancer, lung cancer and melanoma [69]. Emerging research suggests that signaling derived by IL-8 can bias the tumor microenvironment by trafficking MDSC cells and neutrophils with enhanced a proliferation-inducing ligand (APRIL) expression; it can also induce overexpression of VEGF in endothelial cells, which promotes tumor progression by enhancing angiogenesis [69]. IL-8 mRNA expression has been reported in lymphoma. A recent study investigated its potential mechanism of promoting diffuse large B-cell lymphoma (DLBCL) progression at the cellular level. This data showed IL-8 could promote APRIL positive neutrophil infiltration, which results in tumor control [70]. In 1999, experiments measuring the serum IL-8 level in CT and RT patients by ELISA revealed that both groups presented a significantly higher level of IL-8 compared to the control group [62]. In 2002, IL-8 was also observed with a significantly higher level in PV patients not only in peripheral serum but also in bone marrow plasma compared to healthy controls [71]. A study in 2005 showed a significantly elevated serum level of IL-8 in 32 MMM patients with a mean of 60.2 ± 100 pg/ml compared to the control group with a mean of 2.7 ± 2.5 pg/ml. Further investigation of IL-8 mechanism indicates that IL-8 plays a crucial role in megakaryocyte proliferation and differentiation, which is crucial in the pathogenesis of myelofibrosis [72]. A significantly elevated IL-8 level in PV patients compared to that of a secondary group was observed in another study by ELISA [73]. Results from a multiplex cytokine assay in a French group showed the median level of IL-8 was 16.0 pg/ml (range: 8.66–31.6 pg/ml) for 17 PV patients and 26.1 pg/ml (range: 8.66–60.8 pg/ml) for 21 ET patients without healthy controls [41]. Another study that used larger sample sizes revealed a significantly elevated level of IL-8 in PV patients compared to that of the control group (median: 10.2 pg/ml, range: 0–165 pg/ml vs. median: 3.2 pg/ml, range: 0–18 pg/ml) [40]. Similar results were noticed also in 127 PMF patients: a significantly higher level of IL-8 with a median of 14.3 pg/ml (range: 0–1156 pg/ml) in PMF compared to controls with a median of 3.2 pg/ml (range: 0–18 pg/ml). Further analysis regarding IL-8 correlation and clinical features suggests that IL-8 could be a prognostic factor for PMF patients [39]. So far, there have been several pre-clinical and clinical studies with CXCR1/2 inhibitors or anti-IL-8 antibodies in solid cancers as well as in chronic inflammatory diseases such as RA, psoriasis and chronic obstructive pulmonary disease (COPD), as reviewed elsewhere [68,69]. But there are no reports about its clinical applications in hematological diseases. The results presented from the literature above regarding the link between IL-8 and MPN inspires us to explore the possible involvement of IL-8 in MPN development and progression. Future therapies would include the selection of potential patients who could benefit from CXCL8-CXCR1/2 axis blocking treatment.

3.5. IL-11

Interleukin-11 has been identified as a member of the IL-6 family because they share the same receptor protein: gp130 [74]. IL-11 is a multi-functional protein with diverse roles in various cell signaling pathways. It can be produced by a variety of cells in response to inflammatory stimuli. It exerts multiple effects on hematopoietic and non-hematopoietic systems including the liver, gastrointestinal tract, lung, heart, central nervous system, bones, joints and immune system. First, IL-11 works as a hematopoietic growth factor. It has been used to treat thrombocytopenia by activating megakaryocytes and promoting thrombopoiesis. IL-11 can stimulate erythropoiesis as well [75]. Second, IL-11 shows an anti-inflammatory effect with inhibition of

lipopolysaccharide (LPS) mediated release of nitric oxide (NO), TNF- α , IL-1 β , IL-12, TGF- β and IL-6 and downregulation of macrophage by inhibiting the production of IL-1 β , IL-12 and NO [76]. However, a few studies have reported its pro-inflammatory role, while several clinical studies have found overexpression of IL-11 in solid tumors such as gastric carcinoma, colorectal carcinoma and renal cell carcinoma. These studies also demonstrate high IL-11 expression as an independent predictor of poor prognosis in patients with renal cell carcinoma [77].

The relationship between IL-11 and MPN remains unclear. The first study in 1999 reported that only 25% patients with CT (ET = 31, PV = 22, CML = 17) showed detectable IL-11 compared to 63% in the healthy control group; furthermore, the IL-11 median value comparison between these patients showed no significant difference [62]. Later in 2002, another study evaluated IL-11 and IL-8 in peripheral blood serum or bone marrow plasma in 18 PV patients, 14 idiopathic erythrocytosis (IE) patients and 38 health controls. Only 33.3% PV patient revealed detectable IL-11, while 18.4% health control showed detectable IL-11. Further investigation of IL-11 secretion was done by stimulating bone marrow stroma cells (BMSCs) with IL-1B. This *in vitro* study revealed that BMSCs from PV patients secreted high IL-11 after stimulation compared to BMSCs of the control group [71]. Another study evaluated IL-11 levels in 122 patients (ET = 15, PV = 58, secondary erythrocytosis (SE) = 37, IE = 12) by ELISA assay. Results showed that for ET patients, IL-11 is either not detectable or found at a level, which was not significantly different compared to the SE control group. However, a significantly higher level of IL-11 was observed for PV patients compared to the SE group [73]. In our view, there have not been enough studies to accurately evaluate IL-11 levels in MPN patients. Obviously, recombinant IL-11 has no role in treating MPN, but whether its inhibitors could be beneficial to MPN patients or whether IL-11 promotes MPN progression are still factors that should be investigated.

3.6. Tumor necrosis factor (TNF)

The term “TNF” was first used in 1962 and it was well investigated later. Today it comprises 19 superfamily proteins and 29 different receptors [78]. The most well-known family member is TNF- α , which is a pleiotropic pro-inflammatory cytokine. It is mainly produced by activated macrophages. The major function of TNF- α is upregulation of multiple pro-inflammatory proteins such as chemokines, cytokines and adhesion molecules, through activation of NF- κ B and the mitogen-activated protein kinase (MAPK) pathway [79,80]. TNF superfamily proteins have been linked to several diseases including cardiovascular disease, neurological disease, metabolic disease, lung disease, autoimmune disease and lymphoma [78,81]. The first experimental study in 1999 measuring TNF- α levels in MPN patients (ET = 20, PV = 10, CML = 15, MF = 10) by ELISA assay showed a slightly higher level in MPN patients compared to that of the control group; however, statistical analysis was not performed in this study [37]. Later in 2011, Fleischman et al. led a study evaluating TNF- α levels in MPN patients including MF, PV and ET patients. They found that TNF- α plasma concentrations in MF, PV and ET were 10-, 5-, and 4-fold higher than control groups, respectively. These differences were statistically significant. Furthermore, TNF- α concentration was found to correlate with JAK2V617F allele burden. *In vitro* studies culturing MPN CD34+ positively isolated cells with TNF- α showed that TNF- α correlated with JAK2V617F positive MPN cell growth [82]. A French study demonstrated the median TNF- α level was 37.6 pg/ml (range: 23.9–76.0 pg/ml) and 40.4 pg/ml (range: 21.5–108.1 pg/ml) for 17 PV and 21 ET patients, respectively. This subtle difference was not significant. Further analysis based on JAK2 status showed a significant difference in TNF- α level between JAK2 positive PV patients (a median of 13.9 pg/ml, range: 1.64–44.6 pg/ml) and JAK2 negative PV patients (a median of 37.6 pg/ml, range: 23.9–76.0 pg/ml) [40]. Studies in 127 MF and 65 PV patients showed elevated TNF- α in both groups compared to the control group [39,40]. Another study confirmed that significantly higher levels

of TNF- α were observed in MPN patients compared to the control group [42]. Moreover, a TNF- α polymorphism study from Brazil in JAK2V617F positive MPN indicated the potential role of TNF- α in MPN development. It indicated that TNF- α might be strongly correlated with MPN development, especially in patients with JAK2 positive status. Drugs directed by TNF- α have been well-developed as an antagonist against TNF or TNF receptors. Representative drugs include monoclonal antibodies against TNF- α such as infliximab, adalimumab and dacetuzumab, or TNF receptor derivatives such as etanercept [78]. These agents are effective against RA, psoriasis, ulcerative colitis and CD. Studies in animal models revealed that TNF- α inhibited erythropoiesis and negatively regulated the expansion and self-renewal of hematopoietic stem cells [82]. Etanercept, a TNF- α inhibitor, has been used in a clinical trial to treat myelofibrosis. Etanercept relieved patient symptoms, but there was no change in marrow fibrosis after treatment for 12 weeks in 20 evaluable patients [83,84]. In summary, the role of TNF- α in MPN is certain but complex, and the efficacy of its inhibitors still needs further investigation.

3.7. Interferons (IFNs)

IFNs are composed of three types of proteins: type I IFNs (IFN- α and IFN- β), type II IFNs (IFN- γ) and type III IFNs (IL-28A, IL-28B and IL-29). Type I IFNs are produced by almost all cell types in response to pattern recognition receptor (PPR) signaling. They have an anti-inflammatory effect, which can be favorable for controlling virus infections like hepatitis [85,86]. Type I IFNs have been used to treat hepatitis and MPN. Type II IFNs are preferentially produced by activated NK cells and cytotoxic T cells in response to acute virus infection. IFN- γ enhances the adaptive response by helping dendritic cells (DC) to process and present antigens. Blocking IFN- γ has been found to be effective in CD [87]. Type III IFNs can be produced by most cell types stimulated by infectious agents through toll-like receptors (TLR). It is similar to IL-10 [29]. IFN- γ has been reported with diverse effects on both normal hematopoiesis and malignant hematopoiesis [88]. Therefore, IFN- γ levels were evaluated in the previously cited French study with PV and ET patients, and the results showed a median IFN- γ of 209.5 pg/ml (range: 109.8–426.4 pg/ml) for PV patients and a median IFN- γ of 334.7 pg/ml (range: 89.1–921.0 pg/ml) for ET patients [41]. Both IFN- α and IFN- γ serum levels were evaluated in PMF and PV patients compared to healthy controls. Results from IFN- α showed that PV patients present a median of 23.8 pg/ml (range: 0–335 pg/ml) and MF patients present a median of 44.9 pg/ml (range: 0–888 pg/ml) compared to the control group with a median of 27.6 pg/ml (range: 0–90 pg/ml); a significant difference was not noticed for PV. However, a significant difference was observed for MF compared to the control. The median IFN- γ level was 27.1 pg/ml (range: 0–47 pg/ml) and 0 pg/ml (range: 0–683 pg/ml) for PV and PMF patients, respectively, compared to the control group with a median of 5.5 pg/ml (range: 0–23 pg/ml). Both PV and PMF groups presented a significant difference compared to the control group [40,41].

IFN- α has been used to treat MPN patients for more than two decades [88]. Although the discovery of JAK2 inhibitors is a milestone achievement in treating MPN patients with JAK2 mutations, IFN- α is still widely used in MPN treatment in clinics [22,89]. If IFN- α levels are increased in PV and PMF patients, why would it be effective to treat these patients with IFN- α ? We speculate that patients who show no efficacy to IFN- α treatment probably present higher levels of IFN- α . In other words, it might be useful to measure the IFN- α level before IFN- α treatment. Furthermore, since IFN- γ levels are always higher in PV, ET and PMF patients and IFN- γ plays a role in hematopoiesis, it may be beneficial to treat MPN patients with IFN- γ antagonists.

3.8. Vascular endothelial growth factor (VEGF)

VEGF is an important factor for endothelial cell growth as its name

indicates. It is produced by several cell types and it can be regulated by paracrine and autocrine mechanisms [90]. It plays a critical role in physiological angiogenesis such as embryonic and post-natal development, skeletal growth and bone formation [91]. It has also been implicated in some diseases, particularly in tumor development and progression. Several studies concerning VEGF have been done in hematological disease such as AML, MM, lymphoma and MPN. Many of these studies have confirmed its influence in hematopoiesis [92]. A study in 2002 tested VEGF levels in 10 PV patients and 6 SE patients and demonstrated an elevated level of VEGF in 90% of PV patients, which had a positive correlation with platelet levels [93]. Later, in 2003, increased VEGF expression was reported in 10 PV patients, 14 MF patients, 24 ET patients and 12 CML patients by immunohistochemistry with bone marrow biopsy samples [94]. Another study in 2007 reported an increased serum level of VEGF in MMM, ET, PV and CML patients [95]. Another study of angiogenesis evaluated microvessel density (MVD) and VEGF expression in bone marrow biopsy and suggested that VEGF expression in megakaryocytes is significantly higher in MPN patients compared to the control group [96]. It was then confirmed in the following studies by measuring the serum VEGF levels in MPN patients with a multiplex cytokine assay. This French study showed VEGF with a median of 50.8 pg/ml (range: 14.3–381.8 pg/ml) for PV patients and a median of 202.6 pg/ml (range: 22.5–474.3 pg/ml) for ET patients, and the difference between these two groups is significant [40]. The study by Tefferi et al. showed a median level of 9.8 pg/ml (range: 0–44 pg/ml) for PV patients and 2.3 pg/ml (range: 0–47 pg/ml) for PMF patients compared to that of 1.1 pg/ml (range: 0–3 pg/ml) for the control group, and the differences are significant [39,40]. A recent study also showed a significantly elevated serum VEGF in 30 PV patients, 30 ET patients and 30 MF patients by ELISA [97]. Altogether, these studies suggest that VEGF is important for MPN development. Angiogenesis is closely related to the pathogenesis of MPN [98]. Since 1993, targeted therapy against VEGF has been initiated, and there has been substantial progress in this field. Antibodies against VEGF such as bevacizumab, aflibercept and ramucirumab have been widely used in solid tumors such as lung cancer, colorectal cancer and glioma [99,100]. As the importance of angiogenesis in tumor biology including hematological malignancies is well known, it may be possible to apply VEGF antibodies in treating MPN patients in the future.

3.9. Transforming growth factor (TGF- β)

TGF- β is a pleiotropic cytokine regulating cell proliferation, differentiation, migration and survival. It affects multiple biological processes including normal development, carcinogenesis and fibrosis [29]. It can be produced by a variety of cells such as T lymphocytes, B lymphocytes, macrophages and NK cells. It has three isoforms: TGF- β 1, - β 2 and - β 3. Latent forms can be turned into active forms after signal stimulation. TGF- β signaling can be conducted through the Smad-dependent pathway or Smad-independent pathways such as the MAPK and PI3K-Akt pathways [101]. Since important roles of TGF- β in normal hematopoiesis and abnormal hematological malignancies have been established [102], several studies have focused on its implications in hematological malignancies such as leukemia, MM, lymphoma and MPN [103]. The first study focusing on TGF- β in MPN dates back to 1998. TGF- β serum level, TGF- β mRNA and its protein expression were evaluated in idiopathic myelofibrosis (IMF) patients (n = 18) and MF patients including CML (n = 7), PV (n = 3), ET (n = 2) and others (n = 4) [104]. This study used different techniques: CCL64 cell line assay (serum from patients and control was added into the culture medium of CCL64 cells, then the proliferative ability of CCL64 cells was evaluated by Hthymidine assay. The proliferative ability reflect the TGF- β level, because bioactive TGF- β inhibit CCL64 cell proliferation), immunoblots, in situ hybridization and immunohistochemistry. The serum results revealed that there was a detectable level of circulating TGF- β in all samples including the healthy control group. The

differences of total TGF- β in the IMF and MF groups were not significant compared to the control group. However, the differences of the active form of TGF- β in both IMF and MF groups were significantly higher than the control group. Further studies of TGF- β mRNA and its protein expression in bone marrow biopsies also confirmed that there was an elevated level for the IMF and MF groups compared to the control group. In summary, this study provided evidence of TGF- β role in the progression of bone marrow fibrosis [104]. Later, in 1999, another study evaluated the TGF- β level in supernatants from cell culture and examined cytoplasmic TGF- β staining with fluorescence by culturing the CD34 cells from eight MPN patients. The result demonstrated there was no significant difference for MPN patients compared to the control group [105]. A study by Campanelli et al. in 2011 measured both total and bioactive TGF- β levels in MPN patients (PMF = 35, PV = 10, ET = 4) and in the control group (n = 22) by using CCL64 cell line assay. Supernatants of cell cultures of CD34 cells isolated from bone marrow and peripheral blood (PB) were simultaneously used to test TGF- β levels. Both total and bioactive TGF- β levels in the PB and bone marrow were comparable, and they were significantly higher in PMF, PV and ET patients than those in the control group [106]. Another study used two TGF- β antibodies to evaluate latent and active TGF- β in biopsy samples from 39 PMF (19 pre-fibrotic phase and 19 fibrotic phase), 18 ET and 6 controls by immunohistochemical expression. All samples presented mild or no positive staining of active TGF- β , but positive latent TGF- β expression was observed [107]. Another similar study evaluating TGF- β expression in bone marrow biopsy samples from MF patients showed there was a potential relationship between TGF- β and bone marrow fibrosis [108].

A pre-clinical *in vitro* study in 2016 investigated the effect of the TGF- β R1 kinase inhibitor SB431542 on the proliferation and maturation of progenitor and precursor cells from PMF, PV patients and healthy normal adult blood and cord blood. Comparison was done between the cell cultures with and without TGF- β inhibitor, and the results were very interesting. Inhibition of TGF- β signaling promotes proliferation of erythrocytes from adult blood group and PV patients, but no effect was observed in either the cord blood group or PMF patient group. The authors hypothesized that SB431542 inhibits the non-canonical pathway indirectly by reducing the level of expression of TGF- β . They suggest that the continuous administration of the inhibitor is not necessary for effective treatment [109]. There are TGF- β antibodies (fresolimumab and galunisertib), other TGF- β inhibitors (trabedersen) and even TGF- β directed vaccines, which have been tested in clinical trials or are being tested in ongoing clinical trials [110–112]. Among these studies, TGF- β measurements have largely been inconsistent, which may due to methodological discrepancies. However, it is clear that there is a close link between TGF- β and bone marrow fibrosis. Further studies are needed to investigate the potential mechanism of TGF- β in PMF development and progression and the possibility of applying TGF- β inhibitors to treat PMF patients.

3.10. Platelet-derived growth factor (PDGF)

PDGF was purified from platelet extracts and characterized as a mitogen for fibroblasts and cells of mesenchymal origin in the 1970s [113]. Several dimeric isoforms are produced including four homodimeric proteins (PDGF-AA, PDGF-BB, PDGF-CC and PDGF-DD) and one heterodimer protein (PDGF-AB). So in total there are five biologically active PDGF proteins which are coded by four PDGF genes: PDGFA, PDGFB, PDGFC and PDGFD. The PDGF receptors belong to the receptor-tyrosine kinase family, more precisely to the type III group, which also includes c-KIT, FLT3 and the macrophage-colony-stimulating factor receptors. There are two receptors, PDGF- α and PDGF- β , which are more distantly related to the receptors of VEGF and fibroblast growth factors (FGF). The active receptor complex consists of two receptor chains associated with one dimeric ligand. There are two PDGFR genes: PDGFRA and PDGFRB. PDGF is produced by a variety of cell types

including endothelial cells, fibroblasts, vascular smooth muscle cells, osteoblasts, glia, neurons, macrophages and megakaryocytes [113,114]. It plays an important role in normal cell growth, development and tumorigenesis [114]. Patients with PDGFR rearrangements such as FIPIL1-PDGFR and ETV6-PDGFRB are now grouped in a single clinical entity referred to as myeloid neoplasms associated with eosinophilia [115].

Studies focusing on PDGF expression in MPN patients have originated from the 1980s. A representative study by a French group used two different technologies to evaluate the serum PDGF level in MPN patients. Their data revealed an increased level of PDGF in MPN patients compared to the control group [116]. Furthermore, a decreased or a normalized PDGF level was observed in six MPN patients after treatment by IFN- γ for 6 months [117]. However, Dolan et al. reported a decreased level of PDGF in nine MPN patients in their study [118]. In 1989, another study used ELISA to evaluate the PDGF level in urine and serum of MPN patients (PV = 9, ET = 5, MF = 7, CML = 12) and 11 healthy controls. The PDGF levels in serum and urine were both significantly higher than the control group for MF and ET patients, but not for CML and PV patients. Surprisingly, urine PDGF was significantly higher in PV patients compared to healthy controls [119]. Recently, a study evaluating 13 cytokine profiles in PV and ET patients with a multiplex cytokine assay demonstrated that PV patients presented a median PDGF-BB level of 1211 pg/ml (range: 189.1–3716 pg/ml) and ET patients presented a median PDGF-BB level of 2837 pg/ml (range: 592.7–6597 pg/ml), and the difference between these two groups is significant [41]. Clinical trials concerning the PDGF/PDGFR complex have been initiated with PDGFR blocking antibodies such as olaratumab and tyrosine kinase inhibitors (TKI) such as imatinib, dasatinib and nilotinib. Great progress has been made, particularly in solid tumors such as soft tissue sarcoma and prostate cancer [120]. Imatinib has been investigated in MPN patients, particularly in PV patients [121,122]. Combining ruxolitinib and imatinib to treat MPN has initially been used in two patients [123]. Study of PDGF expression or its serum level with large sample sizes of MPN patients is needed. The precise underlying mechanism of PDGF in the pathogenesis of MPN requires further investigation. In the future, new PDGF/PDGFR axis directed drugs such as olaratumab could be an alternative treatment for MPN.

3.11. Macrophage inflammatory protein (MIP1)

MIP1 was discovered in 1988 and has two distinct forms: MIP-1 α and MIP-1 β [124]. They present low levels of constitutive expression but are inducible in most mature hematopoietic cells. MIP-1 α can be produced by monocytes, macrophages, T and B lymphocytes, NK cells, dendritic cells and other cells in response to different stimuli like LPS, IL-1 and virus infections like human immunodeficiency virus (HIV). Less is known about MIP-1 β than MIP-1 α . MIP-1 β can be produced by monocytes, activated T and B lymphocytes and NK cells. So far, three receptors have been proposed for MIP-1: CCR1, CCR3 and CCR5 [125,126]. These have been widely studied in several human diseases: RA, multiple sclerosis, pulmonary fibrosis, ulcerative colitis, glomerulonephritis, sepsis, hypereosinophilia, aplastic anemia, CML, AML, MDS and MM [127]. In particular, MIP-1 α (CCL3) has been reported to play a critical role in leukemogenesis such as AML and CML [128]. However, early animal studies have demonstrated that MIP-1 α could inhibit hematopoietic stem cell proliferation [129]. A recent study in an HIV infection model showed MIP-1 β could promote cell adhesion via ICAM like MIP-1 α and induce reactive oxygen species (ROS) production, which is critical to leukemia development [130]. Tefferi et al. showed significantly elevated serum levels of MIP-1 α and MIP-1 β in PV and PMF patients compared to that of the control group [39,40]. Recent clinical results from the Spanish group showed the MPN group, including PV, ET and PMF patients presented significantly higher levels of MIP-1 α in contrast to the healthy group, while there was no significant

difference of MIP-1 β in PMF patients [42]. This indicates MIP-1 α and MIP-1 β could have different functions, and their roles in MPN pathogenesis still need further investigation.

4. Conclusions

Cytokines regulate functions of the immune system. They are mainly secreted by immune cells after stimulation and they modulate physiological and pathological reactions. Evidence shows that there is an inflammatory state which accompanies MPN, though it is still unclear whether chronic inflammation causes MPN or MPN clonal tumor cells cause inflammation. Several cytokines that have been evaluated by different groups in MPN patients include IL-1 α , IL-1 β , IL-2Ra, IL-6, IL-8, IL-11, IFN, TNF- α , TGF- β , VEGF, PDGF and MIP-1. These cytokines are the most frequently evaluated in studies. Of these, only IL-1 β , TNF- α , IL-6, IL-8, VEGF, PDGF, IFNs and TGF- β show a probable effect in promoting MF progression or present a prognosis predictive value in MPN. A large amount of targeted therapy directed by these cytokines have been tested in clinical trials and some of these show promise in treating MPN. We speculate that there will be more therapeutic options for MPN patients in the future that will effectively relieve symptoms and control the disease.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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