Myeloid neoplasms and autoimmune diseases: markers of association

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Abstract

Objective

To investigate the prognostic significance of concomitant autoimmune diseases (ADs) in myeloproliferative neoplasms (MPNs).

Methods

435 subjects with a diagnosis of MPNs were included in this observational single institution longitudinal study. Of them, 34 patients presented an overt AD at diagnosis of MPN. Clinical presenting features, progression-free and overall survival were compared between MPN subgroups in relation to co-existence of AD at diagnosis of MPN.

Results

Compared to cases without ADs, the subjects with ADs were significantly younger, had lower haemoglobin and haematocrit levels and more frequently presented with splenomegaly. The clinical and biological features associated to progression-free and overall survival were: age, presence of splenomegaly, histotype (MF vs. PV vs. ET), anaemia, high platelet count and presence of any AD at diagnosis of MPN. The age-adjusted hazard ratio (HR) of progression for the presence of AD at diagnosis of MPN was 2.76. Overall survival was not significantly associated to AD at diagnosis, but the HR of progression for the presence of AD at diagnosis of MPN was 2.18.

Conclusion

A possible common genetic predisposition, the inflammatory bone marrow microenvironment and the activation of theJAK/STAT pathway could be considered as responsible for the observed association between MPNs and ADs.

Key words

autoimmune disease, myeloproliferative disease, myeloproliferative neoplasm, Janus kinase, ROCK2

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Introduction

The association between autoimmune disorders (ADs) and haematological malignancies has been reported in the literature, with a particular interest in lymphomas complicating the clinical course of Sjögren's syndrome (SS) and other connective tissue diseases (1-4).

On the other hand, autoimmune derangements have been observed in lymphomas, chronic myeloproliferative neoplasms (MPNs) and myelodysplastic syndromes (MDS), and therefore some possible pathogenetic mechanisms have been hypothesised. In MDS, for example, T-regulatory cells are dysfunctional, with the consequent ineffective control of autoreactive cells and abnormal production of antinuclear autoantibodies (ANA), rheumatoid factor (FR) and antiphospholipid autoantibodies (aPL) in about 50% of patients (5).

In MPNs, an increased prevalence of anti-phospholipid syndrome (APS), immune thrombocytopenic purpura, Crohn's disease, polymyalgia rheumatica, and giant cell arteritis has been reported (6, 7). In some circumstances, the association between haematological neoplasms and ADs has also been explained by the treatment adopted for haematological malignancy, such as interferon (8), fludarabine (9), busulfan (10) tyrosine kinase inhibitors (11) and, more recently, immunotherapy (12).

However, the prognostic significance of ADs in myelodysplastic/myeloproliferative disorders (MDS/MPNs) is still debated and the majority of studies did not find any significant difference in terms of overall survival (OS) between MDS with or without ADs (13). However, other authors have reported for MDS/MPNs patients with systemic vasculitis (14) shorter survival and overall increased probability of death (15).

The aim of our study was to investigate the prognostic role of ADs in MPNs using a series of cases diagnosed at the Haematology Unit of the University of Pisa, Italy. Our objectives were (i) to quantify the prevalence of ADs in MPNs cases at diagnosis; (ii) to identify clinical and biological characteristics distinguishing MPNs cases with and without AD; (iii) to investigate the prognostic role of ADs in terms of progression-free (PFS) and disease-free survival (DFS), considering either AD status at the time of the diagnosis of MPNs or AD status at any time (time-dependent variable).

Patients and methods

Patients

In this observational single institution longitudinal study, 435 subjects with a diagnosis of MPN (*i.e.* essential thrombocythaemia (ET), polycythaemia vera (PV) and myelofibrosis (MF)), performed at the Haematology Unit of the University of Pisa, Italy, between January 2000 and December 2019, were included. All patients agreed to the use of information on their disease and outcome at their first visit by signing an informed consent to donate either leftover samples used for diagnostics and acquired clinical data for every further scientific non-profit purpose. The consent form was approved by the Ethics Committee of the north-western area of Tuscany.

Molecular assays

The assessment of "driver" mutations [Janus-kinase-2 (*JAK2*), MPL-proto-oncogene/thrombopoietin receptor (*MPL*), and CALRETICULIN (*CALR*)] was performed as part of the routine diagnosis/follow-up procedures.

DNA extraction from bone marrow or peripheral blood was made using BioRobot® EZ1 (Qiagen, Milan, Italy). The extracted DNA was maintained at 2–8°C and quantified using the Thermo Scientific NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). Molecular analysis of the *JAK2V617F* mutation was performed by RT-PCR using a TaqMan assay based on specific oligonucleotide probes (Ipsogen, Biotech Luminy, Milan, Italy) and then eventually quantified through the commercial kit "MutaQuant" (Ipsogen, Milan, Italy). The analysis of *CALR* mutations was conducted using the Ipsogene CALR RGQ PCR kit and that of *MPL* by the Ipsogen MPL W515L/K MutaScreen kit, according to the manufacturers' instructions.

Definition of ADs

ADs have been classified according to clinical practice and international guide-

Table I. Clinical and biological characteristics of patients with or without AD at diagnosis of MPN.

	All n=435	No AD at diagnosis n=401	AD at diagnosis n=34	*p*-value [1]
Age (years), median (IQR)	65 (53 to 73)	66 (54 to 74)	57 (42 to 67)	*0.005*
Gender, n (%)		*0.08*		
Female	203 (46.7)	182 (45.4)	21 (61.8)	
Male	232 (53.3)	219 (54.6)	13 (38.2)	
Histotype, n (%) [2]		*0.68*		
MF	131 (30.7)	119 (30.3)	12 (35.3)	
ET	219 (51.3)	204 (51.9)	15 (44.1)	
PV	77 (18.0)	70 (17.8)	7 (20.6)	
JAK2 mutation status, n (%)		*0.60*		
No	45 (14.0)	39 (13.5)	6 (17.6)	
Yes	277 (86.0)	249 (86.5)	28 (82.4)	
CALR mutation status, n (%)		*1.00*		
No	303 (94.1)	271 (94.1)	32 (94.1)	
Yes	19 (5.9)	17 (5.9)	2 (5.9)	
MPL mutation status, n (%)		*0.29*		
No	319 (99.1)	286 (99.3)	33 (97.1)	
Yes	3 (0.9)	2 (0.7)	1 (2.9)	
Splenomegaly, n (%)		*0.02*		
No	176 (44.8)	168 (46.5)	8 (25.0)	
Yes	217 (55.2)	193 (53.5)	24 (75.0)	
WBC (x 10^9/l), median (IQR)	8.9 (7.1 to 11.1)	8.9 (7.1 to 11.0)	9.4 (7.3 to 15.5)	*0.28*
Haemoglobin (g/dL), median (IQR)	14.2 (12.5 to 15.9)	14.3 (12.6 to 15.9)	13.0 (12.0 to 14.2)	*0.04*
Haematocrit (%), median (IQR)	43.0 (38.0 to 49.0)	43.0 (39.0 to 49.0)	39.5 (35.5 to 45.0)	*0.04*
Platelets (x 10^9/L), median (IQR)	602 (372 to 788)	602 (368 to 788)	590 (463 to 793)	*0.71*

[1] *p*-value of the Fisher's exact test for the categorical variables and the Kruskal-Wallis test for the continuous variables.
[2] MF: primary myelofibrosis, post-ET myelofibrosis or post-PV myelofibrosis; ET: essential thrombocytaemia; PV: polycytaemia vera.

lines (22-24). Clinical data concerning ADs have been retrieved from the electronic records stored at the Azienda Ospedaliero-Universitaria Pisana (AOUP). Cases were categorised in two groups according to their AD status at diagnosis of MPN. In order to account for the possible influence of AD that might have been diagnosed during the follow-up, a time-dependent s variable was also created (presence *versus* absence of AD diagnosis during the follow-up).

Statistical analysis

Categorical variables were described through their absolute frequencies and percentages; continuous variables through their medians and interquartile ranges. The heterogeneity of the distribution of variables by MPN histotype and AD status at diagnosis of MPN was assessed with the Fisher's exact test for categorical variables and the Kruskal-Wallis tests for continuous variables. The association between the biological and clinical characteristics of cases and their AD status at diagnosis of MPN was also assessed through the likelihood ratio test resulting from the multiple logistic regression model.

To avoid sparse data, survival analysis was performed for the maximum follow-up duration of 15 years after diagnosis of MPN. For the analysis of progression-free survival (PFS) the endpoint was one of the following events: in MF cases, the reappearance of splenomegaly (at least 5 cm from the costal arch sign), the doubling of spleen longitudinal diameter (SLD), a 50% increase if the baseline SLD >10 cm during treatment or follow-up, transformation into acute leukaemia with over 20% blasts in the marrow or >20% in peripheral blood with a white blood cell count of >10,000 /μL, confirmed two weeks apart, or death; in PV and ET cases, transformation into MF, acute leukaemia or death. For the analysis of overall survival (OS) the event was death. Cases without any of the previous events, for PFS analysis, or alive at the end of follow-up, for OS analysis, were censored at the time of their last visit or at 15 years since diagnosis, whichever came first.

The Kaplan-Meier survival curves for cases with and without AD at time of diagnosis of MPN were compared by the log-rank test. Cox regression models were fitted to estimate the hazard ratio (HR) of progression and death associated either to the presence of AD diagnosis at diagnosis of MPN or to the presence of AD diagnosis at any time. For the last analysis, a time-dependent variable corresponding to the AD status the cases was created and robust statistics were estimated. All HRs were adjusted for age at diagnosis of MPN. Further adjustment for biological and clinical characteristics was also performed. All statistical tests were two-sided at the significance level of 0.05. Statistical analyses were made using the R software (16).

Results

The study included 435 patients, with a median age at diagnosis of 65 years (range: 20 to 89). According to the WHO classification (17), 51% of cases were affected by ET, 18% by PV, and 31% by MF (23% primary MF and 8% post-ET or post-PV MF) (Supplementary Table S1).

The assessment of driver mutations was performed in 322 patients (74% of the whole cohort): 299 cases (93%) carried a mutation in one of the three "driver"

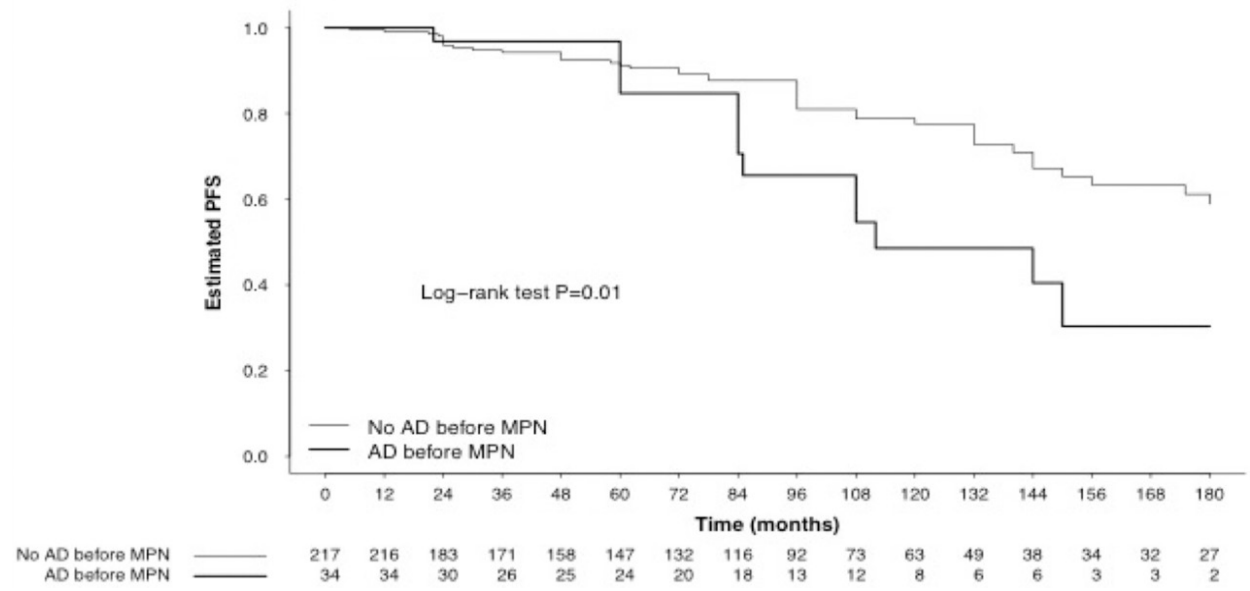

Fig. 1. The PFS curve of MPN cases with (black) or without ADs (grey) is represented. The 10-year PFS was 50% for subjects with ADs *versus* 82% for those without ADs (p=0.01).

genes; 86% carried mutations of *JAK2*, 6% of *CALR*, and 1% of *MPL*, the remaining 7% was "triple negative".

Of the 34 patients with overt ADs at diagnosis of MPN, 17 fulfilled the classification criteria for autoimmune systemic disorder: 10 presented a chronic inflammatory arthritis [*i.e.* rheumatoid arthritis (RA) or spondyloarthritis (SpA)], 6 a connective tissue disease [*i.e.* SS, APS, systemic sclerosis (SSc)], and 1 a vasculitis. Among these patients, no significant association was observed between type of AD and MPN histotype.

In 25/34 (73.5%) subjects with AD, specific autoantibodies were detected: most frequently patients presented anti-nuclear (ANA) antibodies (10/25, 40%), followed by rheumatoid factor (RF) (8/25, 32%), antibodies anti-tyreoperoxidase (7/25, 28%), aPL (5/25, 20%) and anti-extractable nuclear antigen (ENA) (4/25, 16%). No significant association was observed between the antibody specificity and MPN histotype (p=0.19).

AD status at diagnosis of MPN

Table I reports the clinical features of patients, overall and by AD status at diagnosis of MPN. Compared to cases without AD, the 34 cases with AD were significantly younger (median age 57 *vs.* 66 years, p=0.005), had lower haemoglobin levels (median, 13.0 *vs.* 14.3 g/dL, p=0.04) and lower haematocrit (median, 39.5% *vs.* 43.0%, p=0.04); also the percentage of cases with splenomegaly was higher in AD cases than in no-AD cases (75% *vs.* 53%, p=0.02). None of the other variables was significantly different between the two groups.

In the multiple logistic model including all the variables significant at univariable level, only young age and presence of splenomegaly were significantly associated with AD status (p=0.002 and 0.05, respectively).

Progression-free and overall survival

Follow-up data were available for 251 patients; during an average time of follow-up of 84 months we observed 54 progressions (progression-free survival (PFS) probability at 5 years, 0.90; 95% CI, 0.85–0.94) and 43 deaths (overall survival (OS) probability at 5 years, 0.93; 95% CI 0.89–0.96).

The clinical and biological features associated to PFS and OS were: 1) age ≥65 years (p<0.001 for both outcomes), 2) presence of splenomegaly (p=0.001 and 0.009, respectively), 3) histotype (MF *vs.* PV *vs.* ET, p<0.001 for both outcomes), 4) lower haemoglobin levels (p=0.003 and <0.001 respectively), 5) haematocrit (p=0.02 and <0.001 respectively), 6) platelets (p=0.005 and 0.001, respectively) and 7) presence of any AD at diagnosis of MPN (p=0.004 and 0.08, respectively). No other variable was significantly associated to survivals (data not shown).

Figure 1 presents the Kaplan-Meier PFS curve by AD status at diagnosis of MPN (log-rank test p=0.01). The restricted mean survival times at 15 years were 124 months (95% CI, 104–144) and 150 months (95% CI, 142–158) in patients with and without AD diagnosis, respectively (p=0.02). The age-adjusted HR of progression for the presence of AD at diagnosis of MPN was 2.55 (95% CI, 1.36–4.80) and became 3.50 (95% CI, 1.30–9.39) when fully adjusted (Table II).

Overall survival was not significantly associated to AD at diagnosis (log-rank test, p=0.30). The restricted mean survival times at 15 years were 145 months (95% CI, 126–164) and 154 months (95% CI, 147–162) in patients with and without the diagnosis of AD, respectively (p=0.35). The age-adjusted HR of progression for the presence of AD at diagnosis of MPN was 1.97 (95% CI, 0.93–4.15) and became 3.07 (95% CI, 1.00–9.43) when fully adjusted (Table III).

When the presence/absence of AD was included in the survival models as time-dependent variable in order to include among exposed patients those

Table II. Hazard ratios (HRs) of progression for presence of ADs at the time of diagnosis of MPN and for the presence of ADs at any time.

	Hazard ratio of progression – age adjusted [1]				Hazard ratio of progression – fully adjusted [2]			
	Non Events, n	Events, n	HR (95% CI)	*p*-value	Non Events, n	Events, n	HR (95% CI)	*p*-value
ADs at diagnosis of MPNs	194	52	2.55 (1.36 to 4.80)	0.004	159	31	3.50 (1.30 to 9.39)	0.01
ADs at any time[3]	**192**	49	2.76 (1.45 to 5.26)	0.002	159	29	4.29 (1.53 to 12.05)	0.006

[1] HR estimates are from the Cox regression model adjusted for age at diagnosis of MPN.
[2] HR estimates are from the Cox regression model adjusted for age at diagnosis of MPN, splenomegaly, MPN histotype, haemoglobin, haematocrit and platelets.
[3] AD status is entered into the model as time-dependent variable.

Table III. Hazard ratios (HRs) of death for presence of AD at the time of diagnosis of MPN and for the presence of AD at any time.

	Hazard ratio of death – age adjusted [1]				Hazard ratio of death – fully adjusted [2]			
	Non Events, n	Events, n	HR (95% CI)	*p*-value	Non Events, n	Events, n	HR (95% CI)	*p*-value
ADs at diagnosis of MPNs	205	41	1.97 (0.93 to 4.15)	0.08	165	25	3.07 (1.00 to 9.43)	0.05
ADs at any time[3]	202	40	2.18 (1.03 to 4.62)	0.04	165	24	3.70 (1.14 to 12.05)	0.03

[1] HR estimates are from the Cox regression model adjusted for age at diagnosis of MPN.
[2] HR estimates are from the Cox regression model adjusted for age at diagnosis of MPN, splenomegaly, MPN histotype, haemoglobin, haematocrit and platelets.
[3] AD status is entered into the model as time-dependent variable.

who developed AD during follow-up, the age-adjusted HR became 2.76 (95% CI, 1.45–5.26) and 2.18 (1.03–4.62) for PFS and OS respectively (Tables II-III).

Discussion

In this retrospective data collection, a large number of MPN patients screened for ADs offers interesting findings and matter of debate: 1) about 8% of subjects who receive a diagnosis of MPN must deal with an AD in addition to the haematological neoplasm, with the obvious additional problems coming from management of both diseases at the same time; 2) the typical patient with MPN and AD is a young female who present lower haemoglobin levels and higher spleen enlargement; 3) there is not a significant correlation between different ADs and specific MPN histotypes; 4) in the MPN scenario, ADs represent an adverse prognostic factor, especially in terms of PFS.

As described above, association between ADs and MPNs has already been reported in the literature (2-4, 6, 7,18, 19). Nevertheless, the majority of these studies were focused on measuring the risk of developing MPN during ADs. For example, a Danish group reported a statistically significant increased risk of developing chronic myelo-monocytic leukaemia (CMML), a myeloid malignancy overlapping between MDS and MPN, in subjects with a personal history of AD (overall OR, 3.24 *vs.* 2.78 for subjects with non-haematological diseases); this association was also correlated with a worse response to haematological treatment (18). By contrast, our study is focused on "clinically overt" MPN, where we tried to define the phenotype of patient who presents with concomitant ADs. In our series, the prototype of this kind of patient is a young female who presents anaemia and splenomegaly and more probably will develop MF from ET and PV or will undergo to transformation from MF to acute leukaemia, with a probability of progression or death by 15 years, which is at least twice than that reported for subjects without ADs. Several hypotheses could be taken into consideration to explain these findings: 1) the role of microenvironment: indeed, the hyper-inflammatory status that characterises the bone marrow during ADs could induce a deeper genomic instability, thus sustaining the growth of some already present MPN sub-clones, otherwise well suppressed by the efficient immunological control. Indeed, in subjects with pre-existing ADs, this immunological control might fail, thus favouring the onset of haematological malignancies. In addition, we might hypothesise a more direct link between ADs and MPNs. For example, in SLE, psoriasis and arthritis, the Rho-associated coiled-coil containing protein kinase 2 (ROCK2), a cytoplasmic serine/threonine kinase able to hyper-activate the JAK-STAT pathway (20), is over-expressed (21). In a mouse model of spontaneous autoimmunity, ROCK2 sustained the production of IL-17 and IL-21, with consequent inflammatory response and autoantibody production (22). Hyper-activation of ROCK2 has also been reported in myeloid malignancies where constitutive activation of PI3K/Rho/ROCK/myosin light chain pathway promoted leukaemic cell growth and survival (23). Consequently, we might suppose that ADs could sustain the bone marrow genomic instability that could contribute to the onset of the "driver" mutations characterising MPNs. On the other hand, the activation of some pathways, such as that of ROCK2, might increase the activation of the JAK-STAT pathway that could make the MPN more aggressive. Several studies have already well dem-

onstrated that JAK-STAT axis is really active in ADs, as demonstrated by efficacy of JAK inhibitors, such as baricitinib, in patients with RA (24, 25), SLE and other connective diseases (26, 27).

In addition, further AD-related pro-inflammatory cytokines might be responsible for the more aggressive behaviour of MPN in subjects with ADs, including, for example, the TGF-beta higher levels. Indeed, we previously demonstrated by some *in vitro* experiments that in MF the carboxy-terminal pentapeptide of osteogenic growth factor (sOGP10-14) increased bone marrow cellularity through increased levels of TGF-beta (28). Therefore, we might hypothesise that ADs via TGF-beta would further increase bone marrow fibrosis, thus sustaining the further spleen enlargement and the MPN progression.

Finally, we can not exclude the existence of a common genetic predisposition between ADs and MPNs: indeed, the *JAK2* haplotype 46/1, frequently observed also in Crohn's disease (29), has been reported to predispose to mutations in *JAK2* and *MPL*. Analogously, the A3669G polymorphism of the glucocorticoid receptor has been reported to be present in a higher percentage in PV and in MF patients but also in patients with ADs (30).

However, probably the most convincing proof of the strict relationship between ADs and MPNs comes from some more "clinical" considerations: the treatment with ruxolitinib, an anti-JAK1/JAK2 inhibitor, significantly ameliorated the prognosis of patients affected by MF (31) and PV (32), resulting also in the reduction of some pro-inflammatory cytokines, such as TNF-alpha, IL-4, IL-6, and IL-8 (33, 34). In the cohort of the present study, 11 patients with MF and AD who received ruxolitinib experienced a significant improvement of either MPN- or AD-related symptoms in a median of only 7 weeks.

Moreover, the anti-inflammatory effect of ruxolitinib, already shown in the steroid-refractory graft-versus-host disease (GVHD) (35, 36) and in the macrophage activation syndrome (37), came to prominence recently during the pandemic sustained by the SARS-CoV-2. Indeed, in the RESPIRE protocol, 16 out of 18 patients receiving ruxolitinib showed a rapid clinical response and no evolution to mechanical ventilation (38). In the Italian compassionate-use protocol, a significant clinical improvement was observed in 82.4% of cases treated with ruxolitinib (39), analogously with that reported in another German series (40).

In conclusion, our study demonstrates that the coexistence of ADs and MPNs seems to impair the prognosis of myeloproliferative neoplasms. This observation could prompt haematologists to strictly collaborate with rheumatologists and to employ some drugs, such as JAK inhibitors, that might ameliorate the quality of life and the survival of these patients.

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This article is dedicated to all patients and physicians who have to fight MPNs and ADs every day, and to all those who died during the 2020 Coronavirus pandemic.

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