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ORIGINAL ARTICLE

Correlation between JAK2 allele burden and pulmonary arterial hypertension and hematological parameters in Philadelphia negative JAK2 positive myeloproliferative neoplasms. An Egyptian experience

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Abstract Myeloproliferative neoplasms are characterized by a common stem cell-derived clonal proliferation, but are phenotypically diverse. JAK2 is mutated (V617F) in more than 90 % of patients with polycythemia vera (PV) and approximately 60 % of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF). Pulmonary arterial hypertension (PAH) is a major complication of several hematological disorders. Chronic myeloproliferative disorders associated with PAH have been included in group five for which the etiology is unclear and/or multifactorial. The aim of this study is to screen Egyptian Philadelphia negative JAK2 positive myeloproliferative neoplasm patients for the presence of PAH and its correlation with JAK2 allele burden. We also made a review for correlation of JAK2 allele with hematological parameters comparing our results to others. We enrolled 60 patients with Philadelphia negative myeloproliferative neoplasms. All patients enrolled in the study were subjected to laboratory and imaging workup in the form of CBC, liver, kidney profile, bone marrow examination, abdominal ultrasonography, and transthoracic echocardiography. Our results revealed that 7 patients out of 60 (11.67 %) had pulmonary arterial hypertension, 3 patients with PMF, 2 patients with PRV, and 2 patients with ET, and its correlation with JAK2 allele burden was not statistically significant. Correlation analysis between JAK2 V617F allele burden and other parameters revealed: statistical significant correlation with age, HB, HCT, PLT, UA, LDH, and splenic diameter but insignificant correlation with WBCs and PAH. Pulmonary arterial hypertension prevalence in our study was 11.67 % and no significant correlation with JAK 2 allele burden. Our study is the largest one up to our knowledge that studies the association between its prevalence and JAK2 burden.

Keywords Myeloproliferative neoplasm · Pulmonary hypertension · JAK2 mutation

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Introduction

Myeloproliferative neoplasms are characterized by a common stem cell-derived clonal proliferation, but are phenotypically diverse due to differences in genetic rearrangements or mutations, and now it has been clearly understood with the discovery that the protein tyrosine kinase JAK2 is mutated (V617F) in more than 90 % of patients with polycythemia vera (PV) and approximately 60 % of patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF) [1]. The mechanism(s) behind this one allele multiple phenotypes phenomenon has not been fully elucidated. The issue is further confounded by the presence of marked variation in JAK2 V617F allele burden among mutation positive patients [2].

Pulmonary hypertension (PAH) is a major complication of several hematological disorders. The incidence of primary PAH in myeloproliferative neoplasm (MPN), once secondary causes are excluded, seems quite high. As established mainly by transthoracic echocardiography (TTE), the PAH prevalence was 44 % in essential thrombocythemia (ET) [3], 22 % in polycythemia rubra vera (PRV) [4], 37 % in primary myelofibrosis (PMF) [5], and 25 % in chronic myeloid

leukemia (CML) [6], with a global prevalence of 38 %. Increased pulmonary artery pressure in patients with MPN may result from various mechanisms including anemia, and a hypermetabolic state with high cardiac output and left ventricular dysfunction [7]. The possible association between PAH and Philadelphia negative MPN has been suggested by several case reports and small case series [8]. However, the prevalence and incidence of PH in the context of MPN may be underestimated since the clinical signs of disease appear at an advanced stage of the disease and, in some cases, the diagnosis of MPN is difficult to establish in the context of chronic hypoxia [7].

Also, several studies concerned about the relation between hemogram and the presence of JAK2 with controversial results, but only few studies which correlate the JAK2 allele's burden with the different parameters.

This prompted us to perform a more extensive study enrolling a higher number of patients from a single center; 60 patients with Philadelphia negative JAK2 positive myeloproliferative neoplasm and the correlation with JAK2 V617F allele burden.

Patients and methods

The current study was conducted over 20 months starting from May 2013 after approval of the protocol by Research Ethical Committee. In the present study, we enrolled 60 adult Philadelphia negative JAK2 positive myeloproliferative patients from the Hematology Clinic in Kasr Al Aini Hospital; 29 PRV (48.3 %), 18 ET (30 %), and 13 PMF (21.7 %). Patients with known pulmonary hypertension due to specific heart disease were excluded. All patients enrolled in the study after obtaining a written consent. All the patients in the study were subjected to full medical history, complete clinical examination, and laboratory investigations including CBC, ALT, AST, urea, creatinine, uric acid, and LDH. Abdominal ultrasound to assess splenic diameter and transthoracic echocardiography to estimate the tricuspid regurgitation velocity (TRV) (m/s) as well as to assess the probability of pulmonary hypertension were performed as advised by 2015 ESC/ERS Guidelines [9] for the diagnosis of pulmonary hypertension (Tables 1 and 2). The JAK2 allele burden was assessed by JAK-2 MutaQuant kit (IPSOGEN Cancer Profile) in human genomic DNA using ABI PRISM 7300 Sequence Detection System.

Statistics

All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows. Data were statistically described in terms of mean ± standard deviation (±SD), and range. Comparison of non-parametric variables was done using Mann–Whitney *U* test for independent samples when comparing two groups and Kruskal–Wallis test when comparing more than two groups. Comparison of parametric values between two groups was done using *t* test, ANOVA, and post hoc study when comparing more than two groups. For comparing categorical data, Chi-square (χ^2) test was performed. Correlation analysis using Spearman's test *P* values were significant if less than 0.05.

Results

We enrolled 60 Philadelphia negative JAK2 positive MPNs patients; 33 females (55 %) and 27 males (45 %); with the following subtypes 29 PRV (48.3 %), 18 ET (30 %), and 13 PMF (21.7 %).

Table 3 demonstrates all demographic and laboratory results.

Echocardiographic data revealed that 53 patients (88.3 %) had low probability of pulmonary arterial hypertension (PAH); 27 PRV patients (45 %), 16 ET patients (26.6 %), and 10 PMF patients (16.7 %) while the remaining 7 patients (11.6 %) had intermediate probability of PAH; 2 cases of PRV (3.3 %), 2 cases of ET (3.3 %), and 3 cases of PMF (5 %).

Correlation analysis between JAK2 V617F allele burden and other parameters as shown in Table 4, revealing statistical significant correlation with age, HB, HCT, PLT, UA, LDH, and splenic diameter but insignificant correlation with WBCs and PAH.

Comparisons of the two groups of PAH according to different parameters as shown in Table 5, revealing statistical significant difference in WBCs count only.

Table 1 Echocardiographic probability of pulmonary hypertension [9]

Peak TRV (m/s)	Other echo "PAH signs" Table 2	TTE probability of PAH
≥2.8 or not measurable	No	Low
≥2.8 or not measurable	Yes	Intermediate
2.9–3.4	No	
2.9–3.4	Yes	High
>3.4	Not required	

Table 2 Echocardiographic signs suggesting pulmonary hypertension used to assess the probability of pulmonary hypertension in addition to tricuspid regurgitation velocity measurement [9]

A: The ventricles	B: Pulmonary artery	C: IVC and right atrium
Right ventricle/left ventricle basal diameter ratio >1.0	Right ventricular outflow Doppler acceleration time <105 ms and/or mid-systolic notching	IVC diameter >21 mm with decreased inspiratory collapse (<50 % with a sniff or <20 % with quiet inspiration)
Flattening of the interventricular septum (left ventricular eccentricity index >1.1 in systole and/or diastole)	Early diastolic pulmonary regurgitation velocity >2.2 m/s	Right atrial area (end-systole) >18 cm^2
	PA diameter >25 mm	

Comparison between the three groups of myeloproliferative disorders according to PAH revealed insignificant difference $p = 0.11$.

Discussion

Pulmonary arterial hypertension (PAH) is well known in myeloproliferative neoplasms (MPN) [10]. MPN with PAH have been included in group 5 of the clinical classification for PAH. Compared to previous studies of the same concern, the current study has unique feature as we did not only studied the prevalence of PAH but also the correlation between PAH and JAK2 allele's burden in Egyptian patients with Philadelphia negative JAK2 positive MPN.

Right ventricular systolic pressure (RVSP) evaluation by transthoracic echo (TTE) as a utility for diagnosing PAH is good being noninvasive methods and excluding cardiac causes of PAH, but on the other hand PAH may be overestimated by this utility [7], and it was advised in 2015 that PAH probability is to be assessed by tricuspid regurgitation velocity (TRV) utility [9] as done in our study to eliminate a major probability of false positive PAH as possible, also the accurate anamnesis of patient cardiovascular history together with carful elimination of left ventricular dysfunction [11] may eliminate a major proportion of false positive PAH diagnosis.

Among our studied 60 Philadelphia negative JAK2 positive MPN patients, we found 7 patients (11.67 %) with intermediate pulmonary arterial hypertension (PAH). Reisener SA et al. found 4 (13 %) out of 30 MPN patients with PAH unrelated to valvular disease [12]. Garypidou V et al. studied 24 MPN patients, the majority of them (14 out of 24) were ET and found that 10 (41.7 %) patients had PAH [10]. Gupta R et al. described also PAH in 12 (48 %) out of 25 patients with MPNs [13] Altintas A et al. found PAH in 22 (47.8 %) patients out of 46 ET patients [3]. A larger study by Chebrek S et al. who evaluated 68 patients with MPNs (27 ET, 15 PMF, and 26 PV) found lower prevalence of PAH. Only five patients (7 %) were found to have increased pulmonary artery pressure. However, this work did not study the correlation with JAK2 allele burden [14].

The variability in prevalence of PAH in previously mentioned studies and our study make our main concern to investigate the relation between the JAK2 allele's burden and the degree or the severity of PAH as well as the clinical relevance in our studied MPN patients.

Table 3 Demographic and laboratory data of the patients

Parameter	Mean	SD	Minimum	Maximum
JAK2 %	36.0	29.1	5	88
Age (years)	51.7	12.8	19.0	76.0
WBC (×103/cm^3)	11.2	6.4	2.1	30.0
HB (gm/dl)	14.3	4.5	5.0	22.0
PIT (×106/cm^3)	600.7	545.6	16.0	2433.0
HCT %	44.1	13.6	15.3	68.7
AST (u/dl)	27.6	9.9	13.0	62.0
ALT (u/dl)	27.6	10.5	9.0	60.0
Urea (mg/dl)	30.9	13.0	13.0	80.0
Creatinine (mg/dl)	0.9	0.2	0.5	2.3
Uric acid (mg/dl)	5.6	1.4	2.3	11.0
LDH (u/l)	517.6	349.5	180.0	1950.0
Spleen (cm)	16.3	4.6	10.0	31.0

Table 4 Correlation between JAK2 and other parameters

Parameters	*r* value	*P* value
PHT	.086	.513
Age	0.263	.004*
HB	.935	.000*
HCT	.835	000*
WBC	.093	.482
PLT	−.424	.001*
Uric acid	.310	.017**
LDH	.326	.011**
Splenic diameter	.267	.041**

Highly significant (*$p < 0.005$), significant (**$p < 0.05$)

Table 5 Comparisons of the two groups of PHT according to different parameters

	PHT	Mean ± SD	*t* test	*P* value
HCT	Low Intermediate	44.7528 ± 13.32133 39.7429 ± 16.28034	.912	.365
PIT	Low Intermediate	593.9245 ± 531.3556 652.0000 ± 691.1242	−.263	.794
HB	Low Intermediate	14.4887 ± 4.37056 12.9000 ± 5.93914	.867	.390
WBC	Low Intermediate	10.6094 ± 6.05219 16.1429 ± 7.33618	−2.220	.030*
Age	Low Intermediate	50.6981 ± 12.77104 59.5714 ± 10.84523	−1.753	.085
UA	Low Intermediate	5.5865 ± 1.51645 6.1857 ± 1.23886	−.999	.322
LDH	Low Intermediate	498.6604 ± 347.3084 661.5714 ± 359.0608	−1.162	.250
JAK2	Low Intermediate	268.41 ± 1194.289 1111.30 ± 2846.826	−1.440	.155
Spleen	Low Intermediate	16.2075 ± 4.80356 17.5000 ± 3.78153	−.635	.528

Significant (*$p < 0.05$)

In our work, we did not find any correlation between the JAK2 allele's burden and the presence of PAH, and this correlation was not addressed till now by any other studies.

In our study, none of the studied parameters including age, sex, splenic diameter, hemoglobin levels, or platelet count was predictive of the presence of PAH, except for WBCs that was significantly different among two groups of PAH, and this finding was in agreement with both Garypidou V et al. [10] and Gupta R et al. [13], and on the other hand is not consistent with Altintas A et al. [3] and Dingli D et al. [15], who found significant correlation between higher platelets counts and PAH.

Several studies concerned about the relation between hemogram and JAK2 status (absence or presence) with controversial results as shown in Table 6, but only few studies correlated the JAK2 allele's burden with the different parameters.

In our study, we found that there is a positive statistically significant correlation between the JAK2 allele's burden and the hemoglobin level, splenic diameter and LDH, and negative statistically significant correlation with the platelet count. Zohu et al. found positive significant correlation for JAK2 allele's burden with increasing HB but not for platelet count in 222 mutated JAK2 V617F patients [16]. Zahang et al. found that a higher JAK2 V617F load correlated significantly

Table 6 Studies concerned about correlation between JAK2 allele positivity MPNs and hematological parameters

Study name	No. of patients	MPN subtype	Frequency of JAK2 allele positivity	Correlation with HB %	Correlation with WBC	Correlation with PLT	Correlation with LDH
Sultan S and Irfan SM [21]	21	ET	61.9 %	Negative	Positive	No correlation	Positive
Vytrva N et al. [22]	112	52 ET 38 PRV 21 PMF	46 % 97 % 63 %	Positive with higher HB	Positive with higher TLC	Positive with low PLT	–
Duletic AN et al. [23]	106	41 PV 43 ET 9 PMF 13 MPN-U	88 % 58 % 56 % 54 %	–	Positive with higher TLC	Positive with higher PLT	Positive with higher LDH
Chao HY et al. [24]	412		67 %	Positive with higher HB	Positive with higher TLC	–	–
Ilhan G et al. [25]	65	28 PV 29 ET 8 IMF	89 %	Positive with higher HB	Positive with higher TLC	–	–
Pemmaraju N et al. [26]	80	ET	47 %	Positive with higher HB	No significant difference	No significant difference	–
Barosi G et al. [27]	304	PMF	63.4 %	Contributed to hemoglobin variability	Associated with higher white blood cell count	Contributed to platelet count variability	–
Campbell PJ et al. [28]	806	ET		Positive with higher HB	Positive with higher neutrophil counts	–	–
Garcia C et al. [29]	30	ET	44.4 %	No correlation	–	No correlation	Positive

En dash not studied, *ET* essential thrombocythemia, *PRV* polycythemia rubra vera, *IMF* Idiopathic myelofibrosis

with higher HB% level in studied PV patients [17]. Kittur et al. found that JAK2 allele's burden correlated significantly with lower platelet count, palpable splenomegaly at diagnosis, and venous thrombosis occurring after diagnosis in 69 ET patients [18]. Larsen TS et al. found that the JAK2 allele's burden correlated significantly with higher levels of LDH and as well as lower platelet counts in 165 Philadelphia negative MPN patients [19]. Silver et al. found that there is a correlation between the splenic size and the JAK2 allele's burden [20].

On the other hand, studies by Larsen et al. [19], Kittur et al. [18], Zohu et al. [16], and Silver RT et al. [20] determined that higher JAK2 allele's burden correlated with a higher white blood cell count which is different from our finding that there is no significant correlation between JAK2 allele's burden and white cell count in our studied patients.

These differences may be due to the differences among studies regarding the demographic features of studied population, whether studied population on treatment or not, different modalities of treatment, and the number of studied groups. We believe that meta-analysis of similar studies all together is important to know the exact incidence and prevalence of PAH in those patients and its correlation with other parameters.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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