RESEARCH ARTICLE

Evaluation of Inflammation Parameters in Philadelphia Negative Chronic Myeloproliferative Neoplasia Patients

Tuba Hacibekiroglu[1]*, Sema Akinci[2], Abdulkadir Basturk[3], Besime inal[4], Tekin Guney[2], Sule Mine Bakanay[2], Imdat Dilek[2]

Abstract

Background: Chronic myeloproliferative diseases are clonal stem cell diseases which occur as a result of uncontrollable growth and reproduction of hematopoietic stem cells, which are the myeloid series source in bone marrow. Recent studies have suggested that chronic inflammation can be a triggering factor in the clonal change in chronic myeloproliferative neoplasia (CMPN). In our study, we evaluated the existence of a chronic inflammation process in our Philadelphia negative (Ph-)CMPN patients using inflammation parameters in combination with demographic, laboratory and clinical characteristics of the patients. Materials and Methods: Demographic characteristics, clinical and laboratorial data, and thrombosis histories of 99 Ph-CMPN patients, who were diagnosed at our outpatient clinic of hematology in accordance with WHO 2008 criteria, were analyzed retrospectively,with 80 healthy individuals of matching gender and age included as controls. Complete blood counts, sedimentation, C reactive protein (CRP), JAK V617F gene mutations, abdomen ultrasound images and previous thrombosis histories of these patients were retrospectively analyzed. Results: Ph-CMPN and healthy control groups included 99 and 80 cases, respectively. PV, ET and MF diagnoses of patients were 43 (%43.4), 44 (44.4%) and 12 (12.1%), respectively. JAK V617F gene mutation was found to be positive in 64 (71.1%) of all cases and in 27(65.8%), 32 (82%), 5 (50%) of the cases in PV, ET and PMF groups, respectively. Thrombosis was determined as 12 (12%) in the entire group, 12.5% in the JAK V617F negative and 15.3% in the positive patients, with no statistical significance (p=0.758). No significant difference was observed between patients with and without previous thrombosis history in respect to hemogram parameters, sedimentation and CRP (p>0.05), neutrophil to lymphocyte ratio (NLR), erythrocyte distribution width (RDW), mean platelet volume (MPV) and sedimentation levels of the patient.

Keywords: Chronic inflammation - chronic myeloproliferative neoplasia

Asian Pac J Cancer Prev, **16 (13)**, 5159-5162

Introduction

Chronic myeloproliferative diseases are clonal stem cell diseases which occur as a result of uncontrollable growth and reproduction of hematopoietic stem cells, which are the source of myeloid series in bone marrow. According to 2008 classification of World Health Organization, PV, ET and PMF are the primary neoplasia found in Philadelphia negative CMPN (Tefferi, 2012). In pathogenesis, the mutations acquired in the genes which coordinate cell proliferation (mostly those which code tyrosine kinase) are held responsible.

Although the most widely known of these is JAK2 V617F which was discovered in 2005, the existence of a number of other mutations such as JAK2 Exon 12, TET2 and MPL have been proven with conducted studies. JAK2 V617F exon 14 mutation was found positive over 95% in PV, as 55% in ET and as 65% in PMF. JAK-2 Exon 12 mutation was found to be 3% in PV while it was very scarcely detected in ET and PMF (Vannucchi et al., 2008). Elevated numbers of erythrocyte, thrombocyte and leukocyte as secondary to uncontrolled cell reproduction in the myeloid series as a result of a mutation occurring in the gene that codes Janus associated kinase (JAK2), which is a signal protein that activates cell proliferation, are the first observed laboratorial findings. Thrombo-hemorrhagic complications are frequently observed in the clinical data of this patient group. PV and ET are cardiovascular incidences which are the leading causes of morbidity and mortality. Although arterial thromboses are predominant, venous thrombosis are also common (Vannucchi et al., 2013).

Upon detailed examination of thrombosis tables in CMPN patients, a positive correlation of thrombosis

*[1]Hematology, Edirne Public Hospital, Hematology, Edirne, [2]Ankara Ataturk Training and Research Hospital /Hematology, [4]Ankara Zekai Tahir Burak Hospital/Family Medicine, Ankara, [3]Konya Trainig and Research Hospital /Hematology, Konya, Turkey *For correspondence: drkulek@yahoo.com*

particularly with elevated leukocyte levels and JAK2 V617F mutation was indicated. Recent studies have suggested that chronic inflammation can be a triggering factor for the clonal change in CMPN. (Hasselbalch., 2013). It is suggested that chronic inflammation process inflicts damage in the stem cell structure by inducing chronic oxidative stress in bone marrow, and a microenvironment posing high level of risk for mutation can trigger oxidative damage in hematopoietic stem cell DNA ((Hasselbalch., 2013). In addition to sedimentation and C-reactive protein, which are among commonly used chronic inflammation indicators, recent studies have suggested that RDW, MPV and NLR levels, which were associated with inflammation in these studies, are other indicators for its existence (Gasparyan et al., 2011; Guo et al., 2013). In our study, we evaluated the existence of a chronic inflammation process in pathogenesis of chronic myeloproliferative neoplasia by using these 5 inflammation parameters, which have not been studied in combination so far, in CMPN patient group.

Materials and Methods

Demographic, clinical and laboratorial data, abdomen ultrasound imaging and thrombosis histories of 99 regularly followed Ph-CMPN patients, who were diagnosed at our outpatient clinic of hematology in accordance with WHO 2008 criteria, were retrospectively analyzed. 80 gender and age matched healthy individuals were included as control group. JAK V617F mutations, complete blood counts, and levels of sedimentation and CRP at the time of diagnosis were retrospectively analyzed. Additionally, neutrophil to lymphocyte ratio (NLR) was determined for each case. The correlations of obtained data with thrombosis history in cases with CMP were evaluated.

Statistical analysis

SPSS 17 (Chicago IL, USA) was used for all of the statistical analyses. Compliance of data with normal distribution was evaluated with One Sample Kolmogorov Smirnov test. Results were presented as mean±standard deviation in parametric data and as minimum, maximum and median in non-parametric data. Chi-square test for categorical data and Independent Sample T and Mann Whitney-U tests for parametric and non-parametric data comparison between groups were used respectively. Pearson correlation analysis was carried out for correlative laboratorial parameters in CMPN patients. Values below $P < 0.05$ were accepted to be statistically significant. Binary logistic regression analysis was carried out as independent variable analysis.

Results

Out of the 99 patients included in the analysis, 49 cases were female and 50 cases were male with a mean age of 59.8 (26-87). F/M ratio in the healthy control group consisted of 80 individuals was 36/44 with a mean age of 59.8 and there was no significant difference between two groups in respect to age and gender dispersion ($p>0.05$) (Table 4). PV, ET, PMF diagnoses were detected as 43 (43.4%), 44 (44.4%) and 12(12.2%) in patient sub-groups, respectively. Clinical and laboratorial characteristics of patient group and sub-groups were found as specified in Table 1. JAK2 V617F was positive in 64 cases (71.1%) in patient group, and the group with the highest positivity was essential thrombocythemia group with a value of 82%. Splenomegaly and hepatomegaly were most commonly observed in PMF group. Thrombosis was detected in 12 (12.1%) of all patients and its highest incidence was in PMF. (Table 1) Vein thromboses (primarily portal and splenic vein thrombosis) were more common in PMF group while arterial thrombosis (primarily myocardia infarction and cerebrovascular incidents) were more common in PV and ET groups. Thrombosis was detected in 15.3% of the patients with positive JAK2 V617F

Table 1. Clinical and Laboratorial Haracteristics of Ph (-) CMPN Patients

	CMPN n (%)	PV n (%)	ET n (%)	PMF n (%)
Patient number n (%)	99	43 (43.4)	44 (44.4)	12 (12.2)
Female/male	49/50	21/23	24/20	4/8/14
Mean age	59.8	58.5	60	62
JAK2 V617F (+)	64 (71.1)	27 (65.8)	32 (82)	5 (50)
WBC (K/uL)	10897	11173	11530	7583
Hgb (g/dl)	15.2	17.7	13.9	9.2
Plt (K/uL)	620000	413767	819000	219000
MPV (fL)	9.5	9.2	9.7	10.3
RDW (%)	17.05	16.2	17.4	19.3
LDH (U/L)	318	269	286	566
Splenomegaly n (%)	39 (55.7)	20 (57.1)	9 (26.4)	10 (90.9)
Hepatomegaly n (%)	25 (30.1)	9 (25.7)	11 (29.7)	5 (45.4)
Thrombosis n (%)	12 (12.1)	4 (9.3)	4 (10)	4 (33)
Hemmorage n (%)	6 (6)	3 (6.9)	2 (4.5)	1 (8.3)

Table 2. Correlation between JAK2 V617F Mutation and Thrombosis

Diagnosis	n	JAK2 V617F frequency n(%)	Thrombosis frequency n(%) (JAK2+/JAK2-)	p
Total	99	64(71%)	8(12.5%)/4(15.3%)	0.75
PV	43	27(65.8%)	2 (7.4%)/2(14.2%)	
ET	44	32(82%)	4(12.5%)/-	
PMF	12	5(50%)	2(40%)/2(40%)	

Table 3. Hemogram Parameters of Patient Groups with and without Thrombosis

	Thrombosis positive	Thrombosis negative	P value
Number of patients (n)	13	86	
Age	65	59.6	0.1
Gender (F/M)	7/6/14	39/47	0.5
WBC (K/uL)	9269	11143	0.2
Neutrophil (K/uL)	7393	7923	0.7
Lymphocyte (K/uL)	1941	2199	0.4
Hb (g/dl)	14.1	15.4	0.2
Plt (K/uL)	549538	631210	0.5
RDW (%)	18.1	16.6	0.1
MPV (fL)	9.9	9.4	0.1
NLR (%)	4.5	4.2	0.7
Sedimentation (mm/sec)	12.08	11.8	0.9
CRP (mg/dl)	9.9	5.7	0.4

Table 4. Hemogram Parameters in Patient and Control Groups

	Patient (n±SD)	Control (n±SD)	p value
Age (years)	59.8	53.5	0.161
Gender (F/M)	49/50	36/44	0.845
WBC (K/uL)	10.896±5208	6870±1689	<0.001
Neutrophil (K/uL)	7853±4708	1875±611	<0.001
Lymphocyte (K/uL)	2165±1078	4658±1405	<0.001
Hemoglobin (g/dl)	15.2±3.2	14.2±1.4	<0.001
Thrombocyte (K/uL)	620.000± 45.663	271.600± 68.366	<0.001
MPV (fL)	9.5±1.4	7.1±0.76	<0.001
RDW (%)	16.8±3.5	13.3±0.68	<0.001
NLR	4.3±3.6	0.43±0.15	<0.001
Sedim (mm/sec)	14	5	0.005
CRP (mg/dl)	0.5	2	0.1

mutation and in 12.5% of the patients with negative JAK2 V617F, which has no significant difference (p=0.758) (Table 2). Upon comparison of complete blood count parameters, and levels of sedimentation and CRP between patients with and without previous thrombosis, no statistically significant difference was observed between the parameters as it can be seen in Table 3. Laboratorial data of patient and control groups were respectively mean white blood count (WBC) 10.897-6870 K/uL, hemoglobin (Hgb) 15.2-14.2 g/dl, thrombocyte 620.000-271600 K/uL, RDW %16.8- 13.3, NLR 4.3-0.43, MPV 9.5- 7.1 fL, sedimentation 14-5 mm/sec and CRP 0.5-2 mg/dl, all of which excluding CRP were found to be elevated with statistical significance in patient group (Table 4). MPV was determined to be the independent predictive value of CMPN patients.

Discussion

Chronic myeloproliferative diseases are a disease group in hematologic malignities which requires long-term close monitoring. Cause of mortality or morbidity is generally thromboembolic incidents, hemorrhage or leukemic transformation. Positivity of JAK2 V617F mutation and leukocytosis were proven to be factors which increase thrombosis risk by a number of studies. (Takaya et al., 2014; Payzin et al., 2014) The fact that protease and oxygen radicals released with leukocyte activity inflict damage in endothelial cells, leukocyte adhesion molecule release and increased adhesion of leukocyte and thrombocyte on endothelial cell are given as the underlying prothrombotic factors (Kremyanskaya et al., 2013). Conducted researches indicated that JAK2 gene mutation can be correlated with elevated leucocyte activation and activated neutrophils can bind thrombocytes, and the release of tissue factor can be triggered with this binding (Kremyanskaya et al., 2013). In our study, no correlation of JAK2 gene mutation with leukocytosis and thrombosis was observed.

As specified in literature, JAK2 V617F mutation was most commonly found positive in polycythemia vera with 90-95% among Ph- CMPN cases. In our study, it was found positive with 82% in patients with essential thrombocytosis in contrast to the literature.

Recently, chronic inflammation process has been mentioned in chronic myeloproliferative diseases. Conducted studies indicate the increased risk of premature atherosclerosis and thrombosis as a result of the process during which proinflammatory cytokines secreted from increased leukocytes and thrombocytes due to clonal myeloproliferation trigger inflammation. CRP was accepted as inflammatory marker in the literature and their role in thrombosis risk within myeloproliferative diseases was researched (Barbui et al., 2009; Barbui et al., 2011; Landolfi et al., 2011). Furthermore, additional gene mutations induced by cytokines, chemokines and oxygen radicals released in chronic inflammation are thought to play an active role in cancer development and metastasis.

We evaluated inflammation process by comparing sedimentation, CRP, MPV, RDW and NLR levels, which were taken as a basis, not only in the thrombosis group but also within the entire group itself with healthy controls. In contrast to the literature, these values did not have any difference between patients with and without a history of thrombosis. However, the analysis conducted in healthy controls produced significantly elevated levels excluding CRP in the patient group. Several studies conducted in different areas researched the roles of MPV, RDW and NRL in inflammation. Elevated levels of MPV, RDW and NLR in conditions such as infection, thrombosis and cancer which are tables of inflammation are indicated in the literature (Kitaza et al., 2013; Karaman et al., 2013; Unal et al., 2013; Yu Q et al., 2013; Aktas et al., 2014; Gunay et al., 2014). Our findings are not surprising if we consider myeloproliferative diseases as the inflammatory disease of hematopoietic stem cell and evaluate inflammation the trigger of CMPNs as suggested by Haaselbalch et al. Likewise, these indicators, which were demonstrated to be significant in terms of inflammation by conducted studies, were also found to be elevated in our study.

As a result, our study supports that an inflammation process is the triggering and maintaining factor in CMPN. We believe that this hypothesis should be analyzed through extensive multicenter studies with a high number of cases.

References

Aktas G, Alcelik A, Tekce BK, et al (2014). Red cell distribution width and mean platelet volume in patients with irritable bowel syndrome. *Pzr Gastroenterol*, **9**, 160-3.

Barbui T, Carobbio A, Finazzi G, et al (2011). Inflammation and thrombosis in essential thrombocythemia and polycythemia vera: different role of C-reactive protein and pentraxin 3. *Hematologica*, **96**, 315-8.

Barbui T, Carobbio A, Rambaldi A, et al (2009) Perspectives on thrombosis in essential thrombocythemia and polycythemia vera: is leukocytosis a causative factor? *Blood*, **114**, 759-63.

Gasparyan AY, Ayvazyan L, Mikhailidis DP, et al (2011). Mean platelet volume: a link between thrombosis and inflammation. *Curr Pharm Des*, **17**, 47-58.

Günay E, Sarinç Ulasli S, Akar O, et al (2014). Neutrophil-to-lymphocyte ratio in chronic obstructive pulmonary disease: a retrospective study. *Inflammation*, **37**, 374-80.

Guo YZ, Pan L, Du CJ, Ren DQ, Xie XM (2013). Association between C-reactive protein and risk of cancer: a metaanalysis of prospective cohort studies. *Asian Pac J Cancer Prev*, **14**, 243-8.

Hasselbalch HC.(2013). Chronic inflammation as a promotor of mutagenesis in essential thrombocythemia, polycythemia vera and myelofibrosis. A human inflammation model for cancer development. *Leuk Reus*, **37**, 214-20.

Hasselbalch HC. et al (2012). Perspectives on chronic inflammation in essential thrombocythemia, polycythemia vera, and myelofibrosis: is chronic inflammation a trigger and driver of clonal evolution and development of accelerated atherosclerosis and second cancer. *Blood*, **119**, 3219-25.

Karaman H, Karaman A, Erden A, et al (2013) Relationship between colonic polyp type and the neutrophil/ lymphocyte ratio as a biomarker. *Asian Pac J Cancer Prev*, **14**, 3159-61.

Kilinçalp S, Ekiz F, Basar O, et al (2014). Mean platelet volume could be possible biomarker in early diagnosis and monitoring of gastric cancer. *Platelets*, **25**, 592-4.

Kitazawa T, Yoshino Y, Tatsuno K, et al (2013). Changes in the mean platelet volume levels after bloodstream infection have prognostic value. *Intern Med*, **52**, 1487-93.

Kremyanskaya M, Najfeld V, Mascarenhas J, et al (2013). Essential Thrombocythemia Ronald Hoffman, MD, Edward J. Benz eds: Hematology, 6th Edition Basic Principles and Practice, chapter **68**, 1037-8.

Kremyanskaya M, Najfeld V, Mascarenhas J, et al (2013). The polycythemias Ronald Hoffman, MD, Edward J. Benz eds: Hematology, 6th Edition Basic Principles and Practice, chapter, **67**, 1014-5.

Landolfi R, Di Gennaro L (2011). Pathophysiology of thrombosis in myeloproliferative neoplasms. *Haematologica*, **96**, 183-6.

Makay B, Gücenmez OA, Duman M, et al (2014). The relationship of neutrophil-to-lymphocyte ratio with gastrointestinal bleeding in Henoch-Schonlein purpura. *Rheumatol Int*, **34**, 1323-7

Payzin KB, Savasoglu K, Alacacioglu I, et al (2014). JAK2 V617F Mutation Status of 232 Patients Diagnosed With Chronic Myeloproliferative Neoplasms. *Clin Lymphoma Myeloma Leuk*, **14**, 525-33.

Sakalli H, Kal O,(2013). Mean platelet volume as a potential predictor of proteinuria and amyloidosis in familial Mediterranean fever. *Clin Rheumatol*, **32**, 1185-90.

Takaya Y1, Seki R, Kanajii T, et al (2014). Association between Thromboembolic Events and the JAK2 V617F Mutation in Myeloproliferative Neoplasms. *Kurume Med J*, **60**, 89-97.

Tefferi A. (2012). Polycythemia vera and essential thrombocythemia: update on diagnosis, risk stratification, and management. *Am J Hematol*, **87**, 285-93.

Unal D, Eroglu C, Kurtul N, Oguz A, et al (2013). Are neutrophil/ lymphocyte and platelet/lymphocyte rates in patients with non-small cell lung cancer associated with treatment response and prognosis? *Asian Pac J CancerPrev*, **14**, 5237-42.

Vannucchi AM, Antonioli E, Guglielmelli P, et al (2008). Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. *Leukemia*, **22**, 1299-307.

Vannucchi AM, Guglielmelli P.(2013). JAK2 mutation-related disease and thrombosis. *Semin Thromb Hemost*, **39**, 496-506.

Yu Q, Yu XF, Zhang SD, et al (2013). Prognostic role of C-reactive protein in gastric cancer: a meta-analysis. *Asian Pac J Cancer Prev*, **14**, 5735-40.