Research Article
Published: 21 May, 2019

Journal of Hematology and Oncology Forecast

Essential Thrombocythemia Characterization: Mutational, Cytokine, and Thrombotic Profiling

Hernandez-Matias L[1], Maysonet-Cruz J[2], Calzada-Jorge N[1], Pérez-Donato L[1], Torres Rivera W[3], Ramírez-Rodríguez J[1], Laureano-Torres F[1], Méndez LB[3], Ríos O[1], Menéndez-Pérez J[1], Washington AV[1] and Hunter-Mellado R[2]*

[1]Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico

[2]Retrovirus Research Center, Universidad Central del Caribe, School of Medicine, Bayamón, Puerto Rico

[3]Department of Pathology and Laboratory Medicine, School of Science and Technology, Universidad del Este, Carolina, Puerto Rico

Abstract

Essential Thrombocythemia (ET) is characterized by a persistent thrombocytosis associated with mutations in Janus Kinase 2 (JAK2V617F), Myeloproliferative Leukemia protein (MPL), and/or Calreticulin (CALR). Although ET mutational background has been defined for some sub populations, the proportion and diversification of ET mutations have not been defined for the Puerto Rican population. Furthermore, its cytokine profiling and clinical characteristics have also yet to be defined. In order to understand and further provide better treatment for the ET Puerto Rican cohort, this study defines the proportion of common ET mutations, cytokine profiles, plasma levels of Lysyl Oxidase (LOX), soluble Trem-Like Transcript-1 (sTLT-1) and, correlates these levels to the patients' clinical background. Results show that JAK2V617F, CALR, and MPL mutations were present in the ET Puerto Rican cohort in the proportions of 52%, 18%, and 4% respectively. It was also found that TWEAK/TNSF12 was significantly lower and, that MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2, and IL-29/IFNλ1 were significantly higher in ET patients when compared to controls. Interestingly, it was found that ET patients had higher concentrations of sTLT-1, a marker for disseminated intravascular coagulation and that all the interferon's tested were lower in non-diabetic ET patients than in non-diabetic control patients. These findings provide insight into the ET profiling and lay the foundations to provide a better treatment for the Puerto Rican ET sub-population.

Keywords: Essential thrombocythemia; Cytokines; Lysyl oxidase; Puerto Rican cohort; Trem-like transcript-1

OPEN ACCESS

*Correspondence:
Washington AV, Department of Biology, University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico.
Tel: 787-764-0000
Fax: 787-764:0000
E-mail: anthony.washington@upr.edu

Received Date: 03 Mar 2019
Accepted Date: 14 May 2019
Published Date: 21 May 2019

Citation: Hernandez-Matias L, Maysonet-Cruz J, Calzada-Jorge N, Pérez-Donato L, Torres Rivera W, Ramírez-Rodríguez J, et al. Essential Thrombocythemia Characterization: Mutational, Cytokine, and Thrombotic Profiling. J Hematol Oncol Forecast. 2019; 2(1): 1010.

Abbreviations

MPN: Myeloproliferative Neoplasms; ET: Essential Thrombocythemia; JAK2V617F: mutation on Janus Kinase 2; MPL: Myeloproliferative Leukemia Protein; CALR: Calreticulin; sTLT1: soluble Trem-Like Transcript; TLT-1: TREM-Like Transcript-1; LOX: Lysyl Oxidase; CHF: Congestive Heart Failure; CAD: Coronary Artery Disease; CNS: Central Nervous System; MI: Myocardial Infarction

Introduction

Essential Thrombocythemia (ET) myeloproliferative disease is diagnosed in 38-57 out of every 100,000 persons in the United States (2008 to 2010) [1]. This malignant disorder, characterized by thrombocytosis of an autonomous nature, is considered part of the spectrum of entities grouped as Myeloproliferative Neoplasm (MPN). ET neoplasm is predominantly seen in the mid-fifty-year-old population and is often accompanied with signs and symptoms related to bleeding diathesis, cytokine augmentation, thrombotic disorders, thrombocytosis, constitutional symptoms (asthenia, cachexia, satiety, night sweats), and splenomegaly [1-4]. Although, it has been shown that multiple cytokines are increased in ET patients, ET profiling of some interferons, thrombotic factors, and interleukins remain unknown [2,5,6].

ET is associated with three different mutations: Janus Kinase 2 (JAK2V617F), Myeloproliferative Leukemia Protein (MPL), and Calreticulin (CALR) [7,8]. These mutations ultimately lead to the constitutive activation of the JAK2 pathway and are responsible for 86% of documented ET cases.

Table 1: Primer sequences: forward and reverse primer sequences for amplification and sequencing of JAK2, CALR and MPL segments.

Mutation	Forward Primer	Reverse Primer
JAK2	*5'*-ATTGCTTTCCTTTTTCACAAGAT-*3'*	*5'*-GTTTTACTTACTCTCGTCTCCACAaAA-*3'*
CALR	*5'*-CATACGCTGAGGAGTTTGGC-*3'*	*5'*-GAGTGGAGGAGGGGAACAA-*3'*
MPL	*5'*-TAGCCTGGATCTCCTTGGTG-*3'*	*5'*-GCGGTACCTGTAGTGTGCAG-*3'*

The JAK2V617F is responsible for 56%, MPL exon 10 mutation is responsible for 5%, and CALR for 25% of ET mutations [8,9]. The JAK2V617F causes a direct activation of JAK2, while the MPL and CALR mutations affect JAK2 though activation of the thrombopoietin receptor [7-14]. Although these mutations induce ET neoplasms, thrombotic event risk, survival, hemoglobin, and platelet count, differ based on the patients' mutational background [9,15]. For example, patients with CALR mutation have a lower risk of thrombosis, longer survival, higher platelet counts and, lower White Blood Cell (WBC) counts than JAK2 positive patients [9,16]. Patients treated with JAK inhibitors, show a decrease in some constitutional symptoms and pro- inflammatory cytokines [17].

Since thrombotic events are one of the catastrophic manifestations seen in different disorders, including ET patients, a growing amount of research has focused on the identification of molecular markers for thrombotic risk. Lysyl Oxidase (LOX) over expression can increase platelet adhesion to collagen suggesting that higher levels of LOX may be associated with a higher risk of thrombotic events and fibrosis [18-20]. Another potential marker that is involved in thrombus formation is the soluble form of the triggering receptor expressed on myeloid cells (TREM)-Like Transcript-1 (TLT-1) [21]. TLT-1 enhances platelet activation in the presence of low agonist concentrations. Soluble TLT-1(sTLT-1) is a marker for Disseminated Intravascular Coagulation (DIC), a condition that causes thrombosis and bleeding due to an imbalance of platelet and coagulation factor aggregation throughout the body [21,22]. Soluble TLT-1 is also a prognostic factor for survival in acute respiratory distress syndrome [23]. Unfortunately, sTLT-1 levels in ET patients are unknown.

ET mutational proportions have been defined for patients in other countries, but the proportion and diversification of ET mutations have not been defined for the Puerto Rican population nor has its cytokine profiling. In order to understand and further provide a better treatment for the ET Puerto Rican cohort, this study defines the (1) proportion of common mutations, (2) cytokine profile, (3) plasma levels of LOX, and sTLT-1 of these patients, and (4) correlates these levels to the patients' clinical background.

Materials and Methods

Study samples

Blood samples were obtained from ET and control patients after they signed an informed consent agreement. The number of human subjects included in this study is 34; 23 ET patients and 11 controls. For TLT-1 and LOX measurements 22 ET patients were included. None of the controls were diagnosed with MPN. The Institutional Review Board (IRB) at the Universidad Central del Caribe approved all procedures of this study.

Mutational background identification

Whole blood was collected using EDTA tubes and stored at 4°C. DNA extraction was performed using Sigma GenElute Blood Genomic DNA kit. The extraction protocol was followed as suggested by the manufacturer, and samples were stored at -20°C. JAK2V617F, MPL (W515L and S505N), and CALR mutations were identified by Polymerase Chain Reaction (PCR) using primers that took into account each mutation (Table 1). We used primers at 2uM each, 20-30ng of DNA per reaction and NovaTaq Polymerase master mix according to manufacturer's recommendation. Samples were run in duplicate and mutations were confirmed by sequencing. The cycling program was as follows: 40x denaturation at 98°C for 40seconds, annealing for 30seconds and extension at 72°C for 1minute. Each mutation was identified according to the sequences previously published [24,25]. For CALR mutations, CALR type-1, 52-bp deletion (p.L367fs*46), type-2, 5-bp TTGTC insertion (p.K385fs*47), and type- 3, 3-bp deletion (L367fs*48) were evaluated in all samples [24]. Identified proportions of mutations were compared to previously described proportions using Fisher Exact Analysis [8].

Cytokine measurement

Plasma from 23 ET patients and 11 controls was extracted and stored at -80°C until further analysis. The concentration of 37 pro-inflammatory markers was measured with a multiplex solid-based immunoassay (Pro-Human Inflammatory kit, Bio-Rad, Hercules, CA) using the Luminex MAGPIX® system. Each sample was assayed in duplicate following the manufacturer's protocol. Data was analyzed using a 5-parameter logistic curve. Cytokine profile comparisons were not normally distributed and were analyzed using the Mann-Whitney U Test. Comparisons between cell count and cytokine measurements were done using Spearman correlation test.

LOX measurement

The levels of active enzyme LOX and pro-LOX, its glycosylated precursor, were measured in the ET patient plasma using western blot analysis as described previously [25]. Plasma was diluted 1:5 with PBS and disulfide-bond reduction was done using β-Mercapto Ethanol (β-ME). The plasma was separated by PAGE gel and was transferred onto a Poly Vinylidene Di Fluoride (PVDF, Bio Rad) membrane. Anti-Lysyl Oxidase antibody produced in rabbit (Cat.#L4794 from Sigma Aldrich) was used as the primary antibody and peroxidase donkey anti-rabbit IgG horseradish peroxidase (Jackson Immuno research) as the secondary antibody. Total protein was quantified and used for normalization. LOX, and pro-LOX bands (30kDa, 50kDa respectively) intensity was determined by ChemiDoc™ XRS+ and analyzed using Image Lab™ and Graph Pad Prism Software [19,20]. Statistical analysis was completed using the student T-test.

sTLT-1 measurement

The levels of s-TLT-1 in the plasma of patients were measured by slot blot technique as described previously [22]. Plasma was diluted 1:3 with TBS 1X and dotted onto a nitrocellulose membrane. Soluble TLT-1 was detected using antibody 69 anti-human TLT-1 primary antibody (dilution, 1:1000) and peroxidase donkey anti-rabbit IgG (Jackson Inmuno Research) as the secondary antibody (dilution, 1:10000). Soluble TLT-1 recombinant protein (amino acids 1-146; CDI Laboratories) was used to create a standard curve (from 1ng to 20ng). Band adjusted intensity measure was determined by ChemiDoc™ XRS+ and analyzed using Image Lab™ and Graph Pad

Table 2: Essential Thrombocythemia patient profiling, gender, diagnosis and medications. A questionnaire, addressing clinical background, medications, and demographics, was filled out by the participants at the moment of the blood draw. ET N=23 Controls N=11; CHF stands for Congestive Heart Failure; CAD stands for Coronary artery disease; CNS stands for Central Nervous System; MI stands for Myocardial Infarction.

Variable	Experimental Group N (Range)	%	Control group N	%
Age				
Median (range)	68 (21-82)	-	63 (39-76)	-
Hemoglobin				
Median (range)	12.8 (6.4-16.4)	-	-	-
WBC				
Median (range)	7.35 (2.83-31.3)	-	-	-
Platelets				
Median (range)	651 (366-1284)	-	-	-
Female	13	56.5	7	58
Diabetes	6	26.1	6	50
Hypertriglyceridemia	4	17.4	0	0
Hypercholesterolemia	7	30.4	7	58
Hypertension	12	52.2	7	58
CHF	0	0.0	0	0
CAD	2	8.7	0	0
Hemorrhage	0	0.0	0	0
Petechiae	0	0.0	0	0
Splenomegaly	6	26.1	0	0
Renomegaly	1	4.3	0	0
Hepatomegaly	1	4.3	0	0
CNS thrombus	7	30.4	0	0
Heart thrombus	1	4.3	0	0
MI	0	0.0	0	0
Venous thrombus	2	8.7	0	0
Medications				
Aspirin	20	87.0	3	25
Alkylating	0	73.9	0	0
Hydroxyurea	17	0.0	0	0
Anagrelide	3	13.0	0	0
Clopidogrel	1	4.3	0	0
Coumadin	0	0.0	0	0

Prism Software. Statistical analysis was done using T-test.

Results

Mutational background

To identify the proportions of JAK2, CALR, and MPL in our cohort of patients, PCR and Sanger sequencing was used. Results revealed the presence of JAK2 V617F mutation in 12 of the ET patients (52%). CALR mutations were identified in 4 patients (18%); type 1 mutation was found in 2 patients, type 2 in 1 patient, and type 3 in 1 patient. MPL S505N and W515L mutant sequences were evaluated in all samples and S505N mutation was found in 1 patient (4%) for a total of 17 (74%) ET patients with common mutations and 6 (26%) ET patients whose mutational background remained unknown (Figure 1). Control patients did not carry any of the three tested mutations. The proportions of mutations in our population were similar to those previously described (Fisher exact test for: JAK2 p-value: 0.081, CALR p-value 0.580 and MPL p-value: 1) [9].

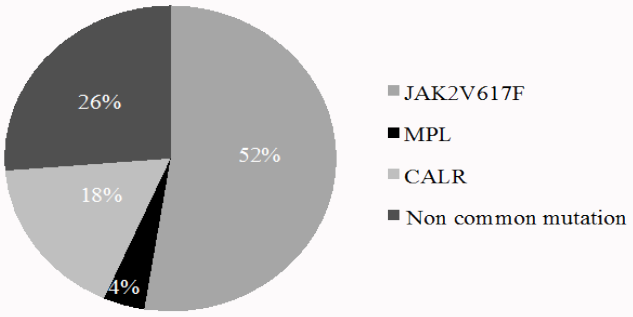

Figure 1: Mutational distribution seen in Puerto Rican ET patients. N=23.

Patient clinical background

From the 23 ET clinical profile evaluations, the most prevalent diagnosis is hypertension (52.2%), followed by hypercholesterolemia (30.4%) and CNS thrombi (30.4%; Table 2). None of the patients suffered hemorrhages, which is considered a more serious effect linked to ET. As to medications, 87% of the patients were taking aspirin. The platelet count was elevated as expected and the gender distribution was 13:10 females to males respectively (Table 1). Noteworthy, 100% of patients with non-common mutations had at least one thrombotic event. Regarding the control group, patients' prevalent diagnosis included hypertension (58%), hypercholesterolemia (58%), and diabetes (50%). None of the controls were diagnosed with MPD. Gender distribution was 7:4 females to males respectively.

ET patient cytokine profile

In order to define ET patient cytokine profile, 37 analytes were measured in the ET and control patient plasma (Table 2). From the 37 analytes tested, the levels of six (IL-34, TNSF14, IL-2, IL-11, IL-20 and IL-27 (p28)) were out of range and subsequently were not included in the analysis. Using Mann-Whitney U analysis, the following 9 analytes showed a significant difference between ET and control patients: TWEAK/TNSF12, MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2 and IL-29/IFNλ1 (Table 2). Since diabetes induces an overproduction of cytokines, only the non-diabetic ET patients and non-diabetic control patients were analyzed. Results showed that non-diabetic ET patients had lower levels of IFN-α2, IFN-β, IFN-γ, IL-2, IL-12 (p40), IL-12 (p70), IL-19, IL-22, IL-28A, IL-35, and MMP1 when compared to control patients (Table 3).

LOX and soluble TLT-1 levels

LOX protein was recently correlated with thrombosis and MPN, but the levels of LOX protein and its precursor were not defined for ET patients [20]. Western blot band intensity analysis revealed that neither ET patient's LOX nor pro-LOX levels were significantly different from control patients. LOX or pro-LOX levels did not differ from ET thrombotic patients and ET non-thrombotic patients (Figure 2A and 2B). Because ET patients do not just suffer from thrombotic events, but also from bleeding we measured sTLT-1, amarker of DIC [4]. The analysis revealed that indeed ET patients had a significantly higher median value (10.95ng/mL) of sTLT-1 than control patients (3.195ng/mL; p= 0.0490) (Figure 2C).

Patients' cytokines levels and clinical background

Subsequently, cytokine levels were analyzed to identify any

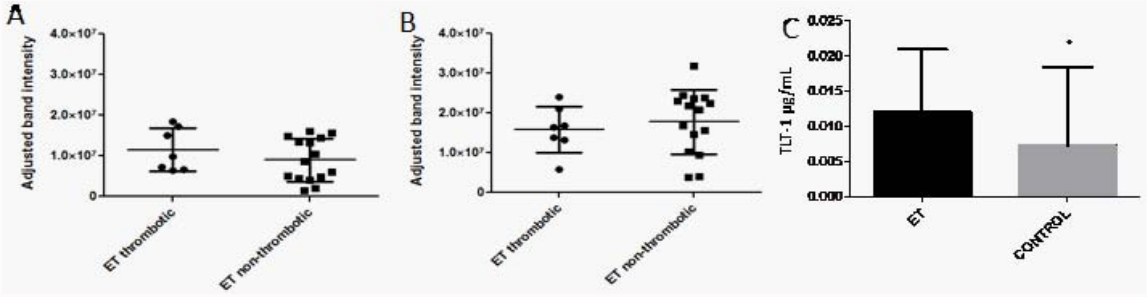

Figure 2: Soluble TLT-1 and LOX levels in ET patients. (**A-B**) Different forms of LOX levels in ET patient plasma. Western blot was used to measure LOX and results were analyzed using T-test. The lines in the scatter dot plot diagrams represent mean with SD, (**A**) pro-LOX (**B**) LOX band intensity is reported. ET thrombotic N= 7 ET non-thrombotic=15. **(C)** Slot-blot technique was used to determine s-TLT-1 levels in ET patient plasma. Optical density of each sample was detected using Image Lab 6.0. Concentration was interpolated from a standard curve. Analysis was done using a Mann Whitney test and *p*-value was established at <0.05. ET N =22 Controls N=11.

Table 3: Profiling of ET patients and controls. Plasma levels of cytokines and other analytes were measured on the ET and control patient plasma using a 37-plex solid-based immunoassay. Analysis was performed using Mann Whitney U test and *p*-value was established at<0.05. Bold values represent significant values. ET N=23, Controls N=11.

Cytokines	Median ET (range) n=23	Median Control (range) n=11	Mann Whitney test P value
TWEAK TNFSF12	43.57(27.57-57.9)	47.77(41.71-69.86)	0.0392
TSLP	7.05(1.79-25.72)	5.89(1.96-10.84)	0.2039
sTNFR2	1166(439-3054)	732.8(367.2-1760)	0.3256
sTNFR1	387.4(123.1-1011)	307.2(256.6-602.1)	0.6433
Pentraxin-3	69.61(36.19-174.2	71.32(32.07-103.1)	0.5372
Osteopontin	12360(6307-27972)	13435(9144-20473)	0.3781
Osteocalcin	508.9(199.2-2491)	590.8(283.8-866.90)	0.717
MMP3	1427(595.6-5695)	1503(680.9-2409)	0.5672
MMP2	5157(2366-9922)	4114(2753-7235)	0.3256
MMP1	664.1(297.4-2279)	399.8(217-1729)	0.006
IL-35	36.15(23.64-99.88)	28.34(17.37-51.74)	0.0059
IL-8	6.93(5.07-11.26)	6.33(-5.29-7.69)	0.0481
April /TNSF13	32374(11095-66721)	27246(22242-59916)	0.4618
BAFF/TNS13B	2074(844.6-9765)	1665(942.6-2400)	0.0608
sCD30 TNFRSF8	77.89(33.59-283.3)	73.21(63.53-122.4)	0.9422
scD163	21379(9011-68511)	25386(12807-54294)	0.4675
Chintase-3 like 1	4378(1629-9420)	5357(2931-7985)	0.1144
gp130 sIL6Rβ	7543(3178-17026)	9103(4964-14364)	0.1427
IFN-α2	14.38 (10.44-30.59)	12.63(9.13-16.56)	0.0154
IFN-β	7.5 (2.55- 19.24)	6.83(4.83-11.59)	0.2032
IFN-γ	4.85(3.03-15.96)	4.36(3.03-7.23)	0.1033
sIL-6Rα	1947(766.9-5206)	1992(1577-4319)	0.2741
IL10	2.79(1.99-3.8)	2.49(1.99-2.81)	0.0509
IL-12(p40)	16.27(7.38-57.08)	13.32(7.38-26.5)	0.066
IL-12(p70)	0.57(0.39-1.12)	0.51(0.48-0.62)	0.1638
IL-19	6.56(4.15-12.6)	5.13(4.15-9.3)	0.0162
IL22(28)	7.38(4.71-14.21)	6.23(5.09-8.34)	0.0153
IL-26	11.7(8.57-15.8)	10.55(7.48-15.45)	0.0886
IL-28A	3.76(2.24-13.79)	2.79(1.9-5.89)	0.0334
IL-29	12.24(6.44-26.8)	10.03(6.44-15.23)	0.0421
IL-32	8.6(2.81-46.13)	4.71(0.38-13.59)	0.0691

Table 4: Comparison between non-diabetic ET and non-diabetic control patients. A total of 17 non-diabetic ET patients and 5 non-diabetic controls were analyzed using Mann Whitney U Test. *p*-value was established at <0.05.

Analyte	P-value
APRIL/TNFSF13	0.9396
BAFF/TNFSF13B	0.2231
sCD30/TNFRSF8	0.8201
sCD163	0.8201
Chintinase-3 like 1	0.1196
gp130/IL-6Rβ	0.3587
IFN-α2	0.0135
IFN-β	0.0233
IFN-γ	0.0491
IL-2	0.0112
IL-6Rα	0.3587
IL-8	0.1241
IL-10	0.1958
IL-12(p40)	0.05
IL-12(p70)	0.0432
IL-19	0.0371
IL20	0.883
IL-22(28)	0.0235
IL-26	0.3086
IL-28A	0.0296
IL-29	0.0739
IL-32	0.0704
IL-35	0.0019
MMP1	0.0373
MMP2	0.1196
MMP3	0.7616
Osteocalcin	0.4008
Osteopontin (OPN)	0.1636
Pentraxin 3	0.4929
sTNFR1	0.8911
sTNFR2	0.5946
TSLP	0.1404
TWEAK/TNFSF12	0.0608

correlation with the patients' clinical background. As a result, it was found that IL-26 levels were significantly higher in patients that showed constitutional symptoms fever, weight loss, visual disturbance, fatigue, headache, dysesthesias. $p< 0.049$). No significant associations were observed between cytokine profile, TLT-1 or LOX, and thrombosis, WBC count, hemoglobin, platelet count, or bleeding.

Discussion

The purpose of this study is to characterize the ET Puerto Rican population to further provide a better treatment for these patients. With this intention, the present study first characterized the ET Puerto Rican cohort proportion of common mutations and found that the mutational profiling was similar to the previously published populations [8,9]. However, the ET cytokine profile was slightly different when compared to the previously reported ones. It was found that MMP-1, IL-35, IL-8, IFN-α2, IL-19, IL-22, IL-28A/IFNλ2 and IL-29/IFNλ1 were significantly higher in ET patients than in control patients. Noteworthy, from the eight different analytes that were significantly higher, just IL-8 was known to be higher in ET patients [26]. Even though MMP-1 and IL-22 were increased in the ET Puerto Rican cohort, previous studies found that ET patients had normal levels for MMP-1 and IL-22. MMP-1 is known to regulate thrombus formation and platelet activation [27]. Interestingly, not just MMP-1 but some of the analytes that were highly expressed in ET patients have been shown to have a direct effect on platelet count. For example, IL-8 was found to impair the proliferation and differentiation of megakaryocyte and myeloid progenitor cells on patients with Myelofibrosis. Moreover, the neutralization of IL-8 receptors had been shown to restore megakaryocytes ploidy [28]. However, mice treated with IL-22 showed a significant increase in platelet count [29]. Previous articles have reported that Interferon alpha (IFN) inhibits the growth of megakaryocyte progenitor cells leading to a reduction of peripheral platelet counts on ET patients [30]. Also, IFN had been shown to down-regulate the expression of IL-8 and enhance the expression of IL-22 [31-33]. Further studies focused on the combination of the effects of IFN, IL-22 and IL-8 on the augmentation of platelet count in ET patients is suggested. Indeed, emphasis should be made on the fact that all the tested Interferons (IFNs) were lower in non-diabetic ET patients than non-diabetic control patients. IFNs are produced in response to a viral infection or by a direct response to inflammation. To our knowledge, there is no association between ET and the capacity of these patients to deal with viral diseases, which could be an interesting point for further research.

In order to further characterize ET patients, we not only define their cytokine profile but also analyze two different molecular markers of thrombotic events: LOX and sTLT-1. Even though LOX protein had been associated with thrombotic events, LOX levels were not able to explain ET patients' thrombotic risk. Instead, sTLT-1 levels were significantly higher in ET patients than the control group. We suggest that the augmentation of sTLT-1 levels may be associated with the hyper activation of platelets in ET patients, but further studies are needed to validate this observation.

We believe that although, ET is a rare disorder and the Puerto Rican population in comparison to other countries is very small, we were able to gather a good representative cross section of ET Puerto Rican patients. This characterization of the ET Puerto Rican cohort described in this study provides multiple insights for further studies and laid the foundations to a better treatment for ET patients.

References

1. Mesa RA, Mehta J, Wang H, Wang Y, Iqbal U, Neumann F, et al. Epidemiology of Myeloproliferative Disorders in US - a Real World Analysis. Blood. 2012; 120: 2834.

2. Pourcelot E, Trocme C, Mondet J, Bailly S, Toussaint B, Mossuz P. Cytokine profiles in polycythemia vera and essential thrombocythemia patients: Clinical implications. Exp Hematol. 2014; 42: 360-368.

3. Passamonti F, Rumi E, Pungolino E, Malabarba L, Bertazzoni P, Valentini M, et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med. 2004; 117: 755-761.

4. Montanaro M, Latagliata R, Cedrone M, Spadea A, Rago A, Di Giandomenico J, et al. Thrombosis and survival in essential thrombocythemia: A regional study of 1,144 patients. Am J Hematol. 2014; 89: 542-546.

5. Tefferi A, Vaidya R, Caramazza D, Finke C, Lasho T, Pardanani A. Circulating interleukin (IL)-8, IL-2R, IL-12, and IL-15 levels are independently prognostic in primary myelofibrosis: a comprehensive cytokine profiling study. J Clin Oncol. 2011; 29: 1356-1363.

6. Geyer HL, Dueck AC, Scherber RM, Mesa RA. Impact of Inflammation on Myeloproliferative Neoplasm Symptom Development. Mediators Inflamm. 2015: 1-9.

7. Boyd EM, Bench AJ, Goday-Fernández A, Anand S, Vaghela KJ, Beer P, et al. Clinical utility of routine MPL exon 10 analysis in the diagnosis of essential thrombocythaemia and primary myelofibrosis. Br J Haematol. 2010; 149: 250-257.

8. Wu Z, Zhang X, Xu X, Chen Y, Hu T, Kang Z, et al. The mutation profile of JAK2 and CALR in Chinese Han patients with Philadelphia chromosome-negative myeloproliferative neoplasms. J Hematol Oncol. 2014.

9. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic Mutations of Calreticulin in Myeloproliferative Neoplasms. N Engl J Med. 2013; 369: 2379-2390.

10. Jatiani SS, Baker SJ, Silverman LR, Reddy P. JAK/STAT Pathways in Cytokine Signaling and Myeloproliferative Disorders: Approaches for Targeted Therapies. Genes Cancer. 2010; 1: 979-993.

11. Zhou Z, Gushiken FC, Bolgiano D, Salsbery BJ, Aghakasiri N, Jing N, et al. Signal Transducer and Activator of Transcription 3 (STAT3) Regulates Collagen-Induced Platelet Aggregation Independently of Its Transcription Factor Activity. Circulation. 2013; 127: 476-485.

12. Varghese LN, Defour JP, Pecquet C, Constantinescu SN. The Thrombopoietin Receptor: Structural Basis of Traffic and Activation by Ligand, Mutations, Agonists, and Mutated Calreticulin. Front Endocrinol (Lausanne). 2017; 8: 59.

13. Moliterno AR, Ankins WD, Pivak JL. Impaired expression of the thrombopoietin receptor by platelets from patients with policythemia vera. N Engl J Med. 1998; 338: 572-580.

14. Staerk J, Lacout C, Sato T, Smith SO, Vainchenker W, Constantinescu SN. An amphipathic motif at the transmembrane-cytoplasmic junction prevents autonomous activation of the thrombopoietin receptor. Blood. 2006; 107: 1864-1871.

15. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood. 2016; 112: 844-848.

16. Bertozzi I, Peroni E, Coltro G, Bogoni G, Cosi E, Santarossa C, et al. Thrombotic risk correlates with mutational status in true essential thrombocythemia. Eur J Clin Invest. 2016; 46: 683-689.

17. Harrison C, Kiladjian JJ, Al-Ali HK, Gisslinger H, Waltzman R, Stalbovskaya V, et al. JAK Inhibition with Ruxolitinib versus Best Available Therapy for Myelofibrosis. N Engl J Med. 2012; 366: 787-798.

18. Eliades A, Papadantonakis N, Bhupatiraju A, Burridge KA, Johnston-Cox HA, Migliaccio AR, et al. Control of Megakaryocyte Expansion and Bone Marrow Fibrosis by Lysyl Oxidase. J Biol Chem. 2011; 286.

19. Matsuura S, Mi R, Koupenova M, Eliades A, Patterson S, Toselli P, et al. Lysyl oxidase is associated with increased thrombosis and platelet reactivity. Blood. 2016; 127: 1493-1501.

20. Tadmor T, Bejar J, Attias D, Mischenko E, Sabo E, Neufeld G, et al. The expression of lysyl-oxidase gene family members in myeloproliferative neoplasms. Am J Hematol. 2013; 88: 355-358.

21. Washington AV, Gibot S, Acevedo I, De La Mota A, Gattis J, Rivera L, et al. TLT-1 (TREM-Like transcript-1) Protects against Hemorrhage Associated with Inflammation by Facilitating Platelet Aggregation. Blood. 2015; 112: 1837.

22. Washington AV, Gibot S, Acevedo I, Gattis J, Quigley L, Feltz R, et al. TREM-like transcript-1 protects against inflammation-associated hemorrhage by facilitating platelet aggregation in mice and humans. J Clin Invest. 2009; 119: 1489-1501.

23. Morales-Ortíz J, Deal V, Reyes F, Maldonado-Martínez G, Ledesma N, Staback F, et al. Platelet-derived TLT-1 is a prognostic indicator in ALI/ARDS and prevents tissue damage in the lungs in a mouse model. Blood. 2018; 132: 2495-2505.

24. Pietra D, Rumi E, Ferretti VV, Di Buduo CA, Milanesi C, Cavalloni C, et al. Differential clinical effects of different mutation subtypes in CALR-mutant myeloproliferative neoplasms. Leukemia. 2016; 30: 431-438.

25. Beer PA, Campbell PJ, Scott LM, Bench AJ, Erber WN, Bareford D, et al. MPL mutations in myeloproliferative disorders: analysis of the PT-1 cohort. Blood. 2008; 112: 141-149.

26. Xie N, Gong H, Suhl JA, Chopra P, Wang T, Warren ST. Reactivation of FMR1 by CRISPR/Cas9-Mediated Deletion of the Expanded CGG-Repeat of the Fragile X Chromosome. PLoS One. 2016; 11: 1-12.

27. Mastenbroek TG, Feijge MA, Kremers RM, van den Bosch MT, Swieringa F, De Groef L, et al. Platelet-Associated Matrix Metalloproteinases Regulate Thrombus Formation and Exert Local Collagenolytic Activity. Arterioscler Thromb Vasc Biol. 2015; 35: 2554-2561.

28. Hermouet S, Bigot-Corbel E, Gardie B. Pathogenesis of Myeloproliferative Neoplasms: Role and Mechanisms of Chronic Inflammation. Mediators Inflamm. 2015; 2015: 1-17.

29. Emadi S, Clay D, Desterke C, Guerton B, Maquarre E, Charpentier A, et al. IL-8 and its CXCR1 and CXCR2 receptors participate in the control of megakaryocytic proliferation, differentiation, and ploidy in myeloid metaplasia with myelofibrosis. Blood. 2005; 105: 464-473.

30. Liang SC, Nickerson-Nutter C, Pittman DD, Carrier Y, Goodwin DG, Shields KM, et al. IL-22 Induces an Acute-Phase Response. J Immunol. 2010; 185: 5531-5538.

31. Seewann HL. Interferon therapy in essential thrombocythemia. Wien Med Wochenschr. 1993; 143: 420-424.

32. Tohyama M, Yang L, Hanakawa Y, Dai X, Shirakata Y, Sayama K. IFN-α Enhances IL-22 Receptor Expression in Keratinocytes: A Possible Role in the Development of Psoriasis. J Invest Dermatol. 2012; 132: 1933-1935.

33. Schnyder-Candrian S, Strieter RM, Kunkel SL, Walz A. Interferon-alpha and interferon-gamma down-regulate the production of interleukin-8 and ENA-78 in human monocytes. J Leukoc Biol. 1995; 57: 929-935.