RESEARCH LETTER

blood advances

TO THE EDITOR:

1.5 million platelet count limit at essential thrombocythemia diagnosis: correlations and relevance to vascular events

Naseema Gangat,[1,2] Natasha Szuber,[3] Yamna Jadoon,[1,2] Faiqa Farrukh,[1,2] Kebede Begna,[1,2] Michelle A. Elliott,[1,2] Alexandra P. Wolanskyj-Spinner,[1,2] Curtis A. Hanson,[1,2] Animesh D. Pardanani,[1,2] Valerio De Stefano,[4] Tiziano Barbui,[5] Alessandro Maria Vannucchi,[6] and Ayalew Tefferi[1,2]

[1]Division of Hematology, and [2]Division of Hematopathology, Mayo Clinic, Rochester, MN; [3]Department of Hematology, Université de Montréal, Montréal, Quebec, Canada; [4]Section of Hematology, Department of Radiological and Hematological Sciences, Catholic University, Fondazione Policlinico A. Gemelli IRCCS, Rome, Italy; [5]Research Foundation, Papa Giovanni XXIII Hospital, Bergamo, Italy; and [6]Department of Experimental and Clinical Medicine, CRIMM, Center Research and Innovation of Myeloproliferative Neoplasms, Azienda Ospedaliera Universitaria Careggi, University of Florence, Florence, Italy

In patients with essential thrombocythemia (ET) with extreme thrombocytosis (ExT $\geq$1500 $\times$ 10^9/L), cytoreductive therapy is recommended in the current National Comprehensive Cancer network and European LeukemiaNet treatment guidelines.[1,2] A recent international physician survey disclosed marked inconsistencies regarding the management of low-risk ET with ExT $\geq$1500 $\times$ 10^9/L, arising from concerns related to an increased risk of thrombosis and/or bleeding.[3,4] The prothrombotic or prohemorrhagic impact of thrombocytosis was studied in a prospective multicenter cohort of 776 patients with ET from the randomized PT-1 trial, and no association between blood counts at diagnosis and future complications was found. However, platelet count outside of the normal range ($>$450 $\times$ 10^9/L) during follow-up was associated with an immediate risk of major hemorrhage but not thrombosis.[5] Conversely, some studies in ET with ExT have implicated aspirin use as the culprit for associations with major hemorrhage,[6-8] whereas other studies have suggested a lower incidence of arterial thrombosis in patients with ExT.[9-11] In addition, interpretation of findings from prior reports is confounded by the variability in platelet count thresholds used to define ExT.[4]

The objectives for the current study in ET included: (1) estimation of prevalence of ExT $\geq$1500 $\times$ 10^9/L at time of diagnosis; (2) phenotypic and genotypic characterization of patients presenting with ExT $\geq$1500 $\times$ 10^9/L; and (3) determination of impact on thrombotic and bleeding events, in the context of other risk factors and specific therapy.

Study patients were recruited from our institutional myeloproliferative neoplasms database after institutional review board approval by the Mayo Clinic institutional review board. The study included 710 patients with ET evaluated over 5 decades (1967-2021) and retrospectively reviewed and confirmed to fulfill the 2016 World Health Organization diagnostic criteria.[12] The study was conducted in accordance with the Declaration of Helsinki. To minimize the inadvertent inclusion of patients with prefibrotic myelofibrosis, cases with anemia defined according to sex-adjusted hemoglobin level $<$11 g/dL in women and $<$12.5 g/dL in men were excluded.[13] All cases were molecularly annotated for driver mutations, with major arterial and venous thromboses and major hemorrhage defined according to conventional criteria.[14] Follow-up information for all patients, including vascular complications and disease evolution, was updated in May 2021 through either review of last clinic visit or medical records, which included access to care delivered outside the institution, or by telephone call to the patient. Analysis considered variables obtained at time of diagnosis. Comparison between categorical variables was performed by using the χ^2 test and continuous variables by using Wilcoxon/Kruskal-Wallis tests. A Cox proportional hazards model was used to compute multivariable analyses. *P* values $\leq$.05 were considered significant. The JMP Pro 16.0.0 software package (SAS Institute, Inc., Cary, NC) was used for all analyses.

Submitted 10 January 2022; accepted 1 March 2022; prepublished online on *Blood Advances* First Edition 10 March 2022; final version published online 1 July 2022. DOI 10.1182/bloodadvances.2022007023.

Presented in abstract form at the 63rd annual meeting of the American Society of Hematology, Atlanta, GA, 11-14 December 2021.

Requests for data sharing may be submitted to Naseema Gangat (gangat.naseema@mayo.edu).

The full-text version of this article contains a data supplement.

Among 710 consecutive Mayo Clinic patients with ET, 41 (6%) displayed ExT ≥1500 × 10^9/L (platelet count range, 1500-3460 × 10^9/L) at time of diagnosis; incidence rates were 15% (20 of 133), 5% (13 of 247), and 2% (8 of 330) for patients aged <40 years, 41 to 59 years, and ≥60 years, respectively ($P < .0001$). ExT ≥1500 × 10^9/L was present in 27 (9%) of 315 conventional "low-risk" and 14 (4%) of 395 "high-risk" patients, based on age >60 years and thrombosis history ($P = .004$). Similarly, the incidence of ExT ≥1500 × 10^9/L was 3% (25 of 986), 5% (42 of 891), and 3% (15 of 558) in 3 independent Italian cohorts from the University of Florence, University of Bergamo, and Catholic University of Rome. Incidence rates of extreme thrombocytosis >1500 × 10^9/L at time of ET diagnosis at their respective institutions were provided by co-authors V.D.S., T.B. and A.M.V. in August 2021 (unpublished data).

Phenotypic and genotypic characteristics of the 41 patients presenting with ExT ≥1500 × 10^9/L were compared with those of their counterparts presenting with a platelet count <1500 × 10^9/L (n = 669) (Table 1). In univariate analysis, patients with ExT ≥1500 × 10^9/L were more likely to be younger (median age, 40 vs 59 years; $P < .0001$) and to display *CALR* mutation (44% vs 26%; $P = .001$), lower hemoglobin level (median, 13.1 g/dL vs 13.9 g/dL; $P = .001$), leukocytosis ≥11 × 10^9/L (43% vs 21%; $P = .001$), and major hemorrhage (15% vs 4%; $P = .001$) at presentation.[15] In multivariable analysis, the significant associations with younger age, lower hemoglobin level, leukocytosis, and major hemorrhage were maintained. No significant associations were noted for incidence of arterial (7% vs 14%; $P = .25$) or venous (12% vs 10%; $P = .58$) thrombosis or microvascular symptoms (23% vs 24%; $P = .87$), between patients presenting with or without ExT ≥1500 × 10^9/L, respectively. Instead, multivariable analysis for events at/before diagnosis identified male sex (odds ratio [OR], 1.8; $P = .01$), *JAK2* mutation (OR, 2.4; $P = .01$), and cardiovascular risk factors (OR, 2.4; $P = .01$) as risk factors for arterial thrombosis and *JAK2* mutation (OR, 2.7; $P = .01$) as a risk factor for venous thrombosis.

Abnormal von Willebrand factor profile at diagnosis defined by ristocetin cofactor activity <30% or loss of high-molecular-weight multimers was documented in 3 of 7 (43%) vs 19 of 57 (33%) evaluable patients with or without ExT ≥1500 × 10^9/L ($P = .62$). Next-generation sequencing performed in a subset of patients (n = 244) depicted a higher incidence of *U2AF1* (7% vs 0.4%; $P = .01$) and *EZH2* (7% vs 0.9%; $P = .04$) mutations among patients with ExT ≥1500 × 10^9/L. Transformation to myelofibrosis was documented in 10 (24%) patients with ExT ≥1500 × 10^9/L at diagnosis vs 86 (13%) without ExT ($P = .05$); this difference was accounted for by longer follow-up of patients with ExT ≥1500 × 10^9/L (11.2 years vs 8.5 years; $P < .001$). Furthermore, myelofibrosis-free survival was similar among patients with ExT ≥1500 × 10^9/L at diagnosis (not reached) vs those without ExT (26 years; $P = .63$).

A comparison of patients presenting with ExT ≥1500 × 10^9/L and those with platelet count between 1 and 1.49 million (n = 83) revealed the following differences: younger age (40 years vs 69 years; $P < .0001$), predominance of female subjects (73% vs 57%; $P = .07$), and *CALR* genotype (44% vs 28%; $P = .02$), with a lower incidence of cardiovascular risk factors (35% vs 76%; $P < .0001$) and arterial thrombosis at/before diagnosis (7% vs 21%; $P = .04$) but a higher rate of hemorrhage at/before diagnosis (15% vs 5%; $P = .07$) in patients with ExT ≥1500 × 10^9/L. Conversely, comparison of patients presenting with a platelet count between 1 and 1.49 million and <1 million revealed predominance of the *CALR* genotype with platelet count between 1 and 1.49 million (43% vs 21%; $P < .0001$), with no differences in age, cardiovascular risk factors, or thrombosis and hemorrhage at/before diagnosis. Given the unique clinical characteristics of patients with ExT ≥1500 × 10^9/L, it was chosen as the cutoff for this study.

CALR mutation was associated with a lower incidence of venous (0% *CALR* vs 22% others; $P = .03$) and arterial (0% *CALR* vs 13% others; $P = .11$) thrombosis at presentation; leukocytosis ≥11 × 10^9/L (24% vs 4% with/without; $P = .07$) was identified as an additional risk factor for venous thrombosis (supplemental Table 1). Neither driver mutations ($P = .77$) nor leukocytosis ≥11 × 10^9/L ($P = .73$) was associated with major hemorrhage at presentation. A total of 14 vascular events (5 arterial thrombosis, 2 venous thrombosis, and 7 major hemorrhage) were documented during median follow-up of 11.2 years as detailed in Table 2. Leukocytosis ≥11 × 10^9/L (24% vs 4% with/without; $P = .06$), and cardiovascular risk factors (23% vs 8% with/without; $P = .11$) were borderline significant for arterial thrombosis–free survival. In terms of major hemorrhage-free survival, presence of *JAK2* mutation (36% *JAK2* vs 8% others; hazards ratio, 5.4; $P = .03$) and leukocytosis ≥11 × 10^9/L (29% vs 9% with/without; $P = .07$) emerged significant/near significant, with the former retaining significance on age-adjusted analysis. Notably, major hemorrhages during follow-up occurred in 3 cases in the absence of antiplatelet/anticoagulant treatments. In the remaining 4 cases, the patients at higher risk of hemorrhage were receiving treatments: high-dose aspirin (325 mg) (n = 1), warfarin (n = 1), and warfarin plus aspirin (n = 2), suggesting caution in adopting such regimens in those patients. Among 19 patients with persistent ExT ≥1500 × 10^9/L, 2 arterial events, 1 venous thrombotic event, and 6 major hemorrhagic events were recorded at follow-up. On univariate analysis for hemorrhage-free survival, age >60 years ($P = .03$), male sex ($P = .02$), leukocytosis ≥11 × 10^9/L ($P = .19$), and *JAK2* mutation ($P = .14$) emerged significant/near significant. The limited number of thrombotic events precluded analyses for thrombosis-free survival.

The presenting clinical features and vascular events for 24 low-risk patients presenting with ExT ≥1500 × 10^9/are provided in supplemental Table 2. Initial treatment details included observation alone (n = 5), aspirin alone (n = 5), cytoreductive therapy alone (n = 7), and aspirin plus cytoreduction (n = 7). At a median follow-up of 15.3 years, 2 arterial thrombotic events were documented; in both instances, cytoreductive therapy but not aspirin was ongoing at the time of event. A single venous thrombotic event was recorded postdiagnosis in a patient who was under observation. Of 12 patients initiated on aspirin at diagnosis, none experienced thrombosis while on therapy, and all 3 thrombotic events occurred in its absence, suggesting a protective effect of aspirin for both arterial and venous thrombosis. Two patients experienced major hemorrhage postdiagnosis; one was associated with acquired von Willebrand syndrome (ristocetin cofactor activity, 32%), in a patient with platelet count of 1520 × 10^9/L who was under observation. Meanwhile, none of the patients on aspirin experienced major hemorrhage postdiagnosis.

Table 1. Presenting clinical and laboratory characteristics of 710 patients with ET stratified according to presence or absence of ExT (≥1500 × 10^9/L) at diagnosis

Variable	All patients (N = 710)	Patients with platelet count ≥1500 × 10^9/L at diagnosis (n = 41)	Patients with platelet count <1500 × 10^9/L at diagnosis (n = 669)	*P*, univariate	*P*, age-adjusted	*P*, multivariate
Age, median (range), y	58 (18-90)	40 (19-86)	59 (18-90)	**<.0001**		**<.0001**
Age >60 y, N (%)	330 (46)	8 (20)	322 (48)	**.0004**		**<.0001**
Female, N (%)	450 (63)	30 (73)	420 (63)	.18		
Hemoglobin, median (range), g/dL	13.9 (11-16.3)	13.1 (11.1-15.6)	13.9 (11-16.3)	**.001**	**.001**	**.005**
Leukocyte count, median (range), 10^9/L	8.6 (3.5-28.1)	10.4 (4.3-28.1)	8.5 (3.5-27.1)	**.0003**	**.0002**	**<.0001**
Leukocyte count ≥11 × 10^9/L, N (%)	153/703 (22)	17/40 (43)	136/663 (21)	**.001**	**.001**	**<.0001**
Cardiovascular risk factors, n/N (%)	340/646 (53)	13/37 (35)	327/609 (54)	**.03**	.49	
Diabetes mellitus	61/648 (9)	2/38 (5)	59/610 (10)	.37		
Hypertension	275/649 (42)	7/38 (18)	268/611 (44)	**.002**	.1	
Smoking	151/642 (24)	10/36 (28)	141/606 (23)	.53		
Palpable splenomegaly, n/N (%)	85/705 (12)	8/40 (20)	77/665 (12)	.11		
Driver mutation status, N (%)						
JAK2V617F	427 (60)	14 (34)	413 (62)			
CALR	191 (27)	18 (44)	173 (26)	**.001**	**.03**	0.1
Type 1 *CALR*	61	5	56			
Type 2 *CALR*	47	10	37			
MPL	20 (3)	0 (0)	20 (3)			
Triple negative	72 (10)	9 (22)	63 (9)			
Next-generation sequencing, N (%)	n = 244	n = 14	n = 230			
SF3B1	6 (3)	0 (0)	6 (3)	.54		
SRSF2	7 (3)	0 (0)	7 (3)	.51		
U2AF1	2 (0.8)	1 (7)	1 (0.4)	**.01**	**.04**	.12
ASXL1	12 (5)	1 (7)	11 (5)	.69		
EZH2	3 (1)	1 (7)	2 (0.9)	**.04**	.08	
IDH1/2	2 (0.8)	0 (0)	2 (0.9)	.73		
TP53	4 (2)	1 (7)	3 (1)	.09		
Major thrombosis at or before diagnosis, N (%)						
Arterial thrombosis*	94/709 (13)	3/41 (7)	91/668 (14)	.25		
Venous thrombosis†	69/709 (10)	5/41 (12)	64/668 (10)	.58		
Major hemorrhage at or before diagnosis,‡ N (%)	32/692 (5)	6/40 (15)	26/652 (4)	**.001**	**.01**	**.02**
Microvascular symptoms,§ N (%)	156/663 (24)	9/40 (23)	147/623 (24)	.87		
Revised IPSET-thrombosis, N (%)	n = 709	n = 41	n = 668			
Very low	161 (23)	19 (46)	142 (21)	**.001**	.08	
Low	155 (22)	8 (20)	147 (22)			
Intermediate	83 (12)	5 (12)	78 (12)			
High	310 (44)	9 (22)	301 (45)			
Treatment instituted at diagnosis, N (%)						
Aspirin	317/400 (79)	22/36 (61)	300/372 (81)	**.01**		
Cytoreductive therapy‖	203/414 (49)	26/38 (68)	183/383 (48)	**.02**		

IPSET-thrombosis, International prognostic score for thrombosis in ET. *P* values in bold are statistically significant, defined as < 0.05.

*Major arterial thrombosis includes myocardial infarction, angina, cerebrovascular accidents, transient ischemic attack, peripheral arterial thrombosis, aortic thrombosis, mesenteric artery thrombosis, and central retinal thrombosis.

†Major venous thrombosis includes deep venous thrombosis, pulmonary embolism, portal/splenic/mesenteric/hepatic vein thrombosis, and cerebral sinus thrombosis.

‡Major hemorrhage includes bleeding events that require red cell transfusion support, resulted in ≥2 g/dL decline in hemoglobin, or involved critical organs.

§Microvascular symptoms include headaches, paresthesia, and erythromelalgia.

‖Cytoreductive therapies include hydroxyurea, anagrelide, and interferon.

Table 2. Details of 14 vascular events among patients with ET and platelet count ≥1500 × 10^9/L at diagnosis of ET

Event	Type of event	Thrombosis or hemorrhage before event	Age at event (y)/sex	Driver mutation	Revised IPSET-thrombosis at diagnosis	Platelet count/leukocyte count at event	CV risk factors	Therapy at the time of event		
								Cytoreduction	Aspirin	Anticoagulation
Arterial thrombosis										
#1	TIA	None	44/Male	*CALR* type 1	Very low	1520 × 10^9/L/ 7.7 × 10^9/L	None	Anagrelide	None	None
#2*	MI	None	55/Female	*CALR* type 2	Very low	NA	HTN	Hydroxyurea	None	None
#3†	CVA	Splenic venous thrombosis DVT	56/Female	*JAK2*	High	1100 × 10^9/L/ 7.9 × 10^9/L	None	Hydroxyurea	81 mg	None
#4	MI	CVA	57/Female	*JAK2*	High	585 × 10^9/L/ 12.7 × 10^9/L	HTN DM	Hydroxyurea	81 mg	None
#5‡	MI	None	87/Male	*JAK2*	High	1185 × 10^9/L/ 19.9 × 10^9/L	HTN	None	81 mg	None
Venous thrombosis										
#6	Portal vein thrombosis	None	50/Female	*CALR* type 2	Very low	299 × 10^9/L/ 7.4 × 10^9/L	None	None	None	None
#7†	PE	Splenic venous thrombosis Post splenectomy DVT CVA	63/Female	*JAK2*	High	565 × 10^9/L/ 8.1 × 10^9/L	None	Hydroxyurea	81 mg	None
Major hemorrhage							**Acquired vWD**			
#8	Lower extremity hematoma	None	46/Female	*CALR* type 2	Very low	1757 × 10^9/L/ 12.1 × 10^9/L	Ristocetin cofactor activity 32%	None	None	None
#9	GI	DVT	73/Female	*JAK2*	Low	489 × 10^9/L/ 4.1 × 10^9/L	NA	None	81 mg	Warfarin
#10*	Postoperative	MI	59/Female	*CALR* type 2	Very low	NA	NA	Hydroxyurea	None	None
#11†	Epistaxis	Splenic venous thrombosis DVT CVA PE	64/Female	*JAK2*	High	1134 × 10^9/L/ 11 × 10^9/L	NA	Hydroxyurea	325 mg	Warfarin
#12	GI	PE	54/Male	*JAK2*	High	NA	NA	None	None	Warfarin
#13	Lower extremity hematoma	None	92/Female	*JAK2*	High	162 × 10^9/L/ 8.8 × 10^9/L	NA	None	None	None

CV, cardiovascular; CVA, cerebrovascular accident; DM, diabetes mellitus; DVT, deep venous thrombosis; GI, gastrointestinal; HTN, hypertension; IPSET-thrombosis, International prognostic score for thrombosis in ET; MI, myocardial infarction; NA, not available; PE, pulmonary embolism; TIA, transient ischemic attack; vWD, acquired von Willebrand disease.

*Same patient experienced events 2 and 10.

†Same patient experienced events 3, 7, and 11.

‡Same patient experienced events 5 and 14.

The current study provides information regarding the phenotype and genotype of patients with ET presenting with ExT at diagnosis. The prospect of controlled studies for further clarification of treatment approach in ET patients with ExT is challenged by the very low incidence of informative cases. Regardless, the information from the current retrospective study is not inconsistent with our current practice of avoiding cytoreductive therapy in otherwise low-risk ET patients with ExT. The current findings require validation in prospective multicenter series.

Contribution: N.G. and A.T. designed the study, collected data, performed analysis, and co-wrote the paper; N.S., Y.J., and F.F. collected data; C.A.H. performed review of bone marrow biopsy samples; and K.B., M.A.E., A.P.W.-S., A.D.P., V.D.S., A.M.V., and T.B. contributed patients; and all authors reviewed and approved the final draft of the paper.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

ORCID profiles: N.S., 0000-0003-1499-6171; K.B., 0000-0003-2730-8593; A.P.W.-S., 0000-0003-1618-5049; V.D.S., 0000-0002-5178-5827; T.B., 0000-0003-2747-6327; A.M.V., 0000-0001-5755-0730.

Correspondence: Naseema Gangat, Division of Hematology, Department of Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 5590; e-mail: gangat.naseema@mayo.edu; and Ayalew Tefferi, Division of Hematology, Department of Medicine, Mayo Clinic, 200 First St SW, Rochester, MN 5590; e-mail: tefferi.ayalew@mayo.edu.

References

1. Barbui T, Tefferi A, Vannucchi AM, et al. Philadelphia chromosome-negative classical myeloproliferative neoplasms: revised management recommendations from European LeukemiaNet. *Leukemia.* 2018;32(5):1057-1069.

2. National Comprehensive Cancer Network. NCCN clinical practice guidelines in oncology, myeloproliferative neoplasms, Version 1.2022. Available at: https://www.nccn.org/professionals/physician_gls/pdf/mpn.pdf. Accessed 28 February 2022.

3. Koren-Michowitz M, Lavi N, Ellis MH, Vannucchi AM, Mesa R, Harrison CN. Management of extreme thrombocytosis in myeloproliferative neoplasms: an international physician survey. *Ann Hematol.* 2017;96(1):87-92.

4. Kuykendall AT, Komrokji R. What's in a number? Examining the prognostic and predictive importance of platelet count in patients with essential thrombocythemia. *J Natl Compr Canc Netw.* 2020; 18(9):1279-1284.

5. Campbell PJ, MacLean C, Beer PA, et al. Correlation of blood counts with vascular complications in essential thrombocythemia: analysis of the prospective PT1 cohort. *Blood.* 2012;120(7):1409-1411.

6. Finazzi G, Carobbio A, Thiele J, et al. Incidence and risk factors for bleeding in 1104 patients with essential thrombocythemia or prefibrotic myelofibrosis diagnosed according to the 2008 WHO criteria. *Leukemia.* 2012;26(4):716-719.

7. Alvarez-Larrán A, Cervantes F, Pereira A, et al. Observation versus antiplatelet therapy as primary prophylaxis for thrombosis in low-risk essential thrombocythemia. *Blood.* 2010;116(8):1205-1210, quiz 1387.

8. Alvarez-Larrán A, Pereira A, Guglielmelli P, et al. Antiplatelet therapy versus observation in low-risk essential thrombocythemia with a CALR mutation. *Haematologica.* 2016;101(8):926-931.

9. Carobbio A, Thiele J, Passamonti F, et al. Risk factors for arterial and venous thrombosis in WHO-defined essential thrombocythemia: an international study of 891 patients. *Blood.* 2011;117(22): 5857-5859.

10. Gangat N, Szuber N, Jawaid T, Hanson CA, Pardanani A, Tefferi A. Young platelet millionaires with essential thrombocythemia. *Am J Hematol.* 2021;96(4):E93-E95.

11. Tefferi A, Szuber N, Pardanani A, et al. Extreme thrombocytosis in low-risk essential thrombocythemia: retrospective review of vascular events and treatment strategies. *Am J Hematol.* 2021;96(6): E182-E184.

12. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood.* 2016;127(20):2391-2405.

13. Barbui T, Thiele J, Passamonti F, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. *J Clin Oncol.* 2011;29(23):3179-3184.

14. Cortelazzo S, Finazzi G, Ruggeri M, et al. Hydroxyurea for patients with essential thrombocythemia and a high risk of thrombosis. *N Engl J Med.* 1995;332(17):1132-1136.

15. Barbui T, Vannucchi AM, Buxhofer-Ausch V, et al. Practice-relevant revision of IPSET-thrombosis based on 1019 patients with WHO-defined essential thrombocythemia. *Blood Cancer J.* 2015;5(11): e369.