



# An underestimated blood parameter before myeloproliferative neoplasm diagnosis: the case of thrombocytosis and the delayed essential thrombocythemia diagnosis

**Background:** Thrombocytosis, i.e. platelet count  $>400.000/\mu\text{L}$  in peripheral blood, is also due to Essential Thrombocythemia (ET), and delaying the diagnosis of thrombocytosis might coincide with a delayed ET diagnosis.

The purpose of our study was to investigate the factors virtually leading to a delay in ET diagnosis.

**Methods:** We analyzed the interval between the first evidence of thrombocytosis and ET diagnosis, and defined it as ET-underestimation-period (ET-uP). We analyzed 280 ET patients followed at our Institution. To verify possible correlations with ET-uP, we investigated patients' ages, gender, history of vascular complications, thrombocytosis, splenomegaly, and marrow fibrosis degree. We also considered their General Practitioners (GP) characteristics: age, personal computer use, number of patient visits in the two years before diagnosis.

Univariate logistic analysis for each parameter was performed using the Spearman's rank correlation coefficient to assess correlation with ET-uP. Multivariate logistic regression analysis was performed on the parameters that resulted significant at the previous analyses. The logistic regression analysis was performed dichotomizing the ET-uP variable using its median value.

**Results:** Median ET-uP was 16 months (range 1-240). The analysis showed that a shorter ET-uP correlated with thrombocytosis ( $p<0.001$ ) and previous cardiovascular events ( $p=0.04$ ). At multivariate analysis, the Area Under the Curve (AUC) of the (Receiver Operating Characteristic) ROC curve was 0.72. No correlation was found analyzing GP data.

**Conclusion:** Our results underline a deficient medical practice, and prompt for corrective actions (use of more restrictive normal range for platelet count—with lower upper limit and warning flags for pathological findings) to timely commence the diagnostic workup of this condition.

**KEYWORDS:** thrombocytosis, platelet count, essential thrombocythemia, general practitioner

## Introduction

In clinical practice, the normal range of platelet count is considered to be between 150.000 and 450.000 thrombocytes per  $\mu\text{L}$  [1]. However, this upper limit is currently being reconsidered following the development of myeloproliferative molecular markers able to refer even mild thrombocytosis (i.e. 400.000/L) to a bone marrow disease [2-8]. Indeed, a notable study carried out on a healthy Italian population reported as upper limit 400.000 and 360.000 thrombocytes per  $\mu\text{L}$  for women and men, respectively [9].

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder [10-13]. It is a clonal pathology of unknown etiology deriving

from the transformation of a pluripotent progenitor cell. ET is characterized by persistent overproduction of platelets ( $>450.000/\mu\text{L}$ ) without marrow fibrosis or equal amounts of other hematopoietic lines [14,15]. As many as 50 to 70% of thrombocytosis diagnoses are occasional findings in patients undergoing blood tests for other conditions or routine checkups. Furthermore, in many cases the diagnosis is made during medical tests following a vascular accident [16,17] and, in our experience, thrombocytosis is already present in the patient's past history even before the event has occurred. Only then is thrombocytosis eventually taken into account.

Due to the wealth of data on the evidence of

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pathological platelet count left uninvestigated in a patient's past history, we analyzed the factors virtually leading to a delay in the diagnosis of ET.

## Materials and methods

The study population comprised 280 consecutive patients diagnosed with ET from January 2000 to June 2015, and referred to the Hematology Unit of the Department of Clinical Medicine and Surgery of the Federico II Medical School in Naples. All patients gave their written informed consent to participate in the study.

ET was diagnosed according to the 2008 WHO criteria [18]. For patients diagnosed before this date, we ascertained that the investigations were nevertheless in line with those standards. The presence of familial thrombocytosis was ruled out. Spleen volume was evaluated by ultrasonography scans [19] at diagnosis in 274/280 patients. Inclusion criteria were normal red cell morphology, absence of blood cell precursors, and CD34 positive cells in peripheral blood within normal ranges. Bone marrow biopsy specimens were available for 229/280 patients. Marrow fibrosis (MF) [20] was absent in 136 and described as grade 0 MF [20] in 93 of them. We did not consider these

data as a limit because our main aim was to understand the reason for the underestimation of thrombocytosis, which can be found in both cases. Patient characteristics were age, gender, history of vascular events, thrombocytosis grade, splenomegaly and bone marrow fibrosis [TABLE 1]. We also collected data regarding the patients' General Practitioners (GP), namely age, checkups (number/year) and Personal Computer (PC) use, by interviewing 249/280 patients. The rationale for the choice of each variable is reported in TABLE 1.

For each patient we identified the time interval between the first finding of high platelet count and ET diagnosis, defining this interval as underestimation period, and this condition as Essential Thrombocythemia underestimation period (ET-uP).

The first finding of high platelet count was obtained through blood count data recorded in the patient's past history, in the absence of any active and concomitant co-morbidity. Furthermore, data on past history were also used to exclude intercurrent pathological events that may have caused thrombocytosis. Furthermore we looked for correlations between patients and GP characteristics, and ET-uP.

**Table 1. Baseline patient characteristics at the time of Essential Thrombocythemia diagnosis.**

Characteristics	No.
Number of patients	280
Male/female	125/155
Age year median (range)	53 (16-95)
Platelets count ( $\times 10^9/L$ ) median (range)	543 (400-2065)
Splenomegaly	162/274 (59%)
Patients with CV event(s)	52 (18.6%)
Reticulin fibre score (20) analysis	229
Absence of fibrosis	136 (59.3%)
MF(20)=0	93 (40.6%)
JAK2v617f mutated/wild type	174/280 (62%)
Presence of CV risk factors	207 (74%)
Overall no. of previous CV events	58 (20.7%)
Ongoing antiaggregant therapy	58 (20.7%)

### Patient and General Practitioner (GP) variables.

		Rationale for the choice of the variable
Patient	Age	Number of visits in relation to age and comorbidity
	Gender	Trend to more frequent visits during pregnancies, or for iron deficiency related to menses
	Previous vascular event	Greater attention to health care, with more frequent blood testing
	Thrombocytosis grade	Greater doctor alert to health condition
	Splenomegaly	Greater attention to health care, with more frequent blood testing
	Bone marrow fibrosis	First diagnosis of thrombocytosis and possible ET reticulum fiber score progression
	Age	Different self-updating modality
GP	PC use	Possible automated warning flags
	Number of visit per year	Greater likelihood of detecting thrombocytosis

Data on the JAK2 mutant allele burden, presence of CAL-R mutation and MPL are not available for this cohort of patients. In any case, thrombocytosis is the common denominator in all these molecular alterations.

## Statistical analysis

Categorical variables were expressed as percentages; median and range were used to describe all continuous variables. Patient- and GP-related variables were examined according to ET-uP by univariate statistical methods. Univariate logistic analysis for each parameter was performed using the Spearman's rank correlation (Rs) coefficient to assess correlation with ET-uP.

Multivariate logistic regression analysis was performed on the parameters that resulted significant at the previous analyses. In order to perform logistic regression, the ET-uP variable was dichotomized using its median value.

The logistic regression model is:

$$P(x_i) = \frac{e^{g(x_i)}}{1 + e^{g(x_i)}}, i = 1, \dots, m \quad (1)$$

With,

$$g_i(x) = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \dots + \beta_n x_{in} \quad (2)$$

Where m is the number of patients,  $x_1, x_2, \dots, x_n$  represent different input variables and  $\beta_0$ ,

$\beta_1, \dots, \beta_n$  the corresponding regression coefficients. For the selection of variables, a stepwise method (Wald-Enter method,  $p=0.05$  for entry into the model and  $p=0.10$  for removal from the model) was performed. Model performance was evaluated by the area under the receiver operating characteristic (ROC) curve (AUC). Statistical analysis was performed with SPSS 18.0.

## Results

Median ET-uP was 16 months (range: 1-240). Results of univariate analysis for the patient variables analyzed are reported in **TABLE 2**. The only variables that resulted significantly correlated with ET-uP were degree of thrombocytosis and a history of cardiovascular (CV) complications. Both variables were inversely correlated with ET-uP [**TABLE 2, FIGURE 1 and 2**], with an increase in the former being associated to a reduced underestimation time of thrombocytosis. None of the GPs' variables was correlated with ET-uP. Applying multivariate logistic regression with a dichotomized ET-uP to a 16-month median value (0 if ET-uP<16, 1 otherwise), both thrombocytemia and history of cardiovascular complications resulted to be independent predictors of the underestimation period (estimated coefficient for CV events: -0.682, Odds Ratio (OR) 0.506  $p=0.042$ ; estimated coefficient for degree of thrombocytosis: -0.418,

**Table 2. Patient characteristics and correlation coefficient (Rs) with ET-up.**

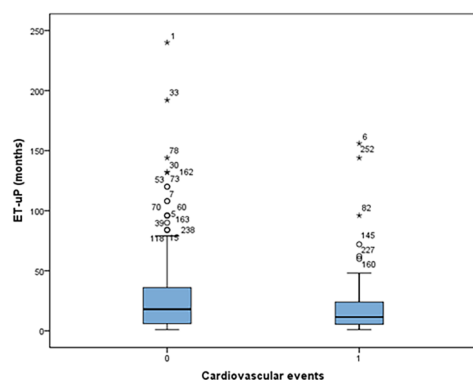
		Univariate analysis	
Characteristics		Rs	p-value
Continuous variables	Median (range)		
Age (years)	53 (16-95)	0.94	0.12
Platelets (10^5)	5.43 (4.00-20.65)	-0.432	<0.001
Categorical variables	N(%)		
Gender			
Female	155 (55.4)	0.054	0.376
Male	125 (44.6)		
Splenomegaly (n=274)			
Yes	162 (59.1)	-0.17	0.779
No	112 (40.9)		
Bone marrow fibrosis <sup>(18)</sup> (n=229)			
Absent	136 (59.4)	-0.69	0.308
Early MF	92 (40.6)		
Cardiovascular risk factor			
Yes	207 (73.9)	-0.38	5.30
No	73 (26.1)		
History of cardiovascular events	273		
Yes	52 (19.0)	-0.122	0.044
No	221 (81.0)		
Ongoing antiaggregating therapy			
Yes	125 (44.6)	-0.150	0.410
No	155 (55.3)		

OR 0.659,  $p < 0.001$ ). The resulting model exhibited a good predictive performance, being characterized by an AUC=0.72 (95% Confidence Interval (CI) 0.66-0.78) [FIGURE 3]. In FIGURE 4, the probability of having an ET-uP greater than 16 months as a function of platelet count for patients with or without a history of cardiovascular events is plotted.

We failed to find any correlation between ET-uP and GP characteristics, (age:  $p=0.3$ , PC use:  $p=0.7$ , number of checkups in the two years prior to diagnosis:  $p=0.7$ ). Nevertheless, it is reasonable to underline the finding of an inadequate number of checkups, including physical examinations per year, and the surprising amount of data on the number of examinations per year in non splenomegalic patients compared to splenomegalic ones: 4 (range, 0-20) vs. 2 (range, 0-14).

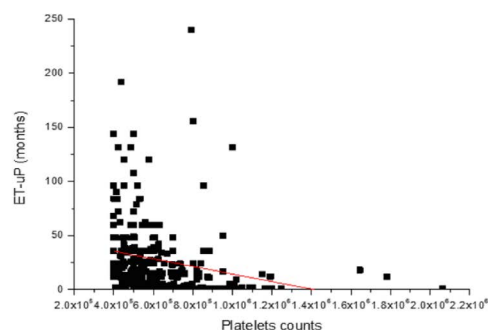
## Discussion

The parameters employed in our study show

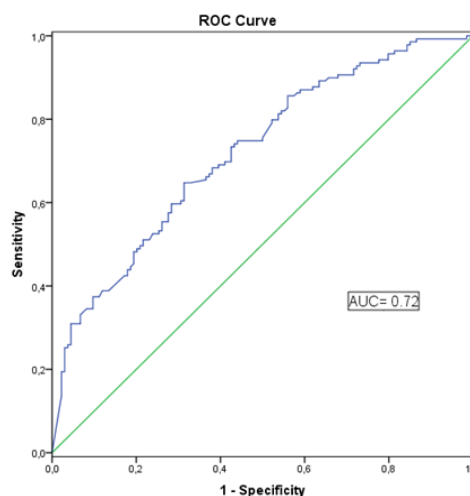


**FIGURE 1. Essential Thrombocythemia underestimation period (ET-uP) and history of cardiovascular (CV) events. Comparison of ET-uP in patients with a history of cardiovascular events (1) and patients without (0).**

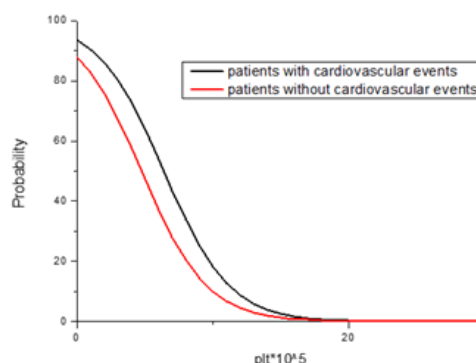
**Patients with CV events: 52. Median ET-uP: 11.5 months (range: 1-156)**



**FIGURE 2. Essential Thrombocythemia underestimation period and thrombocytosis. In red, fitted linear regression model.**



**FIGURE 3. ROC curve of the logistic regression model including as predictive variables thrombocytopenia (platelet counts) and history of cardiovascular complications. The AUC= 0.72 indicates a good predictive performance for ET-uP model prediction.**



**FIGURE 4. Probability of ET-uP > 16 months as a function of platelets counts for patients with a history of cardiovascular events and patients without.**

a statistically significant correlation between ET-uP and thrombocytosis level, and a history of cardiovascular complications.

In our population sample, fifty-two of the 280 patients (18.6%) had experienced mild to severe thrombo-hemorrhagic episode(s) before the diagnosis. In this sub-group, the median ET-uP was 11.5 months (range 1-156). These events cannot be exclusively ascribed to thrombocytosis, as 74% of patients had other cardiovascular risk factors (hypertension, diabetes, smoking status, overweight/obesity) – although thrombocytosis may have played a synergistic role.

To the best of our knowledge, this is the first study analyzing the underestimation period for ET diagnosis in such a large cohort of patients and in a multivariate fashion.

There are two main limitations of our study.

First, it is impossible to discover the real time of the first increase in platelet count, since in common clinical practice not all people perform blood counts every six months or annually and, furthermore, not all people, conventionally, retain old laboratory exams. The first evidence of thrombocytosis could represent a good surrogate of ET insurgence. Secondly, this is a retrospective study, with all the related limitations.

Similar parameters, analyzed in our study, and in particular the presence of cardiovascular risk factors, have been recently employed to study the underestimation of myeloproliferative neoplasms (MPNs) [21]. The multicenter study by Enblom et al. focused on the long under-diagnosis period of MPNs, the possible factors influencing this condition, as well as the possible consequences – such as any cardiovascular episodes. In this study, as many as 272 of the 612 MPNs patients were ET. The authors stressed the concept that an MPNs should always be suspected in patients with unexplained elevated hematocrit, leukocyte and/or platelets counts. At variance, our study analyzed the timing of diagnosis for 280 ET patients. The multivariate analysis was performed using as ideal predictive model either one or two variables, including thrombocytosis and a history of cardiovascular complications. A shorter ET-uP was determined by the degree of thrombocytosis and a past history of cardiovascular complications. The AUC of the ROC curve representing the logistic regression model was 0.72, tending to a statistically significant value.

There is still insufficient knowledge of the factors influencing ET-uP. It seems nevertheless clear how a delay in identifying the altered platelet count could influence the possible development of thrombotic and hemorrhagic episodes during the misdiagnosis period, increasing the patient's risk and thus representing the main cause of morbidity in ET patients [21,22].

Detecting and assessing thrombocytosis is undoubtedly clinically relevant. Consistently with ET Guidelines [22], patients without specific risk factors need only to be kept under observation; they nevertheless undergo additional follow-up visits, paying careful attention to possible ensuing comorbidities.

As additional aim, we analyzed the “General Practitioner's side” to identify possible factors influencing ET-uP; for this purpose, we

considered GP's age, computer use and number of visits the participants to our study had undergone.

Although no relationship was found with the parameters considered, the data collected indicate a relationship between a small number of examinations and a frequent underestimation of splenomegaly and platelet count. Conversely, GP's age and PC usage were found to be irrelevant.

We can only speculate that the underestimation may be related to: (i) little attention to the normal value ranges for platelet count; (ii) frequent asymptomatic thrombocythemia, at variance with thrombocytopenia, (iii) use of anti-aggregant therapy, possibly leading to an improper attention to platelet count.

It is worth underlining that the delayed diagnosis is not due to long waiting lists. In fact, in compliance with the regulations of the Health System of the Campania Region, when a GP or any other accredited physician comes across altered hematological values, referral to a specialist occurs within 2-3 weeks [23]. Nonetheless, as the interval in our case series was even shorter, the delay was irrelevant in relation to ET-uP.

The frequent evidence of long ET-uP deserves attention, as it suggests the need for a timely diagnosis and a more thorough diagnostic workup of this condition. Hematologist should prompt for the use of a more restrictive range for platelets count – especially for the upper limit – and/or a more evident warning flag, especially in relation to age and gender, as a tool of alert.

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## Partnership

VM designed the research study; VM, NP, IC, IS, LM, LS, GC collected clinical data; MP contributed to a diagnostic tool to determine spleen volume; LC analyzed the data; FP supervised the paper; VM and LC wrote the paper.

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## Conflict of Interest

The authors have no conflict of interest to declare.



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