BONE MARROW HISTOLOGY FOR THE DIAGNOSIS OF ESSENTIAL THROMBOCYTHEMIA IN CHILDREN: A MULTI-CENTER ITALIAN STUDY

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Essential thrombocythemia (ET) is a myeloproliferative neoplasm (MPN) mainly affecting middle age patients. While pediatric cases occur, they are rare **and their** molecular features considerably differ from the adult counterparts: *JAK2*V617F mutation occurs in only 25% of cases (1), *CALR* mutations are found in less than 10% of patients (2) and *MPL*W515L mutation is anecdotal (3). Overall, less than 40% of children with unexplained, long-lasting thrombocytosis have clonal marker **of ET** (2).

Following the release of the 2001 WHO classification (4), bone marrow (BM) evaluation has become a cornerstone of ET diagnosis. However, the majority of studies have focused on adults, and little is known about the role of BM biopsy in pediatric ET. In fact, BM biopsy is seldom performed in children with a clinical picture of ET, due to the invasiveness of the procedure. The main objective of this study is to explore the relevance of BM histology in children with high platelet counts in order to identify possible differences in: (i) primary *vs* reactive/secondary thrombocytosis (PedST) of childhood; and (ii) pediatric (PedET) *vs* adult (AdET) cases of ET.

Treatment-naïve diagnostic BM samples were collected from 21 pediatric patients clinically diagnosed as ET according to the 2008 WHO diagnostic criteria in seven Italian **Pediatric** Centers (2011 - 2016). All cases were reviewed (separately and in joint sessions) by two hematopathologists (MP, ES) who were blind to any clinical and/or molecular information. Six BM samples of PedST were used as controls whereby 5 had lymphoma and 1 prolonged spontaneously remitted thrombocytosis. The histological features were compared to those of 36 consecutive AdET, which were strictly diagnosed according to the 2008 WHO criteria and enrolled during the same time period of children. Statistical analyses were performed on data recorded at the time of diagnosis. The study was approved by the local Ethics Committee.

Clinically, PedET was characterized by higher median platelet counts than AdET, (PedET: 1251 x10^9/L; AdET: 502 x 10^9/L), more frequent splenomegaly (PedET: 14/21 cases [70.0%]; AdET: 7/36 cases [13.4%]) and abdominal pain (PedET: 4/21 cases [19.0%]; AdET: 0/36 cases) ($p<0.001$). PedET differed from PedST in key histological parameters (Figure 1: A-B). PedET showed higher megakaryocyte (MK) density (5) (37.5 MK/mm^2; vs 9.2 MK/mm^2; $p<0.001$), loose MK clusters (21/21=100%) and occasional grade-1 reticulin fibrosis (6/21=28.5%), which **was never** documented in PedST (Table 1) (6). Thorough morphological and immunohistochemical evaluation showed similar features in PedET and AdET, despite higher BM cellularity (as commonly seen in children [7]) and higher MK density were reported **in the pediatric group**. **Such increase** in MK density in children was due to higher cellularity values (i.e. the differences in MK density were not

statistically significant after adjusting for cellularity). PedET was also histologically analyzed according to the patients' age and the mutational status, although no differences were found. Histological re-evaluation also identified cases with morphological features suggesting an MPN other than ET. Among the 6 *JAK2*V617F-mutated cases, one showed histological features of polycythemia vera (PV) and another of prefibrotic early primary myelofibrosis (pre-PMF). The remaining 3 mutated cases exhibited a BM picture consistent with ET. Re-evaluation of the 12 **triple-negative** (3NEG) cases revealed features consistent with ET in 9 cases, 2 cases were compatible with pre-PMF (Figure 1 A-C), and one had characteristics of ST.

These results provide insight on the complex scenario of high platelets count in childhood. Thrombocytosis is indeed a common finding in children (8). Most cases are secondary/reactive forms, which spontaneously normalize over time. Rare hereditary thrombocytosis (HT) has also been documented (9). Primary thrombocytosis is extremely rare, with an estimated incidence of approximately 1 per 10 million annually (10).

The differential diagnosis of pediatric thrombocytosis may be challenging in clinical practice and, unlike in adults, molecular biology is of limited value. Children with suspected ET have indeed low rates of driver mutations (2,3,11,12), with lower allele burden than adults (13). Consequently, molecular studies cannot definitively identify the nature of several putative pediatric ET cases. Histological evaluation may prove of greater value, but little has been reported in the literature so far. The only few available studies have either examined single cases or small series of pediatric ET and have reported variable results (14,15). Moreover, another large study about pediatric ET did not specifically address BM importance (16).

Our study is seemingly the largest published study on BM histology in pediatric patients with clinically diagnosed ET to date. Among 21 children, 20 cases had BM findings consistent with MPN (ET n=16; PV n=1; pre-PMF n= 3) and one 3NEG case had a histological picture of ST. The findings of histologically-confirmed ET were distinct from those of PedST, and are thus consistent with the data reported by Thiele *et al* (17) in the adults. Likewise, the BM findings of **PedET** were similar to those of **AdET**, irrespective of the mutational status. Furthermore, the inter-pathologists agreement regarding the final diagnosis and the assessment of each histological parameter was excellent (Kappa index >0.80).

Histological re-evaluation has demonstrated occasional discrepancies between the original clinical diagnosis and the morphologically-integrated one. Critical re-evaluation of the diverging cases reveals the importance of BM evaluation in putative cases of pediatric ET. In particular, one

*JAK2*V617F-mutated case could have been a masked PV (18). The clinical history of this patient revealed transient increases in hemoglobin and hematocrit levels (> 95th percentile for age) and transient ischemic attacks (TIA). TIA occurred in another *JAK2*-mutated ET girl. Similarly, one girl with *JAK2*V617F-mutated MPN (originally interpreted as ET) had a histological picture of pre-PMF. Her clinical history reports Budd-Chiari syndrome during infancy with hepato-pulmonary syndrome, portal thrombosis and progressive splenomegaly (a clinical picture of suspected PMF). The 3NEG cases highlight the importance of BM biopsy for accurate diagnosis, in that 11 out of 12 cases were consistent with an MPN. In particular, two 3NEG cases presented a pre-PMF-like BM picture, supporting the idea that PMF can rarely occur in pediatric patients (19). In the remaining 9 3NEG children with both clinical and histological features of ET, very low mutant allele burdens (20,21) and/or unusual MPN-associated mutations (22) might be present. Of note, histology was consistent with ST in one case, suggesting that a subset of 3NEG ET are indeed mis-diagnosed ST (23). We have recently observed 2 cases of putative 3NEG ET (BM not available for histologic evaluation), who spontaneously achieved hematological remission after 15 years of sustained thrombocytosis. All of these cases illustrate the importance of BM evaluation, possibly in tertiary centers, for diagnosing pediatric MPN (24).

In conclusion, the data presented in this study clearly show that BM evaluation is pivotal for the ET diagnosis among the pediatric population as in adults. BM assessment proves particularly helpful in the differential diagnosis between ET and its clinical mimickers (i.e. PMF, PV and ST) **and should be part of the diagnostic workup of children with long-lasting unexplained thrombocytosis, together with several other clinical, laboratory and molecular parameters.**

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Table 1: Main features in pediatric and adult thrombocytosis.

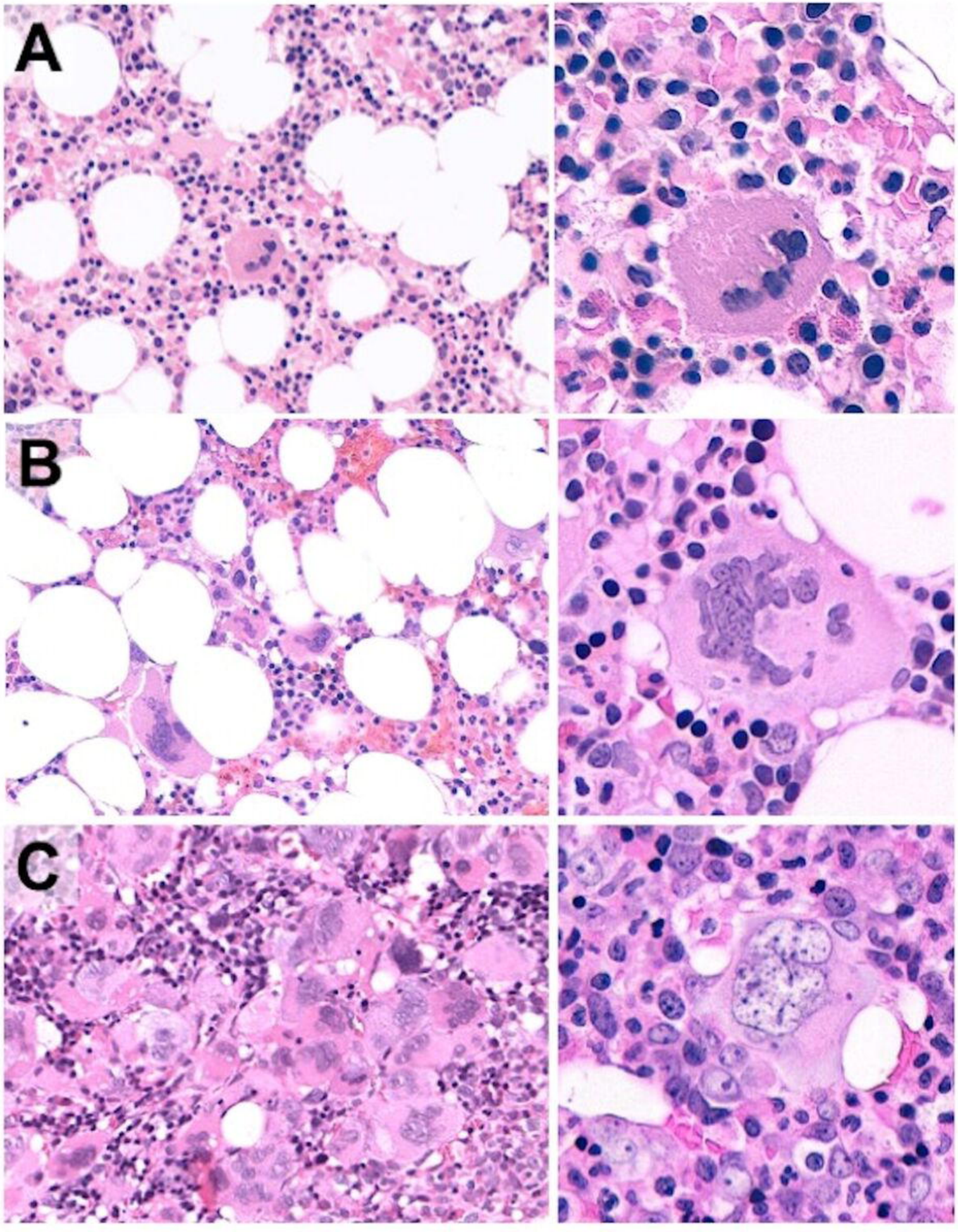
	PedET (n=21)	PedST (n=6)	AdET (n=36)
Age (Y) median (range)	10 (1-16)	7.8 (2-5)	51.8 (30-80)
Sex (M/F)	14/7	2/4	10/26
JAK2V617F CALR (Type 1 / 2) MPLW515L 3NEG	6 2 (1/1) 1 12	NA	14 9 (4/5) 2 11
Cellularity, % Median (range)	80 (55 – 95)	80 (50-80)	55 (20 – 80)
MKD, MK/mm^2 Median (range)	37.5 (10– 107)	9.2 (6-14)	18.3 (9 – 55)
MKD/BM cellularity Median (range)	46.6 (13-134)	15.6 (11-19	45.8 (15-105)
Presence of MK loose clusters, n (%)	21 (100)	0	36 (100)
Presence of MK dense clusters, n (%)	3 (14)	0	0
Presence of BM reticulin-fibrosis, n (%)	6 (28.5)*	0	6 (16.7)

* Two of these cases had histological features consistent with early PMF, 1 patient with masked PV and 3 with ET.

Note: PedET = pediatric essential thrombocythemia, PedST= pediatric secondary thrombocytosis, AdET= adult essential thrombocythemia, MK= Megakaryocyte, MKD= megakaryocytes density, BM= bone marrow, **3NEG= triple-negative**

Legend to the figure

Figure 1. Representative histological features of pediatric cases with: A Essential Thrombocythemia, B Secondary Thrombocythosis, and C early Primary Myelofibrosis (pre-fibrotic stage)
A. loose clusters of megakaryocytes with hyper-segmented (staghorn-like) nuclei **B.** normocellular marrow with scattered non-descript megakaryocytes **C.** tight clusters of atypical megakaryocytes with bulbous (cloud-like) nuclei. (H&E stain, original magnification: 10x; 40x).


A
B
C