bjh research paper

Are all cases of paediatric essential thrombocythaemia really myeloproliferative neoplasms? Analysis of a large cohort

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Summary

Sporadic essential thrombocythaemia (ET) is rare in paediatrics, and the diagnostic and clinical approach to paediatric cases cannot be simply copied from experience with adults. Here, we assessed 89 children with a clinical diagnosis of ET and found that 23 patients (25·8%) had a clonal disease. The *JAK2* V617F mutation was identified in 14 children, 1 child had the *MPL* W515L mutation, and 6 had *CALR* mutations. The monoclonal X-chromosome inactivation pattern was seen in six patients (two with *JAK2* V617F and two with *CALR* mutations). The other 66 patients (74·2%) had persistent thrombocytosis with no clonality. There were no clinical or haematological differences between the clonal and non-clonal patients. The relative proportion of ET-specific mutations in the clonal children was much the same as in adults. The higher prevalence of non-clonal cases suggests that some patients may not have myeloproliferative neoplasms, with significant implications for their treatment.

Keywords: essential thrombocythaemia, paediatric, myeloproliferative neoplasm, JAK2, *CALR*.

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Myeloproliferative neoplasms (MPN) are clonal diseases that usually occur in middle or advanced age and are extremely rare in paediatric patients. Essential thrombocythaemia (ET) is the MPN most commonly seen in children. Overall, about 90% of adults with ET present with markers of clonal origin (Tefferi *et al*, 2014a). An acquired *JAK2 V*617F mutation is the main molecular marker of ET (identified in 50–60% of patients) and 3–5% of ET patients carry acquired mutations in the thrombopoietin receptor gene (*MPL*) (Tefferi *et al*, 2014a). Somatic insertions or deletions in exon 9 of the calreticulin gene (*CALR)* have recently been described in about one in four adults with ET (Klampfl *et al*, 2013; Nangalia *et al*, 2013). *CALR* mutations are mutually exclusive with mutations in either *JAK2* or *MPL* (Klampfl *et al*, 2013). Unlike adults, the majority of children with ET do not reveal clonal markers, such as *JAK2* V617F or *MPL* W515L mutations. Children with ET seem to have different biological characteristics from adult cases, but published data are inconclusive (Fu *et al*, 2013). Familial ET is responsible in many paediatric cases, as seen in a large Italian series (Giona *et al*, 2012). The World Health Organization (WHO) diagnostic criteria for ET (Vardiman *et al*, 2009) therefore do not seem appropriate for children (Teofili *et al*, 2007).

In this study, we report our experience of a large cohort of paediatric patients with sustained high platelet counts, clinically diagnosed with ET, who underwent biological assessment for clonal proliferative status, including *CALR* gene analysis.

Methods

The children with a clinical diagnosis of ET established at any of 14 tertiary haematology centres linked to the Italian Paediatric Haemato-Oncology Association (AIEOP) were consecutively referred to a central laboratory in Padua for biological studies. The present series also included 20 patients already described in a previous report (Randi *et al*, 2006) and 1 case recently published elsewhere (Farruggia *et al*, 2013). All of the children had a sustained increase in platelet count ($>450 \times 10^9$/l) lasting at least 12 months, with no demonstrable reactive or secondary cause, and no family history of MPN or thrombocytosis. None of the children met the WHO criteria for other myeloid neoplasms. Germline *MPL* mutations were studied extensively nonetheless, and one child from a family with the *MPL* S505A mutation was excluded from the present report. Bone marrow histology was consistent with a diagnosis of ET in all the cases tested (49 = 55%), while all the other children only had cytology on bone marrow aspirates due to the need for sedation to perform marrow biopsy in younger children.

Based on European Leukaemia Net recommendations (Barbui *et al*, 2011), in the absence of sufficient data to recommend any treatment, paediatricians individually tailored the treatment to each patient.

The Ethical Committee of Padua Hospital approved the study and informed consent was obtained according to the Helsinki Declaration.

The *JAK2* V617F mutation was genotyped using allele-specific polymerase chain reaction (PCR) in DNA extracted from isolated granulocytes (Randi *et al*, 2006) and the mutant allele burden was measured by quantitative real time-PCR (Teofili *et al*, 2009). The *MPL* (Randi *et al*, 2004; Beer *et al*, 2008) and *CALR* (Klampfl *et al*, 2013) mutations were sought by direct sequencing. The X-chromosome inactivation pattern (X-CIP) in granulocytes was studied in females using the human androgen receptor gene (*AR*, also termed HUMARA) polymorphism method, as described elsewhere (Randi *et al*, 2006).

Differences in the distributions of continuous variables between categories were analysed with the Mann–Whitney test. P values $< 0{\cdot}05$ were considered statistically significant.

Results

We report on 89 subjects (58 females and 31 males) who were under 18 years old at the time of their diagnosis (median age 8·7 years, range 6 months to 16·5 years) with a median follow-up of 6·3 years (range 1–19 years). The clinical findings and the treatments administered are summarized in Table I.

The *JAK2* V617F mutation was found in 14 children (15·7%), with an allele burden of 26·26 ± 9·78%, the *MPL* W515L mutation in 1 and *CALR* mutations in 6 (8%). Four patients had deletions (DEL 52 bp p.L367, type 1) and two had insertions (INS TTGTC p.k385, type 2) that have already been described in adults (Klampfl *et al*, 2013). None of the patients had more than one mutation. On the whole, 68 children (76·4%) were triple wild-type (triple-WT) cases. Clonality assays were available for 21 girls: 6 (28·5%) were found monoclonal, and 4 of them carried *JAK2* V617F or *CALR* mutations, while 2 were triple-WT.

Three different age groups were analysed: younger children (less than 11 years old), young adolescents (up to 15 years old) and older adolescents (15–18 years old). No differences emerged between these three groups in terms of the distribution of clonal *versus* non-clonal cases (Table I).

Three children presented with major thromboses (two Budd–Chiari syndromes and one cerebral vein thrombosis) and they all carried the *JAK2* V617F mutation. Minor haemorrhages occurred in one child with the *JAK2* V617F mutation and two with the *CALR* mutation. Three *JAK2*-mutated and four *CALR*-mutated patients and the one with the *MPL* mutation all had microvascular symptoms, mainly headache (Table II).

Data on the treatments administered were available for 19 clonal and 51 non-clonal cases. Antiplatelet and anticoagulant drugs were commonly used in combination with cytoreductive treatments. In the clonal group, 3 children (15·8%)

Table I. Main clinical findings in children with thrombocytosis.

	Clonal	Non-clonal triple-negative	*P*
Patients, *n* (%)	23 (25·8)	66 (74·2)	
Males/females	8/15	23/43	NS
Median age (range), years	10·25 (0·5–17·5)	8·3 (0·5–16·5)	NS
Age cut-off, *n*			
<11 years	14	46	NS
<15 years	20	59	
15–18 years	3	7	
Median follow-up (range), years	7·55 (1–11·5)	6·14 (1–20)	NS
JAK2 V617F	14	0	
Allele burden mean ± SD (%)	26·26 ± 9·78	–	
MPL W515L (56 patients tested)	1	0	
CALR mutations (74 patients tested)	6	0	
X-CIP positive (23 females tested)	6 (2 *JAK2* V617F; 2 *CALR*)	0	
Major thrombotic events	3 (13%)	0 (0%)	0·03
	2 Budd–Chiari syndrome		
	1 cerebral vein thrombosis		
Minor bleeding episodes	3 (13%)	5 (7·5%)	NS
Patients with microvascular disturbances	8 (34·7%)	19 (28·8%)	NS
Headache	5	18	
Paraesthesia	2	7	
Erythromelalgia	2	1	
Splenomegaly	9 (39·1%)	17 (25·7%)	NS
Median platelet count (range), $\times 10^9$/l	1192 (512–3200)	1239 (503–4440)	NS
Median WBC count (range), $\times 10^9$/l	8·94 (5·5–11·8)	9·02 (5·2–19)	NS
Mean Hb level (range), g/l	138 (105–160)	125 (80–148)	NS
Bone marrow cytology alone, *n* (%)	8 (34·8)	32 (48·5)	NS
Bone marrow histopathology, *n* (%)	15 (65·2)	34 (51·5)	NS
Treatments administered			
Patients with available data	19	51	NS
No treatment	3 (15·8%)	12 (23·5%)	
Aspirin	11 (57·9%)	32 (62·7%)	
LMWH/Warfarin	3 (15·8%)	0 (0%)	
Cytoreductive drugs	13 (68·4%)	20 (39·2%)	
ANA	6	8	
IFN	0	2	
HC	4	4	
Multiple cytoreductive drugs*	3	6	

WBC, white blood cells; Hb, haemoglobin level; X-CIP, X chromosome inactivation pattern; LMWH, low molecular weight heparin; IFN, interferon alpha; HC, hydroxycarbamide; ANA, anagrelide.

*Six patients were given ANA + HC, one ANA + IFN and two were given HC + ANA + IFN.

received no treatment, 11 were given low-dose aspirin (acetylsalicylic acid, ASA), 3 were given anticoagulant therapy, and 13 (68·8%) received cytoreductive drugs [hydroxycarbamide (HC) in 36·4%, anagrelide (ANA) in 47·3% and interferon-alpha (IFN) in 5·2%]. Three of these 13 patients (15·8%) received multiple cytoreductive drugs (2 ANA plus HC; 1 ANA plus IFN and HC). Twelve (23·5%) of the patients in the non-clonal group were given no therapy, while 32 (62·7%) received ASA, 20 (39·2%) were given cytoreductive drugs (8 ANA, 4 HC, 2 IFN), and multiple cytoreductive approaches were used in 6 (11·7%). The types of treatment administered to the two groups were similar (Table I).

Six children had persistently high platelet counts (over 1000×10^9/l) for many years: four of them received low-dose ASA, two were given IFN (for 1 and 2 years), and one girl was treated for less than a year with ANA. Surprisingly, the platelet counts in these patients became normal over the course of a long-term follow-up (median 15 years, range 5–20) without cytoreductive drugs.

Discussion

This study deals with the largest cohort of children with sporadic ET reported to date. In agreement with the literature (Randi & Putti, 2004; Randi *et al*, 2006; Kucine *et al*, 2013),

Table II. Main clinical and laboratory findings in patients with ET by clonal markers.

	JAK2 V617F	*MPL*	*CALR*	Monoclonal
Patients (*n*)	14	1	6	2
Males/females	6/8	1/0	1/5	0/2
Median age (range), years	10·5 (2·5–17·5)	5·05	12·7 (7·9–13·9	4·1 (0·5–7·5)
Median follow-up (range), years	8 (5–9)	3	6·2 (5·4–11·45)	6·55–9
X-CIP positive/studied cases	2/8	–	2/4	2/2
Major thrombotic events	3	0	0	0
Minor bleeding episodes	1	0	2	0
Patients with microvascular disturbances	3	1	4	0
Headache	3	0	2	0
Paraesthesia	0	0	2	0
Erythromelalgia	0	1	1	0
Splenomegaly, yes/no	6/4	0/1	2/4	1/1
Median platelet count (range), $\times 10^9$/l	1042 (512–1710)	1444	1424 (748–3200)	1223–1553
Median WBC count (range), $\times 10^9$/l	9·1 (5·5–11·8)	9·6	10·1 (6·6–16·5)	6·9–7·5
Mean Hb level (range), g/l	137 (111–157)	113	122 (105–160)	125–144

the *JAK2* V617F mutation – which is the main acquired mutation associated with ET – was found in less than one in four cases in the present cohort, and the *MPL*W515L mutation was only identified in one patient recently described elsewhere (Farruggia *et al*, 2013). We found that six children carried *CALR* mutations: four were type 1 and two were type 2 *CALR* mutations (Tefferi *et al*, 2014b). While a recent small study (Langabeer *et al*, 2014) detected no *CALR* mutations in six children with ET, other authors (Giona *et al*, 2014) found eight *CALR*-mutated children in their monocentric series. Given the relatively low sensitivity of sequencing for mutation detection purposes, however, low-level *MPL* and *CALR* mutants may go undetected and future studies will be needed to confirm these findings. While adult patients with the *JAK2* V*617F* mutation are reportedly older, with a male prevalence, higher haemoglobin levels and white cell counts, and lower platelet counts than patients with *CALR* mutations (Tefferi *et al*, 2014a), we found no such differences among our children.

Major venous thromboses at unusual sites (involving the splanchnic veins or cerebral veins) occurred in three children in the present cohort, all carrying the *JAK2* V617F mutation. Among adults with the *JAK2* V617F mutation, higher rates of venous thrombosis at uncommon sites have been reported in large cohorts (Campbell *et al*, 2005; Kiladjian *et al*, 2006). The risk of major thrombotic events is low for *CALR*-mutated adults (Klampfl *et al*, 2013; Rotunno *et al*, 2014; Rumi *et al*, 2014), while our *CALR*-mutated children suffered more often from mild bleeding and minor microvascular disturbances than their *JAK2* V617F-positive counterparts (though the difference was not statistically significant). No other significant differences were found when we compared patients with different mutations.

On the whole, 23 of the 89 patients reported in the present study had clonal disease, while the other 66 had persistent thrombocytosis with no markers of clonality. No significant differences emerged when we compared clonal *versus* non-clonal cases for haematological and biological features. The proportions of cases with clonal *versus* non-clonal disease were much the same for children and adolescents (Langabeer *et al*, 2015). Our children with no clonal markers, who accounted for a larger proportion of our sample than in adult reports (74% vs. 10–15%), may meet the WHO diagnostic criteria for ET. They should not be definitively assumed to be cases of MPN, however, even if other causal factors cannot be identified (Slone *et al*, 2013). It has been repeatedly claimed that ET in paediatric patients differs from its adult counterpart and age-specific diagnostic criteria are needed (Randi *et al*, 2006; Teofili *et al*, 2007; Fu *et al*, 2013). A high incidence of familial ET is finding peculiar to one single-centre series (Giona *et al*, 2012), and has not been confirmed by the present authors.

The existence of clonal markers in some children indicates that clonal ET does occur in paediatric patients, albeit rarely. Considering the mutated cases, the relative proportions of the three known ET-specific mutations in our sample (*JAK2* vs. *MPL* vs. *CALR*: 60% vs. 4·3% vs. 26%) is similar to the proportions found in adults (Klampfl *et al*, 2013; Rotunno *et al*, 2014), so paediatric clonal ET is presumably the same as the disease encountered in adults.

In adults, triple-WT cases are still considered MPN, and some of our non-clonal children also presented with minor bleeding episodes, microvascular disturbances and splenomegaly, suggestive of MPN. But our finding that 75% of children were non-clonal cases seems remarkably high by comparison with adult series (Tefferi *et al*, 2014a), and this incidence did not vary when children were compared with adolescents (Langabeer *et al*, 2014).

It is worth noting that we observed a 'spontaneous remission' in two children with non-reactive sustained thrombocytosis (about 2000×10^9/l) over a period of more than

15 years: they were both triple-WT cases and X-CIP-negative. Two other such cases have already been reported in the literature (Aviner *et al*, 2012). In our series, four other girls with a non-clonal pattern have been followed up for more than 10 years now, and have developed neither new mutations nor a monoclonal pattern.

Our study suggests that a different, non-clonal mechanism underlies megakaryocyte proliferation and excessive platelet production, in some cases at least. Fu *et al* (2013) termed these cases of 'primary thrombocytosis'. The clinical phenotype of children with this form of sporadic 'primary thrombocytosis' is identical to that of clonal ET and it takes a lengthy follow-up to be able to discriminate between children with real triple-WT ET (Tefferi *et al*, 2014a) and non-myeloproliferative patients. The triple-WT cases warrant further investigation with whole exome sequencing or transitional profiling (Nangalia *et al*, 2013; Rampal *et al*, 2014).

Our study identified a small group of children with clonal ET whose biological characteristics were similar to those of adult ET. The therapeutic approaches to such cases need further investigation (Randi & Putti, 2004; Fu *et al*, 2013). We also identified a large cohort of children with non-clonal sustained thrombocytosis diagnosed as ET at advanced haematological centres: we suggest that these patients be approached differently from preceding cases, avoiding labelling these cases as MPN, prolonging their clinical observation, and considering the use of cytoreductive drugs with caution.

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Authorship

MLR and MCP contributed equally to this study: they designed the research, contributed patients, analysed and interpreted the data, and wrote the paper; GB and FF contributed to study design; EP and ED performed biological tests. All other authors contributed patients and took part in the discussion of the data. All authors read and approved the final draft.

Conflict of interest

The authors have no competing financial interests to disclose.

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