Thrombocythemia and polycythemia in patients younger than 20 years at diagnosis: clinical and biologic features, treatment, and long-term outcome

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Sixty-four patients < 20 years of age, investigated for a suspicion of Philadelphia-negative myeloproliferative disease (MPD), were retrospectively evaluated to characterize the different forms and to examine the treatments used and long-term outcome. JAK2 mutations, endogenous erythroid colony growth, and clonality were investigated in 51 children. Mutations of thrombopoietin, the thrombopoietin receptor (MPL), and the erythropoietin receptor and mutations of other genes involved in the pathogenesis of MPD were investigated in JAK2 wild-type patients. Based on our criteria for childhood MPD, we identified 34 patients with sporadic thrombocythemia (ST), 16 with hereditary thrombocytosis (HT), 11 with sporadic polycythemia (SP), and 3 with hereditary polycythemia (HP). JAK2^{V617F} mutations were present in 47.5% of ST and in no HT. The MPL^{S505A} mutation was detected in 15/16 HT patients and in no ST ($P < .00001$). The JAK2^{V617F} mutation occurred in 27% of SP patients diagnosed according to the Polycythemia Vera Study Group or World Health Organization 2001 criteria. Children with ST received more cytoreductive drugs than those with HT ($P = .0006$). After a median follow-up of 124 months, no patient had developed leukemia or myelofibrosis and 5% had thrombosis; the miscarriage rate in thrombocythemic patients was 14%. The low complication rate in our population suggests that children with MPD may be managed by tailored approaches. (*Blood*. 2012;119(10): 2219-2227)

Introduction

Essential thrombocythemia (ET) and polycythemia vera (PV) are typical chronic Philadelphia-negative (Ph^-) myeloproliferative diseases (MPDs) that occur in middle/advanced-age adults.[1] In childhood and adolescence, these disorders are extremely rare[2] and therefore published data on the clinical presentation, biologic features, treatment approaches, and long-term outcome of Ph^- MPD children and adolescents are limited. In the last decade, new insights into the underlying molecular mechanisms of ET and PV have shown their growing clinical relevance and have led to the guidelines published by the World Health Organization (WHO),[3,4] which have replaced those of the Polycythemia Vera Study Group (PVSG).[5] Because the clinical and hematologic findings are similar in children and adults with Ph^- MPD, it has been generally accepted that specific diagnostic criteria developed for adult patients with ET and PV should also be applied to pediatric cases.[3-5] Recently, our group and others have reported a low incidence of JAK2^{V617F} mutations in ET and PV occurring in childhood.[6,7] Further studies have not confirmed these findings in PV patients[8]; however, at least in ET, we clarified that they were due to the MPL^{S505A}-activating mutation mimicking a true MPD.[7] To avoid inappropriate invasive investigations and overtreatment in hereditary forms, we proposed that children suspected of MPD should undergo a specific diagnostic workup.[9,10] In adults, management and therapeutic options are supported by controlled studies, and the clinical outcome of patients treated according to the current clinical practice is predictable.[11,12] In contrast, the treatment approaches in children are highly heterogeneous and are often imported from the adult experience.[8,13-15] With regard to the long-term outcome of MPD, there have been many studies on life expectancy, complications (thrombosis, myelofibrosis, and leukemia),[1,16-23] thrombophilia as an additive risk factor in younger patients,[24] pregnancy course in adults and young individuals,[25-29] whereas few studies have been conducted in children.[6,7,13,30]

The present study was carried out in a series of 64 patients < 20 years of age at diagnosis who were investigated for suspicion of MPD according to diagnostic work-up[9] with the aim of analyzing the clinical and biologic features of the different forms, the treatment strategies used, and the overall outcome, including hematologic evolution, thrombotic events, and pregnancies. Taking advantage of the prolonged period of clinical observation of this cohort of patients, we provide some suggestions for the clinical management of children and adolescents with MPD.

Methods

This retrospective study included 64 consecutive patients suspected of having MPD who were observed at the Hematology Center of the Sapienza University of Rome between December 1981 and March 2009. The study

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was approved by the institutional ethics committee. The inclusion criteria were a diagnosis satisfying the PVSG criteria[5] and, from 2002 on, the WHO criteria.[3,4] Secondary causes of thrombocytosis or erythrocytosis were carefully ruled out in all patients. At diagnosis, cytogenetic analysis revealed a normal karyotype in 49 of 49 tested patients and bone biopsy showed the absence of bone marrow (BM) reticulin fibrosis in 37 of 46 (80%) patients. Clinical and hematologic evaluations were planned at intervals scheduled according to the individual outcome. Since 2002, blood samples from 51 of 64 MPD patients were collected after informed consent in accordance with the Declaration of Helsinki. Cell cultures for endogenous erythroid colony (EEC) growth were performed as described previously.[31] Clonality of hematopoiesis was examined in all female patients by the human androgen receptor assay and by human androgen receptor assay methylation-specific PCR analysis.[32] The presence of JAK2^{V617F} or JAK2 exon 12 mutations were investigated using the method described by Baxter et al[33] and Scott et al,[34] respectively. The mutated allele burden was measured according to the method of Vannucchi et al.[35] As described previously,[10] all patients with hereditary forms were investigated for all those mutations that were detected in hereditary cases of thrombocytosis and polycythemia. Furthermore, in all patients with unidentified genetic defects from both hereditary and sporadic groups, a broad search for several mutations associated with myeloproliferative neoplasms was carried out. Patients with erythrocytosis were generally investigated for mutations of the following genes: hypoxia-responsive element (HRE) of the human erythropoietin gene (Epo), erythropoietin receptor (EpoR), hypoxia-inducible factor-2α and -1α (HIF-2α and *HIF*-1α), von Hippel-Lindau (VHL), prolyl hydroxylase domain protein 1-3 (PHD1-3), STAT5, lymphocyte-specific adaptor protein (LNK), and Ten-Eleven Translocation-2 (TET2). Similarly, patients with *JAK2* wild-type thrombocytosis were investigated for the following genes: 5′-untranslated region of thrombopoietin (THPO), thrombopoietin receptor (MPL), LNK, and TET2. All primers, PCR conditions, and relative references are provided in supplemental Table 1 (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

The presence of thrombophilic conditions was investigated in 40 patients. Functional protein C (*PC*; normal range, 70%-115%) and free protein S (*PS*) Ag (normal range, 64%-124%) were determined by automated latex ligand immunoassay. Functional antithrombin (normal range, 75%-132%) and activated *PC* resistance (normal value, > 0.79) were evaluated by the automated chromogenic method. Lupus anticoagulant was measured using the silica clotting time ratio (normal value, < 1.26) and diluted Russel viper venom time ratio (normal value, < 1.21); homocysteine (normal value, < 15 μM) was monitored by an automated method; methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms, factor V Leiden (*FV*L) and factor II (*FII*) mutations were investigated using gene mutation assay based on the principle of reverse hybridization PCR.[36]

Different therapeutic approaches were used and often varied during the study period because they were based on the physicians' judgment and were usually transferred from the adulthood experience. As a rule, in thrombocythemic patients, the indication for antiplatelet therapy was a platelet count $> 1000 \times 10^9$/L whatever the age and the presence of the symptoms in the first 2 decades of the study, whereas in the last decade, the indication was restricted to patients with symptoms attributed to thrombocytosis. In addition, cytoreductive drugs were started when the platelets were persistently $> 1500 \times 10^9$/L combined or not with splenomegaly and/or symptoms, and treatment was stopped in patients achieving a platelet count persistently lower than 450×10^9/L. In polycythemic patients, the treatment approach was based on existing recommendations for adults,[37] taking into account the low thrombotic risk in childhood; indeed, antiplatelet therapy was used only when symptoms were present. Likewise, the target value of the hematocrit (Hct) varied over the study period: until 1995, phlebotomy was indicated when the Hct was over 52%,[38] and from 1995 on, the target Hct was reduced to 48%.[37]

Statistical analysis

The characteristics of the patients were summarized by cross-tabulations for categorical variables and by standard statistical parameters for continuous variables. Differences in the distributions of prognostic factors in subgroups were analyzed with the χ^2 or Fisher exact test and the Wilcoxon ($K = 2$) or Kruskal-Wallis test ($K > 2$) for categorical and continuous covariates, respectively. The median follow-up time was estimated by reversing the codes for the censoring indicator in a Kaplan-Meier analysis. All tests were 2-sided, accepting $P \leq .05$ as a statistically significant difference. All analyses were performed using SAS Version 9.1.3 software (SAS Institute). Follow-up was updated to February 28, 2010.

Results

Among 64 patients (38 females and 26 males; median age, 14 years; age range, 3 months-20 years), we identified 34 (53%) patients with sporadic thrombocythemia (ST), 16 (25%) with hereditary thrombocytosis (HT), 11 (17%) with sporadic polycythemia (SP), and 3 (5%) with hereditary polycythemia (HP). No familial cases of acquired ET and PV were recorded.

Patients with ST and HT

Clinical, hematologic, and laboratory features. The main features of patients grouped as ST or HT are shown in Table 1. Generally, patients with HT were diagnosed at a younger age than those with ST ($P = .0418$). Except for the significantly higher Hct value in the ST group ($P = .0047$), we did not find any significant difference in the other hematologic features between HT and ST. Similarly, the 2 groups were not distinguishable by BM histology, both being characterized by hypercellularity, giant megakaryocytes, and absent or low (grade 0-1/1) reticulin fibrosis. No significant differences in the clinical features were recorded in the 2 cohorts, although ST patients presented with symptoms or organomegaly at diagnosis more than those with HT. Generally, neither bleeding episodes or thrombotic events were recorded at diagnosis or as an inaugural manifestation of the disease.

Concerning the laboratory features, 10 of 21 (48%) ST patients were JAK2^{V617F} mutated and 7 of 11 (63%) ST female patients exhibited monoclonal hemopoiesis. In contrast, all investigated patients with HT were JAK2 wild-type ($P = .0049$) and all HT female patients showed a polyclonal hemopoiesis ($P = .0128$); moreover, 15 of 16 investigated HT patients harbored the MPL^{S505A} mutation ($P < .00001$ in comparison with ST). No mutations other than JAK2^{V617F} *or* MPL^{S505A} were identified in the 2 cohorts of patients. Among ST cases, no significant differences in clinical, hematologic, and biologic features were found between JAK2^{V617F}-mutated and *JAK2* wild-type patients, as shown in Table 2. In JAK2^{V617F}-positive patients, the burden of mutated alleles ranged from 6%-50% (Table 1) and was not influenced by the administered cytoreductive treatment. In JAK2^{V617F}- and MPL^{S505A}-negative patients, no mutations of the other investigated genes were detected (for details, see supplemental Methods).

Thrombophilic abnormalities were investigated in 31 thrombocythemic patients. As shown in Table 3, a heterozygous *FII*^{G20210A} mutation was found in 3 patients (9.5%), combined with other acquired (prolonged silica clotting time ratio in 1 case) and/or inherited (*MTHFR*^{C677T} polymorphism) abnormalities in 2 patients. Moreover, a heterozygous *FV*L combined with a *MTHFR*^{C677T} polymorphism was detected in 2 patients (6.5%).

Treatment. Based on the clinical features and hematologic findings, ST and HT were indistinguishable until the hereditary form was better defined.[7] Therefore, until a few years ago, the same therapeutic approach was used for ST and HT patients, using different antiplatelet and cytoreductive agents over the years of the study, as detailed in supplemental Table 2. Overall, 10 thrombocythemic children, especially with those HT, did not undergo any

Table 1. Main demographic, hematologic, and clinical findings at diagnosis, biologic markers, treatment, and clinical outcomes of thrombocythemic children analyzed in the present study

Demographic and hematologic characteristics	ST (n = 34)	HT (n = 16)	*P*
Males/females	10/24	7/9	.35
Median age, y (range)	15 (5-19)	12 (3mos-17)	.0418
Median WBCs, $\times 10^9$/L (range)	9.88 (5.62-22.22)	8.43 (5.17-16.06)	.215
Median Hct, % (range)	41.65 (29-53)	36.15 (32.3-42)	.0047
Median platelets, $\times 10^9$/L (range)	1.109 (633-2.800)	990.5 (611-2.950)	.303
BM biopsy, n (%)	34/34 (100%)	5/16 (31%)	
Hypercellularity, n (%)	15/34 (44%)	1/5 (20%)	1.00
Giants megakaryocytes, n (%)	5/34 (15%)	0/5	1.00
Reticulin fibrosis 0-1/1, n (%)	7/34 (20.5%)	1/5 (20%)	1.00
Clinical findings at first presentation			
Symptoms, n (%)	12 (35%)	4 (25%)	.526
Headache	10	3	
Paresthesias	2	1	
Splenomegaly, n (%)	6/32 (19%)	2/15 (13%)	.702
Hepatomegaly, n (%)	2/32 (6%)		
Thrombotic events, n (%)	0	0	
Bleeding events, n (%)	0	0	
Biologic markers	**(n = 21)**	**(n = 16)**	
EEC positive, n (%)	11/19 (58%)	5/12 (42%)	.472
$JAK2^{V617F}$, n (%)	10/21 (48%)	0/13	.0049
V617F allele burden, n	8/10		
Median allele burden (range)	21% (6%-50%)		
Clonal hemopoiesis, no. of females, %	7/11 (64%)	0/7	.0128
MPL mutations, n (%)	0/15	15/16 (94%)	< .00001
Treatment	**(n = 34)**	**(n = 16)**	
Antiplatelet drugs, n (%)	27/34 (79%)	9/16 (56%)	.105
Cytoreductive therapy, n (%)	23/34 (68%)	2/16 (12.5%)	.0006
Antiplatelet drugs at last follow-up, n (%)	6/34 (15%)	3/16 (19%)	1.000
Cytoreductive therapy at last follow-up, n (%)	19/34 (56%)	1/16 (6%)	.0007
ANA	7/19 (37%)		
HU	7/19 (37%)		
IFN-α	4/19 (21%)	1	
IFN-α + HU	119 (5%)		
Clinical outcome			
Splenomegaly + reticulin fibrosis 1-2, n (%)	3/34 (9%)	0	.542
Time to evolution, mo	(37, 108, 119)		
Thrombotic events, n (%)	1/34 (3%)	2/16 (12.5%)	.236
Time to thrombosis, mo	(64)	(28, 64)	
No. of pregnancies/no. of patients	15/6	3/2	
Abortions	6	0	
Spontaneous	2		
Elective	4		
Childbirths	9/15	3/3	.678
Malignancies, n (%)	1 (3 %)	1 (6 %)	
Splenectomy, n (%)	1 (3%)	0	
Lost to follow-up, n (%)	10 (29%)	3 (19%)	
Median follow-up, mo (range)	128 (13-332)	137 (27-327)	

ST indicates sporadic thrombocythemia; HT, hereditary thrombocytosis; n, number of patients; EEC, endogenous erythoid colony; ANA, anagrelide; HU, hydroxyurea; and IFN-α, interferon-α.

treatment. Antiplatelet treatment, mostly acetylsalicylic acid (ASA), was started in 27 of 34 (79%) patients with ST and in 9 of 16 (56%) patients with HT at different times during the observation period. However, the use of ASA progressively decreased over the study period both in newly diagnosed patients and in those on treatment; at the last follow-up, 6 of 34 (15%) patients with ST and in 3 of 16 (19%) patients with HT were still receiving ASA (Table 1). Cytoreductive agents, mostly hydroxyurea (HU), IFN-α, and anagrelide (ANA), were used in 25 of 50 (50%) patients for a mean time of 126 months; 15 of these patients received more than one agent. A significantly greater number of children with ST (68%) received cytoreductive therapy than those with HT (12.5%; $P = .0006$). Over the years, cytoreduction was stopped in 5 patients; nevertheless, at the last observation, 19 of 34 (56%) ST patients were still receiving cytoreductive agents compared to 1 of 16 (6%) children with HT ($P = .0007$).

Clinical outcome. As reported in Table 1, after a median follow-up of 128 months for ST and of 137 months for HT, 3 patients (5%) without any significant thrombophilic abnormalities experienced a thrombotic event that occurred during infectious episodes. A 25-year-old ST male developed a portal thrombosis during HU treatment 1 year after a posttraumatic splenectomy; a 21-year-old HT female had a superficial thrombophlebitis during therapy with low-dose ASA, and a 13-year-old HT male, who had previously undergone posttraumatic splenectomy, experienced a peripheral arterial occlusion while on treatment with low-dose ASA and HU.

Table 2. Main clinical and hematologic features, treatment, and outcome in 21 children with ST grouped according to *JAK2* mutational status

Clinical and hematologic features	JAK2^{V617F}(n = 10)	JAK2 wild-type (n = 11)	*P*
Male sex, n (%)	4 (40%)	4 (36%)	1.00
Female sex, n (%)	6 (60%)	7 (64%)	
Median age, y (range)	18 (6-19)	15 (5-19)	.511
Median WBCs, $\times 10^9$/L (range)	10.8 (7.36-22.22)	10.8 (6.44-18.87)	.468
Median Hct, % (range)	41.8 (29-53)	41.2 (38.1-47.3)	1.00
Median hemoglobin, g/dL (range)	14.2 (9.7-17.5)	13.5 (11.7-15.5)	.533
Median platelets, $\times 10^9$/L (range)	1.020 (633-2.640)	1.400 (794-2.800)	.319
BM biopsy, n (%)			
Hypercellularity,	7/10 (70%)	5/11 (45.5%)	.6499
Giant megakaryocytes	3/10 (30%)	1/11 (9%)	.582
Reticulin fibrosis 0-1/1	3/10 (30%)	3/11 (27%)	1.00
Symptoms, n (%)	4 (40%)	4 (36%)	1.00
Splenomegaly + hepatomegaly, n (%)	3/10 (30%)	2/11 (18%)	.635
EEC positive, n (%)	5/8 (62.5%)	6 (54.5%)	1.00
Monoclonal/female patients, %	5/7 (71%)	2/4 (50%)	.412
Treatment and clinical outcomes			
Antiplatelet drugs, n (%)	9/10 (90%)	10/11 (91%)	1.00
Cytoreductive therapy, n (%)	6/10 (60%)	9/11 (82%)	.3615
Antiplatelet drugs at last follow-up, n (%)	4/10 (40%)	1/11 (9%)	.1486
Cytoreductive therapy at last follow-up, n (%)	6/10 (60%)	8/11 (72%)	.6594
ANA	3/6 (50%)	5/8 (62.5%)	
HU	2/6 (33%)	2/8 (25%)	
IFN-α	1/6 (17%)	1/8 (12.5%)	
Subsequent splenomegaly + reticulin fibrosis 2, n (%)	1 (10%)	1 (9%)	1.00
Thrombosis during follow-up, n (%)	1 (10%)	0	.4762
Malignancies, n	0	1	.4762
Median follow-up, mo (range)	150 (13-289)	193 (31-331)	.6032

EEC indicates endogenous erythroid colony; n, number of patients; ANA, anagrelide; HU, hydroxyurea; and IFN-α, interferon-α.

After a median follow-up of 132 months, none of the thrombocythemic patients developed acute leukemia, whereas 2 untreated patients developed a malignancy: 1 HT male had a localized malignant melanoma and a ST female developed a renal carcinoma. A progressive spleen enlargement combined with an increase of reticulin fibrosis up to grade 1-2 was recorded in 3 of 34 (9%) ST patients (1 of whom was never treated with cytoreductive drugs), whereas no cases of overt post-ET myelofibrosis according to standard criteria[39] were documented among our patients.

Pregnancy. Data on pregnancies are summarized in Table 1. During the follow-up, a total of 18 pregnancies among 6 ET and 2 HT female patients were recorded. Five of the 8 pregnant females had previously received one or more cytoreductive agents, mostly HU. At the time of conception, antiproliferative treatment, namely HU (n = 7), IFN-α (n = 1), and ANA (n = 1), was documented in 9 cases (50%) and was discontinued in all but 2 patients, who were treated with IFN-α. As antiplatelet therapy, an individualized treatment consisting of ASA alone (n = 5), ASA in combination with low-molecular-weight heparin (n = 3), or low-molecular-weight heparin alone (n = 2) was planned in 10 patients. The 18 pregnancies resulted in 12 successful live births and in 6 first-trimester abortions, 4 of them elective. The 2 first-trimester miscarriages occurred in a JAK2^{V617F}-mutated ET patient during treatment with low-dose ASA. Except for a thrombocytosis recorded in a newborn with the inherited MPL^{S505A} mutation from his mother, no other hematologic abnormalities or developmental defects were recorded in the progeny of either male or female thrombocythemic patients.

Patients with SP

Clinical, hematologic, and laboratory features. As shown in Table 4, 5 of the 11 SP patients (45.5%) suffered at diagnosis from symptoms attributed to polycythemia, but 1 patient only presented with peripheral neurologic signs. The Epo serum level was within the normal range in all but 1 patient. BM histology showed hypercellularity with an increased erythropoiesis and myelopoiesis of various degrees in all patients and low (grade 0-1/1-2) reticulin fibrosis in 3 cases, 2 of whom had a concomitant splenomegaly. The most striking observation was that in this series of patients, the JAK2^{V617F} mutation was detected in only 3 patients, all of whom had EEC growth, and, in the case of females, a clonal hematopoiesis. In the 8 JAK2^{V617F}-negative patients, neither the JAK2 exon 12 nor mutations of the other investigated genes (for details, see supplemental Methods) were detected. It is noteworthy that in all JAK2-negative patients, the diagnosis of PV was supported by the detection of a high RBC mass and by histologic criteria,[3-5] and also by the presence of EEC growth in 1 patient. The diagnosis of PV was made according to PVSG or WHO 2001 criteria in all but 1 PV patient. Nevertheless, according to the most recent WHO criteria, all JAK2-negative patients cases could not be defined as PV.

Treatment and clinical outcome. Phlebotomy or erythrocytapheresis were started in 8 of 11 patients (72%); nevertheless, 2 JAK2-mutated patients needed HU administration to reduce the progressive increase of splenomegaly and to decrease the Hct level below 48%. Four SP patients received antiplatelet therapy, namely low-dose ASA or ticlopidine, that was stopped in 3 cases. At the last follow-up, 5 patients needed phlebotomies and 2 JAK2-mutated patients were also on treatment with HU for 29 and 60 months, respectively. Interestingly, among patients requiring periodic phlebotomies, 4 were JAK2-negative patients. After a median follow-up time of 113 months, no thrombotic or bleeding episodes were recorded and no evolution to overt myelofibrosis[39] or leukemia occurred.

Table 3. Inherited and acquired thrombophilic abnormalities and biologic markers in 40 children with MPDs

Thrombophilic abnormalities	Patients with thrombophilic alterations/evaluated patients (%)	Thrombocythemic patients/evaluated patients (n = 31)	Polycythemic patients/evaluated patients (n = 9)
***MTHFR*^{C677T} mutation**	28/40 (70%)	22/31 (71%)	6/9 (67%)
Homozygous	1/28	1/22	
Heterozygous	27/28	21/22	6/6
Combined with:	13/27 (48%)	9/21 (43%)	4/6 (66.5%)
FVL	2/13	2/9	
FII^{G20210A} mutation	1/13	1/9	
FII^{G20210A} mutation + prolonged SCT ratio	1/13	1/9	
Prolonged SCT ratio	1/13		1
Prolonged SCT ratio + high HCY	1/13		1
High HCY	2/13	1/9	1
Low PC	2/13	2/9	
Low PC + PS	1/13		1
Low PS	2/13	2/9	
Heterozygous *FVL* mutation	2/40 (5%)	2/31 (6.5%)	0
Combined with heterozygous MTHFR^{C677T}	2/2	2/2	
Heterozygous *FII*^{G20210A}	3/40 (7.5 %)	3/31 (9.5%)	0
Combined with heterozygous MTHFR^{C677T}	2/3	2/3	
Prolonged SCT ratio	5/39 (13%)	1/30 (3%)	4/9 (44.5%)
Combined with other abnormalities	3/5	1/1	3/4
Prolonged dRVVT ratio	1/38	0	1/9 (11%)
Combined with prolonged SCT ratio	1/1		1/1
Low functional PC level	3/40 (7.5%)	2/31 (6.5%)	1/9 (11%)
Combined with other abnormalities	3/3	2/2	1/1
Low free PS level	5/40 (12.5%)	3/31 (9.5%)	2/9 (22%)
Combined with other abnormalities	3/5	2/3	1/2
High HCY level	3/29 (10%)	1/20 (5%)	2/9 (22%)
Combined with other abnormalities	3/3	1/1	2/2
No thrombophilic abnormalities	7/40 (17%)	7/31 (22.5%)	0

MTHFR^{C677T} indicates methylentethrahydrofolate reductase; PC, protein C; PS, protein S; HCY, homocysteine; SCT, silica clotting time; and dRVVT, diluted Russel viper venom time.

Patients with HP

We observed 3 patients belonging to 2 families with a history of HP. In all of them, the underlying genetic defect was unknown at the time of diagnosis, but during the study period, the HIF2A^{M535} mutation was found in a male patient and, subsequently, in his mother.[40] Interestingly, in none of these patients the Epo serum level was above the normal value. Phlebotomies were regularly carried out in the HIF2A^{M535}-mutated patient, whereas they were seldom performed in the others. None of the HP patients received antiplatelet therapy and no thrombotic episodes were observed.

Discussion

In adulthood, the diagnosis of Ph$^-$ myeloproliferative neoplasms is well-defined and unequivocal, their complications and clinical course are extremely predictable, and the therapeutic approaches are well standardized. In particular, it is known that in adults with ET or PV, the major causes of substantial morbidity and shortened survival are vascular events and evolution to myelofibrosis or acute leukemia. Accordingly, the recent recommendations from the European LeukemiaNet state that the goals of therapy in patients with PV and ET should be to avoid thrombotic and bleeding complications, to minimize the risk of acute leukemia or myelofibrosis, to control systemic symptoms, and to manage risk situations (eg, pregnancy or surgery).[12] Several studies carried out in PV and ET patients identified age older than 60 years and previous thrombosis as major predictors of vascular complications. Indeed, the use of antiplatelet therapy is recommended in all PV and ET patients (except for those with very severe thrombocytosis), whereas antiproliferative therapy should be given only to patients with a high vascular risk for age or previous history of thrombosis.[12] Moreover, in PV patients, even though specific evidences are still lacking, it is widely accepted that the Hct value should be maintained below 45%.[12]

The scenario of Ph$^-$ myeloproliferative neoplasms occurring in childhood is completely different. Indeed, the occurrence of an ET in pre-adolescents and adolescents is extremely rare, whereas PV is quite anecdotic. As a consequence, the precise diagnosis, biologic profile, and optimal treatment of these diseases in younger patients remain uncertain.[6-9,13]

We hereby report the biologic features, clinical findings, treatment, and outcome of a large series of patients with MPD younger than 20 years at diagnosis followed at a single institution for a median time greater than 10 years. Clearly, our study has some intrinsic limitations, mostly because of the length of the enrollment period, the timing, and the different types of treatment (usually planned according to the adulthood experience) used over the years. Moreover, as a single-center study, genetic aspects cannot be widely generalized for the presence in our country of some clusters of HT[41] or polycythemia.[42] Nonetheless, the large amount of data collected and the very long follow-up period allowed us to highlight some important differences between MPDs occurring in adulthood and those occurring in childhood. At first look, the majority of our pediatric patients (67%) were asymptomatic, mainly those with the hereditary forms being diagnosed through a careful history of previously identified family members. However,

from the diagnostic point of view, the first remarkable observation is that a proportion of children suspected of having MPD actually suffer from a hereditary form of thrombocythemia or polycythemia, which are clinically indistinguishable from the sporadic forms in the absence of specific genetic tests. However, it is notable that patients with hereditary forms were often preadolescents or even babies (in HT) and that symptoms were reported less frequently. In our series, with the exception of 1 child, all children belonging to 4 of 5 investigated families exhibited the MPL^{S505A} mutation when tested for HT. The clustering of this autosomal-dominant alteration in our cohort of patients has been demonstrated to be due to a common founder ancestor approximately 23 generations ago.[41]

An important diagnostic challenge is represented by the high prevalence of JAK2 wild-type patients among children suspected of having PV. The very low detection of JAK2-mutated patients in our SP series confirms that this diagnosis is extremely rare and unlikely in childhood; however, it also raises the problem of the actual nature of these cases of "idiopathic polycythemia" presenting with normal levels of serum Epo. Our investigations for an underlying acquired or hereditary genetic abnormality (including members of the O_2-sensing pathway) in these patients was fruitless and highlighted the complexity of the regulation of erythropoiesis homeostasis. The majority of our polycythemic patients required treatment with phlebotomy. Furthermore, during the follow-up, 2 of 3 JAK2-mutated patients needed additional cytoreductive therapy to reduce concomitant hepatosplenomegaly and/or to achieve the Hct target. Biologic markers in our population are very different from those reported by Cario et al in polycythemic children, all of whom were JAK2 mutated.[8] We have no explanation for this difference, although it could be hypothesized that other unknown genetic defects may underlie the JAK2 wild-type forms in our cohort of polycythemic patients. Conversely, the frequency of the $JAK2^{V617F}$ mutation and clonality in our ST patients was similar to that reported in adults with ET.[9,16,17] Recently, several studies have reported that the JAK2 mutational status in adults characterizes 2 populations of ET patients, identifying a subgroup with characteristics similar to those of subjects affected by PV.[24,43,44] On the contrary, we did not find significant differences in clinical and hematologic characteristics at diagnosis, treatment, or disease outcome between $JAK2^{V617F}$-mutated and JAK2 wild-type childhood ST patients.

The final consideration on the laboratory findings in our MPD population concerns the BM histology, because in the last decade, histologic features have emerged as diagnostic parameters for ET, PV, and primary myelofibrosis.[3,4] Most BM biopsies from thrombocythemic patients revealed a marked hypercellularity, a finding more reminiscent of PV or prefibrotic myelofibrosis than of true ET. Nonetheless, after 3 decades of observation, none of our ET patients has shown a progression to an overt PV or to myelofibrosis, suggesting that BM hypercellularity is a normal finding in young patients.

The long period of the study and the variability of therapy timing and type of drugs used in our MPD patients make it difficult to draw definite conclusions. In the early period, antiplatelet therapy was given irrespective of age and the presence of symptoms and regardless of the disease form; thereafter, a progressive decrease in the use of antiplatelet agents has occurred. More recently, low-dose ASA has been given electively only to symptomatic patients and is always avoided in babies when possible. Likewise, the use of cytoreductive agents progressively decreased over the years, considering the low incidence of thrombosis recorded in our patients and the revisited vascular risk factors in adult MPD.[18,21,45-47] The choice of cytotoxic drug has been agreed upon to avoid the risks due to prolonged exposure to myelosuppressive agents; therefore, pipobroman has been replaced by HU and, currently, IFN-α and ANA are used. It should be stressed that discontinuing a treatment that has proven safe and effective has sometimes been an arduous challenge for both the patients and their parents.

In our experience, the most important difference between adult and childhood MPD was observed with regard to clinical outcome, especially vascular events. No patient from our series experienced previous or inaugural thrombosis. In contrast, more than one-third of adult PV patients and more than one-fifth of adult ET patients suffer from an artery or venous thrombosis at diagnosis, and the risk of thrombosis exceeds 20% thereafter in both diseases.[1,16-21] Moreover, a substantial number of adult patients experience vasomotor disturbances, mostly itching in PV cases. Currently, risk factors for thrombosis are considered to be age more than 60 years and a history of previous thrombosis,[45] whereas the relationship between thrombosis and leukocytosis and/or $JAK2^{V617F}$ mutation[24,48,49] needs prospective trials to be validated into a prognostic scoring system. The incidence of thrombotic complications (5%) observed in our population during the follow-up time was lower

Table 4. Demographic and disease characteristics at diagnosis, incidence of biologic markers, treatment, and clinical outcomes of polycythemic children

Clinical and hematologic characteristics	SP (n = 11)	HP (n = 3)
Male sex/female sex	8/3	1/2
Median age, y (range)	16 (6-19)	11 (11-14)
Median WBCs, × 10^9/L, (range)	6.73 (4.86-28.67)	6.76 (5.4-6.77)
Median Hct, % (range)	53.9 (50-72.5)	53.2 (42.4-54.5)
Median platelets, × 10^9/L, (range)	217 (166-945)	207 (202-271)
Symptoms, n (%)	5/11 (45.5%)	0
Headache	4/5	
Paresthesias	1/5	
Splenomegaly, n (%)	3/11 (27%)	0/3
Thrombotic events before/at diagnosis, %	0	0
Bleedings before/at diagnosis, %	0	0
Epo serum level, (range)	4.5 (1.1-16.4)	4.5; 5; 7.4
BM biopsy, n (%)	10/11	1/3
Reticulin fibrosis 0-1/1-2, n (%)	3/10 (30%)	1/1
Biologic markers	(n = 11)	(n = 3)
EEC positive, n (%)	4/10 (40%)	0/3
$JAK2^{V617,}$ n (%)	3/11 (27%)	0/3
V617F allele burden	39%; 43.5%; 48%	
Clonal hemopoiesis, no. females, %	2/3	0/2
Treatment	(n = 11)	(n = 3)
Antiplatelet treatment, n (%)	4/11 (36 %)	0
Cytoreductive therapy, n (%)	8/11 (72%)	1/3 (33%)
Phlebotomy or erythrocytapheresis	6/8 (75%)	1/3
Phlebotomy + HU	2/8 (25%)	
Antiplatelet treatment at last follow-up, n %	1/11 (9%)	0
Cytoreductive procedures at last follow-up, n (%)	7/11 (64%)	1/3 (33%)
Phlebotomy or erythrocytapheresis	5/7 (71%)	1/3
Phlebotomy + HU	2/7 (29%)	
Clinical outcome	(n = 11)	(n = 3)
Splenomegaly + reticulin fibrosis 2, n (%)	2 (18%)	0
Time to evolution, mo	(38, 85)	
Median follow-up, mo (range)	113 (24-210)	28 (28-113)

SP indicates sporadic polycythemia; HP, hereditary polycythemia; n, number of patients; PV, polycythemia vera; and EEC, endogenous erythroid colony.

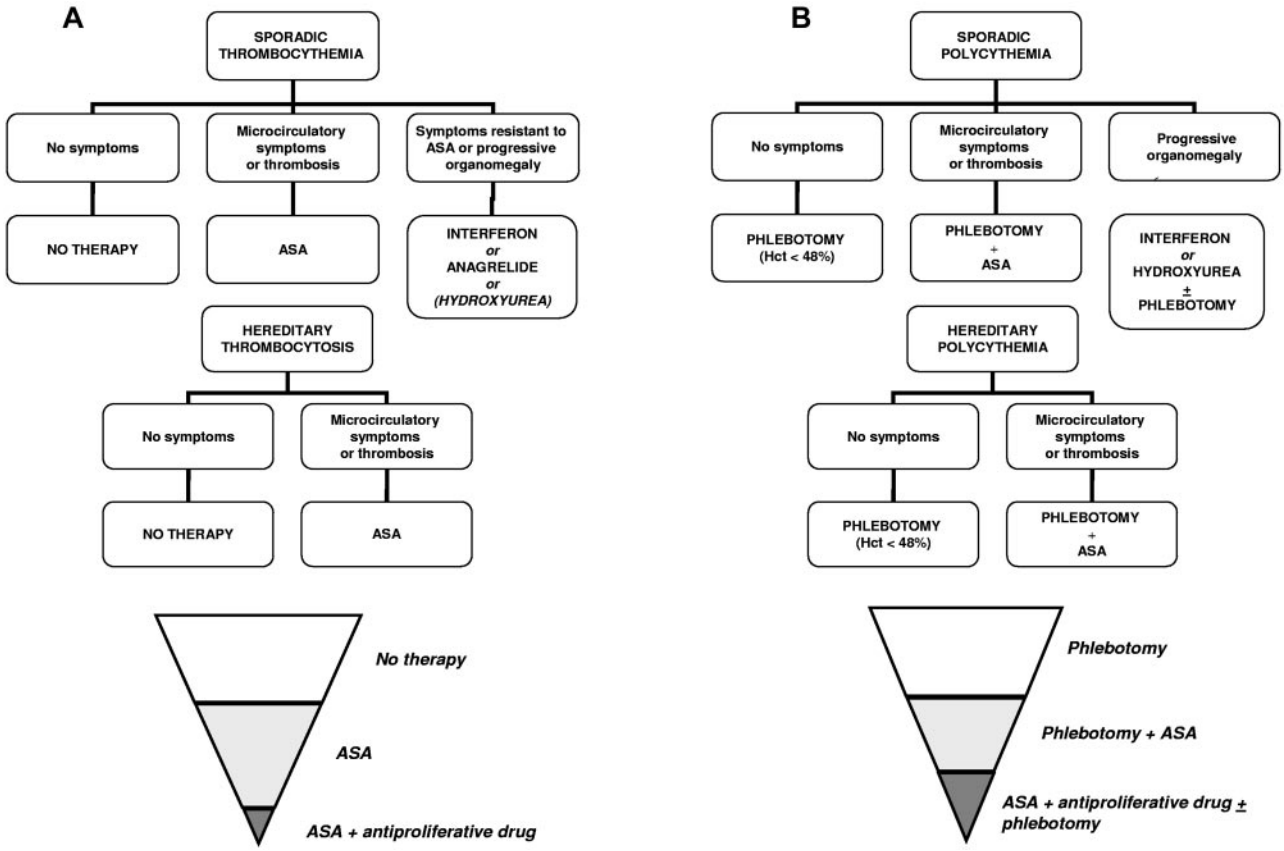

Figure 1. Therapeutic algorithm for thrombocythemic and polycythemic children. The graphics in the lowest section show the number of patients undergoing the different therapeutic options: white sections refer to all asymptomatic patients, the gray sections indicate all symptomatic patients, and the darkest sections are indicative of all symptomatic patients resistant to low-dose ASA and/or with uncontrolled myeloproliferation. As a rule, any treatment, including low-dose ASA, is avoided in infants whatever the platelet number.

than that reported in adults with MPDs,[1,16-21] and was not influenced by JAK2 mutational status or by thrombophilic abnormalities, because none of the patients with hereditary or acquired thrombophilia experienced thrombosis. In contrast, leukocytosis could have an important role in predisposing young patients to thrombosis: in this respect, it is noteworthy that all 3 thrombotic events occurred in the context of an infectious episode combined with leukocytosis. Our findings suggest that the integrity of the vascular tree, typical of young individuals, plays a major role in protecting these patients from thrombosis, whereas it is overcome when additional risk factors, such as flow reduction, surgery, infection, and leukocytosis, occur. In addition, 2 of 3 patients who developed a thrombosis had a simultaneous thrombocytosis because of a MPL^{S505A} mutation, confirming that this mutation highly predisposes to thrombosis.[30]

The outcome of pregnancies in our female thrombocythemic population do not differ from that reported in the general population. Indeed, if the elective abortions are excluded, the overall first-trimester miscarriage rate (14%) in our cohort was similar to that estimated in the general population[50] and lower than that reported in female ET patients (21%-35%) in a larger series.[25-29] The lower rate of spontaneous abortions observed in our study is probably because of the tailored therapeutic interventions performed in the planned pregnancies, when the type of antithrombotic prophylaxis was chosen according to the different thrombophilic risk factors. In addition, the spontaneous decrease of the platelet count during pregnancy, reported as a common finding in pregnant women with ET,[25,26] occurred in most of our pregnancies, so that a cytoreduction with IFN-α was necessary in only 2 patients.

Finally, in our cohort of patients with a median follow-up greater than 10 years, a progressive increase of spleen size was documented in 8% of patients, but none has so far evolved into an overt myelofibrosis. The absence of gradual splenomegaly and/or BM fibrosis detected in our familial forms does not contradict data reported previously in a large series of HT patients older than those included in the present study.[30] Indeed, the detection of splenomegaly and the progression toward a significant BM fibrosis in MPL^{S505A}-mutated patients seemed to be age dependent and related to disease duration. Moreover, in our population, the prolonged exposure to different myelosuppressive agents did not affect the risk of developing second solid cancers.

Our experience suggests that in children suspected of having MPD, the possibility of an inherited form should be considered. Further, in thrombocythemic children, genetic tests on peripheral blood are helpful to avoid the use of invasive procedures in the familial form. It is difficult to derive definite indications on how to treat young patients with MPD on the basis of data collected over a long time period because they do not exist. Nevertheless, for treatment planning, it is important that disorders capable of exacerbating thrombocytosis, such iron deficiency and/or inflammatory status, are not underestimated. It should also be considered that MPD in children has a very favorable course, with some inherited thrombocytosis conferring a thrombotic risk in adults greater than that of true ET.[30] The current therapeutic approach followed at our

institution is illustrated in Figure 1. In both ST and HT patients, low-dose ASA is given electively to symptomatic patients and is carefully avoided in babies, whatever the platelet number. The use of antiproliferative drugs in thrombocythemic patients is strictly reserved to patients with vascular symptoms (often microcirculatory disturbances) resistant to ASA or to those with progressive marked organomegaly. The drug of choice as a first-line cytoreductive treatment is considered to be IFN-α; unfortunately, patients are poorly compliant with this drug and therefore ANA also could be used safely and successfully in these patients.[14] Even though JAK2^{V617F}-mutated PV is an anecdotal diagnosis in infancy, phlebotomy remains the mainstay of therapy for children with polycythemia, probably with a less-stringent Hct reduction than in adults (Hct levels up to 48% might be acceptable). Only polycythemic patients with progressive marked liver and/or spleen enlargement with or without symptoms resistant to ASA are candidates for cytoreductive therapy. We strongly advise against chemotherapy use in the absence of a proven clonal proliferation. Finally, we recommend a thrombophilic screening in MPD women of childbearing age to plan tailored treatment during pregnancy.

In summary, the relative absence of symptoms and the lower incidence of thrombosis compared with adults suggest that children with MPD can be managed in a more conservative manner than adults. A longer observation period will confirm whether this favorable course varies after the fourth decade of life and will enable us to define the lifetime risk for evolution to acute leukemia or myelofibrosis of MPD occurring in infancy. International cooperative studies are necessary to elucidate the molecular etiology of pediatric patients with MPD and to standardize the therapeutic approaches for the different forms.

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Authorship

Contribution: F.G. contributed to the study design; enrolled patients, recorded, analyzed, and interpreted data; and wrote and reviewed the manuscript; L.T. contributed to the study design, coordinated molecular analysis, wrote part of the manuscript, and reviewed the manuscript; M.L.M. enrolled the patients and critically reviewed the manuscript; M.M. and S.C. performed the cell cultures and molecular analysis; G.P. and A.A. enrolled the patients and recorded the clinical data; M.G.M. coordinated the thrombophilic screening and critically reviewed the manuscript; A.M.T. enrolled the patients; P.P. performed the coagulation tests; S.M.O. analyzed the data; M.N. performed the cytogenetic analysis; and G.L., L.M.L., and R.F. critically reviewed the manuscript.

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