Long-term follow-up of essential thrombocythemia in young adults: treatment strategies, major thrombotic complications and pregnancy outcomes. A study of 76 patients

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder typical of middle age. However, it has also been observed in children and young adults.[1-2] Major thrombotic episodes and microvascular disturbances have been described in young ET patients, but the real risk for vascular complications has not been clearly established, and specific therapeutic approaches have been investigated.[3-7] In this study, we retrospectively analyzed a cohort of 76 thrombocytemic patients, younger than 40 years at diagnosis, with the following aims: (1) to evaluate thrombotic and hemorrhagic complications; (2) to specify the treatment adopted; and (3) to report pregnancy outcomes.

Permission was obtained from the institutional review board to examine the medical records of ET patients who were diagnosed at the Institute of Hematology/Oncology "L. and A. Seràgnoli", Bologna. Between 1977 and 2008, 76 consecutive patients under 40 years of age (25 males and 51 females; median age: 32 years, range 18-40; median platelet count: 830×10^9/L, range 452-2045; 23 patients with a platelet count $>1000\times10^9$/L; median leukocyte count: 8.7×10^9/L, range 4-19.6) were recorded. The diagnosis was carried out according to the PVSG[8] or the WHO[9] criteria.

Forty-seven patients (63.5%) were asymptomatic at presentation. Twenty-three (30%) were experiencing vasomotor symptoms, including headache (n=11), dizziness (n=10), visual disturbances (n=5). At diagnosis, major thrombotic and minor hemorrhagic events occurred in 7 (9%) and in 2 (2.6%) patients, respectively. Nine patients had palpable splenomegaly. Cardiovascular risk factors were present in 27 (35.5%) patients.

Median follow-up is 13.5 years (range, 1-32.5). Overall, 3 patients (4%) died (acute leukemia, non-ischemic cardiac disease, second neoplasia).

Timing and type of cytoreductive treatment was based on the discretion of the physician (Table 1). Over the years, a progressive decrease in the use of cytoreductive therapies was observed; concomitantly, busulfan (BU) was replaced with interferon-alpha (IFN-alpha) and anagrelide (ANA).

Ten patients (13%) experienced one or more thrombotic events. A total of 4 arterious (transient ischemic attack, acute myocardial infarction, peripheral arterial thrombosis, stroke) and 16 venous thrombosis (12 deep vein thrombosis and 4 superficial thrombophlebitis) were recorded throughout the follow-up (Table 2). At the time of the first thrombotic episode, 3 patients were on cytotoxic therapy (2 patients with BU and one patient with IFN-alpha) and 6 patients received an antiplatelet drug; after the first episode, all patients started a cytoreductive therapy (3 patients with BU, 2 patients with hydroxyurea and 2 patients with IFN-alpha) but a second thrombotic episode occurred in 4 cases. The cumulative risk of thrombosis during follow-up was 7.5% at ten years and 11.8% at 15 years.

$JAK2^{V617F}$ mutational analysis was performed, as previously described,[10] in 59 patients (78%). The mutation was detected in a heterozygous status in 44% of the patients, an incidence which is inferior to that observed in cohorts of general ET patients.[10,11] $JAK2^{V617F}$ mutation correlated with higher hemoglobin and lower platelets ($P<0.001$) at diagnosis.

No baseline characteristics (previous thrombotic events, platelet/leukocyte count, additional cardiovascular risk factors including smoking, $JAK2^{V617F}$ mutation) correlated with thrombosis; however, the number of the events was relatively small.

Twenty hemorrhagic episodes were reported during follow-up in 10 out of 76 (13%) patients; in 13 cases, bleeding was not associated with concomitant use of antiplatelet drugs. A history of bleeding and extreme thrombocytosis (platelet count $>1000\times10^9$/L) did not predict subsequent bleeding.

Twenty-four pregnancies in 13 females were followed and 15 normal babies were delivered, with 9 abortions (37.5%) and one abruption placentae; in the other cases, delivery was uneventful. During pregnancy, IFN-alpha was administered in 3 cases (in 2 cases, in combination with aspirin) and antiplatelet therapy alone was administered in 9 cases. Treatment at conception did not significantly influence the live birth rate (treated *vs*. untreated: 8/12 *vs*. 7/12, $P=0.99$). The $JAK2^{V617F}$ mutation did not influence pregnancy outcome: in 24 pregnancies the occurrence of fetal loss was comparable (mutated *vs*.

Table 1. Timing and type of cytoreductive and antiplatelet therapy.

Years	N. of evaluable patients	N. of patients receiving antiplatelet drugs (%)	N. of patients receiving cytoreductive therapy (%)	Type of first-line cytoreductive therapy	Type of cytoreductive therapy at last contact	Median follow-up, years (range)
1977-1986	18	14 (78%)	15 (83%)	BU (93%) IFN (7%)	IFN (41%) BU (39%) HU(20%)	27 (20-32.5)
1987-1996	43	38 (88%)	30 (70%)	BU (47%) HU (47%) IFN (6%)	IFN (60%) HU (35%) ANA (5%)	16 (12-22)
1997-2008	76	67 (88%)	54 (71%)	IFN (54%) HU (38%) ANA (8%)	IFN (61%) HU (17%) ANA (22%)	6.5 (1-12)

Patients have been stratified in 3 subgroups according to the date of ET diagnosis. Eighteen patients were diagnosed between 1977 and 1986, 25 between 1987 and 1996, and 33 between 1997 and 2008. BU: busulfan. IFN: interferon-alpha. ANA: anagrelide. Median time from diagnosis to therapy was seven months (0-171). Treatment rate remained quite high, mostly due to the high number of patients with additional cardiovascular factors and with extreme thrombocytosis (over 1000×10^9/L).

Table 2. Clinical and hematologic characteristics at time of thrombotic event during follow-up.

Patient	Age/Sex	Vascular risk factor	Smoking	Previous thrombosis	Interval diagnosis-thrombosis (mos)	Platelets (x 10^9/L)	Treatment	JAK2^{V617F} mutation	Type of event
1	32/M	no	no	no	144	660	ASA	yes	TIA
2	37/M	yes	no	no	194	1000	ASA	yes	Stroke
3	21/F	no	no	no	46	458	–	no	DVT
4	40/M	yes	yes	no	181	360	BU+ASA	n.a.	AMI
5	34/F	no	no	no	4	700	–	n.a.	DVT
6	25/M	no	no	yes	123	441	IFN+ASA	n.a.	DVT
7	38/M	no	no	no	46	491	BU	no	DVT
					240	396	BU		DVT
8	32/F	no	no	yes	29	719	–	yes	DVT
						600	IFN		DVT
						521	–		DVT
9	37/F	yes	no	yes	4	419	ASA	n.a.	DVT
					49	530	BU+ASA		PAT
					70	400	BU+ASA		DVT
					245	311	BU+ASA		DVT
10	24/M	no	yes	no	30	923	ASA	no	STP
					38	764	IFN+ASA		STP
					65	432	IFN+ASA		STP
					73	511	IFN+ASA		DVT
					82	496	IFN+ASA		STP

Median time from diagnosis to the first thrombotic event was 46 months (range, 4-194). ASA: aspirin. BU: busulfan. IFN: interferon-alpha. n.a.: not available. DVT: deep vein thrombosis. PAT: peripheral arterial thrombosis. AMI: acute myocardial infarction. TIA: transient ischemic attack. STP: superficial thrombophlebitis.

unmutated: 3/6 *vs*. 6/18, *P*=0.6).

We report the long-term outcome of a particular cohort of patients, aged from 18 to 40 years old, followed for a prolonged period of time (median, 13.5 years). Only one patient experienced a leukemic transformation, 16 years after diagnosis, and no patient evolved to myelofibrosis. Although it is generally recognized that young age identifies patients at lower thrombotic risk, our cohort of patients presents a prevalence of thrombotic complication (13%) which is higher than that previously reported in other cohorts of young (<50 yrs) ET patients treated with pipobroman[5] or with hydroxyurea,[3] although our population is younger and follow-up is longer. Moreover, this prevalence was comparable to that observed in young, high-risk patients treated with anagrelide[4] and to that observed in general ET studies.[12]

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Key words: ET, young adults, myelofibrosis.

Funding: the study was supported by the Italian Association for Cancer Research (A.I.R.C.), by Fondazione del Monte di Bologna e Ravenna, by European LeukemiaNet funds and by BolognAIL grants.

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Citation: Palandri F, Polverelli N, Ottaviani E, Castagnetti F, Baccarani M, and Vianelli N. Long-term follow-up of essential thrombocythemia in young adults: treatment strategies, major thrombotic complications and pregnancy outcomes - A study of 76 patients. Haematologica 2010;95:1038-1040. doi: 10.3324/haematol.2009.019190

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Prognostic relevance of CD20 in adult B-cell precursor acute lymphoblastic leukemia

We read with great interest the recent article by Maury *et al.* regarding the influence of CD20 expression on prognosis in adult B-cell precursor acute lymphoblastic leukemia (BCP-ALL).[1] This report described their experience with 143 Philadelphia chromosome negative adult BCP-ALL patients treated with GRAALL-2003, a pediatric-like protocol with high dosage of L-asparaginase.[2] Their cohort included 46 (32%) cases with at least 20% of cells expressing CD20. Univariate analysis of CD20 positivity showed no statistically significant effect on overall survival (OS), cumulative incidence of relapse (CIR), and event-free survival (EFS). However, the authors found that CD20 positivity was associated with higher CIR and shorter EFS among the subgroup of patients with high leukocyte count (>30×10^9/L); they also found CD20 expression correlated with higher CIR e in multivariate analysis of the cohort.

We conducted a similar study analyzing the impact of CD20 expression in BCP-ALL. We evaluated 119 patients diagnosed with *de novo* BCP-ALL between the ages of 18 and 60 (median 40 years) at our institute. There were 49 (41%) patients with at least 20% expression of CD20. All patients were initially treated with a modified pediatric protocol including L-asparaginase similar to the one used in the GRAALL-2003 study, as previously described.[3,4] Philadelphia chromosome positive patients received tyrosine kinase inhibitors in addition to this chemotherapy. In total, 32 patients received allogeneic stem cell transplants; these included 14 (29%) of the CD20-positive cases and 18 (26%) of the CD20-negative cases (P=0.83). CD20 positivity was associated with low platelet counts (P=0.004) but not with any other characteristics at diagnosis, including age (median 40.1 years *vs*. 40.0 years) and expression of CD19, CD13, and CD33.

In univariate analysis using the log-rank test, we found no effect of CD20 expression on OS, CIR, or EFS (P=0.18, P=0.40, and P=0.15, respectively). Leukocyte count greater than 30×10^9/L was associated with poorer OS (median 19.9 months *vs*. not reached, P<0.001), CIR (48% *vs*. 26% at two years, P=0.01) and EFS (median 10.6 months *vs*. not reached, P<0.001). Philadelphia chromosome positivity correlated with poorer OS (median 40.8 months *vs.* not reached, P=0.01), CIR (48% *vs.* 23% at two years, P=0.04), and EFS (median 19.3 months *vs*. not reached, P=0.004). Age over 40 had a significant adverse impact on OS (median 80.6 months *vs.* not reached, P=0.05) but not CIR (P=0.41) or EFS (P=0.10). Allogeneic stem cell transplants were associated with shorter OS (median 40.6 months *vs*. 83.0 months, P=0.02), but had no significant difference on cumulative incidence of relapse (P=0.46) or EFS (P=0.10).

Multivariate analysis with Cox's proportional hazards regression was performed on CD20 as well as the four factors identified as significant above: high leukocyte count, Philadelphia chromosome positivity, older age, and allogeneic stem cell transplant. Only high leukocyte count was an independent predictor of an adverse outcome for OS (HR=2.9 [95% CI 1.4-5.8], P=0.003), CIR (HR=2.7 [95% CI 1.1-6.8], P=0.03) and EFS (HR=2.8 [95% CI 1.5-5.22], P=0.002). CD20 positivity did not significantly influence OS (P=0.11) or CIR (P=0.18), and had a trend towards a favorable EFS (HR=0.6 [95% CI 0.3-1.1], P=0.07; Table 1).

Based on the findings by Maury *et al.*, we also performed subgroup analysis of CD20 expression in the low and high leukocyte count groups. Like them, we found no effect of CD20 expression on outcome in the low leukocyte count subgroup (OS: P=0.48, CIR: P=0.35, EFS: P=0.34). However, in contrast to their findings, we found no association between CD20 positivity and adverse outcome among the high leukocyte count subgroup (OS: median 40.2 months *vs.* 12.4 months, P=0.17, Figure 1A; CIR: 47% *vs*. 52% at two years, P=0.63, Figure 1B; EFS: median 33.1 months *vs*. 9.6 months, P=0.18, Figure 1C).

According to the analysis of Maury *et al.*, we then excluded the 14 Philadelphia chromosome positive patients in this high leukocyte count subgroup. Among the 14 remaining patients, there were 5 positive for CD20. All achieved complete remission (CR) on first induction, 2 later relapsed, and one died of relapsed disease. Of the 9 CD20-negative patients, 8 achieved CR on first induction, 2 later relapsed, and 7 died: 3 were disease-related and 4 (in CR) were due to other causes. Four of 5 CD20-positive patients received at least 80% of the planned L-asparaginase dose, as well as 6 of the 8 CD20-negative patients who achieved CR (P=1.00). Three of the 9 CD20 negative patients had the t(4;11) karyotype. Contrary to the results of Maury *et al.*, we found no correlation between CD20 and CIR (P=0.35) or EFS (P=0.16) among this subgroup; surprisingly, CD20-positive patients in this group appeared to have a longer OS (median not reached *vs*. 9.6 months, P=0.023).

The article by Maury *et al.* was the second to report on the prognostic significance of CD20 in adult BCP-ALL.

Table 1. Multivariate analysis for overall survival, cumulative incidence of relapse, and event-free survival in 119 patients with adult BCP-ALL.

Parameter	Overall Survival		Cumulative Incidence of Relapse		Event-Free Survival	
	P	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)	*P*	Hazard Ratio (95% CI)
Age over 40, n=59	0.09	1.76 (0.91-3.41)	0.58	1.27 (0.55-2.94)	0.22	1.46 (0.80-2.67)
Allogeneic stem cell transplant, n=32	0.51	1.29 (0.61-2.74)	0.46	0.67 (0.23-1.93)	0.67	0.85 (0.41-1.78)
CD20 positivity, n=49	0.11	0.57 (0.28-1.13)	0.18	0.55 (0.23-1.32)	0.07	0.56 (0.30-1.05)
Leukocyte count over 30×10^9/L, n=28	0.003	2.89 (1.44-5.81)	0.03	2.71 (1.09-6.75)	0.002	2.76 (1.46-5.21)
Philadelphia chromosome, n=39	0.42	1.36 (0.65-2.86)	0.14	2.11 (0.78-5.70)	0.09	1.83 (0.91-3.69)