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Chronic myeloid leukaemia with extreme thrombocytosis at presentation: incidence, clinical findings and outcome

Chronic myeloid leukaemia (CML) usually presents with marked leucocytosis, but rare cases may present with an isolated, marked thrombocytosis, defined as a platelet count >1000 × 10^9/l (Turakhia *et al*, 2016).

The natural history and the optimal treatment of this entity are not well characterized. We have previously reported our monocentric experience (Sorà *et al*, 2014); based on these results, we evaluated the incidence, clinical characteristics and outcome of this CML variant in a large series of patients from 16 different Italian haematological centres.

From January 2002 to December 2015, 87 of 1591 patients with extreme thrombocytosis were identified, with an estimated incidence of 5·5%. Patient characteristics are shown in Table I. Coagulation tests were available in 59/87 patients (67·8%), of which only three (5%) had an abnormally prolonged activated partial thromboplastin time. These three patients were investigated for the presence of a non-specific inhibitor, such as antiphospholipid antibodies, and the levels of coagulation factors. In one patient, anti-cardiolipin antibodies were also detected and in another patient a protein C deficiency was identified. However, only a few patients had undergone a full investigation for congenital thrombophilia, including *F2* (prothrombin) G20210A mutation and *F5* R506Q (Factor V Leiden).

Only five patients had a bleeding time test, which was prolonged in all cases (median, 10 min; range, 9-17).

All of the 45 (51·7%) patients studied for the *JAK2* V617F mutation were negative. Only seven female patients showed an iron deficiency.

Bone marrow histology was performed in 49 (56·3%) patients and no increase in bone marrow fibrosis was detected. At diagnosis, 9/87 (10·3%) patients had haemorrhagic or thrombotic complications, with only three having a

Table I. Patient characteristics.

Characteristic	
Patients (*n*)	87
Sex (Male/Female)	24/63
Age (years), median (range)	59 (18–87)
White blood cell count ($\times 10^9$/l), median (range)	31·6 (6·34–390)
Haemoglobin (g/l), median (range)	118 (77–133)
Platelet count ($\times 10^9$/l), median (range)	1466 (1054–4720)
Concomitant drugs, *n* (range)	2 (0-8)
Pre-treatment with hydroxycarbamide (yes/no)	73/14
Spleen size (cm below costal margin), median (range)	0 (0–15)
Liver size (cm below costal margin), median (range)	0 (0–3)
Sokal risk, *n* (%)	
Low	6 (7)
Intermediate	23 (26)
High	55 (63)
Not available	3 (4)

grade >2 event according to the National Cancer Institute Common Terminology Criteria v3·0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev3.pdf): specifically, small leg haematoma, mild epistaxis, small ecchymosis, metrorragia, thrombosis of cephalic vein, right leg deep vein thrombosis, femur-popliteal arterial obstruction, retinal vein occlusion and relapse of recent subarachnoid haemorrhage (all n = 1).

In order to reduce haemorrhagic risk, two patients, who had been treated more than ten years ago, received therapeutic thrombocytapheresis with no relevant reduction in their platelet count.

Seventy-three (83·9%) patients received a pre-treatment with hydroxycarbamide, all whom were then treated with first- and/or second-generation tyrosine kinase inhibitors (TKIs) for a median follow-up time of 66 months (range, 3–179). For first-line treatment, 63 (72·4%) patients received imatinib at a median dose of 400 mg/day, 16 (18·4%) received dasatinib and the remaining 8 (9·2%) patients were given nilotinib. Four patients (median age at diagnosis 82 years, range 46–87) developed a solid tumour (1 prostatic, 1 pulmonary, 1 ovarian and 1 breast cancer) at 20, 24, 38 and 60 months from CML diagnosis, respectively. Only 6/87 patients (6·9%) died after a median time from diagnosis of 36 months (range 3–147) and in all these cases the cause of death was not related to haematological disease.

Seventy-seven patients out of 87 were evaluable after 12 months of treatment and 67/77 (87%) obtained a complete cytogenetic response (CCyR) or a major molecular response (MMR) (Hughes *et al*, 2006; Marin *et al*, 2008; Cross *et al*, 2015); 67 were evaluable after 24 months of treatment and 51 (76%) out of 67 were still in CCyR or MMR. After 60 months, the overall survival (OS) and

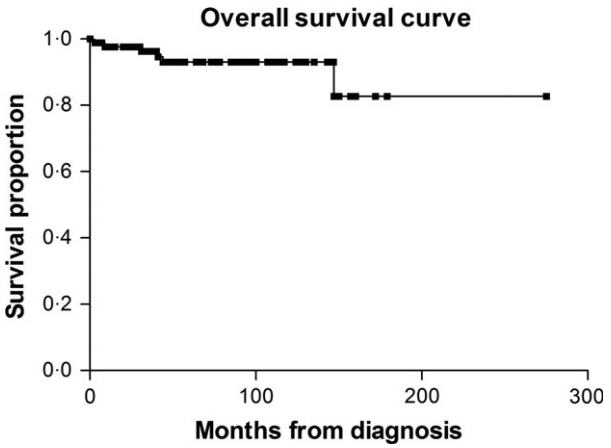

Fig 1. Overall survival curve (median age of follow up 24 months).

progression-free survival (PFS) estimated rates were 93% and 78%, respectively (Fig 1).

Nine patients showed a primary resistance to first-line TKI (7 to imatinib, 2 to nilotinib), but the OS in this group was not significantly different. Two out of the seven patients with resistance to imatinib were positive for an *ABL1* domain point mutation by Sanger sequencing: i.e., V299L and T315I. One primary refractory patient underwent allogeneic transplantation.

No statistically significant difference was found for event-free survival, PFS or OS according to *BCR-ABL1* transcript subtype (e14a2 vs e13a2) or to first- or second-generation TKI use as first-line treatment.

Therefore our study confirms that extreme thrombocytosis in chronic phase CML patients at diagnosis is an infrequent finding. This subset of patients showed the following peculiarities: female predominance, an e14a2 break-point subtype, high or intermediate risk according to Sokal and/or Euro scores, with a favourable outcome in terms of both cumulative cytogenetic and molecular response rate and survival, associated with a low incidence of thrombo-haemorrhagic complications.

According to the World Health Organization criteria, persistent extreme thrombocytosis unresponsive to TKI therapy defines the accelerated phase of CML (CML-AP), whereas this is not the case in the more recent definition of AP of CML by the European LeukaemiaNet 2013 recommendations (Baccarani *et al*, 2013). Similarly, platelet count is included in the variables on which the Sokal and Euro risk scores are based. Therefore, our data challenges the prognostic significance of extreme thrombocytosis in CML patients at diagnosis. Furthermore, considering the European Treatment and Outcome Study (EUTOS) score, which is based only on basophil percentage in the blood smear and spleen size, most of our patients were classified as being low risk. Interestingly, in this subset of CML patients, the cytogenetic and molecular response rates were not significantly different from that reported in the literature for TKI-treated patients without extreme thrombocytosis.

As expected, cytoreductive treatment with hydroxycarbamide did not achieve a relevant reduction in platelet count. In contrast, once TKI therapy was started, the platelet count rapidly returned to the normal range. However, the critical involvement of platelets was not associated with a significant increase in thombo-haemorrhagic complications, which were low in number and severity, and above all, easily manageable.

Due to the inclusion of patients whose CML was diagnosed prior to the acquisition of *JAK2* V617F mutation in the differential diagnosis of thrombocytosis, only half of the patients were assessed for this mutation at diagnosis.

However, it has to be admitted that the coexistence of CML and *BCR-ABL1*-negative myeloproliferative neoplasms is not an infrequent finding (Iurlo *et al*, 2014), indicating the importance of searching for *JAK2* V617F, *CALR* and *MPL* (the latter only if *JAK2* is unmutated) mutations in patients who present with leucocytosis and extreme thrombocytosis. A bone marrow biopsy should always be performed in this particular situation; indeed, an expert haemopathologist is usually able to provide additional information on megakaryocyte morphology and distribution in the bone marrow microenvironment, so indicating the coexistence of two different haematological disorders.

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Author contributions

Federica Sora, Alessandra Iurlo performed the research. Simona Sica, Roberto Latagliata designed the research study. Mario Annunziata,Galimberti Sara, Fausto Castagnetti, Patrizia Pregno, Nicola Sgherza, Francesca Celesti, Monica Bocchia, Antonella Gozzini, Carmen Fava,, Crugnola Monica 11, Enrico Montefusco, Endri Mauro,Capodanno Isabella contributed essential data. Daniele Cattaneo and Federica Sora analysed the data. Federica Sora, Alessandra Iurlo Massimo Breccia wrote the paper.

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HFE mutations in idiopathic erythrocytosis

Absolute erythrocytosis is characterized by persistently raised haemoglobin (Hb) and hematocrit (Ht) levels. The most studied form of absolute erythrocytosis is Polycythaemia Vera (PV), a primary neoplastic disease characterized by the presence of *JAK2* mutations, risk of vascular complications and of evolution to myelofibrosis or acute leukaemia. Secondary erythrocytosis is represented by rare congenital diseases, acquired forms or idiopathic erythrocytosis (IE) (McMullin, 2012). Little is known regarding the clinical characteristics, natural history and best management of patients with IE (Randi *et al*, 2015).

Hereditary Haemochromatosis (HH) is a disease that leads to an iron balance impairment characterized by raised serum ferritin and iron overload in several organs. Type 1, associated with *HFE* gene mutations on chromosome 6 (Yun & Vincelette, 2015) is the most common HH: the most frequent *HFE* mutations observed are H63D and C282Y with allele frequencies in Europe of 13·6% and 3·8%, respectively (Merryweather-Clarke *et al*, 1997). The increased iron bioavailability that results from the defects of molecules involved in iron balance could lead to increased erythropoiesis and hence, erythrocytosis.

Postulating that a possible underlying cause for erythrocytosis could be impairment in iron metabolism, we searched for the presence of *HFE* mutations in IE.

We studied 56 patients with IE: 18 were enrolled in Padua (Italy) and 38 in Belfast (UK). All of the patients had normal serum erythropoietin (EPO) level and none of them carried *JAK2* V617F or *JAK2* exon 12 mutations. Cases of congenital erythrocytosis were excluded because of the absence of a familial increase of red cells and negativity for *EPOR, VHL, PHD2* and *HIF1A* mutations. None of the patients had an overt haemochromatosis or a previous documented *HFE* mutation. Clinical and laboratory data of the patients are shown in Table I.

Table I. Main clinical and laboratory data of patients with erythrocytosis.

	Italian Cohort	UK Cohort	Combined Cohort
N	18	38	56
Males, *n* (%)	17 (94·4)	27 (71·0)	44 (78·6)
Age at diagnosis (years)	54 ± 12	44 ± 14	47 ± 14
Hb at diagnosis (g/l)	178 ± 10	196 ± 19	190 ± 18
Ht at diagnosis (%)	52 ± 3	57 ± 7	55 ± 6
WBC count at diagnosis ($\times 10^9$/l)	8·0 ± 2·5	8·3 ± 2·2	8·1 ± 2·3
MCV at diagnosis (fl)	88 ± 5	96 ± 7	94 ± 7
Platelet count at diagnosis ($\times 10^9$/l)	228 ± 53	220 ± 71	223 ± 65
Erythropoietin at diagnosis (iu)	10·0 ± 5·7	5·9 ± 2·6	7·0 ± 4·1
Ferritin at diagnosis (μg/l)	330·5 ± 239·2	162·9 ± 146·1	227·7 ± 202·5

Values are given as mean ± standard deviation unless otherwise indicated.

Hb, haemoglobin; Ht, haematocrit; MCV, mean cell volume; WBC, white blood cell.

The procedures followed were in accordance with the Declaration of Helsinki and all patients gave informed written consent. We performed the analysis of HFE genotypes using the Lightcycler® 480 (Roche, Burgess Hill, UK).

The frequency of *HFE* mutations among IE patients and the frequency in general population of UK and Italy