

### Bone marrow characteristics predict outcome in a multicenter cohort of primary immune thrombocytopenia patients treated with thrombopoietin analogs

It is well established that immune thrombocytopenia (ITP) results from increased immune mediated platelet destruction (anti-platelets antibodies, autoreactive T cells, and reduction of regulatory T cells) along with impaired production in the bone marrow.<sup>1</sup> The latter has

been attributed to both cellular and humoral mediators that cause suppression of megakaryocyte production and maturation.<sup>2</sup> Current standard first-line therapy consists of corticosteroids, with or without intravenous Ig, achieving about 70-80% response rate. However, a consistent proportion of patients would relapse after corticosteroid discontinuation or tapering, and requires further therapy. Second-line treatments encompass the choice among rituximab, splenectomy, and, more recently, the thrombopoietin analogs (TPOa) romiplostim

**Table 1.** Clinical and bone marrow characteristics of immune thrombocytopenia patients divided according to thrombopoietin analogs treatment.

	All cases N=86	Eltrombopag N=67	Romiplostim N=19
Clinical characteristics			
M, N(%)	35(40.6)	26(38.8)	9(47.4)
F, N(%)	51(59.4)	41(61.2)	10(52.6)
Median age, years (range)	63(6-87)	63(6-87)	61(20-79)
Median Plt, x10 <sup>9</sup> /L (range)	17.5(1-99)	21(1-99)	7(1-49)
*Anti-PLT +, N(%)	28(52)	24(51)	4(17.3)
Bleeding, N(%)	50(58.1)	37(55.2)	13(68.4)
Grade I-II, N	8 – 23	5 – 18	3 – 5
Grade III-IV, N	16 – 3	12 – 2	4 – 1
Therapy lines			
Steroids, N(CR/PR)	86(23/36)	67(15/29)	19(8/7)
Splenectomy, N(CR/PR)	20(9/5)	14(6/3)	6(3/2)
Rituximab, N(CR/PR)	19(2/8)	14(1/6)	5(1/2)
Cyclosporine, N(CR/PR)	16(3/3)	12(1/2)	4(2/1)
Azathioprine, N(CR/PR)	5(0/3)	5(0/3)	0
Danazol, N(CR/PR)	8(1/3)	8(1/3)	0
Bone marrow histology			
Cellularity			
Median % cellularity (range)	40 (8-90)	40(8-90)	47(30-80)
Normal, N(%)	39(45.3)	27(40.3)	12(63.1)
Hyper, N(%)	26(30.2)	21(31.3)	5(26.3)
Hypo, N(%)	18(20.9)	16(23.9)	2(10.5)
Megakaryocytes			
Normal, N(%)	22(25.6)	15(22.4)	7(36.8)
Increased, N(%)	58(67.4)	46(68.6)	12(63.1)
Reduced, N(%)	4(4.7)	4(6)	0(0)
Dysplastic, N(%)	40(46.5)	33(49.2)	7(36.8)
Erythroid precursors			
Hyperplasia, N(%)	9(10.5)	7(10.4)	2(10.5)
Dyserythropoiesis, N(%)	22(25.6)	21(31.3)	1(5.3)
Lymphoid infiltrate			
Median % infiltrate (range)	4(0-18)	1(0-4.5)	3(2-18)
Absent, N(%)	49(57)	34(50.7)	15(78.9)
Mixed, N(%)	12(14)	9(13.4)	3(15.8)
B, N(%)	3(3.5)	2(3)	1(5.3)
T, N(%)	14(16.3)	14(20.9)	0(0)
Fibrosis			
MF0, N(%)	57(66.3)	43(64.2)	14(73.7)
MF1, N(%)	29(33.7)	24(35.8)	5(26.3)

N: number; M: male; F: female; Plt: platelet count; CR: complete response; PR: partial response; MF0: marrow fibrosis grade 0; MF1: marrow fibrosis grade 1. \*Anti-platelets antibodies were tested on 54 patients, 47 on eltrombopag and 23 on romiplostim. Criteria for response were: CR, Plt >100x10<sup>9</sup>/L; PR, Plt >30x10<sup>9</sup>/L.

(ROMI) and eltrombopag (EPAG).<sup>1,3</sup> TPOa have shown high response rates (in up to 90% of cases) in clinical trials with comparable efficacy for both drugs. Their use in the real world showed slightly lower response rates, and interestingly, the possibility of therapy discontinuation or dose reduction in robust responders.<sup>3,4</sup> However, little is known about predictors of response, with the exception of a greater efficacy in early phases of the disease, in non-splenectomized patients, and in those without bleeding signs at diagnosis.<sup>1,5,6</sup> Here we investigated the predictive value of bone marrow features on response to TPOa in a multicentric real world cohort of 86 ITP patients followed a median of 3.8 years (range: 0.4-39.6). Data were retrospectively collected from clinical records and all cases were on active follow up at the time of the study. All the diagnostic procedures and investigations were performed after obtaining informed consent and approval from the Institutional Ethical Committee, and in accordance with the Declaration of Helsinki of 1975. Statistical analysis was performed with Stata15 software. Patients have been treated with EPAG (n=67) and ROMI (n=19) from September 2009 to May 2018, and bone

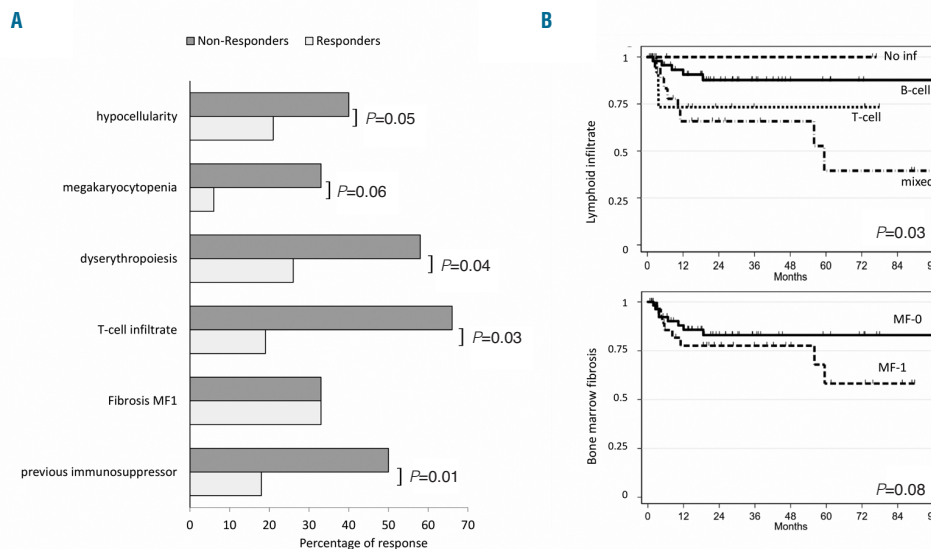
marrow aspirate and biopsy have been performed before TPOa initiation.

As shown in Table 1, patients were mainly middle-aged females presenting with severe thrombocytopenia, bleeding in 58.1% of cases (22% of grade III-IV), and positive anti-PLT autoantibodies in 52% tested cases. Baseline characteristics were comparable between the two groups (ROMI and EPAG). All cases had received first-line treatment with steroids and 43% at least a second line, including splenectomy (23%), rituximab (22%), cyclosporine (19%), azathioprine (6%), and danazol (9%). Response rates confirm those reported in literature (steroids 68%, splenectomy 70%, rituximab 52%, and 40-60% for the others). Bone marrow histology revealed heterogeneous cellularity, with hypocellularity in 20.9% of cases. Concerning megakaryocytes, only 67.4% displayed increased number, and about a half of cases showed dysmegakaryopoiesis. Some dysplastic features were present also in the erythroid lineage, in about one quarter of patients. As regards lymphocyte infiltrate, only 3% of cases had a detectable polyclonal B-cell population, *versus* about 15% with a T-cell or mixed lympho-

**Table 2.** New and known predictors of response to thrombopoietin receptor agonists in immune thrombocytopenia.

Predictor	N	ROMI/EPAG	value	Study type	Follow-up on TPOa	reference
Early phase disease	220	0/220	favorable	retrospective	13 months (5-22)	González-López TJ <sup>6</sup>
Low TPO levels	67	46/37	favorable	retrospective	11 months (0.5-167)	Al-Samkari H <sup>9</sup>
Bleeding	72	72/0	unfavorable	retrospective	24 months (n.a.)	Khellaf M <sup>1</sup>
Splenectomy	124	55/69	unfavorable	retrospective	n.a.	Mazza P <sup>5</sup>
Bone marrow features*	86	19/67	unfavorable	retrospective	16 months (4-130)	Present study

EPAG: eltrombopag; N: number; n.a. not available; ROMI romiplostim; TPO: thrombopoietin; TPOa: thrombopoietin analogs. \*Bone marrow features correlating with lower response rates were bone marrow hypocellularity and fibrosis, megakaryocytopenia, dyserythropoiesis, presence of a Tcell infiltrate and a previous exposure to cyclosporine or azathioprine.



**Figure 1.** Predictors of response and of relapse-free survival in patients treated with thrombopoietin analogs (TPOa). (A) Percentage of responders and non-responders according to bone marrow features or previous treatment. (B) Relapse-free survival (months) by Kaplan-Meier method according to bone marrow megakaryocytes, lymphoid infiltrate, and reticulenic fibrosis. The following hazard ratios emerged: 2.58 (95%CI: 0.61-10.8) for T-cell infiltrate and 4.24 (95%CI: 1.38-13) for mixed infiltrate versus no or B-cell infiltrate, and 1.82 (95%CI: 0.68-4.86) for bone marrow fibrosis grade 1 (MF1).

cyte infiltrate. Notably, one third of patients showed reticular fibrosis bone marrow fibrosis grade 1 (MF-1). Morphological features were comparable between groups treated with the two TPOa. Finally, all patients displayed normal karyotype, except for three cases (1 ROMI and 2 EPAG) showing a chromosome Y deletion. Median time from diagnosis to TPOa was 2.4 years (range: 0.1-28.8 years). Patients were treated for a median of 1.4 years (range: 0.3-10.8 years), and ORR at three months and nine months were 75.6% [Complete Response (CR) 44.2 and Partial Response (PR) 31.4%] and 65.1% (CR 36 and PR 29.1%), respectively. Response rates to EPAG and ROMI were comparable. Regarding predictors of response, bone marrow hypocellularity, megakaryocytopenia, and dyserythropoiesis were significantly more frequent among non-responders to TPOa (Figure 1A). Other factors significantly associated with poor response were the presence of a T-cell infiltrate, or a previous exposure to cyclosporine or azathioprine. At the last follow up, 55 patients were still on treatment, whereas 31 (n= 20 EPAG; n=11 ROMI) discontinued because of lack of response or relapse (n=17), adverse events or intolerance (n=2); the other 12 patients, showing a robust response discontinued the drug, and seven of these are still in remission. Fourteen out of 65 responders (21.5%), ten EPAG and four ROMI, relapsed after a median of 6.2 months (range: 1.8-60 months) and were variably managed (3 splenectomized, 1 switched from ROMI to EPAG, 1 received danazol, 5 were re-treated with EPAG, and the remaining were managed with steroids and supportive treatment). Presence of T-cell or mixed lymphoid infiltrate and reticular fibrosis were associated with shorter relapse-free survival (RFS) (Figure 1B). During EPAG treatment, seven adverse events occurred: two grade IV (1 stroke with platelet counts of about  $30 \times 10^9/L$ , and 1 non-ST elevated myocardial infarction 1 month after EPAG discontinuation for sustained CR), one grade III pneumonia, and four grade I/II transaminase elevation. No events occurred under ROMI.

This is the first paper aimed at evaluating the predictive value of bone marrow features on the response to TPOa (Table 2). In particular, we identified hypoplasia of the megakaryocytic lineage and dysplastic features as factors associated with a poor response to TPOa. As a matter of fact, bone marrow trephine is not routinely performed in ITP. Therefore, it is not possible to exclude the possibility that a proportion of patients enrolled in the registered trials eventually belong to the idiopathic cytopenia/dysplasia of uncertain significance (ICUS/IDUS). The latter are recently described entities, characterized by borderline cytopenia/dysplasia not reaching the diagnostic threshold for myelodysplastic syndrome (MDS) and possibly preceding overt disease.<sup>7</sup> Consistently, bone marrow biopsy monitoring over time has shown a possible evolution of chronic relapsing ITP into ICUS/IDUS.<sup>8</sup> Moreover, an additional category has been described, namely the clonal cytopenia of unknown significance (CCUS), where a marker of clonality is present. A complex genomic landscape has been reported in the ICUS/IDUS mainly involving the erythroid lineage, and a similar workup would be advisable in ITP, since the occurrence and type of somatic mutations are completely unknown. It is well established that baseline endogenous erythropoietin levels predict response to therapy with erythroid stimulating agents in MDS. In this context, it is worth mentioning a recent report showing that low TPO levels predict a greater probability and magnitude of response to TPOa.<sup>9</sup> On the contrary, patients with extreme elevation in TPO are unlikely to respond, suggesting that baseline serum

TPO levels may be clinically useful and give insights in the variability of response to TPOa. Interestingly, EPAG was shown effective in increasing platelets and reducing bleeding in about 40% of low-risk MDS.<sup>10</sup> Likewise, a large randomized phase II study with ROMI in low-risk MDS demonstrated reduced platelet transfusions and hematologic improvement, without increased risk of clonal evolution.<sup>11</sup> Moreover, EPAG induced up to 40% trilineage hematologic improvement in relapsed/refractory aplastic anemia (AA), and ameliorated response to standard immunosuppression (anti-thymocyte globulin and cyclosporine), indicating its effect on the stem cell pool.<sup>12,13</sup> On the whole, although responses are observed in MDS and AA, EPAG and ROMI are more effective in classic ITP, confirming the importance of megakaryocytic reservoir.

As far as bone marrow features are concerned, in TPOa treated cases, bone marrows were characterized by myeloproliferative neoplasm-like features, resembling those of essential thrombocythemia or occasionally early primary myelofibrosis, with increased reticular fibrosis and trilineage hematopoiesis.<sup>14</sup> Here, we found that reticular fibrosis before TPOa therapy predicted shorter RFS, underlining the detrimental role of this morphological feature. This is in line with what has already been reported in several conditions including MDS and autoimmune hemolytic anemia.<sup>15</sup> Finally, we reported that the presence of a bone marrow T-cell infiltrate negatively impacted on response to TPOa and RFS. At the same time, we observed a lower response rate to TPOa in patients previously treated with cytotoxic immunosuppressants (cyclosporine and azathioprine). It can be hypothesized that these cases, notwithstanding immunosuppressive therapy, still show a prominent cellular-mediated immune attack to bone marrow precursors.

In conclusion, there is growing interest in the identification of predictors of response to TPOa in several hematologic conditions. In addition to the previously reported predictors of worse outcome (late phase of the disease, previous splenectomy, bleeding signs at diagnosis, high endogenous TPO), we added bone marrow characteristics such as megakaryocytopenia, dysplastic features, reticular fibrosis, and T-cell infiltrate. Therefore, bone marrow evaluation prior to initiate TPOa in ITP is advisable, as it may help in harnessing therapy and in unraveling the pathophysiological mechanisms underlying TPOa refractoriness.

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