## Elevated plasma thrombopoietin levels and thrombosis in essential thrombocythemia: a preliminary report

Thrombopoietin may activate platelets in vitro in a concentration-dependent manner. We correlated plasma thrombopoietin with symptoms of thrombosis in patients with essential thrombocythemia and found it elevated in 5 symptomatic cases and normal in 7 asymptomatic ones (mean values, respectively: 215.5±49.0 versus 114.4±21.1 pg/mL). Raised thrombopoietin levels might be involved in essential thrombocythemia-associated thrombosis.

Thrombotic complications are common in essential thrombocythemia (ET)[1] and do not necessarily correlate with thrombocytosis and increased concentrations of plasma β-thromboglobulin (βTG) and platelet factor 4 (PF4).[2,3] Thrombopoietin (TPO) has recently been studied in vitro and in vivo in order to determine its biology and to measure its circulating levels, respectively. These studies showed that TPO induces platelet activation in a concentration-dependent manner[2,4] and that the levels of TPO are elevated or normal in clonal thrombocytosis.[5] We, therefore, investigated 12 patients (5 M, 7 F) affected by ET, as defined by the Polycythemia Vera Study Group criteria. We measured their plasma TPO, βTG and PF4 levels and platelet count and tried to correlate these results with a history of thrombosis. The patients had a median age of 68.4 years (range 46-80) and all were treated with hydroxyurea and antiaggregating drugs, either aspirin (ASA) or ticlopidine (TIC) and dipirydamole (DYP). Thrombotic complications had occurred in 5 patients (2 M, 3 F; median age: 68 years) and included two episodes of deep vein thrombosis (DVT), two of transient ischemic attack (TIA) and one of microvascular thrombosis of extremities (erythromelalgia) (E) (symptomatic group). The other group consisted of 7 patients (3 M, 4 F; median age: 68.7 years) who had not experienced thrombosis (asymptomatic group). The presence of thrombotic risk factors was excluded. The mean duration of disease for the whole group of patients considered was 7.25 years (range 1-19; median value: 6 years).

Plasma TPO, βTG and PF4 levels, and platelet counts were determined by ELISA (Quatikine™ Human TPO Immunoassay, R&D Systems, Minneapolis, MN, USA, Diagnostica Stago Boehringer Mannheim, Germany) and by a Sysmex NE-1500 cell counter, respectively.

The results are summarized in Tables 1 and 2.

All the patients had increased levels of βTG, PF4 and platelet count (>600×10⁹/L). The patients (5 cases) who had developed thrombotic complications had elevated plasma TPO levels. Contrariwise, the patients who never had thrombosis (7 cases) had normal plasma TPO values. No significant difference was found between βTG and PF4 levels and platelet count when the two groups of patients with thrombosis (symptomatic) and without thrombosis (asymptomatic) were compared. Conversely, a statistically significant difference in plasma TPO levels was found between symptomatic and asymptomatic thrombocythemic patients ($p<0.01$, Student's t-test).

In accordance with previous studies, we found no association between high values of βTG and PF4, thrombocytosis and thrombosis. We did, however, note a positive correlation between elevated TPO levels and thrombotic events. Therefore, platelet function analysis, which is currently evaluated with measurements of βTG and PF4, may not be the only predictive factor for thrombosis, although at this time its value as a parameter for instigating anticoagulant therapy cannot be excluded. Although preliminary, these findings might suggest that the thrombotic drive in ET could be exerted by raised TPO levels. We, therefore, suggest that serial measurements of TPO concentrations be made in patients with a diagnosis of ET in order to provide useful prognostic information and a rationale for anti-thrombotic therapy. Measurement of plasma TPO levels in essential thrombocythemia is a simple test to discriminate patient subsets with an increased risk of thrombosis. Other authors[6] have reported fewer thrombotic events in patients with polyclonal rather than monoclonal ET. However, clonality status can be difficult to assess and clonal analysis is not common in the clinical field. We did not study clonality in our patients. We think that TPO measurement is a more direct and less costly test to assess the risk of thrombosis in these patients.

These data, if confirmed in larger studies, might also support the rationale for using more effective antithrombotic strategies for the prevention of ASA-TIC-DYP-resistant thrombo-occlusive complications developing in ET patients.

**Table 1. Bioclinical parameters of ET patients.**

| Pts/sex/age | β-TG (10-40IU/mL)* | PF4 (0-5 IU/mL)* | Platelets (150-450 ×10⁹/L)* | TPO (50-150 pg/mL)* | Thrombotic events |
|---|---|---|---|---|---|
| 1/M/74 | 250 | 250 | 215 | 102 | No |
| 2/M/46 | 220 | 188 | 790 | 300 | Yes/DVT |
| 3/M/49 | 260 | 225 | 653 | 101 | No |
| 4/M/67 | 220 | 100 | 467 | 150 | No |
| 5/F/71 | 250 | 214 | 867 | 190 | Yes/TIA |
| 6/F/75 | 240 | 198 | 509 | 102 | No |
| 7/F/82 | 240 | 90 | 701 | 205 | Yes/DVT |
| 8/F/68 | 250 | 95 | 644 | 175 | Yes/TIA |
| 9/F/62 | 230 | 105 | 622 | 207 | Yes/E |
| 10/F/77 | 270 | 104 | 1340 | 140 | No |
| 11/M/80 | 250 | 105 | 574 | 105 | No |
| 12/F/70 | 260 | 223 | 664 | 101 | No |
| Mean° | 245±15.7 | 158.1±62.7 | 668.8±268.1 | 156.5±61.8 | |

*In brackets normal range; °Values shown are means±SD. DVT: deep vein thrombosis; TIA: transient ischemic attack; E: erythromelalgia.*

**Table 2. Bioclinical correlates in ET patients.**

| | Patients with thrombosis (n=5) | Patients without thrombosis (n=7) | Statistical significance |
|---|---|---|---|
| β-TG | 238 ± 13 | 250 ± 16.3 | $p$ = NS |
| PF4 | 138.4 ± 58.1 | 172.1 ± 66.4 | $p$ = NS |
| Platelets | 724.8 ± 102.6 | 628.9 ± 346.7 | $p$ = NS |
| TPO | 215.4 ± 49 | 114.4 ± 21.1 | $p < 0.01$ |

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