Chapter 11

In Multiple Myeloma, Increased Thrombopoietin Production May Be Involved in the Maintenance of Platelet Production
In Multiple Myeloma, Increased Thrombopoietin (Tpo) Production May Be Involved in the Maintenance of Platelet Production

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ABSTRACT

In multiple myeloma (MM), suppression of haematopoiesis occurs as a result of expansion of malignant cells in the bone marrow. Thrombopoietin (Tpo) levels in patients with impaired platelet production are generally found to be highly elevated. To examine the circulating Tpo levels in patients with MM, Tpo levels were measured in 50 serum samples from 34 patients. Tpo levels were subsequently related to disease stage, and cell numbers and markers, i.e. platelet count, leukocyte count and haemoglobin (Hb) concentration. Elevated Tpo levels were found in association with decreased platelet counts (n=8), but also in patients with normal platelet counts (n=14). The latter group included patients without and with signs of impaired haematopoiesis, i.e. with decreased Hb concentration and decreased leukocyte count. These results show that neither platelet counts nor Tpo levels are reliable parameters to judge bone-marrow failure in patients with MM. Furthermore, in patients with MM, increased Tpo levels may play a role in the maintenance of thrombocytopoiesis. The origin of the increased Tpo levels remains to be determined.
INTRODUCTION

In patients with thrombocytopenia resulting from impaired platelet production, such as occurs in patients with congenital amegakaryocytic thrombocytopenia, aplastic anemia and in patients with cancer who are treated with myelosuppressive or myeloablative therapy, thrombopoietin (Tpo) levels are consistently found to be elevated [1-11]. Tpo is the main regulator of thrombopoiesis [12-14]. It is well accepted that the increase in Tpo concentration in patients with a decreased platelet production, results from a diminished clearance of Tpo from the circulation. Normally, Tpo is removed by binding to the Tpo receptor, c-Mpl, present on cells from the megakaryocytic lineage and platelets [15-20]. Absence or diminished numbers of these cells will result in Tpo accumulation in the circulation since Tpo production, with the liver as its main source, is mainly constitutive.

In multiple myeloma (MM), suppression of haematopoiesis occurs as a result of expansion of malignant cells in the bone marrow. Thrombocytopenia is frequently observed in these patients. In the current study, Tpo levels were measured in serum samples from patients suffering from MM either with or without thrombocytopenia and related to markers for disease progression, i.e. clinical staging, haemoglobin (Hb) concentration, leukocyte count and creatinin concentration. In addition, interleukin-6 levels were measured because this cytokine is known to be involved in the pathogenesis of MM [21,22]. IL-6 can support the growth of human myeloma cells and, MM cells can in their turn, induce IL-6 production by stromal cells. Recently, it was reported that IL-6 can increase Tpo production by human liver cell lines in vitro ([23] and our own unpublished observations).

We report that elevated Tpo levels were found in patients with thrombocytopenia, which is in accordance with impaired platelet production. However, elevated Tpo levels were also found in patients with normal platelet counts, both in combination with normal leukocyte counts and Hb levels and in combination with decreased levels of these markers.

PATIENTS AND METHODS

Patient material

In a retrospective study, serum samples from 34 patients with MM were analysed, that were collected and stored between March 1988 and June 1996. In table I, patient characteristics and laboratory test results at the time of sampling are summarised. Samples were collected at different stages of disease progression.

Tpo ELISA

Tpo levels were measured with our previously described solid-phase sandwich ELISA [10]. Results from stored controls that were tested repetitively in time were reproducible (data not shown). Therefore, the storage time of frozen samples did not influence Tpo measurements. Serum Tpo levels in 136 healthy controls ranged from 6-69 Arbitrary Units/ml (AU/ml), which corresponds with 19-221 pg/ml when calibrated against rHuMGDF (MGDF-A), the full-length rHuTpo molecule, which was a generous gift from Amgen (Thousand Oaks, CA,
Table I: Patient characteristics and clinical data

<table>
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<th>Tpo&gt;69 AU/ml</th>
<th>Tpo≤69 AU/ml Tpo&gt;69 AU/ml</th>
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* According to Durie and Salmon (1975)
** x10⁹/L

USA). When calibrated against the rhTpo standard from Research Diagnostics Inc. (Flanders, NJ, USA) 1 A.U. equals 9 pg of Tpo.

**Interleukin-6 ELISA**

For the measurement of IL-6 in serum, the commercially available IL-6 kit (Pelikine kit, CLB, the Netherlands) was used according to the manufacturer's instructions.

**RESULTS**

A total of 50 serum samples were available from 34 MM patients (table I). Figure 1 shows the Tpo levels in these patients compared to controls. Elevated Tpo levels were present in MM patients with thrombocytopenia but also in MM patients with normal platelet counts. There was no correlation between the paraprotein level and Tpo concentration.

Of the 34 patients, 20 patients had elevated Tpo levels (i.e. above 69 U/ml) as detected in 29 serum samples. For 8 samples (npatients=8) platelet counts were decreased, which is in accordance with MM-related thrombopoietic failure. However, for 21 out of 29 samples elevated Tpo levels were related to normal platelet counts [range 181-333] (npatients=14). Nine of the 14 patients in this group either had a decreased Hb concentration, a decreased leukocyte count or both. Four of these 14 patients were classified in clinical stage III. Elevated Tpo levels were also found in combination with normal platelet and leukocyte counts and a normal Hb concentration in samples of seven patients.
Figure 1: Box plots representing the serum Tpo levels of healthy controls and patients with multiple myeloma. Boxes represent the interquartile range containing 50% of all values. The line across the box indicates the median, whereas the whiskers extend to the highest and the lowest value. The circles represent outliers (cases with values between 1.5 and 3 box lengths). The MM group is subdivided in patients with normal platelet (plt) counts and patients with decreased platelet counts, i.e. less than $150 \times 10^9$ platelets per liter.

Among the group with normal platelet counts, there was no significant difference in age, sex, clinical staging, creatinin level, leukocyte count or haemoglobin concentration when comparing patients with normal Tpo levels and elevated Tpo levels ($p>0.05$; Mann-Whitney U test). These parameters were also not different when comparing patients with decreased platelet counts versus patients with normal platelet counts.

Analysis of all samples showed that there was no correlation between platelet count and serum Tpo concentration.

To examine a possible relation between IL-6 and Tpo in MM, IL-6 levels were measured in 34 samples of 34 patients. IL-6 was detectable in 3 patients (24, 25 and 58 pg/ml respectively). All other samples were under the limit of detection (<10 pg/ml). Tpo levels in all three samples were elevated (148, 419 and 111 AU/ml, respectively) and platelet counts were decreased in the first two patients (84 and $48 \times 10^9$/L) and normal in the third patient ($290 \times 10^9$/L).

**DISCUSSION**

In this report it is shown that, compared to healthy controls, elevated serum Tpo levels were present in MM patients with thrombocytopenia, but also in MM patients with normal platelet counts. So far, elevated Tpo levels have mainly been reported in association with thrombocytopenia (i.e. resulting from impaired platelet production [1-11] or increased platelet consumption [24,25]) and hereditary thrombocytosis [26-28].

In analogy with other diseases in which thrombopoiesis is impaired, the elevated Tpo levels in MM patients with thrombocytopenia is most likely caused by a diminished number of platelets and megakaryocytes, resulting in a decreased clearance of Tpo by these cells. In
Tpo levels in multiple myeloma, malignant cells accumulate in the bone marrow, thereby suppressing the outgrowth of haematopoietic progenitor cells, including megakaryocytic progenitors, leading to diminished platelet formation.

The presence of elevated Tpo levels in MM patients with normal platelet counts might suggest that overproduction of Tpo is involved in the maintenance of normal thrombocytopoiesis in these patients. Indeed, normal platelet counts, in combination with elevated Tpo levels, were found in patients with signs of impaired hematopoiesis, i.e. decreased Hb values and/or a decreased leukocyte count and/or classified in clinical stage III. In addition, elevated Tpo levels were also found in patients in whom these parameters were within the normal range, suggesting the presence of normal hematopoiesis. This implies that measurement of platelet count in MM is not always a reliable parameter for bone-marrow suppression and disease progression, because increased Tpo levels might selectively enhance thrombocytopoiesis.

The origin of the increased Tpo concentrations remains to be investigated. It has been described that IL-6 can augment Tpo production by human liver-cell lines in vitro [23]. IL-6 is known to be involved in MM [21,22] and therefore is a likely candidate to be involved in a possible increase of Tpo production. However, no relation between the concentration of Tpo and IL-6 was present in the investigated patient group: Tpo levels were elevated in all three patients in whom IL-6 was detectable, but two of these patients were thrombocytopenic.

In analogy with interleukin-6 [21,22], MM cells may induce Tpo production by bone-marrow stromal cells, or myeloma cells themselves might produce Tpo. Whether Tpo plays a role in the pathogenesis of MM remains a topic of interest. In the current study, no difference was found with respect to markers for disease progression, i.e. clinical stage, creatinin concentration, leukocyte count and Hb concentration, between patients with a normal platelet count and either a high or a normal Tpo level. However, a prospective follow-up study should be performed to judge the possible clinical significance of Tpo measurements in the diagnosis of MM.

REFERENCES

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