Advances in Medical Sciences 59 (2014) 166–171

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Does thrombopoiesis in multiple myeloma patients depend on the stage of the disease?

Joanna Kamińska [a,*], Olga M. Koper [a], Maria Mantur [a], Joanna Matowicka-Karna [a], Jolanta Sawicka-Powierza [b], Jarosław Sokołowski [c], Agnieszka Kostur [c], Agnieszka Kulczyńska [c], Janusz Kłoczko [c], Halina Kemona [a]

[a] Department of Clinical Laboratory Diagnostics, Medical University of Bialystok, Bialystok, Poland
[b] Department of Family Medicine and Community Nursing, Medical University of Bialystok, Bialystok, Poland
[c] Department of Hematology, Clinical Hospital of the Medical University of Bialystok, Bialystok, Poland

ARTICLE INFO

Article history:
Received 21 February 2013
Accepted 17 December 2013
Available online 9 June 2014

Keywords:
Multiple myeloma
Platelet count
Thrombocytopoiesis
Thrombopoietin

ABSTRACT

Purpose: Infiltration of the bone marrow by neoplastic plasmocytes in multiple myeloma (MM) patients might impair megakaryocytopoiesis. The aim of the study was to evaluate stage-dependent platelet count (PLT) and thrombopoietin (TPO) concentration in comparison to the control group. We also wanted to establish whether TPO might be recognized as a marker of the stage of the disease.
Material/methods: The study group consisted of 41 patients (mean age 67.7) with newly diagnosed MM prior to treatment and categorized according to the *Durie and Salmon* diagnostic classification. The control group consisted of 30 healthy subjects (mean age 65.5). PLT, WBC, RBC and Hb were measured with the use of the haematological analyser. TPO was assayed with the use of ELISA and albumin with the use of the immunonephelometry method. The number of plasma cells in the bone marrow was evaluated in bone marrow smears under light microscopy.
Results: PLT was not statistically different as compared the control groups, but was stage-dependent. Thrombocytopenia was observed in the III stage of MM. TPO median was significantly higher in study group than in healthy subjects and it was increasing considerably with the stage of the disease. TPO concentration was negatively correlated with albumin and PLT. AUC for TPO was 0.9764. The number of plasma cells in the bone marrow was considerably increasing with the stage of the disease.
Conclusions: PLT and TPO in MM patients were stage-dependent. Elevated TPO concentration in MM patients might be an unfavourable marker of the stage of the disease.

1. Introduction

Multiple myeloma (MM) is one of the most common haematologic cancers of the haematopoietic system. It is characterized by clonal proliferation of plasma cells in the bone marrow, which overproduce monoclonal immunoglobulins, usually IgG or IgA [1,2]. Accumulation of malignant plasma cells in the bone marrow might impair the three lineages formation of the haematopoietic system, including the production of platelets (within inadequate platelets production) [3–6].

Infiltration of plasma cells to the bone marrow in MM patients may lead to thrombocytopenia, which is the main reason for haemorrhagic incidences [4]. Literature data have shown that antineoplastic treatment may also induce thrombocytopenia [7–9]. Single studies have also indicated that shortened platelet half-life in MM patients could be the reason for a decreased platelet count (PLT) [10,11]. Additionally, thrombocytopenia might also result from the specific interaction of monoclonal paraproteins with platelets leading to the impaired platelet function (thrombocytopathy) and their increased turnover [12,13].

Thrombopoietin (TPO) is the major factor that regulates PLT [14–16]. It is taken from the blood mainly by the haematopoietic stem cells, megakaryocytic progenitors, megakaryocytes and platelets which expressed TPO receptor (c-Mpl). There are approximately 30–60 high-affinity binding sites for TPO per platelet [15]. *In vivo* as well as *in vitro*, the TPO concentration is inversely proportional to PLT and/or megakaryocytes in the bone marrow [17].

* Corresponding author at: Department of Clinical Laboratory Diagnostics, Medical University of Bialystok, Waszyngtona 15A, 15-269 Białystok, Poland. Tel.: +48 85 746 8584; fax: +48 85 746 8584; mobile: +48 508 739 863.
E-mail address: joanna.kaminska@umb.edu.pl (J. Kamińska).

http://dx.doi.org/10.1016/j.advms.2013.12.006
1896-1126/

In the course of neoplastic diseases, the physiological inverse relationship between PLT and TPO concentrations might be disturbed due to the possible production of haematopoietic cytokines by malignant cells and bone marrow stromal cells [18,19].

The aim of our study was to evaluate thrombopoiesis in MM patients based on PLT and TPO concentrations depending on the stage of the disease, compared to the control group. We also wanted to establish whether TPO might be recognized as a marker of the stage of the disease and whether the physiological inverse relationship between PLT and TPO concentrations was maintained in MM patients? White blood cell count (WBC), red blood cell count (RBC), and haemoglobin (Hb), and albumin concentrations as additional auxiliary parameters were assessed in comparison to the control group, taking the stage of the disease into account.

2. Materials and methods

The study group consisted of 41 patients with newly diagnosed MM, prior to treatment (mean age 67.7, ranged 47–86). Patients were diagnosed according to the World Health Organization (WHO) criteria, that included (1) an increased number of abnormal, atypical or immature plasma cells in the bone marrow or histological proof of plasmocytoma, (2) the presence of M protein in the serum or urine, (3) bone lesions [20]. Patients were excluded if they had diabetes mellitus, cardiac failure, coexisting neoplastic disease, thromboembolic disease or chronic infection.

The study group (MM) was categorized depending on the stage of the disease according to the *Durie and Salmon* staging system [21]: I stage – 14 patients (5 F/9 M, mean age 65.9, ranged 48–80); II stage – 17 patients (9 F/8 M, mean age 65.9, ranged 48–87); III stage – 10 patients (4 F/6 M, mean age 75.3, ranged 48–87). The control group (C) consisted of 30 healthy volunteers (mean age 65.5, ranged 45–77).

Patients' samples were collected between 2009 and 2011. Blood samples were drawn from each patient and healthy subjects without stasis between 6 and 7 o'clock in the morning following a fasting period of 10–12 h. Blood samples collected into EDTA-K_2 tubes were analysed within 2 h of venipuncture. Tubes with the blood collected without anticoagulant were allowed to clot for 30 min before centrifugation for 15 min at $1000 \times g$, obtained serum was stored at $-75\ ^{\circ}C$ for further analysis.

The study was approved by the Bioethics Committee on human research of the Medical University of Bialystok (permission number: R-I-002/112/2009).

2.1. TPO ELISA

The TPO concentration in the serum was determined with the use of quantitative sandwich immunoassay technique ELISA (Quantikine Human TPO Immunoassay – for the quantitative determination of human TPO concentrations in cell culture supernates, serum and plasma, R&D Systems Inc., Minneapolis, USA).

According to the manufacturer's information, the TPO minimum detectable dose (MDD) ranged from 2.78 to 18.5 pg/mL. The mean detectable TPO concentration in serum samples in healthy individuals was 74.2 pg/mL.

2.2. Measurement of PLT, RBC, WBC and Hb

The PLT, RBC, WBC and Hb were determined in the blood collected into EDTA-K_2 tubes with the use of ADVIA 2120i haematological analyser (Siemens, Berlin, Germany), according to the manufacturer's instructions.

2.3. Albumin concentration

The albumin concentration was measured with the use of immunonephelometry method on the BN* II (Siemens, Berlin, Germany).

2.4. The number of plasma cells

The number of plasma cells in the bone marrow was evaluated in bone marrow smears under light microscopy.

2.5. Statistical analysis

The results were statistically analysed with the use of the STATISTICA 9.0 PL software (StatSoft Inc., Tulsa, USA). Mann–Whitney's test was used in order to compare two independent samples and ANOVA rank Kruskal–Wallis test was used for the comparison of three samples. The value for each given measured variable is given as median and range. Differences were considered statistically significant for $P < 0.05$. Correlation coefficients were obtained by applying Spearman's rank method. The relationship between sensitivity and specificity for TPO was illustrated with the use of the receiver operator characteristic (ROC) curve.

3. Results

Tables 1 and 2 present statistical data (medians and ranges) concerning PLT, TPO concentration, WBC, RBC, Hb and albumin concentrations, the percentage of plasma cells in MM patients in general and depending on the stage of the disease and in comparison to the healthy controls.

PLT in myeloma patients in general was not statistically different as compared to the control group (Table 1), but significantly decreased with the stage of the disease (Fig. 1). Thrombocytopenia was observed only in III stage patients, however PLT below $150 \times 10^3/\mu L$ and below $100 \times 10^3/\mu L$ were noted in 50% and 30% of III stage patients respectively (data not shown).

There was a sixfold increase in TPO concentration in MM patients as compared to the healthy controls (Table 1) and a considerable increase with the stage of the disease (Fig. 2). Fig. 3 shows the PLT and TPO concentrations in MM patients depending on stage of disease. AUC for TPO was 0.9764, as shown in Fig. 4. Table 3 presents the diagnostic usefulness of TPO in MM patients. In MM patients in general TPO concentration was negatively correlated with the PLT ($r = -0.39$; $P = 0.013$) and with albumin concentration (Fig. 5).

WBC count in MM patients was not significantly different than in healthy subjects, and did not change depending on the stage of the disease. RBC count and Hb concentration were significantly

Table 1
Statistical analysis of data in MM patients in general and in Control (C) group. PLT, TPO, WBC, RBC, Hb and Albumin expressed as medians and ranges.

	MM group ($N = 41$)	Control group ($N = 30$)	P
PLT [$\times 10^3/\mu L$]	212 29–361	230 132–371	0.177
TPO [pg/mL]	157.95 30.26–452.02	29.62 11.09–60.08	**0.0001**
WBC [$\times 10^3/\mu L$]	5.81 1.38–28.21	6.04 4.77–9.25	0.384
RBC [$\times 10^6/\mu L$]	3.59 2.11–4.88	4.88 4.11–5.68	**0.0001**
Hb [g/dL]	10.70 7.1–14.3	14.15 12.1–16.3	**0.0001**
Albumin [g/dL]	3.74 1.69–4.79	4.37 3.79–4.70	**0.0001**