Bone marrow megakaryocytes, soluble P-selectin and thrombopoietic cytokines in multiple myeloma patients.

Dorota Lemancewicz[1,2], Łukasz Bołkun[2], Maria Mantur[3], Jarosław Piszcz[2]
Janusz Kłoczko[2], Janusz Dzięcioł[1]
Departments of [1]Human Anatomy, [2]Hematology, [3]Clinical Laboratory Diagnostics, Medical University of Bialystok, Poland

Background. The expression of adhesion molecules and other cell-surface molecules is substantial in the communication between plasma cells and bone marrow microenvironment. Many of the cytokines involved in multiple myeloma (MM) pathogenesis, e.g. thrombopoietin (TPO) and interleukin-6 play a pivotal role in different developmental stages of megakaryocytopoiesis and thrombopoiesis. The principal aim of our study was to explore the relationship between thrombopoietic cytokines, megakaryocytes (MKs) and soluble P-selectin (sP-selectin) levels in MM patients before and after anti-angiogenic treatment.

Material and methods. Forty-four patients (20 female and 24 male) with a newly diagnosed MM were examined in three groups, following a division based on the International Staging System, ISS. Plasma levels of TPO, IL-6 and sP-selectin were measured by means of ELISA. Bone marrow specimens were studied to determine the number of MKs and the so-called “naked nuclei” (NN), as well as the expression of platelet-derived growth factor (PDGF).

Results. The comparison revealed a significantly higher concentration of cytokines and sP-selectin in newly diagnosed MM patients compared to healthy volunteers: for TPO, p = 0.01, IL-6, p = 0.0005 and sP-selectin, p = 0.00008, respectively. Marked differences were observed in the concentration of sP-selectin, expression of PDGF and MKs counts between patients with MM stage I and MM stage III. Statistically meaningful correspondences were also found between MKs vs TPO, NN vs TPO, as well as MKs vs MPV, p = 0.009, p = 0.004 and p = 0.0005, respectively. Furthermore, the analysis exhibited some statistically meaningful divergences between initial concentrations of sP-selectin in subgroups with different response after chemotherapy. We found a correlation between sP-selectin and IL-6 (rho = 0.57, p = 0.0004), TPO and IL-6 (rho = 0.46, p = 0.001) as well as sP-selectin and TPO (rho = 0.36, p = 0.043), and sP-selectin and PDGF (rho = 0.36, p = 0.03).

Table 1. Clinical features of MM patients.

Number of patients	N- 44
Age	60 (range 52-71)
Stage ISS I II III	 N- 8 N- 20 N- 16
Solitary plasmocytoma	0
HGB [g/dl]	10.34±1.17
Serum M Protein [g/dl]	2.46 ±1.0
Serum Albumin [g/dl]	3.84±0.45
Ca2+ [mmol/l]	2.43±0.54
IgG [mg/dl]	3754.0±1078,50
β2m [mg/l]	3969.30±1645.20
LDH [IU/l]	409.5±65.62
% plasma cells in TB	51.80±22.70
% plasma cell in smear BM	23.50±17.30
Creatine level [mg/dl]	1.08±0.40
PLT [x10^3]	210.8±50.90
MPV [fL]	8.27±0.53

The values are presented as mean ±SD, MM- multiple myeloma, ISS- International Staging System, HGB-hemoglobin, M-monoclonal, Ca- calcium, IgG-immunoglobulin G, β2m- beta-2-microglobulin, LDH- lactate dehydrogenase, TB-trephine biopsy, BM – bone marrow, PLT –platelets count, MPV - mean platelet volume

Table 2. The mean values of chosen parameters of MM patients and healthy volunteers.

Parameter	Untreated patients				After The treatment n=44
	New diagnosed patients n=44	Patients at I ISS n=8	Patients at II ISS n=20	Patients at III ISS n=16	
TPO [pg/ml]	52.0±12.36	34.88±7.35	38.33±0.11	42.85±11.1*	46.47±5.74**
sP-Sel [ng/ml]	33.64±7.06	33.72±1.12	32.06±2.03	37.55±0.75*	27.68±6.51**
IL-6 [pg/ml]	20.30±6.17	17.31±0.68	18.0±0.92	23.36±2.40*	11.61±6.0**
PLT [x10³/l]	210.80±49.30	234.0±26.30	216.2±45.33	192.50±58.82	234.40±32.40
PDGF [%]	68.63±14.15	65.21±16.43	66.0±14.04	73.55±11.30*	35.45±10.35**
MKs [%]	2.89±1.62	3.50±1.85	3.04±1.43	1.33±0.20*	4.08±1.75**
NN [%]	1.09±0.52	1.25±0.72	1.08±0.40	0.83±0.19	1.25±0.40

The values are presented as mean ±SD, ISS - international staging system, TPO-thrombopoietin, sP-selectin - soluble P- Selectin, IL-6 – interleukin 6, PLT - platelets, PDGF - Platelet- Derived Growth Factor, MKs –megakaryocyte , NN – naked nuclei. *p<0.05 between MM patients stage I and III, **p<0.05 between before and after treatment MM patients

Figure 1. The presence of MKs and NN according to ISS in trephine biopsy of untreated MM patients.

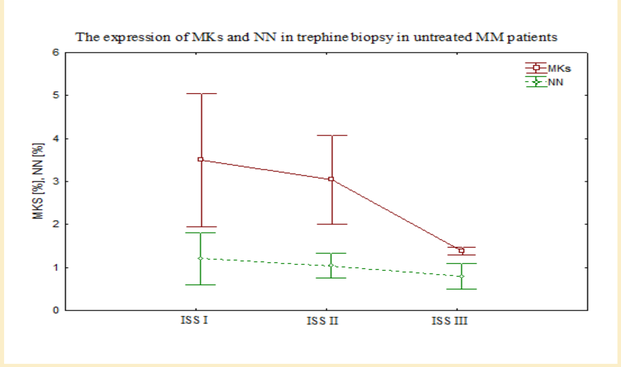
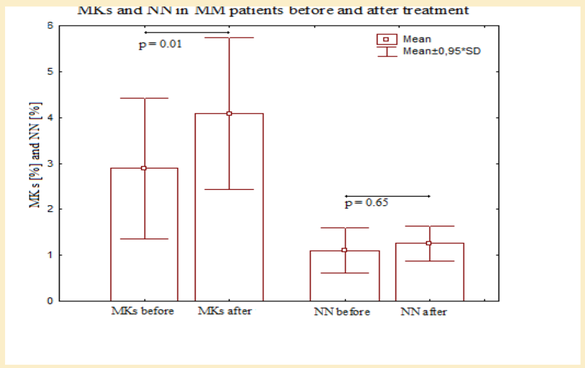


Figure 2. MKs and NN before and after the treatment.



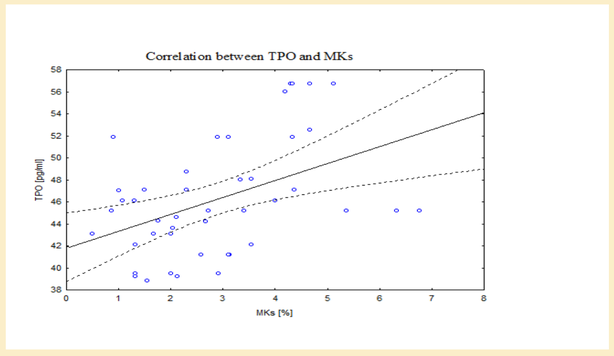
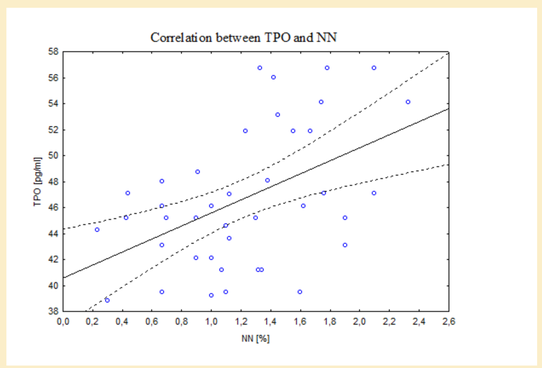


Figure 3. Correlation between TPO and MKs in MM patients

Figure 4. Correlation between TPO and NN in MM patients



ISS- International Staging System, MKs- megakaryocytes, NN- naked nuclei, TPO-thrombopoietin

Conclusions. Our study has eventually demonstrated that sP-selectin, as a marker of platelet activation, could be a useful marker of maximum response to therapy. Its strong association with another marker like PDGF-AB could further lead to the development of the new combinational therapeutic strategies of anti-angiogenic treatment in MM patients.