# Thalidomide and bortezomib overcome the prognostic significance of proliferative index in multiple myeloma

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We analyzed proliferative index of myeloma plasmocytes (PC-PI) in a cohort of 217 patients with multiple myeloma (MM) treated with conventional chemotherapy and biological agents, thalidomide and bortezomib. In the whole group was a difference between overall survival (OS) favoring patients with PC-PI < 2.8% (median overall survival 30 vs 12 months) with a borderline significance (p = 0.06). However, after approximately 40 months from diagnosis the curves merged, suggesting the influence of novel drugs. In patients treated with conventional chemotherapy only, the difference maintained significant even after 40 months (median overall survival 25 vs 10months, p = 0.015), whereas in the group treated with thalidomide and bortezomib was no difference, with medians over 39 months. Even patients with low PC-PI profited from the treatment with novel drugs. Presented results suggest that the treatment of MM with novel agents overcomes the prognostic significance of PC-PI and should be used in all MM patients.

Key words: myeloma – prognostication – proliferative index – biological therapy

Multiple myeloma (MM) is a clonal proliferative neoplasm of the blood with a very heterogeneous prognosis. Several prognostic factors have been established, reflecting the characteristics of the malignant clone as well as the unique features of every individual organism and their mutual interaction. In recent years, stress was put on those parameters that reflect the internal biological properties of myeloma plasmocytes and are therefore regarded as very potent prognostic factors. One of the most respected factors is the proliferative index of myeloma plasmocytes, or the "labeling index" [1–6]. Measurement of the proliferating potential of myeloma cells has been quite an established procedure, significantly dividing patients into groups with different prognosis [2–4, 7–22]. However, most of the studies assessing the proliferation of MM plasmocytes have been from the time of the treatment with the use of conventional chemotherapeutical regimens only and of the general acceptance and validity of "classical prognostic factors". Recent recommendations for the therapy of MM on the other hand include novel treatment using the drugs with "biological effect", such as thalidomide and bortezomib, in which the mechanism of action influences substantially the biological properties of the malignant clone [23–30].

The aim of the presented study was to analyze the prognostic significance of proliferative index of myeloma plasmocytes in patients with MM treated using conventional chemotherapeutic regimens only as well as the novel "biological drugs", thalidomide and bortezomib. The upfront issue was the question of whether the measurement of proliferative index in newly diagnosed MM patients maintains its prognostic significance also in the era of biological based drug therapy.

## Patients and methods

At the Department of Internal Medicine III of the University Hospital in Olomouc, between November 1997 and February 2008, we assessed the proliferative index of myeloma plasmocytes in 217 patients with newly diagnosed MM. In all the patients the proliferative characteristics were evaluated at the time of diagnosis before the start of induction treatment.

All the patients were treated using conventional induction chemotherapy, i.e. regimens VAD, MP, VBMCP, CyVAD and CIDex. Patients treated with high dose chemotherapy with the support of autologous stem cell transplantation were not included in our group and their evaluation will be the contents of a separate paper. The group was then divided into 167 patients who never received any of the novel drugs and a subgroup of 50 patients who were in their first or second relapse treated also with the use of thalidomide (in all pa-

tients) and bortezomib (in 13 patients – 27%). Thalidomide was administered within the treatment regimen CTD senior (cyclophosphamide 50mg p.o. daily, thalidomide 100mg p.o. daily, dexamethasone 20mg p.o. 1.-4. day and 15.-18. day in a 28-day cycle) for senior patients older than 65 years, and within the regimen CTD junior (cyclophosphamide 800mg i.v. 1. day, thalidomide 200mg p.o. daily and dexamethasone 40mg p.o. 1.-4. and 12.-15. day in a 21-day cycle) for patients younger than 65 years [31]. Bortezomib (Velcade) was administered in the regimen VD (Velcade i.v. 1,3mg/m$^2$ on day 1, 4, 8 and 11 together with dexamethasone 40mg p.o. 1.-4. and 8.-11. day) [32]. The induction treatment preceded the relapse treatment with the use of biological drug at least for 4 months.

Both groups had similar age, performance status, distribution of immunochemical type, stage, and similar representation of key prognostic factors, such as beta-2-microglobulin, serum levels of creatinine, calcium, hemoglobin, albumin, serum thymidine kinase and bone marrow involvement. Cytogenetic assessment has not been performed in all the patients, and therefore it has not been considered relevant for the study purposes. The induction treatment was not substantially different in the both assessed cohorts, the only difference was in the inclusion of novel biological agents in the first and/or second relapse in the second group of patients.

The M/F ratio was 1:1 (109 males and 108 females) with an average age of 67 years, in men 65.5 years (33-85) and in women 69 years (44-89).

The diagnosis of MM was defined according to the SWOG and IMWG criteria [33, 34], the staging was estimated according to Durie-Salmon (D-S) and International Prognostic Index IPI [35, 36]. Within the D-S staging, there were 16 patients of stage I (7%), 80 patients of stage II (37%) and 121 patients of stage III (56%). The A and B stadia based on the impairment of renal function at the time of diagnosis were in the ratio 154:63 (71 : 29%). Within the IPI staging system there were at the time of diagnosis 39 patients of stage I, (18%), 60 patients of stage II (28%) and 118 patients of stage III (54%).

Immunochemical type IgG was found in 149 patients (68%), IgA in 39 patients (18%), Bence-Jones type was found in 21 patients (10%), there was one patient with IgM and one patient having an IgD type of MM (both 1%), 6 patients had nonsecretory, respectively hyposecretory form of MM (free light chains assessable only). The kappa:lambda ratio was 141:75 (65% : 35%), in one case we found biclonal type of the disease with the presence of both IgG kappa and IgG lambda chain.

Proliferative activity of myeloma plasmocytes in the aspirate of bone marrow was measured using propidium-iodide index (PC-PI) with the use of flow-cytometry (DNA – Prep Reagents Kit, Coulter, software Multicycle fy. Phoenix), where the measured values represent the percentage of plasma cells in S-phase of the cell cycle [37]. We used the technique of double-staining, where the myeloma plasmocytes were identified using monoclonal antibody against syndecan-1 (CD138) and the S-phase was assessed after the incorporation of propidium-iodide into nuclear DNA. The average number of cells

evaluated by flow-cytometer was 2000-4000. Patients were first divided according to median value of proliferative index (PC-PI = 2.5%), for better differentiation of prognostic groups we used the optimal discriminating level of PC-PI = 2.8%, calculated by the CART analysis at our department [38].

For statistical evaluation we used log rank test and Kaplan-Meier analysis of overall survival.

## Results

The values of propidium-iodide index of myeloma plasmocytes (PC-PI) measured at the time of diagnosis of MM were in the range 0.4 – 4.8% with median 2.5%. As the discrimination level we chose the value of PC-PI =2.8%, which was the optimal discrimination value best differentiating patients with good and poor prognosis, found at our department in previous studies [38]. Within the whole group of 217 patients, those with PC-PI ≥ 2.8 (n=73) had poor prognosis with median overall survival (OS) 12 months, whereas patients with PC-PI < 2.8 (n=144) had substantially better prognosis with median OS 30 months, with borderline significance (p = 0.06), figure 1. The curves, however, closed to each other and after approximately 40 months the OS in both groups was the same.
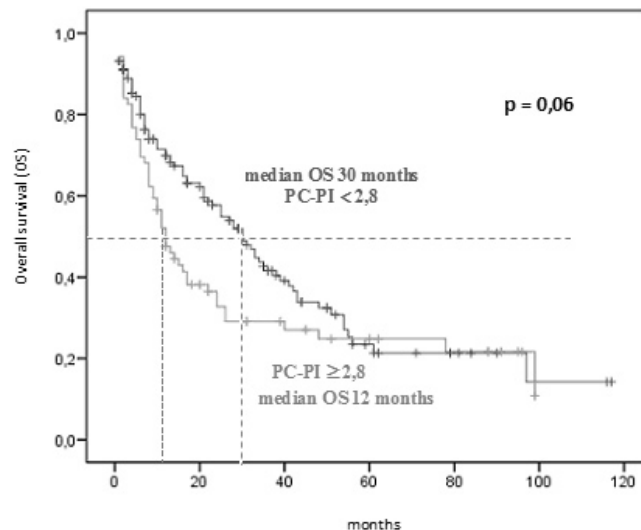


**Figure 1 Overall survival according to proliferative index in multiple myeloma patients treated with conventional chemotherapy and new biological agents**

In patients with multiple myeloma (MM) treated with conventional and biological therapy (n=217) is a difference in the Kaplan-Meier curves of overall survival. Patients with high PC-PI ≥ 2.8% have worse prognosis (median OS = 12 months) than patients with low value of PC-PI < 2.8% (median OS = 30 months),the result is with borderline significance (log rank test p = 0,06). Subsequent closing of both curves is very likely due to the effect of new drugs with biological effect (thalidomide and bortezomib).

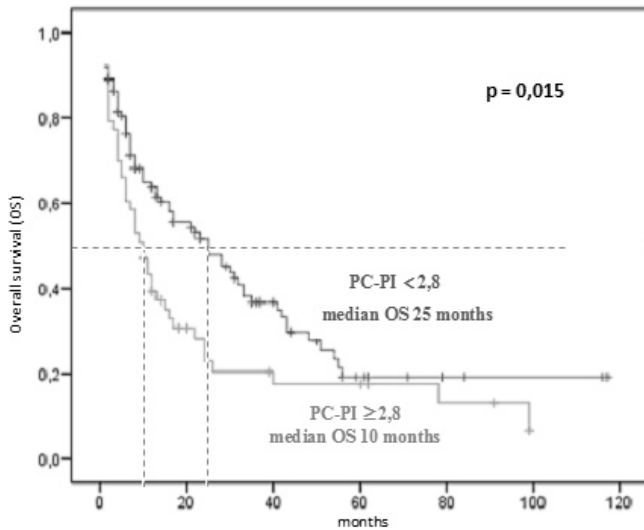PC-PI = propidium iodide (proliferative) index of plasma cells
OS = overall survival

**Figure 2 Overall survival according to proliferative index in multiple myeloma patients treated only with conventional chemotherapy**

The value of PC-PI in patients with multiple myeloma (MM) treated with conventional chemotherapy only (n=167) without the use of new biological agents significantly separates a group of patients with unfavorable prognosis (PC-PI ≥ 2,8%, median OS = 10 months), and a group of patients with better prognosis (PC-PI < 2,8%, median OS = 25 months) , according to Kaplan-Meier curves (log rank test p = 0,015).
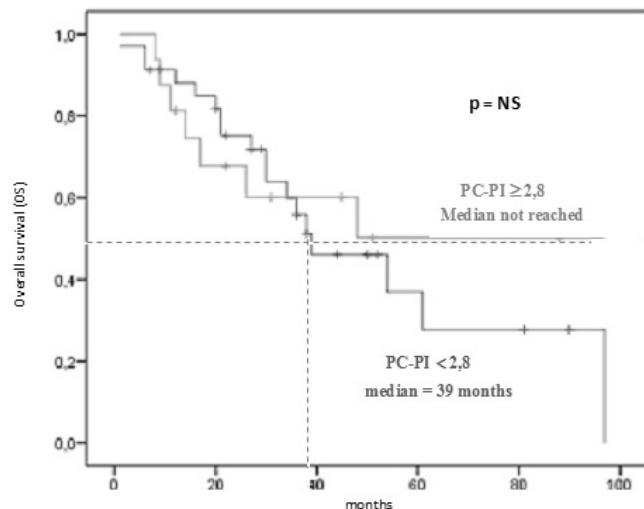PC-PI = propidium iodide (proliferative) index of plasma cells
OS = overall survival



**Figure 3 Overall survival according to proliferative index in multiple myeloma patients treated with novel biological therapy**

In multiple myeloma patients (n=50) treated within the course of the disease with novel drugs with biological mechanism of action (thalidomide, bortezomib) there is no significant difference in the Kaplan-Meier overall survival curves, log rank test p = 0,677, suggesting that novel drugs overcome the prognostic significance of PC-PI.
PC-PI = propidium iodide (proliferative) index of plasma cells
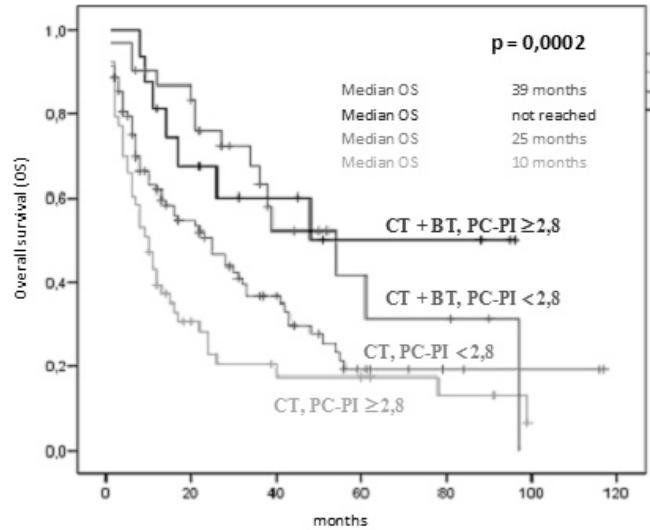NS = statistically non-significant value



**Figure 4  Comparison of the overall survival according to proliferative index in multiple myeloma patients treated with conventional and novel chemotherapeutic approaches**

In comparison of overall survival in multiple myeloma patients (n=217) there is a statistically significant difference in Kaplan-Meier curves in patients treated with conventional chemotherapy (n=167) and patients treated also with the use of novel biological drugs, thalidomide and bortezomib (n=50). The worst prognostic group are those patients witth high PC-PI ≥ 2,8% treated only with conventional therapy (median OS = 10 months), patients with PC-PI < 2,8% treated with conventional chemotherapy have a better prognostic outcome (M OS = 25 months), the best prognosis have those patients who were treated also with new biological agents, regardless of the value of the proliferative index PC-PI (median OS > 39 months), log rank test p = 0,0002.
PC-PI = propidium iodide (proliferative) index of plasma cells
CT = conventional therapy
BT =novel biological treatment (thalidomide, bortezomib)
OS = overall survival

In the group of 167 patients, who were in the whole course of MM treated with the use of conventional chemotherapy only (without the use of new drugs thalidomide or bortezomib) the difference in OS was statistically significant (p = 0.015) separating patients with good prognosis (PC-PI < 2.8, n=110, median OS = 25 months) and poor prognosis (PC-PI ≥ 2.8, n=57, median OS = 10 months), maintaining the difference also after 40 months, figure 2.

In the group of patients treated in the first or second relapse with novel agents, thalidomide and/or bortezomib (n = 50), the OS was without significant difference between the group with lower PC-PI < 2.8 (n=34, median 39 months) and the group with higher values of PC-PI ≥ 2.8 (n=16, median not reached), figure 3.

Within the comparison of the whole group of patients (n = 217) according to the therapy and the value of PC-PI (conventional therapy and PC-PI ≥ 2.8 or PC-PI < 2.8 and novel chemotherapy with PC-PI ≥ 2.8 or PC-PI < 2.8) we

could construct the conglomerate curves of the OS, figure 4. The overall statistical evaluation proved to be significant (p = 0.0002) as well as the differences between individual groups of patients, except of the described lack of statistical difference in all patients treated with novel agents.

## Discussion

Evaluation of proliferative potential of plasma cells in MM is an acknowledged method, although in recent clinical practice not very frequently used despite its undisputed significance in the assessment of the prognosis of the disease [7–22]. The beginning experience using the measurement of proliferative index was targeted at the prognostic evaluation of patients with active MM. Several studies proved strong prognostic significance of proliferative (or later labeling) index, where the higher percentage of proliferation at the time of diagnosis of MM together with a large tumor mass differentiated a group of patients with very poor prognosis and short median OS [3, 11, 39, 40]. Unlike most other prognostic factors in MM, which reflect the tumor mass or the tissue involvement, the assessment of proliferation provides the reflection of actual activity of the disease [20]. Because the first radioactive isotope-based methods (tritiated thymidine or bromdeoxyuridine) were complex and time-demanding, they did not find a wider use in clinical practice [3, 7, 10, 11, 17, 40]. Coherent results of most of the studies and the summaries of multivariation analyses contributed to the development of faster and easier methods of proliferative index evaluation (based mostly on flow cytometry) as well as to a more intensive focus on the molecular biology in MM.

The results from our department confirm the predictive value of PC-PI. Unlike the pilot data of previous studies (including those from our department) which proved a very strong prognostic association [6, 8, 10–13, 17, 22, 38], the presented study shows a difference in OS at a borderline significance only, figure 1. With a closer focus on the Kaplan-Meier curves we can trace an interesting phenomenon of drawing near after approximately 40 months from the diagnosis. This phenomenon suggested the diminishing of the prognostic significance of PC-PI with the length of the disease course, or also the potential influence of new drugs with biological mechanism of action, which were used in a minor portion of the patients. Due to the inclusion of these drugs in the first or second relapse of MM it was very likely that their biological effect might have appeared right after the 40 months from the diagnosis.

To test this hypothesis we sorted out the patients treated with novel agents and carried out the statistical analysis separately in both groups – in patients treated with conventional chemotherapy only (n = 167) and patients treated in their first or second relapse with one or both of the novel drugs, thalidomide and bortezomib (n = 50). In the former, the assessment of PC-PI maintains its strong predictive potential (figure 2) even after 40 months of treatment whereas in the latter the curves of OS were without a significant difference within their whole course despite the value of PC-PI.

The literature dealing with the evaluation of proliferative potential of myeloma plasmocytes is mainly from the time of conventional chemotherapy [1–4, 6–15]. With a more advanced knowledge of the biological properties of the malignant clone, the microenvironment of the bone marrow, and their mutual interactions, it is possible to focus the treatment approach to the interference with these modalities, and a number of previously used prognostic factors (such as the degree of anemia, immunochemical type, bone marrow involvement or some cytogenetic abnormalities and others) lose their prognostic significance [41–45]. From our findings we can assume, that the treatment with novel agents overcomes the prognostic significance of proliferative index. Interestingly, even patients with an unfavorable profile of PC-PI, who are treated with novel biological agents benefit from the administration of novel drugs and have a better prognosis than individuals with favorable value of PC-PI who are treated with conventional chemotherapy only, figure 4.

Measurement of plasma cell proliferative index in MM patients treated with conventional chemotherapy has been performed in several departments using different stains and detection techniques. Apart from the first attempts with radioactive isotopes, most of the studies have been using nonradioactive DNA stains or the expression of certain molecules during the phases of the cell cycle. Up to now, the best known is the bromdeoxyuridine (BrdUrd) or propidium-iodide technique with similar results, and the use of Ki-67 antigen. In order to have comparable results we used the propidium iodide stain, which easily detects the S-phase. The Ki-67 index evaluates all the cell cycle phases [46, 47] and represents rather the "cycling" potential, and it does not necessarily correspond to proliferation detected by the BrdUrd or PC-PI techniques. Other methods such as immunohistochemical analysis of proliferating cell nuclear antigen (PCNA) [48], detection of AgNORs (argyrophillic proteins associated with the nucleolar organizer regions) in histological samples [49], cyclin D1 detection [50] and some others are not widely used in clinical practice. Similarly, the choice of plasma cell identification using surface marker CD138 (syndecan-1) has been derived from the most relevant studies assessing proliferation. Previous detection of cIg and CD38 have been found less specific, as they may be found also in some other cells such as activated T and B lymphocytes, monocytes, and some progenitor cells [51, 52]. In the study of Joshua et al., the best significance for the evaluation of proliferative index had the subpopulation of primitive plasma cells (CD38++, CD45++, CD56+, VLA5-) [13]. Most of the relevant studies have used, however, only the identification of CD138, which was the impulse for our study design.

Our findings are, however, still preliminary as the group of patients treated with novel agents is quite heterogenous and needs to be expanded. None of the patients were treated with thalidomide or bortezomib in frontline therapy. The induction treatment was moderately different within the group, and slightly different responses were noted. The comparison

of patients with conventional chemotherapy only and the introduction of novel agents in second or third line of treatment moreover does not exclude the bias of the influence of very aggressive myeloma causing death in early course of the disease so that the patients did not even survive to the time of administration and/or the effect of the new biological drugs. For more coherent and convincing results we suggest the support of clinical studies by the evaluation of prognostic potential of PC-PI in patients treated with the new drugs with biological mechanism of action, especially in patients with an induction treatment based on these novel drugs.

The presented study has enriched the issue of the assessment of myeloma proliferation also in patients who underwent biologically based treatment, and has extended the approach to include novel drugs that target biological characteristics of myeloma population. We have confirmed the significance of thalidomide and bortezomib in the treatment of elderly patients or patients ineligible for high dose therapy with the support of autologous stem cell transplantation. All patients treated with new agents had a better prognosis with longer OS. It should be emphasized, that even patients with a favorable cytokinetic profile profited from the therapy with novel agents and should therefore be candidates for new biological drugs. The assessment of PC-PI itself is very helpful for the understanding of internal biological properties of myeloma plasmocytes. Our study, however, showed, that within the group of patients treated by novel biological agents, PC-PI lost its prognostic potential, and may not be therefore regarded as a significant prognostic factor in multiple myeloma any longer.

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