specific enzymatic assay. The localization of polyP in the myeloma cell lines was determined by confocal microscopy. The U266 myeloma cell line was used to study whether extracellular polyP affects Ig secretion and survival. Different human cell lines were used to test the specificity of polyP in viability. We analyzed Ig secretion of PC form Bone Marrow and Peripheral Blood after polyP addition. A conventional tetanus toxoid booster immunization was used to increase PC proportion in order to examine the apoptotic effects of polyP. Ig secretion and Apoptosis was determined by ELISA and FACS respectively. *Results*. Micromolar levels of polyP that is present principally as polymers of 75 phosphate units have been found in the U266 and IM9 myeloma cell lines. PolyP is accumulated in intracellular vacuoles similar to the previously reported platelet dense granules and acidocalcisomes of the unicellular eukaryotes. Addition of polyP to human PC produces an unexpected inhibition of Ig secretion and a stimulation of apoptosis. PolyP generates apoptosis specifically in PC, myeloma (malignant PC) cell lines, and B lymphoid cell lines. Normal B cells, T cells, total blood mononuclear cells, and non-lymphoid cell lines are not affected by polyP. In U266 myeloma cell line, polyP induces the externalization of phosphatidylserine, the activation of caspase-3, and the arrest of the cell cycle. Protective effects of IL-6 do not overcome the polyP-induced apoptosis. *Summary/conclusions*. Taken together, our results and suggest for the first time the relevance of polyP for the humoral immune response and open prospects for polyP as a novel therapy for myeloma.

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METHYLATION STATUS OF THE P57KIP2 GENE IN PATIENTS WITH PLASMA CELL DYSCRASIAS

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Background. Oncogenesis is related to cell cycle deregulation. Aberrant DNA methylation, leading to silencing of regulatory genes, has emerged as one of the most frequent molecular changes in haematological malignancies. The p57KIP2 is a tumor suppressor gene that belongs to the CIP/KIP family of cyclin dependent kinase inhibitors that negatively regulate cell cycle progression. *Aim*. We have studied the methylation status of the promoter region of p57KIP2 gene in patients with multiple myeloma (MM) and Waldenström's macroglobulinemia (WM) in order to correlate the methylation pattern with the disease's phenotype. *Patients and Methods*. We have studied bone marrow and paired peripheral blood samples from 12 consecutive MM patients (9 male, 3 female, age range 50-83, median 59) and 2 consecutive WM patients (1 male and 1 female, age 75 and 47 years).

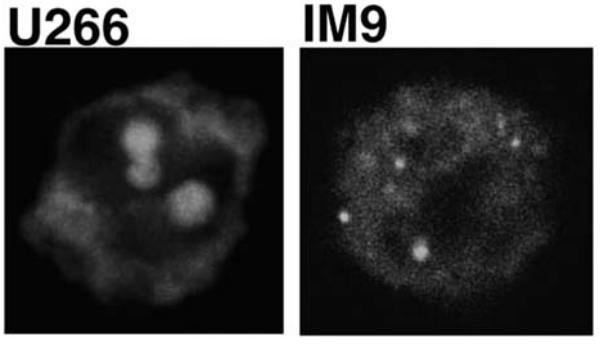

Figure 1. Localization of polyP on U266 and IM9 cells.

Samples from 9/12 MM patients and 2/2 WM patients were taken at diagnosis whereas the remaining 3/12 samples were taken during the course of the disease. Genomic DNA was extracted using standard protocols (Quiamp DNA mini kit). After bisulfite treatment procedure the DNA was PCR amplified with primers specific for the methylated and the unmethylated alleles of the gene. The PCR products were separated on 2% agarose gel. Bone marrow DNA from healthy donors served as negative control. We have also used human male genomic DNA universally methylated for all genes (Intergen Company, Purchase, NY) as positive control. *Results*. Two patients had stage IA disease and did not receive any treatment, five MM patients had stage IIA or more advanced disease and started on VAD chemotherapy, two patients were started on oral melphalan and methylprednisolone, one patient was on plateau, and two patients had progressive disease after having received VAD and were started on bortezomib therapy. One patient with WM was started on cyclophosphamide, dexamethasone and rituximab and the other patient did not receive any treatment. Classical cytogentic analysis was available on 5/12 MM and 1/2 WM patients and the karyotype was reported as normal. All patient samples showed no band corresponding to the methylated allele of the p57KIP2 gene. The band corresponding to the unmethylated allele was clearly visible in all samples. *Conclusion*. To our knowledge this is the first report on p57KIP2 methylation status in patients with plasma cell dyscrasias. Our data show that methylation of p57KIP2 gene is not a frequent event in the patients studied. Further studies are needed to confirm the above results.

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EVALUATION OF THE RELATION BETWEEN ANGIOGENIC CYTOKINES, SELECTED BIOLOGICAL PARAMETERS AND PROGNOSTIC FACTORS IN MULTIPLE MYELOMA

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Background. Multiple myeloma is an unusually heterogenous disease with individually different course, response to therapy and prognosis. Up-to-date diagnostic and stratification systems have, however, an important limitation in their insufficient absorption of those parameters, that express intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. The aim of this study was to evaluate the relation of 10 biological parameters to 6 substantial prognostic factors in multiple myeloma. *Methods*. The analysed group consisted of 66 persons evaluated at the time of diagnosis, before the start of chemotherapy. For the assessment of serum levels of examinated molecules were used following Methods. REA, RIA, ELISA and the technique of sandwich enzymatic immunoassay, for the assessment of proliferative and apoptotic properties were used propidium iodide (PC-PI) and annexin V (PC-AI) indices evaluated with the help of flow-cytometry. Statistical analysis was carried out using Pearson and Spearman test and/or using U-test according to Mann-Whitney. *Results*. High occurence of abnormal serum level of evaluated parameter was found in the case of S-β-2-microglobulin (95,5%), S-thymidinekinase (57,5%), S-sVCAM-1 (78,5%), S-ICTP (87,0%), S-soluble osteoprotegerin (sOPG 76,5%) , S-sSyndecan-1 (56,5%) and low index of apoptosis of plasma cells (PC-AI, 78%). Correlation analysis (Pearson test) revealed a mutual relationship between serum levels of β-2-microglobulin to sVCAM-1 (r=0,39, *p*=0,002), sICAM-1 (r=0,33, *p*=0,011), sOPG (r=0,53, *p*=0,001), sHGF (r=0,34, p=0,006), sSyndecan-1 (r=0,38, *p*=0,003) and sFas (r=0,42, *p*=0,001); of S-albumin to sVCAM-1 (r=-0,29, *p*=0,036), ICTP (r=-0,33, *p*=0,016), sOPG (r=-0,63, p=0,000), sHGF (r=-0,39, p=0,003) and sSyndecan-1 (r=-0,29, *p*=0,042; of S-thymidinekinase to sSyndecan-1 (r=0,46, *p*=0,000) and sFas (r=0,29, *p*=0,019). In neither of the cases was found the relation of PINP and VEGF to any of the evaluated prognostic factors. There was no relation found between any of the analysed parameters and PC-PI or PC-AI. With the use of U-test there was found a relationship of serum levels of sIL-6R (< > 100IU/l) to β-2-microglobulin (*p*=0,001), albumin (p=0,002) and to PC-PI (*p*=0,046). *Conclusion*: The above study established the possibility to enrich the traditional algorithms used in clinical practice for individual characteristics of MM with the parameters sOPG, sHGF, sSyndecan-1 and sFas.

Founded by MSM 6198959205.

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COMPARISON OF SERUM LEVELS OF BIOLOGICAL PARAMETERS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

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Background. The presented work is focused upon the evaluation of the differences between serum levels of selected biological parameters in monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM), especially from the point of view of their potential benefit for clinical practice. *Methods*. Analysed group of 96 patients (30 patients with MGUS and 66 patients with MM) was assessed at the time of diagnosis before the start of therapy. For the

evaluation of serum levels of analysed parameters were used following Methods. radioenzymatic assay (thymidinekinase), radioimmunoanalysis (β-2-microglobulin, ICTP, PINP), method of enzymoimmunoassay (sIL-6R, sVCAM-1, sICAM-1, sOPG and sRANKL) and the technique of quantitative sandwich enzymatic immunoassay (sHGF, sVEGF, bFGF, syndecan-1/CD138 and sFas). Statistical analysis was carried out using Pearson´s and Fischer´s test, χ^2 test and nonparametric U test according to Mann-Whitney (p<0,05). Results. Statistically significant differences were found out between MGUS and MM in case of comparison of serum levels of sIL-6R (p=0,02), ICTP (p=0,001), sHGF (p=0,001) and syndecan-1/CD138 (p=0,001), whereas in case of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF and sFAS there were no statistically significant differences. Within the analysis of the frequency of the occurence of abnormal values in the MM and MGUS group there were significant differences not only in the case of standard parameters such as β-2-microglobulin, thymidinekinase creatinine and albumin, but also in the case of sIL-6R, ICTP, sHGF, and syndecan-1, however not in the case of comparison of the values of sVCAM-1, sICAM-1, PINP, sOPG, sVEGF, and sFas. Measurement of serum levels of sRANKL and soluble form of bFGF was of no avail due to very low values of these parameters. Conclusion: The analysis of the 10 parameters, that are altogether very close related to the biological properties of clonal plasma cells or to the changes of bone marrow microenvironment revealed from the point of the contribution for the differentiation of MGUS from MM, that the only purposeful parameters were only the serum levels of sIL-6R, ICTP, sHGF and syndecan-1 (sCD138), i.e. the parameters with certified significance for the MM prognosis evaluation.

Founded by MSM 6198959205.

0758

RELATIONSHIP OF SERUM FREE LIGHT CHAIN LEVELS TO DEGREE OF MULTIPLE MYELOMA PROGESSION

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Backround: Multiple myeloma is a malignant disease characterised by clonal proliferation and accumulation of neoplastically transformed B-line elements, producing monoclonal immunoglobulin (MIG) demostrable in serum and/or urine. Plasma cells also produce free light chains (FLC) κ and λ, that are not fixed in MIG molecule. Aim of the study was a comparison of serum FLC levels and κ/λ (κ/λ) between stages of Durie' Salmon (D-S), International Prognostic Index (IPI) and South West Oncology Group (SWOG) staging systems. *Methods.* Prospective study included 147 patients with multiple myeloma, examined during one year period. Serum FLC levels were assesed using FREELITE Immunotech system, values of β2-microglobulin were obtained by RIA. Mann - Whitney's U-test was used for statistic evaluation. *Results.* Abnormal values of serum FLC and κ/λ ratio were assesed in 79% and 81%, κ secretion was presented in 67%, λ in 33%. Comparing with each stage of D-S staging system, statistically different levels of dominant chain (p=0,003) and κ/λ ratio (p=0,005) were found between stages II and III in λ group only. Differences in values between other stages were not significant. Comparing substage A and B (serum creatinine over 177 μmol/L), significant differences were found in levels of dominant and alternative chain in κ group (p=0,047 and p=0,014) and also in λ group (p=0,007 and p=0,046), but there was no significant difference between κ/λ ratio values. Using IPI staging system, significantly different levels of dominant kappa chain were found in κ group between stages I and II (p=0,029), between stages I and III in values of κ chain (p=0,029) and κ/λ ratio (p=0,04). In lambda group were also found differences in λ dominant chain and κ/λ ratios between stages I and II (p=0,01 and p=0,011) and also between stages I and III (p=0,0001 and p=0,0013). Differences of FLC values between stages II and III in both groups were not significant. In case of SWOG staging system, in κ group, differences in levels of dominant chain between stages I and II (p=0,038) and I and III+IV (p=0,035) were assesed. In λ group were found different values of dominant chain lambda and κ/λ ratio between stages I and II (p=0,029 and p=0,042) and also between I and III+IV (p=0,002 and p=0,004). Between stages II and III+IV was found a difference in dominant chain in lambda group only (p=0,047). *Conclusions.* Disease progession degree evaluated using dynamic indicators - serum albumine and β2-microglobuline (IPI and SWOG system), correlate with serum FLC levels more expresively, than traditional Durie-Salmon staging system. Serum FLC levels depend on kidney function, but κ/λ ratio values are not affected by impaired renal function.

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VEGF EXPRESSION AND MICROVESSEL DENSITY IN PATIENTS WITH MULTIPLE MYELOMA: CLINICAL AND PROGNOSTIC SIGNIFICANCE

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Background. Angiogenesis or new vessel formation is an essential component in the growth and progression of solid malignancy. However, conflicting data are reported on clinical significance of VEGF deregulation and microvessel density (MVD) in multiple myeloma (MM). *Aim*: The purpose of the study was to evaluate the incidence of VEGF expression and grade of MVD, and to correlate these findings with pathohistological and clinical features of newly diagnosed myeloma patients. *Patients and methods*. We analyzed bone marrow biopsy specimens obtained from 59 patients with MM. The clinical staging was done according to the Durie and Salmon classification (four patients had disease stage I, 15 patients stage II and 39 patients stage III). Expression of VEGF and MVD were analyzed using standard imunohistochemical analysis of B5-fixed and routinely processed, paraffin-embedded bone marrow specimens with antibodies against VEGF and CD34, respectively. MVD was estimated by counting number of microvessels in three *hot spots* at magnification x400, according to the method of Weidner *et al*. VEGF imunoreactivity was estimated on the basis of intensity and percentage of positive plasma cells. *Results*. VEGF was expressed in 47 out of 59 (79.66%) specimens. No statistical correlation could be found between VEGF overexpression and age, clinical stage, degree of osteolytic lesions, types of monoclonal protein, hemoglobin concentration, platelet count, serum concentration of creatinin, calcium and albumins, the extent of bone marrow infiltration, histological grade and proliferative activity (measured with Ki-67 immunoreactivity). In addition, no significant difference regarding overall survival was found between VEGF positive and VEGF negative cases (29 months vs. 34 months, v=0.8). Median MVD was 15 (range: 1-89). We found significant correlation between MVD and histological grade, the extent of bone marrow infiltration and proliferative activity. Although MVD showed prognostic impact on overall survival in univariante analysis (p=0.009), multivariate analysis identified only age, hemoglobin concentration and proliferative activity as independent prognostic factors. *Conclusions*. The upregulated VEGF is seen in plasma cells in the majority of myeloma cases. However, the relationship between this finding and pathogenesis of the disease still remains to be established. The microvessel density can predict poor survival in myeloma and be helpful in choosing optimal therapeutic modality in every individual patient.

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CLONOGENIC CAPACITY OF BONE MARROW CELLS IN PATIENTS WITH MULTIPLE MYELOMA. THE INFLUENCE OF ARSENIC TRIOXIDE AND BORTEZOMIB ON THE PROLIFERATION OF CFU-F AND CFU-GM

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Arsenic trioxide (As_2O_3) and bortezomib were tested as therapeutic agents for a variety of malignancies. The aim of our study was to investigate *in vitro* effects of As_2O_3 and bortezomib on clonogenic capacity of haematopoietic and mesenchymal progenitor cells in patients, with newly diagnosed multiple myeloma and patients with multiple myeloma resistant to standard chemotherapy. *Materials and methods*. Bone marrow samples were obtained from 24 patients with multiple myeloma: 10 before treatment and 14 patients resistant to standard chemotherapy, 11 females and 13 males, 16 with IgG, 6 with IgA, 1 with IgD, 1 with BJ myeloma. Mononuclear cells (MNC) were cultured without As2O3 or bortezomib and with As_2O_3 at a concentration of 0,2 mmol/l. and bortezomib at a concentration of 10 and 20 ng/mL. MNC were plated in a standardized methylcellulose medium (MethoCult 4434, StemCell Technologies) and MesenCult (StemCell Technologies). Colony formation of haematopoietic progenitors (CFU-GM and BFU-E) and mesenchymal progenitor cells (CFU-F) based on morphology of the colonies were assessed on day 14 of cultures. CFU-GM, BFU-E and CFU-F expressed as the percentage of decrease versus control and the mean and standard deviation (SD) of colony inhibition for each concentration of As_2O_3 or bortezomib were calculated across all samples. *Results*. In all patients with resistant myeloma and 2/3 of newly diagnosed patients we