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Correlation between plasma level of urokinase-type plasminogen activator, its receptor in patients with multiple myeloma

Korelacja między osoczowym stężeniem urokinazowego aktywatora plazminogenu i jego receptorem u chorych na szpiczaka plazmocytowego

INTRODUCTION

Urokinase-type plasminogen activator (uPA) is a serine protease, converting cell-bound plasminogen to plasmin and generating the pericellular proteolytic activity needed for degradation of extracellular matrix, basement membranes, and other structural barriers during cellular migration and invasion. uPA is secreted as a single-chain pro-enzyme which is activated by cleavage at lysine 158 into two chains (A and B) connected by a disulfide bridge. Human pro-uPA and two-chain uPA have a molecular weight of 55 kD. Conversion of pro-uPA to uPA is enhanced by binding of pro-uPA to a highly specific receptor (uPAR). uPAR is a single chain, highly glycosylated protein with an apparent molecular mass of 50–60 kD. uPAR is linked to the plasma membrane by a glycosylophosphatidilinositol (GPI)-anchor [1, 2, 3]. uPAR has been shown to accumulate at focal areas of cell-cell and cell-substratum contact and has been shown to be involved in interactions with integrins [4, 5, 6].

Proteolytic activity associated with uPA/uPAR system is very important for cancer invasion. Over-expression of uPA and uPAR has been reported for many carcinoma cell lines and malignant tumors, including: soft-tissue sarcoma [7], osteosarcoma [8], astrocytoma [9], melanoma [10], lung [11], breast [12], ovarian [13], bladder [14], prostate [15], colon [16], esophageal and gastric cancer [17]. In hematological disorders

expression of uPA/uPAR is similar to that, seen in the normal cells with labelling of monocytic and myeloid malignancies (Langerhans cell histiocytosis, histiocytic sarcoma, M2, M4, M5 types of AML) [18, 19, 20]. Overexpression of uPA and uPAR was also detected on lymphoma T cells [21, 22]. Pedersen and al. [13] identified a soluble form of uPAR (suPAR) in the ascitic fluid and serum of patients with ovarian cancer. Then ELISA techniques that allow direct measurement of uPAR in serum have been published. Original anti-uPAR antibody was obtained by Finsen Laboratory Rigshospital in Copenhaggen.

A very interesting and unclear fact so far is engagement of uPA/uPAR system in proteolytic cascade in bone tissue of multiple myeloma patients with following osteolysis.

MATERIAL AND METHODS

Thirty newly diagnosed, untreated patients (19 from Denmark and 11 from Poland) with myeloma were included in the study. There was 14 female and 16 male in this group in the age from 35 to 76 years (mean 58.2). Estimation of uPA and soluble form of uPAR (suPAR) was performed by enzyme-linked immunosorbent assay (ELISA) in Finsen Laboratory Rigshospital in Copenhagen. Results was compared with that achieved in control group. Additionally, we investigated the expression of following plasma cells antigens in 11 patients from Poland: CD45, CD38, CD56, CD19, BB4, CD11A, CD18, CD29, CD49B, CD49E, CD49F, CD54, CD44, CD28, CD21, CD40 using fluorocytometry techniques. In this small group of patients we tried to evaluate correlation between uPA and uPAR levels and above antigens, and also between uPA and uPAR levels and clinical stage of multiple myeloma according Durie-Salmon system, degree of bone lesions, b2-microglobulin and LDH serum levels.

RESULTS

Total concentration of suPAR was 1.49 ± 0.58 ng/ml, and was significantly increased comparing to the control (0.98 ± 0.26 ng/ml). Urokinase level was comparable to the control (0.380 ± 0.18 ng/ml). We found correlation in suPAR and uPA levels (Spearman test, rho 0.55; $p = 0.005$).

Results of correlation between uPA and uPAR levels and expression of selected plasma cells antigens are shown in Table 1 and 2. Significant correlation was found only with CD38 antigen.

Correlation between uPA and uPAR levels and β_2-microglobulin, LDH, clinical stage and degree of osteolysis is presented in Figures 3, 4, 5. Our group of patients was too small and we did not find any correlation up to now.

DISCUSSION

Proteolytic activity generated by uPA-mediated activation of plasminogen is thought to be important for the malignant phenotype in cancer, both for invasion of neigh-

Table 1. Correlation between plasma cell antigens and uPA concentration in serum of myeloma patients

Parameters			R	P
CD45	–	uPA	0.535714	0.215217
CD38	–	uPA	– 0.810844	0.026916
CD56	–	uPA	0.071429	0.879048
CD19	–	uPA	– 0.109109	0.815871
CD138	–	uPA	– 0.222718	0.631210
CD11a	–	uPA	0.178571	0.701658
CD18	–	uPA	0.428571	0.337368
CD29	–	uPA	0.071429	0.879048
CD49b	–	uPA	0.142857	0.759945
CD49d	–	uPA	– 0.127294	0.785646
CD49e	–	uPA	0.074125	0.874507
CD49f	–	uPA	0.428571	0.337368
CD54	–	uPA	– 0.035714	0.939408
CD44	–	uPA	– 0.542857	0.265703
CD28	–	uPA	0.028571	0.957155
CD21	–	uPA	0.085714	0.871743

bouring tissues and formation of distant metastasis. Localisation of uPA and uPAR in carcinoma tissue is still controversial. By some authors uPA/uPAR were reported to be present only in cancer cells, in other studies only in stroma cells, and in recent papers uPA/uPAR expression was described both in cancer and stroma cells [16, 18]. It is probably applying to hematological malignancies and is an element of co-operation between bone marrow cells with participation of integrins [4, 6].

The pathogenesis of multiple myeloma and bone destruction in this disease is still incompletely understood. It is no doubt that osteoclasts play a prominent role in osteolysis [23]. Many cytokines are involved in osteoclasts recruitment and differentiation: Tumor Necrosis Factor (TNF), Hepatocyte Growth Factor (HGF), Macrophage Colony-Stimulating Factor (M-CSF), Inteleukins 1, 3 and 6 [24]. Proteases responsible for extracellular matrix degradation cooperate in a cascade-like system, which first step is binding prourokinase by receptor on the plasma membrane. Activated

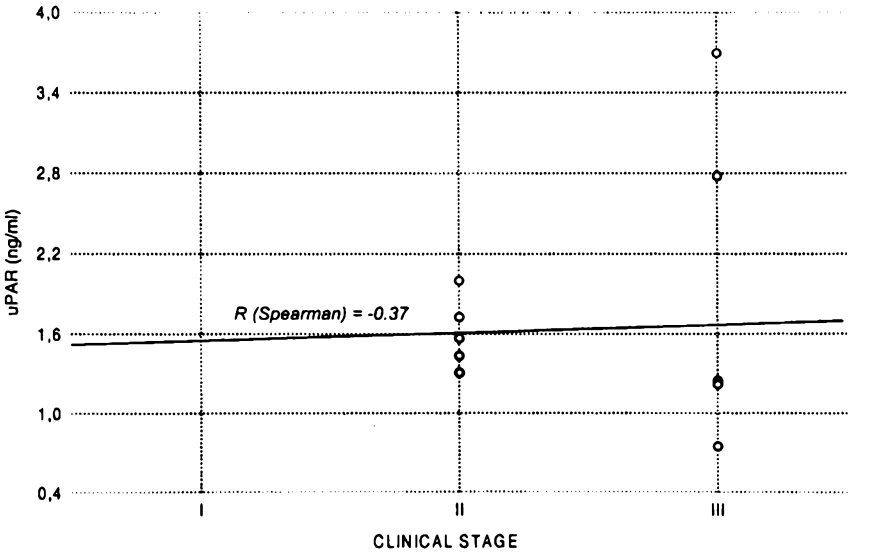
Table 2. Correlation between plasma cell antigens and uPAR concentration in serum of myeloma patients

Parameters			R	P
CD45	–	uPAR	0.450469	0.310429
CD38	–	uPAR	– 0.881818	0.008645
CD56	–	uPAR	– 0.144150	0.757818
CD19	–	uPAR	– 0.055048	0.906689
CD138	–	uPAR	– 0.179787	0.699694
CD11a	–	uPAR	0.522544	0.228878
CD18	–	uPAR	0.522544	0.228878
CD29	–	uPAR	– 0.090094	0.847672
CD49b	–	uPAR	– 0.090094	0.847672
CD49d	–	uPAR	0.238542	0.606461
CD49e	–	uPAR	0.198206	0.335487
CD49f	–	uPAR	– 0.126131	0.670085
CD54	–	uPAR	– 0.202920	0.787572
CD44	–	uPAR	– 0.115954	0.699798
CD28	–	uPAR	0.376851	0.826848
CD21	–	uPAR	0.085714	0.461483

Table 3. Correlation between LDH and β_2-M level and uPA, uPAR concentration in serum of myeloma patients

Parameters			R	P
LDH	–	uPA	– 0.304880	0.933370
LDH	–	uPAR	– 0.341463	0.334218
β_2-M	–	uPA	– 0.194530	0.590207
β_2-M	–	uPAR	– 0.012158	0.973408

Fig.1. Correlation between clinical stage of the disease and uPA or uPAR concentration in serum of myeloma patients



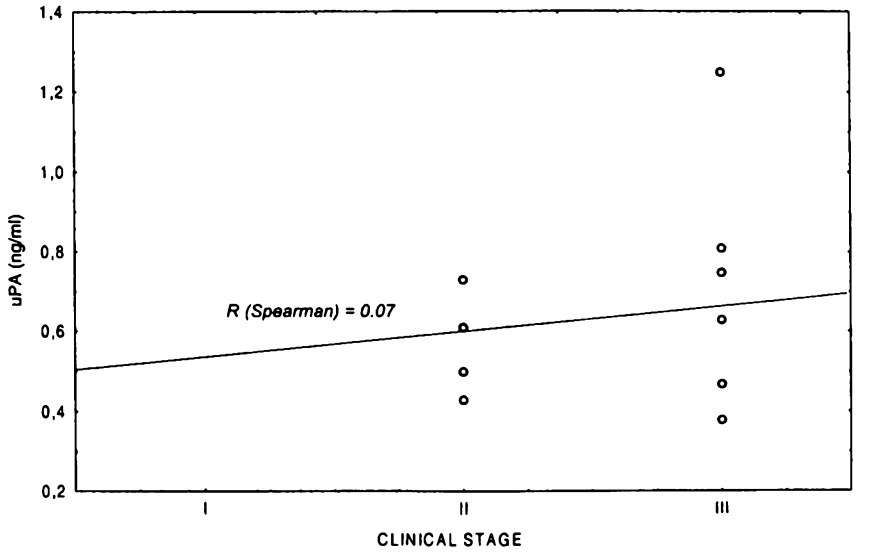
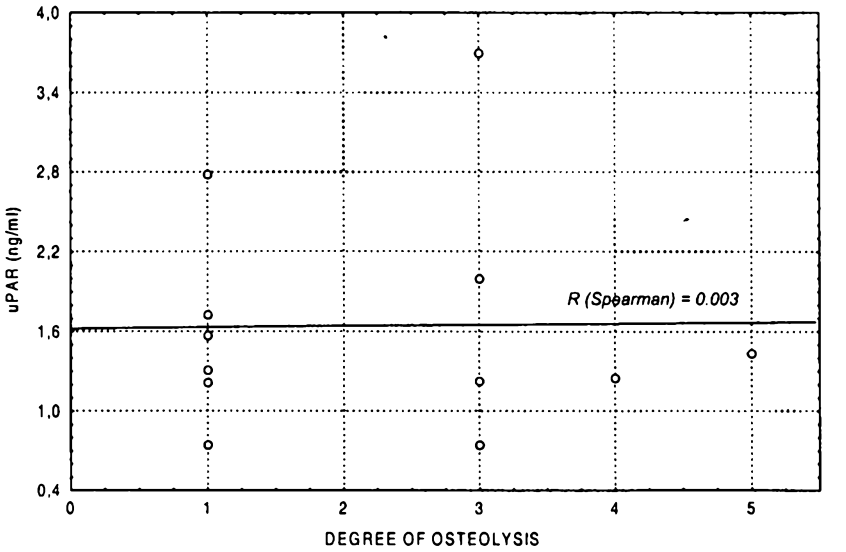
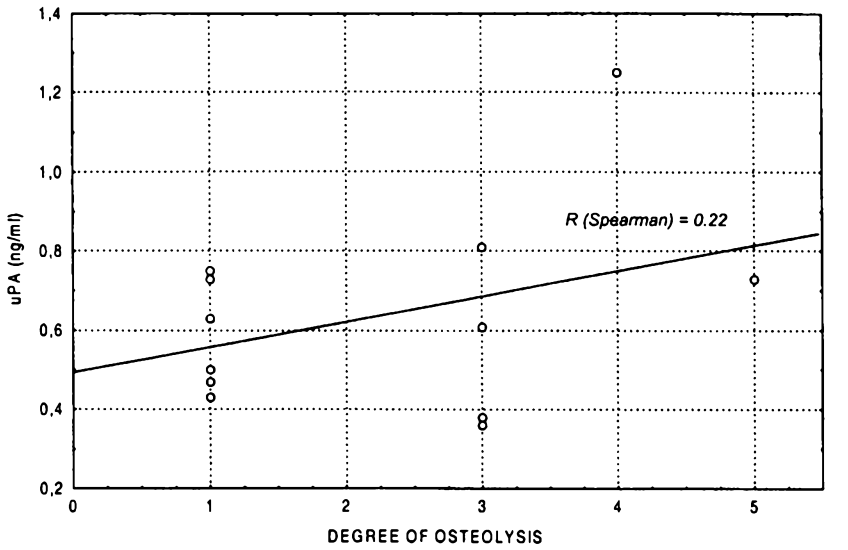


Fig.2. Correlation between osteolysis and uPA or uPAR concentration in serum of myeloma patients





uPA converts plasminogen into proteolytically active plasmin, that additionally initiates activation of cysteine proteases (cathepsin L,N,B) and matrix metalloproteinases (MMP). Recent reports suggest a major role of these enzymes, especially MMP-9 in collagene cleavage [25, 26]. These findings have therapeutic implications, with reference to bisfosfonians so far. We hope, that after the completion of investigated material we would define significance of uPA/uPAR system in prognosis and therapy in multiple myeloma.

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STRESZCZENIE

Do charakterystycznego obrazu klinicznego szpiczaka plazmocytowego należy osteoliza kości. Kaskadę zjawisk proteolitycznych inicjuje układ urokinaza i jej receptor (uPA/uPAR) poprzez aktywację plazminogenenu do plazminy. Plazmina bezpośrednio lub za pośrednictwem sys-

temu metaloproteinaz degraduje macierz kostną. Badanie stężenia uPA i uPAR w surowicy krwi wykonano u 30 uprzednio nieleczonych chorych na szpiczaka plazmocytowego. Całkowite stężenie uPAR wyniosło1,49±0,58 ng/ml i było istotnie statystycznie zwiększone w porównaniu do grupy kontrolnej (0,98 ± 0,26 ng/ml). Stężenie uPA wyniosło 0,380 ± 0,18 ng/ml i nie różniło się w sposób istotny statystycznie od kontroli. W teście Spermana stwierdzono korelację między uPAR i uPA (rho 0,55; p = 0,005). U 11 chorych zbadano ekspresję wybranych antygenów charakterystycznych dla komórek plazmatycznych przy użyciu cytometru przepływowego. Korelację między uPA oraz uPAR wykryto jedynie w przypadku CD38. Nie stwierdzono korelacji między uPA i uPAR, a stadium klinicznym choroby, zaawansowaniem zmian kostnych, oraz stężeniem β_2-mikroglobuliny i LDH.