# The significance of soluble CD138 in diagnosis of monoclonal gammopathies*

V. MAISNAR[1], M. TOUŠKOVÁ[2], M. TICHÝ[3], J. KREJSEK[2], L. CHROBÁK[1], J. VOGLOVÁ[1], J. MALÝ[1]

*[1]2nd Department of Medicine – Department of Clinical Hematology, e-mail: maisnar@fnhk.cz, Charles University Teaching Hospital, 500 05 Hradec Králové, Czech Republic; [2]Institute of Clinical Immunology and Alergology, and [3]Institute of Clinical Biochemistry and Diagnostics, Charles University Teaching Hospital, 500 05 Hradec Králové, Czech Republic*

**Received July 25, 2005**

Report is summary of the results of study designed to ascertain the significance of soluble CD138 (sCD138) assessment in patients with different monoclonal gammopathies. Previous studies have shown that sCD138 is shed from the surface of myeloma cells into serum and that this marker is a new independent prognostic parameter in multiple myeloma.

In presented study was evaluated serum sCD138 level in 14 patients with monoclonal gammopathy of undetermined significance (MGUS) and in 17 patients with multiple myeloma (MM), all MM patients were treated by high-dose chemotherapy regimen with subsequent autologous transplantation of peripheral blood stem cells. To determine the sCD138 level we used a rapid and simple ELISA procedure.

The mean serum sCD138 level of patients with MGUS was 32 ng/ml (range: 5–128). Soluble CD138 levels were elevated in the sera of 10 out of 17 (59%) multiple myeloma patients, the mean baseline sCD138 concentration was 1542 ng/ml (range: 10–17300). In spite of small number of patients the difference between MGUS and MM group was highly statistically significant ($p<0.001$). Multiple myeloma patients with high level of sCD138 at diagnosis (cut-off value: 500 ng/ml) had worse prognosis despite of good response to chemotherapy in some of them ($p=0.029$).

It seems that determination of sCD138 can be recommended as a helpful and reliable marker for differential diagnosis as well as prognosis of monoclonal gammopathies.

*Key words: soluble CD138, syndecan-1, monoclonal gammopathy, multiple myeloma, prognosis*

The term "monoclonal gammopathies" (MG) denotes a very heterogeneous group of disorders characterized by the presence of a monoclonal immunoglobulin (paraprotein) in the serum which is produced by a single clone of plasma cells. Monoclonal gammopathies are classified as monoclonal gammopathies of unknown or undetermined significance (MGUS) and malignant monoclonal gammopathies (MMG) [1, 2]. MGUS are much more frequent than MMG and their occurrence is age dependent [3]. The most frequent and clinically significant MMG is multiple myeloma (MM). In newly diagnosed patients less than 65 years old, high-dose chemotherapy with autologous stem cell support has become the standard care based on significant improvements in progression-free survival and overall survival [4, 5].

Syndecans are heparan sulfate-bearing proteoglycans that are found on the surface of most cells [6]. They are involved in cell-matrix adhesion processes, cell-migration, and growth factor activity [7, 8]. Syndecan-1, newly denoted as CD138, is expressed predominantly on epithelial cells. Within the bone marrow, CD138 is detected solely on cells of the B lymphocyte lineage, and its expression changes at specific stages of differentiation [9].

In the bone marrow of myeloma patients, CD138 is reported to be expressed on myeloma cells only [10]. CD138 antigen is also expressed on malignant plasma cells in peripheral blood [11, 12]. The CD138 molecule mediates specific adhesion of myeloma cells to type I collagen and also mediates cell-cell adhesion between myeloma cells [13–15]. Previous studies have shown that CD138 is shed from the surface of myeloma cells in culture and into human serum [16, 17]. High levels of shed CD138 in the serum are an indicator of poor prognosis in patients with multiple myeloma [18–20].

In this study, we analyzed serum levels of sCD138 in patients with two types of monoclonal gammopathies: MGUS

*This study was supported by grant IGA MH NC/4535-3 and research project MZO 00179906.

and MM. Our aim was to ascertain the usefulness of sCD138 in differential diagnosis of monoclonal gammopathies, and to prove its poor prognostic significance in MM patients even treated with high-dose chemotherapy regimen with subsequent autologous transplantation.

## Patients and methods

Totally we investigated 31 patients (20 men and 11 women) with newly diagnosed monoclonal gammopathies from January 2000 to March 2002: 14 with MGUS and 17 with MM. The basic characteristics of MGUS and MM populations are in Table 1, 2. Plasma cell dyscrasias were defined using the diagnostic criteria of the American SouthWest Oncology Group (SWOG) [2, 21]. All patients with multiple myeloma were treated according to Total therapy I protocol consisting of 2 autologous stem cell transplantations [5]. For one transplantation a minimum of 2.0 x $10^6$/kg CD34+ cells graft was required, as conditioning regimen was applied melphalan 200 mg/m$^2$. The second autologous stem cell transplantation was performed at an interval of 3 to 6 months.

**Table 1. The basic characteristics of MGUS patients group**

| |
|---|
| Male/Female: 9/5 |
| Mean age: 56.6 ± 14.49 years |
| Mean diagnostic plasmacytic infiltration of bone marrow: 6.3 ± 6.00 % |
| Mean diagnostic paraprotein value in serum: 13.0 ± 7.06 g/l |
| Paraprotein types: 9x IgG kappa, 3x IgM kappa, 1x IgG lambda and 1x IgA kappa |

**Table 2. The basic characteristics of MM patients group**

| |
|---|
| Male/Female: 10/7 |
| Mean age: 57.9 ± 6.47 years |
| Mean diagnostic plasmacytic infiltration of bone marrow: 31.3 ± 21.25 % |
| Mean diagnostic paraprotein value in serum: 39.2 ± 27.09 g/l |
| Durie-Salmon stages of MM: 7x IIA, 8x IIIA and 2x IIIB |
| Paraprotein types: 10x IgG kappa, 3x IgG lambda, 2x IgA kappa, 1x kappa-free and 1x lambda-free |

The serum concentration of sCD138 was measured using a commercially available human syndecan-1 ELISA kit (Diaclone Research, Besancon, France; normal range: 0–166 ng/ml). We determined baseline serum levels in both group of patients and further serum level of a second sample 6 months after diagnosis in MGUS patients or 1 month after transplantation in MM patients.

The basic assessment of patient numbers in individual groups was calculated by summary statistics based on binary and ordinary variables. Comparisons between groups of patients were carried out using the Mann-Whitney U-test. The method of KAPLAN and MEIER was used to compute the survival curves [22]. Survival was modeled with the COX regression analysis [23]. Results were considered statistically significant when the p value was less than 0.05.

## Results

First we compare serum concentrations of sCD138 in MGUS and MM patients. The mean serum sCD138 level of patients with MGUS (n=14) was 32 ng/ml (range: 5–128) and we did not observe any increasing tendency of these values after 6-month follow-up period. Soluble CD138 levels were elevated in the sera of 10 out of 17 (59%) multiple myeloma patients, the mean baseline sCD138 concentration in this group of patients was 1542 ng/ml (range: 10–17300) at the time of diagnosis. We observed a significant decrease of sCD138 level after high-dose therapy. One month after transplantation we determined the mean level at 79 ng/ml (range: 12–503). In spite of the small number of patients the difference between MGUS and MM group was highly statistically significant ($p<0.001$). Statistically significant difference was also observed in sCD138 levels before and after therapy in the MM group ($p=0.031$).

In order to evaluate sCD138 as a prognostic marker in multiple myeloma patients, we used a univariate Cox regression analysis. Soluble CD138 was evaluated as a dichotomous variable with respect to survival. When sCD138 was dichotomized by the best cut-off (500 ng/ml), the survival difference between the compared groups was statistically significant: "high" sCD138 group (>500 ng/ml, n=7) had a median survival of 36 months, the median of the "low" sCD138 group (<500 ng/ml, n=10) was not still achieved ($p=0.029$). Kaplan-Meier survival curves for both sCD138 groups are shown in Figure 1.

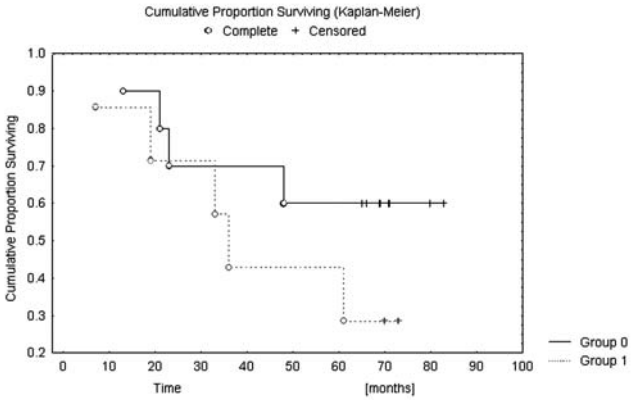


**Figure 1. Kaplan-Meier survival curves for both sCD138 groups of MM patients. Group 0: sCD138 level <500 ng/ml, Group 1: sCD138 level >500 ng/ml.**

## Discussion

Serum sCD138 level was identified as a new and reliable prognostic marker in multiple myeloma. Higher serum levels of soluble CD138 are usually associated with higher levels of serum beta$_2$-microglobulin and elevated plasma cell content in the bone marrow [24,25]. Evaluation of data collected from 138 MM patients showed that the serum sCD138 concentration could serve as an independent prognostic parameter in addition to serum beta$_2$-microglobulin and WHO performance status [20]. A good prognostic system in multiple myeloma should form a reliable basis for selecting the best treatment.

In our study was confirmed that sCD138 provides substantial prognostic value in a Cox regression model. The important finding of this study is that sCD138 does not lose its prognostic value even in patients treated by high-dose chemotherapy with subsequent autologous transplantation as some other MM prognostic factors. We found statistically significant difference between groups with high and low sCD138 levels despite relatively small amount of patients. Our results suggest that sCD138 can identify patients at "high" risk (sCD138 >500 ng/ml). We intend therefore an additional study to determine if these results are reproducible in a considerable population of myeloma patients, with respect to age and treatment regimes.

The CD138 antigen is expressed on most myeloma tumor cells. It seems therefore reasonable testing some new drugs with direct antiCD138 activity [26]. Ongoing studies suggest that CD138 when present on the cell surface exerts a positive effect by promoting tight adhesion and inhibiting invasion [8]. In contrast, once shed from the cell surface, sCD138 may exert a negative effect by promoting tumor growth [27, 28]. Thus therapies designed to block the shedding of CD138, or to block the activity of sCD138, may represent a novel approach to control tumor growth in myeloma patients.

Another important finding of this study is that patients with MM show a substantially higher median level of serum syndecan-1 than did patients with MGUS. The difference between these groups of patients was highly statistically significant. It cannot be done only by different stage of cell differentiation because both plasma cells and myeloma cells bear CD138 on their surfaces. The main reason is probably the usually higher expression of CD138 on myeloma cells than on plasma cells and concurrently because CD138 is lost by apoptotic myeloma cells [10, 17].

We conclude that sCD138 is a good prognostic parameter in MM even in patients treated by autologous transplantation. Through a rapid and simple ELISA procedure, it seems to provide additional prognostic information. Our results also indicate that sCD138 is a helpful marker in differential diagnosis of monoclonal gammopathies.

We are grateful to Mgr. V. BLÁHA for his support with statistical calculations.

## References

[1] ALEXANIAN R, WEBER D, LIU F. Differential diagnosis of monoclonal gammopathies. Arch Pathol Lab Med 1999; 123: 108–113.

[2] DURIE BG, SALMON SE. Multiple myeloma, macroglobulinemia and monoclonal gammopathies. Rec Adv Haematol 1979; 2: 243–261.

[3] KYLE RA, RAJKUMAR SV. Monoclonal gammopathies of undetermined significance. Hematol Oncol Clin North Am 1999; 13: 1181–1202.

[4] ATTAL M, HAROUSSEAU JL, STOPPA AM, SOTTO JJ, FUZIBET JG et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Inter Groupe Francais du Myeloma (IFM). N Engl J Med 1996; 335: 91–97.

[5] BARLOGIE B, JAGANNATH S, DESIKAN KR, MATTOX S, VESOLE D et al. Total therapy with tandem transplants for newly diagnosed multiple myeloma. Blood 1999; 93: 55–65.

[6] CAREY DJ. Syndecans: multifunctional cell-surface co-receptors. Biochem J 1997; 327: 1–16.

[7] SANDERSON RD, SNEED TB, YOUNG A, SULLIVAN GL, LANDLER AB. Adhesion of B-lymphoid (MPC-11) cells to type I collagen is mediated by the integral membrane proteoglycan, syndecan. J Immunol 1992; 148: 3902–3911.

[8] STANLEY MJ, LIEBERSBACH BF, LIU W, ANHALT DJ, SANDERSON RD. Heparan sulfate-mediated cell aggregation. Syndecan-1 and -4 mediate intercellular adhesion following their transfection into human B lymphoid cells. J Biol Chem 1995; 270: 5077–5083.

[9] SANDERSON RD, LALOR P, BERNFIELD M. B lymphocytes express and lose syndecan at specific stages of differentiation. Cell Regul 1989; 1: 27–35.

[10] WIJDENES J, VOOIJS WC, CLEMENT C, POST J, MORARD F et al. A plasmocyte selective monoclonal antibody (B-B4) recognizes syndecan-1. Br J Haematol 1996; 94: 318–323.

[11] BABUSIKOVA O, STEVULOVA L. Analysis of surface and cytoplasmic immunoglobulin light/heavy chains by flow cytometry using a lysed-whole-blood technique: Implications for the differential diagnosis of B-cell malignancies. Neoplasma 2004; 51: 422–430.

[12] WITZIG TE, KIMLINGER T, STENSON M, THERNEAU T. Syndecan-1 expression on malignant cells from the blood and marrow of patients with plasma cell proliferative disorders and B-cell chronic lymphocytic leukemia. Leukemia and Lymphoma 1998; 51: 167–175.

[13] DHODAPKAR MV, ABE E, THEUS A, LACY M, LANGFORD JK et al. Syndecan-1 is a multifunctional regulator of myeloma pathobiology: control of tumor survival, growth, and bone cell differentiation. Blood 1998; 91: 2679–2688.

[14] RIDLEY RC, XIAO H, HATA H, WOODLIFF J, EPSTEIN J et al. Expression of syndecan regulates human myeloma plasma cell adhesion to type I collagen. Blood 1993; 81: 767–774.

[15] VAN RIET I, VANDERKERKEN K, DE GREEF C, VAN CAMP B. Homing behaviour of the malignant cell clone in multiple myeloma. Med Oncol 1998; 15: 154–164.

[16] HOLEN I, DRURY NL, HARGREAVES PG, CROUCHER PI. Evidence of a role for a non-matrix-type metalloproteinase ac-

tivity in the shedding of syndecan-1 from human myeloma cells. Br J Haematol 2001; 114: 414–421.

[17] JOURDAN M, FERLIN M, LEGOUFFE E, HORVATHOVA M, LIAUTARD J et al. The myeloma cell antigen syndecan-1 is lost by apoptotic myeloma cells. Br J Haematol 1998; 100: 637–646.

[18] ANDERSEN NF, STANDAL T, NIELSEN JL, HEICKENDORFF L, BORSET M et al. Syndecan-1 and angiogenic cytokines in multiple myeloma: correlation with bone marrow angiogenesis and survival. Br J Haematol 2005; 128: 210–217.

[19] KYRTSONIS M-CH, VASSILAKOPOULOS TP, SIAKANTARIS MP, KOKORIS SI, GRIBABIS DA et al. Serum syndecan-1, basic fibroblast growth factor and osteoprotegerin in myeloma patients at diagnosis and during the course of the disease. Eur J Haematol 2004; 72: 252–258.

[20] SEIDEL C, SUNDAN A, HJORTH M, TURESSON I, DAHL IMS et al. Serum syndecan-1: a new independent prognostic marker in multiple myeloma. Blood 2000; 95: 388–392.

[21] DURIE BG, SALMON SE. A clinical staging system for multiple myeloma: correlation of measured myeloma cell mass with presenting clinical features, response to treatment and survival. Cancer 1975; 36: 842–854.

[22] KAPLAN EL, MEYER P. Non-parametric estimation from incomplete observations. J Am Stat Assoc 1958; 53.

[23] COX DR. Regression models and life tables. JR Stat Soc 1982; 34.

[24] AREF S, GODA T, EL-SHERBINY M. Syndecan-1 in multiple myeloma: relationship to conventional prognostic factors. Hematology 2003; 8: 221–228.

[25] DHODAPKAR MV, KELLY T, THEUS A, ATHOTA AB, BARLOGIE B et al. Elevated levels of shed syndecan-1 correlate with tumour mass and decreased matrix metalloproteinase-9 activity in the serum of patients with multiple myeloma. Br J Haematol 1997; 99: 368–371.

[26] TASSONE P, GOLDMACHER VS, NERI P, GOZZINI A, SHAMMAS MA et al. Cytotoxic activity of the maytansinoid immunoconjugate B-B4-DM1 against CD138+ multiple myeloma cells. Blood 2004; 104: 3688–3696.

[27] YANG Y, YACCOBY S, LIU W, LANGFORD JK, PUMPHREY CY et al. Soluble syndecan-1 promotes growth of myeloma tumors in vivo. Blood 2002; 100: 610–617.

[28] KLEIN B, TARTE K, JOURDAN M, MATHOUK K, MOREAUX J et al. Survival and proliferation factors of normal and malignant plasma cells. Int J Hematol 2003; 78: 106–113.