Serum syndecan-1: a new independent prognostic marker in multiple myeloma

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Serum samples drawn at diagnosis from 174 myeloma patients were analyzed for the presence of the heparin sulfate proteoglycan, syndecan-1. Syndecan-1 was elevated in 79% of patients (median, 643 units/mL) compared with 40 healthy controls (median, 128 units/mL), \( P < .0001 \). Serum syndecan-1 correlated with the following: serum creatinine, secretion of urine M-component over the course of 24 hours, soluble interleukin-6 (IL-6) receptor, C-terminal telopeptide of type I collagen, \( \beta_2 \)-microglobulin, percentage of plasma cells in the bone marrow, disease stage, and serum M-component concentration. In order to evaluate syndecan-1 as a prognostic marker in multiple myeloma, it was entered into a multivariate Cox regression model. Data from 138 patients were available for this analysis. As a continuous variable, syndecan-1 was an independent prognostic parameter in addition to serum \( \beta_2 \)-microglobulin and World Health Organization performance status. When syndecan-1 was dichotomized by the best cutoff (66th percentile, 1170 units/mL), the survival difference between the groups was highly significant: “high” syndecan-1 group had a median survival of 20 months, and the “low” syndecan-1 group had a median of 44 months (\( P < .0001 \)). We conclude that syndecan-1 is a new independent prognostic parameter in multiple myeloma, and its role in prognostic classification systems should be further investigated.

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Introduction

Multiple myeloma is a B-cell malignancy characterized by the accumulation of clonal malignant plasma cells. It is associated with the production of monoclonal immunoglobulins, bone destruction, anemia, hypercalcemia, and renal dysfunction.

Syndecan is a member of a family of integral membrane heparin sulfate proteoglycans.\(^1\) It is known to participate in cell-matrix adhesion processes by binding to collagens,\(^2,4\) fibronectin,\(^5\) and thrombospondin.\(^6\) Syndecan can also serve as a low-affinity receptor for heparin-binding growth factors.\(^7\)

Within the bone marrow, syndecan-1 is detected solely on cells of the B lymphocyte lineage, and its expression changes at specific stages of differentiation. In mice it is present on the surface of pre-B cells, lost in mature B cells, and re-expressed in plasma cells.\(^8\) In the bone marrow of myeloma patients, syndecan-1 is reported to be expressed on myeloma cells only;\(^9\) it is also expressed on malignant plasma cells in peripheral blood.\(^10\) Syndecan-1 is rapidly lost by apoptotic myeloma cells.\(^11\) Since syndecan-1 is expressed on the surface of viable malignant plasma cells, specific antibodies to syndecan-1 are used for identification and purification of myeloma cells from clinical samples.\(^5,12\)

Previous studies have shown that syndecan-1 is shed from the surface of myeloma cells in culture\(^13\) and into human serum.\(^14\) Measured by a semiquantitative method, syndecan-1 levels in serum of 720 myeloma patients were elevated compared with normal controls. High levels were associated with a high percent of bone marrow plasmacytosis and \( \beta_2 \)-microglobulin levels.\(^14\)

In this study, we analyzed serum levels of shed syndecan-1 in a large well-characterized population of myeloma patients in order to determine its relation to prognosis and other variables at the time of diagnosis.

Patients and methods

Study population

A total of 592 patients were entered in the Nordic Myeloma Study Group (NMSG) randomized trial from June 1990 until November 1992. In this study, patients were randomized to receive melphalan and prednisone with or without the addition of low-dose \( \alpha \)-interferon. The diagnostic and eligibility criteria and results were previously described by NMSG.\(^15\) The following parameters were registered for all patients at the time of diagnosis: age; sex; Durie-Salmon\(^16\) stage; the World Health Organization (WHO) performance status\(^15\); a grading of bone morbidity in 3 stages, as judged by X-ray abnormality (no changes, limited changes, advanced changes); percentage of plasma cells in the bone marrow; immunoglobulin (g) class; serum M-component protein concentration; albumin; calcium; creatinine; total alkaline phosphatase; \( \beta_2 \)-microglobulin; and secretion of urine M-component over the course of 24 hours.

After completion of the study, approximately 400 sera drawn at diagnosis were analyzed for interleukin-6 (IL-6), IL-6 receptor, C-reactive protein (CRP), osteocalcin, C-terminal telopeptide of type I collagen (ICTP), and hepatocyte growth factor (HGF). A manuscript on prognostic factors in the original larger patient material has been published separately.\(^30\) The present study was performed after the closing of this study, and therefore data on syndecan-1 will only be reported in this paper.
The serum syndecan-1 values in patients at the time of diagnosis (20,000 units/mL, ie, over 100 times higher than the median level of normal controls) were 370 units/mL, which is considered above the normal range by conventional criteria. The NMSG study found no significant survival difference between the 2 arms of treatment. Thus it was possible to pool data from the treatment arms to evaluate the prognostic significance for the studied parameter.

Results

Serum analyses

The serum syndecan-1 values in patients at the time of diagnosis and in controls are shown in Figure 1. The distribution of syndecan-1 concentrations was skewed (kurtosis = 15). The median syndecan-1 concentration (25th to 75th percentile) was 643 units/mL (401-2022) in the myeloma and 128 units/mL (76-208) in the control sera. This difference was statistically significant ($P < .0001$). The maximal syndecan-1 level measured in a patient was 20,000 units/mL, ie, over 100 times higher than the median level of normal controls. In 137 patients (79%), the syndecan-1 levels were above the mean level +2SD of syndecan-1 in the control group (> 370 units/mL), which is considered above the normal range by conventional criteria.

Correlation to other parameters

A significant correlation coefficient (r) was obtained with respect to serum creatinine, secretion of urinary M-component over 24 hours, IL-6 receptor, ICTP, $\beta_2$-microglobulin, percentage of plasma cells in the bone marrow, disease stage, and serum M-component concentration (Table 1). By forward selection of these variables, a multiple linear regression yielded creatinine and the percentage of plasma cells in the marrow as the best predictors of syndecan-1 concentration (with an adjusted $r^2$ of 0.18).

Only statistically significant correlations are included. There was no significant correlation between syndecan-1 and pretreatment age, type of serum M-component, radiographic staging of bone destruction, IL-6, CRP, calcium, HGF, alkaline phosphatase, albumin, or osteocalcin.

$r$ indicates correlation coefficient for syndecan-1 and the designated variable.

| Table 1. Correlations between serum syndecan-1 and other variables at diagnosis |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Serum Creatinine | Urine M-Component | IL-6 Receptor | ICTP | $\beta_2$-Microglobulin | % Marrow Plasma Cells | Stage | Serum M-Component |
| N | 174 | 164 | 173 | 157 | 174 | 173 | 174 | 173 |
| $r^*$ | 0.41 | 0.29 | 0.28 | 0.25 | 0.25 | 0.21 | 0.20 | 0.17 |
| $P$ value | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 |

Figure 1. Syndecan-1 in serum by ELISA. Serum syndecan-1 levels at diagnosis in 174 patients with multiple myeloma. Horizontal line denotes median value of 643 units/mL and 40 healthy age- and sex-matched controls (median, 128 units/mL). The difference between the groups is highly significant ($P < .0001$).
between syndecan-1 and pretreatment age, type of serum M-component, radiographic staging of bone destruction, IL-6, CRP, calcium, HGF, albumin, alkaline phosphatase, or osteocalcin (data not shown).

**Relation to treatment response**

When syndecan-1 was evaluated in a univariate logistic regression model, it was a significant predictor of response to treatment ($P = .01$). However, in a multivariate model, it did not retain significance.

**Survival analyses**

When syndecan-1 (transformed by the natural logarithm) was entered in a univariate Cox regression analysis, it was a significant predictor of mortality ($P = .0006$). Syndecan-1 was therefore entered into a multivariate Cox regression analysis involving the other factors in this patient material that held significant ($P < .05$) prognostic information in a univariate Cox regression analysis: serum calcium; soluble IL-6 receptor; $\beta_2$-microglobulin; WHO performance status (0-2 versus 3-4); and ln IL-6, ln CRP, ln creatinine, and ln [ICTP] (data not shown). Patients with missing variables were excluded from the analysis. Complete data from 138 patients were available.

Table 2 demonstrates the result of the multivariate Cox regression. Only 3 factors retained prognostic significance: ln [syndecan-1] ($P = .002$); $\beta_2$-microglobulin ($P = .004$); and WHO performance, 0-1 versus 2-3 ($P = .01$). Thus, when syndecan-1 was included in the model, corrected serum calcium, IL-6, soluble IL-6 receptor, CRP, creatinine, and ICTP added no further prognostic information. Without syndecan-1, the final model included $\beta_2$-microglobulin, corrected serum calcium, and WHO performance status. The difference between the models with and without syndecan-1 was 4.2 $\chi^2$ ($P < .05$).

Syndecan-1 was further evaluated as a dichotomous variable with respect to survival. An evaluation of survival, using different cutoff levels, is summarized in Table 3. The best separation of the curves was with the cutoff point at the 66th percentile of the syndecan-1 values ($\geq 1170$ units/mL). There was a highly significant survival difference ($P = .0001$) between the “high” syndecan-1 group ($\geq 1170$ units/mL, $n = 58$) and “low” syndecan-1 group ($< 1170$ units/mL, $n = 116$). Median survival was 20 and 43 months, respectively, as shown in Figure 2. The follow-up period of surviving patients did not differ significantly between the high and low syndecan-1 groups.

Syndecan-1 high/low grouping ($> 1170$ units/mL) was applied to stratify 2 established classification systems: Durie-Salmon stage I and II, thus these data were pooled. Syndecan-1 separated patients by both classification systems, in all risk categories. The separation was highly significant in the medium-risk and high-risk patient groups.

**Discussion**

The main finding is that in a well-defined population of untreated myeloma patients, the serum syndecan-1 level is a new and powerful prognostic marker. A good prognostic system in multiple myeloma should ideally form the basis for selecting the best treatment, and it should include only variables with independent prognostic information. In order to be useful in clinical practice, these should be available at diagnosis and be measured with simple reproducible techniques. A number of prognostic factors reflecting various aspects of the disease have been identified in myeloma, relating to either the intrinsic malignancy of the tumor, host-tumor interactions, renal function, or tumor mass. Of these, serum $\beta_2$-microglobulin concentration is regarded as one of the most powerful prognostic factors. In our study, we show that syndecan-1 provides substantial prognostic value in a Cox regression model with proven prognostic markers, including $\beta_2$-microglobulin.

An important question is whether syndecan-1 can identify patients at high risk who have a favorable prognosis by other classification systems. Our results, as illustrated in Figures 3 and 4, suggest that this is indeed the case, especially in the medium-risk and high-risk patient groups. Notably, for medium-risk patients, as classified by the Bataille system, with a concomitant high syndecan-1 level, the median survival time was 17 months. This

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**Table 2. Variables with independent prognostic importance for survival according to a multivariate Cox regression analysis, with and without syndecan-1 in the model**

<table>
<thead>
<tr>
<th>Variable</th>
<th>$\beta$ Coefficient</th>
<th>SE</th>
<th>Significance ($P$-value)</th>
<th>$\chi^2$ of the Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model With Syndecan-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ln (Syndecan-1)</td>
<td>0.30</td>
<td>0.10</td>
<td>.002</td>
<td></td>
</tr>
<tr>
<td>$\beta_2$-Microglobulin</td>
<td>0.05</td>
<td>0.02</td>
<td>.004 (4.02)</td>
<td></td>
</tr>
<tr>
<td>WHO status (0-2 vs 3-4)</td>
<td>0.06</td>
<td>0.26</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Model Without Syndecan-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\beta_2$-Microglobulin</td>
<td>0.06</td>
<td>0.02</td>
<td>.0005 (40.2)</td>
<td></td>
</tr>
<tr>
<td>WHO status (0-2 vs 3-4)</td>
<td>0.61</td>
<td>0.26</td>
<td>.02</td>
<td>36.0</td>
</tr>
<tr>
<td>s-Calcium</td>
<td>0.62</td>
<td>0.26</td>
<td>.01</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 3. Survival and pretreatment values with different cutoff values**

<table>
<thead>
<tr>
<th>Syndecan-1 Cutoff Value</th>
<th>33rd Percentile</th>
<th>Median</th>
<th>66th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median survival above cutoff (months)</td>
<td>28</td>
<td>24</td>
<td>20</td>
</tr>
<tr>
<td>Median survival below cutoff (months)</td>
<td>38.3</td>
<td>43</td>
<td>43</td>
</tr>
</tbody>
</table>

$\chi^2$ value, $P$ value

| Median survival above cutoff (months) | 1.45, $P = .23$ |
| Median survival below cutoff (months) | 5.98, $P = .01$ |
| $n$ | 174. |

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**Figure 2. Kaplan-Meier survival curves for 174 myeloma patients.** The curves are separated by: (A) “high” syndecan-1 levels ($\geq 1170$ units/mL, $n = 58$, median survival 20 months) versus (B) “low” syndecan-1 levels ($< 1170$ units/mL, $n = 116$, median survival 43 months). Open circles represent censored patients. The survival difference was highly significant ($P = .0001$).
was shorter than the 19-month median survival of the Bataille
high-risk group as a whole. However, our cutoff point for syndecan-1
was derived from the present set of data. Thus, additional studies
must be performed to determine if these results are reproducible in
other populations of myeloma patients, with respect to age and
treatment regimes.

The NMSG study was a multicenter trial, with limitations in the
variables available for evaluation of prognosis. Thus our analysis
could not include data on some known powerful prognostic
factors in these patients: plasma cell labeling index, the percent-
age of circulating plasma cells, or karyotype abnormalities. Further
studies should be designed to determine if syndecan-1 retains
independent prognostic information when these parameters are
available.

Syndecan-1 correlated significantly with a number of variables
in our study (Table 1). These results are in accordance with
previous suggestions that soluble syndecan-1 reflects tumor mass
(as assayed by the percentage of plasma cells in the marrow, urine
and serum M-component levels, and soluble IL-6 receptor). Also, it may reflect renal failure as determined by increased levels
of serum creatinine. However, the multiple linear regression model
suggests that approximately 20% of the variability in syndecan-1
levels can be attributed to variations in the percentage of plasma
cells in the marrow and serum creatinine. The fact that syndecan-1

Figure 3. Kaplan-Meier survival curves for patients classified by the staging
system of Durie and Salmon. The solid line represents high syndecan-1 levels
(≥ 1170 units/mL), and the dotted line represents low syndecan-1 levels (< 1170
units/mL). Open circles represent censored patients. (A) Patients in Durie-Salmon stages I and II were separated by high syndecan-1 levels (n = 21) and low syndecan levels (n = 68), P = .20. (B) Patients in Durie-Salmon stage III were separated by high syndecan-1 levels (n = 37) and low syndecan-1 levels (n = 48), P = .0006.

Figure 4. Kaplan-Meier survival curves for patients classified by the staging
system of Bataille et al. The drawn line represents high syndecan-1 levels
(≥ 1170 units/mL), and the dotted line represents low syndecan-1 levels (< 1170
units/mL). Open circles (○) represent censored patients. Panels A, B, and C depict the high and low levels of syndecan-1 for patients classified by Bataille: (A) Stadium 1: CRP ≤ 6 mg/L and β₂-microglobulin ≤ 6 mg/L; high levels (n = 64) were separated from low levels (n = 21), P = .10. (B) Stadium 2: CRP ≥ 6 mg/L or β₂-microglobulin ≥ 6 mg/L; high levels (n = 33) were separated from low levels (n = 20), P = .003. (C) Stadium 3: CRP ≥ 6 mg/L and β₂-microglobulin ≥ 6 mg/L; high levels (n = 14) were separated from low levels (n = 14), P = .05.
contains prognostic information which is superior to these variables could indicate that syndecan-1 not only reflects tumor load and renal failure but also other biological aspects of the disease. Syndecan-1 has been found to increase osteoblast development and inhibit osteoclast formation in murine bone marrow cell cultures, suggesting that syndecan-1 may counteract bone destruction. However, syndecan-1 expression does not differ among patients with or without lytic bone lesions. Our study does not support a clear connection between syndecan-1 and the degree of bone affection (as determined by serum calcium levels, alkaline phosphatase, and osteocalcin). In fact, we found a significant positive association between syndecan-1 and serum ICTP, which is a marker of collagen degradation.

In culture, shed syndecan-1 has been shown to induce apoptosis of myeloma cell lines through an unknown mechanism. Also, the positive association between syndecan-1 and serum ICTP, which is an indicator of bone affection (as determined by serum calcium levels, alkaline phosphatase, and osteocalcin), suggests that syndecan-1 may counteract bone destruction. However, syndecan-1 expression does not differ among patients with or without lytic bone lesions. Our study does not support a clear connection between syndecan-1 and the degree of bone affection (as determined by serum calcium levels, alkaline phosphatase, and osteocalcin). In fact, we found a significant positive association between syndecan-1 and serum ICTP, which is a marker of collagen degradation.

We conclude that serum syndecan-1 is a new independent prognostic parameter in multiple myeloma. Through a rapid and simple ELISA procedure, it seems to provide additional prognostic information in some commonly used classification systems. We suggest that syndecan-1 levels should be further explored in the prognostic classification of myeloma to determine its clinical usefulness.

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References

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