Serum Levels of Soluble Syndecan-1 in Hodgkin's Lymphoma

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Abstract. *Background: Syndecan-1 (CD138) is expressed by the Hodgkin-Reed-Sternberg (HRS) cells of classic Hodgkin's lymphoma (cHL), but not in nodular lymphocyte-predominant HL. Syndecan-1 may be involved in the interaction between HRS cells and the cellular and stromal microenvironment typical of nodular sclerosing HL. Patients and Methods: Serum levels of soluble syndecan-1 were determined by ELISA in 66 patients with HL and 14 age- and sex-matched healthy individuals. Results: The levels of syndecan-1 were higher in HL patients than controls (100.2±35.9 ng/ml vs. 67.9±24.5 ng/ml, p<0.001). They marginally correlated with advanced age (p=0.06), male gender (p=0.07) and consequently high IPS (p=0.01), but did not correlate with markers of tumor burden and prognosis, including serum interleukin-10 and soluble CD30. At 6 years, failure-free survival was 70±9% vs. 50±11% (p=0.32) for patients with serum soluble syndecan-1 levels above or below the observed median value of 91 ng/ml. Conclusion: The serum levels of syndecan-1 were elevated in patients with HL, but were not strongly correlated with other potential prognostic factors. Their effect on prognosis deserves further evaluation.*

Hodgkin's lymphoma (HL) represents a B-cell malignancy in the vast majority of cases (1). Syndecan-1 (CD138) is a member of the heparan sulfate transmembrane proteoglycan superfamily, mediating the binding of B lymphocytes to the interstitial matrix. It is expressed in post-germinal center B cells, including plasma cells and immunoblasts, as well as the Hodgkin-Reed-Sternberg (HRS) cells of classic HL, but not in the neoplastic cells of nodular lymphocyte-predominant HL (2, 3). Syndecan-1 may be involved in the interaction between HRS cells and the cellular and stromal microenvironment typical of nodular sclerosing HL through its ligands, namely collagen types I and III and fibronectin.

Elevated serum syndecan-1 is a potent independent prognostic factor in multiple myeloma (4, 5). Syndecan-1 can be shed from the surface of myeloma cells and circulate as a soluble molecule (6). Since syndecan-1 is expressed in various percentages in the HRS cells of all cases of classic HL (2), and plasmacytes are present in the reactive infiltrate surrounding the neoplastic cells, it could be hypothesized that its serum levels would also be elevated in this disease, and might have a role as a prognostic marker.

Given the growing interest in biological prognostic factors in HL (7), the levels of soluble syndecan-1 in serum samples of patients with HL were determined, and possible correlations with clinicopathological characteristics and outcome evaluated.

Patients and Methods

Patients, staging and treatment. The pretreatment serum levels of soluble syndecan-1 were determined in 66 patients with HL, as well as in 14 age- and sex-matched healthy individuals. All patients had histologically confirmed HL, were older than 14 years, HIV-negative and had been treated with ABVD chemotherapy or equivalent regimens, with or without radiotherapy. The process of further selection was as follows: 22 patients, with either primary refractory or relapsed disease, were selected on the basis of pretreatment serum sample availability. Subsequently, we identified 44 patients in their first complete remission (CR$_1$), who were comparable to the previous group with respect to age, gender, stage and year of diagnosis, and also had available pretreatment serum samples.

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All patients were clinically staged according to the Ann-Arbor system, using standard staging procedures. Clinical stages IA and IIA were considered early, while clinical stages IB, IIB, III and IV were considered advanced. The general directions of treatment in our Unit, based on ABVD or equivalent regimens, with or without radiotherapy, have been previously presented (8, 9).

Laboratory assays. Serum levels of soluble syndecan-1 were measured by immunosorbent assay [sCD138 (syndecan-1) ELISA Kit; DIACLONE Research, Besancon, France]. ELISA assays were performed in duplicate and were reproducible. The cut-off limit for the detection of serum soluble syndecan-1 was 2.56 ng/ml. The definition of abnormal values was based on determinations in 14 age- and sex-matched healthy individuals. Serum soluble syndecan-1 levels were considered elevated when they exceeded the mean+2SD observed in this group. The serum levels of β_2-microglobulin, soluble CD30 and interleukin-10 (IL-10) were determined, as previously described (8, 10-12).

Statistical analysis. The Mann-Whitney and Kruskall-Wallis tests were used for non-parametric comparisons of serum soluble syndecan-1 among subgroups of patients defined by their characteristics, as appropriate (13). The association between serum soluble syndecan-1 and sCD30 was evaluated by Spearman's rank correlation coefficient. Failure-free survival (FFS) was defined as the time interval between treatment initiation and treatment failure or last follow-up. Failure was defined as inability to achieve complete or partial remission during initial therapy, requiring a switch to another chemotherapy regimen, or progression after an initial complete or partial remission. The estimation of actuarial FFS was performed by the method of Kaplan-Meier (14). The identification of prognostic factors in univariate analysis was based on the log-rank test (15).

Results

Patients' characteristics. The median age of the patients was 33 years (16-78) and 48 (73%) were males. Thirty patients (45%) had early and 36 (55%) had advanced stage disease, while 27 (41%) had B-symptoms. The histological subtype was nodular sclerosis in 48 patients (74%), mixed cellularity in 12 (18%), nodular lymphocyte predominance in 5 (8%) and unknown in one. Other patient characteristics included: inguinal/iliac involvement in 14/66 patients (21%), bulky disease in 21/56 (38%), involvement of 5 or more anatomic sites in 14/66 (21%), anemia in 25/66 (38%), leukocytes $\geq 15 \times 10^9$/l in 11/66 (17%), severe lymhocytopenia according to the IPS definition (16) in 9/59 (15%), ESR $\geq$50mm in 31/59 (53%), albumin <4 g/dl in 21/62 (34%), elevated LDH in 19/58 (33%), elevated β_2-microglobulin in 30/65 (46%), elevated serum IL-10 levels in 33/66 (50%) and sCD30 $\geq$100 U/ml in 18/66 (27%). The median follow-up of patients, who were alive at the time of the analysis, was 60 months (23-140).

Serum levels of soluble syndecan-1. The patients had higher pretreatment serum levels of soluble syndecan-1 compared
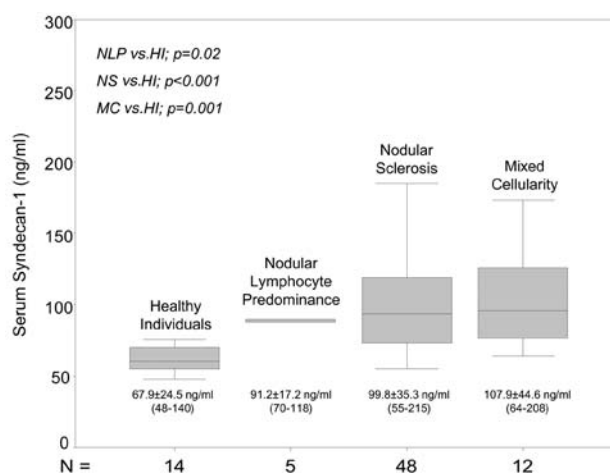

Figure 1. *Comparison of serum levels of soluble syndecan-1 among healthy individuals and patients with different histological subtypes of Hodgkin's lymphoma. Statistical comparisons were based on the Mann-Whitney test. The figures below each box represent the mean±standard deviation and range of serum soluble syndecan-1 values in patients with each subtype and healthy individuals (NLP: nodular lymphocyte predominance, NS: nodular sclerosis, MC: mixed cellularity, HI: healthy individuals).*

with the age- and sex-matched healthy individuals (100.2±35.9 ng/ml *vs.* 67.9±24.5 ng/ml, *p*<0.001). The median values were 91.0 (range 55-215) *vs.* 60.5 (range 48-140) ng/ml, respectively. As shown in Figure 1, patients with either nodular sclerosis, or mixed cellularity, or even nodular lymphocyte predominance, had higher levels of serum soluble syndecan-1 than healthy subjects (99.8±35.3 ng/ml, *p*<0.001; 107.9±44.6 ng/ml, *p*=0.001; 91.2±17.2 ng/ml, *p*=0.02, respectively).

Considering the upper normal limit at 116.9 ng/ml (*i.e.* 67.9+2x24.5), 17/66 (26%) patients showed elevated levels of soluble syndecan-1. Higher levels were marginally correlated with age (*p*=0.06), male gender (*p*=0.07) and elevated β_2-microglobulin (*p*=0.08). They were also significantly correlated with IPS $\geq$3 (*p*=0.01).

No other relationship with baseline clinical and laboratory features was demonstrated. There was also no significant correlation with elevated serum IL-10 levels (*p*=0.70 by Mann-Whitney test). Although there was a trend towards a positive correlation between serum soluble syndecan-1 and sCD30 levels, this was not statistically significant (Spearman's rho 0.17, *p*=0.18).

Failure-free survival. There was no difference in serum levels of soluble syndecan-1 between patients in CR_1 and those with refractory/relapsed disease (98.4±35.8 ng/ml *vs.* 104.1±36.7 ng/ml, *p*=0.48). Similarly, the 6-year FFS was 66±7% *vs.* 51±14% for patients with normal and elevated levels, respectively (*p*=0.81). When survival analysis was conducted at the cut-off of the median value (91.0 ng/ml),

the 6-year FFS was 70±9% *vs.* 50±11% for patients with soluble syndecan-1 levels lower or higher than 91.0 ng/ml, respectively. However, the difference was not statistically significant (p=0.32, Figure 2).

Discussion

We report, here, that serum levels of soluble syndecan-1 are elevated in patients with HL compared to controls. The serum levels of soluble syndecan-1 in patients with HL probably reflect: (a) a normal baseline level, as concluded by the presence of detectable levels in all healthy subjects, (b) the tumor burden, since syndecan-1 is expressed in all cases of classic HL, (c) the percentage of positive HRS cells, which varies widely from case to case (2, 3), and (d) the presence of plasmacytes in the neoplastic infiltrate of HL tissues. The reason for higher mean levels of syndecan-1 in the serum of patients with nodular lymphocyte-predominant HL compared to controls remains unclear, because the neoplastic cells in these cases do not express syndecan-1. However, this might represent a normal variation, since we did not observe marked elevations of syndecan-1 in this small subgroup of patients, who presented an almost absolute overlap with the range of normal values (Figure 1).

One might expect that serum levels of soluble syndecan-1 would behave as a tumor marker in HL. In this study, no association could be demonstrated between soluble syndecan-1 and surrogate markers for tumor burden, such as clinical stage, B-symptoms, number of involved anatomic sites, disease bulk and laboratory markers. Furthermore, the correlation between serum levels of soluble syndecan-1 and sCD30 was not statistically significant. The lack of association between serum soluble syndecan-1 and indices of tumor burden may be due to the high variability of syndecan-1 expression on HRS cells among cases (2). In contrast to tumor burden, serum soluble syndecan-1 showed a borderline correlation with older age and male gender. These associations explain the statistically significant relationship with higher IPS values (16), as well as with higher serum β_2-microglobulin levels, which are known to correlate with advanced age (8).

Despite the relatively large absolute difference in 6-year FFS between patients with serum syndecan-1 below and above the median value, the moderate number of patients and failure events, as well as the shape of the Kaplan-Meier curves, which were superimposable for the initial 2 years of follow-up, prevented the emergence of a statistically significant difference.

Considering the elevated levels of serum soluble syndecan-1 in patients with HL at diagnosis, the biological rationale for a relationship between this molecule and prognosis, and the non-significant trend towards inferior outcome for patients with high levels, further study appears to be justified.
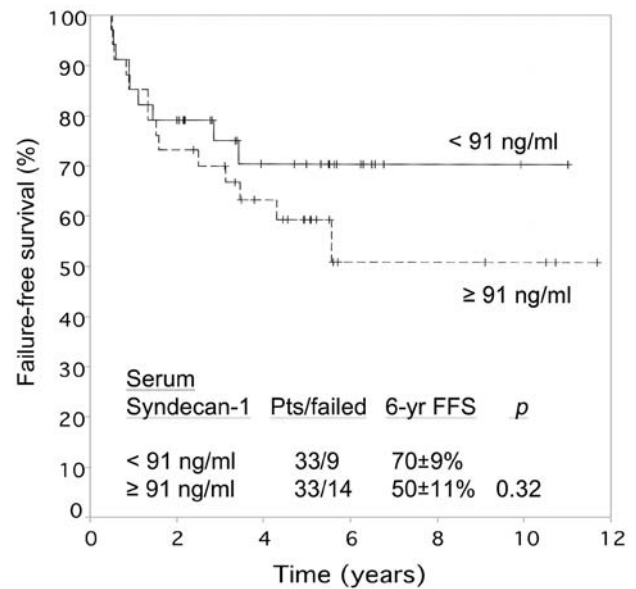

Figure 2. *Effect of serum levels of soluble syndecan-1 on failure-free survival of 66 patients with Hodgkin's lymphoma, treated with ABVD or equivalent regimens, with or without radiotherapy.*

Acknowledgements

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