Clinical Significance of Serum CD44 Measurement in Malignant Lymphoma

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Summary: We monitored the effectiveness of lymphoma therapy by measuring the serum levels of soluble CD44std (sCD44std) and soluble CD44v6 (sCD44v6). Furthermore, we measured the level of soluble interleukin 2 receptor (sIL-2R). A total of 24 patients with non-Hodgkin's lymphoma were enrolled. sCD44std, sCD44v6, and sIL-2R on serum were measured using ELISA system. In all patients, only the sIL-2R level decreased significantly following therapy. However, an analysis of CR and PR showed that the degree of decrease in the sCD44std level was significantly greater than that in the sIL-2R level. Furthermore, among the CS IV cases, only the CD44std level decreased significantly after therapy. These findings suggest that the level of serum sCD44std reflects clinical pathology more closely than the level of serum sIL-2R in CS IV patients and those who respond well to therapy. Moreover, when T-cell and B-cell lymphomas were analyzed separately, the levels of sCD44std and sIL-2R decreased significantly after therapy in patients with B-cell lymphomas, and the degree of decrease in the sCD44std level was very significant with a p-value of 0.0003. This suggests that when sCD44std is used as an index of treatment, it more closely reflects the treatment of B-cell lymphomas. Level of serum sCD44std should prove to be a useful marker for assessing the effectiveness of lymphoma therapy.

Key words malignant lymphoma, CD44, soluble CD44std, soluble CD44v6, soluble interleukin 2 receptor

INTRODUCTION

As lymphocytes migrate from the blood to lymphatic tissue, they bind to high endothelial veins. This step requires several molecules called homing receptors, and CD44 (cell surface glycoprotein) is one such receptor [1]. Although CD44 was first reported to be an adhesion molecule involved in homing [2], it has become clear since then that this molecule is expressed widely in the body and is involved in a wide variety of phenomena in different tissues [3]. In recent years, CD44 has been examined as an adhesion molecule that directly mediates cancer metastases [4].

CD44 is a protein, approximately 85-95 KD in size, and different CD44 mRNA isoforms are formed by alternative splicing of variant exons [4]. Standard CD44 (CD44std) is expressed widely, and one study found that CD44v6, a CD44 variant, was involved in metastasis of certain types of tumor cells [5]. Also, some studies have observed that, among different malignant lymphomas, lymphoma cells having CD44 on their surfaces were more likely to proliferate when compared to those without CD44 [6-8]. CD44std is expressed in all lymphatic tissues, whereas CD44v6 is only expressed in malignant lymph nodes, and is particularly strong in high-grade malignant lymphoma [9].

Therefore, we monitored the effectiveness of lymphoma therapy by measuring the serum levels of soluble CD44std (sCD44std) and soluble CD44v6 (sCD44v6). Furthermore, we measured the level of soluble interleukin 2 receptor (sIL-2R), which has been recently utilized as a marker for adult T-cell
leukemia (ATL) and malignant lymphoma, and compared the clinical significance of these markers.

MATERIALS AND METHODS

Patients
A total of 24 patients with non-Hodgkin's lymphoma were enrolled in the study. Clinical stage (CS) was CS I in 3 cases, CS II in 7 cases, CS III in 4 cases, and CS IV in 10 cases according to Ann Arbor classification [10]. Phenotypes of tumor cells included 5 cases of T-cell lymphoma and 14 cases of B-cell lymphoma. The remaining 5 cases could not be clearly identified.

Measurement of sCD44std, sCD44v6, and sIL-2R
Heparinized peripheral blood was obtained before and after treatment from each patient. sCD44std was measured using sCD44std ELISA (Bender Med Systems, Austria). HRP-conjugate second body was added to the serum sample, and the resultant sample was incubated for 3 hrs. TMB (coloring base quality 3,3',5'-tetramethylbenzidine) substrate solution was added to the sample and incubated for 15 min at room temperature. Stop-solution was then added to the solution. sCD44v6 was measured using sCD44v6 ELISA (Bender Med Systems, Austria), and sIL-2R was measured using sIL-2R ELISA (IMMUNOTECHS A. S., France).

Statistical analyses
All data were analyzed using Student's t-test or Fisher's FLSD (ANOVA).

RESULTS

Analysis in clinical stage
All three cases of CS I achieved CR (complete remission) following treatment. sCD44std and sIL-2R showed a tendency to decrease after treatment, but the trend was not significant (Fig. 1). Among CS II patients, 3 cases achieved CR, 3 achieved PR (partial remission), and 1 case showed a non-response (NR). sIL-2R showed a tendency to decrease after treatment, but the trend was not significant in sCD44std, sCD44v6, and sIL-2R. All 4 CS III cases achieved PR. sCD44std and sIL-2R showed a tendency to decrease after treatment, but the trend was not significant. Among the 10 CS IV cases (CR: 3 cases, PR: 6 cases, uncertain: 1 case), sIL-2R showed a
tendency to decrease after treatment, but the trend was not significant. sCD44std was decreased significantly (p=0.039) after treatment.

**Analysis of PR and CR patients**

In cases achieving CR (Fig. 2) or PR (Fig. 3), sCD44std and sIL-2R were decreased significantly after treatment. In all cases (CSI-IV), sCD44std and sIL-2R were decreased significantly after treatment (p=0.001 and p=0.002, respectively, Fig. 4).

**Analysis of cell phenotype**

In the 15 B-cell lymphoma cases, sCD44std and sIL-2R were decreased significantly after treatment (p=0.0003 and p=0.002, respectively, Fig. 5a, b). In the 4 T-cell lymphoma cases, sCD44std and sIL-2R showed a tendency to decrease after treatment, but the trend was not significant.

**Analysis of cell tissue type**

In diffuse medium (8 cases), diffuse mixed (5 cases), and diffuse large cell type (6 cases), sCD44std and sIL-2R showed a tendency to decrease after treatment, but the trend was not significant (Fig. 6). We were unable to analyze diffuse small cell type and diffuse pleomorphic cell type lymphoma because

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**Fig. 3.** Patients with NHL changed to PR (partial remission) after treatments.

**Fig. 4.** Examination of all cases.

**Fig. 5.** Analysis of cell type. a) B cell type b) T cell type

**Fig. 6.** Construction by forms of NHL.
our study group included only 2 cases of each.

DISCUSSION

CD44 is expressed in various tissues, including white blood cells, red blood cells, fibroblasts, epithelial cells, vascular endothelial cells, skeletal muscles and the central nervous system [3], where it induces lymphocyte proliferation and cytotoxicity [11]. Also, when lymphocytes are stimulated by an antigen, different isoforms of CD44 having variant exons are expressed, and these variants play an important role in the immunological response of B cells [12]. Moreover, the expression of CD44 having variant exons correlates well to the metastasis of human colon and breast cancer, and in rats, a transfectant expressing variant exon 6 is highly metastatic. These facts implicate CD44 in cancer metastasis [5]. Reports on the relationship between malignant lymphoma and CD44 have documented that 51% of lymphomas with high CD44 expression are already advanced at the time of diagnosis [6], and further that the survival time for patients with lymphomas with low or no CD44 expression is significantly longer than that for those with lymphomas with high CD44 expression [7]. The results of lymph node biopsies show that CD44std is expressed in all lymphatic tissues, including healthy ones, and thus the clinical significance of CD44std in lymphoma assessment is low, whereas that of variant types (3v, 6v) is high [9]. Also, CD44 is expressed in approximately 90% of NHL cases, and although there is no significant difference in the level of CD44 expression from low to high grade lymphomas, it reflects the clinical pathology of this condition well, thus suggesting its potential use as a marker of therapeutic effectiveness [19]. As mentioned previously, there are various reports on cell surface CD44. In the present study, we measured the level of serum soluble CD44, not as a surface antigen of lymphocytes, and investigated its relationship to the pathology of lymphomas. It has been documented that the level of serum soluble IL-2 receptor α chain (sIL-2Rα) in patients with lymphoproliferative diseases, such as adult T-cell leukemia or Hodgkin’s disease is elevated [13-17], and an increase in the blood concentration of serum soluble IL-2Rα chain could be an accurate indicator of disease progression [18]. Therefore, we also measured the level of serum sIL-2R, and compared the results between sIL-2R and sCD44.

In the present study, there were no significant differences in relation to grade or origin, but the pre-therapy levels of CD44std and sIL-2R for CS III and IV cases were higher than those for CS I and II cases (data not shown). The levels of these markers for the CS IV cases were lower than those for the CS III cases, and the levels of serum CD44std and sIL-2R were low in the CS IV cases since lymphoma cells had already infiltrated parenchymal organs. Nonetheless, this issue needs to be investigated further since only a small number of cases were studied.

In all patients, only the sIL-2R level decreased significantly following therapy. However, an analysis of CR and PR showed that the degree of decrease in the sCD44std level was significantly greater than that in the sIL-2R level. Furthermore, among the CS IV cases, only the CD44std level decreased significantly after therapy (Fig. 1). These findings suggest that the level of serum sCD44std reflects clinical pathology more closely than the level of serum sIL-2R in CS IV patients and those who respond well to therapy.

There is no conclusive evidence to support differences in prognosis between T-cell and B-cell lymphomas. One study found that there was no difference in prognosis between T-cell and B-cell lymphomas [20], whereas other studies have shown that the rate of recurrence was significantly higher and the disease-free survival time was significantly shorter for T-cell lymphomas when compared to B-cell lymphomas [21,22]. In the present study, when T-cell and B-cell lymphomas were analyzed separately, the levels of sCD44std and sIL-2R decreased significantly after therapy in patients with B-cell lymphomas, and the degree of decrease in the sCD44std level was very significant with a p-value of 0.0003 (Fig. 5). This suggests that when sCD44std is used as an index of treatment, it more closely reflects the treatment of B-cell lymphomas.

Clinical application of sIL-2R as a serological marker for NHL therapy is expected, but the results of the present study determined that the level of sCD44std reflected the clinical pathology of lymphomas comparably or better than the level of sIL-2R. Given that the level of serum sCD44std correlated well to clinical changes in lymphomas, it should prove to be a useful marker for assessing the effectiveness of lymphoma therapy.

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REFERENCES


