Serum Level of the Soluble Form of the CD30 Molecule Identifies Patients With Hodgkin’s Disease at High Risk of Unfavorable Outcome

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THE PROGRESS MADE IN the treatment of Hodgkin’s disease (HD) during the last 20 years offers the chance to cure the majority of patients affected by this disease.1,2 However, with current upfront therapeutic strategies, 20% to 40% of patients either fail to reach a complete remission or subsequently relapse.1,4 A proportion of these patients can still be cured using salvage regimens, which have recently included intensified protocols with or without autologous stem-cell support.5 However, the cumulative toxicity of therapy can induce a variety of long-term adverse effects that eventually affect the quality of life and life expectancy in a consistent proportion of patients.6-8 The availability of reliable indices capable of identifying patients on the basis of their prognostic risk would help to optimize the intensity of treatment, thus avoiding overtreatment of good-risk and undertreatment of high-risk patients.

A number of disease-related and patient-related features have been investigated to assess their possible prognostic relevance.7,9-12 Although some of these features have been recognized and used in therapeutic decision-making,10-13 their role in predicting the individual outcome within patient subgroups is still unsatisfactory.

In a previous report, we demonstrated that the circulating level at presentation of the soluble form of the CD30 molecule (sCD30) in HD represents an independent prognostic factor associated with reduced disease-free survival.14 The rationale for investigating sCD30 in HD derived from the knowledge that this molecule is likely to play a pathogenetic role in this disease. In fact, CD30 is a 120-kD surface molecule, functioning as a transmembrane cytokine receptor, that belongs to the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor family.15,16 which is consistently expressed by Hodgkin’s and Reed-Sternberg (H-RS) cells.17 The interaction between CD30 and its ligand (CD30L) is involved in the growth regulation of HD-derived cell lines.18 sCD30 is an 88-kD molecule released by CD30+ cells in vitro and in vivo.19,20 Its serum concentration increases in different pathologic conditions, including HD, as the result of the release by neoplastic or reactive cells expressing CD30.19,20 In this study, we investigated the prognostic significance of the serum level of sCD30 at diagnosis, in relation to other features of possible prognostic relevance, in a large series of patients with HD observed in three different institutions.
Among cases classified as LP or LD, immunohistologic analysis was available in 15 of 21 and five of six, respectively. In LP, the classic immunophenotypic pattern of atypical cells (L&H) was documented, including the absence of CD30 on paraffin preparations and its faint expression on cryostat sections, the absence of CD15, and the expression of B-cell– and T-cell–associated antigens, in the presence of a consistent number of CD57+ lymphocytes surrounding L&H cells. In cases defined as LD, immunohistologic analysis documented the expression of CD30 and CD15 and the absence of expression of EMA, as well as of B-cell– and T-cell–associated antigens by H-RS cells. Thus, a diagnosis of CD30+ anaplastic large-cell lymphoma (ALCL) was ruled out in these cases on the basis of morphology and immunohistologic phenotype.

**Staging and treatment.** Depending on the results of staging procedures, patients were classified according to the Cotswold’s meeting criteria as follows: stage I = 39, stage II = 163, stage III = 55, stage IV = 46, stage B = 136, and stage A = 167 patients. Eighty-five patients had bulky disease, defined as a mediastinal mass exceeding one third of the thoracic diameter measured at the D5-D6 level, and/or an extramediastinal mass greater than 10 cm. Stage I disease was defined as unfavorable in the presence of extranodal extent, bulky disease, or more than three involved sites, and/or pulmonary hilus adenopathies. Blood and serum levels of hemoglobin and lactate dehydrogenase (LDH) were available for all 303 patients. Erythrocyte sedimentation rate (ESR), albumin, and fibrinogen were available in 214.

Treatment was stage-directed and was based on radiotherapy (RT) alone or vinblastine, bleomycin, and methotrexate (VBM) or ABVD chemotherapy combined with RT for patients with limited disease (stages I to IIA and IIA without risk factors) and on chemotherapy (ABVD, MOPP/ABVD, VEBEP, or MOPP/EBV/CAD) with or without involved-field RT in advanced stages (IIIB, IVA, or IVB) or in the presence of risk factors (IIIB, IIE, IIA bulky, and IIIA unfavorable).

**Patient outcome.** All patients were considered assessable for treatment outcome, since those who received fewer than four cycles of chemotherapy or an incomplete course of RT were not included in this study.

Complete response (CR) was defined as the complete disappearance of all abnormalities (clinical, physical, radiologic, and biochemical) attributable to HD. Partial response (PR) was defined as a reduction of at least 50% for at least 4 weeks in the sum of the products of the perpendicular diameters of all measurable masses with no new lesions appearing and no progression at the original sites of disease. Progression was defined as an increase of any measured lesion or the appearance of new lesions. Follow-up reports were required every 3 months for the first year after treatment and every 6 months thereafter. Failure to achieve a CR, or disease progression, or the occurrence of a relapse after CR, were considered as events.

Among 303 patients, 287 (94.7%) obtained a CR and 16 either failed to reach a CR or progressed during treatment (five initial and 11 relapse after CR, were considered as events. Thirty-three of 303 patients received only RT. The event rate among those patients was 21.2% (seven of 33), similar to that observed in patients treated with chemotherapy alone or combined modality therapy (49 of 270; 18.15%). At the end of the study, 20 patients had died: 18 as a direct consequence of disease and two of causes related to treatment toxicity. None of the patients died before the assessment of response to treatment.

**sCD30 assay.** sCD30 levels were determined in serum samples stored at −70°C by a sandwich enzyme-linked immunosorbent assay (CD30 [Ki-1 antigen] ELISA; DAKO, Glostrup, Denmark), based on the use of two monoclonal antibodies reacting with two different epitopes on the 88-kD soluble form of the CD30 molecule, as previously described. Sera from 113 blood donors (79 males and 34 females; median age, 30 years) served as normal controls.

**Statistical analysis.** As sCD30 values were not normally distributed, results are reported both as the mean (± SEM) and median (range) of sCD30 serum levels. Median levels for different groups of patients were compared using the nonparametric Mann-Whitney U and Kruskal-Wallis tests as appropriate. The cut-off point for sCD30 serum level (100 U/mL) was chosen as the dividing point based on the maximum ratio of the estimated hazard for the groups. Cox’s proportional hazards model was used for univariate and multivariate analysis. The variates fibrinogen, ESR, and LDH were coded as the ratio above the normal value for each participating center. Hemoglobin and albumin were considered as continuous variables, being normal values comparable among the three centers. sCD30 was also evaluated as a continuous variable in the Cox model. Missing data were dealt with by excluding from particular analyses those patients without data on the required variables. Actuarial event-free survival (EFS) curves were constructed by the Kaplan-Meier method and differences were analyzed by the log-rank test. Statistical significance obtained by repeated log-rank testing of subgroups of patients was adjusted by Bonferroni’s correction. EFS was defined as the interval between diagnosis and the achievement either of a PR or the occurrence of disease relapse after CR. For patients who experienced disease progression under treatment, an EFS time of 0 was assigned. All variables found to have a P value ≤.05 were considered to be statistically significant.

**RESULTS**

**Correlations with clinical features.** Serum levels of sCD30 in samples collected at diagnosis before treatment are listed in Table 1. Increased levels (>20 U/mL) were observed in 237 of 303 patients (78.2%). The median value (45 U/mL; range, 1 to 815) was significantly higher as compared with controls (P < .001).

No differences were found between male and female patients (43 and 47 U/mL, respectively; P = .66). Age ≥50 years was associated with higher sCD30 serum levels (43 ± 68 U/mL in patients < or >50 years, respectively; P = .01). Patients with

| Table 1. Serum Levels of sCD30 in HD at Diagnosis According to Presentation Features |
|-----------------------------------------------|----------------|----------------|----------------|---------|
| Feature                                      | No. of Patients | sCD30 U/mL      | P*              |
| Controls                                     | 113             | 5.3 ± 0.53      | <.001           |
| All cases                                    | 303             | 94 ± 7          | 4 ± 0-20        |
| Males                                        | 151             | 94 ± 11         | 43 ± 2-815      |
| Females                                      | 152             | 91 ± 9          | 47 ± 1-612      |
| Age ≥50 yr                                   | 51              | 135 ± 23        | 68 ± 4-815      |
| Age <50 yr                                   | 252             | 86 ± 7          | 43 ± 1-712      |
| Stage I-II                                   | 202             | 69 ± 7          | 37 ± 1-609      |
| Stage III-IV                                 | 201             | 145 ± 16        | 93 ± 2-815      |
| Stage A                                      | 167             | 62 ± 7          | 33 ± 2-609      |
| Stage B                                      | 136             | 133 ± 13        | 66 ± 1-815      |
| Bulky                                        | 85              | 135 ± 15        | 77 ± 2-666      |
| Nonbulky                                     | 218             | 78 ± 8          | 37 ± 1-815      |
| NS                                           | 210             | 92 ± 8          | 44 ± 1-712      |
| MC                                           | 53              | 105 ± 19        | 64 ± 6-815      |
| LP                                           | 21              | 55 ± 14         | 35 ± 6-277      |
| LD                                           | 6               | 309 ± 102       | 297 ± 29-666    |

*P* value refers to Mann-Whitney *U* and Kruskal-Wallis tests.
advanced disease (stages III and IV) had higher sCD30 levels compared with those with more limited disease (stages I and II) (93 vs 37 U/mL, respectively; P < .001). In addition, patients with B symptoms had higher values compared with those with stage A (66 and 33 U/mL, respectively; P < .001). Higher sCD30 levels were detected in patients with bulky disease (n = 85) as compared with patients without bulky mass (77 vs 37 U/mL; P < .001). The analysis of variance performed to compare sCD30 median levels in LP, MC, NS, and LD subtypes showed a significant difference (P = .028), with the highest value being detected in six patients with LD subtype.

Relationship between sCD30 values at diagnosis and outcome. sCD30 values ≥100 U/mL entailed a higher risk of poor outcome, since either failure to reach a CR or a subsequent relapse occurred more frequently in patients with sCD30 values ≥100 U/mL (32 of 78; 41%) than in those with values less than 100 U/mL (23 of 225; 10.2%). The difference between the EFS curves of patients with sCD30 level at diagnosis above and below 100 U/mL was highly significant (P < .001), with a 5-year EFS probability of 59.9% (95% confidence interval [CI], 40.6% to 65.9%) and 87.5% (95% CI, 81.5% to 91.6%), respectively (Fig 1).

Fourteen prognostic parameters, including treatment by each one of the participating centers (center 1, 2, or 3), age (<50 vs ≥50 years), gender, stage (I and II vs III and IV), A versus B symptoms, bulky versus nonbulky presentation, ESR (ratio), albumin (continuous), LDH (ratio), hemoglobin (continuous), fibrinogen (ratio), and sCD30 serum level (continuous), were considered in the univariate analysis by Cox’s regression. As reported in Table 2, sCD30 serum level, advanced stage, presence of B symptoms, and age ≥50 years at presentation were found to be associated with a significantly higher risk of poor outcome. These four variables were then included in the final Cox regression model for multivariate analysis (Table 3).

The variable hemoglobin was also included in this model, because of its borderline statistical significance (hazards ratio, 0.99; P = .064). Only sCD30 serum level and advanced-stage disease retained independent prognostic significance. We therefore combined the sCD30 serum level as a dichotomous variable (<100 U/mL and ≥100 U/mL) with the extension of
disease (stage I and II and III and IV) to maximize their prognostic impact on outcome and to obtain a combined prognostic index (Table 4). Figure 2 illustrates the Kaplan-Meier plot of EFS according to the combination of the two major prognostic factors. The 5-year EFS was 88.7% (95% CI, 82% to 93%) (stages I and II and sCD30 ≤100 U/mL: group 1), 83.1% (95% CI, 67% to 92%) (stages III and IV and sCD30 ≤100 U/mL: group 2), 72.5% (95% CI, 52% to 85%) (stages I and II and sCD30 >100 U/mL: group 3), and 50.5% (95% CI, 35% to 64%) (stages III and IV and sCD30 >100 U/mL: group 4). The overall P value among the four groups of patients was less than .001. In particular, for group 1 versus group 2, P = not significant; group 2 versus group 3, P = not significant; group 1 versus group 3, P = .04; and group 3 versus group 4, P = .05 (Table 4).

**DISCUSSION**

Current treatment strategies are able to cure the majority of patients with HD. However, between 20% and 40% of patients with newly diagnosed HD either fail to respond completely to induction therapy, with subsequent disease progression, or relapse after an initial CR. A proportion of these patients, poor responders to standard treatment, can subsequently be rescued by high-dose therapy associated with autologous hematopoietic progenitor-cell transplantation, which raises the question of the role of such an intensive approach as upfront treatment for high-risk patients. Unfortunately, the search for adverse prognostic features at presentation, which are capable of identifying high-risk patients eligible for more intensive treatment, has so far been largely unsatisfactory.

Our previous observation suggesting that sCD30 evaluation in patients with HD at diagnosis has prognostic significance led us to investigate this issue further in a larger series of patients, treated at three Italian centers during the last 12 years. Although patients were submitted to different regimens, the treatment programs shared a common stage-directed strategy. Patients with localized disease (stages I and II) were treated with RT chemotherapy, depending on the presence of risk factors. Patients with advanced disease experienced slightly different induction chemotherapy regimes (ABVD, MOPP/ABVD, VEBEP, or MOPP/EBV/CAD), with or without consolidation RT, according to the protocol in use in each center during the 12 years of the study. However, in terms of EFS, which is the outcome variable evaluated in this study, no significant difference among groups of patients treated at different centers could be demonstrated (Table 2). In addition, the event rate among patients with localized disease treated with RT alone (seven of 33; 21.2%) was similar to that observed in patients treated with chemotherapy alone or combined modality therapy (49 of 270; 18.15%). Therefore, we assumed treatment modality to be a nonprognostic variable in our cases.

sCD30 levels were increased in the vast majority of patients (78%) and correlated with disease stage, presence of B symptoms, and tumor burden. In addition, higher sCD30 levels were detected in patients with LD histology as compared with the other histologic subtypes. These correlations, which cannot be merely explained on the basis of the number of H-RS cells, as...
previously reported, suggest that serum sCD30 in HD reflects the functional behavior of the neoplastic cells (ie, H-RS) and their relationship with the heterogeneous microenvironment and the complex network of cytokines observed in HD.

Since the final goal of our study was to assess the specific role of sCD30 in identifying high-risk patients, we have evaluated its prognostic significance in terms of EFS in comparison to 13 other presentation features that have been previously reported to hold prognostic significance. At univariate analysis, advanced stage, presence of B symptoms, age ≥ 50 years, and sCD30 serum level (as a continuous variable) were all associated with a significant high risk of treatment failure (Table 2). sCD30 serum level ≥ 100 U/mL at diagnosis was confirmed to have a strong adverse prognostic significance as a single factor, as shown by the difference between the EFS curves of patients with sCD30 levels less than 100 U/mL and ≥ 100 U/mL (Fig 1).

None of the other variables included in the model was significant. Hemoglobin level (evaluated as a continuous variable) showed borderline significance (P = .064), whereas in previous studies, hemoglobin/hematocrit was identified as a prognostic factor that maintained its significance when included in a multivariate analysis. The adverse effect on outcome of advanced age at diagnosis has been reported in different studies. In our series, the risk of treatment failure increased significantly for patients ≥ 50 years of age (hazard ratio, 2.26; P = .007), whereas it was only of borderline significance when we considered a cut-off of 45 years (hazard ratio, 1.69; P = .072). One of the possible explanations for the adverse role of advanced age on outcome is the reduction of treatment intensity, probably related to impaired performance status. In our study, we could not address this issue, since reliable information on performance status at diagnosis and actual dose of drug delivered was available only for a minority of patients. The extent of mediastinal disease has also been reported to be a prognostic factor. Although in our study this parameter was not evaluated separately, it was included in the definition of bulky disease and the presence of high tumor burden did not correlate with a higher risk of treatment failure. This could be related to the fact that patients with localized bulky disease were treated with combined modality therapy, which could have hampered the adverse prognostic effect of tumor burden.

On the basis of the results of the univariate analysis, we included in the final multivariate analysis the four factors with a statistical significance, plus hemoglobin, which had a borderline significant P value. Only sCD30 serum level (continuous) and advanced stage retained an independent prognostic significance in identifying high-risk patients (Table 3). The combined evaluation of sCD30 and stage at presentation clearly showed that patients with both adverse prognostic factors, ie, stages III and IV and sCD30 ≥ 100 U/mL, had an expected EFS at 5 years significantly lower than those with stages I and II and sCD30 less than 100 U/mL. These results are striking if compared with other large cooperative lymphoma studies aimed at detecting possible prognostic factors by including multiple variables and different types of score or numerical indices. In particular, from the combined evaluation of sCD30 and stage at presentation, it is possible to identify patients with a very high and very low risk of treatment failure with standard therapy. This seems to fulfill the requirements of any prognostic modeling, which should provide a reliable and simple guideline to predict patients’ outcome.

A more accurate definition of risk of treatment failure in patients with HD at presentation would help to tailor treatment strategies further in individual cases. This could possibly allow the reduction of initial treatment intensity in low-risk patients, thus reducing early and late treatment-related morbidity and the incidence of long term secondary effects. On the other hand, the reliable identification of high-risk patients could possibly improve their cure rate using more intensive upfront treatment, which has already produced promising results in relapsed HD.

The prognostic usefulness in HD of the combined evaluation of sCD30 and clinical stage emerged from our study now has to be validated in large prospective clinical trials.

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REFERENCES

chemotherapy are the only prognostic factors in supradiaphragmatic Hodgkin’s disease treated by ABVD plus radiotherapy: Ten-year results of the Paris-Ouest-France 81/12 trial, including 262 patients. J Clin Oncol 14:1928, 1996


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