Elevated Serum Levels of CC Thymus and Activation-Related Chemokine (TARC) in Primary Hodgkin’s Disease: Potential for a Prognostic Factor

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Abstract
The CC thymus and activation-related chemokine (TARC) is a protein, which is highly expressed by Reed-Sternberg cells in Hodgkin’s disease and is found in the majority of Hodgkin’s disease patients. Within several trials conducted by the German Hodgkin study group, 62 Hodgkin’s disease patients were elected based on availability of serum samples post and prior therapy to assess TARC levels by ELISA. TARC levels from 33 patients with continuous complete response (CCR), 20 patients with relapse, and nine patients with progressive disease (PD) were correlated with freedom from treatment failure and survival. As defined in healthy donors (mean value ± 2× SD), a TARC level of >500 pg/mL was considered as elevated. The median TARC levels of all patients at baseline and after completed primary treatment were 5,803 pg/mL (range, 116-73,074 pg/mL) and 663 pg/mL (50-24,709 pg/mL), respectively. TARC levels of patients with PD were higher than those of patients with CCR at baseline and after therapy. Baseline TARC correlated significantly with stage (P = 0.019), erythrocyte sedimentation rate (P = 0.004), leukocyte count (P < 0.001), and lymphocyte count (P = 0.026). A TARC level of >2,000 pg/mL after completed treatment was a significant risk factor for poorer survival (P = 0.02) but not for relapse. In conclusion, monitoring serum TARC levels in Hodgkin’s disease patients may add valuable information about therapy success in Hodgkin’s disease patients, especially those with PD and should therefore be prospectively evaluated in future trials. (Cancer Res 2005; 65(13): 5516-9)

Introduction
Whereas patients with Hodgkin’s disease were destined to die because of their malignant disease before the 1970s, over 90% can be cured today by modern polychemotherapy and radiotherapy strategies such as the ABVD or the BEACOPP regimen (1). However, a considerable proportion of patients relapses after therapy or progress during their treatment and have to be salvaged by high-dose chemotherapy and autologous stem cell rescue or treated with a palliation regimen (2, 3). Thus, prognostic markers would be of great value, which identify patients at a higher risk for relapse or which predict for response to current standard chemotherapy regimens.

The CC thymus and activation related chemokine (TARC, CCL17) is a protein which was determined by cloning the D3A gene from peripheral blood mononuclear cells after stimulation with phytohemagglutinin (4). It is produced by antigen-presenting cells, especially dendritic cells (5) and attracts activated TH2 T cells by specifically stimulating the CC chemokine receptor 4 (6, 7). In Hodgkin’s disease, infrequent malignant Reed-Sternberg cells, which only comprise about 1% of the tumor masses are surrounded by an environment consisting of CD4+ cells with a TH2 phenotype and other cells typical for inflammatory diseases (8, 9). In vitro studies have shown that TARC is highly expressed by Reed-Sternberg cells in cell lines (10) and in Hodgkin’s disease lymph nodes (11), which could explain the attraction of TH2 cells by Reed-Sternberg cells. TARC is detectable in human serum and may thus provide important information about an ongoing Hodgkin’s disease before, during, and after treatment.

In this study, we examined the TARC levels of sera from primary Hodgkin’s disease patients, who underwent treatment and investigated the prognostic significance of TARC in this setting.

Patients and Methods
In a retrospective study design, we investigated the prognostic significance of TARC in primary Hodgkin’s disease patients before and after treatment. We included all patients with a relapse or progressive disease (PD) with available serum samples before and after therapy from our Hodgkin’s disease serum bank. In addition, we included all available patients with a continuous complete response with a completed follow-up of at least 44 months, if pretreatment and post-therapy serum samples had been cryopreserved. A total of 62 patients (39 male and 23 female) was included into this study. All patients signed informed consent to participate in one of the following German Hodgkin Study Group (GHSG) protocols, which had been approved by the institutional review board: HD4 (12), HD5 (13), HD7, HD8 (14), and HD9 (1). In addition, they consented to provide additional blood samples for research purposes. A continuous complete response (CCR) was defined as an absence of any signs of Hodgkin’s disease during the entire observation time. PD was defined as the recurrence of Hodgkin’s disease during treatment or within 3 months after the end of completed therapy. A relapse occurred, if the patient showed signs of recurrent Hodgkin’s disease 3 months or later after completed treatment.

All patients with relapse (n = 20) or PD (n = 9) with available TARC levels before and after therapy were selected from our serum database and compared with 33 CCR patients with available serum samples and a follow-up of at least 44 months. The median age was 32 years (range, 17-76 years). Histologies included 39 nodular sclerosis, 18 lymphocyte rich, four mixed cellularity, and one unclassifiable. Six patients were diagnosed with stage I, 25 with stage II, 18 with stage III, and 13 with stage IV disease. Seventeen patients presented with extranodal involvement. B symptoms were reported from 33 patients. According to different study protocols (HD4-HD9), patients received various primary treatment regimes. Seven patients with early-stage Hodgkin’s disease were treated with 40 Gy extended field.
radiation therapy and one received 30-Gy extended field and 10-Gy involved field radiation. Of 24 patients with intermediate stage disease, 22 received two cycles of COPP/ABVD (cyclophosphamide, vincristine, prednisone, procarbazine, Adriamycin, bleomycin, vinblastine, dacarbazine) with radiotherapy (one not assessed), and one received two cycles of ABVD with radiotherapy. Patients with advanced stage Hodgkin’s disease received either four cycles of COPP/ABVD (n = 9), eight cycles of BEACOPP baseline (bleomycin, etoposide, Adriamycin, cyclophosphamide, vincristine, prednisone, procarbazine; n = 14), eight cycles of BEACOPP escalated (n = 6) or COPP/ABV/IMEP (ifosfamide, methotrexate, etoposide; n = 1). Radiation therapy was given to bulk or residual tumors. The patient characteristics are summarized in Table 1.

**Measurement of CC thymus and activation-related chemokine levels.** Serum from patients was stored at −80°C until processing of samples in a standardized ELISA according to the manufacturer’s guidelines (R&D Systems, Wiesbaden, Germany). Briefly, 50 µL of thawed serum sample was added in duplicates to plates precoated with anti-TARC antibodies. After a 2-hour incubation time at room temperature, plates were washed thrice with wash buffer; 200 µL of TARC conjugate were added per well and incubated for 1 hour at room temperature. Plates were washed again thrice. Finally, 200 µL of substrate solution per well were added and incubated for 30 minutes at room temperature in the dark. The absorbance of each well was measured at 450 nm with an ELISA reader. To define normal serum TARC levels, sera from 10 healthy donors were measured.

**Measurement of interleukin-10 and soluble CD30 levels.** Interleukin-10 (IL-10) and soluble CD30 (sCD30) levels were measured to determine whether there was any correlation to TARC. IL-10 and sCD30 ELISA assays were done as described by the manufacturer guidelines (R&D Systems and Bender MedSystem, Vienna, Austria).

**Results and Discussion**

The median serum TARC level in 10 healthy donors was 239 pg/mL with a minimum of 106 pg/mL and a maximum of 431 pg/mL. These values are in the range of previous studies in healthy individuals (15, 16). A level of >500 pg/mL (mean value + 2 SD) was defined as elevated. The median TARC level of all Hodgkin’s disease patients before treatment (baseline) was 5,803 pg/mL with a range from 116 to 73,074 pg/mL. Baseline TARC levels correlated significantly with erythrocyte sedimentation rate (ESR, \( P = 0.004 \)), leukocyte (\( P < 0.001 \)), and lymphocyte count (\( P = 0.026 \)) but not with age, alkaline phosphatase, and hemoglobin. Ann Arbor stage had a significant effect on baseline TARC levels (\( P = 0.019 \)) as well as the risk groups ("early," "intermediate," and "advanced stage" according to the GHSG definitions; \( P = 0.028 \)). Stages and risk groups did not correlate with posttreatment TARC levels. In addition, chemotherapeutic regimen did not have any statistically significant influences on TARC levels after therapy. Thirty-three patients, who maintained a CCR had a median TARC level of 5,180 pg/mL (range, 126-42,084 pg/mL) at baseline. Patients with relapse (\( n = 20 \)) had a median TARC level of 6,734 pg/mL (116-46,131 pg/mL) and patients who suffered from PD showed a median serum TARC level of 15,856 pg/mL (584-73,074 pg/mL) at baseline. Patients with PD had higher TARC levels at baseline than patients with CCR. After completed primary treatment, the median TARC level for all patients was 663 pg/mL (50-24,709 pg/mL). Patients with CCR had a median TARC level of 783 pg/mL (50-3,921 pg/mL), patients with a relapse 493 pg/mL (83-3,786 pg/mL), and patients with PD 945 pg/mL (155-24,709 pg/mL). TARC levels at baseline and after therapy are depicted as a box plot (Fig. 1). There was no correlation between TARC levels at baseline and after therapy.

The initial complete response (CR) rate of all 62 patients was 86% after a median observation time of 88 months (range, 6-112 months). After therapy, 19 of 33 patients (58%) with CCR showed TARC levels above 500 pg/mL and 10 of 33 patients (30%) had TARC levels above 1,000 pg/mL. TARC levels of >2,000 pg/mL were significantly with erythrocyte sedimentation rate (ESR, \( P = 0.004 \)) and leukocyte count (\( P < 0.001 \)), and lymphocyte count (\( P = 0.026 \)) but not with age, alkaline phosphatase, and hemoglobin. Ann Arbor stage had a significant effect on baseline TARC levels (\( P = 0.019 \)) as well as the risk groups ("early," "intermediate," and "advanced stage" according to the GHSG definitions; \( P = 0.028 \)). Stages and risk groups did not correlate with posttreatment TARC levels. In addition, chemotherapeutic regimen did not have any statistically significant influences on TARC levels after therapy. Thirty-three patients, who maintained a CCR had a median TARC level of 5,180 pg/mL (range, 126-42,084 pg/mL) at baseline. Patients with relapse (\( n = 20 \)) had a median TARC level of 6,734 pg/mL (116-46,131 pg/mL) and patients who suffered from PD showed a median serum TARC level of 15,856 pg/mL (584-73,074 pg/mL) at baseline. Patients with PD had higher TARC levels at baseline than patients with CCR. After completed primary treatment, the median TARC level for all patients was 663 pg/mL (50-24,709 pg/mL). Patients with CCR had a median TARC level of 783 pg/mL (50-3,921 pg/mL), patients with a relapse 493 pg/mL (83-3,786 pg/mL), and patients with PD 945 pg/mL (155-24,709 pg/mL). TARC levels at baseline and after therapy are depicted as a box plot (Fig. 1). There was no correlation between TARC levels at baseline and after therapy. The initial complete response (CR) rate of all 62 patients was 86% after a median observation time of 88 months (range, 6-112 months). After therapy, 19 of 33 patients (58%) with CCR showed TARC levels above 500 pg/mL and 10 of 33 patients (30%) had TARC levels above 1,000 pg/mL. TARC levels of >2,000 pg/mL were

**Table 1. Characteristics of 62 primary Hodgkin’s disease patients**

<table>
<thead>
<tr>
<th>Patients</th>
<th>n = 62</th>
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<tbody>
<tr>
<td>Sex</td>
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<tr>
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<tr>
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</tr>
<tr>
<td>Extraneural involvement</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
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</tbody>
</table>

**Primary therapy**

\[4 \times \text{COPP/ABVD} \pm \text{Rx} \]
\[8 \times \text{BEACOPP baseline} \]
\[8 \times \text{BEACOPP escalated} \]
\[2 \times \text{COPP/ABVD} + \text{Rx} \]
\[4 \times \text{COPP/ABV/IMEP} + \text{Rx} \]
\[2 \times \text{ABVD} + \text{Rx} \]
\[40 \text{ Gy EF-Rx} \]
\[30 \text{ Gy EF-Rx + 10 Gy IF} \]
\[\text{Not assessed} \]

Leukocyte count before therapy: \[10.7 \pm 4.7 \times 10^{3}/\mu L\]

Lymphocyte count before therapy: \[1.6 \pm 1.2 \times 10^{3}/\mu L\]

Abbreviations: Rx, radiotherapy; BEACOPP, bleomycin, etoposide, adriamycin, cyclophosphamide, vincristine, prednisone, procarbazine; COPP/ABV, cyclophosphamide, vincristine, prednisone, procarbazine, adriamycin, bleomycin, vinblastine, dacarbazine; IMEP, ifosfamide, methotrexate, etoposide.

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observed in three patients with CCR (9%) and 1 of 20 patients with relapse (5%), but in four of nine patients with PD (44%). In a Kaplan-Meier analysis, patients who presented with TARC levels above 2,000 pg/mL at baseline had a higher risk for relapse than patients with TARC levels below 2,000 pg/mL. Within this cohort of patients, the difference was not yet statistically significant in a log-rank test ($P = 0.068$). However, TARC levels above 2,000 pg/mL after therapy correlated with a worse survival ($P = 0.023$), but the positive predictive values of TARC > 2,000 pg/mL at baseline and after therapy for a relapse/PD were only 53% and 63%, respectively. Kaplan-Meier plots with comparison of patients above and below TARC levels of 2,000 pg/mL are shown in Fig. 2. We also compared

Figure 1. Box plot of TARC levels (pg/mL) at baseline and after therapy for different response groups (whiskers indicate the 10th and 90th percentile).

Figure 2. Kaplan-Meier plots of freedom from treatment failure (FFTF; A and B) and survival (C and D) of Hodgkin’s disease patients at baseline (A and C) and after therapy (B and D). Group differences between patients with baseline TARC levels (TARCb) or post-therapy TARC levels (TARCP) below and above 2,000 pg/mL were calculated by log-rank tests.
TARC with serum levels of IL-10 and sCD30 in a total of 149 sera as these have been reported to have a prognostic effect in Hodgkin's disease (17, 18). However, we did not find any statistically significant correlation between TARC and IL-10 or TARC and sCD30 (data not shown).

The data of this retrospective, explorative study clearly show that TARC is a chemokine that shows pathologic serum levels in almost all primary Hodgkin's disease patients, which has not been reported before. As the average TARC levels drop by ~80%, it seems obvious that TARC correlates with tumor load and disease activity. This is supported by the statistical analysis, which shows a trend towards higher levels of TARC in patients with higher Ann Arbor stages. A TARC level of >2,000 pg/mL after completed therapy indicates a reduced survival probability and indicates a higher risk for progression or relapse, although the positive predictive value is low in this small cohort. We hypothesize that TARC may also be useful to identify patients with PD, who do not respond to therapy and who could profit from an early intervention with a salvage regimen. The strikingly high level of TARC in a few patients with CCR for >6 years needs further exploitation. TARC has recently been associated with disease activity in atopic dermatitis (16) and mycosis fungoides (19). Although not apparent within our patient group, it cannot be ruled out that some of our Hodgkin's disease patients might have had concurrent atopic conditions. Whether this could interfere with the prognostic value of TARC should also be addressed in future prospective trials.

Other prognostic factors are associated with a poorer outcome in Hodgkin's disease. As reported by our group, patients who show detectable levels of the cytokine IL-10 have a worse prognosis than patients without IL-10 (18). However, IL-10 is only expressed in 14% of patients and cannot be generally applied to the great majority of patients. From this perspective, TARC may be a more valuable marker, because it is elevated in ~90% of primary Hodgkin's disease patients. Clearly, TARC is a biological marker for tumor burden in Hodgkin's disease patients. Our explorative study with relatively small patient numbers suggests an effect of TARC on prognosis. As it correlates with other factors predicting outcome (ESR, leukocyte count, and Ann Arbor stage), higher patient numbers are required to determine whether it is an independent prognostic factor. The widely expressed TARC should be monitored in future prospective studies and may contribute to the prognostic score in Hodgkin's disease (20).

References
5. Imai T, Nagira M, Takagi S, et al. Selective recruitment of CCR4-bearing Th2 cells toward antigen-presenting cells by the CC chemokines thymus and activation-regulated chemokine and macrophage-derived chemo-

9. Teruya-Feldstein J, Jaffe ES, Burd FR, Kingma DW, Setsuda JE, Tosato G. Differential chemokine expression in tissues involved by Hodgkin's disease: direct correla-
18. Bohlen H, Kessler M, Sextro M, Diehl V, Tesch H. Poor clinical outcome of patients with Hodgkin's disease and elevated interleukin-10 serum levels. Clinical signifi-

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