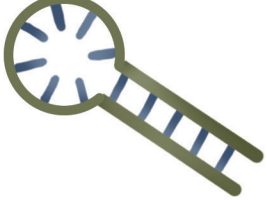

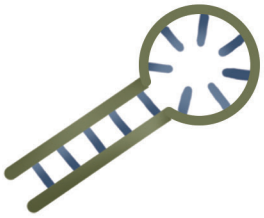

CHAPTER 2

Plasma Thymus and Activation-Regulated Chemokine as an early response marker in classical Hodgkin lymphoma

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Abstract

INTRODUCTION:
Plasma Thymus and Activation-Regulated Chemokine (TARC) is a potential biomarker for classical Hodgkin lymphoma. To define the value of plasma TARC as a marker to monitor treatment response, we correlated serial plasma TARC levels with clinical response in newly diagnosed and relapsed classical Hodgkin lymphoma patients.

DESIGN AND METHODS:
Plasma was collected from 60 (39 early stage and 21 advanced stage) newly diagnosed classical Hodgkin lymphoma patients before, during and after treatment and from 12 relapsed patients before and after treatment. Plasma TARC levels were determined by enzyme-linked immunosorbent assay and were related to pre-treatment metabolic tumor volume as measured by quantification of 2-[18F]fluoro-2-deoxyglucose positron emission tomography images and to treatment response.

RESULTS:
Baseline plasma TARC levels correlated with stage of disease and bulky disease and more closely with metabolic tumor volume. Response to treatment was observed among 38/39 early stage and 19/21 advanced stage patients. Reduction in plasma TARC to normal range levels could be observed as early as after one cycle of chemotherapy in all responsive patients, while plasma TARC remained elevated during and after treatment in the three non-responsive patients. Plasma TARC was elevated in all 12 relapsed patients at time of relapse and remained elevated after salvage treatment in the four non-responsive patients only.

CONCLUSIONS:
Baseline plasma TARC levels correlate with classical Hodgkin lymphoma tumor burden and serial plasma TARC levels correlate with response to treatment in patients with classical Hodgkin lymphoma.

Introduction

In classical Hodgkin Lymphoma (cHL) the neoplastic Hodgkin Reed-Sternberg (HRS) cells are vastly outnumbered by cells in the surrounding reactive infiltrate. This infiltrate is of major importance for the proliferation and survival of HRS cells. Different chemokines and cytokines produced by HRS cells and cells in the reactive infiltrate are responsible for the formation and maintenance of this reactive environment.[1-4]

The CC chemokine ligand 17 (CCL17) or Thymus and Activation-Regulated Chemokine (TARC) is highly expressed by HRS cells in cHL, but not by the tumor cells of nodular lymphocyte predominant Hodgkin lymphoma or other B-cell derived non-Hodgkin lymphomas.[5,6] TARC binds specifically to the CC chemokine receptor 4 (CCR4). CCR4 is mainly expressed on regulatory T and Th2 cells that are both abundantly present in the reactive infiltrate of cHL.[6-8] Approximately 90% of the cHL patients show positive TARC staining in HRS cells by immunohistochemistry (IHC) and about 85% have significantly elevated levels of TARC in their pre-treatment serum or plasma sample compared to healthy controls.[9,10] Although patients with active atopic diseases can also have elevated plasma TARC levels, this is only a modest elevation which is in a significantly lower range than the high plasma TARC levels observed in cHL.[11] Pre-treatment serum TARC levels correlate with stage of disease, erythrocyte sedimentation rate, leukocyte and lymphocyte counts in cHL.[9,10] Niens *et al.*[9] reported TARC levels within the normal range after successful treatment in seven cHL patients and persistent elevated TARC in a single non-responsive patient. Weihrauch *et al.*[10] reported diminished survival rates among patients with higher TARC levels after treatment. However, nothing is known about TARC dynamics during treatment in relation to clinical treatment response.

We therefore prospectively collected serial plasma samples from newly diagnosed and relapsed cHL patients. The aim of the current study was to correlate plasma TARC levels with tumor burden at time of diagnosis and to correlate serial plasma TARC levels during and after treatment with cHL treatment response.

Design and Methods

Patient inclusion and treatment

Serial plasma samples were prospectively collected from all newly diagnosed and relapsed cHL patients that were treated in the University Medical Center Groningen (UMCG) from January 2006 until June 2011.

Inclusion criteria for both newly diagnosed and relapsed cHL patients were (1) receipt of standard treatment regimens, (2) availability of a plasma sample before start of treatment and one or more plasma samples during or after treatment as well as (3) confirmation of TARC expression in diagnostic tissue by immunohistochemistry or by elevated baseline plasma TARC if diagnostic tissue was not available. From 78 newly diagnosed patients treated in the UMCG, 60 were included, while 18 were excluded (1 because of patient refusal, 2 because of receiving palliative treatment, 9 because of lack of a pre-treatment plasma sample, 3 with negative tissue TARC staining and 3 with no available tissue and normal pre-treatment plasma TARC levels). From 17 relapsed patients, 12 patients eligible for DHAP salvage treatment followed by autologous stem cell transplantation were included, while 5 patients were excluded (4 because of receiving only palliative treatment and one because of lack of a plasma sample after treatment).

Permission for this study was obtained from the institutional review board of the UMCG and all participating patients and healthy controls signed informed consent. Routine staging of patients at diagnosis or at relapse included diagnostic Computer Tomography (CT) imaging, 'whole body' 2-[18F]fluoro-2-deoxyglucose positron emission tomography (FDG-PET) imaging, and bone marrow biopsy. Presence of bulky disease was defined as presence of a mediastinal mass greater than one third of the thoracic diameter on chest X-ray (on level Th5-Th6) and/or a nodal mass of more than 10 cm CT imaging. Response to treatment was evaluated according to the revised International Working Group response criteria.[12] Evaluation included FDG-PET/CT scanning which was interpreted according to the International Harmonization Project criteria (IHP).[13] FDG-PET/CT scanning was performed using a Siemens Biograph PET/CT mCT 64 scanner in the vast majority of patients

Patients were treated according to clinical trial (European Organisation for Research and Treatment of Cancer (EORTC)) protocols. Table 1 shows the patient characteristics and data on chemo- and radiotherapy regimens. Briefly, standard treatment for stage I/II (early stage) patients consisted of 3-6 cycles of ABVD chemotherapy with or without 30-36 Gy involved node radiotherapy (IN-RT) according to the EORTC (20051) H10 trial.[14] Standard treatment for stage III/IV (advanced stage) patients consisted of 6-8 cycles of ABVD without radiotherapy or in case of participation in the EORTC 20012 trial, patients randomized between 8 cycles of ABVD and

4 cycles of escalated BEACOPP (eBEACOPP) followed by 4 cycles of base-line BEACOPP.[15] Patients not enrolled in these clinical trials received conventional treatment, which is similar to the standard arm of these trials.

In the relapsed cohort, all patients were scheduled for DHAP-VIM-DHAP salvage chemotherapy, followed by high-dose chemotherapy and autologous stem cell transplantation (ASCT) in case of at least a partial response on salvage re-induction. Three patients who were non responsive to DHAP received second salvage chemotherapy consisting of 2 cycles of mini-BEAM before receiving ASCT (Table 1).

Tissue and plasma collection

Diagnostic formalin fixed paraffin embedded tissue samples were retrieved from the tissue banks of the pathology departments of the UMCG and other regional pathology laboratories affiliated with the hospitals from which the patients were referred for treatment (Martini Hospital Groningen, Sazinon Hoogeveen, Isala Klinieken Zwolle, SSZOG Winschoten and Pathology Friesland Leeuwarden. Immunohistochemistry for TARC was performed to confirm expression of TARC by Hodgkin tumour cells. Immunohistochemistry was performed on formalin fixed paraffin embedded tissue samples mounted on 3-aminopropyltriethoxysilane (APES) coated slides using a goat anti-human TARC antibody (R&D Systems, Minneapolis, USA) after heat-induced antigen retrieval. Ninety-four percent of analyzed tissue samples were positive for TARC. Patients with TARC negative tumour cells also had low plasma TARC at diagnosis and were excluded (see inclusion criteria).

In the newly diagnosed patients, plasma was collected at diagnosis (baseline), after one cycle of chemotherapy, at mid-treatment and after completion of first-line treatment and during routine follow-up. Mid-treatment was after 2 cycles of ABVD in early stage patients and after 4 cycles of ABVD or eBEACOPP in advanced stage patients in parallel with formal response evaluation by FDG-PET/CT. In the relapsed patients, plasma samples were collected before and after salvage treatment. In addition, plasma samples were collected from 107 age (median 31, range 19-62 years) and sex (57% female) matched healthy controls.

Plasma TARC analysis

Ten ml of blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes at each time point. Plasma was obtained after centrifugation at 900xg and stored in aliquots at -20°C. TARC levels were measured by a double antibody sandwich ELISA (R&D systems, Minneapolis, USA). To decrease variation we used mass-calibrated standards and analyzed samples retrieved from a single patient simultaneously. Samples were analyzed without prior knowledge of the corresponding patient or treatment results. To minimize a potential influence of active atopic diseases we set the cut-off for elevated plasma TARC levels at 1,000 pg/ml

Determination of metabolic volume

Baseline FDG-PET studies performed at the University Medical Center Groningen were used for the determination of the pre-treatment metabolic volume. Reconstruction of the FDG-PET studies was performed according to the Netherlands protocol for standardization of FDG whole body PET studies.[16] The four sites with the most intense visual FDG uptake were selected as regions of interest (ROI). All ROIs were analyzed for the maximum Standard Uptake Value (SUVmax) and the corresponding metabolic volume at a fixed threshold of 70% of the SUVmax. The total metabolic volume was calculated by adding up the volumes of the ROIs. All scans were analyzed without knowledge of plasma TARC levels.

Statistics

We used non-parametric analyses because of the skewed distribution of the plasma TARC levels. Baseline plasma TARC levels were correlated to Ann Arbor stage of disease, presence of bulky disease, metabolic volume and SUVmax. Differences in plasma TARC levels between categorical variables were analyzed using the Mann-Whitney U test. Linear correlation coefficients (r) between plasma TARC levels and the metabolic volume were determined using the Spearman rank correlation test. All statistical analyses were performed using SPSS 16.0.

Results

Patient characteristics

Basic characteristics and treatments of the 60 newly diagnosed (39 early stage and 21 advanced stage) and 12 relapsed cHL patients are summarized in Table 1. Median age among the newly diagnosed patients was 33 years (range 16-75) with slightly more females than males. Most patients had nodular sclerosis subtype.

Baseline plasma TARC levels and tumor burden in newly diagnosed patients

Baseline plasma TARC was elevated (>1,000 pg/ml) in 55 out of 60 newly diagnosed patients (92%) and was significantly higher in cHL patients (median 28,013; range 69–269,048 pg/ml) compared to healthy controls (median 118 pg/ml (range: 7-470) (p< .001, Figure 1).

Significant higher levels of baseline plasma TARC levels were observed in patients with stage II-IV disease compared to patients with stage I disease (p< .001, Figure 2a) as well as in patients with bulky disease compared to patients without bulky disease (p=.02, Figure 2b). Baseline plasma TARC levels directly correlated with the metabolic FDG-PET volume (r = .61, p<.001, Figure 2c) and not with IPS score or presence of B-symptoms.

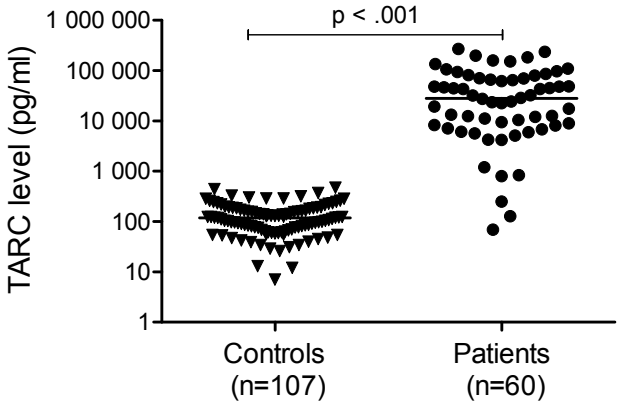


Figure 1. TARC expression in plasma from newly diagnosed cHL patients and healthy controls.
Plasma TARC levels among 107 healthy controls and 60 pre-treatment newly diagnosed cHL patients. The median plasma TARC level was 118 pg/ml (range: 7-470) and 28,013 pg/ml (range 69–269,048) in the healthy controls and the cHL patients respectively. Pre-treatment patient samples were significantly higher compared to the healthy controls (p<.001).

Table 1. Patient characteristics and treatment.

	Newly diagnosed cHL patients (n=60) n (%)	Relapsed cHL patients (n=12) n (%)
Median Age (range)	33 (16-66)	47 (25-64)
Female	36 (60)	5 (42)
Histology		
NS	42 (70)	10 (83)
MC	4 (7)	2 (17)
LR	3 (5)	-
cHL NOS	11 (18)	-
Ann Arbor stage		
I-II (early stage)	39 (63)	3 (25)
III-IV (advanced stage)	21 (39)	9 (75)
B-symptoms present	25 (42)	4 (33)
Bulky disease	22 (37)	1 (8)
Treatment stage I/II patients (n=39):		
ABVD 3-4 cycles + IN-RT	26 (67)	-
ABVD 4-6 cycles	11 (28)	-
ABVD 2 cycles, EscBEACOPP 2 cycles + IN-RT	2 (5)	-
Treatment stage III/IV patients (n=21):		
ABVD 6-8 cycles	15 (71)	-
BEACOPP 5 cycles	1 (5)	-
EscBEACOPP 4 cycles, BEACOPP 4 cycles	5 (24)	-
Treatment relapsed patients (n=12):		
DHAP-VIM-DHAP + BEAM+ ASCT	-	9 (75)
DHAP-VIM-Mini-BEAM (2x) +BEAM + ASCT	-	3 (25)

NS = nodular sclerosis; MC = Mixed cellularity; LR = Lymphocyte Rich; cHL = classical Hodgkin Lymphoma; NOS = not otherwise specified; ABVD = adriamycin-bleomycin-vinblastine-dacarbazine containing chemotherapy regimen; IN-RT = involved node radiotherapy; BEACOPP = bleomycin-etoposide-adriamycin-cyclophosphamide-vincristine-procarbazine-prednisone containing chemotherapy regimen; eBEACOPP = escalated (dose intensified) BEACOPP; DHAP = dexamethason-citarabine-cisplatin containing salvage re-induction regimen; VIM = etoposide-ifosfamide-methotrexate containing regimen; ASCT = autologous stem cell transplantation; BEAM = carmustine-etoposide-cytarabine-melphalan myeloablative chemotherapy.

Plasma TARC as a response marker in early stage patients

Of 39 newly diagnosed patients with early stage disease, 37 achieved a complete response (CR), one a partial response (PR; UPN 38) and one had progressive disease (PD; UPN 39). In all patients with a CR, reduction in plasma TARC levels could be observed as early as after one cycle of chemotherapy (Figure 3a, supplementary Table S1). Thirty-one of 37 patients with a final CR already had a CR at mid-treatment. The other six patients (of which 2 with bulky disease) had a PR at mid-treatment, while TARC levels were already low after one cycle of chemotherapy. Thirty-six out of 37 patients with a CR had a continuous remission (median follow-up 33 months, range 6-66). One patient (UPN 4) relapsed at 27 months follow-up with concomitant re-elevation of plasma TARC.

The single patient with a PR at end-treatment (UPN 38) also showed a reduction of plasma TARC to normal range levels after one cycle of chemotherapy. This patient did not receive additional therapy and remained limited FDG-PET positive at serial post-treatment imaging studies but did not progress (21 months of follow-up).

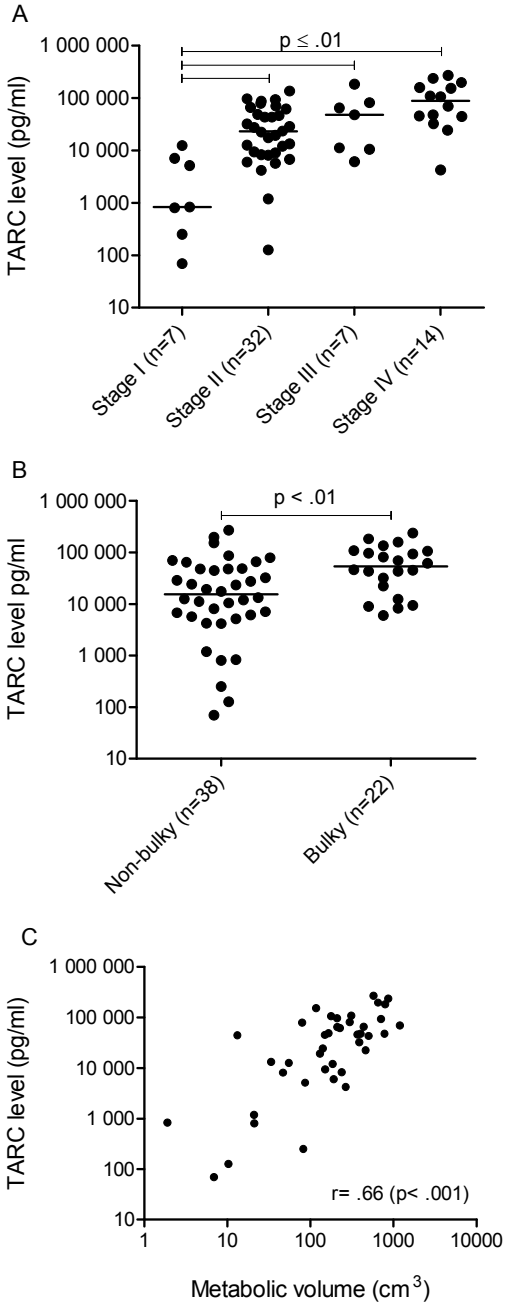
In the patient with PD (UPN 39), plasma TARC was persistently elevated during and after treatment. After progression during salvage chemotherapy this patient died of progressive lymphoma.

Plasma TARC as a response marker in advanced stage patients

Of 21 patients with advanced stage disease, 18 patients had a CR, one a PR (UPN 58) and two had PD (UPN 59 and 60). Similar to early stage patients, reduction in plasma TARC was significant after one cycle of chemotherapy in all CR patients (Figure 3b, supplementary Table S2). Seventeen of 18 patients with a final CR already had a CR at mid-treatment and one patient with a final CR had a PR at mid-treatment (UPN 57). Plasma TARC was slightly elevated at mid-treatment and low at end-treatment in this patient. None of the patients with a CR relapsed (median follow-up of 31 months, range 7-75). The single patient with a final PR (UPN 58) showed low plasma TARC levels during and after treatment. After second line chemotherapy including ASCT and IN-RT this patient remained FDG-PET positive in the same spots as observed after first line treatment. Repeated FDG-PET studies showed that FDG uptake faded out over time and at 10 months post radiotherapy, FDG uptake disappeared.

The two patients with PD (UPN 59 and 60) had persistent high TARC levels during and after treatment. Both patients switched to second line chemotherapy and one of them achieved a CR (UPN 60) with a concomitant reduction of plasma TARC to normal levels. The second patient (UPN 59) is still being treated and response and TARC results have to be awaited.[#1]

[#] Update of this patient after publication: unfortunately this patient progressed after first and second salvage chemotherapy with concomitant high TARC levels. He ultimately died of progressive disease.

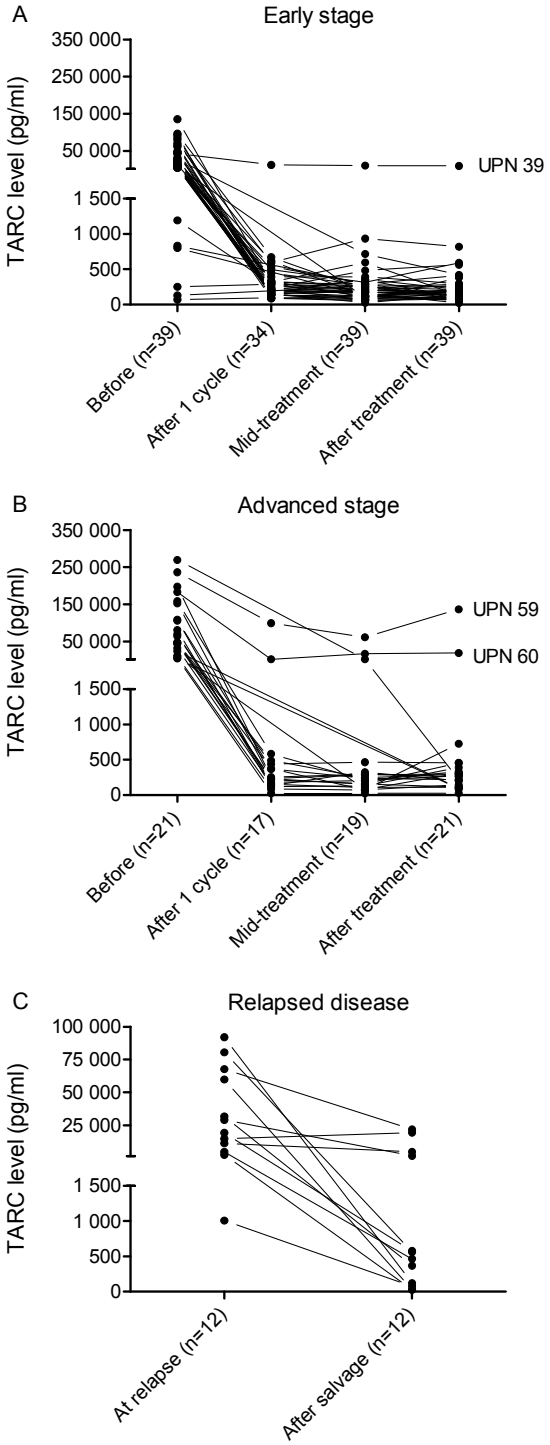

A
1 000 000
100 000
10 000
1 000
100
10
TARC level (pg/ml)
p ≤ .01
Stage I (n=7)
Stage II (n=32)
Stage III (n=7)
Stage IV (n=14)
B
1 000 000
100 000
10 000
1 000
100
10
TARC level pg/ml
p < .01
Non-bulky (n=38)
Bulky (n=22)
C
1 000 000
100 000
10 000
1 000
100
10
TARC level (pg/ml)
r= .66 (p< .001)
1
10
100
1000
10000
Metabolic volume (cm3)

◂ **Figure 2. Baseline plasma TARC levels in relation to tumor burden.**
(A) In newly diagnosed patients, plasma TARC levels in stage II-IV disease were significantly higher compared to stage I disease ($p \leq .01$). (B) Patients with bulky disease had significantly higher baseline plasma TARC levels compared to patients without bulky disease ($p < .01$). (C) Baseline plasma TARC levels significantly correlated with the pre-treatment metabolic volume as determined by quantification of the FDG-PET results.

Plasma TARC as a response marker in relapsed patients

All 12 patients with relapsed disease had elevated levels of TARC at relapse (Figure 4). Eight patients achieved a CR after salvage therapy with a concomitant reduction of plasma TARC to normal range levels after treatment (Figure 4). These eight patients had a continuous remission (median follow-up 28 months, range 3-62). Plasma TARC levels in one patient with SD (UPN 69) and three patients with PD (UPN 70, 71 and 72) remained elevated. Three of these patients died of lymphoma and one patient ultimately achieved a CR (UPN 71) after additional radiotherapy with concomitant normalization of plasma TARC.

Figure 3. Plasma TARC dynamics in early and advanced stage newly diagnosed and relapsed cHL patients. (A) Plasma TARC dynamics before treatment, after one ABVD cycle, at mid-treatment (after 2 ABVD cycles) and after treatment among 39 newly diagnosed early stage cHL patients. All responsive patients had a decline in plasma TARC to normal range levels, while one non-responsive patient (UPN 39) had persistent high plasma TARC levels and could already be identified after one cycle of ABVD. (B) Plasma TARC dynamics before, after one ABVD or (e)BEACOPP cycle, at mid-treatment (after 4 ABVD or 4 (e) BEACOPP cycles) and after treatment among 21 newly diagnosed advanced stage cHL patients. Again, the two non-responsive (UPN 59 and 60) patients could be distinguished from all responsive patients already after one cycle of chemotherapy by persistent high plasma TARC levels. (C) TARC dynamics at relapse and after relapse treatment in 12 relapsed cHL patients. All 12 relapsed patients had moderate to high elevated plasma TARC levels at relapse. Four final non-responsive patients had persistent high plasma TARC levels after second line treatment, while all responsive patients had a decline in plasma TARC to normal range levels. ▸


A
Early stage
TARC level (pg/ml)
350 000
250 000
150 000
50 000
1 500
1 000
500
0
UPN 39
Before (n=39)
After 1 cycle (n=34)
Mid-treatment (n=39)
After treatment (n=39)
B
Advanced stage
TARC level (pg/ml)
350 000
250 000
150 000
50 000
1 500
1 000
500
0
UPN 59
UPN 60
Before (n=21)
After 1 cycle (n=17)
Mid-treatment (n=19)
After treatment (n=21)
C
Relapsed disease
TARC level (pg/ml)
100 000
75 000
50 000
25 000
1 500
1 000
500
0
At relapse (n=12)
After salvage (n=12)

Discussion

In this study we show that plasma TARC levels closely correlate with Hodgkin lymphoma metabolic tumor volume and that serial TARC levels correlate with clinical response to treatment. Interestingly, all responsive patients had a decrease in plasma TARC already after one cycle of chemotherapy while TARC levels remained high in the three non-responsive patients, indicating that plasma TARC might be a potential marker for very early response assessment in cHL. In addition, we show that plasma TARC is also elevated at relapse and again correlates well with clinical response.

Since TARC is specifically produced and excreted by Hodgkin Reed-Sternberg cells, we hypothesized that plasma TARC might closely reflect cHL tumor burden. Consistent with previous studies, we showed that baseline plasma TARC levels correlate with classical clinical parameters of tumor burden such as stage of disease and presence of bulky disease.[9,10] However, considerable overlap in plasma TARC levels was observed between the different groups defined by stage and bulk, probably because these clinical parameters are poor substitutes for total tumor load. Quantified pre-treatment FDG-PET images, reflecting metabolic tumor volume, correlated much better with plasma TARC levels. This indicates that plasma TARC levels indeed reflect cHL tumor burden and might be an ideal marker to determine cHL disease activity.

A proportion of patients with stage I disease had low baseline plasma TARC levels, similar to what we observed in an independent cohort in a previous study.[9] Since we now only included patients with TARC positive HRS cells, the lack of elevated plasma TARC is not caused by lack of TARC production by the tumor cells. Consistent with the correlation of plasma TARC with the metabolic tumor volume, it might be envisaged that in some early stage cases almost all TARC producing tumor cells are removed by the diagnostic lymph node biopsy and/or that the limited (remaining) amount of tumor cells are not capable of producing amounts of TARC that exceed the capture capacity of the surrounding or circulating CCR4+ T cells.

Early interim FDG-PET response is a predictor of final outcome in cHL.[17-24] Treatment adaptation based on the interim FDG-PET result is currently the focus of investigation in several clinical trials. Although the introduction of FDG-PET imaging has been a great breakthrough in response evaluation of malignant lymphoma, a disadvantage of the use of FDG-PET imaging is the relatively high number of false positive scans during treatment.[22,25-30] By applying more sophisticated interpretation methods such as the five point scale, the number of false-positive scans seems to be markedly reduced compared to the dichotomous “negative or positive” evaluation that is currently used in the IHP criteria.[31-35] In contrast to FDG-PET imaging, TARC is specific for cHL tumor cell activity and seems not influenced by concurrent infections or inflammation caused by chemo- or radiotherapy, making plasma TARC an ideal biomarker to assess cHL treatment response.

Early plasma TARC response might be applied in the clinic to determine prognosis or to guide treatment, similar to FDG-PET imaging. In our cohort, all patients with a reduction of plasma TARC after start of treatment had a favorable prognosis, while all patients with persistent high TARC levels failed treatment. Moreover, the six patients who had a PR at mid-treatment (based on FDG-PET/CT imaging) and achieved a CR at end-treatment already had low plasma TARC levels after one cycle of chemotherapy. Therefore, it is tempting to speculate that given current FDG-PET interpretation criteria (IHP criteria), both positive and negative predictive value of interim plasma TARC might even be better than interim FDG-PET imaging. However, inherent to current favorable treatment results in cHL, our small cohort contained only seven non-responsive patients (three newly diagnosed and four relapsed). Therefore, the prognostic impact of plasma TARC could not be directly compared to interim FDG-PET images or other prognostic factors such as the IPS. Future clinical trials including both new FDG-PET interpretation criteria such as the five point scale and plasma TARC evaluation are needed to directly compare the prognostic value of these two response markers.

Our separate cohort of relapsed cHL patients showed that plasma TARC is also elevated at time of relapse and TARC levels after salvage treatment corresponded with clinical response. Monitoring of TARC during follow-up might be a minimally-invasive and effective method for evaluation of disease recurrence, potentially reducing the burden of imaging studies during follow-up. Indeed, two relapsed patients showed elevation of plasma TARC levels months prior to actual clinical diagnosis of relapse (data not shown).

In conclusion, we for the first time show that plasma TARC levels closely reflect cHL tumor load and that serial plasma TARC levels correlate with treatment response. Interestingly, a change in TARC levels could already be observed after one cycle of chemotherapy, indicating its potential to serve as a very early response marker in newly diagnosed patients. However, the true potential of plasma TARC in response evaluation and follow-up of cHL in relation to current evaluation methods needs to be prospectively studied in future studies.

Acknowledgements

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References

1. Kuppers R. The biology of Hodgkin's lymphoma. Nat Rev Cancer. 2009;9(1):15-27.
2. Poppema S and van den Berg A. Interaction between host T cells and Reed-Sternberg cells in Hodgkin lymphomas. Semin Cancer Biol. 2000;10(5):345-350.
3. Maggio E, van den Berg A, Diepstra A, Kluiver J, Visser L, Poppema S. Chemokines, cytokines and their receptors in Hodgkin's lymphoma cell lines and tissues. Ann Oncol. 2002;13 Suppl 1:52-6.
4. Ma Y, Visser L, Roelofsen H, et al. Proteomics analysis of Hodgkin lymphoma: identification of new players involved in the cross-talk between HRS cells and infiltrating lymphocytes. Blood. 2008;111(4):2339-2346.
5. van den Berg A, Visser L, Poppema S. High expression of the CC chemokine TARC in Reed-Sternberg cells. A possible explanation for the characteristic T-cell infiltratein Hodgkin's lymphoma. Am J Pathol. 1999;154(6):1685-1691.
6. Peh SC, Kim LH, Poppema S. TARC, a CC chemokine, is frequently expressed in classic Hodgkin's lymphoma but not in NLP Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and most cases of anaplastic large cell lymphoma. Am J Surg Pathol. 2001;25(7):925-929.
7. van den Berg A, Maggio E, Rust R, Kooistra K, Diepstra A, Poppema S. Clonal relation in a case of CLL, ALCL, and Hodgkin composite lymphoma. Blood. 2002;100(4):1425-1429.
8. Ohshima K, Tutiya T, Yamaguchi T, et al. Infiltration of Th1 and Th2 lymphocytes around Hodgkin and Reed-Sternberg (H&RS) cells in Hodgkin disease: Relation with expression of CXC and CC chemokines on H&RS cells. Int J Cancer. 2002;98(4):567-572.
9. Niens M, Visser L, Nolte IM, et al. Serum chemokine levels in Hodgkin lymphoma patients: highly increased levels of CCL17 and CCL22. Br J Haematol. 2008;140(5):527-536.
10. Weihrauch MR, Manzke O, Beyer M, et al. Elevated serum levels of CC thymus and activation-related chemokine (TARC) in primary Hodgkin's disease: potential for a prognostic factor. Cancer Res. 2005;65(13):5516-5519.
11. Kakinuma T, Nakamura K, Wakugawa M, et al. Thymus and activation-regulated chemokine in atopic dermatitis: Serum thymus and activation-regulated chemokine level is closely related with disease activity. J Allergy Clin Immunol. 2001;107(3):535-541.
12. Cheson BD, Pfistner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25(5):579-586.
13. Juweid ME, Stroobants S, Hoekstra OS, et al. Use of positron emission tomography for response assessment of lymphoma: consensus of the Imaging Subcommittee of International Harmonization Project in Lymphoma. J Clin Oncol. 2007;25(5):571-578.
14. Anonymous Fludeoxyglucose F 18 PET Scan-Guided Therapy or Standard Therapy in Treating Patients With Previously Untreated Stage I or Stage II Hodgkin's Lymphoma. ClinicalTrials.gov. http://www.clinicaltrials.gov/ct2/show/study/NCT00433433. Accessed October 28. 2010.
15. Anonymous Comparison of Two Combination Chemotherapy Regimens in Treating Patients With Stage III or Stage IV Hodgkin's Lymphoma. ClinicalTrials.gov. http://www.clinicaltrials.gov/ct2/show/NCT00049595. Accessed October 28. 2010.
16. Boellaard R, Oyen WJ, Hoekstra CJ, et al. The Netherlands protocol for standardisation and quantification of FDG whole body PET studies in multi-centre trials. Eur J Nucl Med Mol Imaging. 2008;35(12):2320-2333.

17. Hutchings M, Mikhaeel NG, Fields PA, Nunan T, Timothy AR. Prognostic value of interim FDG-PET after two or three cycles of chemotherapy in Hodgkin lymphoma. Ann Oncol. 2005;16(7):1160-1168.
18. Hutchings M, Loft A, Hansen M, et al. FDG-PET after two cycles of chemotherapy predicts treatment failure and progression-free survival in Hodgkin lymphoma. Blood. 2006;107(1):52-59.
19. Gallamini A, Rigacci L, Merli F, et al. The predictive value of positron emission tomography scanning performed after two courses of standard therapy on treatment outcome in advanced stage Hodgkin's disease. Haematologica. 2006;91(4):475-481.
20. Kostakoglu L, Goldsmith SJ, Leonard JP, et al. FDG-PET after 1 cycle of therapy predicts outcome in diffuse large cell lymphoma and classic Hodgkin disease. Cancer. 2006;107(11):2678-2687.
21. Zinzani PL, Tani M, Fanti S, et al. Early positron emission tomography (PET) restaging: a predictive final response in Hodgkin's disease patients. Ann Oncol. 2006;17(8):1296-1300.
22. Gallamini A, Hutchings M, Rigacci L, et al. Early interim 2-[18F]fluoro-2-deoxy-D-glucose positron emission tomography is prognostically superior to international prognostic score in advanced-stage Hodgkin's lymphoma: a report from a joint Italian-Danish study. J Clin Oncol. 2007;20;25(24):3746-3752.
23. Terasawa T, Lau J, Bardet S, et al. Fluorine-18-fluorodeoxyglucose positron emission tomography for interim response assessment of advanced-stage Hodgkin's lymphoma and diffuse large B-cell lymphoma: a systematic review. J Clin Oncol. 2009;27(11):1906-1914.
24. Hutchings M and Barrington SF. PET/CT for therapy response assessment in lymphoma. J Nucl Med. 2009;50:21S-30S,.
25. Avigdor A, Bulvik S, Dann EJ, et al. Combined ESCBEACOPP-ABVD therapy for advanced Hodgkin's lymphoma patients with high IPS score: an effective regimen and low positive predictive value of early FDG-PET/CT. Haematologica. 2007;92(s5):66.
26. Gallamini A, Hutchings M, Avigdor A, Polliack A. Early interim PET scan in Hodgkin lymphoma: where do we stand? Leuk Lymphoma. 2008;49(4):659-662.
27. Markova J, Kobe C, Skopalova M, et al. FDG-PET for assessment of early treatment response after four cycles of chemotherapy in patients with advanced-stage Hodgkin's lymphoma has a high negative predictive value. Ann Oncol. 2009;20(7):1270-1274.
28. Cheson B. The case against heavy PETing. J Clin Oncol. 2009;27(11):1742-1743.
29. Gallamini A. Positron emission tomography scanning: a new paradigm for the management of Hodgkin's lymphoma. Haematologica. 2010;95(7):1046-1048.
30. Cheson BD. Role of functional imaging in the management of lymphoma. J Clin Oncol. 2011;29(14):1844-1854.
31. Meignan M, Gallamini A, Meignan M, Gallamini A, Haioun C. Report on the First International Workshop on Interim-PET-Scan in Lymphoma. Leuk Lymphoma. 2009;50(8):1257-1260.
32. Dann EJ, Bar-Shalom R, Tamir A, et al. A functional dynamic scoring model to elucidate the significance of post-induction interim fluorine-18-fluorodeoxyglucose positron emission tomography findings in patients with Hodgkin's lymphoma. Haematologica. 2010;95(7):1198-1206.
33. Meignan M, Gallamini A, Haioun C, Polliack A. Report on the Second International Workshop on interim positron emission tomography in lymphoma held in Menton, France, 8-9 April 2010. Leuk Lymphoma. 2010;51(12):2171-2180.
34. Barrington SF, Qian W, Somer EJ, et al. Concordance between four European centres of PET reporting criteria designed for use in multicentre trials in Hodgkin lymphoma. Eur J Nucl Med Mol Imaging. 2010;37(10):1824-1833.
35. Meignan M. Interim PET in lymphoma: a step towards standardization. Eur J Nucl Med Mol Imaging. 2010;37(10):1821-1823.

Supplementary

Table S1. Plasma TARC dynamics and response in early stage cHL patients

	plasma TARC level (pg/ml)				Response evaluation		First line treatment
UPN	Baseline (n=39)	After 1 cycle (n=34)	Mid-treatment (n=39)	After treatment (n=39)	Mid-treatment	End-treatment	
1*	96839	671	200	187	CR	CR	4x ABVD + IN-RT
2*	93413	85	118	92	CR	CR	4x ABVD + IN-RT
3	86334	256	219	127	CR	CR	4x ABVD + IN-RT
4	79313	229	482	564	CR	CR	4x ABVD
5	70283	590	933	819	CR	CR	6x ABVD
6	65787	140	85	104	CR	CR	6x ABVD
7*	61624	131	119	150	CR	CR	6x ABVD
8*	43050	205	174	167	CR	CR	4x ABVD + IN-RT
9*	31953	156	178	88	CR	CR	4x ABVD + IN-RT
10	28748	316	594	125	CR	CR	4x ABVD
11	23400	314	281	245	CR	CR	3x ABVD + IN-RT
12*	22436	616	119	220	CR	CR	4x ABVD
13	17540	86	53	79	CR	CR	3x ABVD + IN-RT
14	13350	206	346	101	CR	CR	3x ABVD + IN-RT
15	12614	178	114	61	CR	CR	4x ABVD + IN-RT
16*	12388	497	314	592	CR	CR	4x ABVD + IN-RT
17	12024	393	393	293	CR	CR	4x ABVD + IN-RT
18*	9428	196	173	134	CR	CR	6x ABVD
19*	8967	191	160	22	CR	CR	4x ABVD + IN-RT
20*	8248	169	352	216	CR	CR	6x ABVD
21	8103	227	193	271	CR	CR	3x ABVD + IN-RT
22	7066	-	100	71	CR	CR	3x ABVD + IN-RT
23	6760	135	69	382	CR	CR	4x ABVD + IN-RT
24	5652	444	271	91	CR	CR	4x ABVD + IN-RT
25	5118	139	225	292	CR	CR	4x ABVD + IN-RT
26	4174	153	25	138	CR	CR	4x ABVD + IN-RT
27	834^	-	292	409	CR	CR	4x ABVD + IN-RT
28	800^	-	120	194	CR	CR	4x ABVD + IN-RT
29	250^	285	122	54	CR	CR	3x ABVD + IN-RT
30	127^	-	256	107	CR	CR	3x ABVD + IN-RT
31	69^	95	70	164	CR	CR	6x ABVD

Table S1. Continued

	plasma TARC level (pg/ml)				Response evaluation		First line treatment
UPN	Baseline (n=39)	After 1 cycle (n=34)	Mid-treatment (n=39)	After treatment (n=39)	Mid-treatment	End-treatment	
32*	135486	297	201	160	PR	CR	6x ABVD
33	48721	326	255	78	PR	CR	4x ABVD + IN-RT
34	27277	-	714	421	PR	CR	4x ABVD + IN-RT
35	19313	334	103	212	PR	CR	2x ABVD + 2x eBEACOPP + IN-RT
36*	5977	539	292	112	PR	CR	4x ABVD + IN-RT
37	1193	291	246	212	PR	CR	3x ABVD + IN-RT
38*	46298	220	64	83	PR	PR	2x ABVD + 2x eBEACOPP + IN-RT
39*	43048	12532	10873	9754	PR	PD	6x ABVD

* = patient with bulky disease; ^ = TARC expression by the HRS cells was confirmed by positive TARC staining in tissue samples; UPN = unique patient number; TARC= Thymus and Activation-Regulated Chemokine; CR = complete response; PR = partial response; PD = progressive disease

Table S2. Plasma TARC dynamics and response in advanced stage cHL patients

	plasma TARC level (pg/ml)				Response evaluation		First line treatment
UPN	Baseline (n=21)	After 1 cycle (n=17)	Mid-treatment (n=19)	After treatment (n=21)	Mid-treatment	End-treatment	
40	197,160	254	294	261	CR	CR	8x ABVD
41	152,487	230	153	116	CR	CR	8x ABVD
42*	108,915	134	324	202	CR	CR	6x ABVD
43*	106,003	369	237	215	CR	CR	8x ABVD
44*	81,088	119	129	390	CR	CR	8x ABVD
45*	69,719	179	203	329	CR	CR	8x ABVD
46	64,422	438	465	456	CR	CR	6xABVD
47	47,783	487	246	297	CR	CR	4x eBEACOPP, 4x BEACOPP
48	47,739	233	285	296	CR	CR	6x ABVD
49*	45,183	373	73	725	CR	CR	6x ABVD
50	44,543	217	157	454	CR	CR	6x ABVD
51	32,194	153	105	110	CR	CR	6x ABVD
52	24,225	-	No-	90	CR	CR	8x ABVD
53	11,124	82	72	144	CR	CR	4x eBEACOPP, 4x BEACOPP
54	10,465	-	78	311	CR	CR	8x ABVD
55	6,111	25	24	28	CR	CR	6x ABVD
56	4,221	-	-	88	CR	CR	5x BEACOPP
57	269,048	-	2302	217	PR	CR	4x eBEACOPP, 4x BEACOPP
58*	158,410	583	206	273	PR	PR	4x eBEACOPP, 4x BEACOPP
59*	236,288	99276	61365	136636	PR	PD	4x eBEACOPP, 4x BEACOPP
60*	182,908	2117	16965	19324	PR	PD	6x ABVD

* = patient with bulky disease; UPN = unique patient number; TARC= Thymus and Activation-Regulated Chemokine; CR = complete response; PR = partial response; PD = progressive disease

Table S3. Plasma TARC dynamics and response in relapsed cHL patients

UPN	Plasma TARC level (pg/ml)		Response evaluation	Salvage treatment
	At relapse (n=12)	After salvage treatment (n=12)	End-treatment	
61	91,863	122	CR	DHAP-VIM-DHAP +ASCT
62*	80,432	562	CR	DHAP-VIM-DHAP +ASCT
63	59,785	26	CR	DHAP-VIM-DHAP +ASCT
64	31,705	368	CR	DHAP-VIM-DHAP +ASCT
65	19,149	577	CR	DHAP-VIM-DHAP +ASCT
66	4,731	465	CR	DHAP-VIM-DHAP +ASCT
67	2,524	30	CR	DHAP-VIM-DHAP +ASCT
68	1,008	78	CR	DHAP-VIM-DHAP +ASCT
69	11,179	4,792	SD	DHAP, VIM, Mini-BEAM (2x) +ASCT
70	67,629	21,804	PD	DHAP-VIM-DHAP +ASCT
71	29,085	1,689	PD	DHAP, VIM, Mini-BEAM (2x) +ASCT
72	14,637	19,371	PD	DHAP, VIM, Mini-BEAM (2x) +ASCT

* = patient with bulky disease; UPN = unique patient number; TARC= Thymus and Activation-Regulated Chemokine; CR = complete response; SD = stable disease; PD = progressive disease


TARC