

University of Groningen

Circulating biomarkers in classical Hodgkin lymphoma

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DOI:
[10.33612/diss.97631424](https://doi.org/10.33612/diss.97631424)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Plattel, W. J. (2019). *Circulating biomarkers in classical Hodgkin lymphoma*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen. <https://doi.org/10.33612/diss.97631424>

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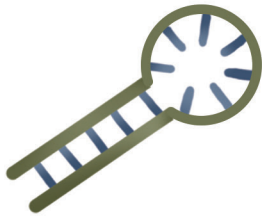
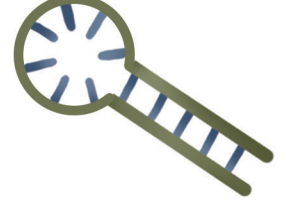
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CHAPTER 3

Biomarkers for evaluation of treatment response in classical Hodgkin lymphoma: comparison of sGalectin-1, sCD163 and sCD30 with TARC

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Published in: Br J Haematol. 2016 Dec;175(5):868-875

Abstract

INTRODUCTION

Soluble Galectin-1 (sGal-1), soluble CD163 (sCD163) and soluble CD30 (sCD30) have been reported to be elevated in plasma or serum of patients with classical Hodgkin lymphoma (cHL). We aimed to determine the clinical utility of these biomarkers for evaluation of treatment response compared to CCL17/Thymus and Activation Regulated Chemokine (TARC).

MATERIALS AND METHODS

Plasma or serum samples were prospectively collected among 103 newly diagnosed cHL patients before and after treatment. Levels of sGal-1, sCD163, sCD30 and TARC were correlated with disease characteristics and clinical treatment response.

RESULTS

Elevated plasma levels of sGal-1, sCD163, sCD30 and TARC were found in 67%, 21%, 91% and 93% of cHL patients respectively. Mean plasma levels of sGal-1 and sCD30 decreased after treatment and sCD163 did not decrease after treatment. There was no correlation with change of these markers and clinical treatment response on individual patient level. TARC levels strongly correlated with disease characteristics and metabolic volume. TARC remained high in 6 out of 7 non-responsive patients and dramatically decreased in 95 out of 96 responsive patients.

CONCLUSION

In summary, elevated pre-treatment levels of sGal-1, sCD163, sCD30 and TARC can be found in patients with cHL. However, only plasma TARC accurately reflects disease activity and correlates with clinical treatment response.

Introduction

Classical Hodgkin lymphoma (cHL) is currently a highly curable disease. More than 85 percent of patients become long-term survivors with current treatment strategies (Ansell, 2012). Current clinical studies focus on preventing long-term toxicity in patients who do not need intensive regimens and on early recognition of patients not optimally responding to initial treatment. Testing for chemosensitivity using [18F]-fluoro-2-deoxy-D-glucose positron emission tomography (FDG-PET) early during treatment is often part of this strategy. Because of a lack of accurate pre-treatment predictive factors, early FDG-PET response is currently the best predictor for final response to treatment.¹⁻⁴

Blood based biomarkers hold the promise to be much more practical, patient friendly and cost-effective and might be used as serial markers during and after treatment to determine early response to treatment and disease recurrence after treatment. However, such markers must have specificity and sensitivity at least comparable or complementary to FDG-PET imaging and must be able to accurately distinguish cHL patients from controls at the individual patient level.

Several candidate biomarkers have been reported to be elevated in patients with cHL compared to healthy controls. These biomarkers can be divided into tumor cell specific markers, secreted by Hodgkin Reed-Sternberg (HRS) cells or markers related to the micro-environment.^{5,6}

We and others have previously shown that the HRS cell specific CC chemokine ligand 17 (CCL17, also Thymus and Activation Regulated Chemokine (TARC)) is a very specific marker for cHL disease activity.⁷⁻¹² TARC levels correlate with metabolic tumor volume as determined by FDG-PET imaging and can already determine response to treatment after one cycle of chemotherapy.^{10,11} Similar to FDG-PET imaging, high TARC levels after treatment correlate with reduced survival.^{12,13}

Meanwhile other groups have demonstrated that the soluble form of tumor cell specific marker Galectin-1 (sGal-1) and the M2 macrophage marker soluble CD163 (sCD163) are elevated in serum of patients with cHL.^{11,14} Also soluble CD30 (sCD30) has been reported to correlate with disease extensiveness and prognosis in cHL.¹⁵⁻¹⁹ However, there are no data on measurements of sGal-1 and sCD30 before and after treatment and data on serial measurements of sCD163 are scarce. The aim of the current study was to compare the clinical value of serial measurements of these markers before and after treatment with TARC for the evaluation response in a well-defined cohort of cHL patients.

Materials and methods

Patient inclusion

We included all newly diagnosed cHL patients in the University Medical Center Groningen (UMCG) from January 2006 until December 2014 in whom plasma or serum samples were collected. In total, 103 cHL patients were included. From 63 of these patients TARC data have been reported previously¹⁰. Permission for this study was obtained from the institutional review board of the UMCG and all participating patients and healthy controls signed informed consent.

Staging en response determination

All patients were staged and evaluated with FDG-PET imaging with or without additional bone marrow biopsy. Response to treatment was evaluated by PET/CT according to the revised International Working Group response criteria.^{20,21} All FDG-PET scans were reviewed and scored according to the Lugano classification including Deauville score.²² In case of doubtful remission status after completion of treatment, suspicious lesions were either biopsied or followed for progression with repeated imaging.

Patients were either included or generally treated according to the standard of arm of European Organisation for Research and Treatment of Cancer (EORTC) clinical trial protocols (Table 1). In short, treatment for early stage patients consisted of 3-6 cycles of ABVD with or without 30-36 Gy involved node radiotherapy (IN-RT) according to the EORTC (20051) H10 trial in the vast majority of patients.²³ Advanced stage patients were mainly treated with 6-8 cycles of ABVD, or 4 cycles of dose escalated BEACOPP (escBEACOPP) followed by 4 cycles of normal dose BEACOPP, or -more recently- with 6 cycles of escBEACOPP. Advanced stage patients with FDG-PET positive disease after chemotherapy received additional involved node radiotherapy (INRT) on PET positive residual disease.

Mid-treatment FDG-PET was planned after 2 cycles of ABVD for early stage patients, or after 4 cycles of ABVD, or 3 or 4 cycles of (Esc)BEACOPP for advanced stage patients respectively. In case of complete metabolic response, i.e. no suspicious uptake on the mid-treatment FDG-PET scan (Deauville 1), FDG-PET scan was not routinely repeated at end of treatment for early stage patients. Determination of metabolic tumor volume has been assessed as previously described.¹⁰

Table 1. Patient characteristics

	cHL patients (n=103) n (%)
Median Age (range)	34 (16-82)
Female	53 (52)
Histology	
NS	64 (62)
MC	12 (12)
LR	5 (5)
cHL NOS	22 (21)
Ann Arbor stage	
I-II (early stage)	61 (59)
III-IV (advanced stage)	42 (41)
B-symptoms present	44 (43)
Bulky disease	30 (30)
Treatment stage I/II patients (n=61):	
ABVD 3-4 cycles + IN-RT	43 (70)
ABVD 4-6 cycles	15 (25)
ABVD 2 cycles, EscBEACOPP 2 cycles + IN-RT	2 (3)
Other	1 (2)
Treatment stage III/IV patients (n=42):	
ABVD 6-8 cycles +/- RT	23 (55)
(Esc)BEACOPP 6-8 cycles +/- RT	12 (29)
Other	7 (17)

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; EscBEACOPP = escalated (dose intensified) BEACOPP.

Sample collection and ELISA

From all 103 patients plasma or serum samples were collected before and after treatment. Plasma samples were obtained from 76 patients before treatment and 75 patients after treatment, serum before and after treatment in 27 and 28 patients respectively. Plasma collected from 107 age and sex matched healthy controls and serum from 25 controls out of the same control cohort, were used for comparison with the pre-treatment patient samples and determination of cut-offs.

Levels of sGal-1, sCD163, sCD30 and TARC were measured by a double antibody sandwich ELISA (all R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. Samples were analyzed without prior knowledge of the corresponding patient or treatment results.

Statistics

Optimal biomarker cut off levels between patients and healthy controls were determined using the Receiver Operating Characteristic method. Differences in biomarker levels between categorical variables were calculated using the unpaired t-test. Baseline biomarker levels were correlated to Ann Arbor stage using the analysis of variance (ANOVA) method and Tukey's Multiple Comparison test as post-hoc test. Correlation between biomarker levels and the metabolic tumor volume determination by FDG-PET were calculated using the Pearson correlation test. All statistical analyses were performed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com). The p-value for statistical significance was defined at .05.

Correlation of biomarker levels with disease characteristics was performed using the results of plasma samples exclusively. The number of serum samples was not sufficient for reliable correlation analysis.

Results

Patient characteristics

Basic characteristics and treatments of the 103 newly diagnosed cHL patients are summarized in Table 1. Median age of the patient cohort was 34 years (range 16-82) and there were slightly more females than males. Most patients had nodular sclerosis subtype.

Pre-treatment biomarker levels compared to controls

We found significantly different levels for sGal-1 and TARC in plasma versus serum in controls (Figure 1). Because of these differences, results for plasma and serum are analyzed and shown separately for all biomarkers.

Compared to controls, cHL patients had significantly higher mean levels for sGal-1 (26.5 vs. 45.8 ng/ml ($p < .001$)), sCD163 (469 vs. 646 ng/ml for sCD163 ($p = .003$)), sCD30 (1.8 vs. 4.6 ng/ml for sCD30 ($p < .001$)) and TARC (134 vs. 54 161 pg/ml ($p < .001$)) in plasma samples at diagnosis (Figure 1, Table 2). Fold difference of the mean for sGal-1, sCD163 and sCD30 were modest with 1.7, 1.4 and 2.6 respectively, whereas the difference of the mean was 404 fold for TARC. ROC analysis performed on plasma samples showed most discriminative values for sCD30 and TARC with areas under the curve for sGal-1, sCD163, sCD30 and TARC of 0.85, 0.55, 0.95 and 0.97 respectively (Table 2, Supporting Figure 1). Cut-off levels with most optimal sensitivity and specificity showed elevated levels of sGal-1 (>35 ng/ml), sCD163 (>617 ng/ml), sCD30 (> 2.7 ng/ml) and TARC (>635 pg/ml) in 67%, 21%, 91% and 93% of patients respectively.

Serum levels were also significantly different in these markers, except for sCD30 in which serum levels of sCD30 were lower compared to controls in a proportion of patients (Figure 1).

Correlation with disease characteristics

Plasma sGal-1, sCD163 and sCD30 levels did not significantly correlate with disease stage or metabolic tumor volume (Figure 2), whereas TARC levels were significantly higher in stage IV compared to stage I or II disease ($p < .001$, Figure 2D). TARC levels also significantly correlated with metabolic tumor volume ($r^2=.40$, $p < .001$; Figure 2H). Higher TARC levels were observed in patients with bulky disease ($p = .003$), B-symptoms ($p = .001$) and early stage unfavorable disease ($p = .03$). No correlation of TARC with International Prognostic Score in patients with advanced disease was found (Table 2, Supporting Figure 2).

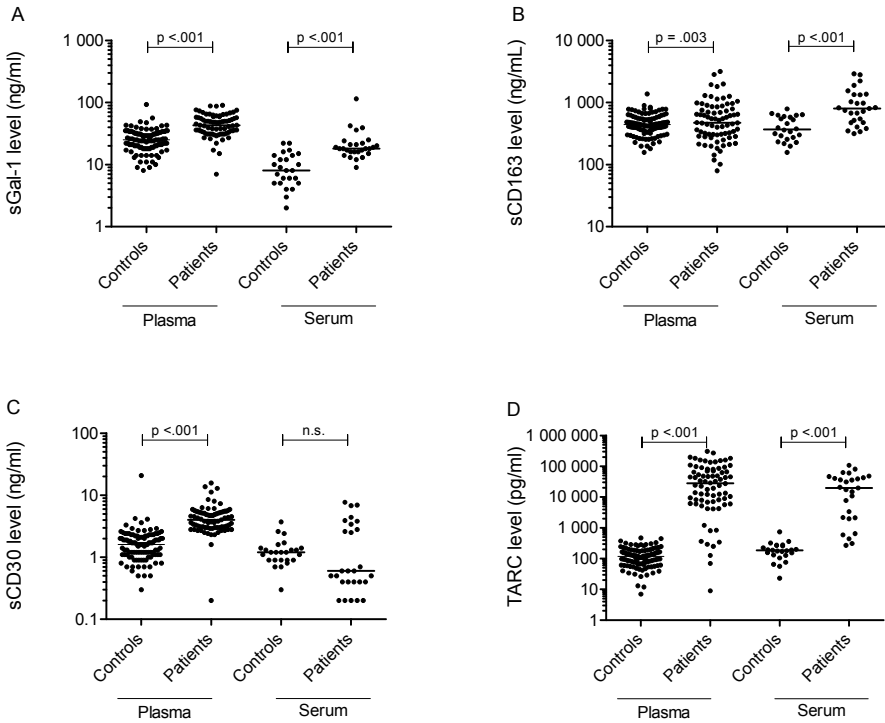


Figure 1. Plasma and serum biomarker levels in cHL patients and healthy controls. sGal-1 (A) and sCD163 (B) levels are significantly elevated in patients with cHL both in plasma and serum samples, with elevation of plasma samples in 67% and 21% of patients respectively. sCD30 (C) showed significant elevation in plasma samples of patients compared to controls, with 91% of samples being elevated (C). Serum samples among a proportion of patients were low for sCD30. TARC levels among patients with cHL were significantly elevated compared to controls in both plasma and serum (D). Ninety-three percent of cHL patients show elevated plasma samples before start of treatment.

For sGal-1 and sCD30 no correlation with bulky disease, B-symptoms, unfavorable disease in early stage patients and high International Prognostic Score in advanced stage patients was found (Table 2, Supporting Figure 2). High sCD163 levels only significantly correlated with presence of B-symptoms ($p < .001$).

Figure 2. Plasma biomarker levels compared to parameters of disease extensiveness. Pre-treatment plasma levels of sGal-1, sCD163, sCD30 and TARC among cHL patients stratified by stage of disease (A-D) and metabolic tumor volume (E-H). Only TARC (D) levels are significantly higher among higher disease stages and correlated with metabolic tumor volume as measured by quantification of pre-treatment FDG-PET images. ▶

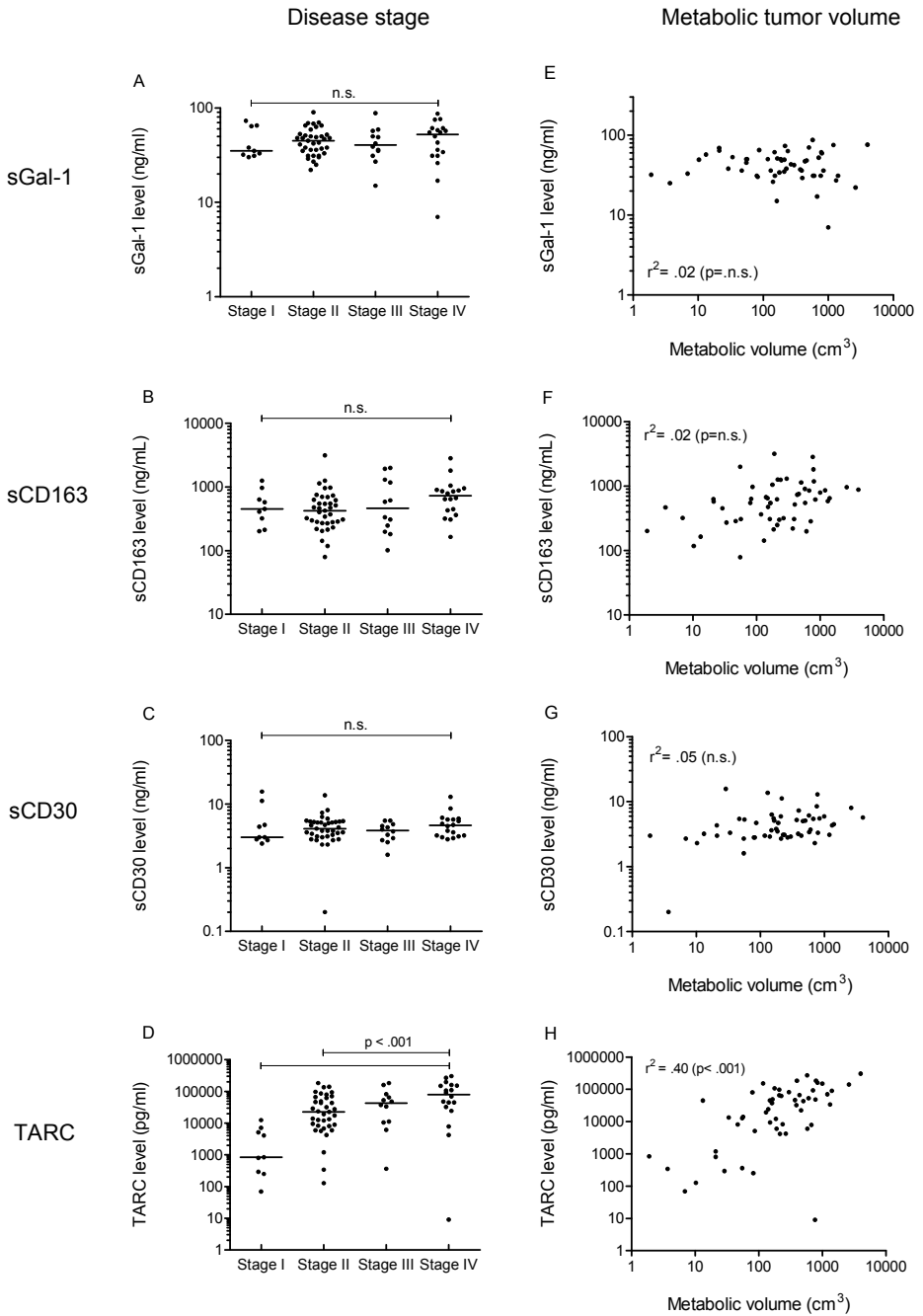


Table 2. Comparison of plasma biomarker characteristics

	sGal-1	sCD163	sCD30	TARC
Mean level controls (SD)	26.5 (11.4)	469 (186)	1.8 (2.0)	134 (90)
Mean level patients (SD)	45.8 (17.3)	646 (561)	4.6 (2.5)	54 161 (65 037)
AUC plasma controls vs. patients	0.85	0.55	0.95	0.97
Upper limit of normal	35	617	2.7	635
Elevated pre-treatment (%)	67	21	91	93
Significantly higher in advanced disease stages	-	-	-	+
Correlation with metabolic tumor volume	-	-	-	+
Significantly elevated in patients with:				
- bulky disease	-	-	-	+
- B-symptoms	-	+	-	+
- unfavorable disease* (early stage patients)	-	-	-	+
- IPS \geq 3 (advanced stage patients)	-	-	-	-
Correlation with treatment response	-	-	-	+

* Unfavorable disease was determined using the European Organization for Research and Treatment of Cancer risk assessment. SD = standard deviation; AUC = Area under the curve; IPS = international prognostic score.

Correlation with treatment response

Out of 103 patients, 96 (93%) achieved a complete remission by first line treatment, and seven patients failed first line therapy: six had an FDG-PET positive (Deauville \geq 4) partial response and one had FDG-PET positive progressive disease (Deauville 5).

Mean plasma levels of sGal-1, sCD30 and TARC significantly decreased after treatment compared to pre-treatment (Figure 3). Plasma levels of sCD163 significantly increased after treatment and serum levels of sCD163 significantly decreased after treatment. Serum levels of TARC also significantly decreased after treatment while changes in serum levels of Gal-1 and sCD30 after treatment were not significant.

sGal-1 levels decreased in almost all patients including four out of seven patients that failed treatment (Fig 3A). sCD163 did not show any consistent correlation with treatment response (Fig 3B) and sCD30 was lower in almost all patients after treatment including four non-responsive patients (Fig 3C). In contrast, TARC dramatically decreased in 95 out of 96 responsive patients and remained high in 6 out of 7 non-responsive patients (Fig 3D). The single patient in complete remission with high post-treatment TARC levels had active atopic dermatitis.

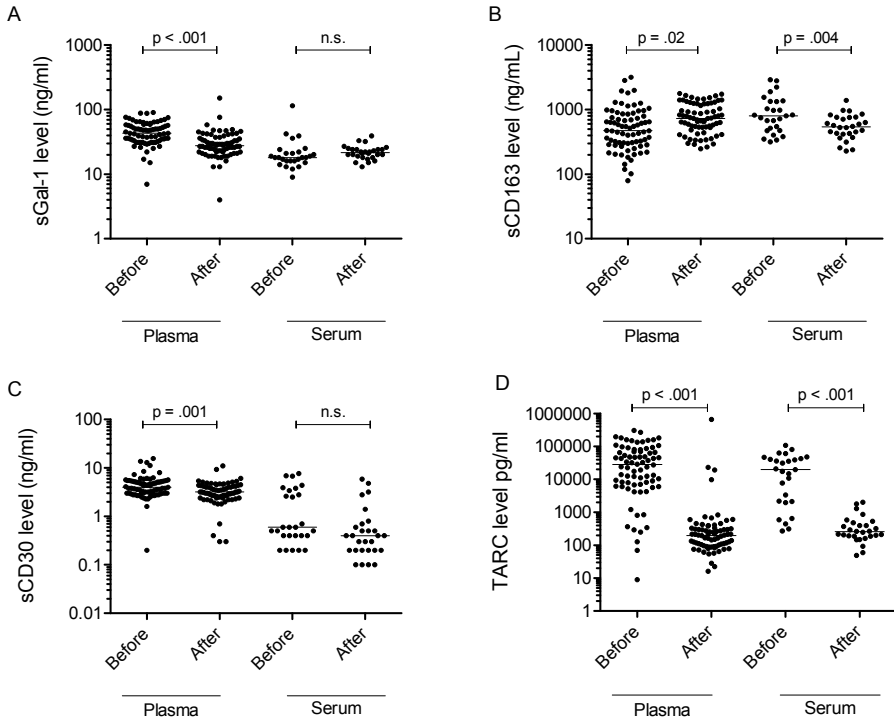


Figure 3. Biomarker levels before and after treatment.

sGal-1 levels significantly decreased after treatment for plasma samples, while there was no significant decrease among serum samples (A). sCD163 plasma levels significantly increased after treatment while serum levels significantly decreased (B). sCD30 levels significantly decreased after treatment in plasma samples while the decrease in serum was not significant (C). TARC significantly decreased after treatment in both plasma and serum samples (D). On individual patient level only TARC levels show clear separation between responsive and non-responsive patients.

Discussion

The aim of this study was to evaluate two recently published blood biomarkers, i.e. sGal-1 and sCD163, as treatment response biomarkers and compare their performance to sCD30 and TARC in a well-defined cohort of cHL patients.

We found that sGal-1, sCD163, sCD30 and TARC levels were elevated in cHL patients compared to healthy controls consistent with previous studies.^{10,11,14-16} sCD30 and TARC had better discriminative power in separating patients from controls compared to sGal-1 and sCD163. Although levels of sGal-1, sCD163 and sCD30 significantly changed after treatment, only TARC corresponded consistently with clinical treatment response at the individual patient level.

Our pre-treatment sGal-1 results are in part consistent with the publication by Ouyang *et al.*¹⁴ They reported elevation of sGal-1 in serum of cHL patients and we confirmed this finding both in plasma and serum samples. We could not confirm the correlation of sGal-1 with clinical parameters of disease extensiveness such as presence of bulky disease, B-symptoms, stage of disease or metabolic tumor volume. Although the results might be influenced by the use of plasma in our study as compared to serum in the study of Ouyang *et al.*, levels in serum were lower compared to plasma in our cohort. This makes it unlikely that serum would be more sensitive than plasma.

For sCD163, part of our results is consistent with a previous report with serial sCD163 measurements.¹¹ They showed a gradual decrease of sCD163 during and after treatment in serum samples of cHL patients. We could observe a similar pattern in our serum samples, while in plasma we observed a slight increase in sCD163 levels in post-treatment samples. This increase might reflect treatment induced inflammatory responses including activation of macrophages.²⁴ sCD163 has been reported to be elevated in several inflammatory conditions such as sepsis, diabetes, liver cirrhosis, rheumatoid arthritis, HIV and macrophage activation syndrome. However, this does not explain the different patterns observed in plasma versus serum samples. Jones *et al.* also found a correlation between serum sCD163 and interim response. It must be stressed out that there was a large overlap between patients in complete compared to partial remission in their cohort. This makes CD163 less useful as a biomarker for response evaluation at the individual patient level.

The sCD30 results are consistent with the studies by Nadali *et al.*^{15,16} In addition to elevated sCD30 levels in cHL patients compared to controls, they reported correlations with stage, bulky disease and B symptoms. We could not confirm these associations but did find a significant decrease in sCD30 levels after treatment.

the current study. Markers that are mainly produced by cells in the infiltrate, like sCD163 by macrophages, are less likely to be disease specific. Elevated levels of sCD163 are for example found in many different types of diseases.²⁵⁻²⁷ Also sGal-1 is found in many tissues and tumor types and elevated sGal-1 levels can be found in serum of patients with head and neck squamous cell carcinoma, glioma, thyroid disease and systemic sclerosis.²⁸⁻³¹ In contrast, sCD30 and TARC are almost exclusively produced by HRS cells. In line with their tumor cells specificity we found elevated levels of sCD30 and TARC in 91% and 93% of patients respectively.

High levels of sCD30 but also high levels of TARC, IL-1RA, ICAM1, IL-6 and IL-2R and others have been correlated with an adverse prognosis.¹⁵⁻¹⁹ Pre-treatment sGal-1 and sCD163 levels might correlate with prognosis as well. The event rate in our cohort was too low to reliably correlate pre-treatment biomarker levels with disease outcome. Well known non-specific blood markers like hemoglobin, lymphocyte count, albumin and erythrocyte sedimentation rate are already included as prognostic factors in the International Prognostic Score (IPS) and future clinical trials should investigate the additive prognostic value of these new biomarkers.

The cohort we used in the current study is an expansion of the cohort that we previously used for TARC measurements.¹⁰ Consistent with our previous study, higher TARC levels correlated with higher disease stage, presence of B-symptoms, bulky disease and metabolic tumor volume. In this larger cohort now containing an additional number of four non responsive patients we could confirm the highly significant correlation of TARC with treatment response. Similar to our study Jones et al also found elevated levels of TARC before treatment and a good correlation of TARC with clinical treatment response.¹¹ A recent study by Moskowitz et al. also showed that interim TARC normalization could predict PET negativity and superior event free survival in patients with relapsed or refractory cHL treated with brentuximab-vedotin.¹²

In conclusion, elevated levels of sGal-1, sCD163 and sCD30 were found in cHL patients but these markers could not discriminate patients from controls as accurately as TARC. Only serial TARC levels accurately reflect disease activity and correlate with clinical treatment response in individual patients. Future studies should elucidate whether TARC might partially replace interim or end-treatment FDG-PET imaging.

Acknowledgements

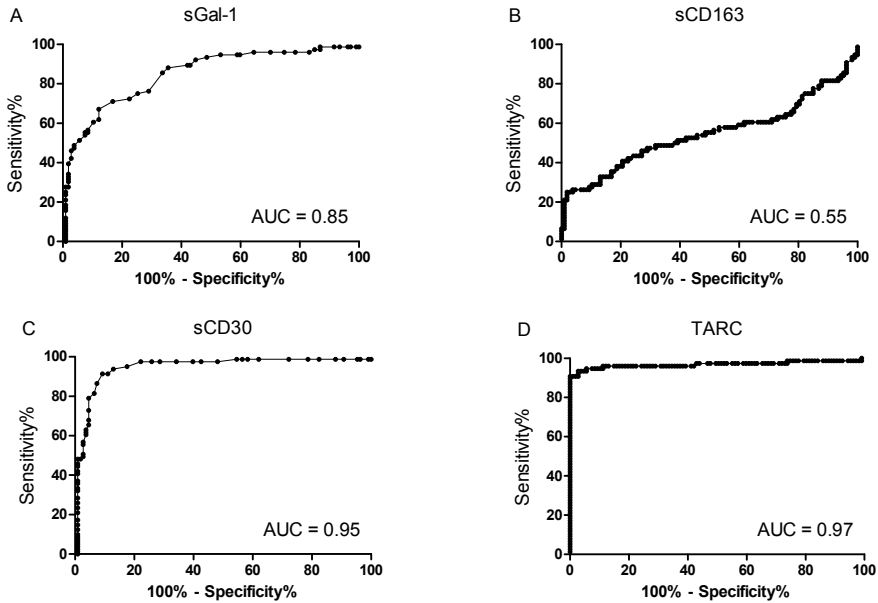
This work was supported by grants from the Dutch Organization of Scientific Research (NWO ZonMW AGIKO, grant no. 92003569) and the Dutch Cancer Society (Grant no. RUG 2010-4860).

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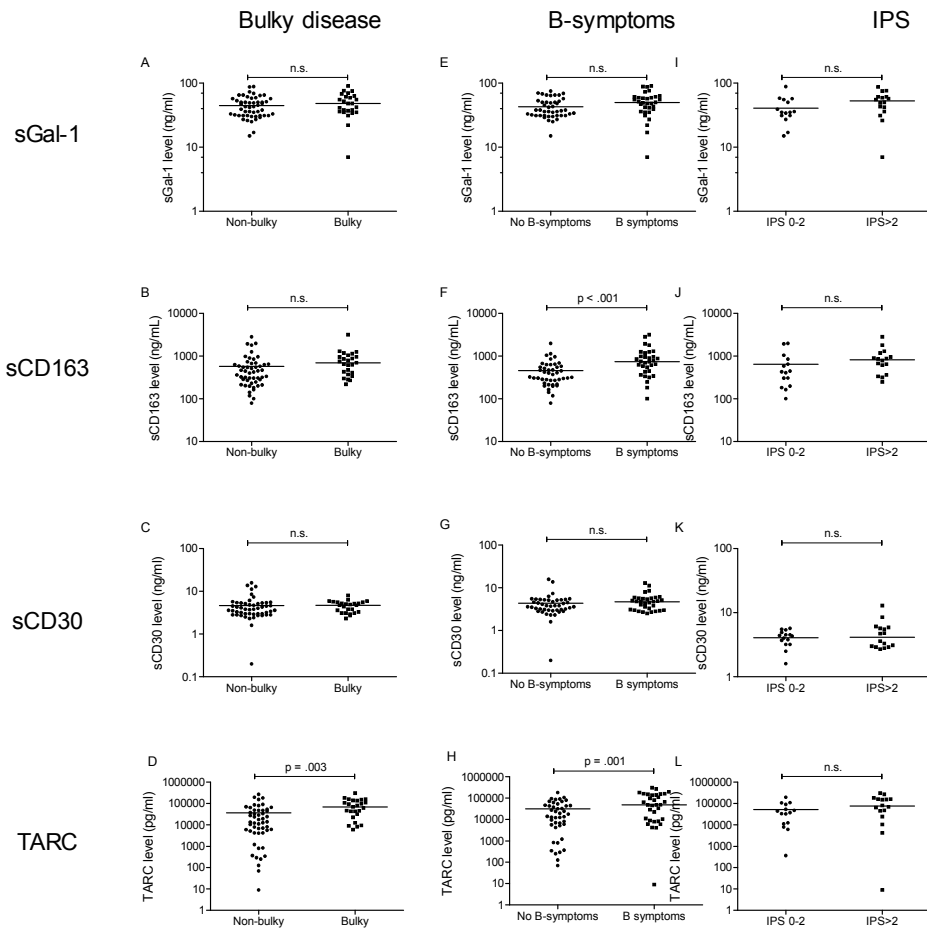
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Supporting figures



Supporting Figure 1. Receiver Operating Characteristic curves for sGal-1 (A), sCD163 (B), sCD30 (C) and TARC (D).



Supporting Figure 2. sGal-1, sCD163, sCD30 and TARC levels according to presence of bulky disease (A-D), B-symptoms (E-H) or IPS score among advanced stage patients (I-L).

