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Brief Report

LYMPHOID NEOPLASIA

Serum levels of TARC, MDC, IL-10, and soluble CD163 in Hodgkin lymphoma: a SWOG S0816 correlative study

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KEY POINTS

- Elevated posttherapy IL-10 and TARC levels were associated with shorter survival, after adjusting for PET results in S0816.
- Exploratory analysis suggests that IL-10 and TARC levels are associated with PFS in PET-negative patients in S0816.

Serum soluble chemokines/cytokines produced by Hodgkin cells and the tumor microenvironment might be of value as biomarkers in classic Hodgkin lymphoma (cHL). We assessed serum thymus and activation-related chemokine (TARC), macrophage-derived chemokine (MDC), interleukin-10 (IL-10), and soluble CD163 (sCD163) levels at baseline, time of interim fluorodeoxyglucose positron emission tomography (PET), and after therapy in cHL patients treated on S0816, an intergroup phase 2 response-adapted study evaluating escalated therapy for interim PET (PET2)–positive patients (www.clinicaltrials.gov #NCT00822120). Epstein-Barr virus (EBV) status was assessed, and 559 serum samples were evaluated for TARC, MDC, IL-10, and sCD163 by immunoassay. EBV positivity correlated with higher sCD163 and IL-10 levels but lower TARC levels. While baseline biomarker levels were not associated with outcome, sCD163 levels at the time of PET2 were associated with favorable progression-free survival (PFS), adjusting for PET2 status. After therapy TARC, MDC, and IL-10 correlated with PFS and overall survival (OS) on univariable analysis, which remained significant adjusting for international prognostic score. When also adjusting for end-of-therapy PET results, TARC and IL-10 remained significantly associated with shorter PFS and OS. Exploratory analysis in PET2-negative patients showed that elevated posttherapy TARC and IL-10 levels were associated with PFS. Serum cytokine levels correlate with outcome in cHL and should be investigated further in risk-adapted cHL trials. (*Blood*. 2019;133(16):1762-1765)

Introduction

Classic Hodgkin lymphoma (cHL) is a B-cell neoplasm characterized by rare neoplastic Hodgkin and Reed-Sternberg (HRS) cells with a microenvironment rich in inflammatory cells that produce a variety of cytokines and chemokines.[1] Thymus and activation-related chemokine (TARC) (CCL17) and macrophage-derived chemokine (MDC) (CCL22) are highly expressed by HRS cells and are involved in the recruitment of immunosuppressive Th2 cells.[2-5] They are elevated in serum in cHL patients, and TARC levels have been associated with treatment failure.[6-11] Interleukin-10 (IL-10), a pleiotropic cytokine with immunosuppressive effects, is expressed by HRS cells and infiltrating T cells. Pretreatment serum IL-10 levels have been associated with survival.[12-17] Gene expression profiling identified signatures that pointed to the importance of macrophages in cHL, and attempts to characterize a gene signature that predicts outcome have been met with variable success.[1,18-20] CD163-positive anti-inflammatory M2 macrophages are associated with poor survival, and soluble CD163 (sCD163) levels are elevated in cHL patient serum and correlate with response.[6,21]

We hypothesized that serum TARC, MDC, IL-10, and CD163 might predict outcome in cHL patients and were particularly interested in the interim positron emission tomography (PET2)–negative patients, given that nearly 20% of these patients relapsed at 2 years.[22]

Study design

Serum biomarkers and Epstein-Barr virus (EBV) status were determined centrally (see supplemental Methods, available on the *Blood* Web site).

Results and discussion

Samples and patient characteristics

A total of 559 samples were analyzed in this study (236 at baseline, 166 at PET2, and 157 after chemotherapy) (supplemental Figure 1). Compared with the entire study cohort, the clinical characteristics and outcome of the 236 patients in this

Table 1. Log2 (posttherapy serum marker level) and survival by univariate Cox regression

Biomarker	Landmark PFS*		Landmark OS†	
	P	HR (95% CI)	*P*	HR (95% CI)
CD163	.3004	0.8 (0.47-1.27)	.5835	0.7 (0.26- 2.13)
IL-10	.0015	1.4 (1.15-1.81)	<.0001	2.0 (1.42-2.77)
MDC	.0430	2.0 (1.01-4.03)	.0198	3.5 (1.22-9.93)
TARC	<.0001	1.9 (1.44-2.49)	.0018	2.2 (1.35-3.69)

*Total samples, 151; number of events, 34.

†Total samples, 151; number of events, 8.

correlative study were not significantly different (supplemental Table 1).

Serum biomarker analysis

As expected, we observed that serum levels of each these markers decreased from baseline to the PET2 time point, with the greatest fold decrease in TARC and MDC. As a population, the levels dropped most after cycle 2, with little change after completing therapy (supplemental Table 2). The change in serum levels from baseline to after cycle 2 and the completion of chemotherapy was statistically significant for each biomarker (Wilcoxon signed-rank tests, $P < .001$). EBV positivity correlated with higher sCD163 and IL-10 levels but lower TARC levels (supplemental Table 3).

Correlations with survival end points

All patients Univariable analysis showed that baseline levels of the 4 markers were not associated with progression-free survival (PFS) or overall survival (OS) ($P > .05$). Similar results were seen at the PET2 time point, with the exception of sCD163. Elevated sCD163 was associated with favorable PFS (hazard ratio [HR], 0.5; 95% confidence interval [CI], 0.33-0.86; $P = .01$). This remained significant after adjusting for PET2 status (HR, 0.5; 95% CI, 0.33-0.85; $P = .009$) and international prognostic score (HR, 0.6; 95% CI, 0.34-0.89; $P = .02$), respectively. Notably, the changes in serum biomarker levels expressed as fold change from baseline to PET2 or after therapy were not associated with PFS or OS. However, Cox regression analysis showed that higher posttherapy levels of IL-10, TARC, and MDC correlated with shorter PFS and OS (Table 1). These remained significantly associated with PFS and OS after adjusting for international prognostic score. Elevated posttherapy IL-10 and TARC levels also remained significantly associated with shorter PFS and OS after adjusting for PET3 status. In order to better understand the most important biomarkers, we performed a multivariate Cox regression analysis of posttherapy serum biomarker levels that included IL-10, TARC, and MDC in the model and found that the best multivariable model included IL-10 and TARC. Higher IL-10 (HR, 1.3; 95% CI, 1.03-1.62; $P = .025$) and TARC levels (HR, 1.8; 95% CI, 1.35-2.37; $P < .0001$) remained significantly associated with shorter PFS. For OS, IL-10 (HR, 1.7; 95% CI, 1.22-2.45; $P = .002$) and TARC (HR, 1.9; 95% CI, 1.10-3.23; $P = .021$) remained significant, with the caveat that only 8 death events were present for this analysis (supplemental Table 4). Eleven patients progressed at or very shortly after PET3, and when we also account for these patients, elevated IL-10 still remained significantly associated with shorter PFS (HR, 1.5; 95% CI, 1.11-1.92; $P = .006$).

To explore whether biomarker levels might assist in identifying interim PET2-negative patients who ultimately progress, we evaluated the log-normalized ratio of PET2 to baseline biomarker levels of each biomarker. No added information was gained from PET2 time-point biomarker ratios.

PET2-negative patients Given that the 2-year PFS was only 82% in the PET2-negative cohort, we were also interested in evaluating the biomarker levels specifically in PET2-negative patients. While baseline and PET2 levels of these biomarkers were not associated with outcome (data not shown) in these patients, higher posttherapy levels of IL-10, TARC, and MDC were associated with shorter PFS (supplemental Table 5). Multivariable analysis including these 3 variables showed that higher posttherapy TARC levels were associated with reduced PFS (HR, 2.1; 95% CI, 1.41-3.00; $P = .0002$). Stepwise selection also

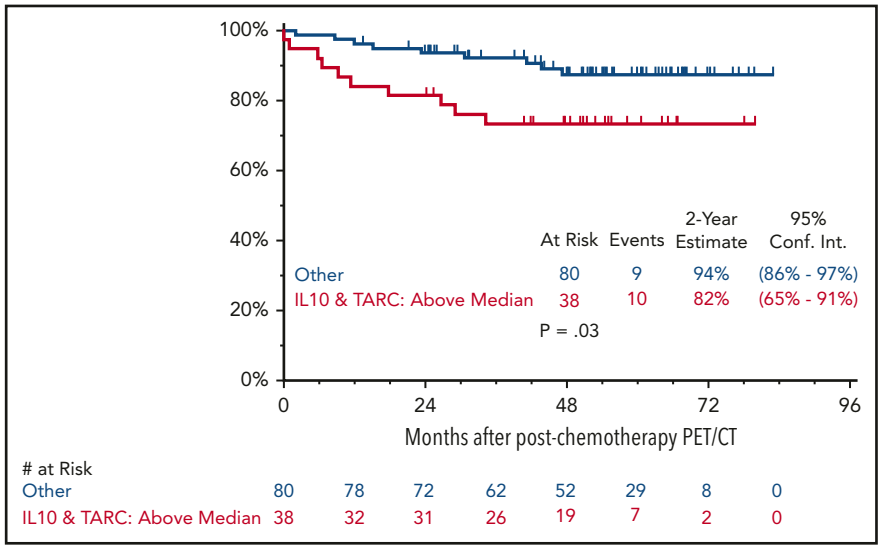

Figure 1. Landmark Kaplan-Meier PFS curves for PET2-negative patients. These curves exclude the 9 patients who were PET3 positive/progressed at time of PET3 or progressed shortly after PET3 assessment using the combined median posttherapy TARC (502.86 pg/mL) and IL-10 (0.2703 pg/mL) levels for these patients as cutoffs, respectively (log-rank test, $P = .03$). CT, computed tomography.

identified posttherapy TARC as the best model for PFS prediction with a similar HR (2.2; 95% CI, 1.59-2.94; $P < .0001$).

Nine patients who were PET3 positive (end of therapy time point) were marked as progressed at the time or shortly after PET3 assessment (all had high TARC levels above the median). In a subset analysis for PFS (too few events were present for OS analysis), we excluded these patients and evaluated the ability of serum biomarker levels to identify poor-prognosis patients. Posttherapy TARC levels were not significant for PFS in a multivariable Cox regression model that considered IL-10, MDC, and TARC (HR, 1.4; 95% CI, 0.87-2.40; $P = .158$). Stepwise selection identified the best prognostic model for PFS included TARC only (HR, 1.5; 95% CI, 1.01-2.37; $P = .0475$). Posttherapy TARC was split at the median, and it was not associated with PFS (2-sided log-rank test $P = .18$), supplemental Figure 2. Given their importance in all patients, posttherapy TARC and IL-10 levels were split at the medians for this time point, and higher levels were associated with shorter PFS when comparing patients with both posttherapy TARC and IL-10 levels above the median to the remaining patients (Figure 1).

End-of-therapy IL-10, TARC, and MDC levels appeared to identify patients with shorter survivals. TARC and, in particular, IL-10 appear of interest, as they remained significant upon multivariable analysis for PFS. Weihrauch and colleagues also found that posttherapy, but not baseline, TARC levels were associated with poor survival.[9] Thus, these 2 markers appear to be of value in identifying patients at risk of relapse after initial risk-adapted therapy. Validation of these findings is required before they should be used in practice.

In this trial, PET2-negative patients had an 82% 2-year PFS, which is arguably lower than anticipated.[22] Identifying risk factors of progression in this presumed favorable group of patients would be desirable. When also accounting for PET3 results, TARC and IL-10 appeared to be prognostic in an exploratory analysis.

EBV status correlated with sCD163, IL-10, and TARC levels. The data for IL-10 are consistent prior studies relating EBV positivity to increased IL-10.[23,24] A prior study showed no relationship between EBV status and sCD163 and TARC levels.[6] However, within EBV-positive cases, plasma EBV DNA did correlate with sCD163.[6] Since EBV status was not associated with outcome, the significance of our observations is uncertain.

Recent attempts to validate or translate gene expression signatures findings into prognostic biomarkers in cHL have not been successful.[19,20] We have provided prospective multicenter evidence that posttreatment serum TARC and IL-10 levels provide important prognostic information in patients with cHL. Future trials incorporating risk-adapted designs should investigate the role of these biomarkers.

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Authorship

Contribution: E.D.H., H.L., A.B.N., H.S., N.L.B., M.L., J.P.L., A.M.E., D.W.S., and J.W.F. designed research, performed research, analyzed data, and wrote the paper; and S.S., B.S.K., and L.M.R. designed research, performed research, and wrote the paper.

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Footnotes

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