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Elevated Pretreatment Serum Levels of Interferon-inducible Protein-10 (CXCL10) Predict Disease Relapse and Prognosis in Diffuse Large B-Cell Lymphoma Patients

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Abstract

While standard clinical prognostic factors predict outcome in diffuse large B-cell lymphoma (DLBCL), predicting the outcome of patients might be further refined using biological factors. We tested whether serum cytokines could provide prognostic information in DLBCL patients. Thirty cytokines were measured in pre-treatment samples from newly diagnosed DLBCL patients using a multiplex ELISA. Sixty-nine patients treated with R-CHOP plus epratuzumab were used in an initial cohort and 185 patients treated with standard R-CHOP served as a subsequent validation cohort. In the initial cohort, elevated serum IL-10 (interleukin-10; HR=6.6, p=0.022), GM-CSF (granulocyte macrophage colony-stimulating factor; HR=10.8, p=0.027) and IP-10 (interferon-inducible protein-10, CXCL10; HR=3.32, p=0.015) were associated with event-free survival (EFS). An identical analysis of the subsequent validation cohort confirmed that elevated serum levels of IP-10 were strongly associated with a poor EFS (HR=2.42, p= 0.0007); and also identified IL-8 (interleukin-8; HR=3.40, p= 0.00002) and IL-2R (interleukin-2 receptor, CD25; HR=2.59, p= 0.0012) as significantly associated with prognosis. The prognostic significance of elevated IP-10 remained significant after adjustment for the International Prognostic Index (IPI; EFS – HR 1.99, p=0.009, overall survival- HR 1.93, p=0.021). Elevated pretreatment serum IP-10 levels are therefore associated with an increased likelihood of disease relapse and an inferior survival in patients with DLBCL.

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AUTHORSHIP CONTRIBUTIONS

SMA designed the study, enrolled the patients, participated in the data analysis and data interpretation, and wrote the manuscript. MJM, SLS, JRC and AJN participated in the data analysis and data interpretation, and participated in writing the manuscript. SCZ performed the assays and participated in writing the manuscript. TMH, BKL and TEW enrolled the patients, participated in the data analysis and data interpretation, and participated in writing the manuscript. WRW and AD reviewed the pathology, participated in the data analysis and data interpretation, and participated in writing the manuscript.

DISCLOSURE OF CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

Keywords

IP-10; CXCL10; cytokines; diffuse large B-cell lymphoma; prognosis

INTRODUCTION

The outcome of patients with DLBCL has significantly improved with the addition of rituximab to CHOP chemotherapy, resulting in R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) becoming the standard initial therapy for newly diagnosed patients.[1] The mean overall survival for R-CHOP treated DLBCL patients at five years in the United States is currently 66.8%, [2] suggesting that a significant number of patients have an excellent outcome with current standard therapy. In contrast, a substantial subset of patients do not achieve a complete remission with initial R-CHOP therapy or relapse shortly after completion of therapy. These patients commonly have a poor long-term outcome.[3, 4] It is therefore important to identify these patient groups at diagnosis, so that novel treatment approaches can be applied to high-risk patients while sparing patients with a good prognosis from the increased risk of toxicity resulting from unnecessary additional treatment. While the standard clinical prognostic factors such as those included in the International Prognostic Index (IPI)[5] continue to predict outcome in R-CHOP treated DLBCL patients, this prediction is incomplete and provides little insight into underlying biologic mechanisms driving outcome.

Cytokines and chemokines are secreted proteins that play a vital role in regulating the normal immune system.[6-9] They control lymphoid cell development, differentiation and migration; regulate the balance between the T-cell subsets including Th1, Th2 and Th17 cells; and support B-cell development, growth, and viability.[6, 9-11] Cytokines and chemokines also play a role in cancer etiology[12, 13] and progression,[14, 15] as well as the host response to the tumor.[16] In lymphoma, previous studies have shown that cytokines measured before disease onset are associated with the subsequent risk of disease development[17, 18] and they also predict disease progression and patient outcome.[19-21] However, no studies have comprehensively measured multiple cytokines simultaneously. We therefore tested whether a panel of 30 serum cytokines could predict prognosis in newly diagnosed DLBCL patients treated with chemo-immunotherapy.

MATERIAL AND METHODS

Study Populations

Participants in the studies provided written informed consent, and the studies were reviewed and approved by the Human Subjects Institutional Review Board at the Mayo Clinic and/or the University of Iowa. Patients in the initial dataset were from the North Central Cancer Treatment Group clinical trial N0489 (NCT00301821), a phase II trial of epratuzumab and rituximab plus CHOP chemotherapy for patients with newly diagnosed untreated DLBCL. Patients in the subsequent validation dataset were from the University of Iowa/Mayo Clinic SPORE Molecular Epidemiology Resource (MER). Briefly, newly diagnosed lymphoma patients were prospectively enrolled into the MER and the diagnosis was confirmed by the study hematopathologist (WRM) in all cases. Patients selected for this analysis had DLBCL, an available pre-treatment serum sample, and initial treatment with R-CHOP chemotherapy given in 21-day cycles. The schedule of laboratory and radiologic tests and follow up was similar between the two cohorts but was not identical as one cohort included patients treated on a clinical trial and the other included patients treated in a community setting.

As a reference population, we used clinic-based controls from an ongoing case-control study of lymphoma.[22] Controls were selected from patients visiting the Mayo Clinic Department of Medicine for a pre-scheduled general medical examination, and eligibility requirements were age 20 years or older and a resident of Minnesota, Iowa or Wisconsin; patients were excluded if they had prior diagnoses of lymphoma, leukemia, or HIV infection. Controls were randomly selected and frequency matched to the patients by 5-year age group, gender, and distance from Rochester, Minnesota. All controls were enrolled over the same timeframe as the patients.

Serum Cytokine Measurements

Serum cytokines were measured from pre-treatment blood draws using a multiplex ELISA (Invitrogen, Camarillo, CA). Thirty cytokines, including pro-inflammatory, Th1 and Th2 associated cytokines, were analyzed using the Luminex-200 system Version 1.7 (Luminex, Austin, TX). The following cytokines were measured – EGF (epidermal growth factor), eotaxin, FGFb (basic fibroblast growth factor), GMCSF (granulocyte-macrophage colony-stimulating factor), GCSF (granulocyte colony stimulating factor), HGF (hepatocyte growth factor), IFN α (interferon alpha), IFN γ (interferon gamma), IL-2 (interleukin-2), IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-15, IL-17, IL-12p40, IL-2R (interleukin-2 receptor), IL-1RA (interleukin-1 receptor antagonist), IL-1 β (interleukin-1 beta), MCP-1 (monocyte chemotactic protein 1), IP-10 (inducible protein 10/CXCL10), MIG (monokine induced by interferon γ /CXCL9), MIP-1 α (CCL3), MIP-1b (CCL4), RANTES (regulated on activation, normal T-cell expressed and secreted/CCL5), TNF α (tumor necrosis factor alpha) and VEGF (vascular endothelial growth factor). Data were acquired using STar Station software (Applied Cytometry, Dinnington, Sheffield, UK) and analysis was performed using the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA). Cytokines measured below the limit of detection were assigned a value of (limit of detection / 2). Inter-assay variation was assessed by inclusion of an internal control serum on all assay plated; the CV for the internal control log2 cytokine values across plates ranged from 0.3% (RANTES) to 12.2% (IL-6). To further control for any variation in the cytokine results due to lot-to-lot variability of the ELISA plates used, separate control specimens were used for each of the cohorts.

Statistical Analysis

The primary analysis was to assess the association of cytokine abnormalities with event-free survival (EFS) and overall survival (OS) for DLBCL. This analysis was first performed on 69 patients treated with ER-CHOP in a clinical trial and an identical analysis was performed in a second cohort of 185 community patients treated with R-CHOP to confirm the results. Each cytokine was examined as a normal vs. elevated dichotomous variable. The upper limit of normal for each cytokine was defined as the 95th percentile from the distribution of the reference population. EFS was defined as the time from diagnosis to disease progression, retreatment, or death due to any cause. OS was defined as the time from diagnosis to death due to any cause. Patients without an event or death were censored at time of last known follow-up. Cox proportional hazards regression models were used to assess the association of individual cytokines and the cytokine profiles with outcome via unadjusted and IPI-adjusted hazard ratios (HR). Kaplan-Meier curves were used to graphically display the association of cytokines with outcome. Analyses were performed using SAS v9.1.3.

RESULTS

Univariate analysis of single cytokines as prognostic factors for DLBCL - Initial cohort

The initial cohort consisted of patients enrolled on a cooperative group trial of epratuzumab in combination with R-CHOP who had pre-treatment sera available for use. One hundred and seven patients were enrolled in the trial and serum was available on 69 patients. The

patient characteristics are shown in Table 1. The median age of these patients was 62 years and 40% were female. The majority of patients (80%) had advanced disease and 46% were low or low-intermediate risk by the IPI. The medium follow-up was 43 months and the EFS and OS at 3 years was 70% and 80% respectively. In a univariate analysis, each of the cytokines was analyzed as a prognostic factor for EFS. When considered as a categorical variable, 10 cytokines were elevated, as defined by greater than the 95th percentile observed in the control population (n=50). The most commonly elevated cytokines were MIG (74%), IL-2R (73%), IL-1Ra (71%), IL-12p40 (42%), and IP-10 (33%). Cytokine elevation (yes vs. no) was associated with EFS at the $p < 0.05$ in 3 cytokines (Table 2), with the association observed in IL-10 (HR=10.8, $p=0.027$), GM-CSF (HR=10.8, $p=0.027$), and IP-10 (HR=3.32, $p=0.015$).

Univariate analysis of single cytokines as prognostic factors for DLBCL – Validation cohort

To validate results from the initial study in an independent cohort, we performed a similar analysis and measured pre-treatment cytokine levels in 185 patients treated off-protocol with R-CHOP during the same period as patients were enrolled in the clinical trial. This group served as a subsequent validation cohort and their clinical characteristics are shown in Table 1. The median age of the patients in this validation cohort was 63 years and both genders were equally represented. The majority of patients (61%) had advanced disease and 60% were low or low-intermediate risk by the IPI. At a medium follow-up of 47 months (range 5-94), there were 62 (34%) events and 50 deaths (27%). In an identical analysis, each of the cytokines was analyzed as a prognostic factor for EFS as a categorical variable. When considered as a categorical variable, 15 cytokines were elevated, as defined by greater than the 95th percentile observed in the control population (n=400). The most commonly elevated cytokines were IL-2R (56%), MIG (48%), IP-10 (31%), and IL-12p40 (27%). Cytokine elevation (yes vs. no) was most significantly associated with EFS with IL-8 (HR=3.40, $p=0.000015$), IL-2R (HR=2.59, $p=0.0012$), and IP-10 (HR=2.42, $p=0.00070$). Of note, IL-8 was elevated in 21% of patients in this cohort but was not elevated in patients in the initial cohort. This may be accounted for by the fact that patients treated with ER-CHOP in the initial cohort were on a clinical trial that required a good performance score (ECOG PS 0-1) and normal lab results. In contrast, patients treated with R-CHOP in this subsequent validation cohort were “all comers” and included patients with poorer performance scores and abnormal lab values. IL-8 may therefore be associated with host factors rather than lymphoma-associated factors. The only cytokine to be significantly associated with EFS in both the initial and subsequent validation cohorts was IP-10 (Table 2). Dot plots of the serum levels of IP-10 in both cohorts are shown in Figure 1 and the association of IP-10 with EFS is shown in Figure 2a.

Association between IP-10 Levels and clinical characteristics of DLBCL patients

When increased IP-10 levels were correlated with clinical features of the 185 patients in the R-CHOP treated cohort, the following significant associations were identified. Increased levels of IP-10 were associated with the presence of B-symptoms ($p=0.002$), bulky disease (tumor mass with a diameter greater than 10 cm, $p=0.0009$) and elevated serum LDH levels ($p=0.001$). IP-10 levels were further compared to other laboratory parameters that have previously been shown to be of prognostic significance. In patients in whom the histologic subtype - germinal center type (GCB) versus non-germinal center type (non-GCB) - had been determined by immunohistochemistry using the Hans algorithm,[23] IP-10 was associated with the GCB subtype ($p=0.047$, $n=77$). IP-10 levels were also significantly associated with elevated pretreatment serum free light chains ($p=0.0026$, $n=185$)[24] and with vitamin D insufficiency ($p=0.0014$, $n=185$).[25] There was no significant association with a decreased pretreatment absolute lymphocyte count ($p=0.077$, $n=170$).[26]

IP-10 levels and Prognosis

Because serum levels of IP-10 were significantly associated with EFS in both cohorts, serum levels of IP-10 were also correlated with OS using the 185 patients in the second R-CHOP treated validation cohort. Compared to patients with normal IP-10 levels, patients with increased serum IP-10 had significantly shorter OS (HR=2.38, $p=0.002$; Figure 2b). Adjustment for the IPI only modestly attenuated the results for EFS (HR=1.99, $p=0.009$) and OS (HR=1.93, $p=0.021$). Importantly, when only low-risk patients (IPI 0-1) were considered, a normal serum level of IP-10 was associated with an excellent outcome. In this group of patients, only 6% of the patients with normal pretreatment IP-10 serum levels relapsed in the first 12 months compared to 27% of patients with elevated IP-10 levels. At a median follow-up of 4 years, only 13% with normal serum IP-10 levels relapsed compared to an event rate of 43% in patients with elevated IP-10 (Figure 2c). Similarly, among the high-risk patients (IPI 2-5), only 11% with normal IP-10 relapsed in the first year compared to an event rate of 36% in the elevated IP-10 group. At 4 years, 37% of patients with a normal pretreatment IP-10 level had progressed compared to 50% of patients with elevated IP-10 (Figure 2d).

DISCUSSION

IP-10 is an inflammatory mediator that belongs to the family of C-X-C chemokines. IP-10 binds to the CXCR3 receptor to induce chemotaxis, apoptosis, cell growth, and also to inhibit angiogenesis.[27, 28] The results of this study show that increased serum levels of IP-10, which might have been expected to modulate the antitumor inflammatory response, are actually associated with a poor prognosis. While a well-coordinated anti-tumor inflammatory response involving upregulation of chemokines such as IP-10 might lead to tumor clearance,[29] chronic inflammation, also mediated by IP-10, can lead to the release of growth and survival factors, promote lymphangiogenesis, facilitate tumor cell invasion, and contribute to evasion of host defense mechanisms.[30] Alterations in IP-10 expression levels have in fact been associated with infectious diseases, immune dysfunction, and tumor development. IP-10 is recognized as a biomarker for various lymphoid malignancies including Hodgkin lymphoma[28] and nasal NK/T cell lymphoma.[27]

In this study, using two independent cohorts, we found that elevated pretreatment serum levels of circulating IP-10 were the dominant predictor of EFS and OS in DLBCL patients in unadjusted models, and the association was also seen following adjustment for clinical factors as defined by the IPI. While both groups received the same chemotherapy, they received different immunotherapy that could influence patient outcome. IP-10 however, demonstrated prognostic importance in both cohorts regardless of the therapy given. Elevated IP-10 levels were also associated with other clinical and laboratory features including the presence of B-symptoms, elevated serum free light chains, and vitamin D insufficiency. However, elevated IP-10 levels were not associated the age of the patient, stage of disease, number of extranodal sites, or bone marrow involvement. To determine whether the activation phenotype of the malignant B-cell in DLBCL accounted for the increased expression of IP-10, we determined whether a germinal center B-cell (GCB) versus non-GCB phenotype was associated with IP-10 levels and found that increased IP-10 levels were associated with a GCB phenotype, suggesting that increased IP-10 expression is not simply evidence of lymphocyte activation.

Rather than merely defining a prognostic marker that correlates with advanced, aggressive disease, and a poor outcome in patients with known high-risk disease, we also tested whether the serum IP-10 levels could identify poor-risk patients with an increased risk of early relapse among those who would typically be expected to do well. The majority of patients who fail R-CHOP treatment do so within the first 2 years[1] and early relapse

(within 18 months) after initial therapy impacts the outcome of patients undergoing stem cell transplantation even if they respond to salvage therapy.[4] We therefore specifically focused on prognostic impact of serum IP-10 levels in patients who are low-risk by the IPI and who typically have a good prognosis when treated with standard therapy such as R-CHOP. We found that elevated serum IP-10 identified patients at significant risk for non-response or early relapse when treated with R-CHOP. When low-risk patients (IPI 0-1) were considered, 6% of the patients with normal IP-10 levels relapsed in the first 12 months compared to 27% of patients with an elevated serum IP-10. With longer follow up, 13% of patients with normal serum IP-10 relapsed at a median follow-up of 4 years, compared to an event rate of 43% in patients with elevated IP-10. This would suggest that low-risk DLBCL patients, with normal IP-10 levels, treated with R-CHOP have an excellent outcome and clinical trials exploring less therapy for this patient group could be considered. On the contrary, low-risk DLBCL with elevated serum IP-10 are at increased risk for non-response or progression and may require additional therapy beyond R-CHOP. Clinical trials investigating new drugs added to R-CHOP, including those that inhibit cytokine and chemokine production, might be appropriate for this higher-risk patient group.

In this study, we have shown that elevated pretreatment serum IP-10 levels are associated with an increased likelihood of disease relapse and an inferior survival in patients with DLBCL. The prognostic significance of IP-10 was found to be relevant in both low and high-risk groups as defined by the IPI. Of particular importance, an elevated serum IP-10 level identifies a subgroup of clinically low-risk patients who are more likely to progress despite standard therapy. Future studies will need to identify the mechanisms that account for the elevated cytokine levels and strategies that decrease cytokine production may result in improved outcomes for DLBCL patients.

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References

1. Coiffier B, Thieblemont C, Van Den Neste E, et al. Long-term outcome of patients in the LNH-98.5 trial, the first randomized study comparing rituximab-CHOP to standard CHOP chemotherapy in DLBCL patients: a study by the Groupe d'Etudes des Lymphomes de l'Adulte. *Blood*. 2010; 116:2040–2045. [PubMed: 20548096]
2. Pulte D, Gonds A, Brenner H. Ongoing improvement in outcomes for patients diagnosed as having Non-Hodgkin lymphoma from the 1990s to the early 21st century. *Arch Intern Med*. 2008; 168:469–476. [PubMed: 18332290]
3. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010; 28:4184–4190. [PubMed: 20660832]
4. Costa LJ, Micallef IN, Inwards DJ, et al. Time of relapse after initial therapy significantly adds to the prognostic value of the IPI-R in patients with relapsed DLBCL undergoing autologous stem cell transplantation. *Bone Marrow Transplant*. 2008; 41:715–720. [PubMed: 18195687]
5. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med*. 1993; 329:987–994. [PubMed: 8141877]
6. Acosta-Rodriguez EV, Merino MC, Montes CL, et al. Cytokines and chemokines shaping the B-cell compartment. *Cytokine Growth Factor Rev*. 2007; 18:73–83. [PubMed: 17336579]
7. Broxmeyer HE. Chemokines in hematopoiesis. *Curr Opin Hematol*. 2008; 15:49–58. [PubMed: 18043246]
8. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med*. 2006; 354:610–621. [PubMed: 16467548]

9. Schaerli P, Moser B. Chemokines: control of primary and memory T-cell traffic. *Immunol Res.* 2005; 31:57–74. [PubMed: 15591623]
10. Ebert LM, Schaerli P, Moser B. Chemokine-mediated control of T cell traffic in lymphoid and peripheral tissues. *Mol Immunol.* 2005; 42:799–809. [PubMed: 15829268]
11. Zwirner NW, Domaica CI. Cytokine regulation of natural killer cell effector functions. *Biofactors.* 2010; 36:274–288. [PubMed: 20623510]
12. Balkwill F. Chemokine biology in cancer. *Semin Immunol.* 2003; 15:49–55. [PubMed: 12495640]
13. Germano G, Allavena P, Mantovani A. Cytokines as a key component of cancer-related inflammation. *Cytokine.* 2008; 43:374–379. [PubMed: 18701317]
14. Kakinuma T, Hwang ST. Chemokines, chemokine receptors, and cancer metastasis. *J Leukoc Biol.* 2006; 79:639–651. [PubMed: 16478915]
15. Raman D, Baugher PJ, Thu YM, et al. Role of chemokines in tumor growth. *Cancer Lett.* 2007; 256:137–165. [PubMed: 17629396]
16. Johrer K, Pleyer L, Olivier A, et al. Tumour-immune cell interactions modulated by chemokines. *Expert Opin Biol Ther.* 2008; 8:269–290. [PubMed: 18294099]
17. Gu Y, Shore RE, Arslan AA, et al. Circulating cytokines and risk of B-cell non-Hodgkin lymphoma: a prospective study. *Cancer Causes Control.* 2010; 21:1323–1333. [PubMed: 20373009]
18. Saberi Hosnijeh F, Krop EJ, Scoccianti C, et al. Plasma cytokines and future risk of non-Hodgkin lymphoma (NHL): a case-control study nested in the Italian European Prospective Investigation into Cancer and Nutrition. *Cancer Epidemiol Biomarkers Prev.* 2010; 19:1577–1584. [PubMed: 20501772]
19. Duletic-Nacinovic A, Stifter S, Marijic B, et al. Serum IL-6, IL-8, IL-10 and beta2-microglobulin in association with International Prognostic Index in diffuse large B cell lymphoma. *Tumori.* 2008; 94:511–517. [PubMed: 18822687]
20. Fabre-Guillevin E, Tabrizi R, Coulon V, et al. Aggressive non-Hodgkin's lymphoma: concomitant evaluation of interleukin-2, soluble interleukin-2 receptor, interleukin-4, interleukin-6, interleukin-10 and correlation with outcome. *Leuk Lymphoma.* 2006; 47:603–611. [PubMed: 16690518]
21. Stasi R, Zinzani L, Galieni P, et al. Detection of soluble interleukin-2 receptor and interleukin-10 in the serum of patients with aggressive non-Hodgkin's lymphoma. Identification of a subset at high risk of treatment failure. *Cancer.* 1994; 74:1792–1800. [PubMed: 8082082]
22. Cerhan JR, Fredericksen ZS, Wang AH, et al. Design and validity of a clinic-based case-control study on the molecular epidemiology of lymphoma. *Int J Mol Epidemiol Genet.* 2011; 2:95–113. [PubMed: 21686124]
23. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood.* 2004; 103:275–282. [PubMed: 14504078]
24. Maurer MJ, Micallef IN, Cerhan JR, et al. Elevated serum free light chains are associated with event-free and overall survival in two independent cohorts of patients with diffuse large B-cell lymphoma. *J Clin Oncol.* 2011; 29:1620–1626. [PubMed: 21383282]
25. Drake MT, Maurer MJ, Link BK, et al. Vitamin D insufficiency and prognosis in non-Hodgkin's lymphoma. *J Clin Oncol.* 2010; 28:4191–4198. [PubMed: 20713849]
26. Wilcox RA, Ristow K, Habermann TM, et al. The absolute monocyte and lymphocyte prognostic score predicts survival and identifies high-risk patients in diffuse large-B-cell lymphoma. *Leukemia.* 2011; 25:1502–1509. [PubMed: 21606957]
27. Moriai S, Takahara M, Ogino T, et al. Production of interferon- γ -inducible protein-10 and its role as an autocrine invasion factor in nasal natural killer/T-cell lymphoma cells. *Clin Cancer Res.* 2009; 15:6771–6779. [PubMed: 19887486]
28. Teichmann M, Meyer B, Beck A, et al. Expression of the interferon-inducible chemokine IP-10 (CXCL10), a chemokine with proposed anti-neoplastic functions, in Hodgkin lymphoma and nasopharyngeal carcinoma. *J Pathol.* 2005; 206:68–75. [PubMed: 15751051]
29. Haabeth OA, Lørvik KB, Hammarström C, et al. Inflammation driven by tumour-specific Th1 cells protects against B-cell cancer. *Nat Commun.* 2011; 2:240. [PubMed: 21407206]

30. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002; 420:860–867. [PubMed: 12490959]

Figure 1. Dot plots of pretreatment IP-10 serum levels in diffuse large B-cell lymphoma patients and normal controls in A) Initial ER-CHOP cohort and B) Subsequent R-CHOP Validation Cohort.

Figure 2.

Outcome of patients with diffuse large B-cell lymphoma (DLBCL) by serum IP-10 levels. (A) Event free survival of all patients by serum IP-10 levels (n=185), (B) Overall survival of all patients by serum IP-10 levels (n=185), (C) Event free survival of low risk (IPI 0-1) patients by serum IP-10 levels (n=65), (D) Event free survival in intermediate and high risk (IPI 2-5) patients by serum IP-10 levels (n=120).

TABLE 1

Patient characteristics

	ER-CHOP Cohort N=69		R-CHOP Cohort N=185	
	N	%	N	%
Age, Median (range)	62 (21-82)		63 (21-92)	
Male	41	59.4	96	51.9
Age > 60	38	55.1	111	60.0
PS 2+	9	13.0	27	14.6
Ann Arbor Stage III/IV	55	79.7	112	60.9
2+ Extranodal Sites	21	30.4	42	23.1
LDH > ULN	46	66.7	91	50.3
IPI				
0-1	17	24.6	65	35.1
2	16	21.7	47	25.4
3	27	39.1	48	26.0
4-5	9	13.0	25	13.5
B Symptoms	28	40.6	38	20.5
Bulky Disease	11	15.9	25	13.6
Immunochemotherapy	69	100	185	100

PS – performance status; LDH – lactate dehydrogenase; ULN – upper limit of normal; IPI – International Prognostic Index

Cytokines associated with Event-Free Survival in the Initial ER-CHOP treated cohort and the Subsequent R-CHOP treated Validation cohort

TABLE 2

Cytokine	ER-CHOP Cohort					R-CHOP Cohort				
	% Elevated	HR*	95% CI	P Value	% Elevated	HR*	95% CI	P Value		
IP-10	33.3	3.32	1.26-8.73	0.015	30.8	2.42	1.45-4.05	0.001		
IL-10	11.6	6.62	1.20-29.1	0.022	14.1	1.50	0.76-2.97	0.243		
GM-CSF	5.8	10.82	1.30-89.9	0.027	5.9	0.92	0.29-2.93	0.882		
IL-8	0	NA	NA	NA	21.1	3.40	1.95-5.92	0.001		
IL-2R	72.5	1.21	0.39-3.70	0.744	55.7	2.59	1.46-4.60	0.001		

* HR is elevated vs. non-elevated cytokine
NA- not applicable