Elevated IL-10 plasma levels correlate with poor prognosis in diffuse large B-cell lymphoma

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ABSTRACT. The aim of the study was to confirm whether plasma levels of interleukin-10 (IL-10) correlate with the prognosis in diffuse, large B-cell lymphoma (DLBCL) patients. Plasma IL-10 levels were determined at the time of diagnosis in a group of 157 consecutively treated, DLBCL patients. Of those, 122 patients (78%) had IL-10 plasma levels below the detection limit (< 5 pg/mL) and 35 (22%) above this value. The median value for patients with detectable IL-10 levels was 35 pg/mL (range, 5 to 2480 pg/mL). Detectable plasma IL-10 levels were significantly associated with age > 60 years, ECOG performance status ≥ 2, Ann Arbor advanced disease stage, bulky tumor mass, elevated serum levels of LDH and β2-microglobulin, presence of anemia and low serum albumin levels as well as the presence of B symptoms. The patients with detectable IL-10 levels had lower probability of CR achievement (OR = 0.23, 95% CI 0.1-0.5, p = 0.0003). In addition, detectable IL-10 levels were significantly associated with shorter PFS (OR = 2.5, 95% CI 1.5-4.4, p = 0.001) and OS (OR = 3.0, 95% CI 1.7-5.2, p = 0.0001). In conclusion, we confirmed in this large group of DLBCL patients that elevated plasma IL-10 levels correlated with adverse disease features and poor prognosis. The plasma concentration of IL-10 may be a useful marker for evaluation of disease activity.

Keywords: interleukin-10, lymphoma, International Prognostic Index

Interleukin-10 (IL-10) is a pleiotropic, immunomodulatory cytokine that plays a crucial role in the ontogenesis and functioning of the immune system. Although generally considered as an immunosuppressive molecule, IL-10 has immunostimulatory properties in several *in vitro* and *in vivo* models. IL-10, as a modulator of adaptive immunity, suppresses Th1 and Th2 type immune responses, but conversely, favors humoral immunity and cytotoxic T lymphocyte (CTL) function [1].

It has been suggested that immune system alterations may be linked to the incidence and clinical course of lymphomas. Numerous studies have shown that IL-10 may be involved in the pathogenesis of lymphoid disorders [1-5]. This cytokine is produced mainly by monocytes and macrophages, B and T cells, but it is also secreted by neoplastic B lymphocytes [1, 2]. In addition, IL-10 may increase bcl-2 expression and protect malignant cells from apoptosis [5]. Elevated IL-10 levels have been found in the plasma of patients with non-Hodgkin's lymphoma (NHL). However, conflicting findings regarding the correlation between cytokine levels and lymphoma prognosis have been published [6-9].

In the present study, we investigated if pretreatment, plasma IL-10 levels correlate with disease features and outcome in a group of 157, consecutively treated patients with diffuse, large B-cell lymphoma (DLBCL).

METHODS

Subjects

The study group consisted of 157 consecutive patients with DLBCL accrued prospectively between 1991 and 1999 and treated with a curative intent at the Department of Hematology of the Centre Hospitalier Lyon-Sud. Plasma samples were taken at the time of diagnosis and were kept at -80 °C. All samples were obtained and coded after informed consent of patients. The study was performed in accordance with the guidelines for studies of human subjects at our institution and French laws.

The initial medical evaluation consisted of a complete history and physical examination, computed tomographic scan of the chest, abdomen and pelvis, blood morphology and chemistry. The extent of the disease was categorized

Table 1
Characteristics of 157 patients with DLBCL and their association with IL-10 serum levels at the time of initial presentation

Characteristics	**Patients with an IL-10 level less than 5 pg/mL, n (%)**	**Patients with an IL-10 level of 5 pg/mL or greater, n (%)**	p[a]
Sex			0.76
Female	65 (79)	17 (21)	
Male	57 (76)	18 (24)	
Age			0.007
60 years or younger	65 (88)	9 (12)	
Older than 60 years	57 (69)	26 (31)	
Performance status (ECOG)			0.001
Less than 2	93 (85)	16 (15)	
2 or more	29 (60)	19 (40)	
B symptoms			< 0.0001
Absent	98 (87)	15 (13)	
Present	24 (54)	20 (45)	
Ann Arbor stage			0.003
I, II	53 (90)	6 (10)	
III, IV	60 (67)	29 (33)	
Unknown	9		
Serum LDH			0.002
1x or less than normal	58 (89)	7 (11)	
Greater than 1x normal	52 (66)	27 (34)	
Unknown	12	1	
Serum β2-microglobulin			< 0.0001
3.0 mg/L or less	83 (87)	12 (13)	
Greater than 3.0 mg/L	23 (50)	23 (50)	
Unknown	16		
Number of extranodal sites			0.3
Less than 2	73 (81)	17 (19)	
2 or more	38 (72)	15 (28)	
Unknown	11	3	
Serum albumin level			0.0001
Greater than 35 g/L	76 (89)	9 (11)	
35 g/L or less	33 (58)	23 (42)	
Unknown	14	2	
Hemoglobin			0.015
Greater than 120 g/L	63 (85)	11 (15)	
120 g/L or less	48 (67)	24 (33)	
Unknown	11		
Bulky tumor (10 cm or greater)			0.03
Absent	69 (84)	13 (16)	
Present	44 (68)	21 (32)	
Unknown	9	1	
International Prognostic Index risk groups			0.0005
Low/intermediate low	66 (54)	8 (23)	
Intermediate high/high	42 (34)	23 (66)	
Unknown	14	4	

[a] The associations were compared with χ^2 test.

according to the Ann Arbor classification, and performance status was assessed using Eastern Cooperative Oncology Group (ECOG) criteria. After completion of the treatment, the patients were followed every 3 months during the first year, then every 6 months for two additional years and yearly thereafter. Clinical characteristics of the patients enrolled in the study are shown in *table 1*. Patients with active bacterial or fungal infection, and those who tested positive for the human immunodeficiency virus, as well as patients with a previous history of autoimmune disease and those who had received recent corticosteroid therapy, were excluded from the analysis.

Treatment

All patients included in this study received anthracyclin-containing regimens, consisting of CHOP (cyclophospha-

mide, adriamycine, vincristine, prednisone) or high-dose CHOP [10] according to age and number of IPI factors, as outlined in GELA prospective trials. No patients received rituximab as a part of the first line treatment. Complete remission (CR) was defined as the disappearance of all disease manifestations, and normalization of all laboratory values. Progression-free survival (PFS) was determined from the onset of treatment until relapse, disease progression, or the last follow-up evaluation. Overall survival (OS) was determined from the onset of treatment until the last follow-up evaluation or death from any cause.

Evaluation of plasma IL-10 levels

Blood samples from 157 newly diagnosed patients were collected before treatment initiation using sterile tubes containing EDTA to prevent further release of cytokine from circulating mononuclear cells. Plasma samples were stored at -80 °C and thawed immediately prior to the determination of IL-10 levels using an human enzyme-linked immunoabsorbent assay (ELISA) (BioSource International, Inc., California, USA). The limit of the test for the quantification of IL-10 levels was 5 pg/mL. In normal controls tested in the laboratory, plasma IL-10 levels were usually undetectable and were always below the limit of 10 pg/mL (unpublished results).

Statistical analysis

Associations between IL-10 levels and clinical or biological variables were assessed using the χ^2 test with Yates's correction when cell frequency was less than 5, unless any expected frequency was less than 20, when Fisher's exact test was used. Logistic regression was used to estimate the influence of various clinical or biological parameters on the CR rate. Survival (PFS and OS) were estimated by the Kaplan-Meier method and compared using the log-rank test. Statistical tests with $p < 0.05$ were considered significant. A multivariate regression analysis with the Cox proportional hazard model was used to adjust the effect of the IL-10 level along with prognostic variables for potential independent factors. Only patients with complete data were entered into the regression procedure (n = 139). Statistical analysis was performed using the Statistica package (StatSoft, Tulsa, OK, USA). Confidence intervals (95%) were calculated.

RESULTS

Plasma IL-10 levels

Plasma IL-10 levels were determined in all 157 DLBCL patients. Of those, 122 patients (78%) had IL-10 plasma levels below the detection limit (< 5 pg/mL) and 35 (22%) above this value. The median value for patients with detectable IL-10 levels was 35 pg/mL (range, 5 to 2480 pg/mL).

Plasma IL-10 levels and other prognostic variables

The clinical prognostic features incorporated in the International Prognostic Index (IPI), as well as other disease features known to correlate with the clinical prognosis of lymphoma, were analyzed in this study.

Detectable plasma IL-10 levels were significantly associated with age > 60 years, ECOG performance status ≥ 2, Ann Arbor advanced disease stage, bulky tumor mass (≥ 10 cm) presence, elevated serum levels of lactic dehydrogenase (LDH) and β2-microglobulin, presence of anemia and low serum albumin levels as well as the presence of B symptoms. IL-10 levels did not correlate with the number of extranodal sites. In addition, IL-10 levels were also associated with low/intermediate low and intermediate high/high risk groups of the IPI *(table 2)*.

Plasma IL-10 levels and other prognostic variables influencing DLBCL outcome

Among the 157 patients, 112 (71%) achieved CR, whereas 45 (29%) did not. Sixty-five patients (41%) experienced disease progression, and 61 (39%) patients died.

The median follow-up for the patients remaining alive was 40.5 months (range: 9-116 months).

We found that detectable plasma IL-10 levels were significantly associated with lower probability of achievement of CR in univariate logistic regression analysis (OR = 0.23, 95% CI 0.1-0.5, p = 0.0003). Other potential prognostic factors that showed an adverse impact on CR in univariate analysis were age > 60 years ($p < 0.0001$), ECOG status ≥ 2 ($p < 0.001$), Ann Arbor advanced disease stage ($p < 0.001$), elevated LDH levels ($p < 0.001$), low/intermediate low and intermediate high/high IPI risk groups ($p < 0.0001$), elevated β2-microglobulin levels ($p < 0.0001$), anemia (p = 0.025), low serum albumin levels (p = 0.001), the presence of B symptoms ($p < 0.0001$), bulky tumor mass (p = 0.03), number of extranodal sites ≥ 2 (p = 0.048). However, only age > 60 years retained its independent prognostic value in multivariate logistic regression analysis (OR = 0.17, 95% CI 0.03-0.9, p = 0.036).

The prognostic parameters associated with shorter PFS and OS by univariate analysis are listed in *table 2*. Importantly, elevated IL-10 plasma levels were significantly associated with shorter PFS and OS (p = 0.001 and p = 0.0001, respectively) *(figure 1)*.

In a multivariate Cox regression model, after incorporating all variables that were significant in univariate analysis, only elevated serum β2-microglobulin levels (HR = 2.9, 95% CI 1.4-6.0, p = 0.005) and age > 60 years (HR = 2.0, 95% CI 1.0-4.0, p = 0.054) predicted shorter PFS. Similarly, elevated β2-microglobulin levels (HR = 2.2, 95% CI 1.0-4.6, p = 0.036) and age > 60 years (HR = 2.5, 95% CI 1.2-5.1, p = 0.012) retained their independent impact on shorter OS. When we entered in the Cox model IL-10 levels and IPI risk groups, which are known to be the most significant predictors of aggressive lymphoma outcome, detectable IL-10 levels (HR = 2.1, 95% CI 1.2-3.8, p = 0.012) and intermediate high/high IPI risk groups (HR = 3.4, 95% CI 1.8-6.4, $p < 0.0001$) were found to be independent variables predictive of shorter OS. Using the same variable for the analysis of progression-free survival, intermediate high/high IPI risk groups retained their independent impact on shorter PFS (HR = 2.7, 95% CI 1.5-5.0, p = 0.001), whereas a trend was observed for detectable IL-10 levels and a shorter PFS (HR = 1.8, 95% CI 1.0-3.2, p = 0.061).

Table 2
Univariate analysis of prognostic factors influencing progression-free survival (PFS) and overall survival (OS)

		PFS			OS	
Variable	***n***	***Risk (OR)***	***95.0% CI***	***p***[a]	***Risk (OR)***	***95.0% CI***	***p***[a]
Sex			0.5-1.3	0.48		0.7-2.0	0.48
Female	82	1.0			1.0		
Male	75	0.83			1.21		
Age			1.8-5.8	< 0.0001		1.6-5.0	0.0005
60 years or younger	74	1.0			1.0		
Older than 60 years	83	3.2			2.8		
Performance status			1.5-4.2	< 0.0001		1.6-4.6	0.0002
Less than 2	109	1.0			1.0		
2 or more	48	2.5			2.7		
B symptoms			1.4-3.9	0.002		1.6-4.7	< 0.0001
Absent	113	1.0			1.0		
Present	44	2.3			2.8		
Ann Arbor stage			1.2-4.0	0.008		1.7-6.1	0.0004
I, II	59	1.0			1.0		
III, IV	89	2.2			3.2		
Serum LDH			1.1-3.3	0.02		1.7-5.8	0.0002
1x or less than normal	65	1.0			1.0		
Greater than 1x normal	79	1.9			3.1		
Serum β2-microglobulin			2.4-7.3	< 0.0001		2.6-8.0	< 0.0001
3.0 mg/L or less	95	1.0			1.0		
Greater than 3.0 mg/L	46	4.2			4.6		
Number of extranodal sites			0.7-2.3	0.35		0.9-2.7	0.1
Less than 2	90	1.0			1.0		
2 or more	53	1.3			1.6		
Serum albumin level			1.1-3.4	0.01		1.2-3.6	0.01
Greater than 35 g/L	85	1.0			1.0		
35 g/L or less	56	2.0			2.1		
Hemoglobin			1.0-2.8	0.06		1.4-4.2	0.003
Greater than 120 g/L	74	1.0			1.0		
120 g/L or less	72	1.6			2.4		
Bulky tumor			1.2-3.4	0.01		1.3-3.9	0.004
Absent	82	1.0			1.0		
Present	65	2.0			2.3		
IL-10			1.5-4.4	0.001		1.7-5.2	< 0.0001
Less than 5 pg/mL	122	1.0			1.0		
5 pg/mL or greater	35	2.5			3.0		
IPI risk groups			1.8-5.6	< 0.0001		2.3-7.5	< 0.0001
Low/intermediate low	74	1.0			1.0		
Intermediate high/high	65	3.2			4.1		

[a] The associations were compared with log-rank test; OR: odds ratio; CI: confidence interval.

DISCUSSION

In this study, we have validated the predictive value of plasma IL-10 levels in the cohort of uniformly treated, 157 DLBCL patients. Two major points can be concluded from this study. Elevated plasma IL-10 levels were observed in 22% of lymphoma patients at the time of diagnosis. These high IL-10 levels were more frequently found in elderly patients and were significantly associated with extended lymphoma disease (Ann Arbor stage > II, bulky tumor mass, elevated LDH and β2-microglobulin levels), or with an alteration of the host status possibly reflecting the host-tumour relationship (poor performance status, presence of B symptoms, low haemoglobin and albumin levels). The association of plasma levels of IL-10 with factors reflecting the host-tumour relationship can be explained by the immune response to the presence of lymphoma and the production of IL-10 by reactive bystander cells [1, 2]. It can be argued that high plasma levels of IL-10 may contribute to the generation of B symptoms, since administration of recombinant IL-10 to normal human volunteers, causes fevers in a dose-dependent manner [11]. Since IL-10 inhibits the proliferation of normal erythroid bursts *in vitro*, elevated plasma IL-10 levels also contribute to the anemia observed in lymphoma patients [12].

The second point of our study is the poor survival of DLBCL patients with detectable plasma levels of IL-10 at

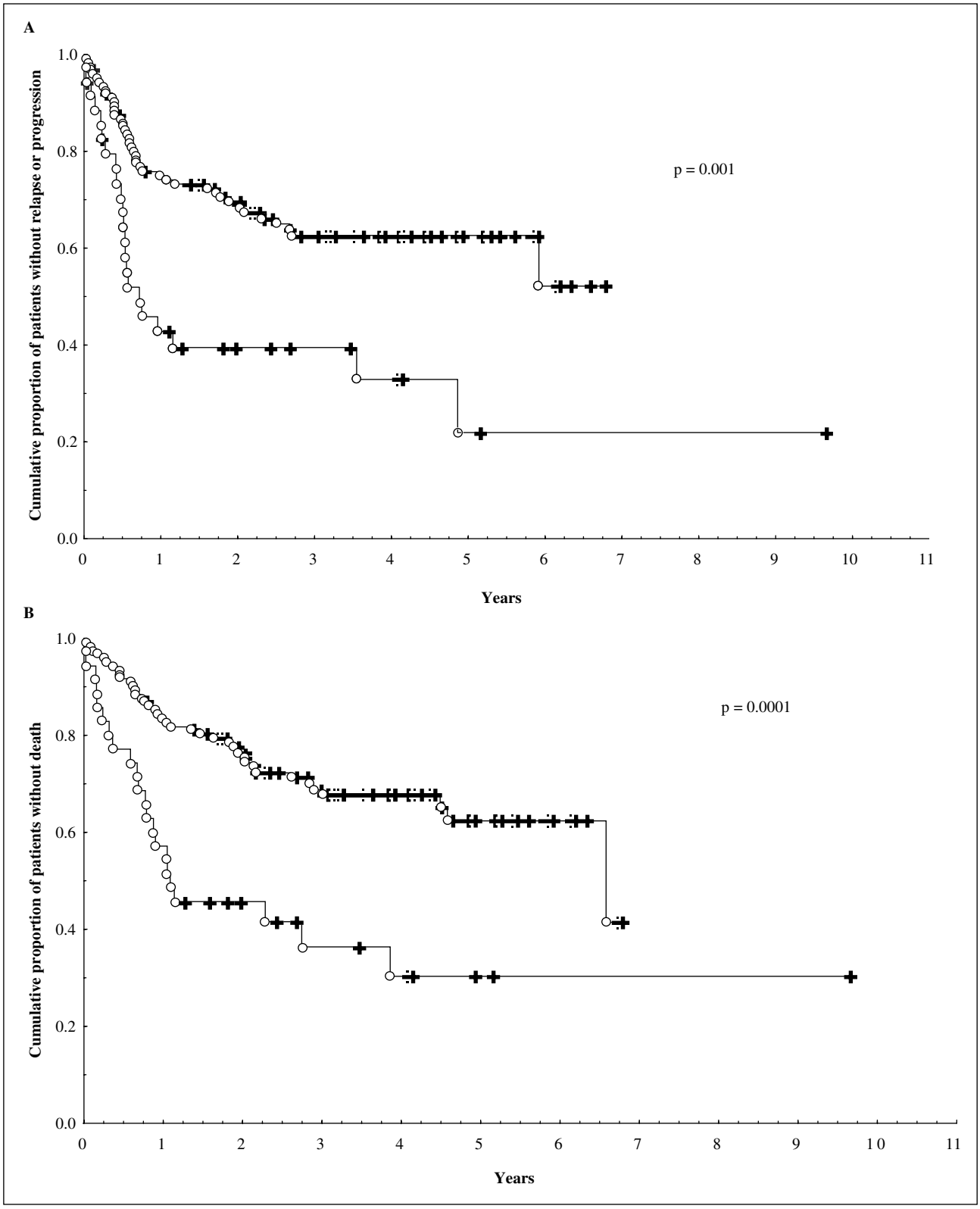

Figure 1
Freedom from progression and overall survival of 157 patients with DLBCL according to the IL-10 serum levels.
The top lines denote the patients with IL-10 levels less than 5 pg/mL (n = 122), whereas the bottom lines denote the patients with Il-10 levels of 5 pg/mL or greater (n = 35); p refers to log-rank test. **A**) Progression-free survival. **B**) Overall survival.

the time of diagnosis. The patients were treated uniformly with anthracyclin-based regimens with a curative intent. The univariate analysis revealed that detectable IL-10 levels were associated with a lower probability of achievement of CR after the induction treatment, as well as with significantly shorter overall and PFS survival. However, in a multivariate Cox regression analysis, when IL-10 levels and other prognostic variables known to be significant predictors of lymphoma outcome were entered, only age and β2-microglobulin levels retained their independent

impact on PFS and OS. IL-10 did not represent an independent influence on DLBCL outcome. Given the strong association between elevated IL-10 levels and many prognostic variables, including age and β2-microglobulin, it is difficult to estimate the relative part played by each of these factors. On the other hand, the lack of predictive value in the multivariate analysis of IL-10 (as well as other known prognostic variables) may be explained by relatively low numbers of events and patients in this model. Of note, when the multivariate analysis was restricted to IL-10 levels and IPI risk groups, which are known to be the most important predictors of aggressive lymphoma outcome, a detectable IL-10 level was found to be an independent variable predictive of shorter OS; a trend towards shorter PFS was also observed.

Recent randomized trails revealed that rituximab in combination with a CHOP regimen is the best treatment in elderly patients with DLBCL [13]. However, our study was designed years ago to examine the outcome of patients treated with anthracyclin-containing regimens and none of them had received rituximab as a part of front line treatment. It has been reported that rituximab in combination with chemotherapy may overcome the prognostic value of some biological markers such as bcl-2 protein [14]. Interestingly, bcl-2 expression can be directly regulated by IL-10, and autocrine IL-10 production may be down-regulated by rituxiamab *in vitro* [15]. This raises the possibility that rituximab may abolish the adverse outcome associated with high plasma IL-10 levels. Therefore, it would be important to further evaluate whether the prognostic value of plasma IL-10 levels remains significant in DLBCL patients receiving a combination of rituximab and chemotherapy.

Plasma IL-10 levels have been reported to influence the clinical course of some infectious, autoimmune, and lymphoproliferative disorders [6-9, 16-28]. Elevated IL-10 levels were correlated with adverse disease features and shorter survival in patients with Hodgkin's disease and chronic lymphocytic leukemia, however, conflicting data concerning non-Hodgkin's lymphoma patients have been published [6-9]. Our results are similar to those obtained by Blay *et al*. [6] but are different from those reported by others [7-9]. This can be explained by the fact that our study population comprised patients with more advanced stages of lymphoma in whom very high levels of IL-10 were shown. Moreover, in our study, the median value and ranges for detectable IL-10 levels were also higher than those observed by Cortes *et al*. [7].

The role of IL-10 in modulating adaptive immune responses towards cancer is still debated. This cytokine may promote the growth of malignant lymphoid cells by inhibiting their apoptosis, by autocrine stimulation or it may counteract the immune response against tumour cells through its immunosuppressive effects [1, 2, 29]. In contrast, the action of IL-10 might also induce apoptosis in certain B-cell subtypes which have been reported to suppress malignant growth by the inhibition of angiogenesis [30, 31]. How circulating IL-10 reflects or contributes to any of these mechanisms *in vivo* is unknown. The genetic influence of IL-10 gene polymorphisms on outcome in lymphoma further emphasizes the key role of this cytokine in lymphoma [28].

In summary, the present analysis indicates that increased IL-10 levels in the plasma of DLBCL patients might reflect an enhanced activation of the immune system towards more aggressive disease, but its potential action as a growth factor for lymphoma cells or as a suppressor of macrophages or T-cell functions should also be taken into account [29].

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