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ORIGINAL ARTICLE

Plasma TNF-α and IL-10 Level-Based Prognostic Model Predicts Outcome of Patients with Diffuse Large B-Cell Lymphoma in Different Risk Groups Defined by the International Prognostic Index

Ewa Lech-Maranda · Jacques Bienvenu · Florence Broussais-Guillaumot · Krzysztof Warzocha · Anne-Sophie Michallet · Tadeusz Robak · Bertrand Coiffier · Gilles Salles

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Abstract Tumor necrosis factor (TNF)-α and interleukin (IL)-10 are key cytokines involved in lymphoma development. Their pretreatment plasma levels were reported to influence the clinical course of non-Hodgkin's lymphoma. In this study the impact of combined elevation of TNF-α and IL-10 on disease features and outcome of patients with diffuse large B-cell lymphoma (DLBCL) were investigated. Plasma TNF-α and IL-10 levels were determined at the time of diagnosis in a group of 106 DLBCL patients uniformly treated with anthracycline-based regimens. Three risk groups depending on the pretreatment levels of the cytokines were identified: low-, intermediate-, and high-risk groups. In univariate analysis, the cytokine intermediate- and high-risk groups were associated with lower probability of achieving a complete remission (odds ratio [OR] = 0.2, 95% confidence interval [CI] 0.06–0.6, $p = 0.006$ and OR = 0.05, 95% CI 0.01–0.2, $p < 0.0001$, respectively) and shorter progression-free survival (PFS) (OR = 4.4, 95% CI 1.9–10.2, $p < 0.001$ and OR = 9.7, 95% CI 4.1–23.0, $p < 0.0001$, respectively) and overall survival (OS) (OR = 4.2, 95% CI 1.7–10.1, $p = 0.002$ and OR = 11.2, 95% CI 4.4–28.4, $p < 0.0001$, respectively) in comparison with the cytokine low-risk group. In multivariate analysis, the cytokine intermediate- and high-risk groups also correlated with shorter PFS (relative risk [RR] = 4.5, 95% CI 1.9–10.9, $p = 0.001$ and RR = 5.8, 95% CI 2.2–15.3, $p < 0.0001$, respectively) and OS (RR = 4.6, 95% CI 1.8–12.0, $p = 0.001$ and RR = 7.5, 95% CI 2.7–20.9, $p < 0.0001$, respectively) regardless of the International Prognostic Index (IPI) scoring system. The TNF-α and IL-10 level-based index may work as an additional model to the IPI for predicting the survival of DLBCL patients. This model may help to identify patients in a given IPI risk group for whom more accurate and risk-adapted treatment could be advised.

Keywords Tumor necrosis factor α · Interleukin-10 · International Prognostic Index · Diffuse large B-cell lymphoma

E. Lech-Maranda · B. Coiffier · G. Salles
Equipe «Pathologie des Cellules Lymphoïdes»,
UMR CNRS 5239, Université Claude Bernard, Lyon, France

E. Lech-Maranda (✉) · T. Robak
Department of Hematology, Medical University of Lodz,
Ciolkowskiego 2 str, 93-510 Lodz, Poland
e-mail: ewamaranda@wp.pl

J. Bienvenu
Laboratoire d'Immunologie, Hospices Civils de Lyon,
Centre Hospitalier Lyon-Sud, Pierre-Bénite, France

F. Broussais-Guillaumot · A.-S. Michallet · B. Coiffier ·
G. Salles (✉)
Service d'Hématologie, Hospices Civils de Lyon,
Centre Hospitalier Lyon-Sud, 69495 Pierre-Bénite, France
e-mail: gilles.salles@chu-lyon.fr

K. Warzocha
Institute of Hematology and Transfusion Medicine,
Warsaw, Poland

Introduction

The formulation of the International Prognostic Index (IPI) has provided generally accepted criteria to identify specific risk groups of aggressive non-Hodgkin's lymphoma (NHL) and to design appropriate therapies (1993). The prognostic factors incorporated in the IPI mostly reflect the tumor mass and the patient's characteristics. However, despite the usefulness of the IPI model, assessment of prognosis of

individual lymphoma patients remains uncertain. Therefore, new biological features that reflect the biological heterogeneity of diffuse large B-cell lymphomas (DLBCLs) have been evaluated for a better determination of different clinical outcomes of patients within the same group defined by the IPI (Shipp 1994).

Recently published studies have indicated that lymphoma development is regulated in part by key cytokines, including tumor necrosis factor (TNF)-α and interleukin (IL)-10 (Gruss and Dower 1995; Hofmann et al. 2002; Mocellin et al. 2004; Moore et al. 2001; Pfeffer 2003). IL-10 is a pleiotropic cytokine that has strong immunosuppressive effects by inhibiting the secretion of pro-inflammatory cytokines, including TNF-α, as well as immunostimulatory effects via the induction of proliferation and differentiation of B cells (Mocellin et al. 2004; Moore et al. 2001). TNF-α is an important pro-inflammatory cytokine that plays a central role in immune responses and acts as an inducer of IL-10 secretion in vitro (Voorzanger et al. 1996; Warzocha and Salles 1998; Younes and Aggarwall 2003). Deregulated production of TNF-α and IL-10 has been observed in NHL (Hofmann et al. 2002; Mocellin et al. 2004; Warzocha et al. 1997a) and reported to influence disease outcome (Blay et al. 1993; Inagaki et al. 2006; Lech-Maranda et al. 2006; Mori et al. 2003; Ozdemir et al. 2004; Salles et al. 1996; Warzocha et al. 1997b). Therefore in the present study we investigated the impact of combined elevation of both TNF-α and IL-10 on disease features and survival of patients with DLBCL. It also seemed interesting to us to test whether this cytokine level-based model may improve the prognostic stratification of the IPI.

Materials and Methods

Subjects

The study group consisted of 106 consecutive patients with DLBCL from whom plasma samples for cytokine evaluation were available at the time of diagnosis. These patients were accrued prospectively between 1995 and 1999 and treated with curative intent at the Department of Hematology of the Centre Hospitalier Lyon-Sud, France. Seven patients with active bacterial or fungal infection, those who were positive for human immunodeficiency virus infection, patients with a previous history of autoimmune disease, and those who had received recent corticosteroid therapy were excluded from the analysis. Plasma samples were taken at the time of diagnosis and were kept at −80°C. All samples were obtained and coded after the patients' informed consent. The study was performed in accordance with the guidelines for studies of human subjects at our institution and with French laws.

Staging

Pathological confirmation of the diagnosis of DLBCL and the clinical history were available in every case. The initial medical evaluation consisted of a complete history and physical examination, computed tomographic scan of the chest, abdomen, and pelvis, and blood morphology and chemistry. The extent of disease and the presence of B symptoms were categorized according to the Ann Arbor classification and performance status was assessed using the criteria of the Eastern Cooperative Oncology Group (ECOG). The IPI system was used to divide the patients into a low-risk (IPI score 0–2) and a high-risk (IPI score 3–5) group. The clinical characteristics of the patients enrolled in the study are presented in Table 1.

Treatment

All patients included in this study received anthracycline-containing regimens consisting of CHOP (cyclophosphamide, adriamycine, vincristine, prednisone) or high-dose CHOP according to age and number of IPI factors, as outlined in GELA prospective trials (Coiffier et al. 1989). Treatment outcome was assessed according to the criteria of the International Workshop to Standardize Response Criteria for Non-Hodgkin's Lymphomas (Cheson et al. 1999). 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. 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 afterwards.

Evaluation of the Level of Cytokines

Blood samples from the 106 newly diagnosed patients were collected before treatment initiation using sterile tubes containing EDTA to prevent further release of cytokines from circulating mononuclear cells. Plasma samples were stored at −80°C and thawed immediately prior to the determination of cytokine levels. All plasma samples were tested by an enzyme-linked immunosorbent assay (ELISA) kit for TNF (Medgenix Diagnostics, Fleurus, Belgium) and a human ELISA kit for IL-10 (BioSource International, Inc., CA, USA). The detection limit of the ELISA test for TNF was 3 pg/ml and for IL-10.5 pg/ml. Samples from 20 healthy blood donors, who had formally given their

Table 1 Characteristics of the 106 patients with DLBCL and their association with TNF-α and IL-10 plasma levels at the time of diagnosis

Characteristic	Patients with TNF-α ≤32 pg/ml *n* (%)	Patients with TNF-α >32 pg/ml *n* (%)	p^a	Patients with IL-10 <5 pg/ml *n* (%)	Patients with IL-10 ≥5 pg/ml *n* (%)	p^b
Sex						
Male	27 (54)	23 (46)	0.6	38 (76)	12 (24)	0.8
Female	27 (48)	29 (52)		44 (79)	12 (21)	
Age						
≤60 years	28 (57)	21 (43)	0.2	45 (92)	4 (8)	0.001
>60 years	26 (46)	31 (54)		37 (65)	20 (35)	
Performance status						
<2	43 (58)	31 (42)	0.004	63 (85)	11 (15)	0.002
≥2	7 (25)	21 (75)		15 (54)	13 (46)	
Unknown	4					
Ann Arbor stage						
I, II	28 (74)	10 (26)	<0.0001	35 (92)	3 (8)	0.004
III, IV	21 (33)	42 (67)		42 (67)	21 (33)	
Unknown	5					
No. of extranodal sites						
<2	32 (55)	26 (45)	0.2	48 (83)	10 (17)	0.2
≥2	16 (41)	23 (59)		28 (72)	11 (28)	
Unknown	9					
Serum LDH						
≤1× normal	32 (71)	13 (29)	<0.0001	40 (89)	5 (11)	0.008
>1× normal	13 (25)	39 (75)		34 (65)	18 (35)	
Unknown	9					
Serum β2-microglobulin						
≤3.0 mg/ml	41 (65)	22 (35)	<0.0001	54 (86)	9 (14)	0.001
>3.0 mg/ml	4 (13)	27 (87)		16 (52)	15 (48)	
Unknown	12					
B symptoms						
Absent	46 (62)	28 (38)	0.001	65 (88)	9 (12)	<0.0001
Present	8 (25)	24 (75)		17 (53)	15 (47)	
Bulky tumor						
Absent	35 (62.5)	21 (37.5)	0.003	48 (86)	8 (14)	0.03
Present	14 (32)	30 (68)		29 (66)	15 (34)	
Unknown	6					
Serum albumin level						
>35.0 g/l	38 (59)	26 (41)	0.002	56 (87.5)	8 (12.5)	0.001
≤35.0 g/l	7 (24)	22 (76)		16 (55)	13 (45)	
Unknown	13					
Hemoglobin						
>12 g/dl	29 (56)	23 (44)	0.1	42 (81)	10 (19)	0.2
≤12 g/dl	18 (38)	29 (62)		33 (70)	14 (30)	
Unknown	7					
IL-10 plasma level						
<5 pg/ml	51 (62)	31 (38)	<0.0001	–	–	–
≥5 pg/ml	3 (12.5)	21 (87.5)				
IPI risk groups						
Low	30 (62.5)	18 (37.5)	0.004	44 (92)	4 (8)	0.002
High	14 (31)	31 (69)		29 (64)	16 (36)	
Unknown	13					

n Number of patients

[a] Associations between groups of patients with TNF-α ≤32 pg/ml and >32 pg/ml, χ^2 test, *p* values are two-sided

[b] Associations between groups of patients with IL-10 <5 pg/ml and ≥5 pg/ml, χ^2 test, *p* values are two-sided

consent, were also assessed for plasma levels of these cytokines.

Statistical Analysis

Associations between the cytokine levels and clinical or biological variables were assessed with the χ^2 test with Yates's correction and Fisher's exact test was used when a cell frequency was less than 5, unless any expected frequency was less than 20. Logistic regression was used to estimate the influence of various clinical or biological parameters on the complete remission (CR) rate. Survival (PFS and OS) was assessed by the Kaplan–Meier method and compared using the log-rank test. 95% confidence intervals (95% CI) were calculated. Multivariate regression analysis with the Cox proportional hazard model was used to adjust the effect of cytokine levels along with the prognostic variables for potential independent factors. Statistical tests with $p < 0.05$ were considered significant. All reported p values are two-sided. Statistical analysis was performed using the SPSS 14.0 package (SPSS Inc., Chicago, IL, USA).

Results

Plasma TNF-α and IL-10 Levels

Pretreatment plasma levels of TNF-α and IL-10 were assessed in all 106 patients with DLBCL. Of these patients, 82 (77%) had IL-10 plasma levels below the detection limit (<5 pg/ml) and 24 (23%) above (median 22.5 pg/ml, range 5–535 pg/ml). In 16 (80%) healthy subjects the IL-10 plasma levels were lower than 5 pg/ml and the median value of quantifiable IL-10 levels was 7.1 pg/ml (range 5–9.8 pg/ml). The detectable IL-10 levels in the healthy controls were significantly lower than in the DLBCL patients ($p < 0.0001$). Both the patients and the healthy controls had plasma levels of TNF-α above the detection limit. The median TNF-α value was 32 pg/ml (range 3–1,836 pg/ml) in the DLBCL patients vs. 10 pg/ml (range 3–14) in the healthy subjects ($p < 0.0001$) (Table 1).

Plasma TNF-α and IL-10 Level-Based Model in DLBCL Prognosis and Outcome

The clinical prognostic features and their associations with plasma TNF-α or IL-10 level are presented in Table 1. Plasma TNF-α levels greater than the median value and detectable IL-10 levels correlated with each other ($p < 0.0001$). Both elevated TNF-α and IL-10 levels predicted a lower probability of achieving a CR (odds ratio [OR] = 0.12, 95% CI 0.05–0.34, $p < 0.0001$ and OR = 0.16, 95% CI 0.06–0.42, $p < 0.0001$, respectively) as well as shorter PFS (OR = 6.9, 95% CI 3.2–14.9, $p < 0.0001$ and OR = 3.2, 95% CI 1.7–5.9, $p < 0.001$, respectively) and OS (OR = 6.7, 95% CI 2.9–15.3, $p < 0.0001$ and OR = 3.8, 95% CI 1.9–7.4, $p < 0.0001$, respectively).

The association of elevated TNF-α and IL-10 levels with numerous adverse prognostic factors as well as shorter PFS and OS allowed us to create a prognostic cytokine model depending on the initial plasma levels of TNF-α and IL-10. Three cytokine risk groups were identified: the low-risk group consisted of 51 patients with TNF-α levels below the median value and undetectable IL-10 levels, the intermediate-risk group comprised 34 patients with only one elevated parameter, and the high-risk group was of 21 patients with TNF-α levels greater than the median value and detectable IL-10 levels. The cytokine high-risk group significantly correlated with age >60 years ($p = 0.001$), ECOG performance status ≥2 ($p = 0.002$), Ann Arbor advanced disease stage ($p = 0.002$), elevated serum levels of LDH ($p = 0.006$), β2-microglobulin ($p < 0.0001$), low serum albumin levels ($p = 0.002$), the presence of B symptoms ($p < 0.0001$), bulky tumor mass ($p = 0.01$), and the IPI high-risk group (IPI score 3–5; $p = 0.001$). In univariate logistic regression analysis, the cytokine intermediate- and high-risk groups were significantly associated with a lower probability of achieving a CR compared with the cytokine low-risk group (OR = 0.2, 95% CI 0.06–0.6, $p = 0.006$ and OR = 0.05, 95% CI; 0.01–0.2, $p < 0.0001$, respectively).

The prognostic parameters associated with shorter PFS and OS by univariate analysis are listed in Table 2. The 3-year PFS rates for the cytokine low-, intermediate-, and high-risk groups were 83.5% (95% CI 69–92), 42.0% (95% CI 26–59), and 21.4% (95% CI 9–44), respectively (log-rank, $p < 0.0001$) (Fig. 1a). The relative risk of disease relapse or progression was 4.4-fold higher (95% CI 1.9–10.2, $p < 0.001$) for the intermediate-risk and 9.7-fold higher (95% CI 4.1–23.0, $p < 0.0001$) for the high-risk group compared with the low-risk group. Three-year OS rates in the cytokine low-, intermediate-, and high-risk groups were 85.5% (95% CI 71–93), 51.3% (95% CI 33–69), and 25.0% (95% CI 10–49), respectively (log-rank, $p < 0.0001$) (Fig. 1b). The relative risk of death was 4.2-fold higher (95% CI 1.7–10.1, $p = 0.002$) in the patients of the intermediate-risk and 11.2-fold higher (95% CI 4.4–28.4, $p < 0.0001$) in the patients of the high-risk group compared with that of the

Table 2 Probabilities of 3-year progression-free and overall survival according to patient- and disease-related prognostic factors

Factor	No. of patients	PFS (%) (95% CI)	p*	No. of patients	OS (%) (95% CI)	p*
Sex						
Male	50	58.3 (43–72)	0.6	50	66.0 (50–79)	0.4
Female	55	55.2 (41–68)		56	60.2 (44–74)	
Age						
≤60 years	49	77.3 (62–88)	<0.0001	49	81.9 (68–91)	0.001
>60 years	56	38.9 (26–53)		57	46.8 (33–61)	
Performance status						
<2	73	66.6 (54–77)	<0.0001	74	71.0 (58–81)	<0.0001
≥2	28	28.6 (15–48)		28	40.6 (24–59)	
Ann Arbor stage						
I, II	37	72.5 (53–86)	0.002	38	85.1 (67–94)	<0.0001
III, IV	63	46.9 (35–59)		63	48.6 (36–62)	
Number of extranodal sites						
<2	57	58.5 (44–71)	0.2	58	68.2 (54–80)	0.1
≥2	39	53.8 (39–68)		39	55.2 (38–71)	
Serum LDH (U/l)						
≤1× normal	44	68.0 (51–81)	0.002	45	77.9 (61–89)	<0.0001
>1× normal	52	43.6 (31–57)		52	46.3 (33–60)	
Serum β2-microglobulin						
≤3.0 mg/ml	62	73.1 (60–83)	<0.0001	63	77.2 (64–86)	<0.0001
>3.0 mg/ml	31	20.0 (9–38)		31	27.4 (14–48)	
B symptoms						
Absent	73	63.1 (50–74)	0.001	74	69.9 (57–80)	0.001
Present	32	43.8 (28–61)		32	48.7 (32–66)	
Bulky tumor						
Absent	55	67.6 (52–80)	0.003	56	74.2 (59–85)	0.002
Present	44	42.7 (29–57)		44	48.5 (34–63)	
Serum albumin						
>35.0 g/l	63	63.6 (50–75)	0.006	64	70.5 (58–81)	0.003
≤35.0 g/l	29	44.6 (28–62)		29	48.7 (31–67)	
Hemoglobin						
>12 g/dl	51	68.9 (55–80)	0.03	52	73.1 (58–84)	0.005
≤12 g/dl	47	42.3 (29–57)		47	48.7 (34–64)	
TNF-α plasma level						
≤32 pg/ml	53	84.4 (70–92)	<0.0001	54	86.3 (73–94)	<0.0001
>32 pg/ml	52	30.3 (19–45)		52	38.2 (25–53)	
IL-10 plasma level						
<5 pg/ml	81	64.5 (52–75)	<0.001	82	70.7 (59–80)	<0.0001
≥5 pg/ml	24	32.1 (17–53)		24	36.0 (19–57)	
TNF-α and IL-10 risk groups						
Low	50	83.5 (69–92)	<0.0001	51	85.5 (71–93)	<0.0001
Intermediate	34	42.0 (26–59)		34	51.3 (33–69)	
High	21	21.4 (9–44)		21	25.0 (10–49)	
IPI risk groups						
Low	47	74.8 (59–86)	<0.0001	48	82.1 (67–91)	<0.0001
High	45	34.8 (22–50)		45	37.9 (24–54)	

* Groups were compared using the log-rank test

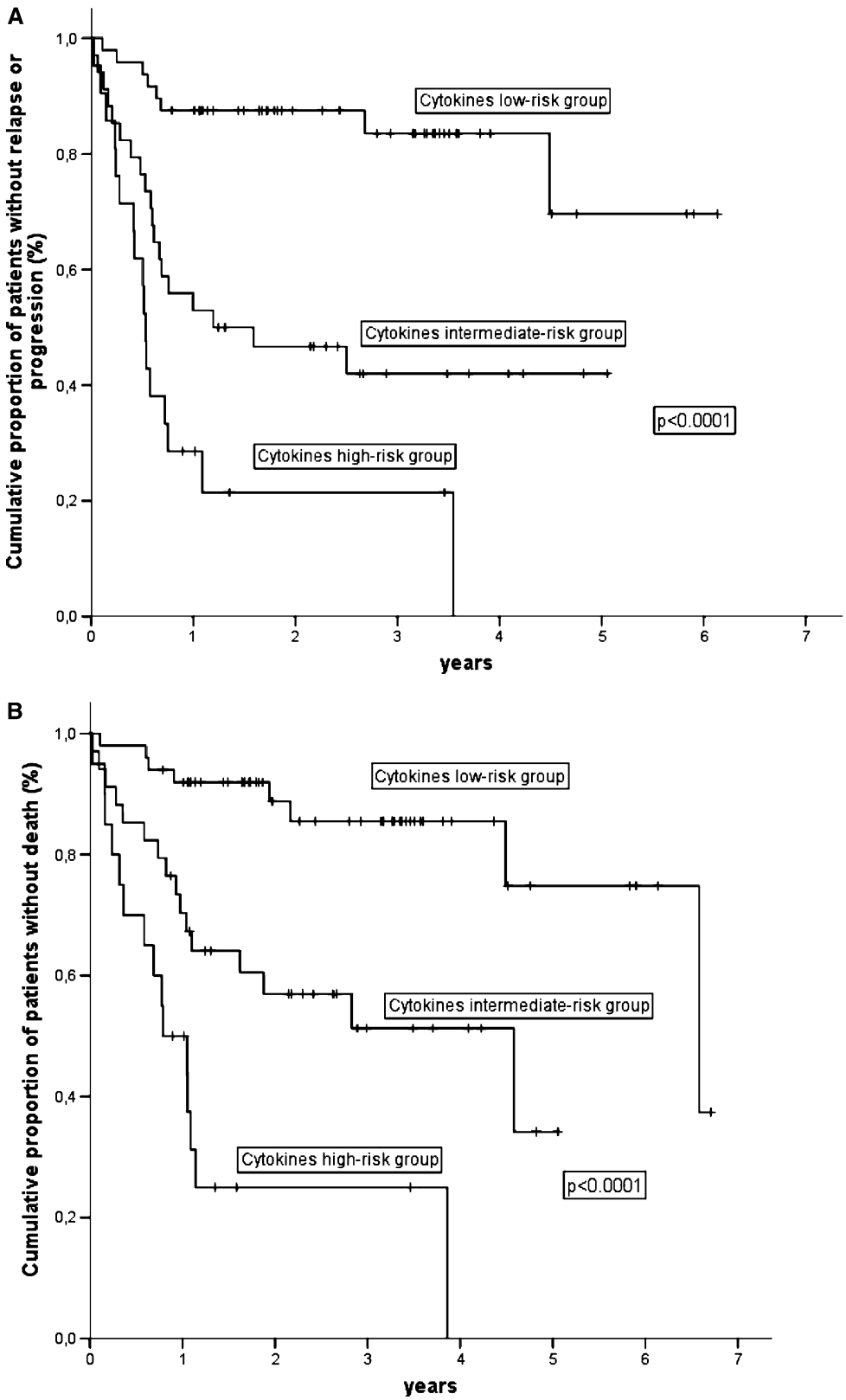

Fig. 1 Progression-free (**a**) and overall (**b**) survival of the 106 patients with DLBCL according to the risk groups defined by the initial plasma levels of TNF-α and IL-10

low-risk group. After incorporating all the variables that were significant in univariate analysis into a multivariate model, the plasma TNF-α and IL-10 level-based system along with age and β2-microglobulin level retained its independent prognostic impact on both PFS and OS (Table 3).

Table 3 Cox model describing progression-free (A) and overall (B) survival

Factor	No. of patients	Relative risk (RR) (95% CI for RR)	*p*
A			
Cytokine risk groups			
Low	38	1.0	–
Intermediate	30	4.1 (1.5–11.5)	0.007
High	15	4.3 (1.7–10.9)	0.002
Age			
≤60 years	37	1.0	
>60 years	46	3.1 (1.4–7.2)	0.006
Serum β2-microglobulin			
≤3.0 mg/ml	57	1.0	–
>3.0 mg/ml	26	2.2 (1.0–4.9)	0.04
B			
Cytokine risk groups			
Low	39	1.0	–
Intermediate	30	4.8 (1.7–13.0)	0.002
High	15	5.7 (1.9–17.2)	0.001
Age			
≤60 years	37	1.0	
>60 years	47	3.5 (1.4–8.4)	0.006
Serum β2-microglobulin			
≤3.0 mg/ml	58	1.0	
>3.0 mg/ml	26	2.2 (1.0–4.8)	0.05

TNF-α and IL-10 Level-Based Model according to the IPI Risk Group

To further investigate whether the plasma cytokine level-based model retains its prognostic impact in different IPI groups, we divided the patients according to the IPI scoring system into a low-risk (IPI score 0–2) and a high-risk (IPI score 3–5) group. In the subgroup with low IPI scores, the 3-year PFS rate was significantly higher in the patients in the cytokine low-risk group compared with those in the cytokine intermediate- and high-risk groups taken together (89.7%, 95% CI 68–97 vs. 55.3%, 95% CI 33–76, respectively, $p = 0.01$) (Fig. 2a). Similarly, the patients of the cytokine low-risk group presented a statistically higher 3-year OS rate than those of the cytokine intermediate- and high-risk groups (95%, 95% CI 76–99 vs. 63.2%, 95% CI 38–83, respectively, $p = 0.01$) (Fig. 2b). In the subgroup with high IPI scores, the patients in the cytokine low-risk group had a significantly higher 3-year PFS rate than those in the cytokine intermediate- and high-risk groups taken together (69.2%, 95% CI 42–87 vs. 19.7%, 95% CI 9–38, respectively, $p = 0.002$) (Fig. 3a). When OS was analyzed, the patients in the cytokine low-risk group presented a statistically higher 3-year OS rate than those in the cytokine intermediate- and high-risk groups (76.9%, 95% CI 50–92 vs. 25.8%, 95% CI 13–44, respectively, $p = 0.002$) (Fig. 3b). When the IPI and cytokine level-based risk groups were included in the Cox model, the cytokine intermediate- and high-risk groups retained their independent impact on shorter PFS (relative risk [RR] = 4.5, 95% CI 1.9–10.9, $p = 0.001$ and RR = 5.8, 95% CI 2.2–15.3, $p < 0.0001$, respectively) and OS (RR = 4.6, 95% CI 1.8–12.0, $p = 0.001$ and RR = 7.5, 95% CI 2.7–20.9, $p < 0.0001$, respectively) (Table 4).

Discussion

In this study we evaluated the influence of a plasma TNF-α and IL-10 level-based index on the prognosis and survival of DLBCL patients treated with anthracycline-containing regimens. The cytokine high-risk group was associated with numerous adverse prognostic variables expressing both increased tumor burden (high serum levels of LDH and β2-microglobulin, advanced disease stage, large tumor mass) and alteration of the host status (poor performance status, B symptoms, low albumin levels). These associations may suggest that elevated plasma levels of TNF-α and IL-10 originate from their autonomous production by malignant and reactive cells (Karin and Greten 2005; Mocellin et al. 2004; Voorzanger et al. 1996; Warzocha and Salles 1998). In addition, the alteration of the host status can be explained by the immune response to the presence of lymphoma and the systemic release of these cytokines (Benjamin et al. 1992; Mocellin et al. 2004; Warzocha et al. 1997a).

Importantly, the cytokine-based model was found to be an IPI-independent predictor of OS and PFS in DLBCL patients. Patients in the cytokine low-risk group presented longer PFS and OS in both the low- and high-risk IPI subgroups. This may help to identify patients in a given IPI risk group for whom more accurate and risk-adapted treatment could be advised. Of note, all patients enrolled in this study were treated uniformly with anthracycline-based regimens with a curative intent and none of them received rituximab as a part of front-line treatment. Recent randomized trials revealed that rituximab in combination with the CHOP regimen is the best treatment for elderly patients with DLBCL (Coiffier et al. 2002). It has been reported that rituximab in combination with chemotherapy may overcome the prognostic value of some biological markers such as bcl-2 protein (Mounier et al. 2003). Interestingly, IL-10 can directly up-regulate bcl-2 expression and autocrine IL-10 production may be down-regulated by rituximab in vitro (Alas et al. 2001). Therefore it would be important to further evaluate

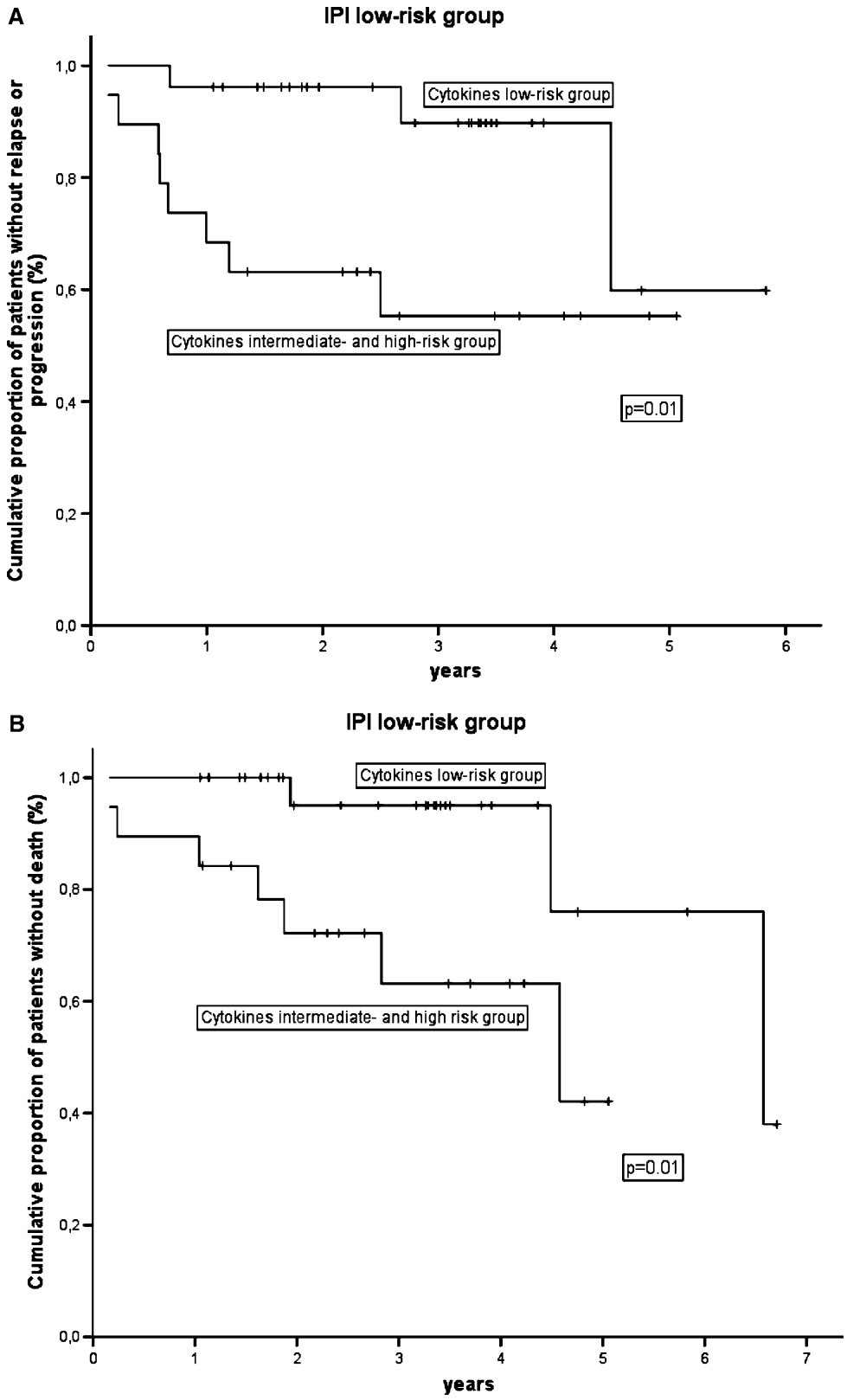

Fig. 2 Progression-free (**a**) and overall (**b**) survival of DLBCL patients with low IPI scores according to the risk groups defined by the initial plasma levels of TNF-α and IL-10. The number of patients at risk was 29 for the cytokine low-risk group and 19 for the cytokine intermediate- and high-risk groups

whether the prognostic value of the cytokine level-based model remains unaffected by the addition of rituximab to the treatment of DLBCL patients.

The possible mechanisms of the contributions of TNF-α and IL-10 to the clinical course of DLBCL are still debated. It is known that TNF-α and IL-10 are key cytokines

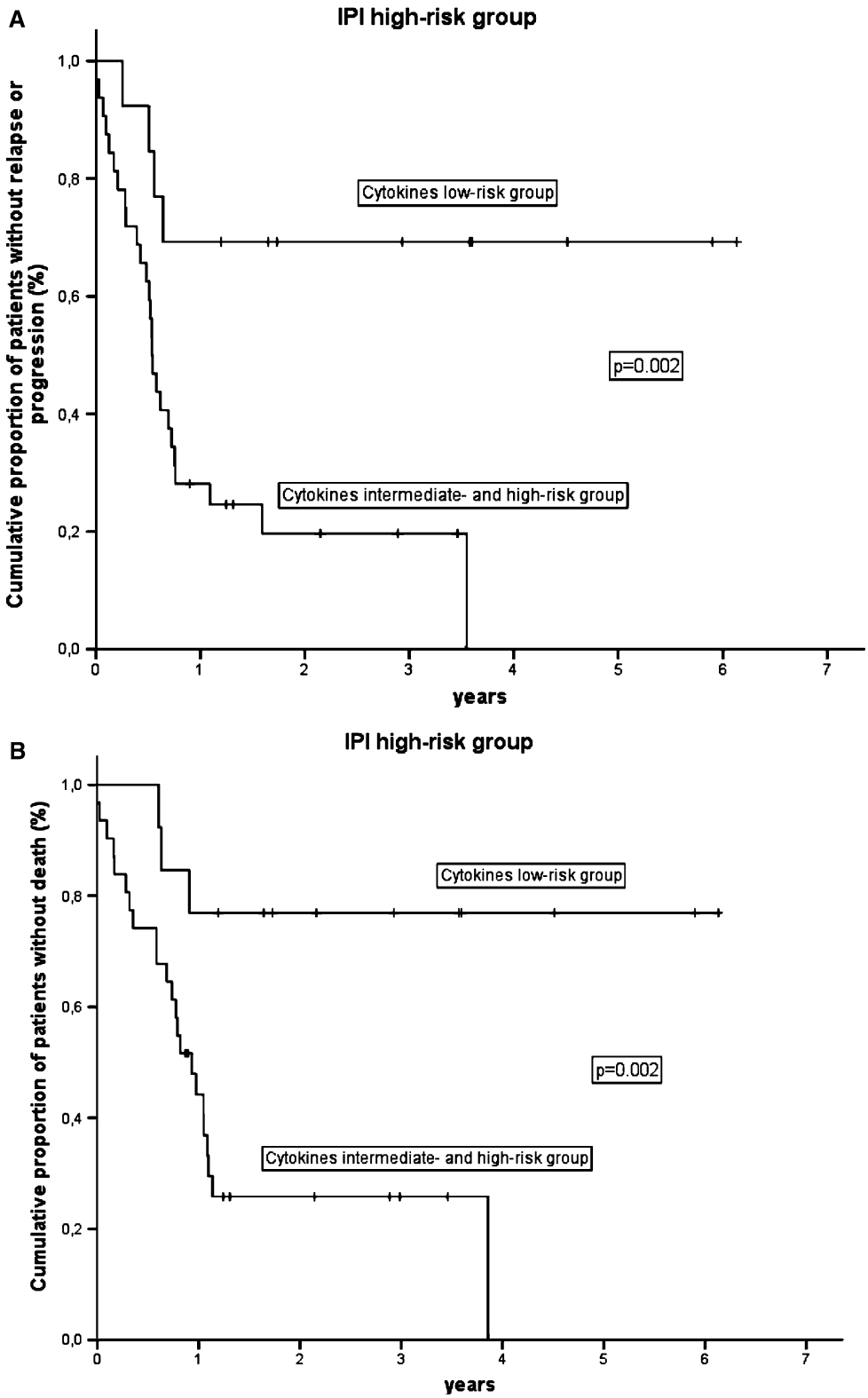

Fig. 3 Progression-free (**a**) and overall (**b**) survival of DLBCL patients with IPI high scores according to the risk groups defined by the initial plasma levels of TNF-α and IL-10. The number of patients at risk was 13 for the cytokine low-risk group and 32 for the cytokine intermediate- and high-risk groups

involved in the modulation of lymphoma development and the balance between cell-mediated and humoral immunity (Aggarwal 2003; Mocellin et al. 2004). IL-10 acts as a growth factor for normal activated B cells as well as neoplastic B lymphocytes; however, it also exerts strong immunosuppressive effects by the inhibition of Th1 lymphocytes (Mocellin et al. 2004; Moore et al. 2001). TNF-α plays a central role in inflammatory and immune responses,

Table 4 Cytokine-based and IPI risk groups in the multivariate analysis of progression-free (A) and overall (B) survival

Factor	No. of patients	Relative risk (RR) (95% CI for RR)	*p*
A			
Cytokine risk groups			
Low	41	1.0	
Intermediate	33	4.5 (1.9–10.9)	0.001
High	18	5.8 (2.2–15.3)	<0.0001
IPI risk groups			
Low	47	1.0	
High	45	3.1 (1.5–6.7)	0.003
B			
Cytokine risk groups			
Low	42	1.0	
Intermediate	33	4.6 (1.8–12.0)	0.001
High	18	7.5 (2.7–20.9)	<0.0001
IPI risk groups			
Low	48	1.0	
High	45	3.8 (1.6–8.9)	0.002

generating a cytokine cascade that includes the production of IL-1, IL-6, IL-10, and other mediators, including TNF itself (Aggarwal 2003; Pfeffer 2003; Warzocha and Salles 1998). Interestingly, TNF-α was found to be the only inflammatory cytokine which induces the production of IL-10 (Wanidworanun and Strober 1993). In contrast, IL-10 has been reported to suppress TNF-α expression, forming a negative-feedback loop that prevents deleterious accumulation of TNF-α (Mocellin et al. 2004; Wanidworanun and Strober 1993). However, it could be hypothesized that in lymphoma patients, especially in those with advanced disease and large tumor mass, such an autoregulatory loop can be deregulated, resulting in uncontrolled production of both TNF-α and IL-10. To support this hypothesis, a strong correlation between high IL-10 and TNF-α expression on tumor cells was observed, suggesting the existence of a local cytokine cascade in the tumor sites (Voorzanger et al. 1996). Recent studies exploring the genetic influence of TNF and IL-10 gene polymorphisms on the susceptibility and outcome of DLBCL patients emphasize the key role of TNF-α and IL-10 in developing lymphoma (Domingo-Domènech et al. 2007; Lan et al. 2006; Lech-Maranda et al. 2004; Rothman et al. 2006; Wang et al. 2007; Warzocha et al. 1998).

In conclusion, this report indicates that TNF-α and IL-10 contribute to the clinical course of DLBCL and may be considered as candidates in the design of a powerful prognostic model in addition to the IPI. This model may be useful in selecting patients for whom new treatment approaches, especially those based on immunomodulation, could be advised. However, our results require further validation in a larger population of patients with DLBCL treated with a combination of rituximab and chemotherapy.

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