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ORIGINAL ARTICLE

Serum Tumor Necrosis Factor-α and Interleukin-10 Levels as Markers to Predict Outcome of Patients with Chronic Lymphocytic Leukemia in Different Risk Groups Defined by the *IGHV* Mutation Status

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Abstract Tumor necrosis factor (TNF)-α and interleukin (IL)-10 are cytokines involved in the balance between cell-mediated and humoral immunity. We investigated whether serum TNF-α and IL-10 levels have any impact on clinical outcome of patients with chronic lymphocytic leukemia (CLL). TNF-α and IL-10 levels were determined in the serum of 160 CLL patients at the time of diagnosis. The cytokine low-risk group consisted of patients with either TNF-α and IL-10 levels below their medians or those with only one elevated parameter. Both TNF-α and IL-10 levels greater than or equal to their medians defined the cytokine high-risk group. The high-risk patients presented a shorter 3-year treatment-free survival (TFS) than low-risk subjects (15 vs. 69.6 %; $p < 0.0001$). The high-risk group ($p = 0.0002$) along with high leukocyte count ($p < 0.0001$) and unmutated immunoglobulin heavy-chain variable region genes ($p < 0.0001$) independently predict the risk of progression in patients with Rai stage 0–II. Furthermore, the high-risk group had an independent prognostic impact on shorter TFS both in patients with mutated (24.3 vs. 78.2 %; $p < 0.0001$) and unmutated (8.2 vs. 49 %; $p = 0.004$) immunoglobulin heavy-chain variable region genes (*IGHV*) as compared to the low-risk group. The estimated 5-year overall survival (OS) of high-risk patients was shorter than those in the low-risk group (83.3 vs. 97.1 %; $p = 0.003$). Multivariate analysis demonstrated the cytokine high-risk group ($p = 0.02$) followed by Rai stage III–IV ($p = 0.048$) to be independent factors predicting shorter OS. At diagnosis, TNF-α and IL-10 may predict the outcome of patients with CLL.

Keywords Chronic lymphocytic leukemia · Tumor necrosis factor-α · Interleukin-10 · Immunoglobulin heavy-chain variable region genes · Outcome

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Introduction

Chronic lymphocytic leukemia (CLL) is a heterogenous disease with a variable clinical course (Kipps 2000). Different prognostic staging systems have been developed to predict a survival of CLL patients including the Rai and Binet classification and novel factors like CD38 and ZAP-70 expression, cytogenetic abnormalities and the mutation status of the immunoglobulin heavy-chain variable region (*IGHV*) genes. Despite the usefulness of these parameters, the prognosis of individual CLL patients nevertheless still remains uncertain.

Recently published studies have indicated that a number of cytokines may be involved in the pathogenesis of CLL

(Gruss and Dower 1995; Moore et al. 2001; Mocellin et al. 2004; Klein and Dalla-Favera 2010). Tumor necrosis factor (TNF)-α and interleukin (IL)-10 are key cytokines involved in the balance between cell-mediated and humoral immunity (Gruss and Dower 1995; Moore et al. 2001; Mocellin et al. 2004). Interleukin-10 is an important immunoregulatory cytokine which has a strong immunosuppressive effect by inhibiting the synthesis of type 1 helper-T cell (Th1) cytokines, including TNF-α, and antigen presentation by reducing MHC class II expression on monocytes (de Waal et al. 1991; Gruss and Dower 1995; Moore et al. 2001; Mocellin et al. 2004). Conversely, IL-10 stimulates the proliferation and differentiation of B and Th2 cells (Gruss and Dower 1995; Moore et al. 2001; Mocellin et al. 2004). There is, however, conflicting data regarding the effect of IL-10 on CLL cells, with some reports suggesting that IL-10 inhibits proliferation and induces apoptosis of malignant cells, while others postulating that IL-10 protects against apoptotic cell death (Fluckiger et al. 1994; Kitabayashi et al. 1995; Voorzanger et al. 1996). TNF-α is a pro-inflammatory cytokine that plays a central role in immune responses and acts as an inducer of IL-10 secretion in vitro (Gruss and Dower 1995; Voorzanger et al. 1996; Warzocha and Salles 1998; Younes and Aggarwall 2003). TNF-α increases the proliferation and growth of CLL cells, but it may also induce cell apoptosis and necrosis (Warzocha and Salles 1998; Younes and Aggarwall 2003). Deregulated production of TNF-α and IL-10 and its influence on disease outcome have been observed in non-Hodgkin lymphoma (Blay et al. 1993; Warzocha et al. 1997; Lech-Maranda et al. 2010) and CLL patients (Fayad et al. 2001; Ferrajoli et al. 2002; Jablonska et al. 2005; Bojarska-Junak et al. 2008). The present study has therefore investigated the influence of combined and elevated levels of both TNF-α and IL-10 on CLL prognosis, especially in association with novel prognostic factors.

Materials and Methods

Patients

It was a retrospective study consisted of 160 CLL patients from whom serum samples for cytokine evaluation were available at the time of diagnosis and who were seen in the Hematology Department in Lodz between 2002 and 2006. The seven patients who had active bacterial or fungal infections, human immunodeficiency virus infection, a previous history of autoimmune disease, or those who received recent corticosteroid therapy were removed from the study. From 14 patients serum samples were not available at the time of diagnosis. The study was approved by the local ethics committee in accordance with the updated Declaration of Helsinki.

Diagnosis of CLL was based on the criteria established by the National Cancer Institute-Sponsored Working Group (NCI-WG) (Cheson et al. 1996). Patient characteristics are presented in Table 1.

Amplification and sequencing of *IGHV* genes were performed according to the protocol described by van Dongen et al. 2003. Sequences were considered as unmutated if there was at least (≥) 98 % concordance between CLL DNA and the closest germline gene and mutated if there was <98 % concordance.

The fluorescence in situ hybridization (FISH) analysis was performed on interphase nuclei of lymphocytes on blood smears at the time of diagnosis as previously reported (Robak et al. 2009). Given that some cytogenetic aberrations occur in combination, Döhner's hierarchical model was used to analyze their associations with cytokines' levels and CLL outcome.

Treatment

Chronic lymphocytic leukemia progression was identified according to the NCI-WG criteria (Cheson et al. 1996). Of all patients, 85 (53 %) experienced disease progression and required chemotherapy. The median time to the initiation of treatment was 9.4 months (range 0–100 months). Fifty-one of those patients (60 %) received cladribine or fludarabine in combination with cyclophosphamide (CC, FC), 26 (31 %) chlorambucil as monotherapy, five (6 %) received rituximab in combination with CC or FC and three patients (4 %) were treated with the COP regimen (cyclophosphamide, vincristine, prednisone) as a first-line treatment (Robak et al. 2009, 2010).

Treatment-free survival (TFS) was calculated from the date of CLL diagnosis to the first treatment, death or the last follow-up if untreated. 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 date of diagnosis until the last follow-up evaluation or death arising from any cause.

Serum TNF-α and IL-10 Levels

In frozen serum samples taken from 160 CLL patients and 20 healthy controls TNF-α and IL-10 levels were measured. All samples had been stored at −80 °C and thawed immediately for the determination of cytokine levels. Serum human IL-10 levels were measured using a high-sensitivity sandwich enzyme immunoassay (Quantikine, R&D Systems, Ltd., USA). The mean of the minimum detectable dose (MDD) for IL-10 was 3.9 pg/mL. Serum

Table 1 The associations of prognostic factors with serum TNF-α and IL-10 levels or cytokine low- and high-risk group at the time of diagnosis in 160 CLL patients

Characteristics	Patients with TNF-α <16.8 pg/mL *n* (%)	Patients with TNF-α ≥16.8 pg/mL *n* (%)	p^a	Patients with IL-10 <17.8 pg/ml *n* (%)	Patients with IL-10 ≥17.8 pg/ml *n* (%)	p^b	Cytokine low-risk group *n* (%)	Cytokine high-risk group *n* (%)	p^c
Age									
≤60 years	31 (39)	21 (26)	0.1	22 (28)	30 (37)	0.3	40 (37)	12 (27)	0.2
>60 years	49 (61)	59 (74)		56 (72)	52 (63)		68 (63)	33 (73)	
Sex									
Female	40 (50)	34 (42.5)	0.4	37 (47)	37 (45)	0.9	55 (51)	17 (38)	0.1
Male	40 (50)	46 (57.5)		41 (53)	45 (56)		53 (49)	28 (62)	
Leukocyte count, 10^9/L	20.5	37		20	32		21	53	
Median (range)	(10–302)	(9–500)	<0.0001	(9–500)	(11–432)	0.0002	(9–500)	(12–432)	<0.0001
Hemoglobin, g/dL	14	13		14	13	0.01	14	12.4	
Median (range)	(8–17)	(4.4–16.2)	<0.0001	(6.7–16.9)	(4.4–17)		(6.7–17)	(4.4–16)	0.0002
Platelet count, 10^9/L	202	165		202	170		201	151	
Median (range)	(94–374)	(21–407)	0.001	(21–407)	(26–374)	0.01	(21–407)	(26–301)	0.0003
Rai stage									
0–II	70 (88)	53 (66)	0.001	67 (86)	56 (68)	0.01	91 (85)	25 (56)	<0.0001
III–IV	10 (12)	27 (34)		11 (14)	26 (32)		16 (15)	20 (44)	
β2-microglobulin level									
Normal	31 (44)	10 (13)	<0.0001	26 (38)	15 (19)	0.01	22 (38)	2 (7)	0.002
Elevated	39 (56)	70 (87)		43 (62)	66 (81)		36 (62)	27 (93)	
LDH level									
Normal	55 (85)	54 (63)	0.003	56 (82)	53 (64)	0.02	46 (78)	19 (59)	0.06
Elevated	10 (15)	32 (37)		12 (18)	30 (36)		13 (22)	13 (41)	
CD38 expression									
Low (≤30 %)	57 (80)	39 (54)	0.001	54 (79)	42 (56)	0.004	74 (78)	18 (43)	<0.0001
High (>30 %)	14 (20)	33 (46)		14 (21)	33 (44)		21 (22)	24 (57)	
ZAP-70 expression									
Low (<20 %)	58 (95)	43 (77)	0.006	50 (89)	51 (84)	0.4	58 (94)	17 (77)	0.03
High (≥20 %)	3 (5)	13 (23)		6 (11)	10 (16)		4 (6)	5 (23)	
Hierarchical cytogenetics groups									
Sole 13q deletion	19 (39)	12 (23)	0.2	15 (34)	15 (28)	0.4	23 (33)	7 (24)	0.3
Normal	12 (24)	9 (18)		11 (25)	10 (18)		16 (23)	5 (17.5)	
Trisomy 12 (no 17p or 11q deletion)	4 (9)	7 (15)		1 (2)	10 (18)		4 (6)	7 (24)	
11q deletion (no 17p deletion)	11 (22)	16 (32)		12 (27)	15 (28)		20 (29)	7 (24)	
17p deletion	3 (6)	6 (12)		5 (12)	4 (8)		6 (9)	3 (10.5)	
IGHV mutational status									
IGHV mutated (homology <98 %)	46 (66)	34 (49)	0.04	40 (63)	40 (52)	0.2	48 (62)	16 (44)	0.07
IGHV unmutated (homology ≥98 %)	24 (34)	36 (51)		23 (37)	37 (48)		29 (38)	20 (56)	

The differences between leukocyte, hemoglobin or platelet count and TNF-α and IL-10 levels or cytokine low- and high-risk groups were calculated using Mann–Whitney *U* test

n number of patients

[a] Associations between groups of patients with TNF-α <16.8 pg/mL and ≥16.8 pg/mL, χ^2 test, *p* values are two-sided

[b] Associations between groups of patients with IL-10 <17.8 pg/mL and ≥17.8 pg/mL, χ^2 test

[c] Associations between patients in the cytokine low- and high-risk groups, χ^2 test, *p* values are two-sided

TNF-α levels were measured by an enzyme-linked immunosorbent assay for human TNF (Quantikine, R&D Systems, Ltd., USA). The mean of MDD for TNF-α was 1.6 pg/mL. Samples from the CLL patients and controls which had not been previously thawed were analyzed for cytokines and run at the same time. All determinations were performed in duplicate and results expressed as means.

Statistical Analysis

Associations between cytokines' levels and categorical variables were assessed using the χ^2 test with Yates's correction when a table cell frequency was less than 20; the Fisher Exact Test being used whenever these were <5. The Mann–Whitney *U* test was used to assess the significance between differences in continuous variables. Logistic regression was used to estimate the influence of various parameters on the complete remission (CR) rate. Since no patient died before treatment, the TFS probability was estimated using the Kaplan–Meier method instead of the Cumulative Incidence Estimation, which takes death before treatment as a competing risk, and the log-rank test was used to evaluate differences between factors. Survival (PFS and OS) was estimated by the Kaplan–Meier method and compared using the log-rank test. 95 % confidence intervals (95 % CI) were calculated. A multivariate Cox regression analysis was used to adjust the effect of cytokines' levels along with prognostic variables for potential independent factors. Since advanced stage (Rai stage III–IV) is an indication for treatment, patients with advanced stage were excluded from the multivariate TFS analysis. Statistical tests with $p < 0.05$ were considered significant. All reported *p* values were two-sided. Statistical analysis was performed using the SPSS 15.0 package (SPSS Inc., Chicago, IL, USA).

Results

TNF-α and IL-10 Levels in CLL Patients and Healthy Controls

In 160 CLL patients, the median TNF-α value was 16.8 pg/mL (range 0.58–133.3 pg/mL) and the median IL-10 value was 17.8 pg/mL (range 1.54–223.9 pg/mL). The medians of both cytokines were used as cut-off points and cytokines' levels greater than or equal to these values were assessed as being high. A correlation between TNF-α and IL-10 levels was observed ($p < 0.0001$, Pearson's test).

In 20 healthy subjects, the median IL-10 level was 9.7 pg/mL (range 0.9–49.5 pg/mL) and the median TNF-α value was 10 pg/ml (range 0.5–14). These levels were significantly lower than those observed in the CLL patients ($p = 0.02$ and $p = 0.01$, respectively).

TNF-α and IL-10 Levels and Prognostic Variables

The association of high TNF-α and IL-10 levels with numerous adverse prognostic factors (Table 1) allowed us to create a cytokine model depending on the initial serum levels of cytokines. First, three cytokine groups, namely low-, intermediate-, and high-risk were identified based on the initial levels of the cytokines. The low-risk group consisted of 46 patients with TNF-α and IL-10 levels below their median values, the intermediate-risk group comprised 66 patients with only one elevated parameter, and the high-risk group was of 48 patients with TNF-α and IL-10 levels greater than or equal to their median values. Since statistical analysis did not reveal any differences between the cytokine low- and intermediate group in terms of adverse prognostic factors relating to the advanced disease, we divided the patients into two cytokine groups (high vs. low). The cytokine low-risk group consisted of 112 patients with either TNF-α and IL-10 levels below their medians or those with only one elevated parameter, and the high-risk group was of 48 patients with both TNF-α and IL-10 levels greater than or equal to their medians.

The cytokine high-risk group was significantly associated with Rai stage III or IV ($p < 0.0001$), elevated β2-microglobulin ($p = 0.002$), high CD38 ($p < 0.0001$) and ZAP-70 ($p = 0.03$) expression, leukocytosis ($p < 0.0001$), low hemoglobin level ($p = 0.0002$) and platelet count ($p = 0.0003$). A trend inclining toward high lactate dehydrogenase (LDH) levels ($p = 0.06$) and the presence of unmutated *IGHV* ($p = 0.07$) was also observed in the cytokine high-risk patients (Table 1).

Cytokine Risk Groups and TFS

The patients in the cytokine high-risk group presented a lower 3-year TFS rate than those in the cytokine low-risk group (15.2 vs. 69.6 %, $p < 0.0001$) (Fig. 1a). In a multivariate analysis, the cytokine risk groups ($p = 0.0002$) along with leukocyte count ($p < 0.0001$) and *IGHV* mutation status ($p < 0.0001$) retained its independent impact on TFS (Table 2).

When the analysis was restricted to the patients with Rai stage 0–II, the estimated 3-year TFS rate was 26.4 % in the cytokine high-risk group and 79.1 % in the cytokine low-risk group of patients ($p < 0.001$) (Fig. 2a).

Cytokine Risk Groups and PFS

Of 85 (53 %) patients receiving chemotherapy due to disease progression, 53 (62 %) achieved a response including 17 (20 %) CRs and 36 (42 %) partial responses. After

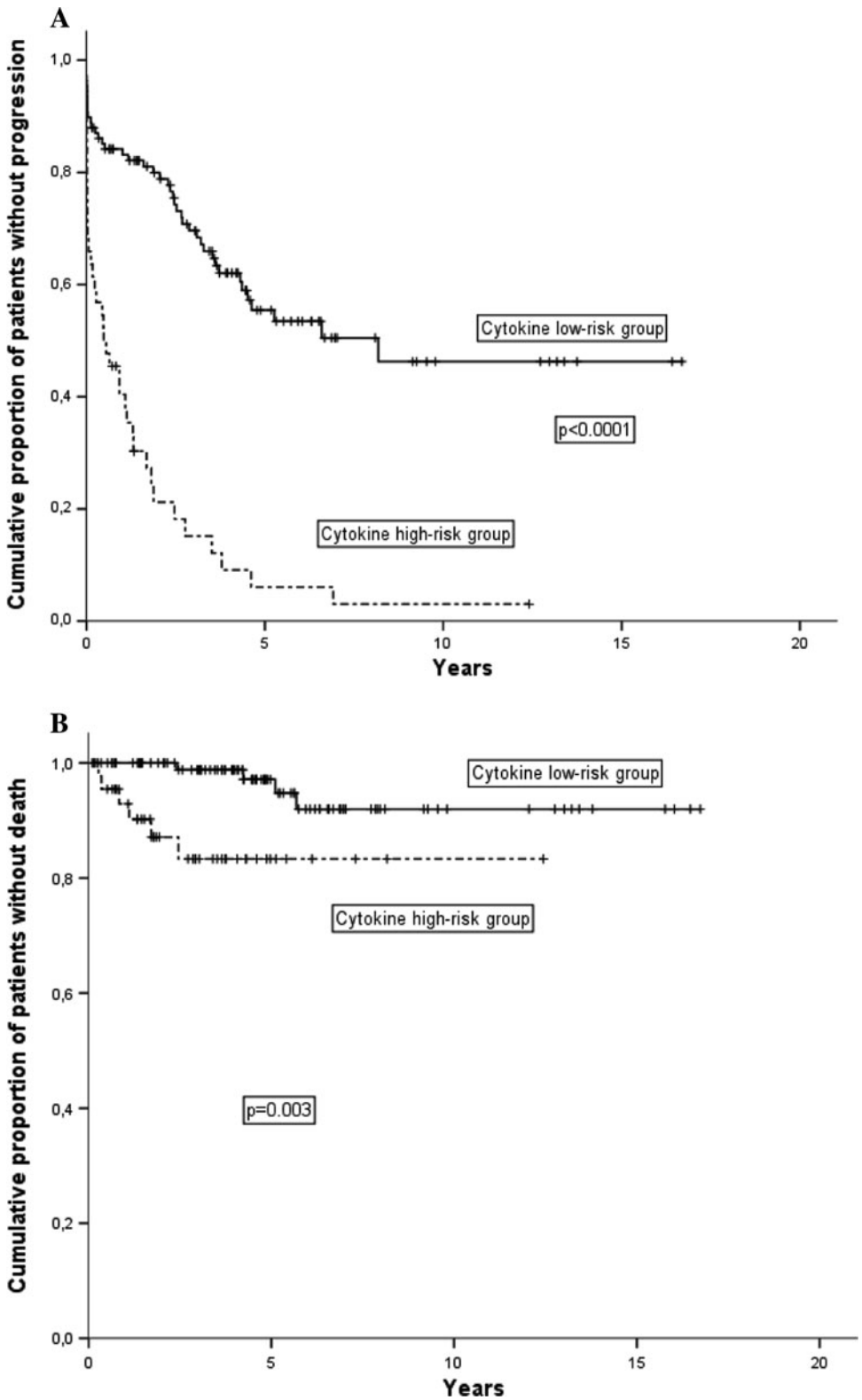

Fig. 1 Treatment-free survival (**a**) and overall survival (**b**) of 160 CLL patients according to cytokine risk groups

completion of the first-line treatment, 20 patients (38 %) experienced subsequent disease relapse or progression. Neither TNF-α nor IL-10 levels correlated with the probability of achieving a CR or PFS (data not shown).

Cytokine Risk Groups and OS

Of all 160 patients, 16 (10 %) died. The median follow-up for the patients remaining alive was 48 months (range

Table 2 Cox model describing treatment-free and overall survival

Factors	TFS	
	RR (95 % CI)	*p*
Leukocyte count × 10^9/L	1.0 (1.01–1.02)	<0.0001
IGHV unmutated versus mutated	3.2 (1.9–5.4)	<0.0001
Cytokine risk groups		
High versus low	2.7 (1.6–4.7)	0.0002
Factors	**OS**	
	RR (95 % CI)	*p*
Cytokine risk groups		
High versus low	4.8 (1.3–7.8)	0.02
Rai stage		
III–IV versus 0–II	3.7 (1.01–14.0)	0.048

RR relative risk, *95 % CI* 95 % confidence interval for RR, *TFS* treatment-free survival, *OS* overall survival

1.2–200.3 months). The estimated 5-year OS rate of patients from the cytokine high-risk group was 83.3 % compared to the 97.1 % 5-year OS rate ($p = 0.003$) in cytokine low-risk group (Fig. 1b). Using a multivariate analysis, only the cytokine high-risk group ($p = 0.02$) followed by Rai stage III/IV ($p = 0.048$) was found to be independent factors predicting shorter OS (Table 2).

When the patients were stratified according to the age at the onset of CLL, in subjects above 60 years the estimated 5-year OS rate was 83.7 % in the cytokine high-risk group and 96.8 % in the cytokine low-risk group ($p = 0.006$). In patients ≤60 years, a trend toward a shorter OS was observed in the subjects from the cytokine high-risk group compared to those in the cytokine low-risk group (82.5 vs. 91.2 %, $p = 0.06$). The cytokine risk groups do not statistically influence OS of patients with Rai stage 0–II, and only a tendency to worse survival was seen in those in the cytokine high-risk group compared to the cytokine low-risk group (88.8 vs. 98.7 %, $p = 0.1$) (Fig. 2b). The OS rates were similar in the cytokine risk groups in relation to the type of the first-line treatment.

Cytokine Risk Groups in Relation to the *IGHV* Mutation Status

We further investigated whether cytokine level-based model retains its prognostic impact in relation to the *IGHV* mutation status in 140 patients from whom DNA samples were available (Table 3). In the subgroup of 80 (57 %) patients with mutated *IGHV*, the 3-year TFS rate was lower in the cytokine high-risk group compared to the low-risk group (24.3 vs. 78.2 %, $p < 0.0001$) (Fig. 3a). In 60 (43 %) patients with unmutated *IGHV*, the 3-year TFS rate was 8.2 % in the high-risk group compared to the 49 % ($p = 0.004$) in the low-risk group (Fig. 3b). When the *IGHV* mutation status and cytokine risk groups were included in the Cox model, the unmutated *IGHV* (relative risk [RR] = 2.8, 95 % CI 1.7–4.7, $p < 0.0001$) and the cytokine high-risk group (RR = 3.7, 95 % CI 2.2–6.2, $p < 0.0001$) retained its independent impact on shorter TFS.

Cytokine risk groups did not influence OS in the mutated *IGHV* patients; however, in unmutated *IGHV* subjects, the trend toward a shorter survival was observed in the cytokine high-risk compared to the low-risk group (Table 3).

Discussion

Two major points can be concluded from this study. First, the cytokine high-risk group was significantly associated with numerous markers of advanced disease. This is consistent with other reports suggesting that elevated serum TNF-α and IL-10 levels may originate from their autonomous production by malignant cells (Foa et al. 1990; Voorzanger et al. 1996; Warzocha and Salles 1998; Mocellin et al. 2004). In addition, it has been recently reported that CD5-mediated signaling is required for constitutive activation of STATs and NFATs in CLL B lymphocytes and the following production of IL-10 (Garaud et al. 2011). However, elevated levels of cytokines may not only reflect their increased production by leukemic cells but also may be derived from T cell dysfunction, deregulation of T cell anti-tumor response and abnormal cytokine synthesis (de Waal et al. 1991; Riches et al. 2010).

Second, the independent impact of cytokine risk groups on the TFS and OS was observed. However, when we restricted the survival analysis to the patients ≤60 years and to those with Rai stage 0–II, only trends toward shorter OS were observed in the subjects in the cytokine high-risk group. It could be explained by the low number of patients and events in this particular group in both analyzed patient populations.

Other authors have reported the influence of TNF-α or IL-10 alone on the clinical course of CLL. Bojarska-Junak et al. (2008) showed the correlation between TNF-α and TFS in univariate analysis and Ferrajoli et al. (2002) demonstrated the association of high TNF-α levels with shorter survival both in univariate and multivariate model. In another study, significant correlations between high serum IL-10 levels and advanced CLL stage, tumor burden or disease transformation were observed but no influence on TFS was observed (Fayad et al. 2001). In addition, these authors showed that CLL patients with elevated IL-10 levels presented worse survival than those with low

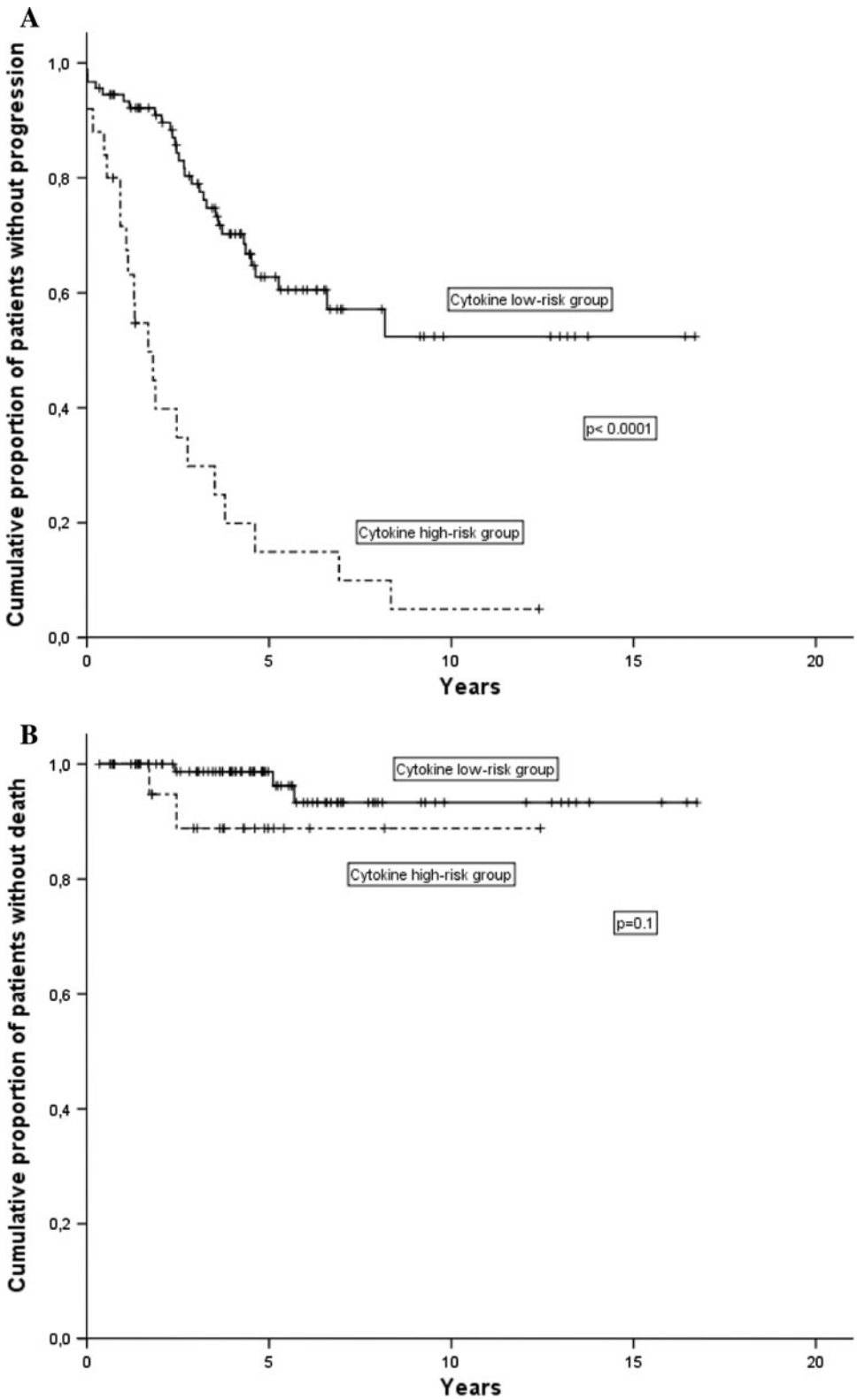

Fig. 2 Treatment-free survival (**a**) and overall survival (**b**) of 116 CLL patients with Rai stage 0–II according to cytokine risk groups

cytokine levels; however, IL-10 did not retain its independent prognostic impact on OS. In our study, we have not observed any influence of cytokines' levels on the CR or PFS. This may be explained by the fact that the patients enrolled in our study were not treated uniformly. Similarly, Ferrajoli et al. (2002) did not report any correlations between serum TNF-α and a CR rate but in the study of Bojarska-Junak et al. (2008), the patients with

Table 3 The influence of cytokine risk groups on the 3-year treatment-free survival and 5-year overall survival of 140 CLL patients in relation to the *IGHV* mutation status

	IGHV mutated		*IGHV* unmutated	
	3-year TFS % (95 % CI)	*p*	3-year TFS % (95 % CI)	*p*
TNF-α				
<The median value	88.4 (74–95)	<0.0001	58.9 (37–78)	0.004
≥The median value	28.7 (13–51)		13.6 (5–33)	
IL-10				
<The median value	81.0 (62–92)	0.007	40.0 (21–62)	0.1
≥The median value	52.5 (35–69)		29.3 (16–48)	
Cytokine risk group				
Low	78.2 (63–88)	<0.0001	49.0 (31–67)	0.004
High	24.3 (9–51)		8.2 (2–33)	
	5-year OS % (95 % CI)	*p*	5-year OS % (95 % CI)	*p*
TNF-α				
<The median value	100	0.4	100	0.04
≥The median value	95.2 (78–99)		86.2 (66–95)	
IL-10				
<The median value	93.8 (71–99)	0.9	87.6 (69–96)	0.5
≥The median value	92.3 (67–99)		66.7 (21–94)	
Cytokine risk groups				
Low	100	0.5	83.3 (44–97)	0.05
High	95.8 (80–99)		80.2 (55–93)	

p Groups were compared using the log-rank test

95 % CI 95 % confidence interval, *TFS* treatment-free survival, *OS* overall survival

hematological remission showed a lower plasma TNF-α level than non-responding subjects. Interestingly, this study demonstrated for the first time that serum TNF-α and IL-10 levels influence CLL outcome in relation to the *IGHV* mutation status. Regarding TFS, the cytokine high-risk group had an independent impact on shorter TFS both in mutated and unmutated *IGHV* patients, suggesting that the cytokine level-based model may work as an *IGHV*-independent predictor of TFS. This may help to identify patients who are at high-risk of disease progression and for whom more accurate and risk-adapted treatment could be advised. The cytokine risk groups did not influence OS in mutated *IGHV* patients, but in the unmutated *IGHV* group it allowed differentiation of patients with poor survival. It should be noted that different methods of treatment were used on the patients which may also have affected the OS in the context of *IGHV* status. These findings, although intriguing, require replication in an independent cohort study of CLL patients.

The possible mechanisms of involving TNF-α and IL-10 in the lymphoid development and disease pathogenesis are still debated. There are inconsistent reports describing their effect on CLL cells which may reflect the complexity of the TNF and IL-10 system in vivo (Foa et al. 1990; Wanidworanun and Strober 1993; Fluckiger et al. 1994; Gruss and Dower 1995; Kitabayashi et al. 1995; Tangye et al. 1998; Rath and Aggarwal 1999). It has been observed that TNF activates both cell survival and cell death mechanisms simultaneously (Rath and Aggarwal 1999). Furthermore, several studies have described the existence of autocrine as well as paracrine loops involving TNF-α and IL-10 in B cell neoplasms (Voorzanger et al. 1996; Ferrajoli et al. 2002; Mocellin et al. 2004; Garaud et al. 2011). TNF-α was interestingly 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 (Wanidworanun and Strober 1993; Mocellin et al. 2004). It could, however, be hypothesized that in patients with B cell neoplasms, 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 (Lech-Maranda et al. 2010).

It is concluded that TNF-α and IL-10 levels along with the known parameters influencing CLL outcome may be considered as a useful prognostic model in predicting the clinical course of disease. It may help in selecting patients who are at high-risk of CLL progression regardless of IGHV mutation status and for whom earlier initiation of risk-adapted treatment or maintenance therapy could be advised.

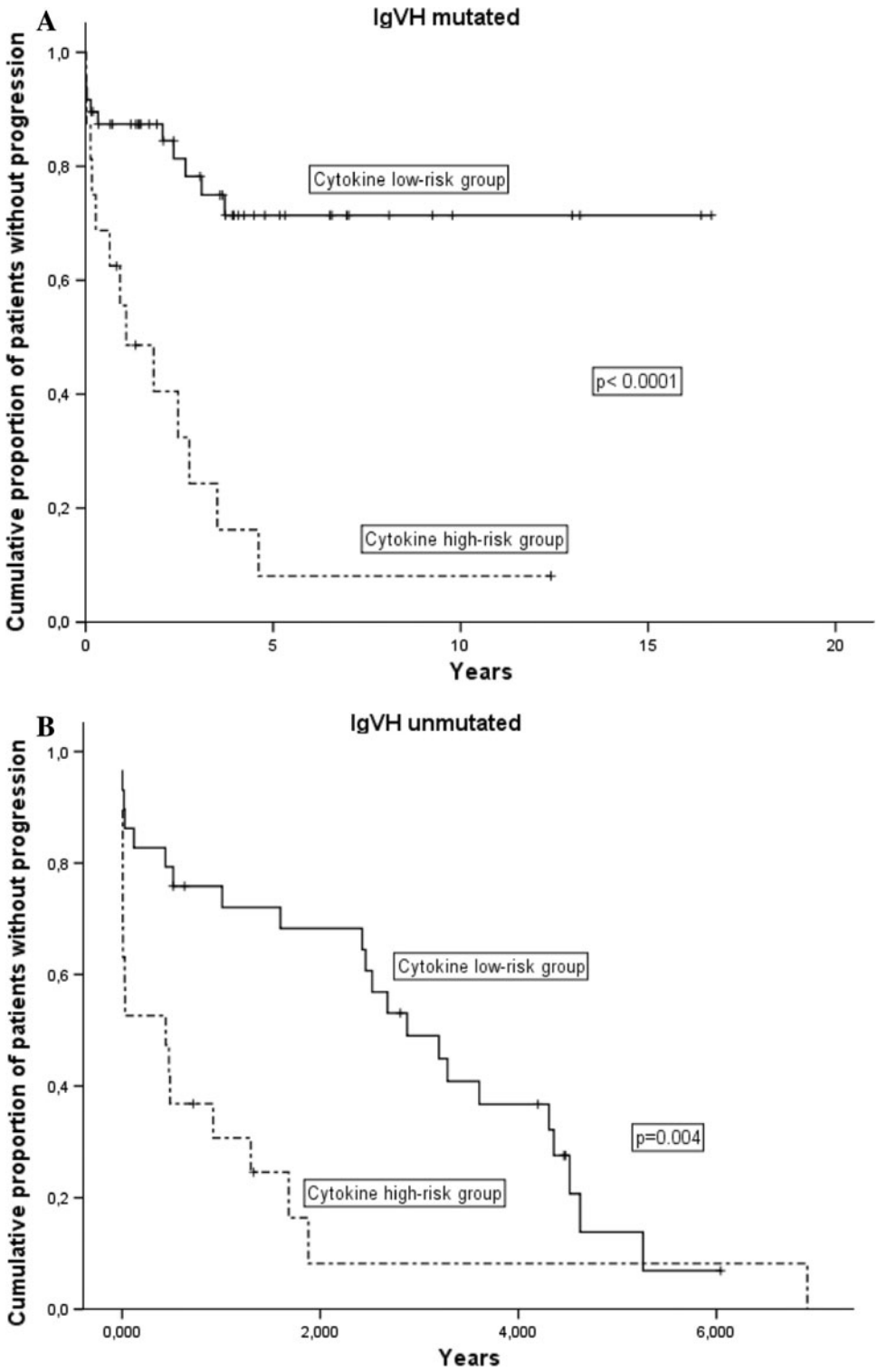

Fig. 3 Treatment-free survival of 140 CLL patients with mutated *IGHV* (**a**) and unmutated *IGHV* (**b**) according to cytokine risk groups

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