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Clinical tumor cell distribution pattern is a prognostically relevant parameter in patients with B-cell chronic lymphocytic leukemia

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Background and Objectives. B-cell chronic lymphocytic leukemia (B-CLL) cells are variably distributed among the major lymphoid compartments contributing to the heterogenous clinical presentation and course of this disease. In order to evaluate this variable distribution we propose a model for its clinical assessment.

Design and Methods. We introduce the model for tumor distribution (TD) assessment based on the total tumor mass (TTM) scoring system, where TD value represents percentage of total tumor mass infiltrating peripheral blood and bone marrow ($TD=TM_1/TTM$). TD in B-CLL can be categorized into 3 subgroups: *pure leukemia* when TD=100%, *predominantly leukemia* if TD=50-99% and *predominantly lymphoma* when TD<50%.

Results. Among 341 B-CLL patients there were 22.6%, 55.1%, 22.3%, *pure leukemia*, *predominantly leukemia* and *predominantly lymphoma* cases, respectively. The TD parameter was strongly associated in univariate analysis with TTM size, Rai and Binet stages, spleen size and β_2 microglobulin. TD was associated with response to therapy and survival, with higher TD values translating into higher response rates and longer survival. However, in univariate and multivariate Cox analysis TD displayed a much stronger relationship with prognosis in female patients, in whom it is the strongest independent predictor of survival along with age and Binet stage.

Interpretation and Conclusions. TD, a quantitative and simple clinical parameter, easily assessed in all patients, offers a reliable tool for evaluation of tumor cell distribution in B-CLL. It has independent and strong prognostic power in females, as opposed to males, possibly unmasking important, as yet unrecognized, biological difference in B-CLL patients.

Key words: CLL, prognosis, gender, tumor distribution pattern, total tumor mass score.

B-cell chronic lymphocytic leukemia (B-CLL) and small lymphocytic lymphoma (B-SLL), although included in the same entity among the indolent B-cell lymphocytic malignancies by the REAL and WHO classifications,[1,2] differ by definition in the distribution of neoplastic cells within body compartments. In the spectrum of B-lymphoproliferative disorders from B-CLL to B-SLL, the neoplastic cells can be distributed solely in the bone marrow (BM) and peripheral blood (PB) compartments, comprising so-called *pure leukemia* cases at one end of the spectrum, or, at the other end, they can solely inflitrate organs, representing *pure lymphoma* cases. However, in the vast majority of cases indolent lymphocytic malignancies involve all compartments, with a variable extent of infiltration. Consequently, there is not only the difference between *pure* leukemias and lymphomas, which are the extremes, but also variety within the *intermediate* cases because of the different extent of involvement of various compartments (Figure 1A). According to both recent classifications, so-called *intermediate* cases and *pure* leukemia cases are classified as B-CLL.[1,2]

Much evidence has recently been gathered concerning lymphocyte trafficking and homing, and

the receptors and mechanisms involved both in normal conditions and in lymphoid neoplasia.[3] B-CLL offers a unique model to study these phenomena in human diseases, since clonal proliferation may express minor, but important intraclonal differences with the different cell subsets having variable homing properties.[4]

The distribution pattern of tumor mass among various compartments depends upon intrinsic properties of neoplastic cells, particularly on their expression of adhesion molecules (such as integrins, selectins, immunoglobulins, CD44 and their ligands), which are essential for the recirculation of lymphocytes and their malignant counterparts.[3,5] This may depend on prior antigen stimulation. It may also be subject to change, spontaneously or after therapeutic interventions, during the course of the disease. It is therefore of interest to study this particular aspect of the disease which has, so far, been rather ignored, possibly because a reliable tool for clinical assessment of this feature was lacking.

It is well known that B-CLL is a disease characterized by a highly variable clinical presentation and course.[6] Some patients may have relatively stable disease and a survival comparable to that of the age-and gender-matched general population, whilst others have a progressive course and a significantly shorter survival. During the past three decades many features have been shown to influence prognosis: the ones currently considered most relevant are simple clinical parameters such as Rai and Binet stages,[6-8] extent of tumor mass (TTM score),[9] age and gender,[10,11] whose prognostic value has been confirmed in many studies. More recently, in order to improve the prognostic assessment of B-CLL cases a number of additional clinical and biological parameters have been introduced. Among the biological parameters, soluble CD23,[12-14] β_2 microglobulin,[15,16] p53,[17] $p27^{kip1}$,[18] CD 38 expression and Ig V gene mutational status,[19-22] bcl-2/bax ratio,[23] and 11q deletion,[24] demonstrate a strong relationship with prognosis but, so far, have not entered the routine definition of different prognostic categories.[25]

In this study we address the biological and clinical relevance of the distribution pattern of the tumor mass in B-CLL taking into consideration peripheral blood versus lymphoid organ compartments, which in the past have not been extensively evaluated.[6] Attention has been focused on individual clinical parameters and adhesion molecules,[4,6,26,27,28] while the profile of adhesion molecules has not yet been related to the tumor distribution pattern assessed by a clinical mea-

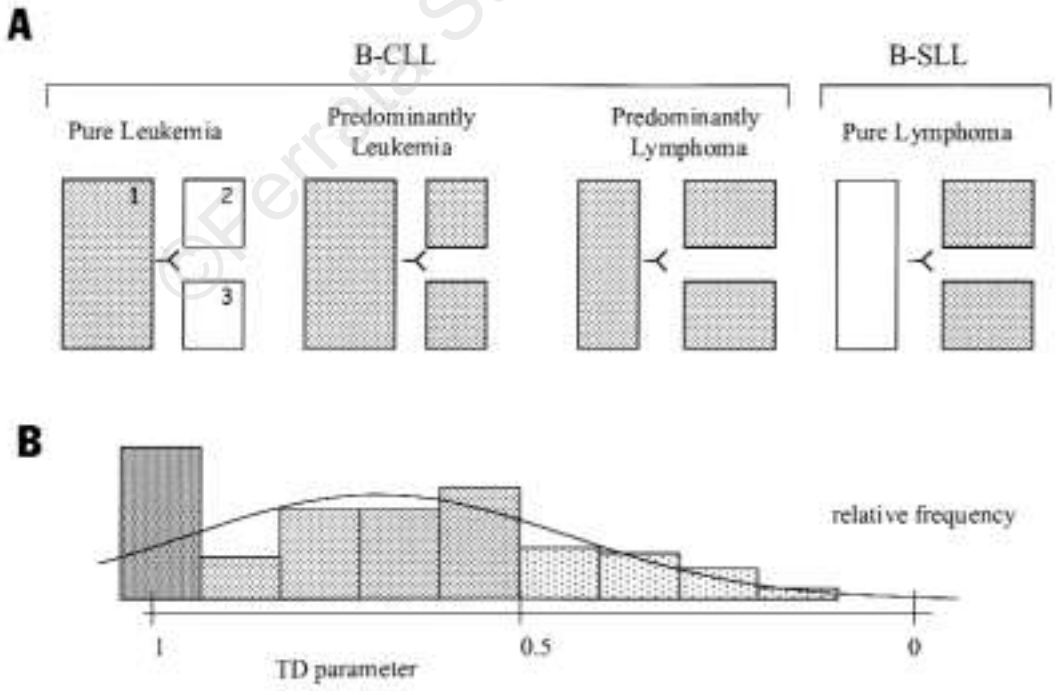

Figure 1A. Different extent of involvement of various compartments in the spectrum of indolent lymphocytic malignancies including B-CLL and B-SLL: (1) peripheral blood and bone marrow compartment; (2) lymph node compartment; (3) spleen compartment. Figure 1B. relative frequencies of B-CLL of cases with different TD parameter.

Table 1. Patient characteristics and prognosis.

		Median survival (months)	*p value*
Number of patients	341		
Gender			
male	223 (65,4%)	51	<0.001
female	118 (34.6%)	76	
Age (years)			
mean±SE/range	61.6±0.6/27-87		<0.001
≤55	96 (28.2%)	74	
>55	245 (71.8%)	51	
TTM score			
mean±SE/range	11.2±0.4/2.3-46.6		
≤9	150 (44.0%)	76	<0.001
>9 to ≤15	111 (32.5%)	54	
>15	80 (23.5%)	42	
Lymphocyte ($\times 10^9$/L)			
mean±SE	56.4±3.7		
≤30	169 (49.6%)	64	NS
>30	172 (50.4%)	58	
Lymph nodes (cm)			
mean±SE	1.9±0.1		
no lymphadenopathy	93 (27.3%)	89	<0.001
lymphadenopathy	248 (72.7%)	54	
Spleen (cm)			
mean±SE	2.7±0.2		
no splenomegaly	177 (51.9%)	72	<0.001
splenomegaly	164 (48.1%)	43	
Hemoglobin (g/L)			
mean±SE	126.3±1.3		
≤100	50 (14.7%)	32	<0.001
>100	291 (85.3%)	66	
Rai stage			
0	72 (21.1%)	96	<0.001
I+II	195 (57.2%)	58	
III+IV	74 (21.7%)	38	
Binet stage			
A	130 (38.1%)	88	<0.001
B	137 (40.2%)	50	
C	74 (21.7%)	38	

sure. It would, therefore, be useful to devise a simple and reproducible clinical tool able to express the tumor distribution pattern. We propose a simple and reliable quantitative model to assess tumor cell distribution in B-CLL. We analyze its relationship with other important clinical and biological features of the disease, as well as its impact on prognosis, with the final purpose of assessing its biological relevance.

Design and Methods

Patients

The present retrospective study was performed on 341 newly diagnosed B-CLL patients followed at Merkur University Hospital, Zagreb, Croatia, and Bianchi-Melacrino-Morelli Hospital, Reggio Calabria, Italy. The diagnosis of B-CLL was based on morphologic and immunophenotypic features, according to the rules employed at the time of the patients' presentation. There were 223 males and 118 females within the group, with a mean age of 61.6±0.6 years (range 27 to 87). Most of these patients were included in randomized trials,[29,30,31] which resulted in a somewhat higher proportion of advanced B-CLL patients.[32] Mean TTM size was 11.2±0.4 (range 2.3-46.6) with 130, 137 and 74 patients in Binet stage A, B and C, respectively (Table 1). To assure that the analyzed series behaved in the prognostic study as a typical B-CLL patient population, an extensive prognostic analysis was performed with known and well-established prognostic parameters. In univariate analysis, classical clinical prognostic factors including TTM, Rai and Binet stages, gender, and age showed strong relationships with prognosis as reported in Table 1. The data show that the analyzed population behaved in the prognostic study like most other published series of B-CLL patients.[6] As far as treatment is concerned, 182 patients were treated with high-dose chlorambucil (HD-CLB), 29 with Binet's CHOP, and the remaining cases either were not treated or were mostly treated with standard-dose chlorambucil (SD-CLB), fludarabine (FAMP), and other regimens.

Fresh or cryopreserved B-CLL cells and sera from 54 patients diagnosed at Merkur University Hospital were available for assessment of surface and intracellular markers and for measurement of soluble marker levels. The institutional ethical committee approved the study and informed consent was obtained from all participants. Samples were obtained from newly diagnosed patients or before new treatment courses for some patients in clinical relapse requiring treatment. These patients were not included in the general prognostic analysis because of their short follow-up. Out of 54 cases, 32 were males and 22 females with a mean age of 63.3 years (range 37-85), 32 were untreated and 22 previously treated. In this subset of cases, mean TTM size was 11.2±6.7, with 26, 8 and 20 patients in Binet stage A, B and C, respectively.

Tumor distribution

The calculation of the tumor mass distribution (TD) was based on the total tumor mass (TTM) score system.[7] TTM is the sum of TM_1 +TM_2 +TM_3 where: TM_1 = square root of the absolute number of peripheral blood lymphocytes/nL, TM_2 = diameter of the largest palpable lymph node in cm, TM_3 = extent of

palpable spleen below the costal margin in cm. Therefore TM_1 takes into account the peripheral blood (and bone marrow) compartment, TM_2 and TM_3 the lymph node and spleen compartment, respectively. For example, a patient with a peripheral blood lymphocytosis of 36×10^9/L, lymphadenopathy with the largest node diameter of 2 cm and a spleen palpable 3 cm below left costal margin has a TTM score of 11 ($TTM= \sqrt{36} + 2 + 3 = 11$).

We propose the following model for the assessment of tumor mass distribution (TD):

$$TD=TM_1/TTM$$

where TD=1 corresponds to a *pure* leukemic form of B-CLL, while TD values approaching 0 correspond to a clinical presentation of *pure lymphoma*. The TD variable has a continuous character and is independent from the absolute amount of infiltration of various compartments because it depends only on the relative amount of infiltration. It represents the percentage of tumor mass within the bone marrow and peripheral blood compartment. For example, TD=1.00 for the *pure* leukemic form of the disease irrespective of absolute lymphocytosis, whereas TD=0.60 occurs in a patient with a lymphocytosis of 81×10^9/L, largest lymph node 2 cm, and spleen palpable 4cm below the costal margin, or in a patient with a lymphocytosis of 36×10^9/L, largest lymph node 3 cm, and a spleen palpable 1 cm below costal margin.

For analytical purposes we converted TD, which has a continuous character into an ordinal variable and divided B-CLL cases into 3 subgroups: *pure leukemia* when TD=100%, *predominantly leukemia* when TD=50-99%, *predominantly lymphoma* when TD=1-49%. Within the spectrum of all indolent B-lymphocytic malignancies a fourth subgroup of patients with *pure lymphoma* can be added, represented by those patients with TD=0%.

Serum analysis

β_2 microglobulin (B2MG) (Immunotech) and soluble CD23 (sCD23) (Serotec) levels were measured by an ELISA method according to the manufacturer's specifications.

Cell analysis

Flow cytometric analysis was performed on a Coulter Epics XL. CD19-PE (DAKO) surface labeling, bcl-2-FITC (DAKO), bax (Santa Cruz Biotechnology) intracellular labeling and determination of mean fluorescence intensity (MFI) of the above-mentioned markers were performed according to previously published methods.[23]

Table 2. Patient characteristics according to the TD parameter subgroups.

TD=	*Pure leukemia 100%*	*Predominantly leukemia 50-99%*	*Predominantly lymphoma 1-49%*	*p value*
Number of patients	77	188	76	–
Gender				
male	49	122	52	NS
female	28	66	24	
Age (years)				
mean±SE	61.2±1.2	61.2±0.8	63.1±1.3	NS
range	38-80	33-86	27-87	
TTM score				
mean±SE	4.6±0.2	12.3±0.5	15.4±0.7	<0.001
range	2.3-12.0	3.5-46.6	5.3-29.5	
Lymphocytes ($\times10^9$/L)				
mean±SE	18.4±2.8	90.2±8.3	42.8±6.4	<0.001
Lymph nodes (cm)				
mean±SE	–	1.9±0.2	3.7±0.4	<0.001
Spleen (cm)				
mean±SE	–	2.5±0.4	6.5±0.8	<0.001
Hemoglobin (g/L)				
mean±SE	140.6±2.6	131.9±2.7	109.0±4.8	<0.001
Rai stage				
0	72	0	0	<0.001
I+II	0	150	45	
III+IV	5	38	31	
Binet stage				
A	72	54	4	<0.001
B	0	96	41	
C	5	38	31	

Statistical analysis

The chi-squared test was used to compare categorical data, and Student's t-test was used to analyze differences between group means. Correlations were assessed between continuous parameters.

In univariate prognostic analysis the log-rank test was used and in multivariate prognostic analysis Cox proportional hazard's model was used.

Probability values (p) below 0.05 were considered statistically significant, and values of Pearson's correlation coefficient (r) greater than 0.5 were considered as markers of strong correlation, provided $p<0.05$. In Cox proportional hazard's model using forward stepwise regression $p<0.05$ was the criterion for entering the model, and $p>0.10$ was the criterion for removing the variate

from the model. BMDP statistical software was used for the analysis.

Results

Characteristics of TD parameter

The pattern of tumor distribution (TD) found in the examined population of patients and the patients' characteristics are reported in Table 2. The majority of cases had a *predominantly leukemia* presentation (55.1%), while *pure leukemia* and *predominantly lymphoma* forms were equally represented (22.6% and 22.3%, respectively) (Figure 1B).

Relationship of TD and other established clinical and laboratory parameters

The TD parameter showed a strong correlation with TTM size (r=-.56), spleen size (r=-.77), β_2 microglobulin (r= .72), and to a lesser extent with erythrocyte sedimentation rate, sizes of lymph nodes and liver, hemoglobin and platelets levels. TD was not correlated with age, lymphocyte count, soluble CD23, or bcl-2/bax ratio. There was, however, a significant although not linear association between lymphocyte count and the TD parameter (Table 2). Lower levels of TD parameter were associated with higher Rai and Binet stage (p<0.05). There was no difference between genders in the distribution of the TD parameter (p=0.30) (Table 2).

TD and response to treatment

Response to treatment was known in 272 patients. TD was related to response observed after first line treatment with a response rate (complete (CR) and partial (PR) response) of 92.0%, 74.1% and 65.0% and CR rates of 74.0%, 43.2%, and 20.0% for TD *pure leukemia*, *predominantly leukemia* and *predominantly lymphoma* TD, respectively. There was no difference between genders regarding response rates.

When we analyzed HD-CLB-treated patients in IGCI CLL-01 and CLL-02 trials,[25,26] CR rates were 93.3%, 66.4%, 48.1% for *pure leukemia*, *predominantly leukemia* and *predominantly lymphoma* TD, respectively. Compared to treatment with SD-CLB and CHOP, HD-CLB therapy was shown to be superior in the *predominantly leukemia* subgroup. In the *predominantly lymphoma* subgroup there was a trend for better response to HD-CLB over SD-CLB (odds ratio=2.7, p=0.12), but there was no difference between response to HD-CLB and CHOP (odds ratio=1.3, NS). Analysis by trial and treatment is reported in Table 3.

TD and prognosis

TD showed a strong relationship with prognosis

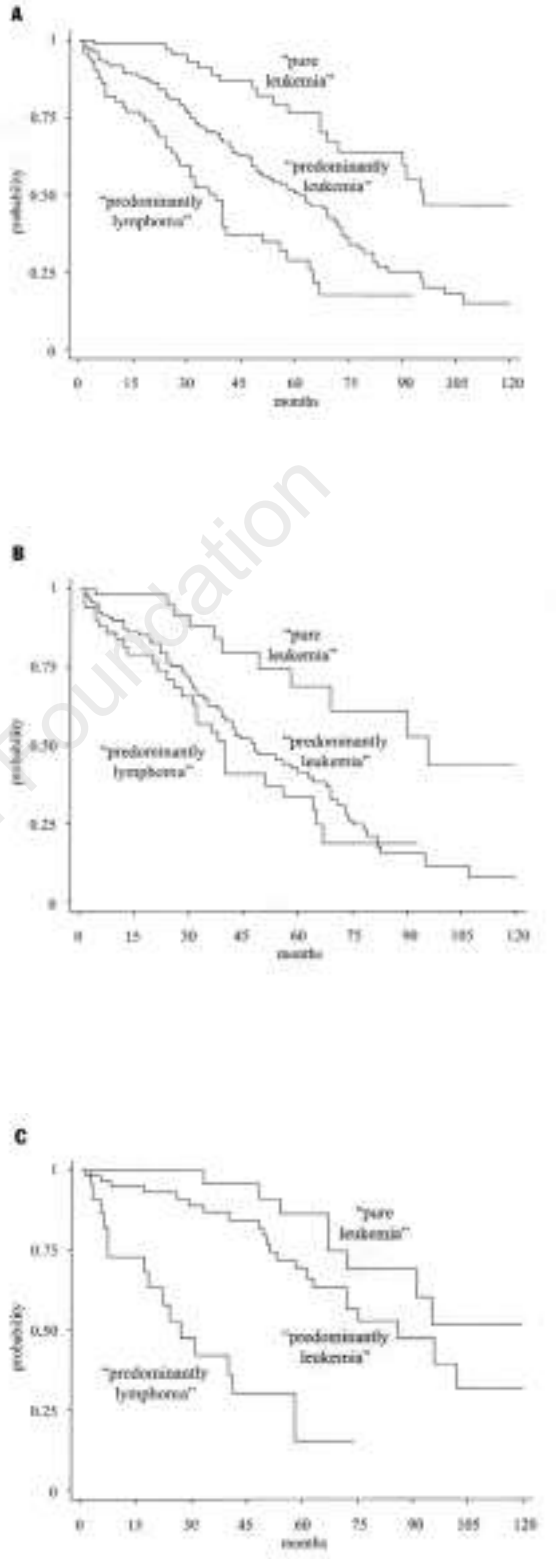

Figure 2. Survival by TD parameter for (A) all patients, (B) males and (C) females.

Table 3. Relationship between TD and clinical response in IGCI CLL-01 and IGCI CLL-02 trials.

	TD	CR+PR	MR+NR	Odds ratio	p value
IGCI-01	100%	26 v 6	0 v 1	–	NS
HD-CLB v SD-CLB	50-99%	64 v 11	9 v 12	11.6	<0.001
	1-49%	25 v 9	7 v 7	2.7	NS
IGCI-02	100%	4 v –	0 v –	v –	–
HD-CLB v CHOP	50-99%	72 v 43	7 v 16	3.82	<0.01
	1-49%	16 v 21	4 v 7	1.3	NS

CR: complete remission; PR: partial response; NR: no response. Response values presented as percentage.

Table 4. Median survival in months in three TD categories by TTM strata, Rai and Binet stages.

	Median survival (months)			
	pure leukemia 100%	predominantly leukemia 50-99%	predominantly lymphoma 1-49%	p value
TTM strata				
≤9	95	62	32	<0.001
>9 to ≤15	48	59	35	
>15	–	54	32	
Rai stage				
0	96	–	–	<0.05
I+II	–	63	40	
III+IV	42	43	20	
Binet stage				
A	96	72	40	<0.01
B	–	53	39	
C	42	43	19	

with a median overall survival of 95, 61 and 36 months for *pure leukemia*, *predominantly leukemia* and *predominantly lymphoma* presentation, respectively ($p<0.0001$) (Figure 2A).

Unexpectedly, the relationship with survival was stronger in females than in males. In the female population (Figure 2B) it clearly distinguished 3 prognostic groups, with median survival not reached for *pure leukemia*, and a median survival of 81 and 26 months in the *predominantly leukemia* and *predominantly lymphoma* groups, respectively. In the male population it failed to discriminate the latter 2 groups (median survival of 48 and 39 months, respectively) (Figure 2C).

Because of its association with TTM size, Rai and Binet stages, we analyzed TD's impact on prognosis across TTM strata and Rai and Binet stages. TD retains its impact on prognosis in each TTM stratum (ie –9, 9.1-15, 15.1-). Also in Binet stages TD clearly distinguishes different prognostic groups within Binet stage A ($p<0.05$) and stage B ($p<0.05$). However, within Binet stage C there is a significant association with survival only in female patients ($p<0.05$). Analysis by TTM strata, Rai and Binet stages is reported in Table 4.

Table 5. Results of the Cox proportional hazards model evaluating prognostic significance of the TD parameter in male and female patients.

Parameter	Estimate (value±SE)	p value	Hazards ratio
All patients			
Binet stage (A,B,C)	0.5329±0.1250	0.0000	1.7039
Age (years)	0.0505±0.0089	0.0000	1.0518
Therapy (HDCLB, other)	0.2983±0.1666	0.0747	1.3476
TD*	0.4078±0.1425	0.0040	1.5036
Male patients			
Binet stage (A,B,C)	0.7188±0.1388	0.0000	2.0520
Age (years)	0.0567±0.0109	0.0000	1.0584
Female patients			
TD*	0.7707±0.2795	0.0042	2.1612
Binet stage (A,B,C)	0.4670±0.2159	0.0334	1.5953
Age (years)	0.0416±0.0169	0.0195	1.0399

**TD parameter divided into 3 categories in the following order: pure leukemia, predominantly leukemia and predominantly lymphoma.*

In multivariate analysis, using Cox proportional hazard's model, TD entered the model as a significant prognostic factor ($p=0.004$) in all patients after Binet stage, age, and therapy (Table 5). However, in the female population TD was the strongest predictor of prognosis ($p=0.004$) followed by age ($p=0.019$), and Binet stage ($p=0.033$) (Table 5), whereas in the male population it failed to enter the model as an independent prognostic factor at a significant level after Binet stage ($p=0.000$) and age ($p=0.000$) had been entered (Table 5).

Discussion

Indolent lymphocytic malignancies include a large variety of clinically different diseases such as indolent non-follicular non-Hodgkin's lymphomas and chronic lymphocytic leukemias. Recently, both the REAL and WHO classifications[1,2] have clearly stated that the lymphocytic neoplastic disorders with or without leukemic spread belong to the same pathologic entity. On the other hand, the heterogeneity of B-CLL presentation and prognosis requires an accurate evaluation of clinical and biological parameters at diagnosis in order to estimate prognosis and to

design the appropriate treatment plan.

In the present study we have introduced a new clinical parameter, the tumor cell distribution pattern - TD, to evaluate the distribution of the tumor burden among the lymphoid compartments in the spectrum of indolent lymphocytic malignancies.[1,2] This study was done and TD validated in a series of patients fulfilling criteria for and behaving in prognostic analysis like a typical B-CLL study population. TD combines simple clinical elements used in other staging systems,[6] focusing on the distribution of tumor cells between peripheral blood and bone marrow compartments and the lymph nodes and spleen compartments. It is a quantitative parameter, is easily assessed in all patients, and includes the two extremes that are *pure leukemia* and *pure lymphoma* forms of the disease. Although the TD parameter was validated in B-CLL patients only, it would be interesting to evaluate its value within the spectrum of all indolent lymphocytic malignancies.

In this series of B-CLL patients we found that the TD parameter was highly associated with TTM size, Rai and Binet stages. *Predominantly lymphoma* B-CLL tends to have a higher tumor mass and more advanced stage. These findings can be explained by different associations of the TD parameter with its own components.[6] TD was not clearly associated with lymphocytosis. A lower level of association was demonstrated with lymph node size and a higher level of association with spleen size.

In a more limited subset of cases, the TD parameter showed a strong correlation with B2MG and failed to show a significant correlation with sCD23 levels or bcl-2/bax ratio. This can explain the difference between B2MG and sCD23. Although both parameters strongly correlate with TTM and clinical stages,[12,15,33,34] and are validated as prognostic factors, they appear as the expression of different features. In our series of patients B2MG was not associated with lymphocytosis, and was highly associated with spleen size and to a lesser extent with lymph node size. On the other hand, sCD23 demonstrates the same level of association with all compartments, likewise TTM size. Thus B2MG and sCD23 appear as biological counterparts of TD and TTM parameters, respectively.[33]

Response to chemotherapy was also correlated with TD. Along with this series of patients mostly treated with HD-CLB, we analyzed the relationship of the TD parameter and response in data from 2 multicenter randomized studies comparing treatment with HD-CLB versus treatment with SD-CLB and CHOP, respectively. The randomized studies demonstrated the superiority of HD-CLB as compared to both SD-CLB and Binet's CHOP.[29,30] When we analyzed the relationship between TD and response to therapy, we first considered only patients treated with HD-CLB in order to rule out the possible impact of different treatments with possibly different efficacies. In this subset of 182 patients, those with both the leukemic forms of the disease demonstrated higher response rates than patients with *predominantly lymphoma* disease (data not shown). On the other hand, taking into consideration the three therapeutic regimens employed in these patients, a higher response rate was obtained after HD-CLB in both leukemic forms, while in *predominantly lymphoma* patients the efficacy of HD-CLB and Binet's CHOP was comparable, in line with the clinically well-recognized efficacy of the CHOP regimen also in indolent lymphoma.[35,36] Finally, monitoring TD during therapy and differentiating the efficacy of chemotherapy at the level of different compartments could be of clinical relevance in designing more effective combination therapy. Moreover, we would like to emphasize the possible importance of TD evaluation when the role of adhesion molecules involved in lymphocyte recirculation[3] is investigated at a clinical level. It may significantly contribute to the explanation of results dealing with the relationship of individual adhesion molecules with clinical presentation, course, response to treatment, and prognosis.[26,27,28,37,38]

The results of the present study demonstrate that TD has a strong prognostic power, differentiating three subgroups with different survival: that is, *pure leukemia*, *predominantly leukemia* and *predominantly lymphoma* presentation. It is evident that the first subgroup, roughly corresponding to Rai stage 0, has a very favorable outcome. The prevalence of disease presentation at organ and lymph node level is associated with a less favorable outcome in terms of both response rate and overall survival. Although significantly associated with TTM size, Rai and Binet stages, the TD parameter maintained its influence on prognosis in TTM strata, and Binet and Rai stage subsets. In all patients with Binet stage A as well as in Binet stage B, TD clearly differentiates 2 subgroups with different prognoses. Within stage C patients, i.e. in the group with very poor prognoses, it can differentiate groups with different prognoses only among females. Very surprisingly, when patients were divided according to their gender, the difference between *predominantly leukemia* and *predominantly lymphoma* patients was no longer significant among males in either univariate or multivariate analysis. In contrast, female patients with *predominantly lymphoma* TD show an extremely

poor prognosis. Their median survival of 26 months is shorter than that of male patients with the same TD (39 months). Moreover it is significantly shorter than that of female patients with Binet stage C (48 months). This information could be of importance in treatment decisions. It is also worth noting that this is the only example in which female patients with roughly the same clinical features show a worse prognosis than male patients.[6] That *predominantly lymphoma* disease may have a different behavior according to gender of the patient may give new insights into the biology, possibly at molecular level, of a gender-specific influence on prognosis.[10]

It may be very interesting to relate this new finding with two recently described biologically different subsets of B-CLL, identified according to CD38 expression and Ig V genes configuration and their different prognoses.[19, 20,21,22] According to one of these reports[19] there was a gender ratio difference, with naive B-CLL occurring almost exclusively in the male population, while memory B-CLL were nearly equally frequent in both genders. Moreover, since antigen stimulation can modify and guide adhesion molecule profile in normal lymphocytes and their malignant counterparts, the role of adhesion molecules in B-CLL cannot be ruled out although, so far, such differences regarding gender have not been reported.

It would also be very interesting to relate TD to 11q23 deletion because patients with 11q deletion have significant lymphadenopathy, and there is a strong impact on prognosis in young patients.[24] However, in our group of patients we did not find any differences in the impact of TD on prognosis between age subsets.

Although it is possible that some of the patients in the *predominantly lymphoma* subgroup previously diagnosed as having B-CLL actually had the leukemic form of mantle cell lymphoma (MCL) with poor prognosis (median survival time 40-50 months), the overall incidence of leukemic MCL is low compared to that of B-CLL (representing only few percent).[39,40] Moreover, there is no difference in terms of prognosis between MCL patients with and without leukemic expression, as well as between genders.[39] We therefore believe that any potential influence of MCL cases on the present study is only minor.

Because of the highly heterogeneous prognosis and clinical presentation of B-CLL it is important to analyze the prognostic power of single factors in defined subsets of patients, since the importance of some parameters relevant in defined group, such as males or females,[10,11] younger or older,[41] naive or memory B-CLL,[19] can be masked when evaluated in larger, composite series.

In conclusion, the present study identifies and validates a new clinical parameter of proven prognostic power for response and survival, with different behavior between genders. Finally, beside the clinical relevance, the biological significance of this parameter could be clarified by further studies on the role of adhesion molecules in the recirculation of neoplastic cells. In this respect, it would be important to confirm the present findings in different series of B-CLL patients and to extend the experience in the correlation with the adhesion molecule profile.

Contributions and Acknowledgments

OJ and BJ conceived, designed and drafted the study, RV, APP, FM, and MB, were the main contributors of patients' data. RK, MMK, and IKS performed the laboratory tests. OJ, VPJ, and BJ analyzed and interpreted the data and critically reviewed the draft. Finally, FM, MB, and BJ reviewed the concepts and conclusions of the study.

OJ conceived and drafted the study. RV, RK, MMK, VPJ, IKS, and APP are listed in order of the amount of data they contributed. FM, MB, BJ are the last names because of their peer review of the data and manuscript.

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Disclosures

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Potential implications for clinical practice

B-CLL is a disease with highly variable clinical presentation and course.[42] The introduction of a quantitative and simple TD parameter, easily assessed in all patients, offers a reliable tool for clinical evaluation of tumor cell distribution in B-CLL. Its independent and strong prognostic power in females, as opposed to males, possibly unmasks important, yet unrecognized biological difference in B-CLL patients.

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