Prognostic relevance of serum β2 microglobulin in patients with follicular lymphoma treated with anthracycline-containing regimens. A GISL study

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ABSTRACT

Background and Objectives

Although serum β2 microglobulin (β2M) is an easy parameter to measure, and over-expressed in a large number of lymphoproliferative diseases, its prognostic value has been largely underestimated. The present study examined the influence of β2M levels on overall survival (OS) of patients with follicular lymphoma (FL).

Design and Methods

The prognostic role of β2M was evaluated in 236 patients with FL identified from the databases of the *Gruppo Italiano per lo Studio dei Linfomi (GISL)* and treated with anthracycline-based regimens from 1993 to 2003.

Results

Elevated serum β2M levels were found in 82 patients (35%). According to multivariate logistic regression analysis, elevated β2M levels were associated with elevated lactate dehydrogenase (LDH) (p=0.021), age (p=0.029), and number of involved nodal areas (p<0.001). The percentage of elevated β2M levels increased progressively with increasing FLIPI scores (17%, 38%, and 63% in the low-, intermediate-, and high-risk groups, respectively). Five-year OS was 61% (95% CI, 47-73%) and 89% (95% CI, 82-93%) for patients with elevated vs normal β2M levels respectively (p<0.001). Cox regression analysis showed that β2M level had an independent and stable prognostic value (HR=3.0; 95%CI, 1.6-5.7). In a multivariate analysis the impact of β2M level on survival was independent of FLIPI score, with a HR of 2.94 (95% CI, 1.54-5.62).

Interpretation and Conclusions

Our results demonstrate that in patients treated in the pre-rituximab era, β2M level was an independent prognostic marker in addition to FLIPI score. We thus suggest that β2M be routinely assessed and tested in future prognostic studies of FL patients treated with combination chemotherapy and anti-CD20 agents.

Key words: β2 microglobulin, follicular lymphoma, GISL, prognosis, survival.

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Follicular lymphoma (FL) is one of the most frequent and well-characterized subtypes of malignant lymphoma in Western countries and accounts for ~10-15% of all malignant lymphomas in adults.[1] The disease is usually characterized by an indolent clinical course, impressive response to initial therapy, frequent relapses, and response to salvage therapy of shorter duration. Despite continuous efforts to develop effective therapies for FL, population-based studies have shown only modest improvements in long-term survival over the last 30 years.[2] As a result, the appropriate initial therapy for FL patients remains a matter of debate. Several treatment options are available, ranging from no initial treatment (i.e., watchful waiting), to high-dose therapy, requiring hematopoietic stem cell support.[3] With such a range of options, it is generally agreed that initial treatment should be chosen according to the risk of disease progression in individual patients.

To date, several attempts have been made to better define the prognosis of patients with FL. A predictive model of survival in FL patients was first proposed by the Italian Lymphoma Intergroup (ILI).[4] The ILI prognostic index is based on six factors: age, gender, B-symptoms, number of extranodal (EN) sites of disease, erythrocyte sedimentation rate (ESR), and serum lactate dehydrogenase (LDH) levels. More recently, the Follicular Lymphoma Prognostic Factor Project developed the Follicular Lymphoma International Prognostic Index (FLIPI) based on age, Ann Arbor stage, hemoglobin (Hb) level, number of nodal areas involved and LDH levels.[5] The ILI and FLIPI indices define three risk categories associated with different survival rates. However, although the indices have demonstrated clinical relevance, they suffer from the limitation of their retrospective nature and share potential biases.[6] For instance, though significantly associated with outcome at univariate analysis, it was decided not to include serum β2 microglobulin (β2M) in the test sample of the FLIPI because of the very high proportion of patients with missing data.

β2M is a low molecular weight, single polypeptide chain that is considered the light-chain molecule of the major histocompatibility complex class I antigens. β2M is found on the membrane surface of almost all nucleated cells. It is particularly plentiful on the surface of white blood cells, and white blood cell membrane turnover is the principal source of serum β2M.[7,8] Increased levels of β2M have been found to be of prognostic relevance in a wide variety of malignancies, including multiple myeloma,[9] chronic lymphocytic leukemia,[10] Hodgkin's lymphoma,[11] and aggressive and indolent lymphomas in general.[12,13]

Here we present the results of a study on the prognostic value of serum β2M levels in FL patients who were treated by the *Gruppo Italiano per lo Studio dei Linfomi* (GISL) in prospective clinical trials for 10 years prior to the advent of anti-CD20 therapy. We analyzed the effects of β2M levels on survival and evaluated the additional prognostic value of β2M levels in FLIPI-identified risk groups.

Design and Methods

The archive of patients enrolled in one of the prospective clinical trials of the GISL group was our source for patient selection. All clinical trials selected for this study were approved by local ethical committees and by the review board of the GISL. All patients signed an informed consent form prior to starting treatment in accordance with the ethical standards of the responsible committee on human experimentation and with the declaration of Helsinki. The possibility of performing future unplanned analyses was explicitly indicated in the study protocol and in the informed consent forms. The current study was considered ethically acceptable by the review board of the GISL. To be considered for the study, patients had to have a diagnosis of FL (any grade) made between 1993 and 2003, be 18-75 years of age, have Ann Arbor stage I-IV disease, have information on serum β2M levels at time of diagnosis and have been treated with anthracycline-containing combination chemotherapy. Patients with stage I-II disease were only enrolled if they had bulky disease or were three or more involved areas. A study data set was defined that included demographics, clinical and laboratory features at diagnosis, treatment details, and follow-up information. *BCL-2* gene status was not routinely assessed; therefore, we did not include it in the data set. All baseline data, including β2M levels, were collected by people who were blind to the patients' treatment and outcome.

The extent of disease was coded according to the Ann Arbor staging system and assessed by clinical examination, chest and abdomen computed tomography scan, and bone marrow trephine biopsy. Response to treatment was determined 1 month after the end of induction therapy with the examinations necessary to verify the absence of abnormal findings at diagnosis. All evaluations of stage and response to treatment were based on data recorded by GISL investigators. Response criteria for non-Hodgkin's lymphoma proposed by the International Workshop were applied.[14] Unconfirmed complete response was combined with complete response (CR) for the purpose of the present analysis.

Statistical analysis

Fisher's exact test was used to evaluate differences between categorized distributed variables, whereas independent factors related to β2M levels were assessed by logistic regression analysis. Survival was calculated from the date of diagnosis using the Kaplan-Meier method. The log-rank test was used to compare subgroups in the univariate analysis of survival. Multivariate analysis of factors related to survival was performed using the Cox's proportional hazards regression method considering variables that were statistically significant according to the univariate analysis. The proportional hazards assumption was checked by the Grambsch-Therneau method. *p* val-

Table 1. Patients' baseline clinical features.

Features		*Total No.*	*No.*	*%*
Gender	Male	236	120	51
Age	> 60 years	236	85	36
Ann Arbor stage	III-IV	236	167	71
No. of extranodal sites	>1	236	52	22
No. of nodal areas	> 4	236	74	31
Performance status	>1	236	5	2
B-symptoms	present	236	33	14
LDH level	> UNL	228	42	18
ESR	≥ 30 mm/h	223	52	23
Hemoglobin level	< 12 g/dL	232	38	16
Albumin level	< 3.5 g/dL	223	28	13
Serum β2M level	> UNL	236	82	35
FLIPI risk				
Low			102	45
Intermediate		225	71	32
High			52	23

LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; β2M: β2 microglobulin; FLIPI: follicular Lymphoma International; Prognostic Index; UNL: upper normal limit.

ues <0.05 were considered statistically significant. The coefficient stability of the Cox proportional hazards regression, obtained by adjusting β2M levels by variables with $p<0.10$ in the univariate analysis, was checked by a bootstrap method.[15] All analyses were performed with Stata Statistical Software Release 8.0 (StataCorp, College Station, TX, USA).

Results

Based on the study inclusion criteria, 236 (81%) out of 291 FL patients were selected from the GISL archives for the present analysis. The median age of the patients was 57 years (range 25-77 years) with 36% of patients over 60 years old. The main clinical characteristics of the study population are shown in Table 1. Eighty-two patients (35%) had serum β2M levels above the upper normal limit (UNL). Finally, according to the FLIPI, 45%, 32%, and 23% of cases were classified at low, intermediate, or high risk, respectively. Clinico-laboratory characteristics and outcome of the 236 patients with available β2M values did not differ from those of the remaining 55 cases (*data not shown*).

All patients received immediate treatment with an anthracycline-containing regimen. One hundred and eighty-seven patients were treated with a CHOP-like regimen, and 49 patients were treated with a PromaceCytaBOM-like regimen. A median of six cycles of chemotherapy (range, 2-8 cycles) was given. The chemotherapy program was completed in 229 (97%) of

Table 2. Univariate and multivariate logistic regression analyses for β-2 microglobulin levels and clinical and laboratory baseline features.

Features *%*	*β-2M > UNL p value*	*Univariate p value*	*Multivariate*
Gender			
Male	38	0.413	
Female	32		
Age			
≤60 years	31	0.154	
> 60 years	41		
Age (continuous form)			
Median	58	0.014	0.029
(range)	(29-75)		
Ann Arbor stage			
I-II	20	0.003	
III-IV	41		
No. of extranodal sites			
0-1	30	0.005	
>1	52		
No. of nodal areas			
0-4	26	<0.001	<0.001
> 4	53		
Performance Status			
0-1	35	>0.5	
2	40		
B-symptoms			
absent	32	0.047	
present	52		
LDH level			
UNL	30	0.004	0.021
> UNL	55		
ESR			
< 30 mm/h	33	0.250	
≥30 mm/h	42		
Hemoglobin level			
≥12 g/dL	30	0.001	
< 12 g/dL	61		
Albumin level			
≥3.5 g/dL	32	0.011	
< 3.5 g/dL	57		

β2M: β2 microglobulin; LDH: lactate dehydrogenase; UNL: upper normal limit; ESR: erythrocyte sedimentation rate.

the patients, and it was interrupted due to complications in seven (3%) patients. Forty-seven (20%) patients received local irradiation after chemotherapy because of initial bulky disease or the presence of residual masses; involved field radiation treatment was administered to 13/21 patients with stage I disease and in 16/48 cases with stage II.

Radiotherapy was always administered as involved field treatment. With induction therapy, 171 patients (72%) achieved a CR, 47 (20%) a partial response, and 18 (8%) patients were classified as non-responders. Fifty patients relapsed after a median interval from diagnosis of 23 months. Salvage treatment for patients with progressive or relapsed disease consisted of chemotherapy with or without radiotherapy (n=53), high dose chemotherapy followed by stem cell transplantation (n=9), radiotherapy

Table 3. Univariate analysis of survival according to baseline clinical and laboratory features.

Features	*5-year survival rate* %	*95% CI*	*Univariate analysis p value*
Gender			
Male	75	64-83	0.145
Female	83	74-90	
Age			
60 years	81	72-88	0.082
>60 years	76	64-85	
Ann Arbor stage			
I-II	87	74-94	0.151
III-IV	76	68-83	
No. of extranodal sites			
0-1	82	75-88	0.024
>1	68	50-81	
No. of nodal areas			
0-4	81	73-87	0.124
>4	76	63-86	
B-symptoms			
absent	84	77-89	<0.001
present	54	33-70	
LDH level			
≤UNL	88	82-93	<0.001
>UNL	42	25-58	
ESR			
<30 mm/h	87	79-92	0.002
≥30 mm/h	61	44-74	
Hemoglobin level			
≥12 g/dL	83	76-88	0.002
<12 g/dL	59	39-75	
Albumin level			
≥3.5 g/dL	83	76-88	0.002
<3.5 g/dL	54	30-73	
Serum β2M level	≤		
≤UNL	89	82-93	<0.001
>UNL	61	47-73	

CI: confidence interval; LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; β2M: β2 microglobulin; UNL: upper normal limit.

alone (n=2), palliative or no treatment (n=10), or unknown treatment (n=11). Rituximab alone or in combination with other therapies was used in 22 patients who received salvage therapy since 1999. Forty-six (20%) patients died at a median interval of 27 months (range 1-88 months) after diagnosis. Thirty-three patients died of disease progression and 13 patients died while off-therapy in CR (3 cases) or in partial response not requiring further therapy (10 cases). After a median follow-up of 46 months (range, 3-140 months) for all cases and 51 months (range, 5-140) for surviving patients, the 5-year survival rate was 80% (95% confidence interval (CI), 73-85%). The mortality rate was 4.5 % per year.

β2M levels at diagnosis

The frequency of elevated β2M levels, according to the main clinical and laboratory parameters, is reported in Table 2. According to univariate analysis with Fisher's exact test, elevated β2M levels were associated with Ann Arbor stages III-IV (p=0.003), >1 extranodal sites of disease (p=0.005), >4 involved nodal areas (p<0.001), the presence of B-symptoms (p=0.047), LDH level >UNL (p=0.004), Hb <12 g/dL (p=0.001), and serum albumin level <3.5 g/dL (p=0.011). Elevated β2M levels were also associated with age when the variable was managed as a continuous variable (p=0.014, Mann-Whitney test). In a multivariate logistic regression analysis, β2M levels were significantly associated with LDH >UNL (p=0.021), number of involved nodal areas (p<0.001) and age (p=0.029).

Survival analysis

According to univariate analysis, >1 extranodal sites of disease, LDH >UNL, presence of B-symptoms, Hb levels <12 g/dL, 1hr ESR ≥30 mm/hr, albumin levels <3.5 g/dL, and β2M levels >UNL had a negative impact on survival (Table 3). Performance status was not included in the univariate analysis because of the low number of patients with a poor performance status. In addition to the above-mentioned parameters, FLIPI was also a strong predictor of outcome with a 5-year survival rate of 90%, 78%, and 59% for patients at low, intermediate, and high risk, respectively (p<0.001).

Patients with β2M >UNL had a 5-year survival of 61% (95% CI, 47-73%) vs. 89% (95% CI, 82-93%) for patients with β2M ≤UNL (p<0.001; Figure 1). The hazard ratio (HR) for β2M in univariate analysis was 4.1 (95% CI, 2.2-7.4). The multivariate Cox regression model also confirmed the prognostic value of β2M (Table 4a). In particular, the discriminating power of β2M was only slightly decreased by the inclusion of potentially confounding factors (HR=3.0, 95% CI, 1.6-5.7, p<0.001), confirming its role as an independent prognostic factor. The prognostic role of β2M levels was also confirmed in an analysis limited to patients with advanced stage disease (*data not shown*).

Relationship between β2M levels and FLIPI

The proportion of patients with β2M >UNL increased progressively with increasing FLIPI scores, being 17%, 38%, and 63% in the low-, intermediate-, and high-risk groups, respectively (Spearman correlation=0.386, test for trend p<0.001). The impact of β2M on survival was measured in each FLIPI risk group. In terms of 5-year survival, the differences were statistically significant in the low (92% vs. 82%, p=0.035) and intermediate (90% vs. 56%, p=0.001) risk groups, but not in the smaller high risk group, although also in this group a trend toward a better outcome for patients with normal β2M values was observed (70% vs. 53%, p=0.264; Figure 2). When both β2M and FLIPI were included in a multivariate analysis of survival, the independent prognostic value of β2M was confirmed, with a HR of 2.94 (95% CI, 1.54-5.62; Table 4b).

Discussion

Despite serum β2M level being an easy parameter to measure, so far its prognostic value has been largely

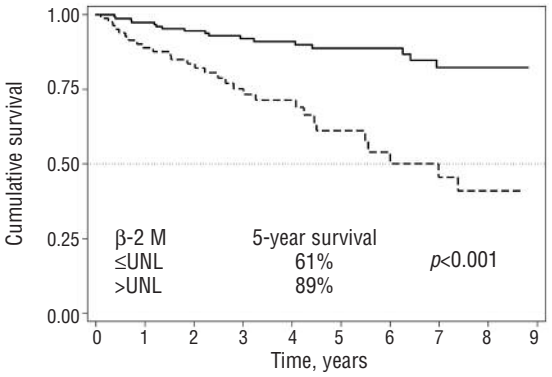

Figure 1. Overall survival according to baseline β2Mlevels. Continuous line, β2M≤UNL; broken line; β2M>UNL; β2M: β2 microglobulin; UNL: upper normal limit.

underestimated. In fact, although β2M was identified more than 30 years ago and was found to be a promising prognostic factor in most lymphoproliferative disorders, few attempts have been made to assess its relevance in a prognostic index. A prognostic index based on serum β2M levels was proposed for patients with diffuse large B-cell lymphoma in the late 1980s, and a simple score based on β2M and LDH levels was able to identify patients with different risks of death.[16]

In a more recent study, baseline serum β2M levels were found to be independent prognostic factors, along with molecular response. Based on this observation, Lopez-Guillermo *et al.* proposed that FL patients could be stratified into prognostic groups according to baseline β2M levels and molecular response to induction therapy.[17]

The prognostic value of β2M serum levels was assessed in two studies dealing with the risk of histological transformation, with apparently conflicting results. In the study by Bastion *et al.*, β2M was the only independent prognostic factor.[18] In the more recent study by Gine *et al.* FLIPI and histological subtype, but not β2M, were found to have independent prognostic roles.[19] However, it is notable that Bastion *et al.* did not analyze FLIPI as a risk factor.

Unfortunately β2M, although significantly associated with survival at univariate analysis, was not included in the test sample of FLIPI and in the final scoring system because of the very high proportion of patients with missing data (available in 17% of cases).[5] Insufficient measurements of β2M also precluded the inclusion of β2M levels in other prognostic models, such as ILI and IPI.[4,20]

We tested the prognostic value of β2M in a large series of FL patients who were homogeneously treated with upfront anthracycline-containing regimens in the context of controlled clinical trials performed by the GISL. In order to be included in these clinical trials patients were required to have an ambulatory Performance Status and in particular to have normal renal function, thus limiting the chance that medical conditions other than lymphoma could lead to altered β2M levels. The almost complete

Table 4. Multivariate Cox regression analysis of overall survival for β2 microglobulin levels and parameters with *p*-values <0.10 from univariate analysis (a) and for β2 microglobulin levels and FLIPI (b).

Features	*HR*	*95% CI*	*p value 95% CI*	*Boot p value*	*Boot*
		Panel (a)			
Serum β2M level	≤				
≤UNL	1.0				
>UNL	3.0	1.6-5.7	0.001	1.4-6.4	0.005
Age					
≤60 years	1.0				
> 60 years	2.0	1.1-3.7	0.027	1.0-4.2	0.059
No. of extranodal sites					
0-1	1.0				
>1	1.2	0.6-2.4	0.537	0.6-2.7	0.610
B-symptoms					
absent	1.0				
present	1.8	0.9-3.6	0.074	0.8-4.3	0.155
LDH level					
≤UNL	1.0				
>UNL	3.0	1.5-6.1	0.002	1.3-6.9	0.009
ESR					
<30 mm/h	1.0				
≥30 mm/h	1.3	0.7-2.7	0.438	0.5-3.2	0.543
Hemoglobin level					
≥12 g/dL	1.0				
<12 g/dL	1.2	0.6-2.5	0.641	0.5-3.1	0.728
Albumin level					
≥3.5 g/dL	1.0				
<3.5 g/dL	1.2	0.6-2.6	0.573	0.5-2.9	0.621
		Panel (b)			
Serum β2M level					
≤UNL	1.00				
>UNL	2.94	1.54-5.62	0.001	1.45-5.97	0.003
FLIPI					
0-1	1.00				
2	1.64	0.76-3.57	0.207	0.72-3.76	0.238
≥3	3.10	1.41-6.73	0.005	1.31-7.26	0.010

HR: hazard ratio; CI: confidence interval; β2M: β2 microglobulin; LDH: lactate dehydrogenase; ESR: erythrocyte sedimentation rate; FLIPI: Follicular Lymphoma International Prognostic Index; UNL: upper normal limit.

compliance with induction therapy (completed in 97% of cases), along with the homogeneity of treatment (all patients were treated with anthracycline-containing regimens), the appropriate observation time (which exceeded 4 years for patients at risk of death), and the limited number of patients lost to follow-up (n=4) allow us to maintain that this series of patients was appropriate for an accurate prognostic evaluation of β2M levels. That is, for patients with FL elevated β2M levels have a strong adverse prognostic value, being associated with a poorer outcome in terms of long-term survival. It is also relevant that β2M retained its prognostic value in a multivariate analysis performed including all the prognostic parameters utilized in the FLIPI, IPI, and ILI indices. Although FLIPI was useful for classifying patients into different risk groups in our study sample, we found that β2M level had an additional, independent value in discriminating prog-

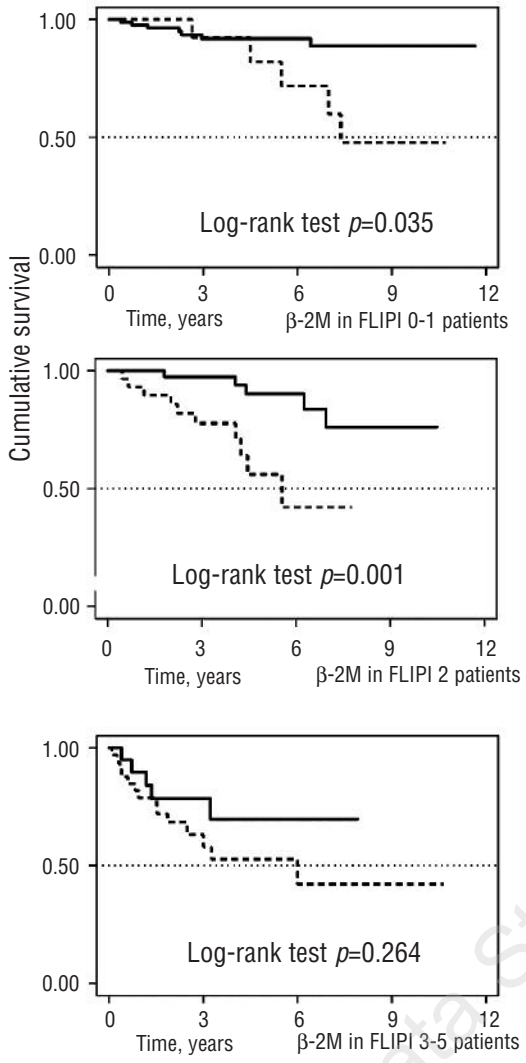

Figure 2. Survival in FLIPI risk groups according to baseline β2 microglobulin levels. Top: score 0-1; middle: score 2; bottom: score 3-5 stratified according to β2M level. Continuous line: β2M ≤UNL; broken line: β2M >UNL. FLIPI: follicular Lymphoma International Prognostic Index; β2M: β2 microglobulin; UNL: upper normal limit.

nosis. Our data suggest that the prognostic effect of β2M is also retained in the three FLIPI groups, although it seems to be more evident in the low and the intermediate-risk groups. However in the high risk group the lack of a statistically significant difference could have depended on the smaller number of cases falling in this category in our study sample. The association between β2M levels and survival suggests that soluble β2M reflects an important biological process that is somehow correlated with tumor mass. In fact, we found that β2M levels were significantly associated with elevated baseline LDH levels and with the number of nodal areas involved. Similarly, in our study, high β2M levels were correlated with anemia, which could be considered an effect of bone marrow involvement by lymphoma. The strong independent effect of β2M on survival suggests that β2M may not be a simple surrogate of tumor mass but could reflect some still unknown biological characteristics of the tumor or of the microenvironment, with an additive effect on the risk of death. With multivariate logistic analysis, we found an association between elevated β2M and age when the latter was analyzed as a continuous variable (Table 2); this association could be explained by an effect of the different *biology* of the elderly patient on β2M (i.e. reduced creatinine clearance). Although correlated, both age and β2M level retained independent roles in predicting overall survival, thus suggesting that in the population of patients with FL analyzed in the present study age had only a limited role, if any, on the increase of β2M.

At present, it is not known whether the prognostic value of β2M in B-cell lymphomas can be altered by treatment. This is particularly relevant for FL patients, for whom the addition of anti-CD20 monoclonal antibody to chemotherapy has been shown to improve survival compared to chemotherapy alone.[21,22] As in the FLIPI, IPI, and ILI studies, none of the patients in our series received rituximab as part of their initial therapy. For this reason, we are cautious about generalizing our results to patients treated with anti-CD20 monoclonal antibody therapies. However, based on the recent study by Buske *et al*. who demonstrated that in patients treated with CHOP plus rituximab, FLIPI maintained its discriminating power,[23] we can expect that the prognostic role of β2M will also be confirmed. Our study demonstrates that information on β2M serum levels may contribute, significantly and independently from FLIPI, to the prognostic definition of FL patients treated upfront with anthracycline-containing chemotherapy. The role of serum β2M levels in the prognosis of FL must be confirmed in future studies investigating FL patients treated with combination chemotherapy plus anti-CD20 agents.

Appendix

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Authors' Contributions

MF, CG and SL conceived and designed the study and drafted the article; CM, LM and MoB collected, analyzed and interpreted the data; MF, CG and SL revised the statistical data; MaB critically reviewed the draft; UG, AM, FM, SP, CS, AL, PGG, LB, SB and VF provided clinical information; MF, CG, SL and MaB finally reviewed the concepts and conclusions of the study. All the authors agreed on the final version of the manuscript.

Conflict of Interest

The authors reported no potential conflicts of interest.

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