Letter to the editor

Absolute Lymphocyte Count Is Unrelated to Overall Survival in Newly Diagnosed Elderly Patients with Multiple Myeloma Treated with Immunomodulatory Drugs

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The absolute lymphocyte count (ALC) has been widely studied in hematologic and solid malignancies as a marker of host antitumor immunity. Its significance has been evaluated in multiple myeloma (MM) and other hematological tumors [1-3] during different clinical stages of disease [4,5]. It has been studied as an independent prognostic factor for survival in patients with acute myeloid leukaemias (AML) [6] and included in several validated prognostic scores, such as the International Prognostic Score (IPS)[7]. Ege et al have reported that ALC, determined as a surrogate marker of host immune status in patients with newly diagnosed MM, is an independent prognostic factor for clinical outcome [8]. However, the role of ALC at diagnosis in patients with MM not eligible for ASCT, treated with regimens containing immunomodulatory drugs (IMIDs), has been less explored and scanty data can only be deduced from few studies on non-Hodgkin's lymphoma [9].

Aim of the current report was to evaluate the role of ALC at diagnosis in older patients with Multiple Myeloma not eligible for ASCT, treated with IMIDs.

We analyzed data on 435 consecutive patients with MM aged $\geq$ 65 y.o., collected between 1998 and 2006. All cases required to be originally diagnosed, followed, and treated at the four participating centers. Patients were excluded if they had a concomitant diagnosis of malignancy or any other plasma cell proliferative disorder. The primary endpoint was to assess the role of ALC on overall survival (OS) at the time of MM diagnosis. We also evaluated the influence of ALC on first complete remission (CR) rate and progression free survival (PFS) at two and four years. Complete remission was defined as follows: paraprotein not measurable by serum proteins electro- phoresis, Bence Jones proteinuria not detectable on urine electrophoresis, and bone marrow aspiration showing less than 5% plasma cells.

Logistic regression (step wise selection) was performed to estimate the significance of ALC on efficacy of first line treatment and we used as prognostic factors age, ALC, sex, type of MM, and

the ISS. ALC was assessed as a continuous variable and dichotomized based on finding the optimal cut-off point.

The overall survival (OS) was analyzed using the approach of Kaplan and Meier[10], and it was calculated from the date of MM diagnosis to the end of study or last available follow-up. Differences between survival curves were tested for statistical significance using the two-tailed log-rank test. Median OS was not reached for one group, so we calculated the mean OS. As a continuous variable, ALC was analyzed also through the Receiver Operating Characteristic (ROC) curve with the calculation of the Area under curve (AUC).

Multivariate analysis was performed using the Cox proportional hazards model [11]. Hazard ratio with confidence interval (CI) was calculated for the survival analysis. As selection criteria of the variable to be included in the model we used the stepwise method with entry probability set at 0.05 and probability to remove set at 0.10. . PFS, the ratio of surviving patients at a specific time, were also calculated in the two groups at 2 and 4 years.All P-values represented were two-sided, and statistical significance was declared when $P < 0.05$.

Overall, 125 patients satisfied the criteria to be included in the current analysis:.
All enrolled patients were treated with regimens containing thalidomide or lenalidomide. The first-line treatment of MM included: melphalan, prednisone and thalidomide (MPT, in 89 patients); vincristine, melphalan, prednisone and thalidomide (VMPT) administered in 24 patients; 5 patients received a combination of thalidomide and dexamethasone (TD) ; 2 patients received lenalidomide and dexamethasone, 2 were treated with cyclophosmamide, lenalidomide and prednisone (CRP), 2 patients were treated with cyclophosmamide, thalidomide and dexamethasone (CTD), 1 with melphalan, dexamethasone and thalidomide (MDT). Patients characteristics and their distribution are reported in Table 1, according to the ALC ($\geq$1.4x109/l vs. <1.4x109/l) at presentation. The

choice of ALC ≥1.4x109/l as the cut-off point was supported by the available data in the literature, furthermore it yielded the greatest differential in survival, from the log-rank test.

In particular, 51 patients presented values of ALC lower than 1.4 and 74 patient higher than 1.4. Comparing the two groups with respect to all the considered parameters we didn't find any significant difference (Table I). Only hemoglobin values are slightly higher in the ALC >1.4 group (p=0.048).

Complete remission (CR) after first-line treatment was observed in 33 (27.7%) patients, very good partial remission (VGPR) in 36 (30.2%) patients and partial remission (PR) in 18 patients (15.1%). A persistent disease was found in 38 patients (30.4 %). Comparing the two groups for the response to treatment, we didn't find any difference in the two groups (chi square test, p=0.47), so ALC did not influence the efficacy of the first line treatment. These data are confirmed also when evaluating at cytofluorimetric assay T lymphocytes subpopulation (CD4+and CD8+). We have also evaluated the relation between ALC and the success of the first line treatment through logistic regression putting sex, age, type of MM (kappa or lambda), ISS and ALC as covariates but no significance was found (sex p= 0.238, age p= 0.678, type of MM p= 0.245, ISS p= 0.929 and ALC p= 0.218). Lymphocytes subpopulation (CD4+and CD8+) count did not vary significantly during treatment with IMIDs. Considering the overall survival probability, we didn't find any difference between the two ALC groups: with a global mean OS of 60.4 months (CI 53.9-63.9, range 1-78), the patients with more than 1.400 showed a mean OS of 58.5 months (CI 50.4-66.6, range 1-78) and the patient with less than 1.400 showed a mean OS of 63.2 (CI 53.5-72.9, range 1-77), (Figure 1, logrank test p=0.60).

We extended the analysis to a multivariate approach , using the Cox regression and considering ALC groups (HR 0.69, CI 0.40-1.18, p= 0.18) as prognostic factor corrected for sex (p= 0.75), age (p= 0.032), type of MM (p= 0.162) and ISS (p= 0.704) and still no significant difference was found with the exception of age of the patients that shows a quite obvious slight higher risk for subjects with increasing of age (HR1.115, CI 1.008-1.235).

The two years-PFS for the group with ALC lower than 1,400 was 90.2% (5 deaths) against 86.3% (8 deaths) of the ALC patients with more than 1,400. The four year-PFS of the two groups was 86.3% (7 deaths) and 82.4% (13 deaths), respectively. "with a value of 0.5787, wefound that ALC values are not able to discriminate between the two groups of survivor and dead". No statistical difference was still found.

In our study, high levels of ALC at diagnosis did not identify, among patients with MM aged > 65 years not eligible for ASCT, those at longer survival. These data are only in part in contrast with previous investigations. We here report on a populationolder than 65 years, treated with immunomodulatory drugs where ALC seems also unrelated to first line treatment response A potential limitation of the use of ALC as a prognostic marker consists in the different cutoff values reported in survival analysis [12]. This suggests that ALC might not be the bestsurrogate marker of host immunity to understand the underling antitumor mechanisms, in specific clinical contexts. The lack of significance of ALC, in patients treated with thalidomide-based regimens [5,13] supports the hypothesis that other prognostic factors are needed in such population.This could be particularly true when usingchemotherapy with immunomodulatory drugs, since these treatments improve CR and OS in MM patients regardless of ISS and chromosomal abnormalities at diagnosis [14].Different reported effects on baseline ALC in studies exploring the action of immunomodulatory drugs on autoimmune or hematological disorders support the hypothesis that still unknown mechanisms, less explored in elderly patients, are able to affect treatment response.

POTENTIAL CONFLICT OF INTERSTS

The authors declare that they have no conflict of interest

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Table I. Baseline characteristics of patients

(According to ALC $\geq$ 1.4 x 10^9/l vs ALC < 1.4 x 10^9 / l at diagnosis of MM)

CHARACTERISTICS	All (n= 125)	ALC < 1.4 x 10^9/l (n = 51)	ALC $\geq$ 1.4 x 10^9/l (n =74)	P Value
Age at diagnosis (years, median and range)	71.5 (50-85)	75 (50-83)	70 (50-85)	0.0503*
Sex (n) • Male • Female	 73 52	 35 16	 38 36	0.0701§
Albumin (g/l, median and range) • All levels • < 3.5 (n=56) • > 3.5 (n=69)	 3.5 (0.8-4.8) 3.1 (0.8-3.5) 4.0 (3.5-4.8)	 3.4 (2.5-4.5) 3.1 (2.5-3.4) 4.0 (3.5-4.5)	 3.7 (0.8-4.8) 3.0 (0.8-3.5) 4.0 (3.5-4.8)	 0.0081* 0.1800* 0.1201*
β2micr (mg/l, median and range) • All levels • < 3.5 (n=42) • > 3.5 (n=83)	 4.3 (1.3-43.1) 2.6 (1.3-3.4) 8.0 (3.5-43.1)	 3.9 (1.3-43.1) 2.8 (1.3-3.1) 6.8 (3.5-43.1)	 4.4 (1.3-14.2) 1.9 (1.3-3-4) 6.9 (3.6-14.2)	 0.5598* 0.0650* 0.6374*
Calcium (mg/dl, median and range)	9.2 (7.3-17.2)	10 (5.8-13.8)	10.6 (6.1-16.5)	0.1292*
Hemoglobin (g/dl, median and range)	10.2 (5.8-16.5)	9.9 (1.5-13.8)	10.9 (7.8-16.5)	0.048*
ISS (n) Stage I Stage II Stage III	 36 45 44	 16 19 16	 20 26 28	0.7430^
LDH (U/L, median and range)	308 (101 3340)	308 (127-3340)	308.5 (101-1022)	0.9478*
Componente M (g/dl), median (range)	3.2 (0.4-7.3)	4 (0.4-7.3)	2.8 (1.0-6.7)	0.4223*
Platelets x 10^9/l, median (range)	187 (71-649)	186 (77-649)	189 (71-393)	0.4201*
Creatinine (mg/dl) media (range)	1.1 (0.5-21.1)	1.0 (0.5-21.1)	1.1 (0.5-8.7)	0.4084*

* Mann-Whitney test (independent samples)
§ Fisher's exact test
^ Chi square test