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Can Infused autograft lymphocyte-to-monocyte ratio predict survival in Multiple Myeloma Post autologous peripheral blood hematopoietic stem cell transplantation?

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ABSTRACT

The infused autograft lymphocyte-to-monocyte ratio (A-LMR) is a prognostic factor for survival in classical Hodgkin lymphoma (cHL), diffuse large B-cell lymphoma (DLBCL) in addition to T cell lymphoma post-autologous peripheral hematopoietic stem cell transplantation (APHSCT). Thus, we check out to investigate if A-LMR is also a prognostic factor for survival post-APHSCT in multiple myeloma (MM).From 2009 to 2015, eighty two MM patients that underwent APHSCT were retrospectively analyzed. The number of lymphocytes and monocyte events were derived from stem cell harvest after mobilization. Cells were analyzed using flow cytometry and A-LMR was calculated by dividing the number of lymphocyte events by the number of monocyte events. Survival outcomes were estimated using Kaplan-Meier method and compared by the log-rank test. Patients with an A-LMR≥1 showed the same progression-free survival (PFS) comparing to patients with an A-LMR<1 and both in disease status (CR versus VGPR) [median PFS was not reached vs 1600 days,1000-days PFS rates of 75% (95%, CI 64–94 %) vs 54% (95%, CI 35–82%), p= 0.23 respectively; [median PFS was not reached vs1600 days, 1000-days PFS rates of 70 % (95% CI 57–85 %) vs 72% (95% CI, 56–93%), p= 0.77]. The infused autograft lymphocyte-to-monocyte ratio can't predict survival in patients with MM undergoing APHSCT. Further study is needed to validate this finding.

Keywords: Autograft lymphocyte-to-monocyte ratio, Survival, Autologous peripheral hematopoietic stem cell transplantation, Multiple myeloma.

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INTRODUCTION

The infused autograft lymphocyte to monocyte ratio (A-LMR) has recently been reported to be a prognostic factor for survival with patients post-autologous peripheral hematopoietic stem cell transplantation (APHSCT) in classical Hodgkin lymphoma (cHL),, diffuse large B-cell lymphoma (DLBCL) and T cell lymphoma [1-3]. However, the relation between A-LMR and clinical outcomes in multiple myeloma (MM) post-APHSCT has not been studied.

A-LMR combines the biomarkers A-ALC (infused autograft-absolute lymphocyte count) and A-AMC (infused autograft-absolute monocytes count) [1], represent respectively, the host immunity that is critically important for survival in MM treated with APHSCT [4,5] and in non-Hodgkin's Lymphoma (LNH) [6].Whereas the second correspond to a surrogate marker of tumor immunosuppression by monocytes and their progeny, as derived myeloid cells (MDSCs) [7,9]. Indeed, these cells are one of the major factors limiting the immune response in cancer [8] by influencing the immune reconstitution and survival which inhibits anti-tumor immunity of the host post-APHSCT [9].

In this study, we evaluated the hypothesis that A-LMR has also an impact on clinical outcomes post-APHSCT of patients with MM from Algerian West.

MATERIALS AND METHODS

Patients

From 2009 to 2015, eighty two patients with MM treated with APHSCT at the Department of Haematology and cell therapy, University Hospital of Oran region (Western Algeria), were included in the study. The ages of the studied patients varied from 35 to 65 years old. An absolute confidentiality of the patients' vital information was maintained for ethical purposes and an ethical approval was obtained from institutions in which the study was carried out.

Peripheral blood stem cells (autografts) collections

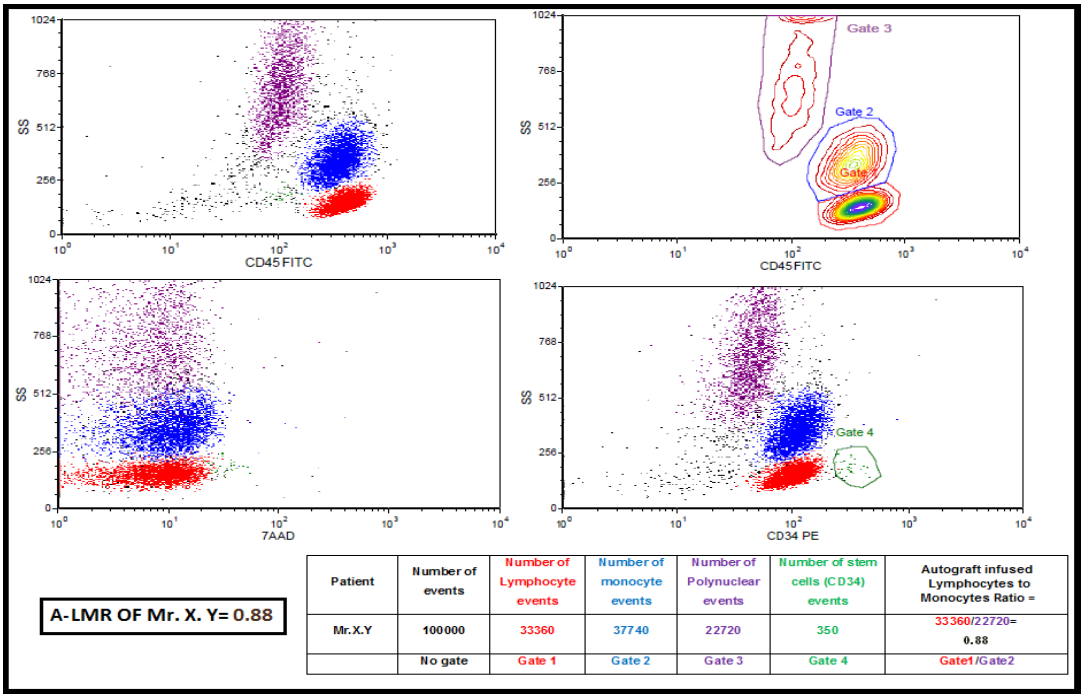

Figure1: The calculate method of A-LMR.

The eighty two patients with MM were mobilized with granulocyte-macrophage colony-stimulating factor (GM-CSF) alone, that was started 4 days before leukapherisis at the dose of 15 µg/kg/d on 07:00 pm.

One or two cytapheresis were performed twelve hours after the fourth injection (07:00 am), the number of CD34+ cells was assessed immediately after the end of the cytapheresis and if a minimum number of 2×10^6 CD34+ cells/kg was not reached another cytapherisis was performed the next morning. The chemotherapy conditioning regiment used was the MEL200 (Melphalan 200 mg/m^2, a 30 minutes injection) [10-11-12].

In A-LMR counts : a lyse no wash procedure was used. Cells were labeled with monoclonals antibodies: anti-CD45 FITC (BD science©) to all the white blood cells, anti-CD34 PE (BD science ©) to stem cells and a viability marker, 7-amino-actinomcycin D (7-AAD) to exclude dead. Cells were analyzed using a FACS CANTO II (EPICS XL (BC), BD Bioscience) to yield an events number. The stored files LMD (List Mode Data) from the archives were reanalysed by the FCS Express 4 (Research Edition Import BD FACSDiva, De Novo Software; Los Angeles, CA, 90010, USA, 2001-2013). The number of lymphocytes, monocytes and polynuclears acquired events was determined from CD45/SSC Dots plot then report to a MS Excel file. A-LMR was calculated as follows in figure 1.

Response and survival criteria

Response criteria were based on the guidelines from International Myeloma Working Group (IMWG)[13-14], Complete response (CR) was defined as a lack of detectable monoclonal protein in serum and urine by immunofixation, accompanied by similar disparition of soft tissue plasmacytomas.Very good partial response (VGPR) was defined as detecting monoclonal immunoglobulin in serum and urine by immunofixation (not electrophoresis) and a reduction in serum monoclonal protein and 24-h urinary light-chain excretion by at least 90%, accompanied by a similar reduction of soft tissue plasmacytomas, if present. Clinical relapse was defined as a 50% increase in the serum of monoclonal protein or 24-h urinary monoclonal protein excretion over the lowest remission level. An increase in the size or number of lytic lesions or soft tissue plasmacytomas constituted progression. In those with CR, any detectable monoclonal protein by immunofixation constituted progression.

PFS (progression-free survival) is the time from the start of treatment until relapse disease or deaths, or last follow-up (whatever the cause of the deaths), whichever occurs first.

Statistical analysis

Concerning the statistical analytical study, PFS was analysed using the method described by Kaplan and Meier [15].The differences between survival curves were tested for statistical significance using the tailed log-rank [16] test via SPSS 20.0 (Statistical Package for the Social Sciences, IBM Corporation; Chicago, IL, USA. August 2011).

Student's t-test was performed via an online calculator and free graphing software [17] to assess the difference between data for A-LMR and those of status of disease. An Error box (mean±2 standard deviation error bars) with data swarm was plotted for comparison. Results were presented using p value and $p<0.05$ was considered significant.

RESULTS

Patients Characteristics

Median age at the time of transplant for the 82 patients with MM was 54 years with ranging between 35-65 years. Among them 30 females and 52 males with a female to male ratio of 1.7:1. The median follow-up for the entire cohort was 908 (range 87–2100) days.

At 100 day post-transplant, 55 achieved a CR (67.1%) and 27 VGPR (32.6%) and mortality was at 0%. The rate of patients with relapse or progression during follow-up was 35.4% (29 patients). A-LMR results are respectively 54 patients (64.6%) with A-LMR≥1 and 28 patients (35.4%) with A-LMR<1. **(Table1)**

Figure 2 shows box plots PFS surviving (days): (A) in patients with an A-LMR<1 and patients with an A-LMR≥1 after APHSCT, the median PFS was not significant (968 vs 831 days, respectively; p=0.81) and (B) in

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patients with status disease after APHSCT (CR, VGPR), the median PFS was significant (980 vs 801 days, respectively; p= 0.014)

Characteristics	Number of cases (N=82)	Percentage (%) (100%)
Age (years)	54	
Range	(35-65)	-
Sex:		
Male	52	63.4
Female	30	36.6
Ratio	1.7:1	
Disease status at 100 Day post-transplant :		
CR	55	67.1
VGPR	27	32.9
Mortality	0	0
Disease evolution during follow-up:		
Relapse, progression	29	35.4
No relapse or progression	53	64.6
A-LMR :		
>=1	54	65.8
<1	28	34.2

Table 1: Baseline characteristics and results of patients.

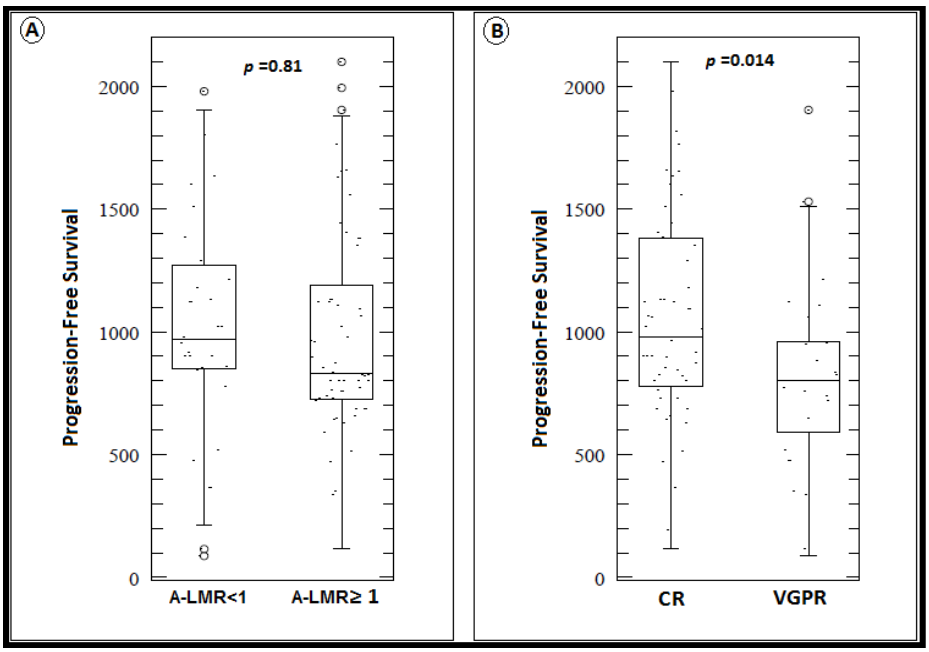

Figure 2: Box plots based on (A) A-LMR data's, (B) disease of status after APHSCT.

Post-transplant survival and infused LMR

Patients with an A-LMR ≥1 showed the same progression-free survival (PFS) comparing to patients with an A-LMR<1 (figure3A))[median PFS was not reached vs 1600 days,1000-days PFS rates of 75% (95% CI 64–94 %) vs 54% (95 % CI 35–82%), p= 0.57 respectively and both in disease status (CR versus VGPR)

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(figure3B); [median PFS was not reached vs 1600 days, 1000-days PFS rates of 70 % (95% CI 57–85 %) vs 72 % (95 % CI, 56–93%), p= 0.26].

By disease status, the same experienced PFS in patients infused with an A-LMR≥1 compared with patients infused with an A-LMR<1 in CR (figure 3C) [median PFS-CR- not reached versus 1600 days,1000-days PFS rates of 78% [95% CI 62–100] versus 71% [95% CI 56–90]), respectively, p=0.63]; and in VGPR (figure 3D) [median PFS-VGPR- not reached versus 957 days, 1000days PFS rates of 61% [95% CI 42–89] versus 49% [95% CI 17–100], respectively, p=0.93].

By A-LMR, same experienced PFS in in patients in CR compared with patients in VGPR, with an A-LMR≥1 (figure 3E) [median PFS– A-LMR≥1-1790 days versus not reached, 1000-days PFS- rates of 74% [95% CI 60–92] versus 41% [95% CI 42–89]), respectively, p=0.44]; and with A-LMR<1 (figure 3F) [median PFS- A-LMR<1- 1600 days versus 975 days, 1000-days PFS– rates of 79% [95% CI 64–100] versus 47% [95% CI 17–100]), respectively, p=0.24].

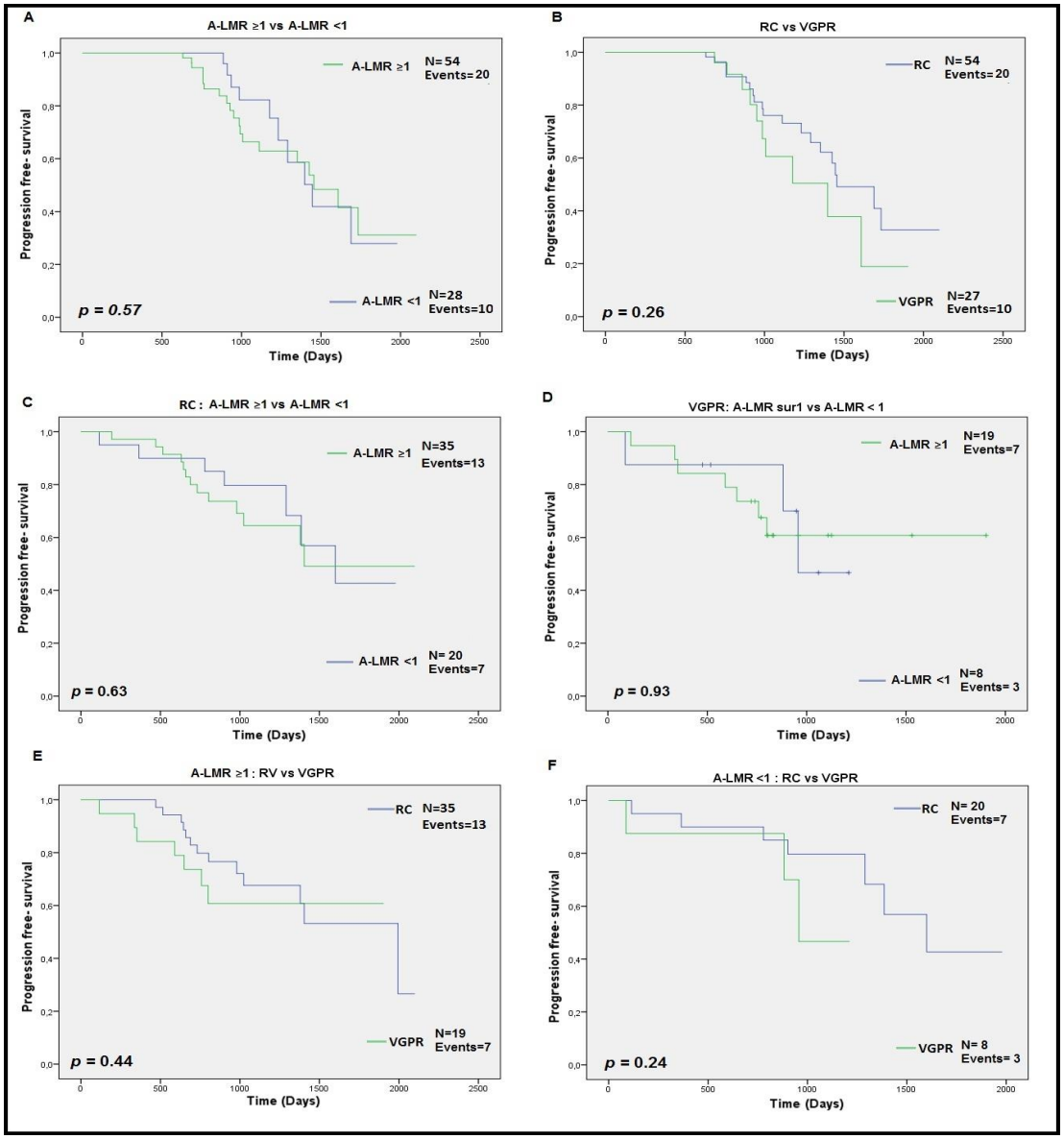

Figure 3: Progression-free survival based on disease status and A-LMR level's: A-LMR level's (A) RC and VGPR (B), RC with A-LMR level's (C) VGPR with A-LMR level's (D) A-LMR≥1 with disease status (E) and A-LMR<1 with disease status (F).

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DISCUSSION

Infused autograft lymphocyte to monocyte ratio has been reported to predict survival after APHSCT in B-cell lymphoma, T cell lymphoma and classical Hodgkin lymphoma [1-3]. Thus, we set out to investigate if A-LMR can have an impact on over survival in patients with multiple myeloma treated with APHSCT.

In MM patients undergoing APHSCT, the infusion of an A-LMR ≥ 1 was not associated with superior PFS. Furthermore, A-LMR was not showed a homogeneous prognostic role between the disease status, as the same survival was observed in patients with an infused A-LMR≥ 1 regardless the disease status in MM. This observation was made in all possible combinations.

It is known that A-LMR combines the biomarkers of A-ALC and A-AMC. The collected and infused dose of A-ALC has been reported as a prognostic factor affecting the immune recovery A-ALC to 15 days was the first correlated factor with clinical results post-APHSCT [5-18,20].

However, some patients were still relapsing post-APHSCT despite being infused with the good dose of A-ALC. The investigations done on the factors counteract survival benefits produced by post-APHSCT A-ALC, have shown the immunosuppressive and tumor growth effects of Myeloid derived suppressor cells (MDSC) (ie, monocytes) [8, 9-20,21].

Many mechanisms have been implicated about the relation to A-AMC that may be associated with post-APHSCT lower survival: immunosuppressive cytokines production; [22] the disruption of the histocompatibility complex specific CD8 + T to a major peptide; [23] the engagement of regulatory T cells; [24] increment of death receptor Fas, leading to apoptosis of T cells [25] and a decrease natural killer cell function [26].

Our results were in contradiction with those reported previously by the literature; further study is needed to validate this finding, but we hypothesize a difference in the experimental methodology.

In the current survey, A-LMR was calculated by flow cytometry: A-LMR= number of lymphocytes events/ number of monocytes events gating on CD45/SSC dot plot. Contrarily, the described method in others studies [1-3] was based on the complete blood cell count for each apheresed unit collection and was calculated as follows: A-ALC = % collection lymphocytes × (absolute white blood cell (WBC) count/kg). The infused A-AMC for each apheresed unit collection was calculated as follows: A-AMC = % collection monocytes × (absolute WBC count/kg). The absolute A-LMR was then calculated by dividing the A-ALC by the A-AMC.

Moreover, one of the limitations of our investigation is that it is a retrospective study including a small cohort of MM despite its homogeneity using factors to minimize selection bias.

This study expands on previous publications on A-ALC and A-AMC stressing the importance of interaction between host immunity and tumor microenvironment, using simple biomarker A-LMR combined into a potential prognostic factor.

CONCLUSION

Infused autograft lymphocyte-to-monocyte ratio can't predict survival in patients with multiple myeloma undergoing APHSCT. Further studies with large cohort and use of other methodology are needed to provide further explanation of this result in multiple myeloma from western Algeria.

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Human Ethics Committee at the appropriate institutions and it conformed to the provisions of the Declaration of Helsinki

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