FLOW CYTOMETRY CONTRIBUTION TO RESPONSE IN WM: VALUATION OF RESIDUAL DISEASE IN THE BONE MARROW AFTER THERAPY.

R. García-Sanz[1,2], E.M. Ocio[1,2], D. del Carpio, A. Caballero[1], J. Alonso[1], B. Paiva[1], L. López-Angalda,[1] T. Villaescusa,[1] R. Pesoa[1], J.M. Hernández,[3] J. Fernández-Calvo,[3] A. Aguilar,[3] A. Martín,[3] R. López,[3] A. Orfao[2], B. Vidriales[1], J.F. San-Miguel[1,2]

1) Hospital Universitario de Salamanca. 2) Centro de Investigación del Cáncer. 3) The Castellano-Leonés (Spain) Study group for the study of Monoclonal Gammopathies. University of Salamanca. Spain

INTRODUCTION: Waldenström's macroglobulinemia (WM) is a B-cell lymphoproliferative disorder in a transition between mutated chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). Due to these intermediate characteristics, many different therapeutic schemes most o them used in either CLL or MM have been explored in WM. Nevertheless, not all patients require therapy and there are no clear definitions about the best combination or even the optimal length of the therapy. This is especially important when we have to decide between gentle approaches with single agents such as alkylators or MoAbs and more intensive approaches that include the use of nucleoside analogues, polychemotherapy, and new agents, or even more, high dose therapy followed by stem cell transplantation. Thus, when a WM patient initiates treatment, the clinician will benefit from the use of sensitive tools to assess treatment efficacy. Moreover, clinical trials also require strict but reproducible criteria for evaluating new drugs and therapeutic strategies. Survival, especially overall survival, is the most objective parameter upon evaluating the efficacy of a new treatment scheme. However, due to the favorable outcome of WM, this will take many years and if we want to compare two treatment strategies this will require a very large number of patients to show statistically significant differences. Accordingly, response measures that can serve as adequate and accurate surrogates for overall survival are critically needed. Uniform response criteria in WM were adopted in past international workshops (Weber et al, 2003; Kimby et al, 2006), and are mainly based on the reduction of the immunoglobulin M. However, these criteria were based on panel expert consensus, with few evidences to support that a certain response was better than other, with the exception of less than stable disease. Actually, very recent data have demonstrated that patients who achieve a minor response

have a similar overall survival, progression free survival, and time to progression as patients who achieve an objective response. In addition, the use of Rituximab can result in initial increases in the IgM monoclonal component without meaning a poor outcome, and long term reductions can be expected several months (even more than one year) after the end of the therapy.

Other parameters for the response evaluation in WM include: the use of FLC and hematimetric assessments, image measurements with or without metabolic evaluation and bone marrow exams. However, data are scanty to obtain definite conclusions. Bone Marrow Evaluation was only considered for CR assessment, requiring the absence of malignant cells by morphologic evaluation. By contrast, bone marrow aspirate and biopsy are not required to confirm PR. In addition, data on immunophenotypic analysis by flow cytometry (FCM) or immunohistochemistry is very scanty.

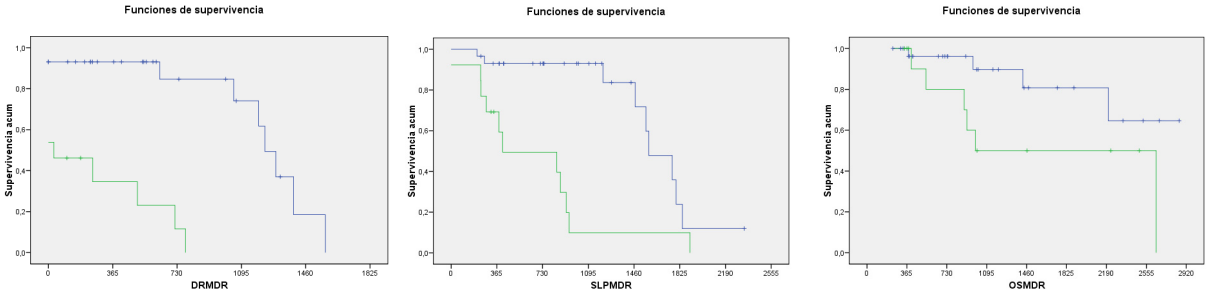
AIM: to determine the value of bone marrow assessment by FCM during the evolution of the disease in order to know if it could predict the clinical outcome of WM.

PATIENTS AND METHODS: we reviewed our database for IgM related disorders which now includes 424 patients, to select those patients with follow-up BM assessment by FCM. Among them, we selected 61 patients with enough data available. 42 of them were symptomatic ands required a therapeutic intervention.

RESULTS: 42 patients were included in the study; the median age of 71 years (48-86) and the male/female ratio was 29:13. Residual disease (changes in BM tumor burden) were evaluated after 1^{st} line in 26 cases, and 2^{nd} or subsequent therapy in 13. The median time between diagnosis and chemotherapy was 10 days (1-186) for previously untreated patients, and 31 months for previously treated patients. Treatment was based on low dose alkylating agents in 19 cases and semi-intensive polychemotherapy (CHOP) or nucleoside analogues (2CdA or Fludarabine plus cyclophosphamide) with or without rituximab in 23 cases. A complete response (irrespective to immunofixation) was attained in 19% of patients, partial in 50%, minor in 9·5%, stable disease in 9·5% and progressive disease in 12%. The mean monoclonal B-cell lymphocyte infiltration at pre-therapy evaluation was 17.8%±12.1%, and after treatment it was decreased to 5.4%±0.7% ($p=0.049$). However, there were important differences according to the response:

	BM monoclonal B-cells (Residual disease)				
Moment of evaluation	CR (n=8)	PR (n=22)	MR (n=4)	SD (n=4)	PR (n=4)
Pre-Therapy (%)	17,98	18,47	34,25	8,96	6,58
Post-Therapy (%)	0,098%	1,41	13,44	9,68	25,75
Ratio (Pre/Post)	1172,17	221,64	3,37	1,03	0,56

More interestingly, patients who achieve a bone marrow status with less than 5% monoclonal B lymphocytes after therapy has a longer duration of response ($p<0.001$), progression free survival and ($p=0.001$) and overall survival ($p=0.05$).



CONCLUSION: Residual Disease assessment by FCM at the BM level was highly predictive of response and survival in WM in this retrospective analysis. These results merit future investigation of FCM in prospective studies in order to asses if it can be used a surrogate marker for individual in WM and whether or not it could be of help to tailor the therapy and the length of therapy in these patients.