Flow cytometry contribution to response in Waldenström's Macroglobulinemia: valuation of residual disease in the bone marrow after therapy.

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INTRODUCTION: Waldenström's macroglobulinemia (WM) is a B-cell lymphoproliferative in which the target tumor cell is thought to be placed in between mutated chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). The specific characteristics of this cell allow the use of sensitive techniques, such as Multiparametric Flow Cytometry (MFC) and allele specific oligonucleotide PCR, for monitoring disease evolution and treatment efficacy assessment. Moreover, clinical trials also require strict but reproducible criteria for evaluating new drugs and therapeutic strategies. Survival, especially overall survival (OS), is the main aim for evaluating the benefit provided by any therapeutic strategy. However, OS assessment requires too many years to show differences between differentially treated groups. Accordingly, response to therapy has been evaluated as a surrogate for overall survival. Uniform response criteria in WM adopted in other workshops (Weber et al, 2003; Kimby et al, 2006), are mainly based on the reduction of the M component. However, apart from panel expert consensus, few evidences can support any benefit for a certain response respect to other. Actually, recent data have demonstrated that patients with objective and minor responses have similar overall and progression free survival. In addition, some therapeutic approaches, especially those including the use of Anti-CD20 can result in initial increases in the M-component without meaning a poor outcome, and long term reductions can be expected long time before end of the therapy. Even more, there are some descriptions of good M-component responses with new therapies that are not accompanied by parallel cell reductions in the bone marrow. Actually, bone marrow evaluation was only considered for CR assessment, requiring the absence of malignant cells by morphologic evaluation and no bone marrow aspirate or biopsy are required to confirm PR. MFC has been pointed out as a possible valuable tool to evaluate bone response to therapy in WM.

AIM: to determine the value of bone marrow responses by MFC after therapy in WM in order to consider it for a better evaluation of different therapeutic strategies.

Patients and methods: Sixty-eight patients with IgM monoclonal gammopathies and sequential MFC analysis were included in his study. 49 of them were symptomatic and required a therapeutic intervention that was evaluated for immunophenotypic response.

RESULTS: median age of 70 years (36-86) and the male/female ratio was 45:23. Residual disease (changes in BM tumor burden) were evaluated after 1st line in 28 cases, and 2nd or subsequent therapy in 21. 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 26 cases and semi-intensive polychemotherapy (2CdA or Fludarabine plus cyclophosphamide, R-CHOP or BDR) in 23 cases. A complete response (irrespective to immunofixation) was attained in 16·3% of patients 10·2 of patients, partial in 55·1%, minor in 12·2%, stable disease in 6·1% and progressive disease in 10·2%. The mean monoclonal B-cell lymphocyte infiltration at pre-therapy evaluation was 19·7% (SD 15·9), and after treatment it was decreased to 2·39 (SD 1·2) ($p<0.001$). Differences according to the response:

	BM monoclonal B-cells (Residual disease)				
Moment of evaluation	CR (n=8)	PR (n=26)	MR (n=6)	SD (n=3)	PR (n=5)
Pre-Therapy (%)	12·9±7·0	21·7±16·6	33·3±19·9	11·0±3·8	8·6±5·2
Post-Therapy (%)	0·27±0·41	2·27±6·00	11·46±8·61	7·00±1·87	25·00±20·33
Ratio (Pre/Post)	530,7	272,7	3,56	1,67	0,60

Patients who achieve a reduction in the bone marrow clonal lymphocytes did better than patients without such reduction. Any cut-off point was able to discriminate between different prognosis, and thus patients with less than 5%, 1%, 0·1% or 0·01% monoclonal B lymphocytes after therapy had a longer survival that their corresponding groups of patients with higher numbers. Differences could be observed independently of the type of survival curve that was evaluated (Response duration, Progression free survival, Specific Disease Survival and Overall Survival, $p<0·05$). However, the probability of error for differences was higher in case of overall survival because it requires more time of follow-up.

Cases achieving a negative MFC bone marrow status (less than 10^{-4} tumor cells or "Immunophenotypic Stringent Complete Response" -ISCR) were a special group. Ten patients achieved this status: nine of them did it with intensive approaches and one with long-term chlorambucil. None of the intensive patients has relapsed yet while the patient treated with the gentle approach relapsed three years after MRD negativity achievement. Conventional response was complete in 4 cases and partial in 6. Only two of these patients have died and due to associated morbidity (urological neoplasia and ischemic cardiopathy),

rendering a projected specific disease survival of 100% at more than 8 years (Figure 1).

CONCLUSION: Residual Disease assessment by MFC at the BM level was highly predictive of response and survival in WM. Achievement of ISCR by FCM was associated with very long term complete response or important reductions to the M-component to a status similar to a pseudo MGUS situation. Prospective studies in order to asses if it can be used a surrogate marker for individual outcome prediction in WM and whether or not it could be of help to tailor the therapy and the length of therapy in these patients.