Brief Report

CLINICAL TRIALS AND OBSERVATIONS

Critical analysis of the stringent complete response in multiple myeloma: contribution of sFLC and bone marrow clonality

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Key Points

- In MM patients, stringent CR criteria, in particular the sFLC ratio, do not predict significantly better outcome among MM patients in conventional CR.

Stringent complete response (sCR) criteria are used in multiple myeloma as a deeper response category compared with CR, but prospective validation is lacking, it is not always clear how evaluation of clonality is performed, and is it not known what the relative clinical influence is of the serum free light chain ratio (sFLCr) and bone marrow (BM) clonality to define more sCR. To clarify this controversy, we focused on 94 patients that reached CR, of which 69 (73%) also fulfilled the sCR criteria. Patients with sCR displayed slightly longer time to progression (median, 62 vs 53 months, respectively; P = .31). On analyzing this contribution to the prognosis of sFLCr or clonality, it was found that the sFLCr does not identify patients in CR at distinct risk; by contrast, low-sensitive multiparametric flow cytometry (MFC) immunophenotyping (2 colors), which is equivalent to immunohistochemistry, identifies a small number of patients (5 cases) with high residual tumor burden and dismal outcome; nevertheless, using traditional 4-color MFC, persistent clonal BM disease was detectable in 36% of patients, who, compared with minimal residual disease-negative cases, had a significantly inferior outcome. These results show that the current definition of sCR should be revised. (*Blood*. 2015;126(7):858-862)

Introduction

Achieving deeper levels of tumor debulking in multiple myeloma (MM) represents a surrogate marker for survival.[1-4] To discriminate different outcomes among patients in conventional complete response (CR), the International Myeloma Working Group (IMWG) introduced more stringent CR (sCR) criteria[5] by adding a normal free-light chain ratio (sFLCr) plus the absence of clonal plasma cells (PCs) in bone marrow (BM) by immunohistochemistry (IHC) to the preexisting European Society for Blood and Marrow Transplantation CR criteria.[6] In 2011, the evaluation of BM clonality by low-sensitivity multiparametric flow cytometry (MFC) was included as an alternative methodology to IHC to define sCR.[7] Despite its wide use as a clinical end point, only 1 study[8] has reported a benefit of sCR over CR, whereas other studies suggested that the κ/λ values do not provide additional prognostication.[9-11] Furthermore, the term sCR is widely used without a clear description of how BM clonality was evaluated, nor the individual influence of the

sFLC and BM clonality to define sCR criteria. Here, we report on the value of achieving sCR among patients in conventional CR included in 2 consecutive Grupo Español de Mieloma Múltiple/Programa para el Estudio de la Terapéutica en Hemopatías Malignas (GEM/PETHEMA) clinical trials. We also studied the individual contribution to the prognosis of patients in CR of each of these parameters: sFLCr, BM clonality by low-sensitivity MFC, and minimal residual disease (MRD) monitoring by conventional 4-color MFC.

Study design

This study focuses on 94 patients in CR: 50 who were transplant eligible and were treated according to the GEM2005MENOS65 (median follow-up, 70
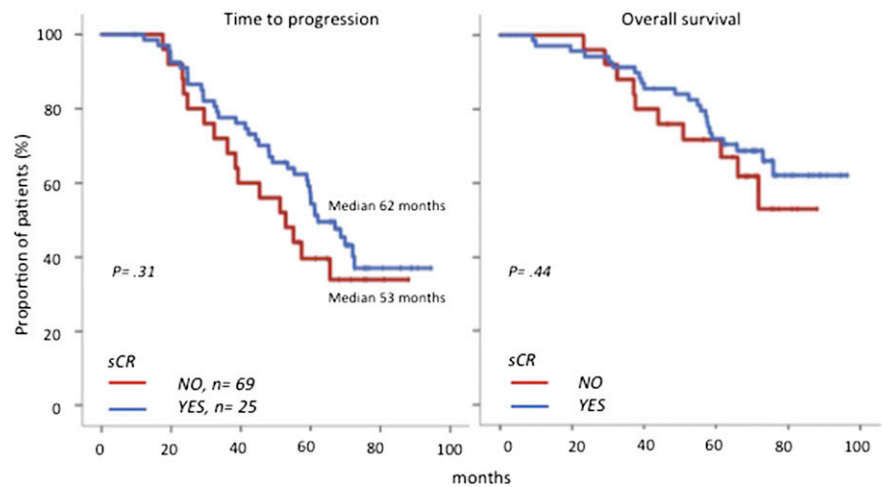
Figure 1. Time to progression and overall survival of patients in conventional CR according to their status for the sCR criteria.

months), and 44 elderly MM patients included in the GEM2005MAS65 (median follow-up, 65 months) trials.[12,13] After 6 induction cycles or after transplantation in younger patients, all were in CR as strictly defined according to the EBMT criteria.[6] In all cases, sFLC (FREELITE assay; Binding Site Ltd.) was measured by immune-nephelometry, and sFLC κ/λ ratios were classified as normal (0.26-1.65) or abnormal (<0.26 if the patient was λ; >1.65 if the patient was κ, following the IMWG guidelines).[5] BM clonality was defined by IHC when the κ/λ ratio was $>4{:}1$ or $<1{:}2$ for κ and λ patients, respectively, after counting ≥100 PCs. Here, we used an alternative method to IHC based on a low-sensitivity MFC approach to define clonality. Thus, for patients with a κ isotype, a 4:1 ratio of clonal/polyclonal PCs was defined by the presence of 80% phenotypically aberrant clonal PCs within the BM PC compartment. For patients with the λ isotype, a ratio of 1:2 polyclonal/clonal PCs was defined by the presence of 50% clonal PCs within the BM PC compartment. The low-sensitivity MFC-based assessment of clonality adapted to the IHC ratios as proposed by the IMWG criteria was also compared with MRD monitoring using conventional 4-color MFC as described elsewhere[14,15]: >20 clonal PCs after measuring $\geq200\,000$ nucleated cells, at a sensitivity level of 10^{-4}.

Briefly, erythrocyte-lysed whole BM samples were immunophenotyped using the 4-color antibody combination CD38-fluorescein isothiocyanate/CD56-phycoerythrin/CD19-PerCP-Cy5.5/CD45-allophycocyanin, with the exception of selected cases in which other antigens rather than CD56 (e.g., CD28, CD81, and/or CD117) were more useful to discriminate clonal from normal PCs (patient-specific approach). Data acquisition was performed in FACSCalibur and FACSCantoII flow cytometers (Becton-Dickinson, San Jose, CA); Infinicyt software (Cytognos, Salamanca, Spain) was used to analyze flow data.[16] Time to progression (TTP) and overall survival (OS) curves were plotted by the Kaplan-Meier method, and the log-rank test was used to estimate the statistical significance of differences observed between curves.

Results and discussion

Patients achieving CR showed superior outcome compared with those failing to reach CR, regardless of the induction therapy or patients' age (data not shown).[12,13,17] The rate of sCR was of 73% in transplant-eligible patients and 79% in elderly cases; overall, 69 (73%) of 94 cases in CR fulfilled the sCR criteria, whereas the remaining 25 cases were not considered in sCR because they failed to accomplish 1 of the 2 criteria: abnormal sFLCr (n = 21; 84%) or BM PC clonality (n = 5; 20%); 1 patient had both abnormal

sFLCr and BMPC clonality. On comparing the 69 patients in sCR with the 25 in CR, the former showed a nonsignificantly longer TTP (median, 62 vs 53 months, respectively; $P = .31$) and OS (both medians not reached [NR]; $P = .44$; Figure 1). Interestingly, patients with abnormal vs normal sFLCr showed superimposable TTP (median, 57 vs 61 months; $P = .98$; Figure 2A) and OS (both medians, NR; $P = .90$). By contrast, the few patients (n = 5) in whom BM clonality was detected by the low-sensitivity IHC-adapted MFC method had significantly shorter TTP (median, 36 vs 62 months, respectively; $P < .001$; Figure 2B) and OS (44 months vs NR; $P = .002$) than patients in whom BM clonality was undetectable or detected at levels below the threshold proposed for IHC assessment (i.e., MRD). On using our traditional MRD method (that albeit limited at the time by 4 colors, was 2-log more sensitive than IHC), persistent MRD was detectable among 34 of the 94 (36%) patients who, compared with MRD-negative cases, had significantly inferior TTP (median, 45 vs 68 months, respectively; $P = .03$; Figure 2C) and OS (median, 76 months vs NR, respectively; $P = .07$). The prognostic value of MRD was equally observed among patients in sCR (data not shown; $P = .03$). As expected, the outcome of MRD-positive patients by MFC was not as dismal compared with cases with high residual disease by low-sensitivity MFC, because the former method also included patients with low MRD levels; nevertheless, sensitive and quantitative MRD monitoring can also discriminate the high-risk population by stratifying patients into 3 risk categories: high, intermediate, and low risk according to MRD levels ($>0.1\%$, 0.1-0.01%, and $<0.01\%$, respectively).[1,18]

Because the sFCL test is insensitive to the monoclonal or polyclonal nature of light chains, and the ratio κ/λ is frequently altered by the oligoclonal bands[19] that emerged in the context of immune regeneration,[20] the lack of clinical relevance of κ/λ ratios reported herein is not surprising and agrees with previous observations.[9-11,21] However, the absence of significant differences for TTP and OS between patients in stringent vs conventional CR differs from that reported by Kapoor et al,[8] which showed highly significantly survival benefit for patients in sCR compared with those in conventional CR. Although the number of patients in this study and follow-up of both series are similar, unfortunately, the Kapoor et al study does not mention the individual contribution of the sFLC ratios or BM clonality assessments to understand the origin of the discordant results. In the present study, only the IHC-adapted MFC-based BM clonality (low-sensitivity MFC) assessment (and not the sFLCr)
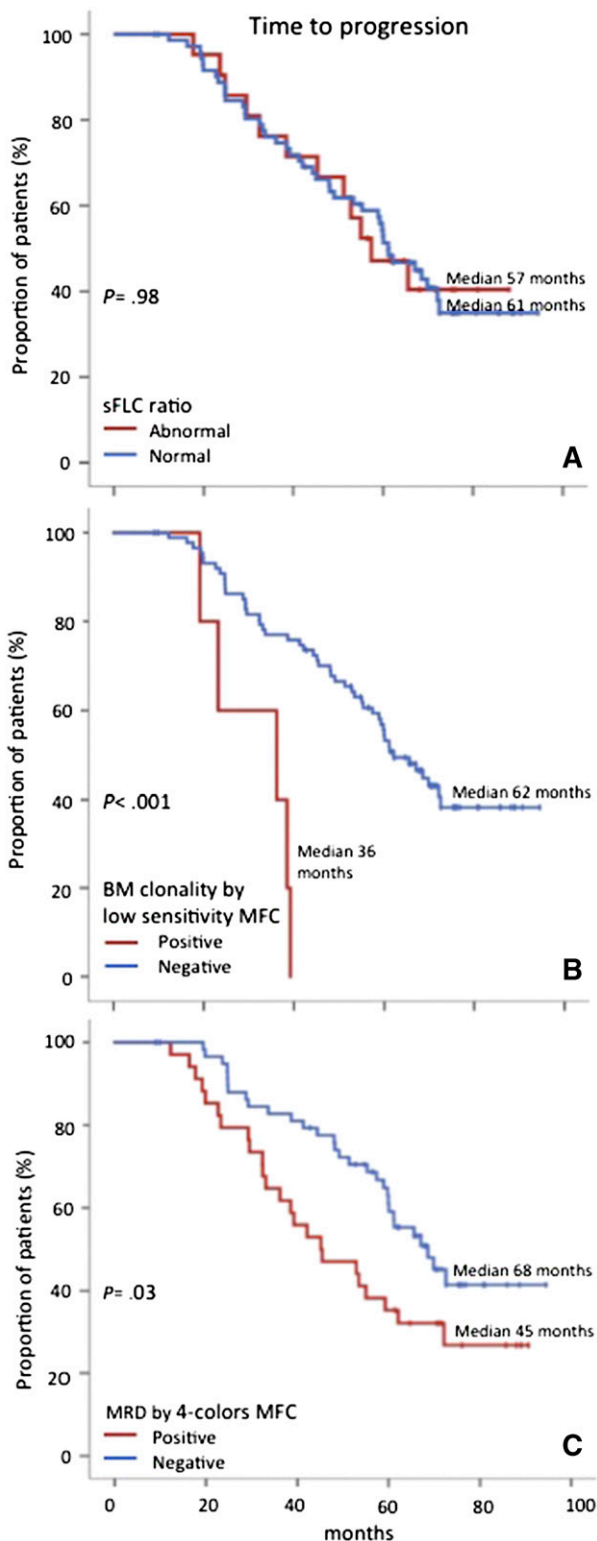
Figure 2. Survival regarding every component of sCR definition. Time to progression of patients in conventional CR according to (A) normal vs abnormal sFLC ratios; (B) BM clonality by low-sensitivity MFC; and (C) conventional MRD monitoring by 4-color MFC.

BM biopsies are not the standard of care to monitor the response in the GEM/PETHEMA clinical trials, we cannot perform a direct comparison between IHC and low-sensitivity MFC; however, it is likely that the multicolor (4-color instead of single or 2-color staining) and higher cellular input ($\geq$200 000 nucleated cells) of the IHC-adapted MFC method should render higher sensitivity and specificity compared with IHC. Conversely, using MRD monitoring by conventional MFC on the same population revealed that the percentage of MRD positivity increased to 36%, and these patients had significantly inferior outcomes. These results highlight the limitations of IHC when low numbers of clonal PCs are masked by polyclonal (κ and λ) normal PCs and confirm that attaining deeper levels of remission does translate into prolonged survival.[1]

In summary, our results show that for MM patients in CR, response assessment according to the stringent CR criteria does not predict a different outcome. In particular, the sFLCr does not identify patients in CR at distinct risk, whereas low-sensitivity MFC immunophenotyping only identifies a small number of patients with high residual tumor burden and dismal outcome; MRD monitoring using conventional MFC identifies a complementary group of patient with shorter survival. These results should stimulate the scientific community to perform a large (meta)-analysis and corroborate the exact role of the sCR criteria in MM.

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Authorship

Contribution: J.M.-L., B.P. A.Orfao, J.B, J.F.S.M, and J.J.L. provided the concept and design; J.M.-L., B.P., M.-V.M., T.C., M.-B.V., M.A.S.-G., L.P., A.Oriol, F.d.A., J.B., J.F.S.F., and J.J.L. provided the study material and patients; J.M.-L., B.P., and L.L.-A., collected and assembled the data; B.P., T.C., L.B., and A.Orfao provided data analysis and interpretation; J.M.-L., B.P., L.L.-A., J.F.S.M., and J.J.L. wrote the manuscript; and all authors provided final approval of the manuscript.

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identified patients in CR with a different outcome; however, it should be noted that only 5% of the patients (probably with a nonsecretory high tumor burden) showed residual disease by this method. Because

Appendix: study group members

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