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

Running head: Significance of stringent CR in Multiple Myeloma

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Abstract

Stringent complete response (sCR) criteria is used in multiple myeloma as a deeper response category compared to CR, but prospective validation are lacking, it is not always clear how evaluation of donality is performed, nor is it known what is the relative clinical influence of the serum free light chain ratio (sFLCr) and BM donality to define more stringent CR. For clarify this controversy we have focused on 94 patients that reached CR within PETHEMA trials, of which 69 (73%) also fulfilled the sCR criteria. Patients in sCR displayed slightly, longer time-to progression (medians of 62 vs. 53 months, respectively; P = .31) and similar overall survival (P = .44). Upon analyzing the contribution to the prognosis of sFLCr or donality, it was found that the sFLCr does not identify patients in CR at distinct risk; by contrast low-sensitive 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 colors-MFC, persistent clonal BM disease was detectable in 36% of patients, who, compared to MRD-negative cases had significantly inferior outcome. These results show that the current definition of sCR should be revised.

Key Point:

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

Achieving deeper levels of tumor debulking in multiple myeloma (MM) represents a surrogate marker for survival. In order to discriminate different outcomes among patients in conventional CR, the IMWG introduced more stringent CR (sCR) criteria by adding a normal free-light chain ratio (sFLCr) plus absence of clonal plasma cells (PCs) in bone marrow (BM) by immunohistochemistry (IHC) to the pre-existing EBMT CR criteria. 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. Despite its wide use as a clinical end-point, only one study has reported a benefit of sCR over CR, while other studies suggested that the κ/λ values do not provide additional prognostication. Furthermore, the term sCR is widely used without 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 two consecutive GEM/PETHEMA clinical trials. We have 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.

Patients and Methods

This study focuses on 94 patients in CR: 50 transplant-eligible and treated according to the GEM2005MENOS65 (median follow-up, 70 months), and 44 elderly MM patients included in the GEM2005MAS65 (median follow-up, 65 months) trials. After six induction cycles or after transplantation in younger patients all were in CR strictly defined according to the EBMT criteria. In all cases, sFLC (FREELITE assay; Binding Site Ltd) were 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). BM clonality is defined by IHC when the κ/λ ratio is >4:1 or <1:2 for κ and λ patients, respectively, after counting ≥100 PCs. Here, we have used an alternative method to IHC based on a low-sensitivity MFC approach to define clonality. Thus, for patients with 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 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 to MRD monitoring using conventional 4-color MFC as described elsewhere: >20 clonal PCs after measuring ≥200,000 nucleated cells, at a sensitivity level of 10^-6.
Briefly, erythrocyte-lysed-whole-BM samples were immunophenotyped using the 4-color antibody combination CD38-FITC/CD56-PE/CD19-PerCP-Cy5.5/CD45-APC, 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 FACSCantoll flow cytometers (Becton-Dickinson; San Jose, CA), and the Infinicyt software (Cytognos; Salamanca, Spain) was used to analyze flow-data. 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 as compared to those failing to reach CR, regardless of the induction therapy or patients' age (data not shown). 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, while the remaining 25 cases were not considered in sCR because they failed to accomplish one of the two criteria: abnormal sFLCr (n=21; 84%) or BMPC clonality (n=5; 20%); one patient had both abnormal sFLCr and BMPC clonality. Upon comparing the 69 patients in sCR vs. the 25 in CR, the former showed a non-significantly longer TTP (median of 62 months vs 53 months, respectively; \( P = .31 \)) and OS (both medians not reached –NR–; \( P = .44 \)) (Figure 1). Interestingly, patients with abnormal versus normal sFLCr showed superimposable TTP (medians of 57 months 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 (medians of 36 months 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). Upon 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 out of the 94 (36%) patients in which, compared to MRD-negative cases, had significantly inferior TTP (median 45 months 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 show, \( P = 0.03 \)). As expected, the outcome of MRD-positive patients by MFC was not so dismal as compared to cases with high residual disease by low-sensitivity MFC because the former method also includes patients with low MRD-levels; nevertheless, sensitive and quantitative MRD monitoring can also discriminate the high-risk population by stratifying patients into three risk-
categories: high-, intermediate- and low-risk according to MRD levels (>0.1%, 0.1% - 0.01%, and <0.01%, respectively).

Since the sFCL test is insensitive to the monoclonal or polyclonal nature of light chains, and the ratio $\kappa/\lambda$ is frequently altered by oligoclonal bands$^{20}$ emerged in the context of immune regeneration$^{21}$, the lack of clinical relevance of $\kappa/\lambda$ ratios reported herein is not surprising and agrees with previous observations$^{9-11,22}$. 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, that showed highly significantly survival benefit for patients in sCR compared to those in conventional CR$^8$. While the number of patients in this study and follow-up of both series are similar, unfortunately, Kapoor study does not mention the individual contribution of the sFLC ratios or BM clonality assessments in order 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) identified patients in CR with different outcome; however, it should be noted that only 5% of the patients (probably with a non-secretory high tumor burden) showed residual disease by this method. Because BM biopsies are not standard of care to monitor response in GEM/PETHEMA clinical trials, we cannot perform a direct comparison between IHC and low-sensitivity MFC; however, it is likely that the multicolor (4-colors instead of single or 2-color staining) and higher cellular input (≥200,000 nucleated cells) of the IHC-adapted MFC method should render higher sensitivity and specificity as compared to IHC. Conversely, using MRD monitoring by conventional MFC on the same population revealed that the percentage of MRD-positivity increased up 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 ($\kappa$ and $\lambda$) 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, while 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.
References


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Figure legends

Figure 1. Time to progression and overall survival of patients in conventional complete remission (CR) according to their status for the stringent CR (sCR) criteria.

Figure 2. Time to progression of patients in conventional complete remission (CR) according to 
A: normal vs. abnormal serum free-light chain (sFLC) ratios. B: bone marrow (BM) clonality by low-sensitivity multiparametric flow cytometry (MFC). C: conventional minimal residual disease (MRD) monitoring by 4-colors MFC.
Figure 1.

Time to progression

- Proportion of patients (%)
- Median 62 months
- Median 53 months

- sCR: NO, n=69
- sCR: YES, n=25

- P = .31

Overall survival

- Proportion of patients (%)
- P = .44

- sCR: NO
- sCR: YES
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