Assessment of response to therapy in multiple myeloma by multiparameter flow cytometry. Usefulness of an eight-color single tube with monoclonal antibodies in dried formulation

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Abstract

Objectives. Multiparameter flow cytometry is considered the gold standard to evaluate minimal residual disease in multiple myeloma (MM) and patients in complete remission can achieve “Flow MRD-negative” status (i.e. immunophenotypically abnormal plasma cells not detectable). In the current study we report the usefulness of an eight-color flow cytometric method with a 10^-5 sensitivity, using monoclonal antibodies in dried formulation.

Material and methods. Forty-six patients with MM were treated with bortezomib-based regimens and, when eligible, with autologous stem cell transplantation. Response to therapy was assessed according to the criteria validated by the International Myeloma Working Group. Multiparameter flow cytometry was carried out with an 8-color panel validated by the Euroflow Consortium. A commercially available single 8-color tube in dried formulation was used and almost 2,000,000 events were acquired, in order to obtain a 10^-5 sensitivity.

Results. Sixteen patients achieved stringent complete remission and another three patients achieved complete remission. In these groups of patients, the “Flow MRD-negative” status was achieved in sixteen cases. In patients who had a different degree of response (very good partial response, partial response, minimal response) immunophenotypically abnormal plasma cells were always detected.

Conclusion. Using a single eight-color tube in dried formulation, and an acquisition strategy able to obtain a 10^-5 sensitivity, not only is it possible to detect a deep response to modern therapy in patients who obtained at least complete remission, but it is also always possible to detect minimal residual disease in patients with either complete remission or stringent complete remission.

Key Words: Minimal residual disease, Monoclonal antibodies in dried formulation, Multiparameter flow cytometry, Multiple myeloma

Introduction

Treatment of multiple myeloma (MM) has dramatically changed over time (1,2). Until 2000, standard therapy for this disease was based on the use of alkylating agents and corticosteroids, followed by high dose therapy and infusion of autologous hematopoietic stem cells (ASCT) in selected patients. At the beginning of this century, new drugs, such as thalidomide, bortezomib and lenalidomide, were approved for MM treatment. In 2012 carfilzomib was approved, followed by panobinostat, daratumumab, elotuzumab, and ixazomib in 2015 (3).

The current standard therapy is a 3-drug combination based on bortezomib. Complete remission (CR) or very good partial response (VGPR) rates of 60% or more can be obtained by MM patients following first-line therapy with the most common therapeutic regimens, such as VTD (bortezomib, thalidomide, dexamethasone) (4) or VCD (bortezomib, cyclophosphamide, dexamethasone) (5) for patients younger than 65 years of age, and VMP (bortezomib, melphalan, dexamethasone) for patients older than 65 (6). ASCT after first-line therapy is considered the standard of care for newly diagnosed and fit MM patients (i.e. patients < 65 years old and < 70 years old in good clinical conditions) (7). Both single and tandem ASCT are characterized by improvements in response rates, progression-free survival and overall survival, even in patients aged 65-70 (8-11).

As a consequence of the improved results obtained with modern therapeutic regimens, the definition of response has recently been updated. In 2016 the International Myeloma Working Group (12) established additional response subcategories, beside the well-established CR, VGPR, PR. Indeed, since deeper responses can be obtained with modern therapy, the subcategory of “Flow MRD-negative”, i.e. negative for immunophenotypically abnormal PCs (ia-PCs), was added to the classic criteria for response assessment.

Multiparameter flow cytometry (MFC) is a very powerful method to assess deep response to MM therapy. In the past, standard 4-6 color methods were used, with a 10^-4 sensitivity, to detect minimal residual disease (MRD), i.e. persistence of ia-PCs. Although applicable in more than 95% of cases, such methods were less sensitive than PCR-based methods which, in turn, had the limitation of being applicable in fewer cases (50-90%) (13).

More recently, modern MFC approaches have been proposed for highly sensitive and standardized analyses with the aim of detecting MRD in MM. Methods based on
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status, as evaluated by MFC methods with a 10^{-5} sensitivity, showed better outcomes with regards to time to progression and overall survival, when compared to patients evaluated by less sensitive MFC methods (16).

In the present paper we report our experience of MFC implementation to assess response in MM patients treated with first line therapy, who had undergone induction treatment with Bortezomib-based regimens, followed by ASCT when eligible. MFC was carried out by using an eight-color single tube containing MoAbs in dried formulation, which is available commercially. This method was able to reach a 10^{-5} sensitivity, in agreement with the IMWG guidelines and with the EuroFlow Consortium evidences (15). In addition to the single eight-color tube in dried formulation, a second tube, with MoAbs in liquid phase, was used to evaluate PC clonality.

Materials and methods

Forty-six patients suffering from MM were studied (M: 28; F: 18; age: 43-80). The isotypes of the monoclonal components were: IgGk, 18; IgG\lambda, 2; IgAK, 10; IgA\lambda, 3; IgD\lambda, 1; micromolecular K, 10; micromolecular \lambda, 2. Our patients had been treated with: VTD, 19; VMP, 10; VTD + ASCT, 16; VCD + ASCT, 1.

All patients underwent re-evaluation of disease 12-16 weeks after termination of either first-line therapy (with VTD or VMP), or ASCT after first-line therapy, by: bone marrow trephine biopsy analysis, myeloaspirate morphologic observation and immunophenotyping, and serological exams which included immunoglobulins and free light chains (FLC) ratio.

Response to treatment was determined in agreement with the criteria established by the IMWG (12). Therefore, the following categories of outcome were identified: CR (complete remission), sCR (stringent CR), VGPR (very good partial response), PR (partial response), MR (minimal response), Flow MRD-negative.

The smears obtained from myeloaspirates were stained by May-Grunwald-Giemsa. The percentage of PCs was calculated for each sample based on observation using an optic microscope. Bone marrow trephine biopsies were processed with standard methods and the PC percentage were calculated for each sample.

Immunophenotyping was carried out with a multicolor technique, using a FacsCanto II cytometer (Becton Dickinson, MA, USA), following the instructions by the manufacturer. The MoAb panel included in this tube consists of: CD38-FITC, CD28-PE, CD27-PerCP-Cy5.5, CD19-PE, Cy7, CD117-APC, CD81-APC-H7, CD45-Horizon V450, CD138-Horizon V500.

An amount of 200\mu L of bone marrow sample was added to the tube and incubated at room temperature for 20 minutes. After doublet exclusion by plotting FSC-A vs FSC-H, a total of 2,000,000 events were acquired and PCs were identified as CD138+CD38+ events. A well-defined cluster of 20 PCs was considered sufficient for the analysis. Thus, the sensitivity of our method was 0.001% (i.e. 10^{-5}). The criteria for considering PCs as ia-PCs were: down-regulation of antigens expressed by normal PCs (CD19, CD45, CD27, CD81); up-regulation of CD28 and/or CD117. A minimum of 2 aberrant phenotypes were required to define a cluster of clonal PCs (16). PCs positive for CD19, CD45, CD27 and CD81, and negative for CD117 and CD28 were considered as immunophenotypically normal (in-PCs). Patients with ia-PCs < 0.001% were defined as having Flow MRD-negative response.

The determination of PC clonality was carried out as an additional assay, using MoAbs in liquid phase, according to routine procedures established in our laboratory. The MoAb panel used to assess PC clonality included: CD138, CD38, CD19, CD45 (all purchased from BD), cytoplasmic immunoglobulin K chain, cytoplasmic immunoglobulin \lambda chain (both evaluated by means of rabbit F(ab\prime)\), polyclonal antibodies purchased from Dako (Agilent Technologies, Santa Clara, CA, USA). Cells were permeabilized by means of the Fix & Perm kit (ThermoFisher Scientific, Waltham, MA, USA), following the instructions by the manufacturer. At least 2,000,000 events were acquired.

Results

Sixteen patients (34.7%) achieved sCR. In this subset of patients, 14 fulfilled the criteria for Flow MRD-negative status, while the remaining 2 achieved a flow MRD-positive status.

Three patients fulfilled the criteria of CR, but not of sCR, since they did not achieve normalization of the FLC ratio, and in one case MRD was detected, while the remaining two patients achieved the status of Flow MRD-negative. Therefore, Flow MRD-negative status showed a significant correlation with the sCR/CR status (16/19 patients; 84.2%).

Tables 1 and 2 show, respectively, one case of Flow MRD-negative status, while the remaining 2 achieved a flow MRD-negative status and one case of persistence of MRD.

Eight patients achieved VGPR, and in all cases variable percentages of ia-PCs were detected.

Variable percentages of ia-PCs were also found in all remaining poor responders (14 PR, 5 MR). Table 1 summarizes the results.

We found that ia-PCs always displayed clonal restriction for the corresponding immunoglobulin light chain, while in-PCs always showed polyclonal expression of cytoplasmic immunoglobulin light chains (not shown).

Discussion

Our study shows that MFC is a very powerful method to assess deep response to therapy in cases of MM. Since methods based on eight-color MFC, with 10^{-5} sensitivity, have been validated by EuroFlow Consortium (14) and have...
Fig. 1. Flow cytometric analysis in a case of sCR with Flow MRD-negative status.

Fig. 2. Flow cytometric analysis in a case of positive MRD. The analysis shows the persistence of 0.006% plasma cells (arrows) with abnormal immunophenotype (i.e. negative for CD19, CD45, and CD81, and positive for CD117).
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been shown to add important information about the prognosis of patients treated with bortezomib-based regimens and with ASCT after first-line therapy, we used this method to assess response in our patients who had undergone first line therapy, with or without ASCT. In addition, we used a commercially available eight-color single tube containing MoAbs in dried formulation.

By applying our method, we were able to identify patients achieving Flow MRD-negative status and to detect MRD in patients with either CR or SCR. In all the other degrees of response to therapy ia-PCs were always detected. Therefore, the most useful application of highly sensitive MFC methods appears to be the evaluation of patients achieving at least CR status.

Some recent papers have shown the great usefulness of using dried reagent formulations to analyze both peripheral blood and bone marrow samples from either normal subjects or patients with hematologic disorders or primary immunodeficiencies (17,18). In addition, we recently used the same BD OneFlow PCD tube to detect leptomeningeal myelomatosis in one symptomatic patient suffering from MM with good results (19).

Our experience shows that the use of a single eight-color tube containing MoAbs in dried formulation is particularly suitable for routine evaluation of patients with MM undergoing modern therapy. The most apparent advantages of lyophilized reagents are: long stability at room temperature; no need to pipet and create liquid cocktails of MoAbs; fast sample preparation, acquisition and analysis; highly sensitive ia-PC detection; more straightforward laboratory organization; possibility to compare results with other laboratories involved in MM sample evaluation; increased staining reproducibility, with reported CV values below 20% (17).

Finally, K/Λ analysis did not provide additional information about the quality of response to therapy. Therefore, the use of the BD OneFlow PCD tube appeared to be sufficient to identify both in-PCs and ia-PCs, thus facilitating the work of the flow cytometry laboratory in a routine setting.

References


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Table 1. Results: percentage of plasma cells as enumerated by trephine biopsy, myeloaspirate and flow cytometry analyses, and immunophenotyping outcome.

<table>
<thead>
<tr>
<th>Disease status after therapy</th>
<th>n</th>
<th>PCs % at bone marrow biopsy</th>
<th>PCs % at myeloaspirate</th>
<th>Total PCs % by flow</th>
<th>% of ia-PCs</th>
<th>Flow MRD-negative***</th>
</tr>
</thead>
<tbody>
<tr>
<td>sCR</td>
<td>16</td>
<td>2 - 5</td>
<td>0.5 - 3</td>
<td>0.008 – 0.16</td>
<td>0 – 0.05</td>
<td>14</td>
</tr>
<tr>
<td>CR</td>
<td>3</td>
<td>1 - 4</td>
<td>0.5 – 1.5</td>
<td>0.012 – 0.085</td>
<td>0 – 0.026</td>
<td>2</td>
</tr>
<tr>
<td>VGPR</td>
<td>8</td>
<td>1 - 6</td>
<td>0.5 -5</td>
<td>0.006 – 0.5</td>
<td>0.0011 – 0.48</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>14</td>
<td>7 - 25</td>
<td>1 - 8</td>
<td>0.022 – 6</td>
<td>0.0185 – 5.98</td>
<td>0</td>
</tr>
<tr>
<td>MR</td>
<td>5</td>
<td>20 - 70</td>
<td>20 - 70</td>
<td>5.3 – 16.7</td>
<td>5.29 – 16.96</td>
<td>0</td>
</tr>
</tbody>
</table>

PCs: plasma cells. ia-PCs: immunophenotypically abnormal plasma cells. *Percentages are expressed as range values. **: % of total acquired events. *** Samples with less than 10⁻⁶ ia-PCs.


