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Minimal residual disease testing for multiple myeloma

Ocena minimalnej choroby resztkowej w szpiczaku plazmocytowym

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Abstract

Minimal residual disease (MRD) in a patient with multiple myeloma (MM) is defined as the minimum levels of pathological plasma cells remaining after treatment when a patient is in complete response (CR). The ultimate aim of studying MRD is to identify patients with different prognosis and to tailor treatment for individual patients. MRD studies in MM should be recommended in young patients in CR after autologous hematopoietic stem cells transplantation and in older patients in CR after regimens including proteasome inhibitors. Bone marrow is the only recommend location to assess MRD in MM. The recommended methods of MRD testing include next generation sequencing of immunoglobulin genes or multiparametric flow cytometry (MFC), depending on the experience of each center and the possibility of study samples being available in the first 24 hours for MFC analysis. MRD should be considered as a therapeutic objective. However, there is not enough evidence for taking clinical decisions based on MRD status alone, and for this reason we encourage the design of new clinical studies to address these questions.

Key words: multiple myeloma, minimal residual disease, complete response, flow cytometry, next generation sequencing

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Streszczenie

Minimalną chorobę resztkową (MRD) u pacjenta z rozpoznaniem szpiczaka plazmocytowego definiuje się jako populację nowotworowych komórek plazmatycznych, która pozostała w organizmie chorego po osiągnięciu odpowiedzi całkowitej (CR). Ostatecznym celem badań MRD jest dążenie do identyfikacji chorych o odmiennym rokowaniu i indywidualizacji leczenia na tej podstawie. Ocenę MRD u chorych na szpiczaka plazmocytowego zaleca się u młodszych pacjentów, którzy osiągną CR po przeszczepieniu autologicznych krwiotwórczych komórek macierzystych oraz u chorych starszych osiągających CR po chemioterapii opartej na inhibitorach proteasomu. Powinno się oznaczać MRD wyłącznie w szpiku kostnym. Do rekomendowanych metod oceny MRD w szpiczaku plazmocytowym zalicza się sekwencjonowanie następnej generacji genów immunoglobulinowych oraz wieloparametryczną cytometrię przepływową, przy czym wybór jednej z tych metod powinien zależeć od doświadczenia danego ośrodka oraz możliwości wykonania badania cytometrycznego w czasie 24 godzin od pobrania próbki szpiku. Eradykację MRD powinna się obecnie uważać za

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istotny cel terapii szpiczaka plazmocytowego. Jednak, ze względu na brak wystarczających danych do podejmowania decyzji klinicznych wyłącznie na podstawie wyniku badania MRD, istnieje potrzeba dalszych, dobrze zaprojektowanych badań klinicznych w tym zakresie.

Słowa kluczowe: szpiczak plazmocytowy, minimalna choroba resztkowa, odpowiedź całkowita, cytometria przepływowa, sekwencjonowanie następnej generacji

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Complete response and minimal residual disease in multiple myeloma: a comprehensive vision

Minimal residual disease (MRD) is defined as the minimum levels of pathological plasma cells remaining after treatment when a patient is in complete response (CR). The ultimate goal of studying MRD is to identify patients with different prognosis and to adapt the treatment to individual patients; a means to precision medicine.

The importance of MRD was first addressed in acute lymphoblastic leukemia (ALL) in children and chronic myeloid leukemia [1–3]. It is very well documented that patients who achieve MRD negativity in ALL have longer survival; this leads to tailored treatment in the case of MRD status after induction, as Sant Jude or the German Acute Leukemia Group have shown [4, 5].

In multiple myeloma (MM), the last decade has seen an unprecedented increase in the survival of patients; due to impressive improvements in understanding the biology of the disease, and the treatments available. The remarkable increase of survival along with an increase of responses indicates that deepest responses are one of the best surrogate markers of longer survival in MM [6–10]. In this scenario, the proportion of patients who achieve CR has improved significantly in the last decade, from 30% to 70% in young patients with the newest combinations [11–13], and from < 5% to 30% in older patients [14, 15].

The first step in curing MM is achieving CR. This has increased to 50–70% with the newest therapeutic strategies [13, 16]. However, a majority of patients relapse, in part due to the persistence of MRD levels. It should be noted that although a patient achieves a CR, more than 10^8 pathologic plasma cells could sometimes persist (Figure 1) [17]. In addition, residual cells have a heterogeneous clonal architecture and undergo evolution, which means that the techniques employed for routine assessment of MRD cannot identify clonal evolution [18–20].

Nevertheless, MM hassingular characteristics confounding some aspects related to response as surrogate markers of survival: 1) some patients who fail to achieve CR have a good outcome, and return to a monoclonal gammopathy of undetermined significance (MGUS) phenotype after treatment [21]; 2) some patients in CR do not sustain CR showing reduced survival [9, 22–24]. In part, this could be due by the fact that the sensitivity of criteria used to define CR may be suboptimal (Figure 2). Despite this limitation, CR in MM is considered as a very good surrogate marker of survival in all clinical situations: both in younger and older patients, after new drug treatment (in several combination) and in relapsed patients [25, 26].

In classifying the response, several efforts have been made to incorporate new categories of deep response. First, stringent CR (sCR), immunophenotypic response (IR) and molecular response (MR) were defined (Table 1) [27, 28]. There is some controversy about the clinical impact of normalizing serum free light chains (sFLC) [29–32]. The first two analyses reflected the benefit in progression free survival of normalizing sFLC within the overall patient population, and not exclusively among patients in CR. The third study, from the Mayo Clinic, showed a significant prolongation of PFS and OS for those patients in CR with a simultaneous normal sFLC ratio, but there was not data about bone morrow (BM) clonality evaluation. However, the study of Martinez-Lopez et al. [32], demonstrated no clinical impact of normalizing the sFLC, contrary to the previous studies. In our opinion, this category of response (sCR) must be revised, and it should not be included in the categories of MRD in multiple myeloma. Also, the new serologic test HevyLite to study heavy light chain pairs, has no role as a technique for MRD assessment, although it could improve the definition of CR [33].

Several studies have demonstrated the use of multicolor flow cytometry (MFC) in detecting MRD in the bone marrow and showed that

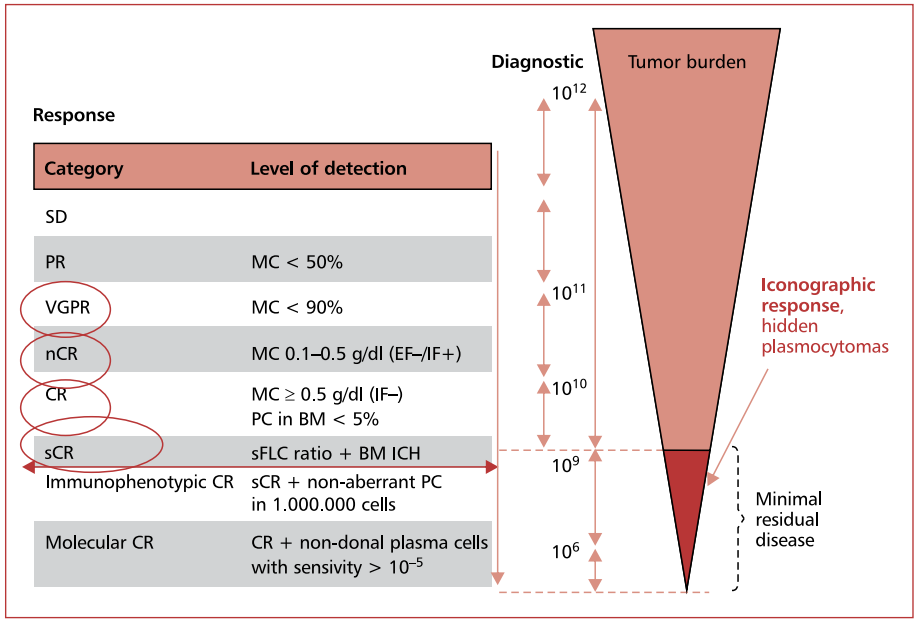

Figure 1. Response categories in multiple myeloma regarding tumor burden (based o n Rajkumar SV et al. *Blood* 2011, Durie B et al. *Leukemia 2006*, Blade J et al. *Br J Haematol.* 1998); SD — stable disease; PR — partial remission; MC — mast cell; VGPR — very good partial response; nCR — near complete remision; sFLC — serum free light chains; PC — plasma cells; BM — bone morrow; sCR — stringent complete remision; – — negative ; + — positive

Rycina 1. Zależność między uzyskaną kategorią odpowiedzi w szpiczaku plazmocytowym a liczbą pozostałych po leczeniu komórek nowotworowych (na podstawie Rajkumar S.V. i wsp. *Blood* 2011, Durie B. i wsp. *Leukemia 2006*, Blade J. i wsp. *Br. J. Haematol.* 1998); SD — stabilizacja choroby; PR — remisja częściowa; MC — komórki tuczne; VGPR — bardzo dobra odpowiedź częściowa; nCR — prawie całkowita remisja; sFLC — wolne łańcuchy lekkie w surowicy; PC — komórki plazmatyczne; BM — szpik kostny; sCR – przekonująca całkowita remisja; – — ujemne; + — dodatnie

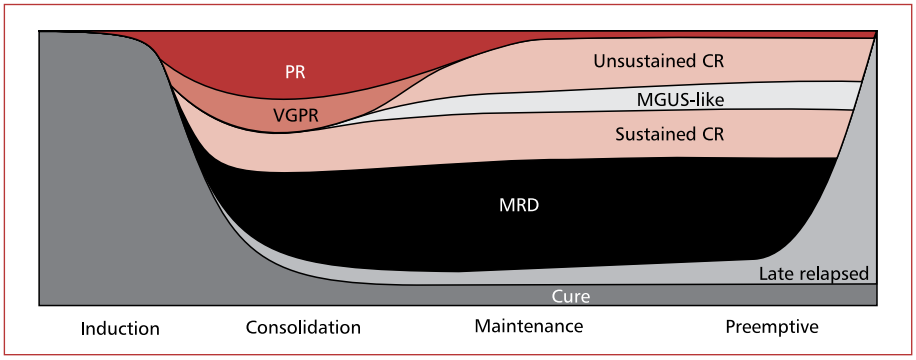

Figure 2. Dynamic evolution of the different patterns of response in multiple myeloma; PR — partial remission; CR — complete remision; VGPR — very good partial response; MGUS — monoclonal gammopathy of undetermined significance; MR — molecular response

Rycina 2. Ewolucja szpiczaka plazmocytowego w zależności od głębokości uzyskanej odpowiedzi na leczenie; PR — remisja częściowa; CR — całkowita remisja; VGPR — bardzo dobra odpowiedź częściowa; MGUS — gammapatia monoklonalna o nieokreślonym znaczeniu; MR — odpowiedź molekularna

MRD was one of the most important predictors of outcome [6, 8, 23, 34–37]. Of note, in all these studies, three to six-colour MFC approaches with a sensitivity of one in 10^4 myeloma cells were used. Moreover, although MRD evaluation by allele specific oligonucleotide – quantitative polymerase

Table 1. Response criteria for multiple myeloma are given according to the International Myeloma Working Group guidelines (source [28])

Tabela 1. Kryteria odpowiedzi na leczenie szpiczka plazmocytowego według wytycznych Międzynarodowej Grupy Roboczej ds. Szpiczaka (źródło [28])

CR	Stringent complete response (sCR)	Immunophenotypic CR	Molecular CR
Negative immunofixation of serum and urine, *and*	CR as defined, *plus*	Stringent CR *plus*	CR plus negative ASO-PCR, sensitivity 10^{-5}
Disappearance of any soft tissue plasmacytomas, *and*	Normal FLC ratio *and*	Absence of phenotypically aberrant PCs (clonal) in BM with a minimum of 1 mln total BM cells analyzed by multiparametric flow cytometry (with > 4 colors)	
< 5% PC in bone marrow	Absence of clonal PC by immunohistochemistry or 2- to 4-color flow cytometry		

CR — complete remission; sCR — stringent complete response; ASO-PCR— allele specific oligonucleotide polymerase chain reaction; PC — plasma cells; BM — bone marrow

Table 2. Categories of minimal residual disease (MRD) responses according to the International Myeloma Working Group (IMWG) guidelines (source [28])

Tabela 2. Kategorie odpowiedzi uwzględniające minimalną chorobę resztkową (MRD) według wytycznych Międzynarodowej Grupy Roboczej ds. Szpiczaka (IMWG) (źródło [28])

Sustained MRD-negative	MRD negativity in the marrow (NGF or NGS, or both) and by imaging as defined below, confirmed minimum of 1 year apart. Subsequent evaluations can be used to further specify the duration of negativity (e.g. MRD-negative at 5 years)
Flow MRD-negative	Absence of phenotypically aberrant clonal plasma cells by NGF on bone marrow aspirates using the EuroFlow standard operation procedure for MRD detection in multiple myeloma (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Sequencing MRD-negative	Absence of clonal plasma cells by NGS on bone marrow aspirate in which presence of a clone is defined as less than two identical sequencing reads obtained after DNA sequencing of bone marrow aspirates using the LymphoSIGHT platform (or validated equivalent method) with a minimum sensitivity of 1 in 10^5 nucleated cells or higher
Imaging plus MRD-negative	MRD negativity as defined by NGF or NGS plus disappearance of every area of increased tracer uptake found at baseline or a preceding PET/CT or decrease to less mediastinal blood pool SUV or decrease to less than that of surrounding normal tissue

NGF — next generation flow; NGS — next generation sequencing; DNA — deoxyribonucleic acid; PET — positron emission tomography; CT — computed tomography

chain reaction (ASO-qPCR) is a very sensitive (one in 10^5 myeloma cells) and specific approach, it is only applicable in a low proportion of patients with MM due to technical limitations [27, 38]. Thus, it was important to define new response categories to identify deeper responses in MM patients as the ones proposed lately by the International Myeloma Working Group. (Table 2) [28]. The new criteria include a more sensitive molecular complete response to detect very low levels of pathologic plasma cells, supporting the use of several methodologies and techniques for MRD assessment in MM. In this way different high-sensitivity quantification methods are being developed and improved using flow cytometry, gene sequencing, and sensitive imaging approaches [37, 39–42].

In which patients and when should MRD be tested?

Most studies of MRD have been carried out in young patients after transplantation with or without novel agents [7, 8, 10, 43–50]. Recently, several studies in older patients treated with new drugs, particularly bortezomib after induction therapy have been published [6, 9, 39, 51].

There are only two studies analyzing the effect of maintaining MRD levels [8, 52]. The first employing thalidomide as maintenance, demonstrated that 28% of MRD-positive patients who received maintenance thalidomide became MRD negative, and only a 3% in the non-maintenance group. The second employing lenalidomide in a small

number of patients which improved response in eight patients (27%), and four (13%) became MRD negative [8, 52].

Although achieving CR after the first relapse is unlikely (less than 10%); there is only one study in this situation which found that those patients, who achieved MRD negativity after first relapse had longer progression free survival in a small series of patients [53]. So, in patients in relapse with CR, performing MRD could be indicated.

Which patients should be studied for MRD? It does not make sense to study patients who do not achieve CR, if there is already measurable disease. However, some studies found that a small fraction of patients in near CR (nCR) could achieve the status of MRD negative (5–7%) [6, 39]. If this small number of patients is analyzed, most of them achieve CR in a few months, this phenomenon could be due to the slow clearance of monoclonal immunoglobulins.

As an exception, there is a small group of patients who have an oligoclonal pattern in the immunofixation tests after therapy. In these patients, it is very difficult to determine CR status and they would therefore be candidates for the study and assessment of MRD [54].

Based on this evidence, we would recommend performing MRD studies in MM after transplantation in young patients in CR and in older patients in CR after regimens including proteasome inhibitors. At this moment, we would encourage studying the effect of maintenance and consolidation on MRD levels.

What is the optimal location to study MRD?

Although bone marrow BM infiltration in MM is patchy; BM aspiration has been the location classically used in MM to assess MRD. BM assessment has its pitfalls: 1) the pattern of BM infiltration in MM is not uniform, so the possibility of residual MM plasma cells in another region different from the one analyzed cannot be excluded (false negative results); 2) only BM is analyzed, thus extra-medullary relapses are not assessed. Nevertheless, MM is a bone marrow disease and pathological plasma cells are mostly in this niche.

In addition, to solve BM limitations, the use of imaging techniques to study hidden plasmocytomas has been postulated [55]. A study suggests that normalization of PET-CT receiving novel-agent based therapy can predict outcome in young MM patients [56, 57]. At this moment, bone marrow is the only recommended location to assess MRD in MM.

What is the recommended method of MRD assessment?

The two classical methods used for MRD assessment are multiparametric flow cytometry (MFC) and deep sequencing techniques. Other techniques such as serologic analysis of immunoglobulins: Hevylite or sFLC are not sensitive enough to be considered MRD techniques. What should be the ideal method for MRD assessment? 1) universally applicable; 2) easy to do; 3) minimally invasive; 4) cheap, and 5) fast results. However, at present such ideal technique does not exist.

Multiparametric flow cytometry

Detecting phenotypically aberrant clonal PCs through MFC can be performed in > 95% of MM patients, and with multidimensional staining (≥ 8--colors). It does not require information on the diagnostic samples, although this may be helpful. From a clinical point of view, achieving an immunophenotypic CR (CR plus no residual aberrant plasma cells in 10,000 normal cells; sensitivity of 10^{-4}) predicts extended survival both in young patients receiving intensive therapy and elderly patients treated with novel agents [6–8, 37, 58]. Patient risk-stratification can be further improved by combining cytogenetic baseline evaluation plus MRD monitoring in order to identify those cases at risk of unsustained CR [23]. Conventional flow cytometry has two particular disadvantages: limited sensitivity compared to molecular techniques, and lack of standardization [59]; however, novel multidimensional (digital ≥ 8--color) flow is already monitoring MRD levels in the same sensitivity range as ASO-PCR (10^{-5}), and current efforts by the EuroFlow consortium [60] and the Black Swan Research Initiative promoted by the International Myeloma Foundation are aiming to develop a fully automated MRD immunophenotypic method (Table 1). Recently, an ultrasensitive flow cytometry methodology has been published, termed next regeneration flow cytometry that reached sensitivity of 10^{-5}. This methodology is based on: automated analysis, eight color flow cytometers and studying more than 5 million cells of the same sample.

Gene sequencing

Since MM does not have a specific molecular marker, analyzing MRD relies on studying

immunoglobulin gene rearrangements. Using this strategy and following the recommendations of the BIOMED concert action, it is possible to identify a molecular marker in most of the 90% of MM patients. There are three major techniques to analyze immunoglobulin gene rearrangements: 1) fluorescent PCR (F-PCR) using family primers of immunoglobulin genes that, despite low sensitivity (10^{-3}), identifies patients in CR with longer survival [9]; 2) ASO-PCR has been the most employed molecular technique to define molecular response in MM, and most studies have shown that achieving complete molecular response improves progression free survival [38, 45, 46, 61], 3) high throughput sequencing or next generation sequencing (NGS). However, two first methods have important limitations such as low applicability (around 37–70%) [61] complexity, expertise needed, specific reagents and cost. These drawbacks prevent their use in the clinical setting. A promising novel methodology for analyzing immunoglobulin genes is high throughput sequencing; it has superior applicability compared to ASO-PCR (> 90% of patients) [62], it is highly sensitive — even 10^{-6} depending of the quality of the sample — and adequately stratifies patients with longer survival [39, 55, 63, 64]. However, it also has some pitfalls such as availability, lack of experience and cost (Table 1).

Based on the experience of Spanish Myeloma Group, ASO-qPCR or F-PCR of immunoglobulin genes should no longer be used. High-sensitive MFC and NGS of immunoglobulin genes will certainly be widely employed in the future. MFC or NGS will be used depending on the experience of each center and the possibility of study samples in the first 24 hours for MFC analysis. For clinical trials and studies for MRD assessment, we would recommend at this moment using both methods in parallel.

Summary

In conclusion MRD should be considered as a therapeutic objective. However, there is enough evidence to take clinical decisions depending on MRD status, and for this reason we would encourage the design of clinical studies to address such questions.

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