MODERN APPROACHES TO ASSESSMENT OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA (PLASMA CELL MYELOMA) CASES

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The treatment of multiple myeloma is inextricably linked to the need for assessment and monitoring of the minimal residual disease (MRD). Assessment of the MRD allows evaluating the efficacy of therapy and obtaining significant prognostic information; it is an indicator of the degree of eradication of the tumor clone. The methods for detecting residual tumor cells evolve constantly, which translates into updates of the criteria reflecting the scale of response to therapy. There is no single MRD detection technique; common recommendations suggest seeking for pathological cells both intramedullary and extramedullary. This review describes current MDR determination methods, including imaging, next generation multiparametric flow cytometry, and methods based on DNA analysis — allele-specific oligonucleotide polymerase chain reaction and next generation sequencing. We compare their advantages, limitations, disadvantages, clinical significance, and show the necessary sensitivity thresholds of the described methods and the conditions that make this or that approach ideal in the context of detection of MRD.

Keywords: multiple myeloma, minimal residual disease, methods of assessment, flow cytometry, next generation sequencing

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СОВРЕМЕННЫЕ ПОДХОДЫ К ОЦЕНКЕ МИНИМАЛЬНОЙ ОСТАТОЧНОЙ БОЛЕЗНИ ПРИ МНОЖЕСТВЕННОЙ МИЕЛОМЕ (ПЛАЗМОКЛЕТОЧНОЙ МИЕЛОМЕ)

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Лечение множественной миеломы (ММ) неразрывно связано с необходимостью оценки и мониторирования минимальной остаточной болезни (МОБ). Определение МОБ является важной задачей, позволяющей более глубоко оценить эффективность терапии, получить значимую прогностическую информацию, и является определяющим критерием степени эрадикации опухолевого клона. Это обусловливает необходимость совершенствования методов выявления остаточных опухолевых клеток и приводит к обновлению критериев определения глубины ответа в соответствии с уровнем МОБ. В настоящее время не существует единого метода обнаружения МОБ, рекомендуется использовать как интрамедуллярную, так и экстрамедуллярную детекцию патологических клеток. В обзоре описаны современные методы определения МОБ, включая методы визуализации, выявление остаточных опухолевых клеток в образцах костного мозга и периферической крови с использованием многопараметрической проточной цитометрии (МПЦ), в том числе нового поколения (NGF), и методы, основанные на анализе ДНК — аллель-специфичная олигонуклеотидная полимеразная цепная реакция (АСО-ПЦР) и секвенирование нового поколения (NGS). Проведен сравнительный анализ их преимуществ, ограничений, недостатков и, соответственно, клинической значимости. Показаны необходимые пороги чувствительности описываемых методов и ситуации, в которых применение того или иного метода является оптимальным для диагностики МОБ.

Ключевые слова: множественная миелома, минимальная остаточная болезнь, методы оценки, проточная цитометрия, секвенирование нового поколения

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Multiple myeloma (MM) is a B-cell malignant tumor, the morphological substrate of which are plasma cells producing monoclonal immunoglobulin. In 2017, World Health Organization (WHO) replaced "multiple myeloma" with "plasma cell myeloma" in its registers. However, in the context of the 5th Edition of the World Health Organization Classification of Hematolymphoid Tumors (2022), experts discussing mature lymphoid and histiocyte-dendritic cell neoplasms strongly supported the term "multiple myeloma" rather than "plasma cell myeloma," and thus it was adopted in the International Consensus Classification of Mature Lymphoid Neoplasms [1]. Therefore, in this article, we call the considered disease "multiple myeloma," as is habitual for hematologists.

It is generally recognized that monitoring of minimal residual disease (MRD) in multiple myeloma cases, which aims at detecting subclinical amounts of myeloma cells after successful antitumor therapy, is an important task that allows a more in-depth assessment of the said therapy's efficacy, adds significant prognostic information regarding overall survival (OS) and progression-free survival (PFS) of MM patients, and yields data needed to establish the degree of eradication of the tumor clone. In this connection, improvement of the methods for detecting residual tumor cells is a continuous effort, and the categories of degree of response in accordance with the MRD level are being constantly updated [2–4].

In recent years, MRD detection methods have been developing rapidly, and their sensitivity and applicability have expanded significantly. To improve the sensitivity of myeloma cell detection, there were developed new high-performance bone marrow (BM) aspirates evaluation methods, including multiparametric flow cytometry (MFC), allele-specific oligonucleotide qualitative polymerase chain reaction and next-generation sequencing (NGS). These methods enable quick examination of several thousands to a million BM cells or the

corresponding amount of DNA in a single test, thus quantifying the residual tumor cells in BM.

It is known that MRD-negative (MRD(–)) patients will inevitably relapse, and in some of them, neither MFC nor PCR can detect tumor cells, which supports the need for further efforts to standardize and improve MRD diagnostics.

A lower MRD detection cutoff value peculiar to the sensitive types of examination, such as NGS or highly sensitive MFC, will further improve disease prediction capabilities [5, 6]. For example, using NGS and allocating patients into 3 groups by time to progression (TTP), a group of researchers has shown that people with high (< 10^{-3}), intermediate (10^{-3}–10^{-5}), and low (> 10^{-5}) levels of MRD can have significantly different TTP (27, 48 and 80 months, respectively) [5]. Thus, currently, 10^{-5} is the threshold for affirmation of an MRD-negative status.

In 2016, the International Myeloma Working Group (IMWG) published the following MRD(–) status criteria [7]:

- persistent MRD(–) status, i.e., MRD negative results of BM cells examinations with NGF and/or NGS and PET-CT, persisting for 1 year;
- MRD(–) status confirmed by flow cytometry, that is, absence of aberrant phenotype clonal plasma cells (PCs) in BM aspirates according to NGF that follows the standard EuroFlow operating procedure (or an equivalent validated protocol), minimum sensitivity of 10^{-5} or higher;
- MRD(–) status confirmed by sequencing, i.e., absence of clonal PCs in the results of NGS of BM aspirates, with clone presence defined as less than two identical readings in BM aspirates' DNA sequences established with a minimum sensitivity of 10^{-5} or higher;
- MRD(–) status confirmed by NGF or NGS plus disappearance of each area of increased absorption of the marker that was detected initially or by previous PET-CT, or a drop of the number thereof below the mediastinum SUV value, or below normal.

This review aims to comparatively analyze the advantages, limitations, disadvantages, and clinical significance of the current MRD assessment methods, and describe conditions making this or that method optimal in a given clinical situation.

MRD assessment methods in multiple (plasma cell) myeloma cases

Serological methods of identification of tumor clone

In MM cases, tumor load is diagnosed and monitored through identification of free light chains (FLC) in serum and urine [8]. Currently, assessment of serum FLC κ and λ is one of the routine tests, especially for patients with nonsecretory and oligosecretory myeloma and AL-amyloidosis [9].

Back in 2006, IMWG group included normalization of the FLC level and absence of clonal myeloma cells in BM biopsies sampled from MM patients, as established by immunohistochemistry and/or immunofluorescence, in the list of more stringent criteria defining complete response (CR) [10]. In diagnostics, FLC ratio is an independent prognostic factor of aggressiveness of the disease [11], which also helps stratify patients into risk groups [12]. However, there is still no single opinion about inclusion of the FLC test into routine monitoring of MRD in MM patients, because some studies report contradictory results, even in the context of treatment response [13, 15]. For example, it was shown that normalization of the FLC level is not associated with better survival rate in patients whose CR meets the traditional criteria. In addition, it was suggested to replace identification of FLC with that of heavy chains, which should be considered more a surrogate marker of immune system recovery than an MRD monitoring item; moreover, FLC testing was criticized as reliable method of assessment of MRD in myeloma, although FLC ratio is one of the response evaluation criteria.

Morphological study

Morphological study of BM is the most common method for determining tumor load in MM cases. Several large-scale studies have shown the independent prognostic value of BM microscopy [16, 17], however, the sensitivity of this method is limited by the number of cells sampled and variability of sampling conditions.

Visualization methods

Multiple myeloma differs from other hematological diseases in the patterns of infiltration of BM with MM cells, which vary depending on the type of the disease and sampling location. Moreover, dilution of BM aspirates with peripheral blood can lead to false negative results. These problems, along with the possible extramedullary (EM) lesions, complicate interpretation of results of all MRD tests relying on BM. Therefore, affirmation of the MRD(–) status may be false. Alternative methods, such as imaging [18, 19], monitoring of clonogenic MM progenitor cells [19, 20] or circulating myeloma tumor cells can give additional information about MRD [2]. Sensitive imaging techniques enable reliable assessment of small EM lesions due to the high frequency of EM recurrences in MM cases. Magnetic resonance imaging (MRI) is the most sensitive non-invasive method of detection of skeletal bone foci, assessment of prevalence and nature of soft tissue lesions, and identification of the type of BM infiltration. Inter alia, MRI is the study indicated in cases of monoclonal gammapathies of undetermined significance (MGUS) and smoldering myeloma, as it detects foci measuring 5 mm and, thus, clarifies progression of the tumor process. However, in the presence of necrosis and inflammation, focal lesions may remain over-intense in both responding and non-responding patients, therefore, an unambiguous CR conclusion based on the results of MRI may be impossible.

While MRI does not allow correctly assessing active foci after myeloma therapy, positron emission tomography (PET) has proven its prognostic significance [18, 21] and may be the most effective method for monitoring MRD in MM cases. The specific advantage of PET is the ability to identify both bone marrow and EM lesions, and to separately show tumor and necrotic tissues. Despite the PET/CT combination being common in clinical practice, it has a number of problems: not all MM patients have detectable foci, and interpretation of data is complicated by heterogeneity of the imaging criteria and insufficient reproducibility in various studies. Moreover, PET/CT is not always sufficiently informative because of spatial resolution limit of 0.5 cm and potential for false negative results when the level of absorption of fluorodeoxyglucose is very low. For repeated examinations, it is necessary to factor in radiation exposure, which is higher than peculiar to radiography and CT [22, 23].

A more specific PET/CT with fluorodeoxyglucose (^{18}F-FDG) is considered a standard imaging method for assessment of efficacy of treatment. Persistence of significant abnormal ^{18}F-FDG uptake after treatment is an independent negative prognostic factor, which substantiates the importance of this MRD diagnostic method when used before starting maintenance therapy. The definition of complete metabolic

response as detected by PET has recently been standardized, and interpretation criteria harmonized. Researchers note promising results shown by innovative radiopharmaceuticals (small molecules targeting CXCR4 chemokine receptors, isotope-labeled CD38 antibodies) as potential theranostics that are both diagnostic and antitumor agents [24].

Allele-specific oligonucleotide PCR (ASO PCR)

A relapse in an MM patient means that not all clonogenic malignant cells were destroyed, and there persist residual tumor cells not detected by the above methods. In this connection, it is important to use more accurate monitoring techniques during remission and relapse, namely, molecular biological methods, including ASO PCR and quantitative real-time PCR. The tumor marker selected in MM cases for MRD assessment is the hypervariable region of rearrangement of immunoglobulin heavy chain genes (IgH). Location of this region and analysis of the sequence require synthesis of allele-specific oligonucleotide primers and probes of specific design [25].

In the context of identification of clonal rearrangements of IgH, ASO PCR allows detecting very small amounts of tumor PCs with sensitivity of 1×10^{-5}. Unlike qualitative or semi-quantitative PCR methods, ASO PCR accurately quantifies MRD. The method involves synthesis of primers complementary to the junctional region of rearranged IgH genes; they are used to learn the depth of response in BM samples taken at various times, which also requires a baseline (taken before treatment) diagnostic sample.

The advantages of PCR methods of MRD diagnosing are their sensitivity, accuracy, reproducibility, low DNA amount requirements, and indispensability in the context of retrospective studies. On the other hand, they are more complex, expensive, take longer and reveal only the initial tumor clone. Nevertheless, detection of tumor markers with the help of PCR is a common practice in clinical testing of patients for early recurrence or tumor contamination of hematopoietic stem cells (HSCs) during autologous transplantation (autoTHSC). Thus, with fully patient-specific primers/probes, ASO PCR is effective in >90% of MM patients; the method allows detection of dynamic changes of MRD during autoTHSC, regardless of the CR established by traditional accepted methods [26].

NGS

NGS is another technique used to establish the MRD status in cases of malignant lymphoid neoplasia. It is a quantitative method based on the use of consensus primers for universal amplification with sequencing of all rearranged segments of Ig genes found in the clonal myeloma cells [5, 27]. NGS is applicable in more than 90% of cases; its sensitivity is $\leq 10^{-6}$. Utilizing automated data analysis and requiring no expert interpretation relying on knowledge of the tumor clone's characteristics, this method can be used in most laboratories. Moreover, such molecular studies are not affected by genetic heterogeneity and changes in the clonality of malignant cells occurring during treatment. The results of NGS can also be interpreted with the aim to identify subclones and clonal evolution at the MRD stage [4]. However, applicability of this test in the context of stratification of patients into risk groups requires additional validation.

MFC

Currently, MFC is one of the main methods for diagnosing malignant neoplasms, detecting their PCs in BM by aberrant expression of surface markers in approximately 90% of patients. The sensitivity of 6-color MFC is 1×10^{-4} myeloma cells; 8 and more colors, or markers, increase it up to 1×10^{-6} tumor cells, and make the test more specific. The method can also differentiate the expression of light k or λ chains of Ig (IgL) [28, 29]. In recent years, the sensitivity of MFC has increased to $\geq 10^{-5}$ thanks to simultaneous assessment of 8 or more markers in one tube, which allows identifying aberrant PC phenotypes while assessing MRD and counting the sufficient number of cells ($\geq 5 \times 10^6$) [30–32]. Invention of flow cytofluorometry that can detect up to 30 markers simultaneously increased the number of fluorochromes that can be used in one tube, as well as the number of cells examined.

MFC also allows evaluating the role of tumor microenvironment in plasma cell diseases [33] and identifying the possible therapeutic targets on malignant PCs [34].

There have been described many surface markers signaling difference between tumor PCs from normal ones. The most common are CD138, CD38, CD45, CD56, CD19, and cytoplasmic κ and λ Ig light chains. Additional diagnostic markers, many of which are characterized by aberrant expression on the PC, are CD20, CD27, CD28, CD81, CD117 and CD200 [35]. In the context of monoclonal antibodies therapy against CD38 or CD138, CD54, CD229, CD319 may be useful. However, heterogeneity of the expression of these markers, differences in the number of studied events and analysis strategy complicate interpretation of results of various studies and add contradictions thereto [36].

MFC has known value in prediction of results of autoTHSC. Many researchers note that MFC-confirmed 100th day MRD(–) status of patients after autoTHSC is one of the most important predictors of disease outcome, and it is associated with a statistically significant improvement of the PFS indicator regardless of the cytogenetic characteristics [6, 37, 38].

According to a study, 58% of the patients who underwent autoTHSC and received lenalidomide maintenance therapy for 1 year achieved CR, and 68% of them were MRD(–) according to the results of MFC. At the three-year mark, PFS was 77%, and OS 100%. None of the patients who became MRD(–) had a relapse after 39 months (median value) [35].

However, there are factors that limit efficacy of MFC: quality of BM samples (should be high), no standard MFC protocols and variable sensitivity, contents of the monoclonal antibody panels and level of execution in various laboratories [39]. Moreover, first generation MFC is not as sensitive as ASO PCR and NGS.

Next generation MFC

Considering the many options of execution of MFC test, the unified MRD definition criteria should be established by a consensus [40]. A consortium of EuroFlow and IMWG have developed next generation MFC, or NGF (next generation flow), which is more sensitive, relies on a new design, and allows counting larger number of cells. There was created and validated eight-color antibody panel for MM diagnostics: 1st tube — CD45/CD138/CD38/CD56/β2 microglobulin/CD19/cyIgkappa/cyIglambda, 2nd tube — CD45/CD138/CD38/CD28/CD27/CD19/CD117 [41], with 4 basic markers (CD38, CD138, CD45, CD19) and 8 additional ones for subsequent identification, counting and characterization of tumor PCs. This method allows simultaneous analysis of up to 10^6 cells. Software algorithms have also been developed for automatic identification of clonal PCs (i.e. MRD) in BM samples.

International Myeloma Working Group approved NGF as a reference method for establishment of immunophenotypic CR in MM cases. Its sensitivity is up to 2×10^{-6}, surpassing that of the previous MFC tests (10^{-4}–10^{-5}), but it strongly

Table. Comparison of MRD assessment methods utilizing BM samples [7]

	ASO PCR	MFC	NGS
Applicability	60–70%	About 100%	≥ 90%
Need for baseline sample	Yes, requires synthesis of patient-specific probes	No, tumor PCs can be identified in any sample by their phenotypic differences with normal PCs	Baseline samples are needed for identification of the dominant clone; alternatively, the initial state can be learned from stored samples with tumor cells
Sample requirements	< 10^6 cells	> 5 × 10^6 cells	< 10^6 cells, greater amount increases sensitivity
Sample processing	May be delayed; works with fresh and stored samples	Study within 24–48 hours after sampling	May be delayed; works with fresh and stored samples
Sample quality control	Impossible. Requires additional studies	Immediate, with global analysis of BM cell	Impossible. Requires additional studies
Sensitivity	≥ 1 in 10^5 cells	≥ 1 in 10^5 cells	≥ 1 in 10^5 cells
Additional information about contents of the sample	None	Detailed information on the content of leukocyte populations	Information about the repertoire of Ig B-cell genes in the studied samples
Duration and complexity of execution	Requires synthesis of patient-specific primers/probes; may take several days	Takes a few hours, relies on an automated data processing system	May take several days, requires significant bioinformatics support
Standardization	Completed for other diseases (EuroMRD), can be done for MM	Standardized by EuroFlow	Work in progress
Availability	Widely available, there are about 60 EuroMRD member laboratories that undergo quality control twice a year	Most clinics have flow cytometers (4 or more colors). Many laboratories use EuroFlow protocols and kits.	Limited to one company/platform

depends on the correctness of identification of the pathological immunophenotype, which translates into the need for highly qualified specialists [42].

Next generation flow cytometry was shown to perform better than NGS, although on a small amount of data [40]. In a series of experiments, researchers compared the two methods: they used both to test for MRD samples from MM patients that underwent autoTHSC 3 months ago. The specific protocols were LymphoTrack® (NGS) and EuroFlow (NGF). The experiment has shown high correlation between the methods (r = 0.905), although it was concluded that NGF was the preferred one for the task. Three-year PFS, according to NGS and NGF, was higher in MRD(–) than in MRD(+) patients (NGS: 88.7 vs. 56.6%; NGF: 91.4 vs. 50%; p < 0.001 for both comparisons), which translated into better 3-year OS (NGS: 96.2 vs. 77.3%; NGF: 96.6 vs. 74.9%, p < 0.01 for both comparisons). In the Cox regression, MRD(–) status meant similar results of both NGS and NGF tests, but the latter was the preferred one considering PFS (RR: 0.20, 95% CI: 0.09–0.45, p < 0.001) and OS (RR: 0.21, 95% CI: 0.06–0.75, p = 0.02). These results confirm that sensitivity of MFC can be on par with that of molecular methods [43].

Currently, NGF enables the shift to the new phase of quantification of residual disease, replacing "minimal" with "measurable" in MRD [44].

The use of therapeutic drugs based on CD38 antibodies, such as daratumumab [45], which weaken the expression of CD38 antigen on PCs, gave rise to the need for alternative markers enabling identification of normal or neoplastic PCs. For this purpose, CD269, CD319, CD229 and CD54 markers proved to be informative, as they allowed identifying PCs in more complex samples, including long-stored ones [29]. It should be noted that monoclonal antibody therapy does not have such an effect on the results of NGS.

Comparison of methods

Each of the described MRD assessment methods (based on the PC phenotype and/or genotype) has both advantages and disadvantages that should be taken into account (Table).

There is a study [46] that compares applicability, sensitivity and prognostic significance of ASO PCR and MFC for MRD assessment, which involved 170 MM patients who responded to therapy at least partially [46]. Ultimately, data from only 42% of PCR tests were used, the reasons being lack of detected clonality (18%), sequencing failures (10%), and suboptimal characteristics of the ASO PCR results (30%). The comparison of MRD assessments by PCR and MFC revealed a significant correlation of the results delivered by both methods (r = 0.881). The results of PCR allowed allocating patients with CR into 2 risk groups with different PFS (49 vs 26 months, p = 0.001) and OS (not achieved vs 60 months, p = 0.008). Although less widely applicable than MFC, ASO PCR enables evaluation of the effectiveness of therapy and stratification of MM patients into risk groups [46].

The prognostic capacity of these methods has also been compared in the context of the emerging new approaches to MM therapy and novel drugs [47]. The survival curves produced by both methods were almost identical, with very high MRD assessment prognostic values for both intensively and non-intensively treated patients, which confirms the significance of both methods in prediction of results of the therapy. However, neither method can detect EM relapses in 100% of cases.

Thus, ASO PCR and MFC are reliable methods for monitoring the effectiveness of treatment. They can support accurate predictions of the outcomes for patients who underwent autoTHSC and those who did not. ASO PCR has greater sensitivity, but MFC is more common. MFC should be considered the method of choice for assessment of MRD in MM cases, and molecular methods can be regarded as additional tools until clear demonstration of their comparative advantages [48].

Real time PCR has greater sensitivity compared to MFC, but the latter is simpler and faster, so they can complement each other in MRD testing. A study [49] has shown a significant correlation between MRD assessment with the help of real-time PCR and by CD138 expression.

Results of the RV-MM-EMN-441 study show that in patients who underwent autoTHSC, the value of MRD is lower than in those who received cyclophosphamide + lenalidomide + dexamethasone. The progression of MRD was preceded by clinical manifestations of a relapse with a median of 9 months, and biochemical signs thereof with a median of 4 months. The assessment of MRD by both MFC and real-time PCR allowed allocating patients to a low-risk group and improving characterization of the effect of therapy [50].

An ideal MRD testing method should meet a number of requirements, including: high degree of applicability (usable in most cases), high sensitivity and specificity, good executability, availability, short duration, low sample requirements (low amount thereof, simple transportation), reproducibility, proven clinical significance, and cost-effectiveness. A significant disadvantage of the sequencing-based molecular methods is the need for a baseline sample, which is used to establish tumor-specific sequences. Currently, there are no methods that fully satisfy these ideal criteria, but the NGS and NGF meet most of the given requirements [5, 27, 51].

MRD assessment using peripheral blood

Typically, clonal PCs are localized in BM, but sensitive methods can detect small amounts of them in the peripheral blood of most MM patients. Circulating tumor cells usually mean worse PFS and OS. MFC-enabled test for PCs in peripheral blood returned negative for patients with CR and positive in those who suffered a relapse [52].

Small amounts of tumor cells circulating in peripheral blood can also be detected by molecular genetic methods. Although ASO PCR was shown to give significantly lower MRD values in preipheral blood tests than BM studies, patients that underwent autoTHSC and whose test returned negative, 3 months after the operation had better PFS (median 15 months vs 4 months) and OS (median 52 months vs 17 months) values [53]. Sequencing-enabled monitoring of clonotypic cells in peripheral blood helped detect MM recurrence at its early stage. Results of another study of ASO PCR's capabilities showed that this method allows detection of myeloma cell clones with occurrence of less than one cell per 10^6 leukocytes; all in all, the researchers found myeloma cells in the peripheral blood of 96% of patients [54]. Despite the correlation between MM clone value in parallel studies of BM and peripheral blood samples, none of the patients in the described studies reached complete remission. Several studies investigated DNA of circulating cells, searching for small amounts of residual tumor cells, which enables tracking of individual tumor clones [55, 56].

CONCLUSION

Given the importance of determining the MRD status of MM patients in the context of production of novel drugs, improvement of HSC transplantation programs and therapy in general, it becomes especially important to use the most sensitive and informative methods for detecting residual tumor cells in clinical practice.

The ideal MRD monitoring test should detect pathological plasma cells relying on a sensitive, predictive, non-invasive, standardized, cost-effective and affordable approach. Along with the evolution of immunological approaches, there are many new additional ways being developed that are designed to identify residual tumor cells in bone marrow and beyond.

Imaging techniques, such as PET-CT or MRI, can detect residual disease, including extramedullary foci and foci in bone marrow. Moreover, recent studies show that whole body diffusion-weighted MRI (WB-DWI-MRI) can give a more accurate MRD assessment than PET-CT with FDG [57]. Another important MRD test method is NGS with sequencing of IgH/IgK/IgL loci for the purpose of identification of rearrangements of the Ig gene in MM cells. NGS data can be further interpreted to identify subclones, clonal evolution, and growth of individual clones at the MRD stage. MRD should be part of the array of clinical tests, assessed on bone marrow samples using proven and standardized procedures with a high sensitivity threshold, ideally 10^{-6}; currently, the list of such methods includes NGF and NGS.

Based on the analysis of pros and cons of each MRD assessment method, it can be concluded that in general, by sensitivity, the rating starts with NGS or NGF, followed by MFC, then ASO PCR, and by applicability — MFC or NGF, then NGS, then ASO PCR, since the latter requires diagnostic samples to identify patient-specific sequences of clonotypes [4].

Combining NGF, NGS and PET CT under a complex approach to MRD assessment is a promising trend, since MFC or NGS can assess MRD from the intramedullar perspective, and WB-DWI-MRI or PET-CT — from extramedullar one, which, combined, grants more accuracy to the overall assessment of deep remission [58]. Currently, several laboratory and preclinical studies revolve around new methods, such as matrix laser desorption/ionization mass spectrometry, high-performance liquid chromatography mass spectrometry, detection of circulating extracellular DNA, and RNA sequencing at the single cell level [59, 60]. Inclusion of the new alternative methods in the testing array for MM patients may radically change the assessment of MRD in the future.

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