Multiple Myeloma MRD

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Minimal residual disease is important in evaluating response in multiple myeloma. Either NGS or NGF could be done. Laboratory experience and standardization are needed in order to give high quality Analysis.

Keywords: myeloma, minimal residual disease, NGF , NGS

1. Introduction

Multiple myeloma (MM) is caused by monoclonal plasma cells that proliferate in the bone marrow causing anemia, hypercalcemia, kidney failure, or bone skeletal lesions (i.e., CRAB criteria)[1]. The protein found in the serum or urine of these patients, called the monoclonal protein, is produced by myeloma plasma cells. MM patients presented with a median age above 70 years, and MM incidence has increased in the last 20 years[2], representing 10% in all hematological neoplastic diseases. In the last few years, great improvement has been seen in MM patient survival[1][2][3][4]. This improvement was due to the approval of novel drugs such as proteasome inhibitor (PI) bortezomib (2003) and immunomodulatory drugs (IMIDs) thalidomide and lenalidomide (2006) associated with high-dose therapy represented by autologous stem cell transplant (ASCT)[5][6]. These new drugs associated with novel strategies such as consolidation therapy after transplant and maintenance with lenalidomide (2017) have led to survival prolongation. Extramedullary MM, a peculiar aggressive form of MM related to hematogenous spread of monoclonal plasma cells, also seemed to be improved by the use of novel drugs[7][8][9][10][11]. In the last few years, other drugs have become available, such as second- and third-generation PIs (i.e., Carfilzomib in 2012, Ixazomib in 2018), second-generation IMIDs (Pomalidomide in 2013), and drugs that have new mechanisms of action that were first used in clinical trials and are now commercially available[12], such as the monoclonal antibodies Daratumumab (that targets CD38 on monoclonal plasma cells) and Elotuzumab, which targets “signaling lymphocytic activation molecule F7” (SLAMF-7). Despite this great progress, a small percentage of patients, that is, high-risk cytogenetics patients (10–15%) carrying del 17p and t(4;14), have particularly poor survival and together with patients with other aggressive features (extramedullary disease, renal insufficiency, disease refractoriness, elevated LDH) represent unmet clinical needs[13][14].

2. Complete Remission (CR) and Minimal Residual Disease

Novel drugs have improved complete MM responses that are considered survival surrogates. However, it was difficult to deeply define a complete response until a few years ago. In fact, CR was considered when plasma cells were in less than 5% of the bone marrow, and the absence of monoclonal protein was shown in serum or urine by immunofixation or disappearance of soft tissue plasmacytomas. Since most patients relapse even after achieving CR, it became obvious that better techniques are needed to identify minimal residual disease at the lowest possible level. Bone marrow or bone core biopsy analysis can only detect one monoclonal plasma cell out of 100–300 cells with a specificity of 10^{-2}. Nowadays, this is not enough considering the depth of response achieved with novel and more efficient drugs. Recent data suggested that more sensitive assays like eight-color next-generation flow (NGF) or next-generation sequencing (NGS) could much further improve minimal residual disease (MRD) detection. International Myeloma Working Group (IMWG) guidelines for response definition identified that a cut-off of 10^{-5} should be considered as the target for the definition of MRD negativity, detected by either NGS or NGF[15][16].

3. Next-Generation Sequencing (NGS) for Evaluation of Multiple Myeloma MRD

Clonal immunoglobulin (Ig) gene rearrangements represent the main molecular target in MM MRD detection[17]. Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) was utilized as the first technique to assess MM MRD. However, its applicability is limited by the high rate of somatic hypermutations (SHMs) within Ig heavy chain complementary determinant regions (IgH-CDR). NGS technology, through the parallel sequencing of millions of reads,

overcomes technical ASO-PCR pitfalls, allowing for MRD measurement with 10^{-6} sensitivity[18]. The advantage of this approach is represented by the ability to also identify clonality in MM patients with a low tumor burden, offering an improvement in the knowledge of MM biology, providing useful information regarding therapeutic choices and disease management. Moreover, in contrast to ASO-PCR, NGS does not require designing patient-specific primers and a standard curve for “MRD quantification”. Obtained data need to be elaborated through specific bioinformatic tools designed to analyze millions of reads. Two identical sequencing reads were defined as clonotypes, and the >5% frequency of a clonotype, established as the cut-off value for MRD evaluation in follow-up samples, is referred to as clonality[19].

During the last few years, several NGS platforms for MRD detection in MM were tested. Two of these, ImmunoSEQ (Adaptive Biotechnologies, Seattle, WA, USA) and LymphoSIGHT® (Adaptive Biotechnologies, Seattle, WA, USA) both from Adaptive Biotechnologies; the latter, currently known as ClonoSEQ® (Adaptive Biotechnologies, Seattle, WA, USA), was the first licensed by the FDA in 2019, and it is currently the most frequently adopted. These commercial kits are characterized by two-stage and single-reaction PCR amplifying IgH VDJ rearrangements, respectively. Additionally, the LymphoTrack-MiSeq platform was compared to ASO-PCR, as documented by Yao and colleagues; this NGS technology allowed the quantification of those cases defined as positive but not quantifiable (PNQ) by ASO, and it was consequently suggested for MM MRD monitoring [19]. Despite the high sensitivity of NGS, the feasibility of this approach is limited by high costs, long turnaround time, and required expertise. For these reasons, more affordable techniques, such as digital PCR (dPCR), were proposed to be used in daily MM patient management. dPCR does not require a standard curve, leading to the absolute quantification of the target gene, and it is also less laborious than NGS regarding data interpretation. On the other hand, its applicability is not yet standardized, and further studies are needed to confirm it[20][21][22]. Moreover, sample quality is another critical aspect: the hemodilution and patchy nature of MM BM samples may interfere in molecular MRD evaluation, leading to possible false-negative results. Several clinical trials investigated the prognostic value of NGS technology in MM management. Obtained results by a French group (2009 IFM study) on MM patients treated with lenalidomide, bortezomib, and dexamethasone (RVD) showed higher sensitivity for NGS compared with MFC in MRD detection, with MRD negativity established at $<10^{-6}$[23]. The CASSIOPEIA trial demonstrated that NGS and NGF are superimposable techniques for MRD evaluation. In the PETHEMA study, MRD analysis by deep sequencing at distinct levels was able to identify three MM groups with different time to tumor progression (TTP)[24][25]. In conclusion, on the basis of this evidence, the NGS approach was demonstrated to be a powerful tool for MRD detection, considering the key role of the achievement of MRD negativity in the clinical management of MM patients. In this scenario, the most appealing prospective in MM patients is the use in the clinical practice of this reliable and sensitive technique for MRD assessment. The strong deep-sequencing prognostic role by NGS could lead clinicians to better treatment strategies.

3.1. Quality of Bone Marrow Aspirates May Influence MRD Analysis

Both NGF and NGS require high amounts of standardized starting material and sample preparation protocols to optimize the procedure across laboratories. Performing a count of the starting materials, before processing the sample, could help to estimate the number of cells at disposition; however, it is not possible to predict through cell numbers the level of sensitivity we could acquire by NGF, neither is possible to estimate the quality and quantity of DNA/RNA that could be extracted from the sample. Low-quality BM aspirates should be interpreted with caution, especially if they are to be used for specific applications such as MRD quantification[26][27][28]. First, a frequently encountered pitfall in MRD evaluation is BM hemodilution by peripheral blood that may lead to important changes in the distribution of cell populations and to underestimation of neoplastic cells percentages. Different methods were recommended to accurately evaluate the degree of hemodilution. These methods were based on an automated lymphocyte count, PB contamination indices that took account of PC percentages, CD34+ cells, and CD10+ neutrophils[29], or numbers of CD16 bright neutrophils[30]. Moreover, in the case of flow cytometric analysis, NGF can also provide the qualitative assessment of patient samples by allowing for analysis of normal B-cell compartments and non-PC BM cells, such as mast cells or RBCs, which can give us an idea of the hemodilution of analyzed BM samples. The other major problem in MM MRD evaluation is the necessity to acquire a high cell number, especially in the case of flow cytometric analysis, where an acquisition of at least 10^7 cell/sample is suggested. This problem may be overcome by optimizing the preanalytical procedure through red blood cell (bulk) lysis with ammonium chloride, which gives better results than those of previously employed Ficoll stratification. The use of immunomagnetic CD138+ bead enrichment that isolates neoplastic cells may be helpful in molecular biology tests to selectively and independently analyze the bad quality of BM aspirates; moreover, flow-activated cell sorting (FACS) is preferable, especially in the case of low-infiltrated BM samples, because it provides better recovery of both normal and neoplastic plasma cells[31]. However, we must consider that these two methods could somehow introduce manipulation of BM cells causing problems in MM MRD quantification. Working on high-quality BM samples for MRD evaluation could ameliorate assays, and it is crucial to have good coordination between clinicians and laboratories to improve the accuracy, sensitivity, and specificity of MM MRD detection in MM patients.

3.2. MRD in Peripheral Blood

Some studies are exploring the option of alternatively detecting MRD in MM PB samples, overcoming the invasive BM biopsy procedure and with the possibility to detect myeloma plasma cells in peculiar situations such as extramedullary localization. Another reason to search for MRD in PB is to avoid false-negative results due to the patchy distribution of myeloma cells in the bone marrow. NGS, NGF, or analysis of circulating cell-free tumor DNA (ctDNA) in liquid biopsy, in combination with imaging methods for MM MRD monitoring[32], could represent a valid strategy. However, all these efforts did not show so far a clear advantage for PB analysis with respect to BM. In fact, a recent study[33] showed that in 137 naïve MM patients, after treatment 55 (40%) still had detectable disease with NGF in the BM, while MRD was not detectable in PB. NGS in another study was utilized to detect myeloma cells in peripheral blood of 27 MM patients, and there was no difference in responding patients vs. nonresponders[34]. In a French study, ctDNA in PB was negative in 18/26 MM patients that still had disease in BM [35]. At the moment, PB MRD needs to be further investigated in trials that compare BM and PB techniques.

References

1. Gozzetti, A.; Candi, V.; Papini, G.; Bocchia, M. Therapeutic advancements in multiple myeloma. Front. Oncol. 2014, 4, 241.
2. Howlader, N.; Noone, A.M.; Krapcho, M.; Miller, D.; Brest, A.; Yu, M.; Ruhl, J.; Tatalovich, Z.; Mariotto, A.; Lewis, D.R.; et al. (Eds.) SEER Cancer Statistics Review, 1975–2017; National Cancer Institute: Bethesda, MD, USA, 2020. Available online: https://seer.cancer.gov/csr/1975_2017/ (accessed on 15 July 2020).
3. Mohty, M.; Terpos, E.; Mateos, M.V.; Cavo, M.; Lejniece, S.; Beksac, M.; Bekadja, M.A.; Legiec, W.; Dimopoulos, M.; Stankovic, S.; et al. EMMOS Investigators. Multiple Myeloma Treatment in Real-world Clinical Practice: Results of a Prospective, Multinational, Noninterventional Study. Clin. Lymphoma Myeloma Leuk. 2018, 18, e401–e419.
4. Brenner, H.; Gondos, A.; Pulte, D. Recent major improvement in long-term survival of younger patients with multiple myeloma. Blood 2008, 111, 2521–2526.
5. Cini, M.; Zamagni, E.; Valdré, L.; Palareti, G.; Patriarca, F.; Tacchetti, P.; Legnani, C.; Catalano, L.; Masini, L.; Tosi, P.; et al. Thalidomide–dexamethasone as upfront therapy for patients with newly diagnosed multiple myeloma: Thrombophilic alterations, thrombotic complications, and thromboprophylaxis with low-dose warfarin. Eur. J. Haematol. 2010, 84, 484–492.
6. Kumar, S.K.; Rajkumar, S.V.; Dispenzieri, A.; Lacy, M.Q.; Hayman, S.R.; Buadi, F.K.; Zeldenrust, S.R.; Dingli, D.; Russell, S.J.; Lust, J.A.; et al. Improved survival in multiple myeloma and the impact of novel therapies. Blood 2008, 111, 2516–2520.
7. Gozzetti, A.; Cerase, A.; Lotti, F.; Rossi, D.; Palumbo, A.; Petrucci, M.T.; Patriarca, F.; Nozzoli, C.; Cavo, M.; Offidani, M.; et al. GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) Myeloma Working Party Extramedullary intracranial localizations of multiple myeloma and treatment with novel agents: A retrospective survey of 50 patients. Cancer 2012, 118, 1574–1584.
8. Jurczyszyn, A.; Grzasko, N.; Gozzetti, A.; Czepiel, J.; Cerase, A.; Hungria, V.; Crusoe, E.; Silva Dias, A.L.; Vij, R.; Fiala, M.A.; et al. Central nervous system involvement by multiple myeloma: A multi-institutional retrospective study of 172 patients in daily clinical practice. Am. J. Hematol. 2016, 91, 575–580.
9. Gozzetti, A.; Cerase, A. Novel agents in CNS myeloma treatment. Cent. Nerv. Syst. Agents Med. Chem. 2014, 14, 23–27.
10. Castillo, J.J.; Jurczyszyn, A.; Brozova, L.; Crusoe, E.; Czepiel, J.; Davila, J.; Dispenzieri, A.; Eveillard, M.; Fiala, M.A.; Ghobrial, I.M.; et al. IgM myeloma: A multicenter retrospective study of 134 patients. Am. J. Hematol. 2017, 92, 746–751.
11. Jurczyszyn, A.; Olszewska-Szopa, M.; Hungria, V.; Crusoe, E.; Pika, T.; Delforge, M.; Leleu, X.; Rasche, L.; Nooka, A.K.; Druzd-Sitek, A.; et al. Cutaneous involvement in multiple myeloma: A multi-institutional retrospective study of 53 patients. Leuk. Lymphoma 2016, 57, 2071–2076.
12. Ocio, E.M.; Richardson, P.G.; Rajkumar, S.V.; Palumbo, A.; Mateos, M.V.; Orlowski, R.; Kumar, S.; Usmani, S.; Roodman, D.; Niesvizky, R.; et al. New drugs and novel mechanisms of action in multiple myeloma 2013: A report from the International Myeloma Working group (IMWG). Leukemia 2014, 28, 525–542.
13. Rajan, A.M.; Rajkumar, S.V. Interpretation of cytogenetic results in multiple myeloma for clinical practice. Blood Cancer J. 2015, 5, e365.

14. Gozzetti, A.; Le Beau, M.M. Fluorescence in situ hybridization: Uses and limitations. Semin Hematol. 2000, 37, 320–333.

15. Landgren, O.; Rajkumar, S.V. New Developments in Diagnosis, Prognosis, and Assessment of Response in Multiple Myeloma. Clin. Cancer Res. 2016, 22, 5428–5433.

16. Kumar, S.; Paiva, B.; Anderson, K.C.; Durie, B.; Landgren, O.; Moreau, P.; Munshi, N.; Lonial, S.; Bladé, J.; Mateos, M.V.; et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016, 17, e328–e346.

17. Lionetti, M.; Neri, A. Utilizing next-generation sequencing in the management of multiple myeloma. Expert Rev. Mol. Diagn. 2017, 17, 653–663.

18. Bai, Y.; Orfao, A.; Chim, C.S. Molecular Detection of Minimal Residual Disease in Multiple Myeloma. Br. J. Haematol. 2018, 181, 11–26.

19. Yao, Q.; Bai, Y.; Orfao, A.; Chim, C.S. Standardized Minimal Residual Disease Detection by Next-Generation Sequencing in Multiple Myeloma. Front. Oncol. 2019, 9, 449.

20. Della Starza, I.; Nunes, V.; Cavalli, M.; De Novi, L.A.; Ilari, C.; Apicella, V.; Vitale, A.; Testa, A.M.; Del Giudice, I.; Chiaretti, S.; et al. Comparative Analysis Between RQ-PCR and digital-droplet-PCR of immunoglobulin/T-cell Receptor Gene Rearrangements to Monitor Minimal Residual Disease in Acute Lymphoblastic Leukaemia. Br. J. Haematol. 2016, 174, 541–549.

21. Korde, N.; Mailankody, S.; Roschewski, M.; Faham, M.; Kotwaliwale, C.; Moorhead, M.; Kwok, M.L.; Manasanch, E.E.; Bhutani, M.; Tageja, N.; et al. Minimal Residual Disease (MRD) Testing in Newly Diagnosed Multiple myeloma (MM) Patients: A Prospective Head-to-Head Assessment of Cell-Based, Molecular, and Molecular-Imaging Modalities. Blood 2014, 124, 2105.

22. Perrot, A.; Lauwers-Cances, V.; Corre, J.; Robillard, N.; Hulin, C.; Chretien, M.L.; Dejoie, T.; Maheo, S.; Stoppa, A.M.; Pegourie, B.; et al. Minimal residual disease negativity using deep sequencing is a major prognostic factor in multiple myeloma. Blood 2018, 132, 2456–2464.

23. Avet-Loiseau, H.; Bene, M.C.; Wuilleme, S.; Corre, J.; Attal, M.; Arnulf, B.; Garderet, L.; Macro, M.; Stoppa, A.M.; Delforge, M.; et al. Concordance of Post-consolidation Minimal Residual Disease Rates by Multiparametric Flow Cytometry and Next-generation Sequencing in CASSIOPEIA. Clin. Lymphoma Myeloma Leuk. 2019, 19, E3–E4.

24. Martinez-Lopez, J.; Lahuerta, J.J.; Pepin, F.; Gonzalez, M.; Barrio, S.; Alaya, R.; Puig, N.; Montalban, M.A.; Paiva, B.; Weng, L.; et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. Blood 2014, 123, 3073–3079.

25. Oliva, S.; Gambella, M.; Gilestro, M.; Muccio, V.E.; Gay, F.; Drandi, D.; Ferrero, S.; Passera, R.; Pautasso, C.; Bernardini, A.; et al. Minimal residual disease after transplantation or lenalidomide-based consolidation in myeloma patients: A prospective analysis. Oncotarget 2017, 8, 5924–5935.

26. Rawstron, A.C.; Orfao, A.; Beksac, M.; Bezdickova, L.; Brooimans, R.A.; Bumbea, H.; Dalva, K.; Fuhler, G.; Gratama, J.; Hose, D.; et al. European Myeloma Network. Report of the European Myeloma Network on Multiparametric Flow Cytometry in Multiple Myeloma and Related Disorders. Haematologica 2008, 93, 431–438.

27. Brooimans, R.A.; Kraan, J.; Van Putten, W.; Cornelissen, J.J.; Löwenberg, B.; Gratama, J.W. Flow cytometric differential of leukocytes populations in normal bone marrow: Influence of peripheral blood contamination. Cytom. B Clin. Cytom. 2009, 76, 18–26.

28. Gupta, R.; Bhaskar, A.; Kumar, L.; Sharma, A.; Jain, P. Flow Cytometric Immunophenotyping and Minimal Residual Disease Analysis in Multiple Myeloma. Am. J. Clin. Pathol. 2009, 132, 728–732.

29. Delgado, J.A.; Guillén-Grima, F.; Moreno, C.; Panizo, C.; Pérez-Robles, C.; Mata, J.J.; Moreno, L.; Arana, P.; Chocarro, S.; Merino, J. A simple flow-cytometry method to evaluate peripheral blood contamination of bone marrow aspirates. J. Immunol. Methods 2017, 442, 54–58.

30. Loken, M.R.; Chu, S.C.; Fritschle, W.; Kalnoski, M.; Wells, D.A. Normalization of bone marrow aspirates for hemodilution in flow cytometric analyses. Cytom. B Clin. Cytom. 2008, 76, 27–36.

31. Paiva, B.; Puig, N.; Cedena, M.T.; De Jong, B.G.; Ruiz, Y.; Rapado, I.; Martinez-Lopez, J.; Cordon, L.; D'Alignani, D.; Delgado, J.A.; et al. Differentiation stage of myeloma plasma cells: Biological and clinical significance. Leukemia 2017, 31, 382–392.

32. Sanoja-Flores, L.; Flores-Montero, J.; Puig, N.; Contreras-Sanfeliciano, T.; Pontes, R.; Corral-Mateos, A.; García-Sánchez, O.; Díez-Campelo, M.; de Pessoa Magalhães, R.J.; García-Martín, L.; et al. Blood monitoring of circulating tumor plasma cells by next generation flow in multiple myeloma after therapy. Blood 2019, 134, 2218–2222.

33. Oberle, A.; Brandt, A.; Voigtlaender, M.; Thiele, B.; Radloff, J.; Schulenkorf, A.; Alawi, M.; Akyüz, N.; März, M.; Ford, C.T.; et al. Monitoring multiple myeloma by next-generation sequencing of V(D)J rearrangements from circulating myeloma cells and cell-free myeloma DNA. Haematologica 2017, 102, 1105–1111.
34. Biancon, G.; Gimondi, S.; Vendramin, A.; Carniti, C.; Corradini, P. Noninvasive molecular monitoring in multiple myeloma patients using cell-free tumor DNA: A pilot study. J. Mol. Diagn. 2018, 20, 859–870.
35. Mazzotti, C.; Buisson, L.; Maheo, S.; Perrot, A.; Chretien, M.L.; Leleu, X.; Hulin, C.; Manier, S.; Hébraud, B.; Roussel, M.; et al. Myeloma MRD by deep sequencing from circulating tumor DNA does not correlate with results obtained in the bone marrow. Blood Adv. 2018, 2, 2811–2813.