JMS 2023;Vol 6(1):143-148
UDC:616.419-006.448:[601.4:575.224.232.3
DOI: 10.55302/JMS2361143kb

IMPLICATIONS OF CHROMOSOMAL ABNORMALITIES AND BENCE-JONES PROTEINS IN MULTIPLE MYELOMA

Svetlana Krstevska-Balkanov[1,3], Sanja Trajkova[1,3], Aleksandra Pivkova-Veljanovska[1,3], Dejan Spasovski[2,3], Nevenka Ridova[1,3], Goce Kalcev[4], Irina Panovska-Stavridis[1,3]
[1]University Clinic for Hematology, Clinical Center Mother Therese, Skopje, North Macedonia
[2]University Clinic for Rheumatology, Clinical Center Mother Therese, Skopje, North Macedonia
[3]Faculty of Medicine, Ss. Cyril and Methodius University in Skopje North Macedonia
[4]University of Cagliari, Cagliari, Italy

Abstract

Introduction: Translocations are among the main genetic events responsible for multiple myeloma (MM). Also, in two thirds of MM cases, the presence of Bence Jones proteins is observed. The aim of this study was to demonstrate the role that chromosomal abnormalities and Bence-Jones proteins play as crucial biomarkers in MM patients.

Materials and Methods: This retrospective study was carried out at the University Clinic for Hematology in Skopje, North Macedonia, between January 2009 and December 2019. MM patients were divided into different treatment groups: those younger than 65 years old, without comorbidities, and eligible for autologous peripheral blood stem cells (PBSCT) were included in the Cyclophosphamide-Thalidomide-Dexamethasone (CyThalDex) protocol group.

The Melphalan Prednisone Thalidomide (MPT) protocol was used in patients over the age of 65 who were unsuitable for aggressive treatment options such as PBSCT due to comorbidities and renal failure. The third group's treatment did not include new immunomodulators like thalidomide.

Results: Molecular and chromosomal analyses were performed in 46 MM patients. The survival time of patients with MM concerning molecular and chromosome stratification showed that 20% of them were high-risk [hypodiploid (gain1q, loss1p) Del17p, Del13q, t(11;14) t(4;14) and multiple mutations] who survived 60 months, and the median survival time in these patients was 20.8 months. In patients with MM who had a standard risk, death outcomes were not registered during the observation period. Taking into account all MM patients included in our study, Bence Jones proteins in the urine were present in 35.8% of patients, while their presence was not observed in 64.2%. The percentage difference was statistically significant.

Conclusion: The use of these critical biomarkers in the clinical background of this disease in the future can only be achieved through careful evaluation and validation in clinical trials.

Keywords: multiple myeloma; chromosomal aberrations; Bence-Jones protein; molecular investigation

Introduction

A hematological cancer known as multiple myeloma (MM) is characterized by the growth and accumulation of plasma cells that have undergone terminal differentiation in the bone marrow [1].

Patients with MM may also express atypical plasma cells (also known as myeloma cells) in the peripheral circulation [2]. As a primary genetic consequence of chromosomal aberrations, this disease develops in a multi-step process from premalignant diseases like monoclonal gammopathy of undetermined significance (MGUS) and smouldering multiple myeloma (SMM) [3].

With the development of novel agents over the past decade, the outcomes of patients with MM have significantly improved. The disease does, however, exhibit significant heterogeneity in terms of clinical course, presentation, and survival. Translocations are connected to the main genetic events responsible for MM [4], including those involving immunoglobulin heavy chains, fibroblast growth factor receptor 3 (FGFR3), and cyclin D1. Nearly 14% of MM patients have translocation

t(11;14), which results in an up-regulated expression of cyclin D1 (a key molecule in the progression of the cell cycles). The translocations t(6;14), t(14;16), and t(14;20), as well as the well-known translocation t(4;14) linked to FGFR3 overexpression, were also found to be associated with MM [5].

Bence-Jones protein (BJP), on the other hand, is a biomarker for multiple myeloma in urine. The term "Bence-Jones protein" refers to an uncertain category of proteins with a molecular weight of about 22–24 kDa and the recognizable behaviour of precipitating out of solution at temperatures between 45 and 58°C. These proteins fall into two distinct identified and studied classes, Kappa and Lambda. [6, 7]. Bence-Jones proteins in the urine of patients with multiple myeloma have long been detected by this unusual action upon heating [8].

In target organ manifestations of multiple myeloma like kidney failure, lytic bone lesions, anemia, or high numbers of plasma cells in the bone marrow of patients, Bence Jones proteins are critical diagnostic markers. In two thirds of cases of multiple myeloma, Bence Jones proteins are present [9, 10].

The aim of this study was to demonstrate the role that chromosomal abnormalities and Bence-Jones proteins play as crucial biomarkers in multiple myeloma patients.

Materials and methods

This retrospective study was carried out at the University Clinic for Hematology in Skopje, North Macedonia, between January 2009 and December 2019. The cohort group consisted of 296 newly diagnosed MM patients, 146 (49.3%) of whom were female and 150 (50.7%) were male. Patients ranged in age from 31 to 88 (mean age of 62 years). Diagnostic criteria for MM were established by the International Myeloma Working Group (IMWG) [11]. Patients were divided into different treatment groups based on disease staging (by the IMWG), age, comorbidity status, and renal impairment. Patients younger than 65 years, without comorbidities, and eligible for autologous peripheral blood stem cells (PBSCT) were included in the Cyclophosphamide-Thalidomide-Dexamethasone (CyThalDex) protocol group. Only 46 patients had their molecular and/or cytogenetic analyses performed. The Melphalan PrednisoneThalidomide (MPT) protocol was used in patients over the age of 65 who were unsuitable for aggressive treatment options such as PBSCT due to comorbidities and renal failure. The third group's treatment did not include new immunomodulators like thalidomide. Salvage therapy, consisting of chemotherapy and corticosteroids, was preferred. All patients provided written informed consent prior to the start of the study. All medical history information was obtained from the University Clinic for Hematology - patients (from Skopje) record database.

Results

Molecular and chromosomal analyses were performed in 36 MM patients. The highest proportion, 41.3%, had a normal result. Hyperdiploidy (standard risk) was found in 13.0% of patients. 54.3% of patients with normal and hyperdiploid findings had a standard risk.

Hypodiploidy (high risk), including gain 1q, loss 1p, and del13q, was found in 10.9% of MM patients, while t(11; 14) and t(4; 14) were found in 6.5%. Furthermore, only one patient had the del 17p mutation. It is important to note that 45.7% of MM patients in our study were at high risk (*Table 1*).

Furthermore, we evaluated the survival time of patients with MM in relation to molecular and chromosome stratification, and it was found that 20% of them were at high risk (patients with hypodiploid (gain 1q, loss 1p), Del17p, Del13q, t(11;14) t(4;14) and multiple mutations) who survived 60 months, with a median survival time of 20.8 months. Death outcomes were not recorded in patients with MM who had a standard risk during the observation period. The Log-Rank test (WW=4.6275, Sum=8.1880, Var=2.0767, Test statistic=3.211191, p=0.00132) revealed a statistically significant difference between MM patients at low and high risk.

Bence-Jones proteins were found in the urine of 35.8% of MM patients in our study, while they were not found in the urine of 64.2% of them. The percentage difference was statistically significant for $p < 0.05$ ($p = 0.0000$) (*Table 2 and Figure 1*)

Table 1. Molecular and chromosomal abnormalities in patients with multiple myeloma

Molecular and genetic changes	**Number of patients**	**%**	**Level of risk**	**Number of patients**	
2. Hyperdiploidy	6	13,0	Standard	25	4,3
6. Normal	19	41,3			
1. Hypodiploidy (gain 1q, loss 1p) (high risk)	5	10,9	High	21	45,7
3. del 13q	5	10,9			
4. t(11;14) t(4;14)	3	6,5			
5. del 17p	1	2,2			
7. Multiple changes	7	15,2			
Total	46	100,0	Total	46	100,0

Table 2. Distribution of MM patients according to Bence-Jones in the urine

Bence-Jones proteins	**Number of patients**	**%**
Negative	190	64.2
Positive	106	35.8
Total	296	100.0

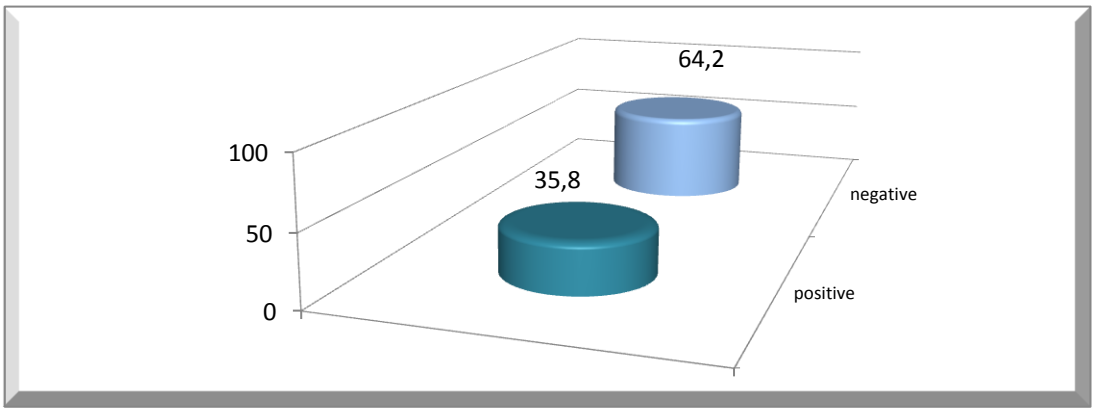

Figure 1. Distribution (in percentage) of MM patients according to Bence-Jones in the urine.

Discussion

Multiple myeloma is a clonal malignancy disease characterized by terminally differentiated plasma cells, and it is the second most common hematological malignancy (10% of all hematological malignancies) after non-Hodgkin lymphoma, with an increase in incident cases over the last 25 years [12].

Until now, an advanced growth of insight has been observed pertaining to genomic and molecular characterization of MM, spreading from metaphase karyotyping and Fluorescent In Situ Hybridization (FISH) to more strong technologies, such as gene expression profiling (GEP) and next-generation sequencing (NGS) [13]. The IMWG consensus statement indicates that MM is classified as high risk if cytogenetic analysis of bone marrow samples indicates monosomy 13 (-13) or del(13q), del(17p), t(4;14), or t(4;16); or if interphase FISH identifies t(4;14), t(14;16), or del(17p) in MM cells. FISH identification of the 13/del(13q) condition is not linked to high-risk status.

However, the primary goal of risk stratification is to predict treatment decisions based on the criteria established in the diagnosis of the symptomatic form of multiple myeloma (hypercalcemia, anemia, renal dysfunction, and bone lesions). In addition, clinical validation of the routine use of FISH markers in prognosis is required.

The advancement of genomic techniques has resulted in a one-step ahead appreciation of the underlying genetic abnormalities of MM, not only at the chromosomal level, but also at the single gene level, indicating that multiple myeloma is a sublimate of diseases with a recurrent clinical phenotype [3].

Using metaphase cytogenetics and FISH, the main genetic abnormalities in MM are translocations and trisomies, which frequently involve odd-numbered chromosomes and are found in about 40% of patients with some overlap [15].

The immunoglobulin heavy chain (IgH) gene locus on chromosome 14 (14q32.33) and one of the specific partner chromosomes, including chromosomes 4, 6, 11, 14, and 20, are frequently involved in key translocations (>90%) in MM. Chromosomes 12 and 8 are less common chromosome partners. Primary trisomies are characterized by the presence of odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and/or 21), indicating a hyperdiploid karyotype.

Chromosomes 12 and 8 are less common chromosome partners [16]. Primary trisomies are characterized by the presence of odd-numbered chromosomes (3, 5, 7, 9, 11, 15, 19, and/or 21), resulting in a hyperdiploid karyotype. In general, monosomy of chromosome 13 and del 13q are the most common minor MM cytogenetic abnormalities, accounting for 35-40% and 6-10% of patients, respectively. Additional abnormalities seen in MM include del 1p, gain 1q, del 17p, and monosomy 17 [17].

Despite the poor prognosis associated with t(4;14), it has been observed that early treatment of such patients with a proteasome inhibitor may lead to an improved outcome. Significantly, genetic changes are influenced by clinical parameters such as the International Staging System (ISS) and serum lactate dehydrogenase (LDH). Patients with t(4;14) and ISS1 and normal LDH, for example, are expected to thrive better than patients with t(4;14) and ISS3. The revised ISS is focused on this. The revised ISS records deletion 17p, t (4;14), and t (14;16) as cytogenetic abnormalities are thought to be associated with high-risk diseases [18].

The Bence Jones protein detected in urine is the second most important biomarker for MM, but it is no less important than the first. When there is a suspicion of plasma cell disorders, Bence Jones proteins should be analyzed.

Other indications for testing these proteins include hypercalcemia, anemia, renal involvement, and bone manifestations in the context of painful lytic lesions and vertebral crushes, as well as long bone fractures. BJP is found in the urine of more than 60% of patients with classical myeloma. Hyperviscosity symptoms such as headaches, blurred vision, epistaxis, and increased susceptibility to infection may also raise suspicion for disorders such as multiple myeloma [19]. Excessive secretion of Bence Jones proteins causes tubular obstruction and tubulointerstitial inflammation, resulting in tubular nephropathy [20].

In this regard, kidney failure is a common complication of multiple myeloma.

Conclusions

While the disease complexity and diversity continue to make personalized medicine challenging for myeloma patients, we believe that the genomic revolution will undoubtedly lead to precision medicine in myeloma in the near future. Future studies will only be able to use these key

biomarkers in terms of clinical background if they are thoroughly evaluated and validated in clinical trials.

Funding
This study received no external funding.
Acknowledgments
No acknowledgments.
Conflicts of Interest
The authors declare no conflict of interest.

References

1. Podar, K.; Leleu, X. Relapsed/Refractory Multiple Myeloma in 2020/2021 and Beyond. Cancers 2021, 13, 5154, https://doi.org/10.3390/cancers13205154.
2. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer Statistics, 2021. CA: A Cancer Journal for Clinicians 2021, 71, 7-33, https://doi.org/10.3322/caac.21654.
3. Kumar, S.K.; Rajkumar, V.; Kyle, R.A.; van Duin, M.; Sonneveld, P.; Mateos, M.V.; Gay, F.; Anderson, K.C. Multiple myeloma. Nature reviews. Disease primers 2017, 3, 17046, https://doi.org/10.1038/nrdp.2017.46.
4. Manier, S.; Salem, K.Z.; Park, J.; Landau, D.A.; Getz, G.; Ghobrial, I.M.; Genomic Complexity of multiple myeloma and its clinical implications. Nature Reviews Clinical Oncology 2017, 14, 100-113, https://doi.org/10.1038/nrdp.2017.46.
5. Furukawa, Y.; Kikuchi, J. Molecular basis of clonal evolution in multiple myeloma. International journal of hematology 2020, 111, 496-511, https://doi.org/10.1007/s12185-020-02829-6.
6. Bhole, M.V.; Sadler, R.; Ramasamy, K. Serum-free light-chain assay: clinical utility and limitations. Annals of clinical biochemistry 2014, 51(Pt 5), 528-542, https://doi.org/10.1177/0004563213518758.
7. Sewpersad, S.; Pillay, T.S. Historical perspectives in clinical pathology: Bence Jones protein-early urine chemistry and the impact on modern day diagnostics. Journal of clinical pathology 2021, 74, 212-215, https://doi.org/10.1136/jclinpath-2020-206675.
8. Gupta, N.; Sharma, A.; Sharma, A. Emerging biomarkers in Multiple Myeloma: A review. Clinica Chimica Acta 2020, 503, 45-53, https://doi.org/10.1016/j.cca.2019.12.026.
9. Delgado, J.C. Value of Urinary Free Light Chain Testing for Monitoring of Bence Jones Proteinuria. The journal of applied laboratory medicine 2019, 3, 1059-1060, https://doi.org/10.1373/jalm.2018.027862.
10. Long, Y.; Aljamal, A.A.; Bahmad, H.F.; Yedla, N.; Herrera, G.A.; Schwartz, M.A.; Layka, A. Multiple myeloma presenting as acute tubulointerstitial nephritis. Autopsy & case reports 2021, 11, e2021328, https://doi.org/10.4322/acr.2021.32.
11. Rajkumar, S.V.; Dimopoulos, M.A.; Palumbo, A.; Blade, J.; Merlini, G.; Mateos, M.V.; Kumar, S.; Hillengass, J.; Kastritis, E.; Richardson, P.; Landgren, O.; Paiva, B.; Dispenzieri, A.; Weiss, B.; LeLeu, X.; Zweegman, S.; Lonial, S.; Rosinol, L.; Zamagni, E.; Jagannath, S.; Sezer, O.; Kristinsson, S.Y.; Caers, J.; Usmani, S.Z.; Lahuerta, J.J.; Johnsen, H.E.; Beksac, M.; Cavo, M.; Goldschmidt, H.; Terpos, E.; Kyle, R.A.; Anderson, K.C.; Durie, B.G.; Miguel, J.F. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. The Lancet. Oncology 2014, 15, e538-e548 https://doi.org/10.1016/S1470-2045(14)70442-5.
12. van de Donk, N.W.C.J.; Pawlyn, C.; Yong, K.L. Multiple myeloma. Lancet 2021, 397, 410-427, https://doi.org/10.1016/S0140-6736(21)00135-5.
13. Saxe, D.; Seo, E.J.; Bergeron, M.B.; Han, J.Y. Recent advances in cytogenetic characterization of multiple myeloma. The International Journal of Laboratory Hematology 2019, 41, 5-14, https://doi.org/10.1111/ijlh.12882.
14. Bergstrom, D.J.; Kotb, R.; Louzada, M.L.; Sutherland, H.J.; Tavoularis, S.; Venner, C.P.; Myeloma Canada Research Network Consensus Guideline Consortium. Consensus Guidelines on the Diagnosis of Multiple Myeloma and Related Disorders: Recommendations of the

Myeloma Canada Research Network Consensus Guideline Consortium. Clinical Lymphoma, Myeloma & Leukemia 2020, 20, e352-e367, https://doi.org/10.1016/j.clml.2020.01.017.
15. Prideaux, S.M.; Conway O'Brien, E.; Chevassut, T.J. The genetic architecture of multiple myeloma. Advances in hematology, 2014, 864058, https://doi.org/10.1155/2014/864058.
16. Kumar, S.K.; Rajkumar, S.V. The multiple myelomas-current concepts in cytogenetic classification and therapy. Nature Reviews Clinical Oncology 2018, 15, 409-421, https://doi.org/10.1038/s41571-018-0018-y.
17. Boyle, E.M.; Deshpande, S.; Tytarenko, R.; Ashby, C.; Wang, Y.; Bauer, M.A.; Johnson, S.K.; Wardell, C.P.; Thanendrarajan, S.; Zangari, M.; Facon, T.; Dumontet, C.; Barlogie, B.; Arbini, A.; Rustad, E.H.; Maura, F.; Landgren, O.; Zhan, F.; van Rhee ,F.; Schinke, C.; Davies, F.E.; Morgan, G.J.; Walker, B.A. The molecular make up of smoldering myeloma highlights the evolutionary pathways leading to multiple myeloma. Nature Communications 2021, 12, 293, https://doi.org/10.1038/s41467-020-20524-2.
18. Palumbo, A.; Avet-Loiseau, H.; Oliva, S.; Lokhorst, H.M.; Goldschmidt, H.; Rosinol, L.; Richardson, P.; Caltagirone, S.; Lahuerta, J.J.; Facon, T.; Bringhen, S.; Gay, F.; Attal, M.; Passera, R.; Spencer, A.; Offidani, M.; Kumar, S.; Musto, P.; Lonial, S.; Petrucci, M.T.; Orlowski, R.Z.; Zamagni, E.; Morgan, G.; Dimopoulos, M.A.; Durie, B.G.; Anderson, K.C.; Sonneveld, P.; San Miguel, J.; Cavo, M.; Rajkumar, S.V.; Moreau, P. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. The Journal of Clinical Oncology 2015, 33, 2863-2869, https://doi.org/10.1200/JCO.2015.61.2267.
19. Long, Y.; Aljamal, A.A.; Bahmad, H.F.; Yedla, N.; Herrera, G.A.; Schwartz, M.A.; Layka, A. Multiple myeloma presenting as acute tubulointerstitial nephritis. Autopsy & case reports 2021, 11, e2021328, https://doi.org/10.4322/acr.2021.328.
20. Bridoux, F.; Leung, N.; Belmouaz, M.; Royal, V.; Ronco, P.; Nasr, S.H.; Fermand, J.P.; & International Kidney and Monoclonal Gammopathy Research Group. Management of acute kidney injury in symptomatic multiple myeloma. Kidney international 2021, 99, 570-580, https://doi.org/10.1016/j.kint.2020.11.010.