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Chromosomal Aberrations and Bence-Jones Proteins as a Significant Biomarkers in Multiple Myeloma

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Abstract: Multiple Myeloma (MM) is a hematological malignity associated with the proliferation and accumulation of bone marrow terminally differentiated plasma cells. The outcomes of patients with MM have dramatically improved over the past decade with the establishment of novel agents. Nonetheless, the disease presents considerable heterogeneity in clinical course, presentation, and survival. Molecular and chromosomal analyses were performed on 46 patients with MM. The survival time of patients with MM concerning molecular and chromosome stratification showed that 20% of them were with 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 outcome was 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 MM patients, while in 64.2%, their presence was not observed. The percentage difference is statistically significant The utilization of these crucial biomarkers in the clinical background for this disease in the future can only be achieved through thorough evaluation and validation in clinical trials.

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

1. Introduction

Multiple Myeloma (MM) is a hematological malignity associated by the proliferation and accumulation of bone marrow terminally differentiated plasma cells. [1]. Atypical plasma cells (or myeloma cells) might also be expressed in the peripheral circulation in MM patients [2]. This genetic disease develops in a multistep process from premalignant diseases, such as monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM), as a prime genetic consequence arising as a result of chromosomal aberrations. [3]. The outcomes of patients with MM have dramatically improved over the past decade with the establishment of novel agents. Nonetheless, the disease presents considerable heterogeneity in clinical course, presentation, and survival. Translocations, including immunoglobulin heavy chains, fibroblast growth factor receptor 3 (FGFR3), and cyclin D1, are linked to the primary genetic events settled in MM [4]. Translocation t(11;14), an up-regulated

expression of cyclin D1 (a principal molecule in the progression of the cell cycles), is found in nearly 14% of MM patients. Another well-known translocation, t(4;14), related to over-expression of FGFR3, together with translocations t(6;14), t(14;16), and t(14;20), have also been established to be linked with MM. [5].

On the other hand, Bence-Jones protein (BJP) is a biomarker in urine for multiple myeloma. The term "Bence-Jones protein" develops an ill-defined set of proteins with a molecular weight of around 22–24 kDa, and the typical effects of precipitating out of solution when warmed to 45° C to 58° C. Kappa and Lamda are two different identified and investigated classes of these proteins. [6, 7]. This atypical action on heating has long served as a significant test for Bence-Jones proteins in the urine of patients with multiple myeloma [8]. Bence Jones proteins are crucial diagnostic of multiple myeloma in target organ manifestations such as kidney failure, lytic () bone lesions, anemia, or large numbers of plasma cells in the bone marrow of patients. Bence Jones proteins are present in 2/3 of multiple myeloma cases [9, 10].

The objective of this study is to show the significant role of the chromosomal aberrations and Bence-Jones proteins as crucial biomarkers in patients with multiple myeloma.

2. Materials and Methods

This retrospective study was carried out at the University Clinic for Hematology in Skopje, North Macedonia, in the period between January 2009 and December 2019. The cohort group was made up of 296 lately diagnosed patients with MM, of which 146 (49.3%) were female, and 150 (50.7%) were male. The age ranged from 31 to 88 years (mean age of 62 years). Diagnostic criteria of MM were established by the International Myeloma Working Group IMWG [11]. The division of the patients into various treatment groups was after the staging of the disease (by IMWG), age, comorbidity status, and renal impairment. In the group which was treated with Cyclophosphamide-Thalidomide-Dexamethasone (CyThalDex) protocol were patients younger than 65 years, without comorbidities, eligible for autologous peripheral blood stem cells (PBSCT). Molecular and/or cytogenetic analyses were done on only 46 patients. Patients over 65 years of age, unsuitable for aggressive treatment options such as PBSCT, comorbidities, and renal failure, were treated with Melphalan-Prednisone-Thalidomide (MPT) protocol. The third group's management was without include new immunomodulators such as thalidomide. For preference, a salvage therapy that consisted of chemotherapy and corticosteroids was given.

Before starting the study, written informed consent was obtained from all the patients. All medical history data were taken from patients' record database at the University Clinic for Hematology-Skopje.

3. Results and Discussion

Molecular and chromosomal analyses were performed on 46 patients with multiple myeloma. The highest percentage, 41.3%, has a normal finding. On the other hand, hyperdiplodia (standard risk) was registered at 13.0%. The percentage of patients with normal and hyperdiploid findings with standard risk was 54.3% (Table S1). Hypodiploidy (high risk), including gain 1q, loss 1p, and del13q, was reported in 10.9% of patients with MM. t(11; 14), and t(4; 14) was registered in 6.5%. Furthermore, del 17p was registered in only one patient. It is important to emphasize that 45.7% of MM patients included in our study were high risk. (Table 1).

Additionally, we assessed the survival time of patients with MM in relation to molecular and chromosome stratification, and it was shown that 20% of them were at high risk (patients with 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 outcome was not registered during the observation period. Log-Rank test (WW=4.6275, Sum=8.1880, Var=2.0767, Test statistic=3.211191, p=0.00132) perceived a statistically significant difference between MM patients with standard-risk and those patients with a high risk.

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			
6. Normal	19	41,3	Standard	25	54,3
1. Hypordiploidy (gain1q, loss1p) (high risk)	5	10,9			
3. del 13q	5	10,9			
4. t(11;14) t(4;14)	3	6,5	High	21	45,7
5. del 17p	1	2,2			
7. Multiple changes	7	15,2			
Total	46	100,0	Total	46	100,0

Taking into account all MM patients included in our study, Bence-Jones proteins in the urine were present in 35.8% of them, while in 64.2%, their presence was not observed. The percentage difference is statistically significant for $p < 0.05$ ($p = 0.0000$) (Table 2 and Figure 1).

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

Bence-Jones proteins	Number of patents	%
Negative	190	64,2
Positive	106	35,8
Total	296	100,0

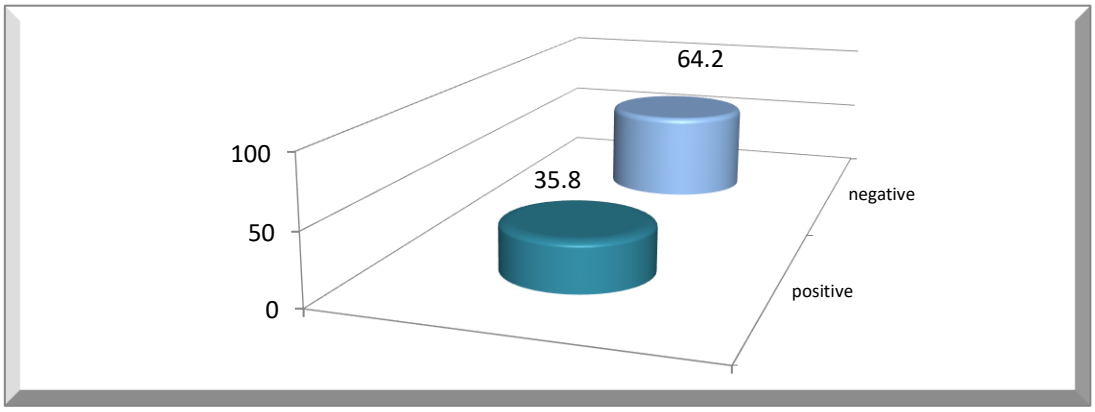

Figure 1. Distribution (in percentage) of MM patients according to Bence-Jones in the urine.

Multiple myeloma represents a clonal malignity disease of terminally differentiated plasma cells expressing the second most frequent hematological malignancy (10% of all hematological malignancies) after non-Hodgkin lymphoma, with an overall increase in incident cases over the past 25 years [12]. Until now, has been observed an advanced growth of knowledge pertaining to genomic and molecular characterization of MM spreading from metaphase karyotyping and Fluorescent In Situ Hybridization (FISH) to more high-throughput technologies, such as gene expression profiling (GEP) and next-generation sequencing (NGS) [13]. According to the IMWG consensus statement, classification of the MM as a high risk is

in case the cytogenetic analysis of bone marrow samples shows 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 13/del(13q) condition alone is not linked with high-risk status.

However, the main purpose of risk stratification is not to decide whether a patient will be treated or not but to predict the treatment decision based on the criteria set in the diagnosis of the symptomatic form of multiple myeloma (hypercalcemia, anemia, renal dysfunction, and bone lesions). Furthermore, clinical confirmation of routine use of FISH markers in prognosis is required. The development of genomic techniques has led to a one step ahead appreciation of the underlying genetic abnormalities of MM, not only at the chromosomal level but at the single gene level, indicating that multiple myeloma is not a single disease but a sublimate of diseases with a recurrent clinical phenotype [3].

Using metaphase cytogenetics and FISH, the principal genetic abnormalities in MM include translocations and trisomies often involving odd-numbered chromosomes, each of which are noted in about 40% of patients with some overlap [15]. Mainly, the key translocations (>90%) in MM often include the immunoglobulin heavy chain (IgH) gene locus on chromosome 14 (14q32.33) and one of the particular partner chromosomes, including chromosome 4, 6, 11, 14, and 20. Less common chromosomes partners include chromosomes 12 and 8. Primary trisomies generally involve the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and/or 21, indicating a hyperdiploid karyotype. Less common chromosomes partners incorporate chromosomes 12 and 8 [16]. Primary trisomies typically assumed the odd-numbered chromosomes 3, 5, 7, 9, 11, 15, 19 and/or 21, leading to a hyperdiploid karyotype. In general, monosomy of chromosome 13 and del 13q are the most frequent minor MM cytogenetic abnormalities identified in 35-40% and 6-10% of patients. Supplementary abnormalities commonly observed in MM include del 1p, gain 1q, del 17p, and monosomy 17 [17].

It is worth mentioning that despite the poor prognosis related to t(4;14), it comes to light that early treatment of such patients with a proteasome inhibitor may result Significantly, genetic alterations are further harmonized by clinical parameters such as the international staging system (ISS) and serum lactate dehydrogenase (LDH) to influence prognosis. Thus, patients with t(4;14) and ISS1 and normal LDH are expected to thrive better than patients with t(4;14) and ISS3, for example. This is the basis of the revised ISS. According to the revised ISS, deletion 17p, t (4;14), and t (14;16) are among the cytogenetic abnormalities that are believed to be associated with high-risk diseases. [18].

The second crucially important biomarker for MM, but no less important than the first, is the Bence Jones protein detected in the urine. When there is suspicion of plasma cell disorders, examination of Bence Jones proteins is suggested. Other signs and symptoms, including hypercalcemia, anemia, renal involvement, and bone manifestations, in view of painful lytic lesions and vertebral crushes, and long bone fractures are also indications for testing these proteins. More than 60% of patients with classical myeloma present BJP in their urine. . Raised suspicion for disorders such as multiple myeloma may also arise from symptoms of hyperviscosity like headaches, blurred vision, epistaxis, and increased susceptibility to infection [19]. Excessive secretion of Bence Jones proteins causes acute kidney injury from tubular obstruction and tubulointerstitial inflammation, termed tubular nephropathy [20]. Regarding this, kidney failure is a common complication of multiple myeloma.

4. Conclusions

While the complexity and diversity of the disease continue to make personalized medicine a challenge for myeloma patients, it is our opinion that this genomic revolution certainly will lead to precision medicine in myeloma in the near future. In future studies, the usage of these key biomarkers in terms of clinical background can be achieved only through thorough evaluation and validation in clinical trials.

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Conflicts of Interest

The authors declare no conflict of interest.

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