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DOCTORAL THESIS

CLINICAL, BIOLOGICAL, HEMATOLOGICAL AND IMMUNOLOGICAL FACTORS THAT INFLUENCING THE RESULTS OF THERAPY AND THE EVOLUTION IN MULTIPLE MYELOMA

ABSTRACT

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OVERVIEW

A. Rationale of the topic

Multiple myeloma (MM) is a heterologous hematological malignancy that involves the proliferation of plasma cells. Despite the improvement of the the treatment strategies in MM over the last decade, this disease remains incurable, although the overall survival of patients has increased significantly in the past few years. All of the current efforts focus on developing new diagnostic and treatment modalities, with the hope of transforming this disease into a curable one. In the past 15 years, new techniques of prognostic marker identification have become available, also supported by new imaging techniques. The stratification rate of the MM is essential in order to understand the prognosis and the treatment response. Patients with MM stratified into the high-risk group, such as the ones with 17p13 deletion, generally have poor outcomes in terms of current treatment strategies, and all the efforts are currently focused on establishing alternative strategies for the management of these patients. For low-risk patients, they have at least 50% chance of surviving more than 10 years.

B. Significance and contemporaneousness of the topic

The American Cancer Society estimated 26,850 new cases of multiple myeloma in the United States in 2015, with approximately 11,240 deaths. The average age of affected people is 62 years old for men (75%> 70 years old), and 61 years old for women (79%> 70 years old). The 5-year survival rate reported in the SEER database increased from 25% in 1975 to 34% in 2003, due to the newer and more efficient treatment options available.

MM is typically sensitive to a variety of cytotoxic drugs, both as initial treatment, and as relapse treatment. Unfortunately, the responses are transient, and MM is not considered curable even with the current approaches. However, the MM treatment has evolved rapidly due to the introduction of new drugs, such as thalidomide, lenalidomide and bortezomib. Studies of associated cytogenetic abnormalities indicate that MM is a heterogeneous disease, suggesting that risk-adapted approaches and individualization of treatment will further contribute to improving the patients' management.

SPECIAL PART

3.1. PERSONAL CONTRIBUTIONS AND VALUE OF RESULTS

The purpose of the conducted study is to identify the negative prognostic factors influencing the type of treatment, evolution under treatment, as well as the survival rate in patients diagnosed with multiple myeloma. In order to determine the prognosis of MM it is necessary to know the host organism, as well as the tumor factors.

Our study identified several negative prognosis factors, which allowed the classification of the patients as per the ISS and DS staging systems; they supported the individualization of the treatment and influenced the rate of survival in patients with multiple myeloma.

3.2. PURPOSE

The purpose of this work is to analyze the influence of therapy outcomes and the evolution of multiple myeloma, as per the clinical, biological, hematological and immunological factors considered as prognosis factors in this pathology.

3.3. GOALS

In order to attain the goal, we set the following objectives:

- Identification of clinical, biological, hematological and immunological factors that could influence therapy, and determination of therapy outcomes in multiple myeloma
- Analysis of the influence of these factors
- Correlation of the identified factors with the treatment outcome and survival rate

3.4. MATERIAL AND METHOD

3.4.1. STUDY MATERIAL

We performed a retrospective-prospective study between January 1st, 2013 and December 31st, 2017. The study group included 105 patients who were diagnosed with multiple myeloma per primam in the Hematology Clinic of the Municipal Emergency Clinical Hospital of Timisoara. The study was retrospective between 2013 - 2015, the data being obtained from the general clinical observation sheets of the patients diagnosed with multiple myeloma in our clinic during this time window; the study became prospective between 2015 - 2017, and it included patients who came to the clinic for diagnosis per primam and were followed throughout the evolution.

3.4.2 STUDY METHODOLOGY

In order to create the study group, the general clinical observation sheets were reviewed for the patients included retrospectively in the study. The study became prospective between 2015 - 2017, and it included patients who came to the clinic for diagnosis per primam and were followed throughout the evolution.

We conducted the following assessments for each patient:

- Clinical exam
- Biological (biochemical) exam

- Hematological exam (complete blood count with peripheral blood smear, bone marrow aspiration, osteomedullary biopsy)
- Immunological exam
- Imaging exam

3.4.3 PARAMETERS TAKEN INTO CONSIDERATION FOR DIAGNOSIS

In order to establish the diagnosis, clinical and paraclinical examinations were taken into consideration, and the following investigations were carried out:

- Complete blood count with peripheral blood smear;
- ESR;
- Fibrinogen;
- FAS;
- LDH;
- PCR;
- Total proteins;
- Immunoglobulins;
- Albumin;
- Beta 2 microglobulin;
- Urea;
- Creatinine;
- Serum calcium;
- Serum potassium;
- D-dimer;
- Imaging tests (X-rays, CT, MRI);
- Urine protein electrophoresis (Bence-Jones protein);
- Serum protein electrophoresis;
- Serum and urine assessment for monoclonal protein, imunofixation, quantitative immunoglobulins.

Kappa and Lambda chains, as well as the level of plasmocytes in the bone marrow, were determined.

In order to determine the prognosis of MM it is necessary to know the host organism, as well as the tumor factors. The research pertaining to the stratification of MM in various stages began in the 1960s and continued into the 1970s, when a number of clinical and laboratory parameters were identified, including the hemoglobin level, serum calcium, serum creatinine, and the severity of bone lesions. In 1975, Durie and Salmon developed the Durie-Salmon Staging (DS) system as a prognostic model, using the following parameters: hemoglobin level, serum calcium level, serum creatinine level, urine light-chain concentrations, number of bone lesions on the bone X-ray, as well as the level and type of monoclonal protein.

The DS system was adopted as a standard method for MM staging for several years, and has become the most commonly used prognostic scheme for the newly diagnosed patients with MM. ISS is a simple staging system, based on serum beta-2 microglobulin and albumin.

Subsequent to the conduct of the investigations and after the diagnosis had been established, the treatment protocol used as a first-line pretransplant therapy may consist in the administration of the VAD regimen. Bortezomib - Dexamethasone regimen may also be used as a first-line treatment.

The secondary therapeutic lines administered are: Bortezomib-Dexamethasone regimen with the addition of Cyclophosphamide; Bortezomib-Dexamethasone regimen with the addition of Caelyx; Carfilzomib - Dexamethasone regimen; Melphalan (Alkeran) – Prednisone regimen, and Melphalan - Prednisone - Thalidomide regimen.

3.5. STATISTICAL ANALYSIS

The data were collected from the observation sheets for each patient, as per the anthropometric parameters, stage of the disease, occurred adverse events, type of treatment and treatment response. Statistical analysis of the data was performed with SPSS20.0. The descriptive statistics expressed the results in percentages and absolute values. Kaplan Meyer analysis was used to analyze the survival curve in the 2 groups.

3.6. RESULTS

Our study included 105 patients, and the best represented age groups were between 41-60 years old (37 patients) and 61-75 years old (45 patients). 3 patients were under 40 years of age, and 20 patients were over 75 years of age, the gender distribution being approximately equal.

The hematological determination showed that the majority of the patients, i.e., 41.9%, had the hemoglobin value between 7-10 mg/dl. 84 patients had the ESR values greater than 40 mm; fibrinogen values were increased in only 25.7% of the patients, and PCR values were increased in in 38.1% of patients. 55.2% of patients had elevated LDH values. D-dimer levels were increased in 43.8% of patients. 48.5% of patients had elevated serum creatinine values (above the normal value). 51.4% of patients had beta-2-microglobulin levels higher than 5.5, where 22.9% showed values between 3.5 - 5.5, and only 18.1% showed values lower than 3.5. The bone marrow aspiration showed more than 60% plasmocytes in the bone marrow for 42 patients, whoch is considered a negative prgnosis factor. The serum calcium levels were increased in 27.6% of patients.

The presence of Kappa chains was found in 65.7% of the patients, and Lambda chains were present in 34.3% of patients, which is a negative prognostic factor, even though the difference in survival between the two categories of patients is not statistically significant.

At the time of diagnosis 14.3% of patients were in stage I, 20% in stage II and 65.7% in stage III.

The treatment response showed that 24 (22.9%) of the patients had complete remission, 24 partial remission (25.7%), and 19 stationary disease (18.1 %). 32 of the patients, approximately 30.5% had progressive disease, and 3 died before the end of the first line of treatment.

The longest survival is seen in patients with complete remission, approximately 70 months, followed by the ones with partial remission. Those with progressive disease have the shortest survival time.

Complications in multiple myeloma are determined both by the illness itself, as well as by the toxicity of the chemotherapy treatment. It is difficult to differentiate which of these are manifestations of the disease or complications of the treatment. The most common, which also carry a negative impact on survival, are infections, myelosuppression and pathological fractures.

IV. DISCUSSIONS

Multiple myeloma is a neoplastic dyscrasia of the plasma cells, where survival of patients ranges between a few months and several years, being influenced by several factors.

In 2020, the global age-standardized incidence rates for multiple myeloma (MM) were 2.2/100,000 for males and 1.5/100,000 for females, with an age-standardized mortality rate of 1.1/100,000.

Out of the 105-patient group that underwent our analysis, 55 were women and 50 were men, even though the literature shows that multiple myeloma mainly affects men.

As for the age of the patients included in the study group, the age range over 60 years old was predominant. The majority were in the sixth decade of life, consistent with the literature data.

In order to establish the negative prognostic factors for survival in our study, we performed a regression analysis that showed us the predictors of a low survival time out of the reviewed factors which had a statistically significant influence on the treatment response and survival duration, namely: age over 60 years old, hemoglobin level <10g/dl, platelet level under 150000/mm^3, creatinine >2mg, serum calcium level >10mg/dl, increased level of beta-2-microglobulin, total serum proteins, increased level of LDH, fibrinogen and D-dimers, tumor burden, advanced stages of the disease at the time of diagnosis.

Randomized phase 3 studies show that patients treated with carfilzomib and dexamethasone had a longer progression-free survival compared to the patients treated with bortezomib and dexamethasone. Progression-free survival was also longer for patients in the group with carfilzomib than the ones in the group with bortezomib, irrespective of the previous transplant status. Establishing that the proportion of patients with a complete response or a partially better and very good response was greater in the group with carfilzomib compared with the group with bortezomib is encouraging, because studies have shown that an association between depth of response and improved survival in patients with multiple myeloma.
In our study, the assessment of survival according to the type of treatment showed that survival does not differ very much: in the VAD group the survival duration was approximately 45 months, vs. Bortezomib + Dexamethasone, where survival duration was 38 months, with an average of 37 months.

Patients treated with Carfilzomib and Dexamethasone, and patients treated with Bortezomib, Cyclophosphamide and Dexamethasone showed a similar survival (40.188 months). The lowest survival duration is found in patients treated with Melphalan + Thalidomide + Prednisone. The longest survival is seen in patients with complete remission, approximately 70 months.

The first-line treatment with VAD and Bortezomib + Dexamethasone indicates a statistically significant increase of the survival duration. Second-line therapeutic regimens showed no statistically significant differences for survival. The treatment response is a statistically significant negative prediction factor, the survival being lower in those with progressive and stationary disease.

The analysis of the mean survival time of all patients was 29.462±2.037 months in patients treated with bortezomib and dexamethasone, compared with the patients treated with VAD, who had a mean survival time of 22.481±3.328 luni.

Most common complications occurred in our study after initiating polychemotherapy were anemia, chronic kidney failure and pathological fractures. Kidney failure was associated with an increased frequency of early death and a short survival, but was not associated with any modification of the response rate, nor the remission period.

In order to follow up the evolution of the patients with multiple myeloma the same parameters are monitored, namely: CBC with peripheral blood smear, urea and serum creatinine, serum calcium, protein electrophoresis with immunoelectrophoresis and immunofixation, beta2M, LDH level and level of plasmocytes in the bone marrow, as well as imaging testing.

Study 2 was designed as a cross sectional investigation pilot study, and the patients were recruited during the second wave of the COVID-19 pandemic. Our aim was to assess the nutritional knowledge and quality of diets in a cohort of patients with MM, and prepare an intervention study to increase general and specific nutrition knowledge, including nutrition counseling. Although the relationship between nutrition knowledge, food choices and food intake is complex, little is known about the level of nutrition knowledge in patients diagnosed with MM și calitatea dietelor acestora după diagnosticand the quality of their diets after diagnosis. To the best of our knowledge, neither the low carb diet score, nor the nutrition knowledge has been assessed for patients diagnosed with MM. This score has been used for several years in relation to the risk of chronic diseases and mortality.

Anemia is often associated with and aggravated by chronic kidney disease. In our sample, patients from the high carbohydrate diet tertile have lower hemoglobin and albumin levels and higher D-dimers, calcium, uric acid, percentage of plasmacytes in the bone marrow and beta-2microglobulin levels, compared to patients from the medium carbohydrate tertile.

The literature shows a long list of prognosis factors useful for MM, indicating that no single factor can estimate accurately the survival of these patients. Thus, a series of factors, that can be easily determined, are taken into consideration at the time of diagnosis, in order to help us assess the risk of unfavorable treatment response. These factors must include beta2M and percentage of percentage of plasmacytes in the bone marrow. A panel of negative prognosis factors influencing the evolution and treatment response in multiple myeloma would allow an individualized therapy for each patient.

V. CONCLUSIONS

5.1. PERSONAL CONTRIBUTIONS

The literature indicates that MM is a heterogeneous disease, suggesting that risk-adapted approaches and individualization of treatment will further contribute to improving the patients' management. Research in various primary regimens focused on the improvement of complete remission rates in both categories of candidates for transplant and non-transplant, as well as on the significance of assessing the primary therapy response after two administered cycles.

5.2. ATTAINED GOALS AND FUTURE RESEARCH DIRECTIONS

This paper focused on the influence of several (clinical, biological, hematological and immunological) prognosis factors on the evolution and therapeutic decision in patients with multiple myeloma.

The parameters with prognosis significance were:

- Age over 60 years old,
- Low level of hemoglobin,
- Increased level of LDH,
- Increased level of fibrinogen,
- Increased level of D-dimers,
- Increased levels of urea and creatinine,
- Hypercalcemia
- Increased levels of Beta 2 microglobulin,
- Tumor burden,
- Advanced disease stages at the time of diagnosis

The study also showed the benefits of certain lines of treatment.

Future research directions consisted in establishing a prognosis algorithm for patients who had relapses and therefore needed a different therapeutic approach.

5.3. LIMITS OF THE STUDY

The study was conducted in a group of patients who where diagnosed with multiple myeloma per primam. The study did not include patients with relapses, which did not allow the assessment of the influence of the identified prognosis factors on the patients' rate of survival.

5.4. TECHNICAL AND ECONOMICAL ADVANTAGES AND DISADVANTAGES

The classification of patients in groups of risk based on the identification of negative prognosis factors would allow the administration of an individualized therapy. All these would probably determine a reduction of the hospital admission duration for these patients, an increase of the patients' survival rate, conducive to an improvement of the population health status on the long term.

5.5. CONCLUSIONS OF THE STUDY

- The assessment of survival according to the type of treatment showed that survival does not differ very much: in the VAD group the survival duration was approximately 45 months, vs. Bortezomib + Dexamethasone, where survival duration was 38 months, with an average of 37 months.
- We can see that the second line treatment showed a longer survival duration for the patients who were treated with Alkeran+PDN (46.343 months), as well as for the patients who were treated with Bortezomib+Dexamethasone+ Caelyx (45.941 months). Patients treated with Carfilzomib and Dexamethasone, and patients treated with Bortezomib, Cyclophosphamide and Dexamethasone showed a similar survival (40.188 months). The lowest survival duration is found in patients treated with Melphalan + Thalidomide + Prednisone.

- In order to establish the negative prognostic factors for survival in our study, we performed a regression analysis that showed us the predictors of a low survival time: age over 60 years old, decreased hemoglobin level, increased LDH, fibrinogen and D- dimers, elevated values of urea and creatinine, hypercalcemia, elevated values of beta-2-microglobulin, tumor burden, advanced stages of the disease at the time of diagnosis.
- The first-line treatment with VAD and Bortezomib + Dexamethasone, respectively, indicates a statistically significant increase of the survival duration. Second-line therapeutic regimens showed no statistically significant differences for survival. The treatment response is a statistically significant negative prediction factor, the survival being lower in those with progressive and stationary disease.
- The cross sectional investigation pilot study conducted during the second wave of the COVID-19 pandemics aimed to assess the nutritional knowledge and quality of diets in a cohort of patients with MM, and prepare an intervention study to increase general and specific nutrition knowledge, including nutrition counseling.
- Beta-2microglobulin, a severe prognosis factor and a clinical indicator in our sample, was associated with a high carb diet.
- Better knowledge of food types and nutritional value of foods, combined with personalized nutritional advice, could encourage patients with MM to make healthier decisions, which might extend survival.

List of publications

1. **Ema-Cristina Borsi**, Adina Bucur, Cristina Potre Oncu, Ovidiu Potre Oncu, Bianca Cerbu, Dan Costachescu, Ioana Ionita, Constantin Tudor Luca, Hortensia Ionita. - First Line Therapy in Multiple Myeloma: VAD vs Bortezomib-Dexamethasone, REV. CHIM, Bucharest, Volum 70, Issue 3, pag. 1017-1022, 2019, ISSN-0034-7752, https://doi.org/10.37358/RC.70.19.3, IF: 1,605.

2. **Borsi E**, Serban CL, Potre C, Potre O, Putnoky S, Samfireag M, Tudor R, Ionita I, Ionita H. High Carbohydrate Diet Is Associated with Severe Clinical Indicators, but Not with Nutrition Knowledge Score in Patients with Multiple Myeloma. Int J Environ Res Public Health. 2021 May 19;18(10):5444, doi: 10.3390/ijerph18105444, IF: 2,849.