IJHOSCR

Original Article

International Journal of Hematology-Oncology and Stem Cell Research

Clinical, Laboratory, Cytometry and Cytogenetic Characteristics of a Cohort of Patients Diagnosed with Multiple Myeloma for the First Time in a Third-Level Hospital in Medellín, Colombia, Survival after 8 Years of Follow-Up

Carlos Atencia-Flórez[1], Catalina Quintero-Valencia[2], María Mondragón-Arismendy[2], Andrés Cardona-Arias[3], Carlos Regino-Agamez[1], Julián Vélez-Urrego[4]

[1]Department of Internal Medicine, University of Antioquia School of Medicine, Medellín, Colombia
[2]Laboratorio Integrado de Medicina Especializada – LIME, Medellin (Antioquia), Colombia
[3]University of Antioquia School of Medicine, the SURA Healthcare Insurance Company, Medellin (Antioquia), Colombia
[4]San Vicente Foundation University Hospital, Medellin (Antioquia), Colombia

Corresponding Author: Carlos Andrés Regino Agamez, Department of Internal Medicine, University of Antioquia School of Medicine, Medellín, Colombia
E-mail: carlos.regino@udea.edu.co

Received: 18, Jan, 2021
Accepted: 12, Feb, 2022

ABSTRACT

Background: Multiple myeloma is the second most common hematologic malignancy after lymphomas. Few studies have characterized significant and full variables at the time of diagnosis of multiple myeloma in Colombia, and there is no data evaluating patients for follow-up.

Materials and Methods: A retrospective cohort study is presented, describing the clinical, laboratory, cytometric, and cytogenetic characteristics of patients with a *de novo* diagnosis of multiple myeloma evaluated in a reference hematology laboratory attached to a highly complex hospital in Medellín, Colombia. We follow them until death as a main outcome.

Results: A total of 170 patients with a *de novo* diagnosis of multiple myeloma were collected from a database of 421 patients with different monoclonal gammopathies. Mainly, it was found that 50.8% of the patients were men; the median age was 62 years; 65.4% had secretion of the IgG kappa; half of the patients presented International Staging System (ISS) Stage III. The β2 macroglobulin >4 mg/L and creatinine >2 mg/dl were the main variables significantly associated with survival (Hazard Ratio (HR) 2.4 and 2, respectively). Eighty-five percent of patients presented with bone lytic lesion involvement and less than 3% with extramedullary involvement. Conventional Banding Karyotype (CBK) genetic risk assessment yield was poor, compared with although scarce data regarding Cytogenetic risk assessment based on Fluorescence in-situ Hybridization (FISH).

Conclusion: The clinical profile of the patients with a *de novo* diagnosis of multiple myeloma in our cohort is similar to that described in international studies. The diagnosis of multiple myeloma was documented at younger ages, with more advanced stages, anemia, and a high percentage of bone disease. ISS provides an excellent tool for prognosis purposes. Cytogenetic risk assessment based on FISH should be done for all MM patients from therapeutic implications. We need standardized protocols for bone marrow sample manipulation and processing in order to guarantee good correlation for plasma cells count methods.

Keywords: Multiple myeloma; Cohort study; Survival; Colombia

INTRODUCTION

Cancer is the second leading cause of death worldwide and it is estimated that 70% of these deaths occur in countries with medium to low incomes. Multiple myeloma (MM) is a type of plasma cell cancer. It represents 1% of all cancers, 10% of hematologic malignancies, and is second only to lymphomas. In western countries, the age-adjusted annual incidence is six cases per 100,000 inhabitants per year in the United States and Europe, with a median age at diagnosis of 70 years, with two-thirds of the cases in men. The situation in Colombia does not differ from the rest of the world, since – in 2015 – it was the second most-frequent hematological cancer in prevalence, represented by 23.2% of the cases according to statistics from the National Cancer Institute[1].

The prognosis of MM is related to the stage of diagnosis, the patient's age, functional status, and other clinical and laboratory variables [2,3]. Only one study carried out at the *Fundación Santa Fe de Bogotá* has collected the characteristics of the diagnosis of patients with multiple myeloma in our country, the findings of which did not differ much from two large studies carried out in Brazil and throughout Latin America, in which a large percentage of patients with advanced stages at diagnosis was evidenced [4-6].

The main objective of this study is to describe the most relevant clinical, laboratory, cytometry, cytogenetic and imaging characteristics at the time of diagnosis of multiple myeloma in a cohort of patients from a tertiary-level hospital in Colombia and to explore whether some of them are associated with less or greater survival.

MTERIALS AND METHODS

The database of monoclonal gammopathies of the University of Antioquia (UdeA) Hematology Laboratory, is located at the San Vicente Fundación University Hospital (HUSVF, in Spanish), a departmental and regional reference center. It has a high level of complexity care facility and is where patients with malignant hematological diseases are referred. The database was made by one of the UdeA laboratory analysts and by an internal medicine specialist working with HUSVF. From this database, patients older than 18 years diagnosed with multiple myeloma for the first time during a hospital stay at the HUSVF between January 14, 2009 and June 12, 2017 were included.

The MM diagnosis was established with the criteria of the International Myeloma Working Group Consensus [7]: 1) presence of clonal plasma cells in bone marrow ≥10%; 2) Bone or extramedullary biopsy with the presence of a plasmacytoma with one or more of the following myeloma defining events (Increased Calcium level, Renal dysfunction, Anemia, and destructive Bone lesions, CRAB): serum calcium >1 mg/dL of the upper limit of normal or >11 mg/dL; creatinine clearance <40 mL per minute or serum creatinine >2 mg/dL; hemoglobin >2 g/dL below the normal lower limit or <10 g/dL; one or more lytic lesions evidenced by radiography, tomography, emission tomography positron or magnetic resonance imaging; 3) in case none of the criteria of Point 2 were met, the patient should have a percentage of clonal plasma cells in bone marrow ≥60%, the ratio of free/implied free light chains ≥100 or more than one focal injury evidenced by magnetic resonance. Non-secretory MM was defined with the previously mentioned criteria in the absence of monoclonal protein in serum and urine. Those who had previously been diagnosed with MM or did not have sufficient data in their medical history to corroborate the diagnosis of MM, those who were diagnosed with monoclonal gammopathy other than MM, patients with monoclonal gammopathy of undetermined significance, latent multiple myeloma were excluded, as well as extramedullary solitary plasmacytoma and plasma cell leukemia and those who had solid tumors, autoimmune diseases, lymphomas or metastases from solid tumors to the bone marrow that produced an abnormal reaction in plasma cells.

Only laboratory reports and diagnostic images obtained at the time of the MM diagnosis were taken into account. If the patient had more than one available result, the first one that had been taken was recorded. Frequency and distribution were determined for each laboratory value or diagnostic imaging result obtained. The dates of death were obtained from the national database of the Single Register of Affiliates to Social Protection of Colombia

by searching the system. The HUSVF Research Ethics Committee approved the research project.

Quantitative variables are presented as medians and interquartile ranges. Qualitative variables are presented in percentages. Age was expressed in years; hemoglobin in grams per deciliter (g/dL); leukocytes and platelets in their absolute count per microliter (µL); creatinine in milligrams per deciliter (mg/dL); calcium in mg/dL; albumin in g/dL; and β2 microglobulin in milligrams per liter (mg/L). We obtained CBK with R and G banding and FISH (standard risk defined as 3/3 normal markers del(17)(p13) t(4;14), and gain 1q21 and high risk as del(17)(p13) (loss of TP53 gene); t(4;14); t(14;20), gain 1q21) and the percentage of plasma cells from myelogram, and Flow cytometer immunophenotype (CD45+, CD138+, CD38+, CD56+, CD19+, CD27, CD28+, CD117+, CD81+, CyIgκ and CyIgλ) based on PCST Euroflow protocol from the bone marrow biopsy. A univariate analysis of global survival was performed with the Cox Proportional Hazards Regression and time to event, plotted on Kaplan-Meier curves.

RESULTS

The original database had 421 patients with monoclonal gammopathies, of whom 170 met the inclusion criteria; the others were excluded because they had a diagnosis other than MM, because there was no access to their medical history to confirm the diagnosis of MM or because they were patients referred to the UdeA Hematology Laboratory without an episode of hospitalization at the HUSVF.

Half were men with a median age of 62 years; only 2.6% were under 40 years of age (Table 1). Lymphadenopathy, splenomegaly, and hepatomegaly were rare findings (Table 2). According to the median, the patients presented anemia, hypoalbuminemia and elevation of β2 microglobulin, but normal creatinine and calcium values. According to the International Staging System (ISS) the patients were classified 22 (21.6%) as Stage I, 29 (28.4%) as stage II and 51 (50%) as stage III (Table 1). CBK were reported as "Normal Karyotype" in 92 patients (54.1%). FISH were obtained only from 36 patients (21%): 19 classified as standard risk and 17 as high risk. Comparing CBK with FISH, the former classified correctly only 25% of the patients.

Most of the patients (85.5%) had bone disease, defined as lytic lesions, at the time of diagnosis; pathological fractures or vertebral collapses detected by plain radiography, plain or contrasted tomography, and simple or contrasted MRI. The presence of plasma cell proliferation of a clonal origin in conjunction with a defining event of MM in areas other than the iliac crest was described as bone plasmacytoma. This finding occurred in 25% of cases at the time of diagnosis.

The majority of myelomas were IgG kappa-type heavy and light chain secretors; we only had two cases of IgD myeloma and one non-secretory myeloma. The median concentration of compromised immunoglobulins G, A and M was 4229 mg/dl, 3487 mg/dl and 4605mg/dl, respectively. The predominant light chain was Kappa (by immunofixation) and its concentration in relation to Lambda was only quantified in seven patients in the sample (by free light chain).

Bone marrow plasma cells were count using 3 methods: bone marrow smear myelogram (read by an expert Hematologists), bone marrow trephine biopsy (read by an expert Pathologists) and flow cytometry (read by PCST Euroflow protocol). Mean plasma cells counts were 35%, 30% and 20% respectively (mean 28,2%). We perform correlation analysis between plasma cells percentage by these 3 methods. (Figure 1)

The survival curve according to the Salmon-Durie prognostic classification (Figure 3) shows the best prognosis for patients with Stage I compared to Stages II and III, with no difference between Stages II and III. In the case of ISS survival (Figure 2), differences are observed among the three stages and ISS model could predict higher mortality among patients in stages II and III. (HR 2,49. IC95% 1,21-5,13)

In the univariate analysis (Table 4), hemoglobin ≥10 and albumin ≥3 are factors related to greater survival, while decreased platelets, increased calcium, creatinine, and β2 microglobulin are statistically significantly associated with a lower survival (Figure 4). Age, albumin, and the percentage of plasma cells in the bone marrow did not

demonstrate statistically significant differences with respect to mortality. A normal karyotype could not distinguish patients with good or poor prognosis based on standard and high risk category by FISH analysis (HR 1,12. IC95% 0,58-2,18) (Figure 5).

Table 1: Demographic characteristics, laboratory data, and prognostic stage of 170 patients with multiple myeloma diagnosed for the first time

Variable	n (%) or median (IQR*)
Male gender	86 (50.6%)
Age in years (n=156)	62 (54 – 72)
Age in categories	
<40	4 (2.6%)
40-49	15 (9.6%)
50-59	48 (30.7%)
60-69	38 (24.4%)
70-79	39 (25%)
≥80	12 (7.7%)
Laboratory Exams	
Hemoglobin (n=163)	9.6 (7.9 – 12)
Leukocytes (n=163)	7100 (5080 – 9500)
Platelets (n=162)	234000 (164000 – 315000)
Creatinine (n=163)	1.1 (0.8 – 2.5)
Calcium (n=163)	9.2 (8.4 – 10)
Albumin (n=160)	3 (2.5 – 3.7)
β2 microglobulin (n=102)	5.6 (3.5 – 9.9)
Salmon-Durie Staging (n=164)	
I	3 (1.8%)
II	17 (10.4%)
III	144 (87.8%)
ISS Staging (n=102)	
I	22 (21.6%)
II	29 (28.4%)
III	51 (50%)

*Interquartile range

Table 2: Presence of bone disease, plasmacytoma and findings on physical examination in patients with multiple myeloma

Variable	n (%)
Bone disease	121 (85.8%)
Bone plasmacytoma (other than iliac crest)	36 (25%)
Extramedullary involvement	
Lymphadenopathy	3 (1.9%)
Hepatomegaly	4 (2.5%)
Splenomegaly	4 (2.5%)

Table 3: Classification of the Type of Multiple Myeloma, Based on the Immunoglobulin Secretion Pattern and Serum Immunoglobulin Concentration

Variable	n (%) or median (IQR*)
Type of Myeloma	
Non secretory Light chain secretory Heavy and light chain secretory	1 (0.58%) 42 (24.7%) 127 (74.8%)
Heavy Chain (n=127)	
IgG IgA IgM IgD	83 (65.4%) 35 (27.6%) 7 (5.5%) 2 (1.6%)
IgG (n=83)	4229 (1929 – 5765)
IgA (n=35)	3487 (1693 – 4975)
IgM (n=7)	4605 (3316 – 5786)
Light Chain (n=165**)	
Kappa Lambda	116 (70.3%) 49 (29.7%)
Light Chain Ratio (n=7)	37.7 (5.7 – 289)
% Plasma cells in Bone Marrow (mean of all thre methods)	28.2 (15 – 35)

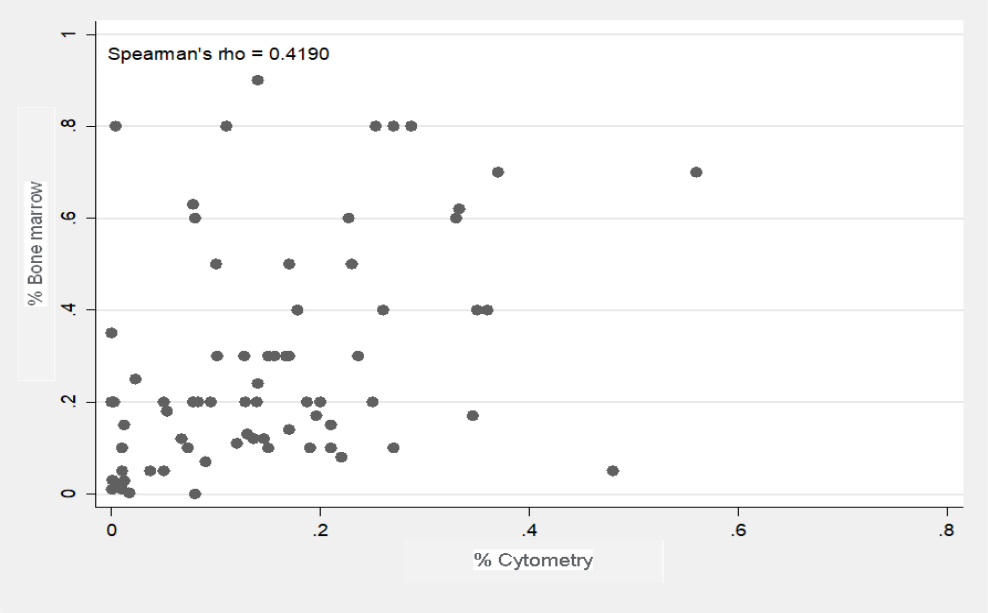
* Interquartile range

** Four patients with heavy chain secretion had no light chain secretion data

Table 4: Univariate analysis with cox regression of factors associated with overall survival in patients with multiple myeloma

Variable Studied	Median Survival Time in the Absence against the Presence of the Variable Studies (Months)	Overall Survival	
		HR (CI* 95%)	*p*-Value
Age ≥70 years	24 vs. 20	1.2 (0.8 – 1.8)	0.375
Hemoglobin ≥10	17 vs. 25	0.6 (0.4 – 0.8)	0.003
Platelets <150000	24 vs. 11	2.0 (1.3 – 3.0)	0.001
Calcium ≥11	23 vs. 9	1.9 (1.3 – 3.0)	0.003
Creatinine ≥2	25 vs. 13	2.0 (1.3 – 2.9)	<0.001
Albumin ≥3	14 vs. 22	0.8 (0.5 – 1.1)	0.192
β2 microglobulin >4	31 vs. 21	2.4 (1.3 – 4.4)	0.004
Plasma cells in bone marrow ≥50%	29 vs. 23	1.5 (0.8 – 3.0)	0.213

* Confidence Interval



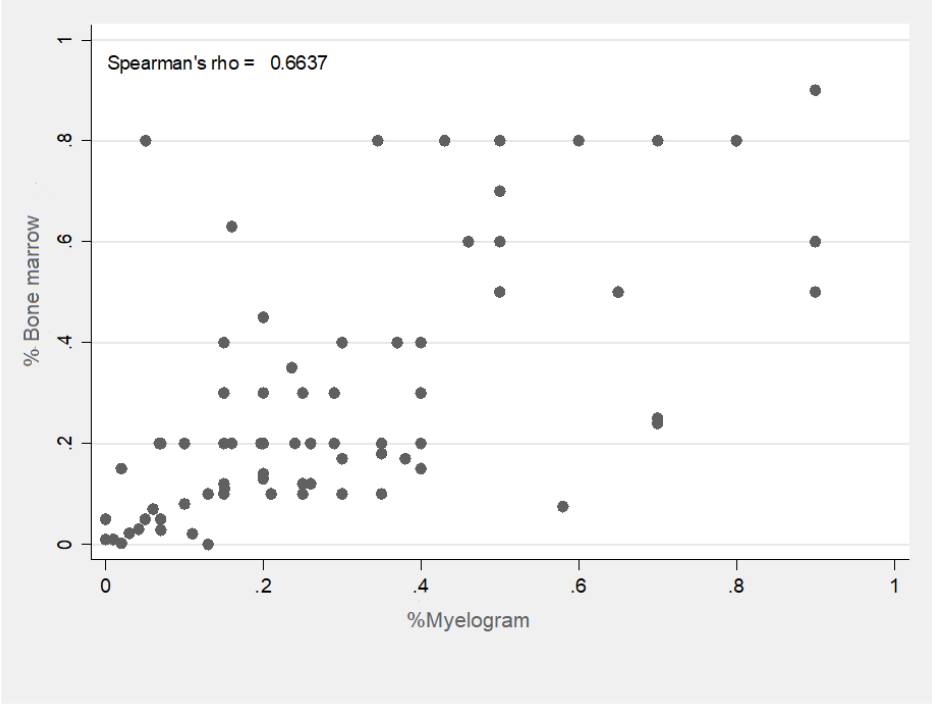


Figure 1. Disperssion plots A) between bone marrow plasma cells detected by trephine biopsy and bone marrow smear myelogram; B) between bone marrow plasma cells detected by trephine biopsy and bone marrow flow cytometry

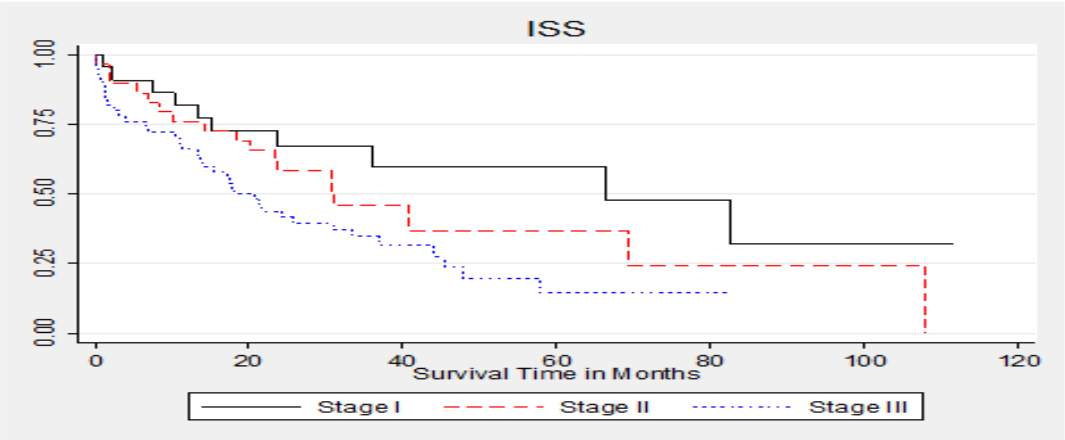


Figure 2. Survival in months according to the stage of multiple myeloma with the ISS classification at the time of diagnosis

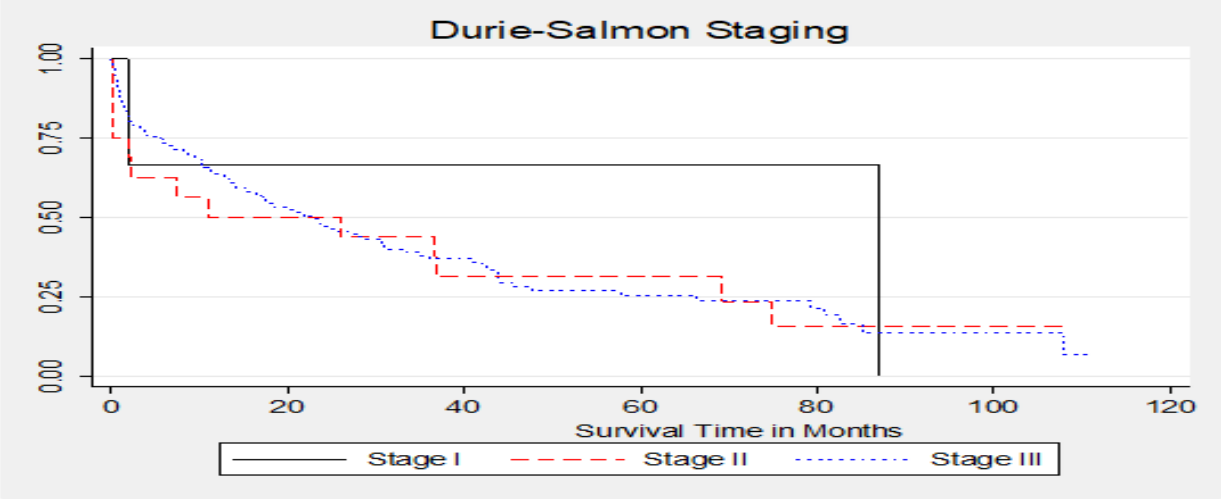


Figure 3. Survival in months according to the stage of multiple myeloma with the salmon-durie classification at the time of diagnosis

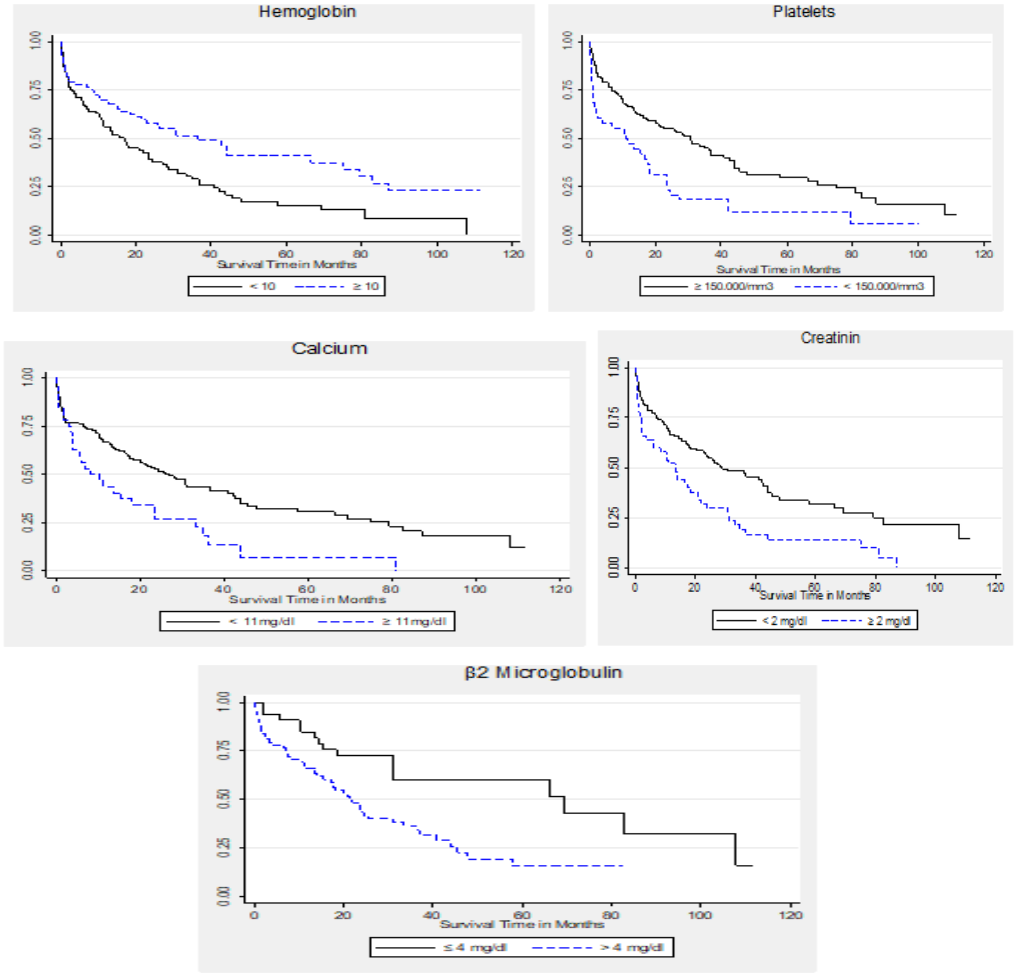


Figure 4. Survival in months according to hemoglobin (gr/dl), platelet count, calcium, creatinine and β_2 microglobulin at the time of diagnosis of multiple myeloma

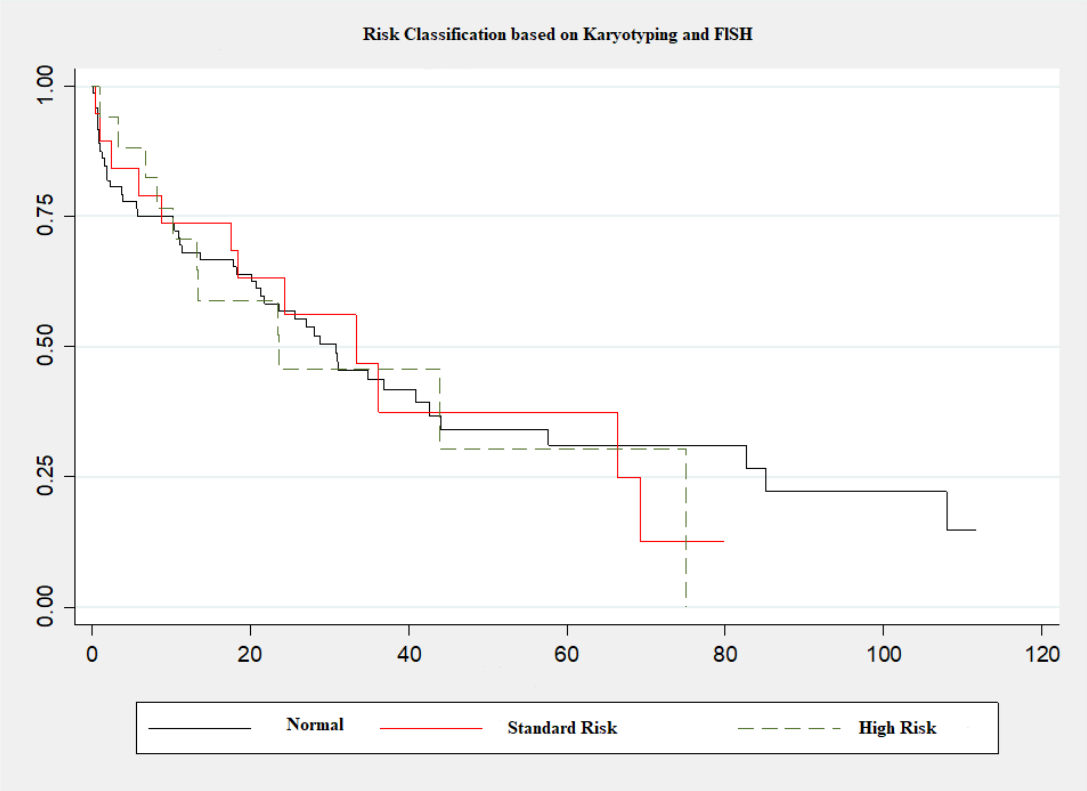


Figure 5. Risk Clasification based on Convention Karyotyping and Flourescence in-situ Hybridization

*Normal based on cbk

**Standard risk. Fish with 3/3 normal markers del(17)(p13) t(4;14), and gain 1q21

***High risk: fish del(17)(p13) (loss of tp53 gene); t(4;14); t(14;20), gain 1q21

DISCUSSION

This is a retrospective cohort we presented the clinical, laboratory, cytometry, cytogenetic and imaging characteristics of patients diagnosed for the first time with multiple myeloma high complex care institution. According to this study, patients with MM have a median age of 62 years and half of them are men, findings that contrast with those of the world literature, in which the median age is 70 years and almost two thirds are men [2, 6]. However, in the study by the *Fundación Santa Fe de Bogotá*, the average age was 60 years [4]. In agreement with other series, the age of presentation below 40 years is a very rare finding, a fact that underlines the fact that the disease is characteristic of older adults and the elderly [2, 4-6]. On physical examination, it is unusual to find lymphadenopathy, hepatomegaly and splenomegaly, as has been demonstrated in other series [2, 5, 6].

In the laboratory, the most frequent finding is anemia, with a median hemoglobin of 9.6 g/dL, almost 1 g/dL lower than the median hemoglobin in the Mayo Clinic series [2]. Creatinine and calcium are similar to those reported in other series[2,4-6]. Decreased albumin and increased β2 microglobulin, as occurred in most of our patients, are associated with a more adverse prognostic classification[8-10]. 87.8% of cases were considered Stage III of Salmon-Durie, and a half of the patients were considered Stage III of the ISS. In our cohort, there seems to be a better correlation of the ISS with mortality; however, this would require a prognostic study with this hypothesis *a priori* to confirm it.

Most of the subjects presented bone disease, represented in the form of lytic lesions, pathological fractures, or vertebral collapses, and 25% had a proliferation of plasma cells of clonal origin in parts other than the iliac crest, a situation that was recorded as bone plasmacytoma. In the Mayo Clinic

series, and in others, about 80% of patients presented with some of the bone lesions previously described, which is similar to our findings[2, 11-13]. In the case of the *Fundación Santa Fe de Bogotá* series, radiological abnormalities were demonstrated in 79% of the cases [4].

Almost all cases of MM were secretory with the IgG Kappa combination as the most frequent; there were only two cases of IgD secretion and only one case of non-secreting myeloma, although we cannot completely assure it since it did not have free light-chain measurements in serum. This distribution is usually observed in other series and clinical studies[2, 4, 14, 15].

In this center, the request for serum free light-chain concentrations was scarce and the result was only available in seven patients.

In MM patients, only the hemoglobin value ≥10 g/dL was statistically significantly associated with longer survival, while the platelet count, creatinine, calcium, and β2 microglobulin were significantly associated with lower survival. Age ≥70 years, a variable that we expected to be associated unfavorably with survival, did not have statistically significant differences; however, it was available only in 156 records, since in 2011 there was a change in the system of recording medical records in HUSVF and we lost several demographic data.

The mean survival of patients with MM according to ISS was 771 days for stage I, 744 days for stage II and 500 days for stage III. Hypothetically, we propose that mortality in our series of patients with MM was so high because a large proportion of our patients came from remote rural areas, had more unfavorable socioeconomic conditions (which limits their access to new therapies), did not have timely access to health services. They presented serious complications of the disease and had long delays in their diagnosis. However, we cannot affirm this categorically, since most of the patients had outpatient follow-up in centers other than ours. All the variables previously described have been related to a less-favorable prognosis [16-19]. The median age of presentation of MM in our population was slightly earlier at the time of diagnosis[2], and could mean a more aggressive form of presentation of the disease, although other population reports have already evidenced this finding.

To our knowledge, this is the first analysis of the presentation of patients with MM in Medellín, Colombia, and is the series with the largest number of patients analyzed in this country where the survival of the cases is described. There is only one similar study in our country from the *Fundación Santa Fe de Bogotá*, but the variables related to survival were not analyzed [4]. As strength, we had no loss to follow-up, as the vital status of all patients was available until the cut-off date of February 2019 that we proposed. In our center, it was not frequent that the concentration of free light chains in serum was quantified.

There was poor correlation between bone marrow plasma cell counts between the 3 methods we used to measure them: bone marrow smear myelogram, bone marrow trephine biopsy, and flow cytometry (Spermann´s Rho 0,663 and 0,419) This could be explained by their intrinsic yield and analytical variation but also this could reflect the absence of standardized protocols after the bone marrow in undertaken and starts the sample manipulation and processing.

We found only 92 patients did undergo cytogenetics analysis based on Conventional Banding Karyotype. But less patients (n=39) went FISH analysis in bone marrow. Normal CBK could not discriminate patients with high nor standard death risk and its accuracy was 25%. This could be due the scarce of patient sample, but calls the attention to obtain FISH analysis in all patients. In the future, we also suggest systematically seeking for quantifying free light chains in serum and chemotherapeutics treatments in order to better characterize the population, assessment of the genetic risk and determine in future research whether they are related to survival.

CONCLUSION

In conclusion, the clinical profile of patients with a *de novo* diagnosis of multiple myeloma in our cohort is similar to that described in international studies. The diagnosis of multiple myeloma was documented at younger ages, with more advanced stages, anemia and a high percentage of bone disease. The best prognostic model for mortality appears to be the ISS;

however, this needs to be confirmed in a validation study.

Statement of Ethics

The Ethics Committee approved the protocol and study in Act file N° 17-2017 from September 15 – 2017 at the Hospital Universitario an Vicente Fundacion.
comite_etica_investigacion@sanvicentefundacion.com

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

Funding Sources

All Authors have not received support for the work under consideration for publication. All funding nedded for research come from personal budget.

REFERENCES

1. Pardo C. Anuario estadístico 2015. Instituto Nacional de Cancerología.2015; 13:1–129.
2. Kyle R, Gertz M, Witzig T, et al. Review of 1027 patients with newly diagnosed multiple myeloma. Mayo Clin Proc. 2003;78(1):21–33.
3. Palumbo A, Rajkumar S, San Miguel J, et al. International Myeloma Working Group consensus statement for the management, treatment, and supportive care of patients with myeloma not eligible for standard autologous stem-cell transplantation. J Clin Oncol. 2014;32(6):587–600.
4. Segovia J, Duarte M, Restrepo J, et al. Mieloma múltiple en el Hospital Universitario Fundación Santa Fe de Bogotá (1983-2006). Acta Med Colomb. 2008; 33(4): 276-281.
5. Hungria V, Maiolino A, Martinez G, et al. Confirmation of the utility of the International Staging System and identification of a unique pattern of disease in Brazilian patients with multiple myeloma. Haematologica. 2008;93(5):791–2.
6. Hungria V, Maiolino A, Martinez G, et al. Observational study of multiple myeloma in Latin America. Ann Hematol. 2017;96(1):65–72.
7. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol. 2016;17(8):e328–46.
8. Durie B, Salmon S. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975;36(3):842-54.
9. Greipp P, Miguel J, Dune B, et al. International staging system for multiple myeloma. J Clin Oncol. 2005;23(15):3412–20.
10. Rossi D, Fangazio M, De Paoli L, et al. Beta-2-microglobulin is an independent predictor of progression in asymptomatic multiple myeloma. Cancer. 2010;116(9):2188–200.
11. Rasch S, Lund T, Asmussen J, et al. Multiple myeloma associated bone disease. Cancers (Basel). 2020;12(8):2113.
12. Tosi P. Diagnosis and treatment of bone disease in multiple myeloma: spotlight on spinal involvement. Scientifica (Cairo). 2013;2013:104546.
13. Croucher PI, Apperley JF. Bone disease in multiple myeloma. Br J Haematol. 1998;103(4):902-10.
14. Nair B, Waheed S, Szymonifka J, et al. Immunoglobulin isotypes in multiple myeloma: Laboratory correlates and prognostic implications in total therapy protocols. Br J Haematol. 2009;145(1):134–7.
15. Krejci M, Buchler T, Hajek R, et al. Prognostic factors for survival after autologous transplantation: A single centre experience in 133 multiple myeloma patients. Bone Marrow Transplant. 2005;35(2):159–64.
16. Remes K, Anttila P, Silvennoinen R, et al. Real-world treatment outcomes in multiple myeloma: Multicenter registry results from Finland 2009-2013. PLoS One. 2018;13(12): e0208507.
17. Weide R, Feiten S, Chakupurakal G, et al. Reality of Care for Patients with Multiple Myeloma 1995-2016. New Drugs in Routine Care Lead to Improved Survival. *Blood.* 2017; 130(Suppl 1): 5628.
18. Hameed A, Ali J, Munawar K, et al. Characteristics and outcomes of patients with multiple myeloma: Data from a developing country. Med J Islam Repub Iran. 2018;32:1.
19. Cowan AJ, Allen C, Barac A, et al. Global burden of multiple myeloma: A systematic analysis for the global burden of disease study 2016. JAMA Oncol. 2018;4(9):1221–1227.
20. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29.