

# Epidemiological and biological parameters of monoclonal plasma cell dyscrasias in thirty one patients consulting a Moroccan Pasteur Institute.

## Abstract

**Aims:** To describe epidemiological and biological features of patients with monoclonal plasma cell dyscrasias. Our study also aims to compare our results with those from similar institutions in Morocco and other countries and to evaluate the degree of awareness of patients of the importance of treatment follow up. The patients were seen at the Moroccan Pasteur Institute in Casablanca during a period of two years and four months.

**Place and duration of study:** Laboratory of immunochemistry, Pasteur institute, Casablanca, Morocco. From April 2006 to July 2006.

**Methodology:** Thirty one case notes of patients who had a serum or urine monoclonal gamma or beta globulin spike were assessed.

**Results:** The mean age of the patients was  $54.7 \pm 10.7$  years (range, 26–72 years) and there were more females with a sex ratio 0.82. 66.7% of the patients had a monoclonal gamma globulin peak revealed by electrophoresis. According the results of performed agar gel immuno-electrophoresis, 48% of them had monoclonal immunoglobulin G (IgG) antibodies, followed by class IgA with 29% of cases. Moreover, 55% of the patients were Kappa-chain positive, while 45% were Lambda-chain positive. Our study showed that the majority of patients didn't follow up the progression of their monoclonal gammopathies by biological tests. However, the assessment of prognostic factors of some patients demonstrated an increased erythrocyte serum rate (66.7% of cases), anemia (50% of cases), a raised of calcium serum levels (37.5% of cases) and  $\beta_2$ -microglobulin serum levels were higher than 3.5 mg/l (33.3% of cases). In addition, the mean concentration of proteins was 91 g/l (58.6% of cases), low levels of albumin below 36 g/l were observed (63% of cases) and the monoclonal component levels were above 30 g/l (55.6% of cases).

**Conclusion:** Some of our records were different from those of other series: our patients were younger with a slight predominance of female individuals. Others were consistent mainly with the more frequently observed monoclonal gamma-globulin peak, and also with the assessed levels of IgG, which is known as the most common isotype in plasma cell disorders. Our study also showed the usability of electrophoresis and immuno- electrophoresis for detection of monoclonal gammopathies. However, it was revealed that the two techniques weren't sufficient for diagnosis. On the other hand, a rise of prognostic factors levels was also noted except for C-reactive protein. Nevertheless, our study pointed out that the majority of patients didn't have a rigorous monitoring of their disorders by carrying out hematological and serological examinations.

**Comment [u1]:** Also delete this. You will require all the patients results in other institutions to be able to do this.

**Comment [u2]:** You cannot do this in a retrospective study using patients case notes.

**Comment [u3]:** This was not part of the objectives of the study. This study being a retrospective study cannot be used to compare 2 laboratory methods, more over you never stated you wanted to do this in your aims and objectives.

36

37 **Keywords:** *Plasma cell dyscrasias, electrophoresis, immuno-electrophoresis, prognostic factors.*

38 **Abbreviations:** **PCD:** *plasma cell* dyscrasias, **MG:** monoclonal gammopathy, **MM:** multiple  
39 myeloma, **WM:** Waldenström macroglobulinemia, **MGUS:** monoclonal gammopathy of undetermined  
40 significance, **CLL:** chronic lymphocytic leukemia **CRP:** C-reactive protein, **Ig:** immunoglobulin, **β2-m:**  
41 beta-2-microglobulin.

42

## 43 1. Introduction

44 Plasma cell dyscrasias (PCDs) are a variety of disorders characterized by monoclonal proliferation  
45 and accumulation of lymphoplasmacytic cells in the bone marrow and, sometimes, with a tissue  
46 deposition of monoclonal immunoglobulins or their components [1]. These monoclonal gammopathies  
47 (MG) include a wide range of hematological conditions ranging from benign disorders such as  
48 monoclonal gammopathy of undetermined significance (MGUS) to malignant pathologies for example-  
49 For instance, multiple myeloma (MM), smoldering multiple myeloma (SMM), Waldenström  
50 macroglobulinemia (WM), amyloid light-chain (AL) amyloidosis, POEMS syndrome, non- Hodgkin  
51 lymphoma (NHL) and B-cell chronic lymphocytic leukemia (B-CLL) [1-3].

52 These diseases may be difficult to diagnose because they affect many tissues and exhibit generally  
53 non-specific symptoms [4]. Thus, the basis of the diagnosis is the detection of an abnormal  
54 monoclonal paraprotein in the serum and/or urine, by means of electrophoresis and immuno-  
55 electrophoresis [2]. Since then, the definitive diagnoses of PCDs require a multidisciplinary approach  
56 including bone marrow biopsy, clinical assessment, radiology and clinical laboratory assays [4].  
57 Furthermore, plasma cell labeling index, karyotypic abnormalities molecular alterations, analysis of  
58 beta-2-microglobulin and C-reactive protein levels are laboratory prognostic parameters, related to the  
59 intrinsic malignancy of the plasma cells, which indicate the risk of transformation from benign to  
60 malignant forms, and therefore designate the follow-up and treatment of each individual patient [5].

61 In the current retrospective study, we reviewed the epidemiological and biological features of patients  
62 with plasma cell dyscrasias, established during the period between January 2004 and April 2006 in the  
63 Moroccan Pasteur Institute (Casablanca), and we compared our results with those from similar  
64 institutions in Morocco and other countries.

65

## 66 2. Materials and methods

### 67 2.1. Study location and patients

**Comment [u4]:** The diagnosis of PCD is based on the detection of more than 20% monoclonal plasma cells in bone marrow or tissues. This should not be mixed up with presence of gammopathies.

68 | ~~The current~~This retrospective study was conducted at Pasteur Institute, Casablanca, Morocco, by  
69 reviewing case notes of patients consulting immunochemistry laboratory during the period from 1  
70 January 2004 to 30 April 2006.

71 This laboratory received requests for urine or serum protein electrophoresis and immuno-  
72 electrophoresis for various diseases: myeloma, immune deficiencies, hepatic insufficiency, systemic  
73 diseases and rheumatology, etc.

74 Only patients who had a gamma or beta (rarely alpha 2) monoclonal peak and a monoclonal  
75 immunoglobulin demonstrated by urine or serum protein electrophoresis and immuno- electrophoresis,  
76 respectively, were retained. The choice of these two techniques was based on the necessity for  
77 detection of monoclonal gammopathies affection.

Comment [u5]: This statement doesn't make sense. Please re-write.

78 During the period of study, 1758 electrophoreses and 99 immuno-electrophoreses were gathered.

79 Any double immuno-electrophoresis or non-coupled to electrophoresis was excluded. Thus, our  
80 population was limited to 31 patients. Their distribution was done by taking in consideration the age,  
81 sex, nature of spike, as well as the type of immunoglobulin heavy and light chains.

82 Moreover, to detect whether patients had followed-up the evolution of their diseases, some prognostic  
83 factors were also evaluated such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP),  
84 serum levels of calcium,  $\beta$ 2-microglobulin ( $\beta$ 2-m), hemoglobin and some other proteins.

85 It should be noted that bone marrow biopsies were performed in the clinic that sent the patients' sera  
86 for testing in the laboratory. We didn't get the results of these analyses.

87 Statistical analysis was performed by calculation of percentages and arithmetic averages.

88

### 89 3. Results

90 Our current investigation was a retrospective study that included 31 Moroccan patients having a risk to  
91 develop plasma cell dyscrasias consulting Pasteur Institute, Casablanca, between January 2004 and  
92 April 2006.

93 Detailed epidemiological and clinical characteristics of 31 patients are shown in table 1.

94

95 Table 1. Epidemiological and clinical characteristics of 31 patients.

| P | A  | S | Type of<br>protein<br>ep and<br>iep | M. Ig<br>type | Proteins<br>Level (g/l) | Protein fractions levels (g/L): |      |      |       |       | ESR (mm) |    | Levels of:    |              |                      |                |
|---|----|---|-------------------------------------|---------------|-------------------------|---------------------------------|------|------|-------|-------|----------|----|---------------|--------------|----------------------|----------------|
|   |    |   |                                     |               |                         | Al                              | A1   | A2   | B     | G     | FH       | SH | CRP<br>(mg/l) | Hg<br>(g/dl) | $\beta$ 2m<br>(mg/l) | Ca<br>(mmol/l) |
| 1 | 45 | M | ser prot                            | IgM/K         | 90                      | 33.12                           | 3.96 | 8.82 | 29.52 | 14.58 | NP       | NP | NP            | NP           | NP                   | NP             |

|    |    |   |          |       |      |        |       |        |        |        |     |     |    |      |       |      |
|----|----|---|----------|-------|------|--------|-------|--------|--------|--------|-----|-----|----|------|-------|------|
| 2  | 55 | M | ser prot | IgG/L | 80   | 40.64  | 2.65  | 10.08  | 11.84  | 14.88  | 10  | 22  | <6 | 14.6 | 1.56  | 2.35 |
| 3  | 60 | F | ser prot | IgM/K | 110  | 41.25  | 2.86  | 8.8    | 8.47   | 48.62  | NP  | NP  | NP | NP   | 1.31  | NP   |
| 4  | 46 | F | ur prot  | LLC   | 0.4  | -      | -     | -      | -      | -      | NP  | NP  | NP | NP   | NP    | NP   |
| 5  | 57 | M | ser prot | IgA/K | 76   | 37.012 | 2.356 | 7.524  | 23.408 | 5.7    | NP  | NP  | NP | NP   | NP    | NP   |
| 6  | 59 | F | ser prot | IgG/K | 106  | NF     | NF    | NF     | NF     | NF     | NP  | NP  | NP | NP   | NP    | NP   |
| 7  | 50 | F | ser prot | IgG/L | 84   | 38.163 | 2.772 | 9.072  | 8.148  | 25.878 | 64  | 105 | NP | 12.8 | 1.05  | 2.24 |
| 8  | 71 | M | ser prot | IgG/L | 80   | 23.2   | 4.16  | 14.88  | 3.68   | 34.08  | NP  | NP  | NP | NP   | NP    | NP   |
| 9  | 56 | F | ser prot | IgG/L | 84   | 39.06  | 2.856 | 9.576  | 8.232  | 24.276 | 26  | 54  | NP | 11.6 | NP    | NP   |
| 10 | 61 | F | ser prot | IgG/L | 120  | 30.72  | 2.4   | 6.96   | 5.28   | 74.64  | NP  | NP  | NP | NP   | NP    | NP   |
| 11 | NF | M | ser prot | IgG/L | 120  | 34.92  | 2.88  | 7.56   | 6.96   | 67.68  | NP  | NP  | NP | NP   | NP    | NP   |
| 12 | 52 | M | ur prot  | IgG/K | 2    | -      | -     | -      | -      | -      | NP  | NP  | NP | NP   | NP    | NP   |
| 13 | 56 | M | ser prot | IgG/L | 80   | 36.24  | 3.12  | 12.88  | 13.04  | 14.72  | 8   | 18  | <6 | 11.5 | 1.56  | 2.3  |
| 14 | 50 | F | ser prot | IgA/K | 116  | 29     | 1.6   | 5.68   | 3.9    | 74     | NP  | NP  | NP | NP   | 7.76  | NP   |
| 15 | 63 | F | ser prot | LLC   | 72   | 36.43  | 3.24  | 12.96  | 11.304 | 8.064  | NP  | NP  | <6 | 12.4 | NP    | 2.54 |
| 16 | 72 | F | ur prot  | KLC   | 1.85 | -      | -     | -      | -      | -      | -   | -   | -  | -    | -     | -    |
|    |    |   | ser prot | KLC   | 70   | 35.42  | 3.08  | 13.44  | 9.31   | 8.75   | 42  | 80  | <6 | NP   | 14.16 | NP   |
| 17 | 49 | M | ser prot | IgG/K | 94   | 36.472 | 3.478 | 10.81  | 9.776  | 33.464 | NP  | NP  | NP | NP   | NP    | NP   |
| 18 | 67 | F | ser prot | IgA/L | 120  | 29.64  | 3.12  | 4.44   | 80.04  | 2.76   | NP  | NP  | NP | NP   | 7.98  | NP   |
| 19 | 71 | M | ser prot | LLC   | 58   | 32.712 | 3.306 | 7.424  | 9.744  | 4.814  | NP  | NP  | NP | 7.9  | NP    | NP   |
| 20 | 54 | F | ser prot | IgA/K | 110  | 26.29  | 2.09  | 7.04   | 9.13   | 65.45  | NP  | NP  | NP | 6.4  | 2.25  | NP   |
| 21 | 45 | M | ser prot | IgA/L | 90   | 41.58  | 3.42  | 10.89  | 9.45   | 24.66  | NP  | NP  | NP | NP   | NP    | NP   |
| 22 | 66 | F | ser prot | IgM/K | 74   | 30.118 | 2.738 | 7.844  | 19.092 | 14.208 | NP  | NP  | NP | NP   | NP    | NP   |
| 23 | 30 | F | ser prot | IgG/K | 102  | 31.212 | 2.652 | 6.834  | 8.772  | 52.53  | NP  | NP  | NP | 10.8 | NP    | NP   |
| 24 | 58 | M | ser prot | IgG/K | 80   | 31.28  | 4.08  | 9.2    | 8.72   | 26.72  | 81  | 115 | NP | 10.8 | NP    | NP   |
| 25 | 45 | F | ser prot | IgA/K | 72   | 37.152 | 2.52  | 7.92   | 9      | 15.408 | 105 | 132 | NP | 9.6  | NP    | NP   |
| 26 | 51 | F | ser prot | IgG/L | 74   | 33.152 | 2.738 | 9.102  | 8.51   | 20.498 | 64  | 105 | NP | 12.3 | 1.05  | 2.24 |
| 27 | 59 | M | ser prot | IgA/K | 96   | 35.424 | 2.016 | 6.528  | 49.344 | 2.688  | NP  | NP  | NP | NP   | 6.77  | 2.65 |
| 28 | 44 | F | ser prot | IgG/K | 106  | 29.468 | 3.922 | 12.508 | 6.996  | 53.106 | NP  | NP  | NP | NP   | NP    | 2.92 |
| 29 | 57 | M | ser prot | IgG/L | 70   | 30.87  | 4.2   | 12.11  | 9.94   | 12.88  | 10  | 22  | <6 | 14.2 | 1.56  | 2.35 |
| 30 | 66 | F | ser prot | IgA/K | 84   | 33.768 | 2.268 | 8.4    | 31.08  | 8.484  | NP  | NP  | NP | NP   | 1.07  | NP   |
| 31 | 26 | M | ser prot | IgA/K | 121  | NF     | NF    | NF     | NF     | NF     | NP  | NP  | NP | NP   | NP    | NP   |

97 P: patient; A: age; S: sex; Ep: electrophoresis; IEP: immuno- electrophoresis; M: monoclonal; Ig:  
 98 immunoglobulin; Al: albumin; A1: alpha 1; A2: alpha 2; B: beta; G: gamma; ESR: Erythrocyte  
 99 sedimentation rate; FH: first hour; SH: second hour; CRP: C-reactive protein ; Hg: Hemoglobin; β2m:  
 100 β2-microglobulin; Ca: serum calcium; Ser prot: serum protein; Ur prot: urine protein; M: male; F:  
 101 female; K: kappa; L: Lambda; LLC: Lambda light chain; KLC: Kappa light chain; NP: not performed;  
 102 NF: not found.

### 104 3.1. Epidemiological data

105 The mean age was 54.7 ± 10.7 years with extremes ranging from 26 to 72 years. Fifty-five percent of  
 106 the patients were female and forty-five were male with a sex ratio 0.82.

107 The two third of our study population were located in the range between 40 and 60 years with equality  
 108 of reaching for both sexes. However, a female predominance in patients over 60 years was observed  
 109 (Fig. 1). It's noteworthy to mention that the data about the age of one patient had not been found.

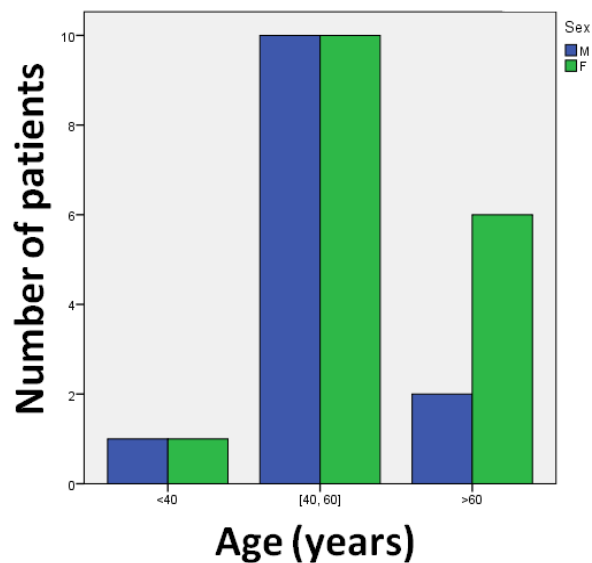


**Fig. 1: Distribution of patients according to age and sex (n=30).**

M: male; F: female

**Comment [u6]:** Remove figure 1, it's not necessary

### 3.2. Immunochemical data

According to the electrophoresis results, 66.7% of patients had a gamma-globulin monoclonal peak, against 33.3% had a monoclonal peak in beta-globulin zone.

The data from immuno-electrophoresis confirmed that the tested population in the current study had a monoclonal band in serum. In fact, we found that immunoglobulin G monoclonal antibody (Ig G) was placed in the forefront. It was observed in 15 patients (48%), Ig A was found in the second place with 9 cases (29%) followed by 4 patients (13%), who had free light-chains and 3 patients (10%) had Ig M, while in none of them Ig D and Ig E were detected (Fig. 2).

Besides that, 17 of the patients (55%) were Kappa-chain positive and 14 (45%) were Lambda-chain positive.

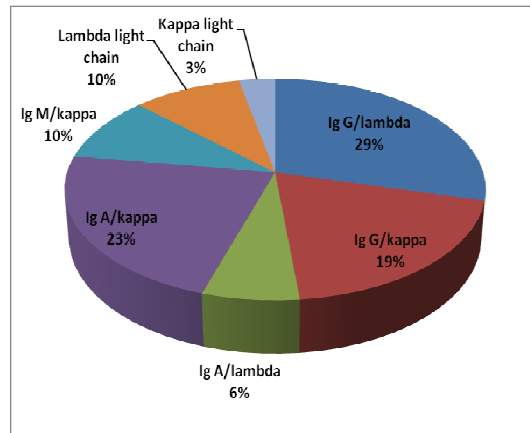


**Fig. 2: Percentage of different immunoglobulin subtypes of monoclonal plasmaproliferative disorders (n=31).**

### 3.3. Biological data

In some patients, prognostic factors as ESR, CRP, serum levels of calcium,  $\beta$ 2-microglobulin, hemoglobin and other proteins, were also taken in consideration. According the data in table 2, the majority of patients didn't follow up the progression of their malignant disorders by biological tests.

Nevertheless, patients requesting for these analyzes demonstrated an acceleration-elevation of ESR (66.7% of cases), anemia (50% of cases), a raised of serum level of calcium (37.5% of cases) and  $\beta$ 2-microglobulin serum level was greater than 3.5 mg/l (33.3% of cases). Moreover, the mean concentration of proteins was 91 g/l ranged in values varying from 58 to 121 g/l (normal values of serum proteins are between 60 and 80 g/l). It was noted that serum protein electrophoresis had been performed only for 29 patients, while the others had urine electrophoresis. Thus, we found that 58.6% of patients had a hyperproteinemia against 38% having normal levels of serum proteins, whereas 3.4% of cases having a hypoproteinemia, corresponding to a patient with a monoclonal light Lambda chain.

With respect to the spike size, 44.4% of the cases were below 30 g/l, and 55.6% above 30 g/l, respectively. The mean concentration of the monoclonal component was 37 g/l.

On the other hand, the average level of albumin was 33.86 g/l with extremes ranging from 23.2 g/l and 41.58 g/l.

In seventeen patients (63%) having low levels of albumin, 15 patients had higher serum monoclonal protein levels. The remaining two patients had normal concentrations of beta- or gamma- globulin fractions, which were probably associated with light chain MG. However, there was a moderate

negative correlation between the parameters investigated (Pearson's correlation,  $r = -0.37$ ,  $n = 17$ ,  $p=0.12$ ).

**Comment [u7]:** Which of the parameters exactly are you referring to ? Please state them.

**Table 2. Number and percentage of patients requesting for prognostic analyzes.**

| Prognosis factor                                                                                                     | Erythrocyte serum rate | CRP             | Level of:       |                          |                  |
|----------------------------------------------------------------------------------------------------------------------|------------------------|-----------------|-----------------|--------------------------|------------------|
|                                                                                                                      |                        |                 | Calcium         | $\beta 2$ -microglobulin | Hemoglobin       |
| Number of patients who requested for each analysis divided by total number of the studied population (%)             | 9/31<br>(29%)          | 5/31<br>(16.1%) | 8/31<br>(25.8%) | 12/31<br>(38.7%)         | 12/31<br>(38.7%) |
| Number of patients having anomalies in analysis divided by number of patients requesting for prognostic factors (%). | 6/9<br>(66.7%)         | 0/5<br>(0%)     | 3/8<br>(37.5%)  | 4/12<br>(33.3%)          | 6/12<br>(50%)    |

#### 4. Discussion

The presented investigation was the first study conducted in 2006 at Pasteur Institute in Casablanca (Morocco) aimed to highlight the epidemiological and biological features of thirty one patients having a risk to develop plasma cell dyscrasias.

In the current period, only five immunochemistry laboratories in Casablanca, performing electrophoresis and immuno-electrophoresis, existedexists. Among them, figures Pasteur Institute covering, not only 50% of Casablanca population, but also 20% from the population of other four Moroccan cities (Settat, Jadida, Mohammedia and Berrechid).

**Comment [u8]:** Is your study on immuno-electrophoresis in Morocco or Plasma cell dyscrasias ? You have to focus your discussion on the patients and their features.

Few years later, many studies, performed in Morocco and other countries, were directed to the same target. Some of the results from those investigations were consistent with our data, but others were different. In fact, our patients were younger (54.7 years), in comparison with the data, which were reported by other surveys. Beginning with a Moroccan investigation which had been executed in Mohamed V Military Hospital of Rabat (2012) [6], the mean age was 60.21 years. In other countries, the average age was a bit closer to that found by Moroccan institutions. For instance, in India [7], Egypt [1], Tunisia [8] Lebanon [9] and Saudi Arabia [10], it was 56, 58.5, 62.7, 65 and 65.6 years, respectively. However, it was much higher in Western nations especially in Iceland [11], Spain [12] and France [13], with mean ages of 70, 73 and 79 years, respectively. The observed differences,

171 | ~~concerning in~~ mean ages of patients with MG between Eastern and Western nations, may be due to  
172 | the quality of life, health services and medical scientific researches, which are different in the  
173 | developed and underdeveloped countries. Ideally, to the best of our knowledge, no epidemiological  
174 | study has explored the detection of MGUS in Moroccan population and prediction of its progression to  
175 | myeloma or related malignancy. Such an investigation could help to prevent development of a benign  
176 | disease, which requires just a close follow-up to a malignant cancer in patients. Consequently, it might  
177 | be more cost effective and probably could increase the life expectancy among patients with MG.

178 | Besides that, our outcomes had demonstrated that 93.3% of our patients were above 40 years old, but  
179 | it was important to underline that only two patients were 26 and 30 years. These results were  
180 | corroborated with ~~that presented in inquire, carried out~~ the study by Kassem et al. (2014) [1], who  
181 | ~~proved-showed~~ that 96% of patients having PCD were of ~~mature age~~ the older age group. This  
182 | confirmed some literature data [1, 4, 7, 14, 15], according which monoclonal gammopathies as CLL,  
183 | MM and MGUS are diseases of older adults.

184 | Similarly ~~to the results of other studies, we found~~ showed that the prevalence of MG increased with the  
185 | age [1, 7, 13, 15-17]. Nevertheless, the main difference between the cited and our data was that we  
186 | noted a slight predominance of female individuals with a sex ratio 0.82 and an equality of reaching for  
187 | both sexes in patients aged between 40 and 60 years, ~~meanwhile~~, other authors found that men were  
188 | affected more frequently than women by PCD with different sex ratios 1.08 [11], 1.2 [13, 18], 1.24 [9],  
189 | 1.43 [1, 10], 1.71 [8], 1.94 [14] and 2.3 [6], respectively. The established predominance in female  
190 | individuals can be explained by the ~~eventual~~ influence of geographical distribution of Casablanca  
191 | population, which was presented by 50.7% of females and 49.3% of males according to 2005 and  
192 | 2006 statistics performed in Casablanca [19].

193 | On the other hand, the most frequent type of immunoglobulin in ~~the current~~ study was IgG. It was  
194 | found in 48% of patients, followed by Ig A (29%), monoclonal light chains (13%), and the isotype Ig M  
195 | (10%) which came in the last place. This distribution coincided with that reported by the clinical  
196 | oncology department of Cairo University (Egypt) [1], not in point of view ~~the percentage~~, but ~~the~~ order.  
197 | In ~~the~~ latter inquiry, 63.6% of the patients were assessed with prevalence of IgG against 17.5% of IgA,  
198 | while 13.4% had monoclonal light-chains and 5.5% had an Ig M monoclonal band. Nonetheless, our  
199 | findings were not in concord with those reported by French studies [13]. Effectively, the first  
200 | investigation was led in internal medicine department of Rennes university hospital [13], which  
201 | revealed that isotypes repartition was: Ig G (42.8%), Ig M (31.9%), Ig A (8.9%), biclonal gammopathy  
202 | (9.8%) and monoclonal light chains (6.6%), respectively. Whereas, the second research, conducted in  
203 | all ~~the department s of~~ General Hospital of Blois [13], showed that this distribution was Ig G (59.7%),  
204 | Ig M (27.5%), Ig A (11.8%) and monoclonal light chains (2.7%), respectively. In contrast, no  
205 | monoclonal light chain was detected in an Indian investigation [18], while in Singh et al. survey [7], no  
206 | IgM type paraprotein was observed.

207 | Moreover, electrophoresis showed a monoclonal peak in gamma globulin fraction in 66.7% of the  
208 | patients. Our ~~current~~ findings were in agreement with those previously reported [8, 18, 20], outlining a

**Comment [u9]:** Monoclonal gammopathy (MG) and plasma cell dyscrasias are not the same. You seem to be discussing MG and not plasma cell dyscrasias as you stated in your topic and aim. I advice you stick to your aim. MGUS is a type of PCD, but several other PCDs exists.

209 monoclonal spike in the gamma zone in 78%, 84.8% and 85.5% of cases, respectively. These  
210 observations could be explained by the fact that the gamma fraction contains the largest portion of  
211 immunoglobulins. Therefore, monoclonal gammopathies are the most frequently encountered in this  
212 portion of the electrophoresis. Additionally, since most IgG monoclonal proteins are cationic, they  
213 migrate to the gamma zone of the electrophoretic gel [21]. This statement may justify the  
214 predominance of the isotype IgG in all the studies, discussed above.

215 In addition, the association of IgG with the lambda light chain was established in 29% of cases. In the  
216 opposite, the study of Dasse et al. [22] conducted in Abidjan (Ivory Coast), indicated that the  
217 association Ig G/kappa was the most found monoclonal form in 60% of cases.

218 Alternatively, analysis of the association between heavy and light chains revealed that kappa light  
219 chain was more linked than was lambda light chain (55% against 45%, respectively). This statement  
220 joined the outcomes of many researches [1, 7, 20, 22].

221 In this series of 31 patients, all the detected heavy chains were associated to light chains in  
222 monoclonal gammopathy (intact immunoglobulin). This result was compatible with the data presented  
223 by Dasse et al. (2000) [22]. Nevertheless, the presence of immunoglobulin free light chains had been  
224 proven in 13% of cases.

225 With respect to biological tests, the hyperproteinemia touched 58.6% of our patients. A similar result  
226 was obtained from the study of Gaougaou et al. [23], in which increased serum protein levels in 63%  
227 of the cases with MM have been noticed. It was pointed out that PCDs were not always related with  
228 hyperproteinemia. Indeed, cases of MG, presented in the current investigation, indicated free light  
229 chains with normal or low levels (hypoproteinemia) of serum proteins. A similar finding was described  
230 in another study [21] reporting that the mean serum total protein concentration was significantly higher  
231 in IgG, IgA, and IgM, compared with free light chain monoclonal gammopathies, where this value was  
232 within normal limits. This could be explained by the effect of low molecular weight of the light-chain  
233 secreted by malignant plasma cells about 22500 Dalton versus 40000 to 70000 Dalton, which shows  
234 the variation of molecular weight of the heavy chains. It was important to mention that 17 of 21  
235 patients (80.9%), having total protein level above or equals to 80 g/l, had monoclonal paraprotein  
236 concentration above 20 g/l. There was a strong positive relationship between the two variables under  
237 study (Pearson's correlation,  $r = 0.712$ ,  $n = 21$ ,  $P < 0.001$ ). As was previously reported, in a study,  
238 performed by van Hoesen et al., [21], monoclonal proteins often increase serum total protein level. In  
239 fact, it was found that paraprotein levels were reflected in significantly increased mean serum total  
240 protein in IgG, IgA, and IgM [21].

241 An increase in  $\beta 2$ -micro-globulin levels in 33.3% of cases was as well noted. It has already been  
242 reported that this molecule is a small membrane protein, associated with the heavy chains of class I  
243 major histocompatibility complex proteins. It's a powerful prognostic factor, correlating with the survival  
244 in monoclonal disorders [24]. Indeed, according to the results of Di Giovanni et al., [25], in the group of  
245 patients suffering from MM or WM, the mean level of the same protein has been assessed to be  
246 significantly higher than in the group with MGUS. Values above 3 mg/L were highly indicative of a

247 neoplastic process. They were observed in all the WM patients and in greater than 90% of myeloma  
248 patients. It was noticed, in the current study, that the patients having  $\beta_2$ -m concentrations above 5.5  
249 mg/l, had albumin levels below or equals to 35 g/l. This finding is in keeping with a previous  
250 investigation [26], demonstrating that serum  $\beta_2$ m values had a negative correlation with serum  
251 albumin. Our data were in concordance with those recorded by Charafeddine et al., [9], demonstrating  
252 that  $\beta_2$ -m and albumin were related to spike size. These patients had as well serum levels of  
253 monoclonal protein above 30 g/l except for one patients suffering from kappa light chain MG. There  
254 was a strong positive association between monoclonal component (MC) above 30 g/l and serum  $\beta_2$ m  
255 levels (Pearson's correlation,  $r = 0.703$ ,  $n = 5$ ). This correlation could be explained by the fact that  $\beta_2$ -  
256 m reflects the tumor mass [24], which is detected by hyperproteinemia due to the elevation of serum  
257 monoclonal protein in beta- or gamma- globulin zone. This increase often leads to the decrease of  
258 others fractions levels such as serum albumin. Additionally, it was previously described that the  
259 concentration of the serum monoclonal protein at diagnosis and the serum albumin value were  
260 independent predictors of progression from MGUS to lymphoma or a related disorder with a  
261 multivariate analysis [20]. In contrary, normal or low (hypoproteinemia) levels of proteins were  
262 associated with normal concentrations of beta- or gamma- globulin fractions in patients suffering from  
263 light chain MG, but presenting high levels of  $\beta_2$ -m and low values of serum albumin concentrations.  
264 This result joined that reported by Charafeddine et al., [9], finding that most of the patients, diagnosed  
265 with light chain MG had a spike size below or equals to 20 g/l. Thus, the association of albumin and  
266  $\beta_2$ -m serum levels could be good prognosis factors for detection of PCDs. However, there was a weak  
267 negative correlation between the parameters investigated (Pearson's correlation,  $r = -0.34$ ,  $n = 12$ ,  $P =$   
268  $0.309$ ). This, maybe, was due to the small number of patients carrying out the biological test  $\beta_2$ -m.  
269 Therefore, the current data should be proven by investigating the involvement of both  $\beta_2$ -m and  
270 albumin serum levels in MG prognosis in a large-scale of Moroccan patients.

271 In assessment of serum calcium levels, according to the results of the current study 37.5% of the  
272 patients had hyper-calcemia, leading to an increased risk of bone lesions and collapse of the vertebral  
273 body. This was supported by the deduction reached by Pagano et al., [27], who noted that 27% of  
274 patients suffering from plasma cell leukemia had an elevation in serum calcium level. Hyper-calcemia  
275 is one of the important factors besides renal insufficiency, anemia, and lytic bone lesions (CRAB  
276 criteria), that can distinguish between MM and MGUS [1, 28, 29], since patients with the pre-malignant  
277 plasmatic cell disorder are known by absence of hyper-calcemia [20]. However, because MGUS is a  
278 condition of the elderly, concomitant diseases can confound the distinction [30]. For instance,  
279 hypercalcemia may be caused by hyperparathyroidism that should be considered in the absence of  
280 skeletal lesions [30]. It was also made out in the current investigation that the patients with hyper-  
281 calcemia had high values of  $\beta_2$ -m serum concentrations, albumin levels below or equals to 35 g/l and  
282 serum levels of monoclonal protein above 30 g/l, with exception of one patient, suffering from kappa  
283 light chain MG. As was discussed above, high concentrations of calcium, as well as high  $\beta_2$ -m and low  
284 albumin serum levels, could be considered as prognostic factors for PCDs. Statically, there was a  
285 moderate negative correlation between albumin levels and calcium concentrations (Pearson's  
286 correlation,  $r = -0.48$ ,  $n = 8$ ,  $P = 0.226$ ).

**Comment [u10]:** NO SIR I, CRAB distinguishes between symptomatic and asymptomatic myeloma.

287 It was also established that the emergence of anemia was more thrust. It was perceived that 50% of  
288 patients had a drop in rate of hemoglobin which may reveal the impact of the plasma cell infiltration on  
289 the bone marrow production. In other surveys [7, 27, 18], anemia features were also detected, but only  
290 in 48%, 25%, and 7.2% of patients, respectively. It should be noticed that our six patients with anemia  
291 might not be affected by solitary plasmacytoma. In fact, the decrease of serum hemoglobin level isn't a  
292 criterion for this disease, which is also characterized by a low serum or urinary level of monoclonal  
293 immunoglobulin [31]. In contrast, these patients could have MM or WM, for which the anemia is a  
294 common complication [4]. In the current study, it was also observed that 4 of 6 patients with anemia  
295 had albumin levels below 35 g/l, but had variable levels of serum monoclonal paraprotein (below or  
296 above 30 g/l regardless of the immunoglobulin isotype). There was a moderate positive correlation  
297 between albumin levels and hemoglobin concentrations (Pearson's correlation,  $r = 0.606$ ,  $n = 6$ ).  
298 Various studies had demonstrated a positive correlation between hemoglobin and albumin levels in  
299 patients with MG [9, 17]. However, according to our investigation, these two factors might not be  
300 indicators of the increase of MC levels of non-IgM MG. In fact, as was previously reported [20, 30],  
301 anemia may be multi-factorial in the elderly. It could be caused by conditions, different from MG, such  
302 as renal insufficiency, iron deficiency, nutritional imbalance, occult gastrointestinal bleeding, or, less  
303 frequently, myelodysplastic syndrome. On the other hand, our observation didn't corroborate with an  
304 investigation, performed by Charafeddine et al. [9], carried out in the clinical laboratory of the  
305 American University of Beirut Medical Center with 540 patients, in which low serum concentrations of  
306 hemoglobin and albumin showed significant correlation with high M spike size above 20 g/l. The  
307 difference between the two inquiries resides in the size of the population under study. Thus, our  
308 outcome should be confirmed or rejected after study the impact of prognostic factors in a large number  
309 of patients.

310 Furthermore, it was explored that 66.7% of our patients had a higher erythrocyte sedimentation rate  
311 (ESR). In others surveys, an elevation of this biological test was observed in all seven reported cases  
312 with plasma cell leukemia [32] and in 94.6%, 17.9%, 25.5% of patients with MM [23], MGUS and SMM  
313 [33], respectively. In the current investigation, whatever were the ESR levels, MC concentrations were  
314 lower than 30 g/L. Probably; there is no clear relationship between ESR levels and the size of MC in  
315 patients with MG as was previously reported [33], suggesting variability in the interaction of individual  
316 MCs with red blood cells to create rouleaux formation [33]. Besides that, due to the small number of  
317 patients followed-up, we can't judge whether or not ERS is a prognostic factor for PCDs.

318 Unlike the study, performed by Salonen and Nikoskelainen [34], displaying elevated CRP levels in  
319 patients with hematological malignancies (around 152 mg/l), all our five patients had a normal CRP, a  
320 prognosis index, correlated with survival and activity of proliferative cells [24]. It is also an infection  
321 indicator as a cause of death in patients with PCDs [34]. Despite of this positive outcome, the majority  
322 of serological and hematological examinations allowing to patients to have a rigorous monitoring of  
323 their MG, had not been carried out by all of them. Additionally, the differential diagnosis between  
324 different disorders may not be done. Effectively, findings of Ig M MG, plasma cell proliferation on bone  
325 marrow biopsy and clinical findings consistent with myeloma (CRAB signs) classically distinguished  
326 the rare diagnosis of Ig M MM from the more common WM, characterized with a monoclonal Ig M and

known by lymphadenopathy, organomegaly, lymphoplasmacytic lymphoma and hyper-viscosity (related to the absolute level and structure of the Ig M pentamer) [28]. Conversely, some patients may not have all of these findings. Both diseases could share clinical and biological manifestations such as hyper-viscosity, which may lead to diagnostic errors [28, 35, 36]. Recent advances in cytogenetic and immuno-phenotyping of bone marrow plasma cells can help further define the differences between IgM MM and WM [28, 37]. Myeloma plasmatic cells lack CD19, CD45, and CD27 expressions and are positive for CD56 (75%), CD117 (30%), and CD20 (30%) according to the report of Rawstrom et al. (2008) [38], while in WM, clonal B-cells express CD19, CD20, CD45 [36]. In contrast to WM, MM is characterized by chromosome translocations, which include not only the t(11;14) but also the t(4;14) and t(14;16) located in the genes coding for immunoglobulin heavy chains [37].

It was noteworthy that without having results of bone marrow biopsy and the lack of performing biological examinations in the current study, the type of MG couldn't be determined. Moreover, electrophoresis and immuno-electrophoresis assays could predict MG affection, but they should not be considered sufficient testing when contemplating a diagnosis of a PCD [39]. For this reason, the diagnostic of each disorder requires the performance of bone marrow biopsy, for confirmation of diagnosis with clinical investigations, radiological studies and laboratory tests.

Importantly, a definitive diagnostic allows the choice of the adequate treatment for each PCD by taking in consideration age, gender, race, family history.

Our study had several limitations: the laboratory data of the MG cases were included without their clinical diagnosis and many factors affecting prognosis were not assessed in the univariate and multivariate analyses due to the limited number of patients. Moreover, the current study was Pasteur Institute based and thus cannot be generalized for the entire Moroccan population.

## 5. Conclusion

The purpose of the current study was to evaluate epidemiological and biological characteristics of patients with monoclonal gammopathies, detected by the association of electrophoresis and immuno-electrophoresis. Our data were very similar to those reported by others investigations but slightly different in the aspect of mean age and sex ratio. It is also important to mention that biological tests weren't performed by all members of our cohort, probably because of financial constraints, that limit the opportunity for monitoring of their disorders progression and response to the respective treatment.

## Ethical approval

Ethical committee of Pasteur Institute of Casablanca Morocco approved the study.

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