



Monoclonal gammopathy in a tertiary referral hospital

Waleed Tamimi^{a,*}, Ahmad Alaskar^b, Mohammed Alassiri^a, Waleed Alsaeed^a, Saud A. Alarifi^c, Faris Q. Alenzi^d, Dunia Jawdat^e

^a Department of Pathology and Laboratory Medicine, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

^b Division of Hematology, Department of Oncology, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia

^c College of Science, King Saud University, Riyadh, Saudi Arabia

^d College of Applied Medical Sciences, Al-Kharj University, Saudi Arabia

^e King Abdulla International Medical Research Center, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:

Received 2 February 2010

Accepted 21 February 2010

Available online 3 March 2010

Keywords:

Monoclonal gammopathies

Dyscrasia

Multiple myeloma

MGUS

Monoclonal immunoglobulin

Waldenström's macroglobulinemia

M-protein

Serum electrophoresis

Urine electrophoresis

Immunofixation

ABSTRACT

Objective: Monoclonal gammopathies reflect conditions of plasma B-cell disorders. Our objective was to identify the prevalence and types of these gammopathies in our population.

Methods: A 10 year retrospective study was conducted. Serum and/or urine protein electrophoresis were performed on 6624 samples. Positive bands were further tested by immunofixation (IFE).

Results: Homogenous bands were detected in 7% of the patients. IFE method confirmed 6.3% in which 59% were males and 41% were females. The mean age was 64.7 for females and 66.5 for males. The sensitivity and specificity were 91% and 99% respectively. The most common protein was IgG kappa 41%, followed by IgG lambda 19%. Sixty-eight percent of these patients had monoclonal gammopathy of undetermined significance and 14.6% had multiple myeloma.

Conclusion: The majority of the studied population had MGUS. This observation is in concord with other western populations. The sensitivity and specificity of protein electrophoresis is diagnostically and reasonably acceptable.

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Introduction

Monoclonal gammopathies are a group of disorders characterized by uncontrolled proliferation and accumulation of plasma cells with the overproduction of monoclonal immunoglobulin proteins [1]. These proteins are commonly referred to as monoclonal proteins, M-proteins, M spikes, paraproteins or immunoglobulinopathies which can be detected in the serum or urine of the patients. They consist of 2 heavy chains of the same class and 2 light chains of either kappa (κ) or lambda (λ). In some cases, the plasma cells produce only free light chains (FLC) mostly detected in the urine and known as Bence-Jones proteins [2].

In a study conducted in 2003 it was shown that plasma cell disorders account for 7% of all hematological malignancies, with approximately 14,000 newly diagnosed cases in the United States annually [3]. The median age of diagnosis is approximately 65 years, and 2% to 4% less than 40 years. Males are diagnosed approximately twice as frequently as females.

In some cases monoclonal gammopathy is a result of an underlying malignancy known as plasma cell dyscrasia. This type is usually associated with multiple myeloma (MM), amyloidosis (AL), Waldenström's macroglobulinemia (WM) or solitary plasmacytoma. In other cases it is a result of a benign clonal expansion with no symptoms. This type is referred to as monoclonal gammopathy of undetermined significance (MGUS) [4]. It has been reported that MGUS, MM and amyloidosis AL are the most common monoclonal gammopathies [5,6].

MM accounts for a small percentage of all cancers, however the costs associated with myeloma are among the highest. In addition, it has been estimated that among the 1,437,180 of new cancer cases projected for 2008, approximately 19,920 individuals will be diagnosed with MM. The median age at diagnosis is 70 years, and myeloma occurs in males (7 per 100,000) at a rate 56% higher than females (4.5 per 100,000). The highest prevalence rate occurs in African-Americans, especially aged 80–84 years and older [7]. There has been a significant improvement in the overall 5-year survival in patients with MM since the 1960s: 12% from 1960 to 1963 for whites to 34% from 1996 to 2003 for all races. Approximately 10,790 deaths from myeloma are anticipated this year [8].

The signs and symptoms of MM are nonspecific, and sometimes, the diagnosis is suspected due to abnormal laboratory tests alone. Asymptomatic or minimal symptoms but abnormal laboratory tests

* Corresponding author. Division of Clinical Chemistry, Department of Pathology and Lab Medicine, National Guard Health Affairs, King Saud Bin Abdulaziz University for Health Sciences, PO Box 22490, Riyadh 11426, Saudi Arabia. Fax: +966 1 2520130.

E-mail address: tamimiw@ngha.med.sa (W. Tamimi).

were reported as around 11% by Kyle [9]. Finding a monoclonal immunoglobulin in the blood might be the first step in making the diagnosis of MM. Hyperproteinemia was first demonstrated in MM in 1928 by Perlzweig et al. [10].

To detect monoclonal proteins a reliable sensitive method needs to be applied. Tiselius and Kabat [11] initially demonstrated antibody activity in the gamma globulin fraction using the moving-boundary method of electrophoresis. The moving-boundary electrophoresis apparatus, using a U-tube, became commercially available, but it was very cumbersome. In 1951, filter papers as a support permitted the separation of protein into discrete zones, which could be stained with a variety of dyes [12]. Later on immunoelectrophoresis was described by Grabar and Williams in 1953 [13]. Eleven years later, immunofixation was introduced by Wilson [14]. Nowadays serum and/or urine protein electrophoresis using agarose gel is classically performed in most laboratories to detect the M-proteins in the samples and this can be confirmed by immunofixation.

A critical milestone was the concept of monoclonal versus polyclonal gammopathies presented in the Harvey Lecture Series by Jan Waldenström in 1961 [15]. He described patients with a narrow band of hypergammaglobulinemia on electrophoresis as having a monoclonal protein. Many of these patients had MM or macroglobulinemia, but others had no evidence of malignancy and he considered them to have “essential hypergammaglobulinemia” or a “benign monoclonal protein.” Today, the preferred term is MGUS, because MM, macroglobulinemia, light chain amyloidosis AL, or a related disorder may subsequently develop [16]. Waldenström regarded the broad band in hypergammaglobulinemia as a polyclonal increase in proteins. A distinction between monoclonal versus polyclonal gammopathies is important because patients with a monoclonal gammopathy either have a neoplastic process or may develop a malignancy, whereas patients with a polyclonal gammopathy have an inflammatory or reactive process [16].

Methodology

Patients

The records of 6624 patients in whom either serum and/or urine protein electrophoresis were performed at the King Fahad National Guard Hospital, Riyadh, Saudi Arabia, from January 1, 1996, to December 31, 2006, were reviewed.

The diagnosis of MM was based on the following findings: (1) increased numbers of abnormal, atypical, or immature plasma cells in the bone marrow or histological proof of plasmacytoma; (2) presence of an M-protein in the serum or urine; or (3) bone lesions consistent with those of MM.

Patients with MGUS, smoldering MM, solitary plasmacytoma, and plasma cell leukemia were also included. Patients with primary amyloidosis AL were included only if features of MM predominated.

Initial values were derived from laboratory tests performed within 1 month of the diagnosis of MM. Radiographs and other test results were also obtained and evaluated. The MM patients studied fulfilled the South-West Oncology Group (SWOG) and International Myeloma Working Group (IMWG) criteria for MM diagnosis [17,18]. All patient demographical data were recorded and evaluated.

Samples

Blood samples were collected using vacutainer gel separator tubes (Becton Dickson, USA) and left to clot for 15–30 min at room temperature before centrifugation at 3000 RPM for 10 min. Primary sample tubes were transferred immediately to the chemistry and immunonephelometer analyzers to determine the total protein and immunoglobulin levels respectively. Serum samples were then separated in secondary plastic tubes and stored frozen (–20 °C) for future electrophoresis testing.

Urine samples were collected for 24 h by patients and delivered to the laboratory where the total volume was measured. Samples were then delivered to the chemistry laboratory in which total protein electrophoresis was performed by the same analyzers described for serum samples.

Experimental

Protein electrophoresis (PE) using Beckman (USA) agarose gel was performed on all samples, which was then treated with acid blue stain to visualize proteins and scanned with a Beckman apprise densitometer. Each gel contained 10 lanes wherein which 8 samples and two normal and abnormal controls were included in each run.

Immunofixation (IFE) method using gel and anti sera fixation samples from Beckman (USA) was carried out on all suspected samples and also on those samples from controls that had an indistinct band on protein electrophoresis. Every IFE gel contained 6 lanes which were each occupied by one patient sample. Hospital medical records were reviewed for all patients regarding diagnosis, paraprotein type and date of diagnosis.

The relative amount of each protein fraction was determined by multiplying the relative amount by the total serum protein concentration. Total protein was measured on a clinical chemistry automated analyzer, by the use of a colorimetric Biuret assay. Quantization of immunoglobulin IgA, IgG, and IgM was performed on Dade Behring BNII immunonephelometer.

Results

The flowchart in Fig. 1 shows that out of 6624 patients, homogenous bands were found in 7% ($n=468$) of the patients. IFE method, confirmed that 6.3% ($n=416$) of the patients have a monoclonal gammopathy. Among the negative electrophoresis results which equals 93% ($n=6156$), only 0.7% ($n=41$) cases were confirmed positive for MM. The sensitivity and specificity of gel electrophoresis in the studied population were found to be 91% and 99% respectively.

Fig. 1 also shows the different types of the monoclonal immunoglobulins observed. They were identified as 41% ($n=171$) IgG kappa, 19% ($n=79$) IgG lambda, 7% ($n=29$) IgM kappa, 3% ($n=13$) IgM lambda, 2.6% ($n=11$) IgA kappa, 2.9% ($n=12$) IgA lambda, 14.7% ($n=61$) free light chain kappa and 9.6% ($n=40$) free light chain lambda.

By reviewing other tests, radiological, and bone marrow reports, the differential diagnosis of these monoclonal gammopathy cases has revealed that 68% of these cases were MGUS, 14.6% MM, 6.5% lymphoma, 4% WM, 2.1% chronic lymphocytic leukemia and 4.8% of unknown causes which could be of IgD or mixed cases.

Bi-colonel bands were observed in the blood of twenty-eight (6.7%) cases. Twenty-five were identified as two heavy chains with their corresponding light chains and the other three were only isolated heavy chains and separate free light chains. Three cases were MM and four were lymphoma, five WM, and sixteen MGUS. There were also four cases identified as having tri-colonel antibodies and all were MM.

Some patients have developed or complicated one or more secondary conditions for instance, 75 cases were associated with liver diseases, 45 lung diseases, 45 renal diseases, 48 metabolic diseases, 41 cardiac diseases, and 33 infectious diseases.

Table 1 shows the age and gender distribution among MM patients. There were 245 (59%) males and 171 (41%) females. The age ranged from 9 to 100 years with a mean of 66.5 for males and from 11 to 116 years with a mean of 64.7 for females.

Discussion

Monoclonal gammopathies can be an indicative of several diseases that are difficult to diagnose due to the involvement of several tissues

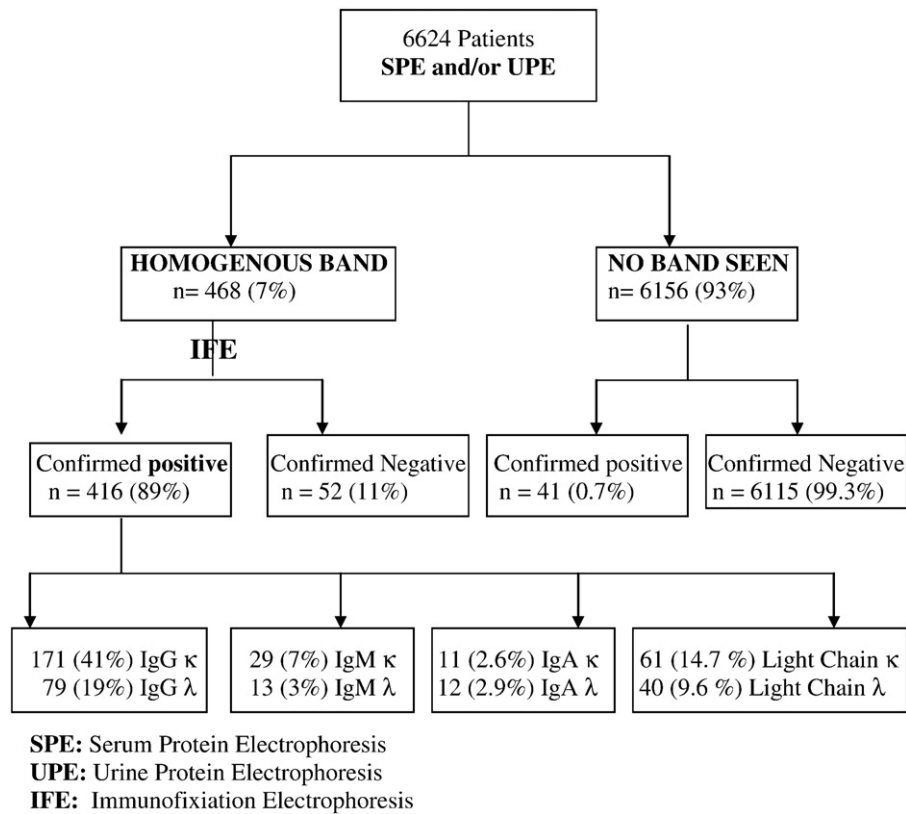


Fig. 1. Flowchart of immunoglobulin proteins by SPE and UPE followed by IFE.

and the nonspecific symptoms. Therefore identifying immunoglobulin proteins in the serum or urine can be very helpful to assess in the diagnosis of a disease such as MM and amyloidosis AL.

MM represents 1% of all cancers diagnosed in the United States and 10% of all hematological malignancies. The median age at diagnosis of MM is 62 years. Only 2% to 3% of the cases were reported in patients younger than 30 years [19]. The prevalence of monoclonal gammopathy in the geriatric population is as high as 8% [20]. Kyle R.A. et al., have retrospectively reviewed the records of 1027 patients in whom MM was initially diagnosed at the Mayo Clinic in Rochester, Minn, USA from January 1, 1985, to December 31, 1998. Their results have showed that 2% of the patients were younger than 40 years, and 38% were 70 years or older. The median age was 66 years [21]. They also reported that of these 1027 patients, 59% were males.

In another study of the Iowa Surveillance, Epidemiology, and End-Results Registry (SEERR) [22], 1% of 4041 patients with MM from 1973

to 1998 were younger than 40 years. Other studies, have found that the average patient age at diagnosis was 68 years, and 99% of patients diagnosed were older than 40 years [23,24]. These data agree with our finding in which 35% of our myeloma patients are above the age of 70 years, but contrarily indicate that we may have a higher prevalence (15%) of myeloma in patients younger than 40 years; however, this cannot be confirmed without conducting a large-scale study. In the two previous studies, investigators have found that the median age for both females and males was the same and this also agrees well with our data.

Noboru H. et al. investigated M-proteins detected by electrophoresis in 12,531 Japanese patients between 1995 and 2000 [25]. They have detected M-proteins in 0.8% ($n=106$) of the patients with a mean age of 69.1 years. There was no significant difference between the frequency of males and females. Their findings suggest that the frequency of detecting M-protein increases with advanced age, and the ratio of MM in the diagnosis of M-proteins is high. In our study, we have shown a higher incidence of monoclonal proteins in our patients compared to that of Noboru H. et al.'s study. This might be due to the different types of population used in the study, as in our study we used a hospital-based population whereas in Noboru H. et al.'s study they used a community-based population.

In a study by Kyle et al. M-protein was detected in the serum of 93% of their patients, which is slightly higher than our finding (89%). They also found that 63% of their patients had a kappa M-protein, and 37% had a lambda M-protein [26], which is similar to our data of 65%, and 35% respectively. They also identified the type of monoclonal proteins as 34% IgG kappa, 18% IgG lambda, 0.3% IgM kappa, 0.2% IgM lambda, 13% IgA kappa, 8% IgA lambda, 9% free light chain kappa and 7% free light chain lambda. They have also identified 2% of bi-colonels in their population. These data are slightly similar to our IgG data but lower compared to our IgM and higher compared to our IgA.

Table 1

The age and gender distribution among patients with myeloma between 1996 and 2006.

Age	No. of males (%)	No. of females (%)
<20	3 (1.2)	3 (1.8)
20–29	12 (4.9)	9 (5.3)
30–39	18 (4.3)	16 (9.4)
40–49	26 (10.6)	21 (12.3)
50–59	47 (19.2)	29 (17.0)
60–69	51 (20.8)	39 (22.8)
70–79	36 (14.7)	23 (13.5)
80–89	29 (11.9)	15 (8.8)
90–99	21 (8.6)	15 (8.8)
>99	2 (0.8)	1 (0.6)
Total	245 (59%)	171 (41%)

In a study by Bradwell, it was found that more than 50% of their patients had IgG protein, which is similar to our data, and 20% had IgA proteins, which is more compared to the data in our population. They also revealed that the monoclonal light chain comprises up to 15% of all the new cases of MM [27].

Previous studies have indicated that MGUS is less prevalent in Asian countries than in Western countries [28,29]. In our studied population, we found a prevalence of 4.3% for MGUS. Studies comparing hospital-based studies to population-based studies show higher prevalence of MGUS in hospital-based population with more than 5% of these patients older than 70 years [30,31]. The most accurate population-based study indicated that the prevalence of MGUS was 3.2% among 50 years or older people [27]. Racial differences were also reported in some articles [32–35].

In a study of inpatients from US Veterans Affairs hospitals, the age-adjusted prevalence rate for MGUS was 3 times higher among African-Americans [33]. A previous small-scale study reported that MGUS was less prevalent among elderly Japanese than among Americans [34]. Moreover, in a study on 52,781 people, MGUS was identified in 1088. The overall prevalence was 2.1%. Among those individuals 2.4% were 50 years or older. The prevalence was significantly higher in men than in women (2.8% versus 1.6%; $p < 0.001$). The heavy chain isotypes were IgG in 73.6% of the patients, IgA in 17.7%, IgM in 7.5%, and oligoclonal gammopathies in 1.1%. They concluded that the prevalence of MGUS is lower in the Japanese population than that reported in Western countries among people older than 60 years, especially among women [36].

Based on our observation, the most common immunoglobulin found in our patients with MGUS was IgG (58%). This value is slightly lower compared to what is reported in the American studied population (68.9%) [37] and in the French studied population (68%) [38]. In contrast, IgM was found to be 8% in our MGUS patients. This value is similar to what is found in the Swedish studied population (8%) [39] but is lower than what was found in the American studied population (17%) [37] and French studied population (24%) [38]. The reason for this difference is poorly understood.

The exact etiology of MM remains unclear. Occasionally it is identified within members of the same family, although no evidence suggests a hereditary basis for this disease [40]. The risk of developing MM appears to be higher in populations of lower socioeconomic status, particularly where diagnostic services are unavailable [41]. In addition, operating within nuclear industry, agriculture, or where workers are exposed to wood dust increase the risk of developing MM [42].

We conclude that the prevalence of monoclonal gammopathies is found to be 6.3% among clinically suspected population in this institute. The prevalence in younger population is higher compared to other countries; however, a large-scale data needs to be collected to confirm this hypothesis. The most common monoclonal immunoglobulin observed in our studied population was IgG which concurs with other ethnic groups. The sensitivity and specificity of the agarose gel electrophoresis is diagnostically and reasonably acceptable.

Acknowledgments

We would like to thank the staff of the chemistry laboratory of King Fahad National Guard Hospital for their technical support. We would like also to thank King Abdullah International Medical Research Center (KAIMRC), King Saud bin Abdulaziz University for Health Sciences (KSAU-HS) for their editing support of the manuscript. Authors would also like to thank Dr. Abdulkareem Almomen, Clinical hematologist for reviewing this manuscript. Part of this work was presented as a poster in the Euromedlab, Innsbruck, Clinical Chemistry and Laboratory Medicine Vol. 47 Special Supplement, Poster # M-A195, 2009.

References

- [1] Attalmanan Mohammed, Levinson Stanley S. Understanding and identifying monoclonal gammopathies. *Clin Chem* 2000;46:1230–8.
- [2] Bradwell AR. Serum free light chain analysis. 4th ed. Birmingham: The Binding Site; 2006. p. 12–21.
- [3] Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer Statistics 2003. *CA Cancer J Clin* 2003;53:5–26.
- [4] Keren DF, Alexanian R, Goeken JA, Gorevic PD, Kyle RA, Tomar RH. Guidelines for clinical and laboratory evaluation of patients with monoclonal gammopathies. *Arch Pathol Lab Med* 1999;123:106–7.
- [5] Katzmann JA. *ClinLab News*. June 2006.
- [6] Katzmann, et al. *Clin Chem* 2005;51:878–81.
- [7] Kyle RA. Sequence of testing for monoclonal gammopathies. *Arch Pathol Lab Med* 1999;123:114–8.
- [8] Cook Richard. Economic and clinical impact of multiple myeloma to managed care. *J Manag Care Pharm* 2008;14(7)(suppl S):S19–S25.
- [9] Kyle RA. *Mayo Clin Proc* 2003;78:21–33.
- [10] Perlzweig WA, Delrue G, Geschichter C. Hyperproteinemia associated with multiple myelomas: report of an unusual case. *JAMA* 1928;90:755–7.
- [11] Tiselius A, Kabat EA. Electrophoretic study of immune sera and purified antibody preparation. *J Exp Med* 1939;69:119–31.
- [12] Kunkel HG, Tiselius A. Electrophoresis of proteins on filter paper. *J Gen Physiol* 1951;35:89–118.
- [13] Grabar P, Williams CA. Methode permettant l'etude conjuguee des proprietes electrophoretiques et immunochimiques d'un melange de proteines; application au serum sanguin. *Biochim Biophys Acta* 1953;10:193–4.
- [14] Wilson AT. Direct immunoelectrophoresis. *J Immunol* 1964;92:431–4.
- [15] Waldenström J. Studies on conditions associated with disturbed gamma globulin formation (gammopathies). *Harvey Lect* 1961;56:211–31.
- [16] Kyle RA. Monoclonal gammopathy of undetermined significance: natural history in 241 cases. *Am J Med* 1978;64:814–26.
- [17] International myeloma working group criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Hematol* 2003;121:749–57.
- [18] Jacobson JL, Hussein MA, Barlogie B, Durie BGM, Crowley JJ. A new staging system for multiple myeloma patients based on the Southwest Oncology Group experience. *Brit J Hematol* 2003;122:441–50.
- [19] Ishida T, Dorfman HD. Plasma cell myeloma in unusually young patients. *Skeletal Radiol* 1995;24:47–51.
- [20] Boccadoro M, Pileri A. Diagnosis, prognosis, and standard treatment of multiple myeloma. *Hematol Oncol Clin North Am* 1997;11:111–31.
- [21] Kyle RA, Morie AG, Thomas EW, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, Plevak ME, Therneau TM, Greipp PR. Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc* 2003;78:21–33.
- [22] Iowa Surveillance, Epidemiology, and End-Results SEER Registry. 1973–1998.
- [23] Hideshima T, Mitsiades C, Tonon G, Richardson PG, Anderson KC. Understanding multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. *Nat Rev Cancer* 2007;7:585–98.
- [24] Schwartzberg L. Managed care considerations in the treatment of multiple myeloma. *Managed Care Oncol Spring* 2007;3(14–16):47.
- [25] Noboru H, Sachiko H, Junko T, Kumiko N, Toshio S, Minoru T, Hiroya H. Total monoclonal gammopathy detected by serum protein electrophoresis for last five years. *Jpn J Med Technol* 2001;50(8):1037–41.
- [26] Kyle RA, Morie AG, Thomas EW, Lust JA, Lacy MQ, Dispenzieri A, Fonseca R, Rajkumar SV, Offord JR, Larson DR, Plevak ME, Therneau TM, Greipp PR. Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc* 2003;78:21–33.
- [27] Bradwell A. Serum free light chain analysis 4th ed. Birmingham, UK: The Binding Site Ltd.; 2006.
- [28] Bergsagel D. The incidence and epidemiology of plasma cell neoplasms. *Stem Cells* 1995;13(Suppl 2):1–9.
- [29] Morgan GJ, Davies FE, Linet M. Myeloma aetiology and epidemiology. *Biomed Pharmacother* 2002;56(5):223–34.
- [30] Kyle RA. Monoclonal gammopathy of undetermined significance (MGUS). *Baillieres Clin Haematol* 1995;8(1):761–81.
- [31] Anagnostopoulos A, Evangelopoulou A, Sotou D, Gika D, Mitsibounas D, Dimopoulos MA. Incidence and evolution of monoclonal gammopathy of undetermined significance (MGUS) in Greece. *Ann Hematol* Jul 2002;81(7):357–61 [Electronic publication 2002 Jul 11].
- [32] Cohen HJ, Crawford J, Rao MK, Pieper CF, Currie MS. Racial differences in the prevalence of monoclonal gammopathy in a community-based sample of the elderly [published correction appears in *Am J Med*. 1998;105(4):362]. *Am J Med* 1998;104(5):439–44.
- [33] Landgren O, Gridley G, Turesson I, Caporaso NE, et al. Risk of monoclonal gammopathy of undetermined significance (MGUS) and subsequent multiple myeloma among African American and white veterans in the United States. *Blood* Feb 1 2006;107(3):904–6 [Electronic publication 2005 Oct 6].
- [34] Bowden M, Crawford J, Cohen HJ, Noyama O. A comparative study of monoclonal gammopathies and immunoglobulin levels in Japanese and United States elderly [published correction appears in *J Am Geriatr Soc*. 1993;41(6):622]. *J Am Geriatr Soc* 1993;41(6):11–4.
- [35] Bergsagel D. The incidence and epidemiology of plasma cell neoplasms. *Stem Cells* 1995;13(Suppl 2):1–9.
- [36] Masako Iwanaga, Masuko Tagawa, Kunihiro Tsukasaki, Shimera Kamihira, Masao Tomonaga. Prevalence of monoclonal gammopathy of undetermined significance: study of 52,802 persons in Nagasaki City, Japan. *Mayo Clin Proc* 2007;82(12):1474–9.

- [37] Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med* 2006;354(13):1362–9.
- [38] Saleun JP, Vicariot M, Deroff P, Morin JF. Monoclonal gammopathies in the adult population of Finistere, France. *J Clin Pathol* 1982;35(1):63–8.
- [39] Axelsson U, Bachmann R, Hallen J. Frequency of pathological proteins (M-components) in 6,995 sera from an adult population. *Acta Med Scand* 1966;179(2):235–47.
- [40] George ED, Sadosky R. Multiple myeloma: recognition and management. *Am Fam Physician* April 1 1999.
- [41] Koessel SL, Theis MK, Vaughan TL, Koepsell TD, Weiss NS, Greenberg RS, et al. Socioeconomic status and the incidence of multiple myeloma. *Epidemiology* 1996;7:4–8.
- [42] Fritschi L, Siemiatycki J. Lymphoma, myeloma and occupation. *Int J Cancer* 1996;67:498–503.