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INCREASED MONOCLONAL COMPONENTS: PREVALENCE IN AN ITALIAN POPULATION OF 44 474 OUTPATIENTS DETECTED BY CAPILLARY ELECTROPHORESIS

POVIŠENE MONOKLONSKE KOMPONENTE: PREVALENCIJA U SKUPINI 44.474 ITALIJANSKIH NEHOSPITALIZOVANIH ISPITANIKA DETEKTOVANA KAPILARNOM ELEKTROFOREZOM

Arialdo Vernocchi[1], Ermanno Longhi[1], Giuseppe Lippi[2], Silvia Gelsumini[3]

[1]Laboratory Medicine IRCCS »MultiMedica«, Milan, Italy
[2]Laboratory of Clinical Chemistry and Hematology, Academic Hospital of Parma, Parma, Italy
[3]Laboratory Medicine, University Hospital »Ospedale di Circolo e Fondazione Macchi«, Varese, Italy

Summary

Background: Identification, quantification and typing of M-Proteins (MP) play an important role in the diagnosis, classification and monitoring of monoclonal gammopathies both of malignant origin (eg. Multiple Myeloma) and of unknown origin. Previous evidence attests that MGUS (Monoclonal Gammopathy of Undetermined Significance) detected by agarose gel electrophoresis has a prevalence of 3.2% in the general population. Therefore, our study aimed to verify this data by means of capillary zone electrophoresis (CZE).

Methods: CZE was performed to evaluate the prevalence of M-Protein (MP) in 44.474 consecutive outpatients of all ages with a prescription for serum protein electrophoresis over a 2-year period (2008 and 2009). All MPs that were identified were then typed by immunofixation electrophoresis on agarose gel (IFE).

Results: In subjects aged over 50 (23.408, i.e., 52.6% of the whole cohort) MP ≤30 g/L (MGUS) was identified in 6.0% of cases, with a frequency nearly double than that previously reported. The population was then divided into ten-year age groups: the 71–80 age group had the highest percentage of MP (29%), followed by 61–70 (27%), 51–60 (18%), 81–90 (12%), 41–50 (8%), 31–40 (3%), >90 (2%) and <30 (1%). The frequency of MP types (IFE) was the same in each age group, with IgG Kappa being the most represented class.

Kratak sadržaj

Uvod: Identifikacija, kvantifikacija i tipizacija M-proteina (MP) imaju važnu ulogu u dijagnostikovanju, klasifikovanju i praćenju monoklonskih gamapatija kako malignog (npr. multipli mijelom) tako i nepoznatog porekla. Prethodni dokazi pokazuju da monoklonska gamapatija neodređenog značaja (MGNZ) koja se otkriva elektroforezom na agaroznom gelu ima prevalenciju od 3,2% u opštoj populaciji. Stoga je cilj ove studije bio da verifikuje navedene podatke putem elektroforeze kapilarne zone.

Metode: Kapilarna elektroforeza je izvršena da bi se utvrdila prevalencija M-proteina (MP) kod 44.474 uzastopnih kliničkih pacijenata svih uzrasta kod kojih je u toku dve godine (2008. i 2009) prepisana elektroforeza proteina u serumu. Identifikovani su svi MP a zatim tipizirani imunofiksacionom elektroforezom na agaroznom gelu.

Rezultati: Kod ispitanika starijih od 50 godina (23.408, tj. 52,6% ukupnog broja) MP ≤30 g/L (MGNZ) identifikovan je u 6,0% slučajeva, sa gotovo dva puta većom učestalošću nego što je prethodno procenjeno. Populacija je zatim podeljena na starosne grupe raspona po deset godina: grupa 71–80 imala je najveći procenat MP (29%), a slede grupe 61–70 (27%), 51–60 (18%), 81–90 (12%), 41–50 (8%), 31–40 (3%), > 90 (2%) i < 30 (1%). Učestalost tipova MP (imunofiksaciona elektroforeza na agaroznom gelu) bila je ista u svim starosnim grupama, dok je najzastupljenija klasa bila IgG Kappa.

Address for correspondence:

Silvia Gelsumini
University Hospital »Ospedale di Circolo e Fondazione Macchi«,
Viale Borri 57, 21100, Varese, Italy
E-mail: silvia.gelsumini@ospedale.varese.it
Laboratory phone number: +390332539315;
mobile: +393886118996
Laboratory fax number: +390332539318

List of Abbreviations: SPE, Serum Protein Electrophoresis; MP, M-Protein; MGs, Monoclonal Gammopathies; MGUS, Monoclonal Gammopathy of Undetermined Significance; CRAB, absence of hypercalcaemia, renal failure, anaemia and bone lesions; FLCr K/Λ, Free Light Chain ratio; CZE, capillary zone electrophoresis; IFE, Immunofixation Electrophoresis on agarose gel.

Conclusions: According to the high MGUS prevalence observed in this study, these results may be useful especially for general practitioners, because the identification even of small MP (analytical sensitivity: 0.5 g/L) may help optimize clinical management.

Keywords: MGUS, monoclonal component prevalence, capillary electrophoresis

Zaključak: Na osnovu velike uočene prevalencije MGNZ, zaključujemo da ovi rezultati mogu biti naročito korisni za lekare opšte prakse, pošto identifikacija čak i malih MP (analitička osetljivost: 0,5 g/L) može doprineti optimizaciji kliničkog menadžmenta.

Ključne reči: monoklonska gamapatija neodređenog značaja, prevalencija monoklonske komponente, kapilarna elektroforeza

Introduction

The leading clinical and diagnostic benefit of serum protein electrophoresis (SPE) is its ability to identify the presence of M-Protein (MP), which can be found in the serum either randomly or as the consequence of a clinical suspicion (1). The presence of monoclonal immunoglobulins in the serum and/or in the urine is regarded as a marker of monoclonal gammopathies (MGs), a cluster of pathologies characterized by hyperproliferation of a B lymphocytes clone in the hematopoietic marrow which, in the absence of an appropriate antigenic stimulus, produces a highly homogeneous population of antibodies. The identification, quantification and typing of MPs play an important role in the diagnosis, classification and monitoring of MGs of both malignant (e.g., Multiple Myeloma) and unknown origin. The so-called MGUS (Monoclonal Gammopathy of Undetermined Significance) is characterized by the presence of an MP ≤ 30 g/L in serum, negative CRAB criteria (absence of hypercalcaemia, renal failure, anaemia and bone lesions) and the presence of a percentage of plasma cells in the bone marrow lower than 10%. This laboratory diagnostics is particularly useful not only due to frequent absence of symptoms in MGUS patients, but also for monitoring its potential evolution towards malignancy. The progression from MGUS to Multiple Myeloma is the greatest risk condition in such patients, which supports the need for regular and long-lasting monitoring (2). Indeed, the rate of progression from MGUS to Multiple Myeloma or a related malignant condition approximates 1%/year. Since this percentage does not significantly decrease over time, a patient with MGUS should not only receive a timely diagnosis, but will also need unlimited follow-up. Three leading risk factors are the well-known and recognized conditions predisposing to malignancy, including MP of at least 15 g/L, MP mounting a heavy chain different from IgG, and serum with an abnormal free light chain ratio (FLCr kappa/lambda) of >3.5 (3, 4). Early identification and typing of MGUS is therefore of utmost clinical importance, because no definite proof of whether or not it will evolve towards Myeloma has been provided so far. Recent studies showed that the three predisposing conditions can efficiently predict the risk of progression. The level of the MGUS is the first indicator of progression. Twenty years after a diagnosis of MGUS the risk of malignancy is 58% in patients with all three risk factors, 37% in patients with two risk factors, 21% in patients with a single risk factor and 5% in patients with no risk factors, respectively. Therefore, this classification is an effective approach to identify patients with the highest progression risk towards Multiple Myeloma or a related malignant disease, who would benefit from tailored treatments. Moreover, 20 years after the first identification of MGUS, patients with MP >25 g/L have a progression risk 4.6–fold higher compared to those with MP lower than 5 g/L (2). Patients with IgM MP, and to a greater extent those with IgA MP have a higher progression risk towards Multiple Myeloma than those with IgG MP. The estimation of FLCr has been successfully used in the past few years to assess progression risk, wherein patients with an abnormal FLCr carry a significantly higher risk than those with a normal ratio (2). Therefore, in the absence of tests for differential diagnosis between MGUS and the malignant forms, the most effective strategy is to monitor the evolution of the monoclonal gammopathy over time, along with the above-mentioned factors and the CRAB criteria. Previous evidence attests that MGUS detected by agarose gel electrophoresis has an overall prevalence of 3.2% in the general population (2). Therefore, this study aimed to verify these data by means of capillary zone electrophoresis (CZE).

Materials and Methods

CZE technique was used to estimate the prevalence of monoclonal components in all consecutive outpatients referred to the Department of Laboratory Medicine of the Treviglio Hospital (BG – Italy) over a 2-year period (i.e., between January 2008 and December 2009) with a prescription for serum protein electrophoresis. The analysis of data was carried out in the year 2012, after obtaining approval from the Hospital Ethics Committee, in accord with the Declaration of Helsinki and under the terms of relevant local legislation. The analysis was arbitrarily limited to the outpatient population since the prevalence of MP is greater in hospitalised patients (i.e., the percentage of MGUS can be as high as 6.1% in hospitalised patients aged over 50) (2). Moreover, the presence of MP in hospitalised patients is frequently associated with a variety of disorders. Blood samples were collected according to standard laboratory procedures (ISO 9001:2008 certified and accredited according to the International Joint Commission standards) from fasting patients. Specifically, blood was drawn in primary blood tubes with no separator gel or additives,

centrifuged and processed within 4 hours from collection. Electrophoresis was performed with the CZE technique on the Capillarys 2 (SEBIA). All the MPs were typed by immunofixation electrophoresis on agarose gel (IFE) using the Hydrasys instrument (SEBIA). A database was created for each subject, containing blood sampling date, date of birth, sex, barcode identification, total protein concentration, electrophoresis performed by using the CZE technique, comment on the electrophoretic pattern, MP concentration, MP type, IFE scan, along with values of IgG, IgA and IgM, presence or absence of Bence Jones protein in urine (IFE urine, SEBIA), presence of cryoglobulinemia or rheumatoid factors. The Bence Jones protein was assessed in the second samples of the morning.

Results

Overall, the population cohort consisted of 44,474 outpatients of all ages examined during the 2-year period of the study, 1,606 of whom with the presence of an MP (3.6%; 1.85% males and 1.76% females; median age 69 years). An MP <30 g/L (i.e., MGUS) was detected in 6.0% (95% confidence interval, 5.7–6.3%) of subjects aged over 50 years (23,408/44,474, i.e., 52.6% of the total study population) while the frequency of MP <30 g/L was much lower (i.e., 0.8%) in those aged 50 or younger (21,066, i.e., 47.4% of the total study population). In all subjects in whom MP was detected, the frequency of MGUS was 98.4%, with a median concentration of 4 g/L. The presence of MGUS was identified in 733/10,304 men and 677/13,104 women (7.1% vs. 5.2%; p<0.001) (*Table I*). After stratification of the

Table I MGUS Prevalence (6.0%) according to Age Group and Sex among outpatients referred to the Department of Laboratory Medicine of the Treviglio Hospital (BG – Italy). The percentage was calculated as the number of patients with MGUS divided by the number of those who were tested.

Age	Men	Women	Total
	Number/Total number (percent)		
51–60	145/3540 (4.09)	144/3849 (3.74)	289/7389 (3.91)
61–70	244/3577 (6.82)	190/4008 (4.74)	434/7585 (5.72)
71–80	250/2431 (10.28)	211/3514 (6)	461/5945 (7.75)
81–90	85/702 (12.11)	109/1536 (7.09)	194/2238 (8.67)
>90	9/54 (16.66)	23/197 (11.68)	32/251 (12.75)
Total	733/10304 (7.1)	677/13104 (5.2)	1410/23408 (6.02)

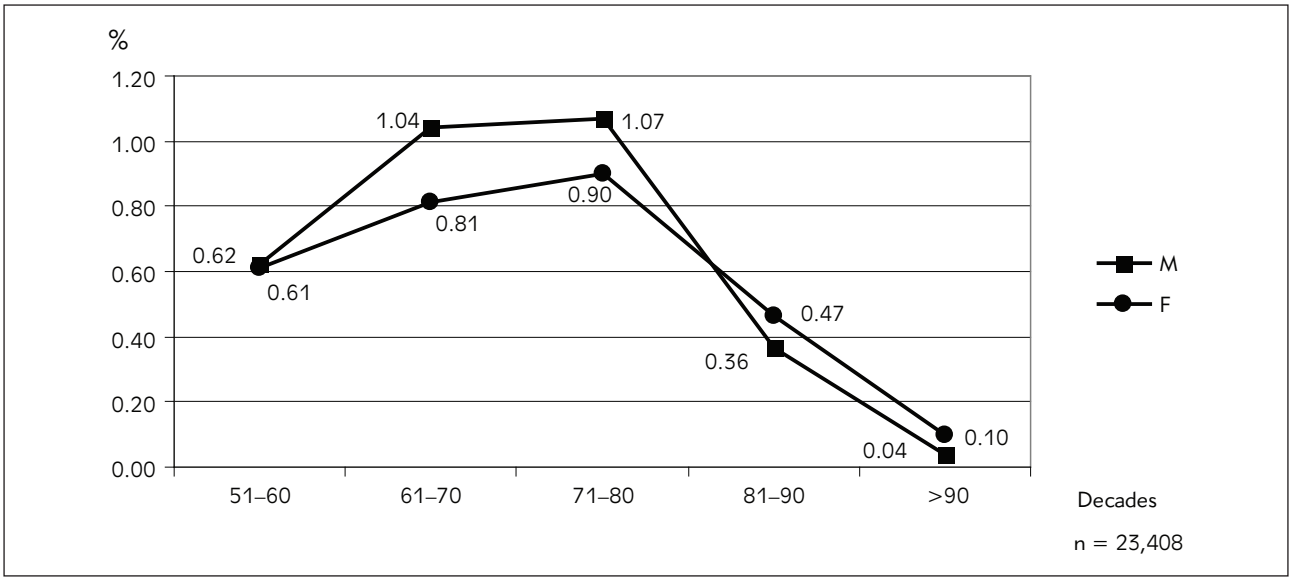

Figure 1 MGUS Prevalence in outpatients population aged over 50 (6.0%).The outpatients have been divided into decades and gender [(Males (M) and Females (F)] and compared to all the outpatients aged over 50.

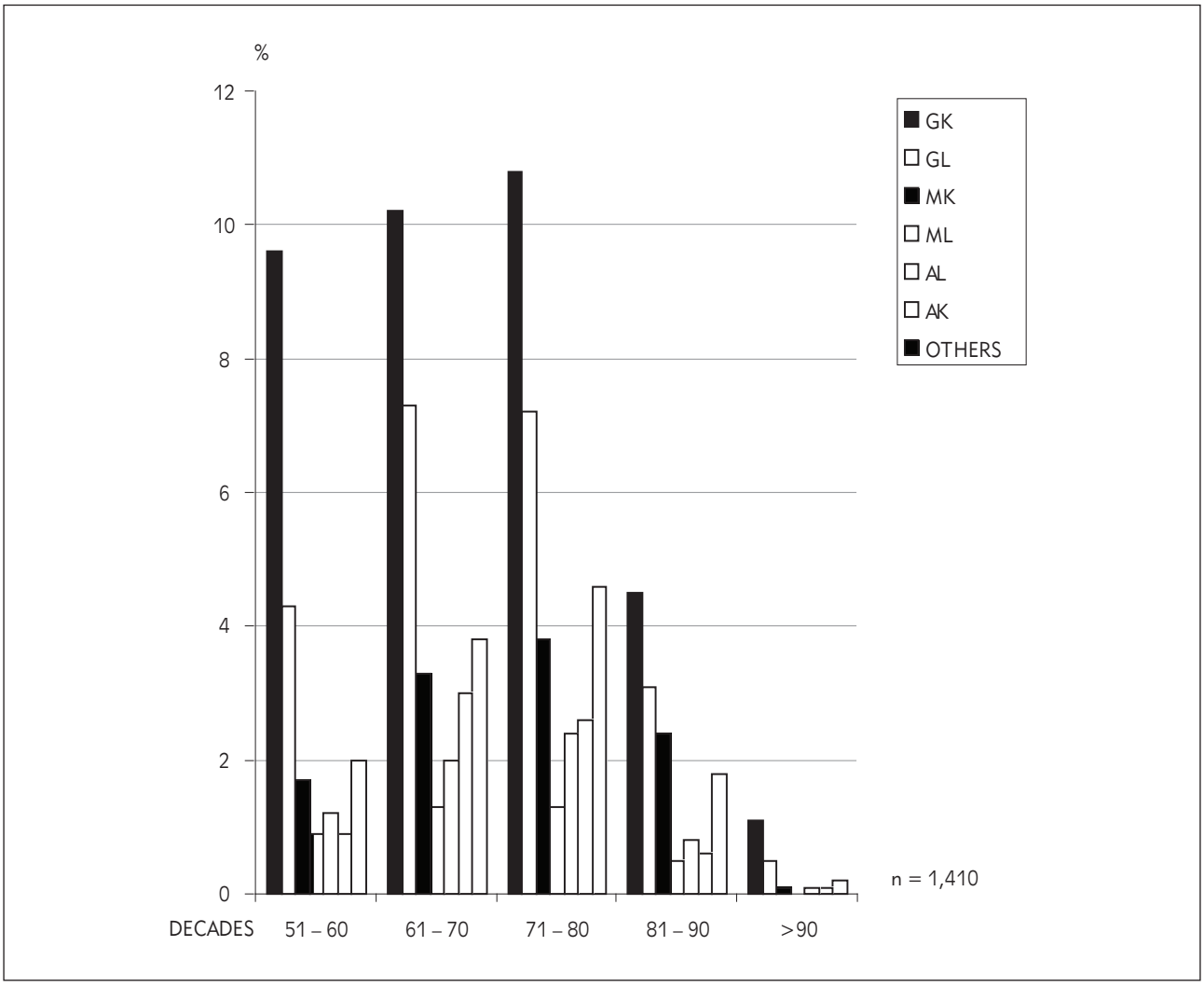

Figure 2 MP typings in MGUS outpatients aged over 50. The MPs have been divided into decades (»Others« include the percentage of Kappa and Lambda free).

entire population into 10-year age groups, the highest frequency of MP was found in the 71–80 age group (29%), followed by the 61–70 group (27%), the 51–60 group (18%), the 81–90 group (12%), the 41–50 group (8%), the 31–40 group (3%), those aged 91 or older (2%) and, finally, those aged 30 or younger (1%). Interestingly, the frequency of MP in each age group was found to be higher in males than in females until the age of 80. After this age, the male to female ratio was inverted, with a greater prevalence in women (*Figure 1*). The frequency of MP types (IFE) was virtually overlapping in each age group, with IgG Kappa being the most frequent class (i.e., IgG Kappa 40.8%, IgG Lambda 25.3%, IgM Kappa 12.9%, IgA Kappa 8.1%, IgA Lambda 7.4%, IgM Lambda 4.5%, Kappa Free 0.5%, Lambda Free 0.5%) (*Figure 2*). The values of MP were found to be similar in each group, but exhibited an incremental trend in parallel with ageing. The Bence Jones protein was measured in 710/1,410 outpatients with MGUS aged 50 or older, and was found to be positive in 228 cases (32%), 153 of whom were positive for Kappa free (67.1%) and 75 for Lambda free (32.9%), respectively.

Discussion

The identification of MPs is very frequent around the globe, and it is now considered as one of the most prevalent conditions in people aged 50 years or older. The available data on the prevalence of MP in the general population were almost solely based on the agarose gel technique, whereas little information has been obtained using more sensitive techniques, such as CZE. In the present study, the frequency of MP in a general population of subjects aged 50 years or older was as high as 6.0%, thus being almost twice that reported by Kyle et al. (5–7). Indeed, this data is probably attributable to the greater sensitivity of the CZE used in our study compared to the agarose gel tech-

nique previously employed by Kyle et al. (2). We have also observed that the prevalence of MGUS in all subjects in whom MP could be identified was as high as 98.4%, thus confirming by means of a highly sensitive technique such as CZE that the incidence of this condition considerably increases with ageing (i.e., the median age of MGUS patients was 69 years in our study population). It is also noteworthy, however, that MGUS could also be identified at earlier ages, since the youngest patient with MGUS was 25 years old. Due to the importance of identifying and monitoring MGUS patients, the results of our population study seemingly attest that the advantage of the higher sensitivity compared to conventional agarose gel electrophoresis would make it the preferable mean to detect and quantify MP, notwithstanding some methodological problems that remain, including an ameliorable analytical variability in total protein measurement and the still unmet accuracy of the peak boundaries positioning (1). Indeed, the laboratory should also provide interpretative comments in the laboratory report, to assist the specialist in patient management. The primary clinical objective is to establish whether or not the serum protein pattern is suggestive of a MP, and then to assess its concentration and biochemical characteristics. It is hence noteworthy that the patient should be followed using an identical method and, preferably, by the same laboratory. The laboratory report should also be informative about the progress of disease, thus including data about complete, almost complete, partial, ongoing or stable remission.

In conclusion, the results of this population study support the concept that routine identification of MP should be carried out in all patients aged 50 years or older by using an analytically sensitive technique, such as the CZE was proven to be (8). In particular, the high prevalence of MGUS found in our investigation provides valuable information to general practitioners, because the identification of even small MP allowed by CZE (i.e., analytical sensitivity as low as 0.5 g/L) may be effective for achieving an early and accurate diagnosis, as well as in the follow-up and clinical management of patients (9–10).

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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