Patterns of monoclonal components and their correlation with different analytical parameters

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From a laboratory perspective, monoclonal immunoglobulins are recognized as bands of restricted migration on serum or urine protein electrophoresis, usually named M-component, M-protein, or M-spike. It is the result of an overproduction of a single clone of plasma cells or B lymphocytes.[1] The condition is commonly seen in patients above 50 years of age.[2] It may be benign as in monoclonal gammopathy of undetermined significance (MGUS), or may reflect a disease process as in multiple myeloma (MM).[1] Excessive production of monoclonal immunoglobulins in MM can cause organ and tissue destruction, mainly bone lesions and subsequent fractures, hypercalcemia, anemia, and renal tissue damage.[3] The increase in M-spike size correlates with tumor burden in the bone marrow (corresponds to an increase in number of plasma cells), and therefore entails a worse prognosis.[4] According to Kyle et al,[1] MGUS accounts for 59% of patients with serum M-protein, MM in 15%, amyloidosis (AL) in 12%, lymphoproliferative disorders in 3%, smoldering multiple myeloma (SMM) in 5%, Waldenstrom's macroglobulinemia (WM) in 2%, and 4% in others. Due to their heterogenous clinical presentation and outcome, monoclonal gammopathies lack clear prognostic factors that predict survival of patients and their treatment response. Male gender, age older than 70 years, percent of plasma cells in bone marrow, serum β_2-microglobulin (β_2M), albumin, calcium, hemoglobin, creatinine, C-reactive protein (CRP), M-protein isotype, labeling index (percentage of malignant cells at the S phase of DNA synthesis), chromosomal abnormalities, presence of plasmablastic subtype, IL-6 and IL-6R are used as prognostic factors.[4]

In this retrospective study, we report the incidence and characteristics of M-components encountered at the clinical laboratory of the American University of Beirut Medical Center (AUBMC) from January 1996 to December 2006 in 540 patients. Primary analysis performed at the time of diagnosis was included for all patients with an M-component. In addition, correlation of different immunoglobulin (Ig) types with creatinine levels and M-spike size were explored. We also investigated the effect of creatinine concentration on β_2M. The M-spike size was used to assess tumor burden.[4]

Agarose gel electrophoresis followed by immunofixation were applied for recognition and identification of an M-protein in serum or urine, using Paragon Electrophoresis (Beckman Coulter Inc, CA, USA), and Sebia Hydrasys (Sebia Electrophoresis, Evry Cedex, France) systems. The study was approved by the Institutional Review Board at AUBMC.

Two levels of statistical analysis were performed in this study using the Statistical Package for Social Sciences version 15 (SPSS Inc, Chicago, IL, USA). The data was summarized using descriptive statistics. Comparisons between categorical data were carried out using the χ^2 test or Fisher exact test. The logistic and Cox regression models were used to assess the relationship between levels of M-spike and various laboratory parameters. A *p*-value of ≤0.05 was considered significant.

On average, 50 new M-component cases (11.8%) were detected out of 422 (range: 373-473) investigated yearly with a median age of 65 years. The occurrence rate of M-components was 4% in individuals younger than 40 years, reached 6.6% at 50-54 years, and almost doubled (14.0%) at 55 years and above. Male preponderance was evident with male/female ratio of 1.24:1. The most common region of appearance in serum electrophoresis (SEP) was the gamma followed by the beta, and rarely the alpha 2 region. On the other hand, SEP was normal in 1.4 % of the cases in which the M-component was detected by immunofixation only. The most common isotype was IgG (59.2%), followed by IgA (15.0%), IgM (11.8%), and IgD (0.5%). Multiple well-defined M-proteins (either of the same or different types) were detected in 8.4% of SEP, and 5.2% of the cases were light chains alone (**Table 1**). The light chain distribution varied among the different immunoglobulin classes. Lambda light chain was encountered in all IgD, in 34% of IgG, in 33.3% of IgM, in 52.5% of IgA, and 61.9% of light chain disease cases (**Table 1**). With respect to the spike size, 23.6% of the cases were <5 g/L or not detected, 38.7% were <10 g/L, and 36.5% were >20 g/L. Of the 219 cases investigated for the presence of urine Bence Jones proteins (BJP), 55.3% were positive. The BJP were present in all light chain disease cases, and varied between 18.8% and 23.2% in the other isotypes. The lowest median age was that of IgD group (46 years), whereas the highest was of the IgM group (72 years) (**Table 1**). While, the male/female ratio varied between 1.22 and 2.13 for the different isotypes, the 2 IgD patients were males. In our study, plasma cells >10% was present in 47.5%, erythrocyte sedimentation rates (ESR) ≥20 mm/hour in 77.8%, ESR ≥50 mm/hour in 51.2%, creatinine ≥106.1 µmol/L (≥1.2 mg/dL)

in 38.5%, and calcium ≥2.63 mmol/L (≥10.5 mg/dL) in 8.1% of the cases. Low serum albumin (≤30 g/L) was noted in 19.5%, and hemoglobin (≤12.0 g/dL) in 61.7%. Furthermore, these findings according to M-protein isotypes are shown in **Table 1**. Using χ^2 test, hemoglobin ≤12.0 g/dL and albumin ≤30 g/L, ESR ≥50 mm/hour and plasma cells ≥10% showed significant correlation with high M-spike size >20 g/L (p=0.000), while gender, creatinine ≥106.1 mmol/L (≥1.2 mg/dL), and calcium ≥2.63 mmol/L (≥10.5 mg/dL) did not. Concerning age, a cutoff value of 55 years did not show a correlation with spike size (p=0.19), while a cutoff of 70 years was significant (p=0.028). Patients with normal creatinine and β_2M level above 4.0 mg/L had higher M-spike levels than those with β_2M under 4.0 mg/L (p=0.016). This correlation was lost when adding patients with creatinine ≥106.1 µmol/L (≥1.2 mg/dL) (p=0.968). Using logistic and Cox regression models albumin ≤30 g/L (odds ratio (OR)=3.67; p=0.004), and ESR ≥50 mm/hour (OR=4.236; p=0.000) were identified as independent indicators of higher M-spike size, and consequently of tumor burden. However, gender (p=0.582) and hemoglobin (p=0.07) failed to show this correlation. Creatinine varied significantly between different isotypes (with an overall p=0.019). Normal creatinine levels dominated in patients with IgA and IgG, whereas levels ≥106.1 µmol/L (≥1.2 mg/dL) were more common in patients with IgD. Light chain and IgM were present almost equally in normal and elevated creatinine groups. The M-spike size varied significantly between different isotypes (with an overall p=0.000). Most of the patients diagnosed to have light chain disease, IgM or IgD had a spike size ≤20 g/L. On the other hand, most of the IgA patients had a spike size >20 g/L. The IgG patients were approximately equally distributed between the 2 groups.

In our study population, the median age at diagnosis of 65 years was comparable to studies from Mayo Clinic and China.[1,2] A slight male preponderance was compatible with some reports.[1,2] In comparison to previously published data, slight variations in isotype distribution were reported. In 962 patients from Mayo Clinic, IgG was detected in 55.5% of cases versus 59.2% in our study; IgA was approximately 10% versus 15.0%; IgM at 20% versus 11.8%; multiple monoclonal bands at 8% versus 8.4%; IgD in <0.5% versus 0.5%; and light chain at 6% versus 5.2% in our patients.[1] Opposite to the normal occurrence, our data showed a lambda/kappa ratio of 1.6:1 that is consistent with other studies.[2] In our setting, protein electrophoresis and immunofixation were only requested, once the disease was clinically suspected, and was not used to screen healthy individuals. The overall laboratory findings at diagnosis showed that a good proportion had evidence of other organ involvement, and only 23.6% of the patients had a spike size <5 g/L. Moreover, the concentration of M-component was <10 g/L in 38.7% of cases, and >20 g/L in 36.5%; whereas when 21,463 residents of Olmsted County, Minnesota were screened for an M-protein, 63.5% (of 694 cases detected) had a spike <10 g/L.[5] Usually, MGUS accounts for most (approximately two-thirds) of monoclonal gammopathies found, with approximately 20 times greater prevalence than MM,[1] increasing incidence with age (3.2% at over 50 years, and 5.3% at over 70 years),[5] and an overall incidence of progression to MM of 1% per year.[1] The risk of progression continues even after 25 years of a stable M-protein.[1] The M-protein spike size (15 g/L), number of plasma cells in the bone marrow, abnormal serum free light-chain ratio, and non-IgG isotype are risk factors for progression. It is worthy to note that in 3% of cases, the abnormal plasma cell does not secrete an

Table 1 - General characteristics of different M-protein isotypes.

Plasma protein	Light chain	IgA	IgG	IgM	IgD	Multiple monoclonal
			(%)			
Incidence	(5.2)	(15.0)	(59.2)	(11.8)	(0.5)	(8.4)
(K)/(λ)/(K and λ)	(38.1)/(61.9)/(0)	(47.5)/(52.5)/(0)	(66.0)/(34.0)/(0)	(66.7)/(33.3)/(0)	(0)/(100)/(0)	(36.4)/(18.2)/(45.5)
Bence Jones proteins	(100.0)	(19.7)	(23.2)	(18.8)	-	(20.6)
Median age, years	61	65	64	72	46	62
Male/female ratio	1.63	2.13	1.35	1.22	100% male	1.56
Male	(6.1)	(18.8)	(58.2)	(10.8)	(0.9)	(5.2)
Female	(5.5)	(13.1)	(63.5)	(13.1)	(0.0)	(4.8)
Hemoglobin ≤12.0 g/dL	(4.7)	(16.0)	(60.4)	(10.4)	(7.6)	(0.9)
Creatinine ≥106.1 µmol/L	(8.2)	(15.3)	(52.0)	(18.4)	(2.0)	(4.1)
Calcium ≥2.63 mmol/L	(14.3)	(33.3)	(47.6)	(0.0)	(0.0)	(4.8)
ESR ≥50 mm/hour	(3.0)	(25.4)	(53.6)	(7.5)	(1.5)	(9.0)

Ig - immunoglobulin, K - Kappa, λ - lambda, ESR - erythrocyte sedimentation rate

M-component, and can only be detected by abnormal free light chain ratio.[3]

In our patients, tumor burden is reflected by the direct association between increased M-spike size and %plasma cells (p=0.000). Chombart et al[4] showed that gender, age, serum albumin, serum creatinine, CRP, hemoglobin and β_2M were factors associated with overall survival in a univariate analysis; only β_2M and CRP were independent factors affecting the overall survival in a multivariate analysis. Our data showed that %plasma cells, β_2M, ESR, albumin, and hemoglobin were related to spike size/tumor burden in monoclonal gammopathies at diagnosis in concordance with Chombart et al[4] using the univariate analysis. In contrast, gender and age (cutoff of 55 years) were not related to spike size, and only the age with a cutoff of 70 years or above showed a significant association. The multivariate analysis showed that only serum albumin and ESR were independent factors of tumor burden. It is important to mention here that significance of β_2M was lost once creatinine level exceeded 106.1 µmol/L (1.2 mg/dL). This is possibly due to the fact that an increase in β_2M in patients with normal creatinine level is an indicator of tumor burden, but in patients with kidney disease, β_2M increases because of decreased clearance.[4] Furthermore, although hemoglobin was significantly associated with spike size in the univariate model, its effect was lost when taking into account other factors (albumin and ESR) possibly because hemoglobin was a confounding factor for these other parameters. Moreover, approximately 34% of patients diagnosed later to have MM are asymptomatic at presentation with incidental abnormalities in total protein, creatinine, or hemoglobin.[3]

When comparing the immunoglobulin isotypes versus the M-spike size, most patients with light chain disease, IgM or IgD were more likely to have an M-spike ≤20 g/L, thus indicating less end organ damage than for the other isotypes (p=0.000). This contradicts our finding in patients with IgD who were more likely to have renal failure than the others (p=0.019). Delayed detection of these cases at a stage when organ damage had already occurred,[6] is a possible explanation, in contrast with other isotypes (mainly IgA and IgG) with normal serum creatinine at diagnosis. Additionally, the light chain type (kappa or lambda) did not confer any significant difference in renal failure (p=0.193). Patients with IgD type accounted to less than 2% of all myeloma cases.[6] Reviewing our data for the last 10 years, only 2 patients were of this type (0.5% of cases). These patients were diagnosed at a younger age (44 and 48 years) with renal damage, smaller M-spike size, and lambda light chain. This is consistent with findings from other reported cases, where most of the patients were younger, had aggressive clinical course, renal impairment, a smaller M-spike size, and lambda light chain.[6]

One limitation of our study is the fact that we only included the laboratory data of the M-component cases without their clinical diagnosis. In addition, β_2M and %plasma cells were not included in the multivariate analysis due to the limited number of patients. Many factors affecting prognosis were not also assessed due to the retrospective nature of the study.

In conclusion, in patients with an M-component seen at AUBMC, 23.6% had a spike size <5 g/L and 36.5% had a spike size >20 g/L with evidence of other organs involvement. The observed frequency reflected the local pattern of patients admissions and diagnostics at AUBMC. Recommendation for screening the general population remains to be addressed in a community-based study where the actual incidence of M-proteins is determined. Other than the high percentage of patients with increased M-spike size at diagnosis, our data, coming from a developing country is comparable to those originating from developed countries. Moreover, some routine laboratory parameters mentioned above are important early indicators of tumor burden in conditions involving monoclonal gammopathies. In summary, given the natural history of the disease, earlier diagnosis at the fifth decade of life with regular follow up could improve patient survival, and be more cost-effective and simple.

Received 8th September 2010. Accepted 27th December 2010.

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