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Poor performance of the total kappa/lambda light chain quantification in the diagnosis and follow-up of patients with multiple myeloma

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ABSTRACT

Background. The gold standard for paraproteinemia screening in plasma cell disorders has been serum protein electrophoresis (SPE) with immunofixation electrophoresis (IFx); serum total and free light chain quantifications have also been used. **Objective.** To define the role of SPE, IFx and serum total light chain (sLC) determinations in patients with multiple myeloma (MM), both at diagnosis and at maximum response during treatment follow-up. **Material and methods.** These serological studies were performed in a group of 62 patients with MM at diagnosis, and in a subset of 29 patients at the point of maximum response to treatment. **Results.** At diagnosis, we found an abnormal SPE in 58%, an abnormal IFx in 92% and an abnormal sLC in 45% of the 62 patients; 64% had simultaneously abnormal results in all three serological studies. IFx alone proved to be the most sensitive of all three assays, followed by SPE, which was redundant in most instances with sLC and IFx. At maximum response, the abnormal SPE normalized in 7 cases, the abnormal IFx in 7 cases and the abnormal sLC in 7 cases. There were 12 instances in which an abnormal IFx was found despite normal sLC, and one case in which a normal IFx was found in the presence of abnormal sLC. The association between IFx and sLC was highly significant ($r = 0.9274611$, $p < 0.000001$), despite instances where a positive result for IFx was associated to a normal sLC. **Conclusions.** All three serological methods should ideally be simultaneously performed in patients with MM both at diagnosis and throughout therapy. In this series, the total sLC assay was not more sensitive than IFx neither at diagnosis nor during follow-up.

Key words. Multiple myeloma. Electrophoresis. Immunofixation. Light chain.

Capacidad insuficiente de la cuantificación de cadenas ligeras totales en el diagnóstico y seguimiento de pacientes con mieloma múltiple

RESUMEN

Antecedentes. *Los estándares de oro para identificar la paraproteinemia en padecimientos inmunoproliferativos malignos son la electroforesis de proteínas séricas (EPS) y la inmunofijación (IF); la cuantificación de cadenas ligeras totales y libres también se ha empleado.* ***Objetivo.*** *Definir el papel de la EPS, de la IF y de la cuantificación de cadenas ligeras totales (CLT) en pacientes con mieloma múltiple (MM) tanto al diagnóstico como a lo largo del tratamiento.* ***Material y métodos.*** *Estudios serológicos se aplicaron en un grupo de 62 pacientes con MM en el momento del diagnóstico, así como en un subgrupo de 29 pacientes en el punto de máxima respuesta al tratamiento.* ***Resultados.*** *Al diagnóstico de los 62 pacientes se encontró EPS anormal en 58%, IF anormal en 92% y CLT anormales en 45%. En 64% de los pacientes, las tres pruebas serológicas fueron simultáneamente anormales. La IF fue la más sensible de las tres pruebas, seguida de la EPS. En el punto de máxima respuesta terapéutica, la EPS se normalizó en siete casos, la IF en siete casos y las CLT también en siete casos. En 12 casos con IF anormal, las CLT fueron normales y en un caso, la IF fue normal en presencia de CLT anormales. La asociación entre IF y CLT fue muy significativa ($r = 0.9274611$, $p < 0.000001$) a pesar de los casos con IF anormal y CLT normales.* ***Conclusiones.*** *Idealmente deben obtenerse resultados de los tres métodos para detectar la paraproteinemia en suero de pacientes con MM, tanto al diagnóstico como a lo largo del tratamiento. En este grupo de pacientes, la prueba de CLT no fue más sensible que la IF, ni al diagnóstico ni durante la evolución.*

Palabras clave. *Mieloma múltiple. Electroforesis. Inmunofijación. Cadenas ligeras.*

INTRODUCTION

The monoclonal plasmaproliferative disorders encompass a broad spectrum of diseases ranging from the often benign monoclonal gammopathy of undetermined significance (MGUS) and the potentially curable solitary plasmacytoma, to life-threatening multiple myeloma (MM) and light chain amyloidosis (AL). All these conditions have a racial distribution[1-6] and in Mexican mestizos, the incidences of AL,[1] MGUS,[2-4] MM,[5] and Waldenström's macroglobulinemia[6] are substantially lower than in Caucasians. In each of these diseases, quantification of serum immunoglobulins has been the mainstay of diagnosis, prognosis and management. Until the 1990s, the repertoire of tests used to document and measure monoclonal immunoglobulins included serum protein electrophoresis (SPE), immunoelectrophoresis, immunofixation electrophoresis (IFx) and nephelometric measurement of immunoglobulin chains in serum. In the early 2000s, assays to quantify serum immunoglobulin total light chains (sLC) were developed; more recent tests differ from prior light chain-measuring reagents in that novel polyclonal antibodies react with epitopes that are blocked when associated with heavy chains, thus measuring serum immunoglobulin free light chains (sFLC);[7] this latter test is not yet available in our country. We herein analyze the results of assessing the presence of paraproteinemia in patients with MM using three different serological studies: SPE, IFx and sLC.

MATERIAL AND METHODS

Patients

The records of all sequential patients diagnosed with MM at the Centro de Hematología y Medicina Interna de Puebla (Clínica Ruiz) in Puebla, México, between July 2002 and December 2012, were analyzed. The diagnosis of MM was based on the following findings:

- Increased numbers of abnormal, atypical or immature plasma cells in the bone marrow or histological proof of plasmacytoma.
- Presence of a monoclonal M-protein in the serum or urine; or
- Bone lesions consistent with those of multiple myeloma. Individuals with plasma cell reactions to connective tissue disorders, liver disease, metastatic carcinoma or chronic infections were not included, nor patients with MGUS, smoldering MM, solitary plasmacytoma or plasma cell leukemia. Only patients with symptomatic myeloma were studied. Informed consent was obtained from all patients; the study was approved by the Ethics Committee of the Clínica Ruiz.

Methods

Serum protein electrophoresis (SPE) was performed according to Alper.[8] Briefly, serum proteins are fractionated on the basis of their electrical charge at a given pH into five classical fractions: albumin, alpha 1, alpha 2, beta and gamma proteins.[8] An abnormal pattern was defined when a monoclonal spike in the beta or gamma fractions was identified.

Immunofixation electrophoresis (IFx) was conducted according to Ritchie and Smith.[9] Briefly, serum proteins are first resolved by electrophoresis, while in a second step, soluble antigen and antibody are allowed to react into insoluble immune complexes that precipitate in the gel matrix. The use of heavy and light chain-specific antisera allows the identification of the isotype(s) involved in the monoclonal paraproteinemia. An abnormal pattern is defined when the presence of a typically monoclonal heavy and/or light chain is identified.

Serum total light chain (sLC) quantification was determined according to Lievens.[10] In an immunochemical reaction, kappa and lambda light chains in serum form immune complexes with specific antibodies; these complexes scatter a beam of light as it passes through the sample. The intensity of the light scatter is directly proportional to the concentration of either kappa or lambda chains.[10] An abnormal result was defined when the kappa / lambda ratio was either below 1.35 or above 2.65.

Statistical analysis

Association between individual results of the K/L ratio and of IFx, as well as the sensitivity of individual or combined tests, was analyzed in 2 x 2 tables. Significance was determined by Fisher's exact test.

RESULTS

Patients

A total of 62 patients with MM were diagnosed at the Centro de Hematología y Medicina Interna de Puebla in Puebla, México, between July 2002 and December 2012. In a subset of 29 patients, SPE, IFX

and sLC were assessed every three months, at least in two instances, and results were recorded upon maximum therapeutic response; this subset of individuals was diagnosed, treated and followed by one of us (GJRA).

Serological studies at diagnosis

Among the 62 individuals with MM, the following results were obtained at diagnosis: 36 (58%) had an abnormal SPE, 57 (92%) had an abnormal IFx and 28 (45%) had abnormal sLC (Figure 1). In 57 cases (92%), at least one of these tests was abnormal, and 21 (34%) patients had simultaneously abnormal results in all three serological studies at diagnosis. In 5 individuals (8%) with normal serological studies, the paraproteinemia was found in the urine by urine IFX, while two patients had normal results in serum and urine studies, representing truly non-secretory myelomas (3%). SPE alone would have missed 42% of cases, IFx alone would have missed 8% of cases and sLC alone would have missed 55% of cases; accordingly at diagnosis, IFx was found to be more sensitive than the total sLC assay in this group of MM patients. As shown in table 1, all 62 patients except for 2, had at least one abnormal result. IFx alone proved to be the most sensitive of all three assays, followed by SPE, which was redundant in most instances with sLC and IFx results. Figure 1 depicts the sensitivity of each one of the assays alone, or in association with the other two disease markers. sLC alone, proved to be the least sensitive of all three assays.

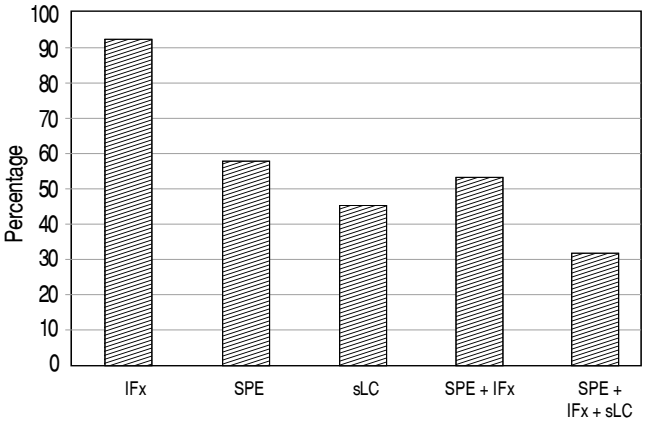

Figure 1. Percent of abnormal tests at diagnosis in the group of 62 patients with multiple myeloma. IFx: immunofixation electrophoresis. SPE: serum protein electrophoresis. sLC: serum total light chain quantification.

Evolution of the serological studies

In the 29 patients with repeated and sequential serological studies throughout treatment, the SPE became normal in 7 cases, the IFx in 7 cases and the sLC in another 7 cases during maximum response to treatment. All cases with normal IFX and sLC had normal SPE patterns. There were 12 instances in which an abnormal IFx was found despite normal sLC, and one case in which a normal IFx was found in the presence of abnormal sLC. In nine individuals, paraproteinemia completely reverted as assessed by these three methods.

Statistical analysis

The association between IFx and sLC was high despite many instances in which a positive IFx was associated to a normal K/L ratio. Although IFx cannot be considered a reference method or a gold standard, it is evident that it outperformed the detection of serum whole light chains, which lacks sensitivity when compared to sLC (Tables 1 and 2).

DISCUSSION

Historically, the gold standard when screening for plasma cell disorders has been SPE with IFx in serum and urine. In the Mayo Clinic's experience with 428 patients studied at diagnosis and assessed by means of SPE, IFx and serum free light chains (sFLC), the latter study was more sensitive than the others.[11] On the other hand, throughout the treatment of these diseases, the International Myeloma Working Group (IMWG) has published updated response criteria that incorporate the sFLC assay;[12] in MM, a stringent complete remission requires negative SPE, IFx and sFLC. However, there have been no formal studies that have validated these criteria[7] nor validating the usefulness of serial sLC measurements;[7,13] the serum free light chain assay throughout treatment follow-up may not be as useful, because of the frequently observed treatment-related immunosuppression of the uninvolved free light chain during chemotherapy.[7]

We have found that at diagnosis, the combination of SPE, IFx and total sLC was able to identify 92% of cases of MM, thus practically precluding the information provided by urine studies, specifically urine IFx, positive in only 8% of cases. We must emphasize that the method we employed to conduct the sLC assay is not the most recent, as it assesses total light chains and not free light chains;[7] measurement

Table 1. Number and proportion of patients with different combinations of positive/abnormal tests at the time of diagnosis.

sLC	IFx	SPE	Positive (%)
+	+	+	20 (32.2)
-	+	-	16 (25.8)
-	+	+	14 (22.6)
+	+	-	7 (11.3)
-	-	+	2 (3.22)
-	-	-	2 (3.22)
+	-	-	1 (1.61)

Immunofixation outnumbered the other two tests. IFx: Immunofixation electrophoresis. SPE: serum protein electrophoresis. sLC: serum total light chain quantification.

Table 2. Association between the three analyzed tests.

Association	r	r^2	P
sLC and IFx	0.927	0.861	< 0.000001
sLC and SPE	0.934	0.872	< 0.000001
IFx and SPE	0.900	0.810	< 0.0004

IFx: immunofixation electrophoresis. SPE: serum protein electrophoresis. sLC: serum total light chain quantification.

of free sLC is considerably superior to the measurement of total sLC, but the method is not available in México yet.

This study also established that the total sLC assay was not more sensitive than IFx in the identification of a paraproteinemia, since in treated MM patients, we were able to identify patients (n = 12) with an abnormal IFx in the presence of a normal sLC assay. It is possible that the treatment of MM results in the suppression of both the normal and the abnormal light chains, thus rendering the assessment of the predominance of one of the light chains difficult. Determining whether a monoclonal component is present or absent in the serum proteinogram or IFx gel, depends on both the platform and matrix used but most importantly, on the expertise of the observing professional. This could be a disadvantage when compared to sLC determinations, in which the technician's adroitness does not impact the test's result as significantly.

CONCLUSION

In conclusion, in our experience, all three serological methods (SPE, IFx and sLC) should ideally be performed in patients with MM both at diagnosis and throughout treatment. Since there is a subset of MM patients with normal serological studies, it is still advisable to obtain urine studies, specifically urine IFx to both identify the paraproteinemia at diagnosis and to search for it throughout the therapeutic period. Because the method which we employed to conduct the sLC assay was not the most up-to-date, these observations may be different if other methods are used to assess total or free serum light chains. The interpretation of an IFx result relies on the expertise of the analyst, and it is well known and accepted that the more the reliability of a laboratory test depends on individual personal skills, the less robust the test is for general application. Unfortunately as of yet, methods to measure serum free light chains are not yet available in our country; we feel that switching to this technology will allow a more accurate identification and follow-up of individuals with monoclonal gammopathies. A study comparing methods to assess both free and total light chains in this scenario is necessary.

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