Practical Laboratory Medicine 26 (2021) e00240

Contents lists available at ScienceDirect

Practical Laboratory Medicine

journal homepage: www.elsevier.com/locate/plabm

Immune subtraction for improved resolution in serum protein immunofixation electrophoresis and antibody isotype determination in a patient with autoantibody

Asad Ullah, Nivin Omar, Natasha M. Savage, Roni J. Bollag, Gurmukh Singh *

Department of Pathology, Medical College of Georgia at Augusta University, Augusta, GA, 30912, USA

ARTICLE INFO

Keywords:
Monoclonal gammopathy
Immune subtraction
Multiple myeloma
Neuropathy
Immunofixation electrophoresis
Autoantibody
Isotype determination

ABSTRACT

Heavy chain isotypes of low level monoclonal immunoglobulins are sometimes obscured in serum immunofixation electrophoresis (SIFE) by a heavy background of polyclonal immunoglobulins. However, accurate determination of the heavy chain isotype is essential for a complete diagnosis, as isotype determination of autoantibodies may have relevance in determining therapeutic procedures. Immune subtraction (IS) was employed in a patient with neuropathy and GD1a autoantibody. IS allowed identification of the cognate heavy chain related to a lambda light chain restriction noted on initial SIFE as well as isotype determination of the autoantibody.

Antisera specific to individual heavy and light chains were used for depletion of specific immunoglobulin types. Depletion of kappa light chain associated immunoglobulins allowed unequivocal determination of the isotype of lambda light chain-associated low level monoclonal band to be IgG Lambda. Selective depletion of kappa, lambda, gamma and mu heavy chain immunoglobulins was employed to determine IgG Kappa isotype of the auto-antibody.

1. Introduction

Serum protein electrophoresis (SPEP) and serum immunofixation electrophoresis (SIFE) along with similar electrophoretic analysis of urine (UPEP and UIFE) are instrumental in the diagnosis of monoclonal gammopathies including plasma cell myeloma/multiple myeloma [1–3]. Neoplastic monoclonal gammopathies span three disorders of increasing severity, namely: monoclonal gammopathy of undetermined significance (MGUS), smoldering or asymptomatic multiple myeloma (SMM), and the malignant entity of multiple myeloma or plasma cell myeloma (MM) [4–9]. Monoclonal immunoglobulins are also present in serum of patients with a number of other disorders, including autoimmune disorders [3]. The monoclonal immunoglobulin secreted by neoplastic plasma cells is usually an intact immunoglobulin with IgG being the most common and IgE isotype being the rarest. In MM, about 85% of the lesions produce intact immunoglobulins in decreasing order of frequency as IgG kappa, IgG lambda, IgA kappa, IgA lambda, and IgD and IgE myelomas. About 13% of the myeloma lesions produce light chains only [3]. Dyscrasias producing heavy chain only are usually lymphomas, as are IgM producing lesions [10]. A recently described entity of light chain predominant multiple myeloma (LCPMM) constitute about 18% of intact immunoglobulin producing myelomas, and the affected patients have a shorter survival by about 2 years [11].

* Corresponding author. Department of Pathology, Medical College of Georgia at Augusta University, 1120, 15th Street, BI 2008A, Augusta, GA, 30912, USA.
E-mail address: gusingh@augusta.edu (G. Singh).

https://doi.org/10.1016/j.plabm.2021.e00240
Received 16 December 2020; Received in revised form 18 April 2021; Accepted 26 May 2021
Available online 30 May 2021

Virtually all cases of the MM are thought to evolve via sequential stages of MGUS and SMM, though these precursor conditions may go undiagnosed as these are often clinically silent and may be discovered incidentally. However, it is important to accurately characterize MGUS lesions, since 1–2% of patients with MGUS progress to MM each year [4,5].

Occasionally autoantibodies present as low level monoclonal immunoglobulins [3]. Such monoclonal immunoglobulin autoantibody lesions may be associated with neuropathy, which may be amenable to treatment by plasmapheresis and/or immune suppression. However, presence of monoclonal light chains has not been associated with auto-immune disorders and the apparent lambda light chain monoclonal gammopathy, noted in this patient warranted further characterization. Isotype determination of an autoantibody is important if plasmapheresis is considered as part of the treatment, because, IgM antibodies are predominantly intravascular given their large size, they are more amenable to treatment by plasmapheresis [12]. Therefore, accurate characterization of even low-level monoclonal immunoglobulins, especially apparent light chain monoclonal bands, is important in order to determine optimal patient care.

Instances of lambda light chain restriction on SIFE, without a recognizable heavy chain, are seen not infrequently. It is generally correct to assume that the associated heavy chain is gamma but accurate determination is important to exclude free light chain lesions. Moreover, accurate sub classification of monoclonal proteins allows for better patient follow-up, particularly in patients undergoing stem cell transplant who may develop an oligoclonal pattern over the course of their treatment [13,14]. An instance of autoantibody to GD1a in a patient with neuropathy and other features of auto-immune disorder, warranted isotype determination in order to ascertain the practicality of plasmapheresis as a therapeutic modality and is presented as an example of the efficacy of immune subtraction for identification of the isotype of auto-antibody.

2. Methods

This study was carried out at a 480 bed medical center affiliated with a medical school in Southeastern USA. The study was reviewed and approved by the Institutional Review Board.

SPEP and SIFE were performed by using Helena SPIFE Touch equipment and Helena immunofixation kits, as described previously [9,15–17]. The antibody preparations in the immunofixation kits, obtained from Helena, were used for immune-subtraction (IS). SPEP always preceded study of the serum by SIFE. The SIFE protocol recommended by the manufacturer includes tenfold dilution of serum for immunofixation for gamma and kappa chains and fivefold dilution for alpha, mu and lambda chains. These recommendations were considered in immune-subtraction maneuvers for this patient. The antibodies used for immune-subtraction were the same polyclonal reagents provided by Helena Laboratories for SIFE analysis.

Case report: A 69-year-old female physician presented with a sensory neuropathy, affecting lower extremities, without any obvious causative factors. She also experienced weakness that was worse in the morning. She also exhibited focal deep fat loss and loss of vibratory sensation. Other indicators of auto-immunity included asymptomatic antibodies to parietal stomach cells, antithyroglobulin and antithyroidperoxidase antibodies. These auto-antibody tests were done at different institutions and were not repeated, as these were not considered germane to the clinical circumstances. There was history of surgical removal of parathyroid adenoma. Patient was in Zika endemic area in 2016 but did not experience any symptoms relevant to this virus. Guillen Barre syndrome was considered but not pursued.

SPEP and SIFE were conducted at other sites and were repeated, among other studies. A lambda light chain restriction was noted on SIFE without an apparent cognate heavy chain. Kappa chain associated immunoglobulins were depleted by precipitation with Helena antiserum to kappa light chains. Briefly; in two separate tubes, 90 μL or 190 μL of antiserum was added to 10 μL of patient serum, the mixture was vortexed and incubated overnight at 4 °C. The immune complexes were removed by centrifugation in an Accuspin Micro 17 Fischer-Scientific centrifuge at 13,000 rpm (16,600 g) for 5 minutes. The amount of antisera used were in consonance with the dilution of serum recommended by the manufacturer, for SIFE. The supernatant was processed for SIFE without further dilution. Following immune subtraction of kappa chain associated immunoglobulins, a gamma chain band was evident at the same location as noted for lambda light chains. Thus the apparent lambda restriction was identified as monoclonal IgG lambda. Similar protocol was used for depletion of lambda, mu, and gamma chain associated immunoglobulins, by adding appropriate antisera and centrifugal removal of immune complexes. Dilution of serum with saline alone did not result in improvement in resolution that was noted by depletion of kappa light chain associated immunoglobulins.

The serum was also referred for autoantibody panel for neuropathy (ARUP reference laboratory). Based on appropriate titer evaluations, antibody to only GD1a ganglioside was detectable. The method measures titers of combined IgG and IgM antibodies to

Table 1
Titers of antibody activity to GD1a are shown. The titers shown are the values reported by the reference laboratory. The figures in parentheses are values corrected for dilution of the specimen due to addition of antibody in immune-subtraction.

Specimen (dilution)	Titer of GD1a antibody (Corrected for dilution)
Serum (0)	122
Lambda depleted serum (10)	19 (190)
Kappa depleted serum (10)	7 (70)
Gamma depleted serum (10)	5 (50)
Mu depleted serum (5)	31 (155)
	Reference range 0–50

neuropathic antigens. Selective depletion of kappa and lambda light chains and gamma and mu heavy chains was conducted followed by autoantibody testing to ascertain the isotype of auto-antibody. The titers reported by the reference laboratory were corrected for the dilution of the specimen induced by addition of antisera. For example, 90 μL of anti-kappa antiserum was added to 10 μL of serum, incubated overnight and subjected to centrifugation as described for SIFE. The reported titer was multiplied by 10 to correct of dilution and the corrected results are given in parenthesis in Table 1.

3. Results

The change in resolution of SIFE results, before and after IS, are shown in Fig. 1.

In Fig. 1, SP refers to staining for all serum proteins. The letters on top of the lanes under SIFE represent the antisera to gamma, alpha, mu, kappa and lambda chains respectively. In this figure, a lambda light chain band/restriction is present in SIFE, shown on the left side of the figure. The corresponding heavy chain is not discernible. Patient's serum was treated with antiserum to kappa light chains to deplete kappa light chain associated immunoglobulins. Two different dilutions, namely 9:1 and 19:1 were employed, as described in methods. The right side of the figure shows the results of SIFE following immune subtraction. The ratio of antiserum to patient serum is indicated. –K represents depletion of kappa chain associated immunoglobulins. The letters G and L, at the bottom of IS SIFE side of the figure denote antisera to gamma and lambda chains used for immunofixation. A gamma heavy chain band is evident at the same migration site as the lambda light chain band. Thus the apparent lambda restriction is identified as monoclonal IgG lambda. It is reiterated that the serum in lanes G and K, on the left side of the figure were diluted 10-fold with saline, to match the dilution wrought by the addition of anti-kappa antiserum in the lanes under 9:1. The gamma heavy chain corresponding to the lambda light chain restriction is clearly evident after subtracting kappa chain associated immunoglobulins. The resolution did not improve on dilution with saline alone.

The autoantibody specificity results to GD1a for the whole serum and various fractions are shown in Table 1. The described patient presenting with sensory neuropathy was observed to have autoantibody to GD1a. In an attempt to ascertain if the monoclonal IgG lambda was the responsible autoantibody, aliquots of serum were depleted of kappa and lambda light chain associated immunoglobulins by precipitation with respective antisera. Based on the IS results, the GD1a-specific antibody was found to be associated with the kappa chain immunoglobulin fraction. The next attempt was to ascertain if the autoantibody was of IgM isotype, as intravascular IgM is more susceptible to depletion by plasmapheresis. Gamma and mu heavy chain associated immunoglobulins were depleted from aliquots of serum and tested for autoantibody reactivity with GD1a. Antibody activity was associated with IgG fraction. Thus the monoclonal IgG lambda happened to be an incidental finding and not associated with autoantibody activity. Depletion of gamma and mu heavy chain associated immunoglobulins followed by repeat epitope specificity testing for the neuropathy autoantibody panel established that the autoantibody was IgG kappa. Given the IgG kappa isotype of the autoantibody and other considerations, plasmapheresis was not pursued in this patient.

4. Discussion

The lesions in neoplastic monoclonal gammopathies may secrete intact immunoglobulins, light chains only, biclonal or triclonal immunoglobulins, or in about 1% of cases may not secrete any immunoglobulins [3,15,17,18]. We observe apparent light chain only bands, usually lambda monoclonal bands, more frequently than would be expected based on the frequency of free light chain monoclonal gammopathies. This suggested that the apparent monoclonal light chain-only bands were being misinterpreted due to abundance of a polyclonal background obscuring the cognate heavy chain band. This corresponded with the observed preponderance of apparent lambda light chain only bands, as IgG kappa is the predominant immunoglobulin and was obscuring the gamma chain associated with lambda chain bands. This IgG kappa over-representation is further accentuated in patients with polyclonal increase in gamma globulins as evidenced by kappa dominant kappa/lambda ratio in more than 90% of the patients with an abnormal

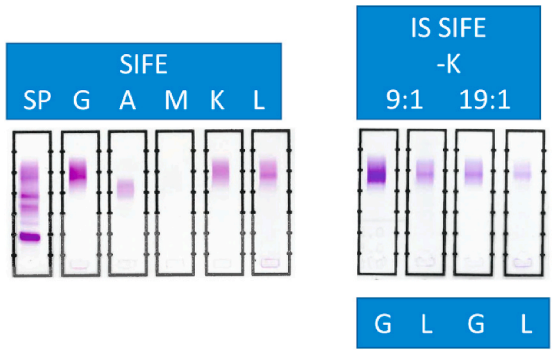

Fig. 1. Conventional SIFE is shown on the left side of figure. A lambda restriction is noted, without an obvious cognate heavy chain. Depletion of kappa associated immunoglobulins revealed a monoclonal IgG lambda band (right panel). The monoclonal gamma chain is more evident in the specimen with greater depletion of kappa associated immunoglobulins –K 19:1).

kappa/lambda ratio associated with polyclonal hypergammaglobulinemia [3,9,17]. Oligoclonal pattern in patients status-post stem cell transplantation may complicate the detection of the original malignant clone; and IS has anecdotally been observed to assist in identification of an original malignant clone [13,14].

In order to determine whether a heavy background of polyclonal kappa species was obscuring the expected gamma heavy chain band associated with low-abundance apparent monoclonal lambda light chain bands, we depleted kappa chain-associated immunoglobulins as described above and ascertained the band to be due to monoclonal IgG Lambda. IS analysis for determining the isotype of an autoantibody may have clinical relevance if the autoantibody is a monoclonal antibody and/or if the antibody is IgM isotype. Monoclonal autoantibody neuropathies warrant treatment with plasmapheresis in addition to other modalities such as intravenous immunoglobulin treatment and immunosuppression. IgM autoantibodies may be more susceptible to depletion by plasmapheresis due to predominantly intravascular location of IgM immunoglobulins. Larsen et al. used a similar technique in resolving the apparently multiple monoclonal bands in a patient with IgM kappa monoclonal protein that exhibited rheumatoid factor activity [19].

This procedure can be easily performed in any laboratory performing gel based immunofixation electrophoresis and is relevant for accurate identification of monoclonal gammopathies. The antibodies usually used in staining SIFE gels can be used for IS. The suitability of this maneuverer for capillary zone electrophoresis was not evaluated in this study. The results of this investigation did not affect the treatment of the patient. We recognize that before using such procedures in clinical care one would need to establish a validation process of sufficient rigor to meet requirements for a laboratory developed test.

Declaration of competing interest

Dr. Singh serves as a consultant to Diazyme Inc and HealthTap.

References

[1] M. Dimopoulos, R. Kyle, J.P. Fermand, S.V. Rajkumar, J. San Miguel, A. Chanan-Khan, et al., Consensus recommendation for standard investigative workup: report of international myeloma workshop consensus panel 3, Blood 18 (2011) 4701–4705.

[2] R.A. Kyle, (For the 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. Haematol. 121 (2003) 749–757.

[3] G. Singh, Serum and urine protein electrophoresis and serum-free light chain assays in the diagnosis and monitoring of monoclonal gammopathies, J Appl Lab Med 5 (2020) 1358–1371, https://doi.org/10.1093/jalm/jfaa153.

[4] R.A. Kyle, T.M. Therneau, S.V. Rajkumar, D.R. Larson, M.F. Plevak, J.R. Offord, et al., Prevalence of monoclonal gammopathy of undetermined significance, N. Engl. J. Med. 354 (2006) 1362–1369.

[5] R.A. Kyle, D.R. Larson, T.M. Therneau, A. Dispenzieri, S. Kumar, J.R. Cerhan, S.V. Rajkumar, Long-term fellow-up of monoclonal gammopathy of undetermined significance, N. Engl. J. Med. 378 (2018) 241–249.

[6] S.V. Rajkumar, M.A. Dimopoulos, A. Palumbo, J. Blade, G. Merlini, M.V. Mateos, et al., International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma, Lancet Oncol. 15 (2014) e538–e548.

[7] R.A. Kyle, E.D. Remstein, T.M. Therneau, A. Dispenzieri, P.J. Kurtin, J.M. Hodnefield, et al., Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma, N. Engl. J. Med. 356 (2007) 2582–2590.

[8] S.V. Rajkumar, O. Landgren, M.-V. Mateos, Smoldering multiple myeloma, Blood 125 (2015) 3069–3075.

[9] G. Singh, Serum free light chain assay and κ/λ ratio: performance in patients with monoclonal gammopathy-high false negative rate for κ/λ ratio, J. Clin. Med. Res. 9 (2017) 46–57.

[10] G. Bianchi, K.C. Anderson, N.L. Harris, A.R. Sohani, The heavy chain diseases: clinical and pathologic features, Oncology 28 (2014) 45–53.

[11] G. Singh, H. Xu, Light chain predominant intact immunoglobulin monoclonal gammopathy disorders: shorter survival in light chain predominant multiple myelomas, Lab Med. Lab Med. (2020), https://doi.org/10.1093/labmed/lmaa057.

[12] S. Ramchandren, R.A. Lewis, An update on monoclonal gammopathy and neuropathy, Curr. Neurol. Neurosci. Rep. 12 (2012) 102–110.

[13] G. Singh, Concentrations of serum free light chains in kappa and lambda lesions in light-chain myelomas, Lab. Med. 50 (2019) 189–193, https://doi.org/10.1093/labmed/lmy067.

[14] G. Singh, R. Bollag, Quantification by Ultrafiltration and Immunofixation Electrophoresis Testing (QUIET) for monoclonal serum free light chains, Lab. Med. 51 (2020) 592–600, https://doi.org/10.1093/labmed/lmaa012.

[15] G. Singh, Serum free light chain assay and κ/λ ratio: performance in patients without monoclonal gammopathies – high false positive rate, Am. J. Clin. Pathol. 146 (2016) 207–214.

[16] G.R. Shaw, Nonsecretory plasma cell myeloma-becoming even more rare with serum free light-chain assay: a brief review, Arch. Pathol. Lab Med. 130 (2006) 1212–1215.

[17] S.L. Hall, J. Tale, D. Gill, P. Mollee, Significance of abnormal protein bands in patients with multiple myeloma following autologous stem cell transplantation, Clin. Biochem. Rev. 30 (2009) 113–118.

[18] G. Singh, Oligoclonal pattern/abnormal protein bands in post-treatment plasma cell myeloma patients: implications for electrophoresis and serum free light chain assay results, J. Clin. Med. Res. 8 (2017) 671–679.

[19] R. Larsen, S. Allen, T. Thompson, R. Bollag, G. Singh, Challenges in interpreting multiple monoclonal bands on serum protein electrophoresis and serum immunofixation electrophoresis: an illustrative case report, J Appl Lab Med 4 (2019) 455–459.