Occurrence of Double Monoclonal Bands on Protein Electrophoresis: An Unusual Finding

Vishrut K Srinivasan¹ · Priyanka Bhagat¹ · Frainey Bansal¹ · Seema Chhabra¹

Received: 19 August 2015 / Accepted: 17 November 2015 / Published online: 28 November 2015
© Indian Society of Haematology & Transfusion Medicine 2015

Abstract Various techniques of protein electrophoresis are used for detection of monoclonal proteins/paraproteins in serum and/or urine of patients with monoclonal gammopathies. These are detected as the so-called ‘M’ bands (monoclonal bands) on serum protein electrophoresis and/or immunofixation electrophoresis. In most cases, a single M-band is detected. However, more than one M-band can be detected in the samples of a minor proportion of patients. This condition is termed as ‘double gammopathy’ or ‘biclonal gammopathy’. A knowledge of such an unusual occurrence is essential for recognition and appropriate interpretation of this entity.

Keywords Monoclonal gammopathy · Double gammopathy · Biclonal · Monoclonal proteins

Introduction

Monoclonal gammopathy (MG) represents the presence of monoclonal protein (M-protein) in serum and/or urine, which occurs as a result of overproduction of immunoglobulins (Igs) by a clone of abnormally proliferating

B-lymphocytes and/or plasma cells. This can be found mainly in monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), or Waldenström’s macroglobulinemia. However, monoclonal proteins may also occur in primary amyloidosis, cryoglobulinemia, lymphoproliferative disorders, solitary plasmacytoma, certain infections such as Hepatitis C virus (HCV) infection, and in even rare conditions like, POEMS syndrome and scleromyxedema [1].

Longworth et al. [2] were the first to demonstrate a tall, narrow based spike by serum protein electrophoresis (SPEP) which was later termed as “M” component/peak by Moore et al. [3]. On performing serum protein electrophoresis, monoclonal Igs appear as single intense, discrete band on agarose gel and a sharp peak in the densitometer tracing. This band/peak can be noted in the gamma-, beta-gamma-, or beta-region depending on the type of monoclonal Igs being secreted.

Following detection of ‘M’ band/peak on serum protein electrophoresis (SPEP), immunofixation electrophoresis (IFE) is recommended for definitive identification of monoclonal proteins and characterization of heavy chain (HC) and light chain (LC) being secreted. IFE has greater sensitivity than SPEP in detecting small amounts of ‘M’ protein in plasmacytoma, primary amyloidosis or in treated cases of multiple myeloma/macroglobulinemia, where SPEP does not show any monoclonal band. It is also helpful for differentiating a monoclonal from a polyclonal increase in Igs. Polyclonal increase of Igs is usually associated with an inflammatory or reactive process, whereas monoclonal Igs result from neoplastic proliferation of plasma cells. In addition, cases with normal SPEP, in which strong clinical suspicion of multiple myeloma, or a related disorder exists, IFE is necessary for confirmation of diagnosis.

✉ Seema Chhabra
drseemachhabra@gmail.com

Vishrut K Srinivasan
vishrut.ks@gmail.com

Priyanka Bhagat
drpriyankabhagat@gmail.com

¹ Department of Immunopathology, Level 4, Research Block A,
Postgraduate Institute of Medical Education and Research,
Sector-12, Chandigarh 160012, India

Double Gammopathy

In a certain subset of patients with monoclonal gammopathies, more than one M-protein can be found in serum and/or urine. This category of gammopathies are called ‘double gammopathies’. The term ‘biclonal gammopathy’ has been used interchangeably in literature, in lieu of ‘double gammopathy’. Double gammopathy may present with two separate distinct M band/peak on SPEP or a single M band on SPEP which further resolves into two separate bands on IFE. The double gammopathies are further subdivided depending on the type of HC and LC detected. IFE in these cases can reveal either different HC isotypes with same LC isotype or same HC isotype with dissimilar LC isotypes (Figs. 1, 2, 3). The subset of cases with different HC isotypes with different LC isotypes has been distinctively referred to as ‘true biclonal gammopathy’ [4].

Conditions Associated with Double Gammopathy

Double gammopathies are known to be associated with multiple myeloma, certain lymphoproliferative disorders, as well as with cases of MGUS. In the latter situation, the condition is alternatively called ‘double/biclonal gammopathy of undetermined significance’ (DGUS/BGUS) [5].

Origin of Double Gammopathies

Double gammopathies are either detected at initial presentation or during the course of the disease after initial diagnosis has been established. Double gammopathies can result from proliferation of “two or more distinct plasma cell clones” producing more than one monoclonal protein. Alternatively, “clonally related” plasma cells which have

undergone class switch recombination at a later stage in disease pathogenesis can also give rise to double gammopathies [4, 6]. During disease progression or following the treatment, the level of the ‘double’ M-proteins may rise or fall in concordant or discordant manner [5]. The causative mechanisms for the aforementioned processes have not yet been properly elucidated.

Incidence of Double Gammopathies

The incidence of double gammopathies among various cohorts of gammopathies in reported literature is 2–6 % [5, 7–11]. IgG–IgA has been reported as the most common HC isotype combination by Kyle et al. [7]. However, Riddell et al. [5] observed IgG–IgM to be the most common HC isotype combination. Certain other studies have found occurrence of IgG–IgG as the most common combination [10, 11]. The differences between these case series may be the result of difference in population distributions, racial differences, differences in sensitivity of detection method used, or selection bias during case selection for inclusion in the series. Among the light chains, kappa light chain was most commonly detected [5, 8, 11].

True Biclonal Gammopathies

True biclonal gammopathies may result either from neoplastic proliferation of two different “clonally unrelated” malignant plasma cell lines or from the existence of a single clone of B-lymphoid cells, which subsequently diversifies into two independent clones after a process of antigenic selection [1]. Accordingly, the clonal proteins being secreted always have a “different variable region and differ in both light chain and heavy chain class”. This

Fig. 1 A case of multiple myeloma with 36 % plasma cells in bone marrow presenting as “Double gammopathy with IgG kappa and IgA kappa monoclonal bands” (*LC isotype matched/HC isotype dissimilar*) showing **a** SPEP: Two bands in gamma region, **b** Densitometer

tracing: two peaks in gamma region, **c** IFE: one band each in IgG and IgA heavy chain lanes with two bands in kappa light chain lane corresponding to each of the bands seen in heavy chain lanes

Fig. 2 A known case of Non-Hodgkin lymphoma presenting as “Double gammopathy with IgM kappa-lambda monoclonal bands” (*HC isotype matched/LC isotype dissimilar*) showing **a** SPEP: One band in gamma region; **b** Densitometer tracing: single sharp peak in

gamma region; **c** IFE showing one band in IgM heavy chain lane, one band in kappa light chain lane and one band in lambda light chain lane

Fig. 3 A case of multiple myeloma with 19 % plasma cells in bone marrow presenting as “Monoclonal gammopathy with IgA kappa and IgA kappa monoclonal bands” (*Both LC & HC isotypes matched*)

showing **a** SPEP: Two bands in gamma region; **b** densitometer tracing: two sharp peaks in gamma region; **c** IFE: two bands each in IgA heavy chain lane and kappa light chain lane

Fig. 4 A known case of lymphoplasmacytic lymphoma with bone marrow infiltration presenting as a ‘true’ biclonal gammopathy with IgG kappa and IgM lambda monoclonal bands (*Both LC & HC isotypes dissimilar*) showing **a** SPEP: Two bands in gamma region,

b Densitometer tracing: two sharp peaks in gamma region, **c** IFE: one band in IgG heavy chain lane and a corresponding band in kappa light chain lane along with one band in IgM heavy chain lane and a corresponding band in lambda light chain lane

provides conclusive evidence that the two M-bands seen are actually the product of two separate clones and represent a ‘true’ biclonal gammopathy (Fig. 4). The detection of a single LC isotype in majority of double gammopathies, as mentioned earlier, is suggestive of a single clonal origin of M-proteins in these cases, indicating that ‘true’ biclonal gammopathies form a minor subset of double gammopathies.

Does a Double Band Always Signify Biclonal/Double Gammopathy?

In certain situations, two distinct bands on SPEP yield only a single band in one of the HC (IgG/IgM/IgA) lanes along with two bands in a single light chain lane i.e. either kappa or lambda on IFE (Fig. 5). One of the two bands seen in light chain lane is due to the presence of excess free LCs which migrate faster compared to LCs associated with Ig heavy chains. This could be due to asynchronous production of the components of the Ig molecules, leading to synthesis of an intact monoclonal Ig plus excess monoclonal free LCs [12].

Similarly, resolution of the subtypes causing two bands may also be complicated especially in case of IgA gammopathies. Monoclonal IgA molecules have a tendency to dimerize, and the resulting dimer often has a different mobility than the monomer parent molecule, thereby, yielding two bands on SPEP in some cases of IgA monoclonal gammopathies. In these circumstances, IFE will yield two bands in IgA lane at different positions along with corresponding 2 bands in one of the LC lanes i.e. IgA kappa IgA kappa (Fig. 3). Pre-treatment of sera with 2-mercaptoethanol depolymerizes the multimers and helps in better distinction of double gammopathies [13]. On the

other hand, the pentameric IgM can break down into 7 s subunits, which show up on electrophoresis as one or more ‘extra’ monoclonal bands. These multiple bands are most likely due to various molecular forms of a monoclonal protein derived from a single plasma cell clone [1].

In certain cases of IgA myeloma, there may not be any corresponding band in LC lane at the level of IgA band in the HC lane. This finding can be reported as “IgA with no apparent or discernible light chain attached”. This occurs due to structural properties of IgA molecule that hinders the reaction between LC epitopes and the antibodies against them [13]. This may also occur due to prozone phenomenon where there is excess of antigen or low antibody concentration [14].

Presence of additional band in beta-gamma region on SPEP can sometimes interfere with analysis and maybe misinterpreted as a monoclonal band/peak. This band is caused by presence of fibrinogen which can occur due to insufficient clotting of sample. However, this ‘fibrinogen’ band disappears on IFE as it does not bind to any specific antisera used. Thus, in such situations, IFE is helpful in reaching a diagnostic conclusion.

Utility of Nephelometry

Serum protein electrophoresis may show subtle or no abnormalities in cases, such as non-secretory multiple myeloma, light chain MM, primary amyloidosis, and light chain deposition disease. In these cases, however, monoclonal free light chains (FLC) are secreted which can be detected and quantified in serum/urine. The quantitative FLC assay by nephelometry is more sensitive than electrophoretic techniques for detection of serum FLC [15]. In addition to LCs, HCs can also be quantified with the help

Fig. 5 A case of multiple myeloma with 54 % plasma cells in bone marrow presenting as “Monoclonal gammopathy with IgG lambda with free excess lambda light chains” showing **a** SPEP: one band in

gamma region, **b** Densitometer tracing: one sharp peak in gamma region, **c** IFE: one band in IgG heavy chain lane and two bands in lambda light chain lane

of nephelometric methods. This may aid in better identification of separate Ig classes in cases of biclonal/double gammopathies.

Implication of Biclonal Gammopathy

Clinically, there is no difference between double/biclonal gammopathy and monoclonal gammopathy in terms of outcome for the patient [7]. However, recognition of this entity not only increases diagnostic accuracy and credibility, but also helps in assessing treatment response during follow-up of patients. Measurement of both clonal proteins, at diagnosis and during follow-up, helps in identifying synchronicity of response to both clones. The difference in response of different clones can aid in driving further treatment decisions in such cases. Additionally, the recognition of the different monoclonal components can serve as a tool for further deciphering disease pathogenesis and for defining a distinct subset of monoclonal gammopathies.

Compliance with Ethical Standards

Conflict of interest None.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

1. Katzmann J, Kyle RA, Lust J et al (2013) Immunoglobulins and laboratory recognition of monoclonal proteins. In: Wiernik PH, Goldman JM, Dutcher JP et al (eds) *Neoplastic diseases of blood*, 5th edn. Springer, New York, pp 565–588
2. Longsworth LG, Shedlovsky T, Macinnes DA (1939) Electrophoretic patterns of normal and pathological human blood serum and plasma. *J Exp Med* 70:399–413
3. Moore DH, Kabat EA, Gutman AB (1943) Bence-jones proteinemia in multiple myeloma. *J Clin Invest* 22:67–75
4. Tschumper RC, Dispenzieri A, Abraham RS et al (2013) Molecular analysis of immunoglobulin genes reveals frequent clonal relatedness in double monoclonal gammopathies. *Blood Cancer J* 3:e112
5. Riddell S, Traczyk Z, Paraskevas F et al (1986) The double gammopathies. Clinical and immunological studies. *Medicine (Baltimore)* 65:135–142
6. Bakkus MH, Schots R, Gomez La Fuente PB et al (2000) Clonally related IgA- and IgE-secreting plasma cells in a myeloma patient. *Eur J Haematol* 65:348–355
7. Kyle RA, Robinson RA, Katzmann JA (1981) The clinical aspects of biclonal gammopathies. Review of 57 cases. *Am J Med* 71:999–1008
8. Kyle RA, Therneau TM, Rajkumar SV et al (2002) A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med* 346:564–569
9. Kyle RA, Gertz MA, Witzig TE et al (2003) Review of 1027 patients with newly diagnosed multiple myeloma. *Mayo Clin Proc* 78:21–33
10. Guastafierro S, Ferrara MG, Sica A et al (2012) Serum double monoclonal components and hematological malignancies: only a casual association? Review of 34 cases. *Leuk Res* 36:1274–1277
11. Garcia-Garcia P, Enciso-Alvarez K, Diaz-Espada F et al (2015) Biclonal gammopathies: retrospective study of 47 patients. *Rev Clin Esp* 215:18–24
12. Kunkel HG (1968) The “abnormality” of myeloma proteins. *Cancer Res* 28:1351–1353
13. Osterland CK, Chaplin H Jr (1966) Atypical antigenic properties of a gamma-A myeloma protein. *J Immunol* 96:842–848
14. Sun T, Peng S, Narurkar L (1994) Modified immunoselection technique for definitive diagnosis of heavy-chain disease. *Clin Chem* 40:664
15. Bradwell AR, Carr-Smith HD, Mead GP et al (2001) Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 47:673–680