Broad Bands Observed in Serum Electrophoresis Should Not Be Taken *Lightly*

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CASE DESCRIPTION

A 78-year-old woman was referred to our hospital for further investigation of a bicytopenia. She had a history of rheumatoid arthritis and Sjögren syndrome for which she was treated with methotrexate and etanercept. She had no complaints, and during physical examination, no lymphadenopathy, masses, or visceromegaly was found. Hematologic evaluation showed the following results: hemoglobin, 9.7 g/dL (reference, 12.1–16.1 g/dL); white blood cells, 1.6 X 10^9/L (reference, 4.0 –10.0 X 10^9/L); and thrombocytes, 156 X 10^9/L (reference 150–400 X 10^9/L). A peripheral blood smear did not show blasts or dysplastic features of the white blood cells. The patient had normal ferritin, vitamin B12, and folic acid concentrations without evidence of hemolysis. Serum protein electrophoresis (SPE)[4] and immunofixation electrophoresis (IFE) with pentavalent antiserum were performed as M-protein screening. Both techniques demonstrated a broad band in the β/γ region (Fig. 1A). Further IFE analysis, shown in Fig. 1B, identified the abnormal pattern as a broad IgG band (blue brackets) with no corresponding light chain band (red brackets). These data suggested the presence of an IgG heavy chain M protein (γ-HC).

Capillary electrophoresis (CE) combined with immunosubtraction (IS) analysis (Fig. 1C) confirmed that the abnormal pattern was caused by a γ-HC. IgG-IS (Fig. 1D) illustrated that the γ region consisted of polyclonal IgG-κ and IgG-λ (green). The β2 region largely consisted of IgG not associated with light chains (γ-HC, represented in red), and the remaining fraction (blue) was a combination of polyclonal IgA, polyclonal IgG, and nonimmunoglobulins.

The serum Hevylite immunoassay has been recommended both for confirmation and quantification of a monoclonal heavy chain (*1*). The Hevylite reagents specifically target the unique junctional epitope between the immunoglobulin heavy chain and light chain combination. An (IgGκ + IgGλ)/IgG_{total} ratio lower than 0.8 is indicative of the presence of a γ-HC (*1*). In our patient, the ratio was 0.6 (IgG_{total} = 15.6 g/L, IgGκ = 4.4 g/L, and IgGλ = 4.9 g/L). Furthermore, the γ-HC concentration could be estimated as follows: [γ-HC] = [IgG_{total}] - [IgGκ] - [IgGλ]. The γ-HC concentration in our patient measured using the Hevylite reagents was 6.2 g/L. This corresponds to the γ-HC concentration determined from the CE pattern and total serum protein concentration (β2 region is 16.5% of 58 g/L = 9.5 g/L). From Fig. 1D, it is estimated that approximately two-thirds of the β2 region comprises the γ-HC (two-thirds of 9.5 = 6.3 g/L).

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Bone marrow analysis showed the presence of approximately 2% plasma cells, of which about 30% were atypical or binucleated (Fig. 1E). No other abnormalities were observed within the bone marrow. Using flow cytometry, a small population of plasma cells was detected. Eighty percent of these plasma cells expressed cytoplasmic IgG, and 50% of the cells did not express either cytoplasmic κ or λ (Fig. 1F). Interestingly, these κ/λ- negative cells expressed multiple aberrant markers ($CD45^{high}CD138^{high}CD20^{+}CD56^{+}$). All together, we observed a low percentage (approximately 1%) of malignant plasma cells expressing a γ-HC.

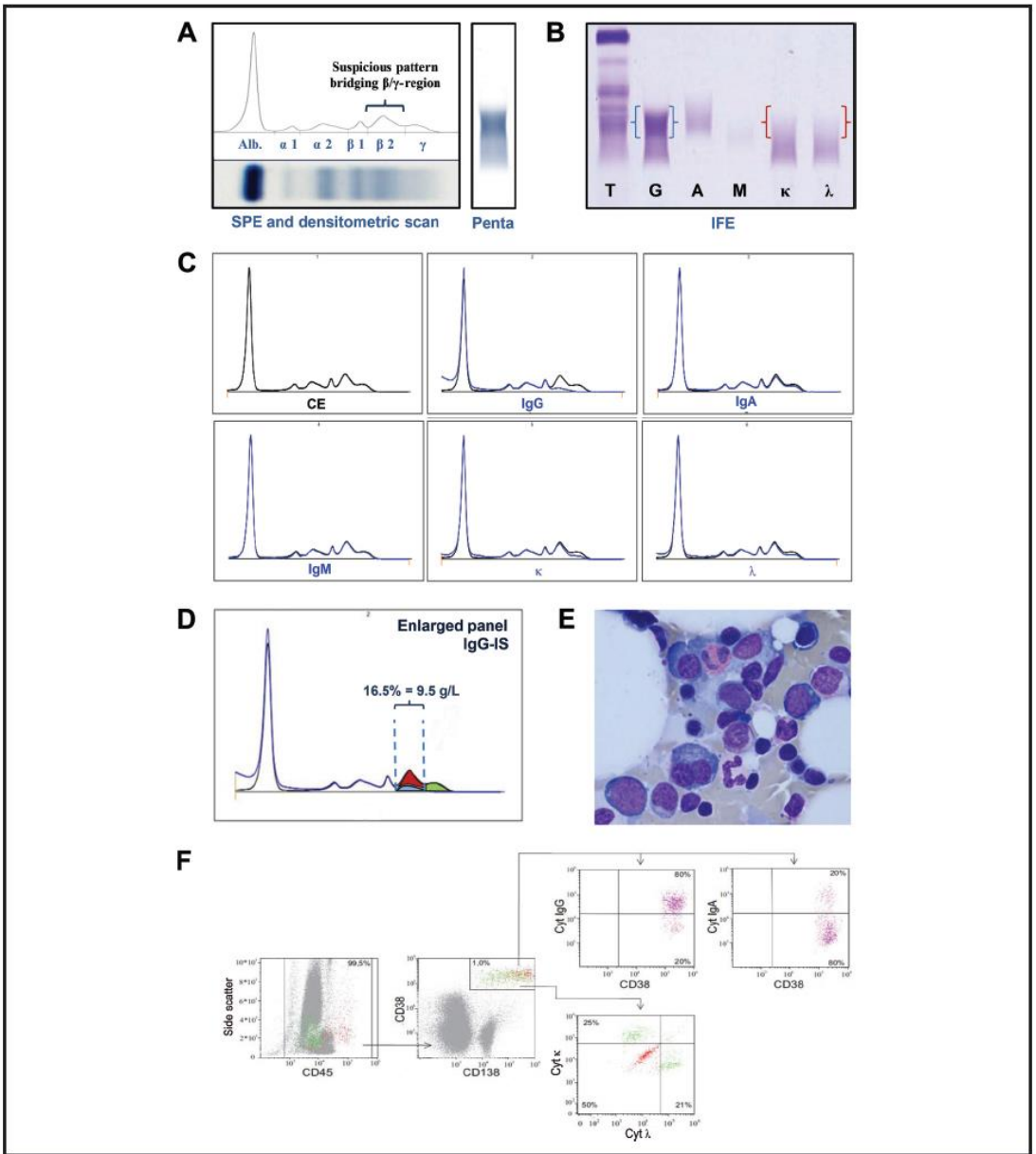

Fig. 1. Laboratory findings associated with γ-HC.

(A), SPE and the corresponding densitometry scan show an abnormal broad band bridging the β/γ region. The same abnormal dense region is observed in the pentavalent screen. (B), IFE analysis demonstrates that the abnormal dense region corresponds to IgG (blue brackets) and has no associated light chains (red brackets). (C), CE combined with IS analysis (indicated by blue lines in each panel) confirms that the abnormal pattern is caused by an IgG heavy chain. (D), Enlarged panel of IgG-IS illustrates that the γ region consists of polyclonal IgG-κ and IgG-λ (green). The β2 region largely consists of IgG not associated with light chains (red), and the remaining fraction (blue) is a combination of polyclonal IgA, polyclonal IgG, and nonimmunoglobulins. (E), Bone marrow aspirate showing normal and binucleated plasma cells. (F), Flow cytometry of the bone marrow aspirate shows a small population of plasma cells that are mainly IgG-positive. Approximately 50% of the IgG-positive plasma cells do not express κ or λ (indicating the heavy chain clone in red).

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QUESTIONS TO CONSIDER
• Which electrophoretic features are unique for a heavy chain protein?
• What alternative methods can be used to confirm the presence of a heavy chain protein?
• Would your diagnostic laboratory be able to recognize a heavy chain?

Reference

1. Kaleta E, Kyle R, Clark R, Katzmann J. Analysis of patients with γ-heavy chain disease by the heavy/light chain and free light chain assays. Clin Chem Lab Med 2014;52:665–9.

Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the May 2019 issue of *Clinical Chemistry*. To view the case and comments online, go to http://www.clinchem.org/content/vol65/issue5 and follow the link to the Clinical Case Study and Commentaries.

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