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Clinical Case Study

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, 1.6×10^9/L (reference, $4.0–10.0 \times 10^9$/L); and thrombocytes, 156×10^9/L (reference $150–400 \times 10^9$/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.

QUESTIONS TO CONSIDER

1. Which electrophoretic features are unique for a heavy chain protein?
2. What alternative methods can be used to confirm the presence of a heavy chain protein?
3. Would your diagnostic laboratory be able to recognize a heavy chain?

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 $(IgG_\kappa + IgG_\lambda)/IgG_{total}$ 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, IgG_κ = 4.4 g/L, and IgG_λ = 4.9 g/L). Furthermore, the γ-HC concentration could be estimated as follows: $[\gamma\text{-HC}] = [IgG_{total}] - [IgG_\kappa] - [IgG_\lambda]$. 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).

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.

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[4] Nonstandard abbreviations: SPE, serum protein electrophoresis; IFE, immunofixation electrophoresis; γ-HC, IgG heavy chain M protein; CE, capillary electrophoresis; IS, immunosubtraction; HCD, heavy chain disease; MGUS, monoclonal gammopathy of unknown significance; EQA, external quality assessment.

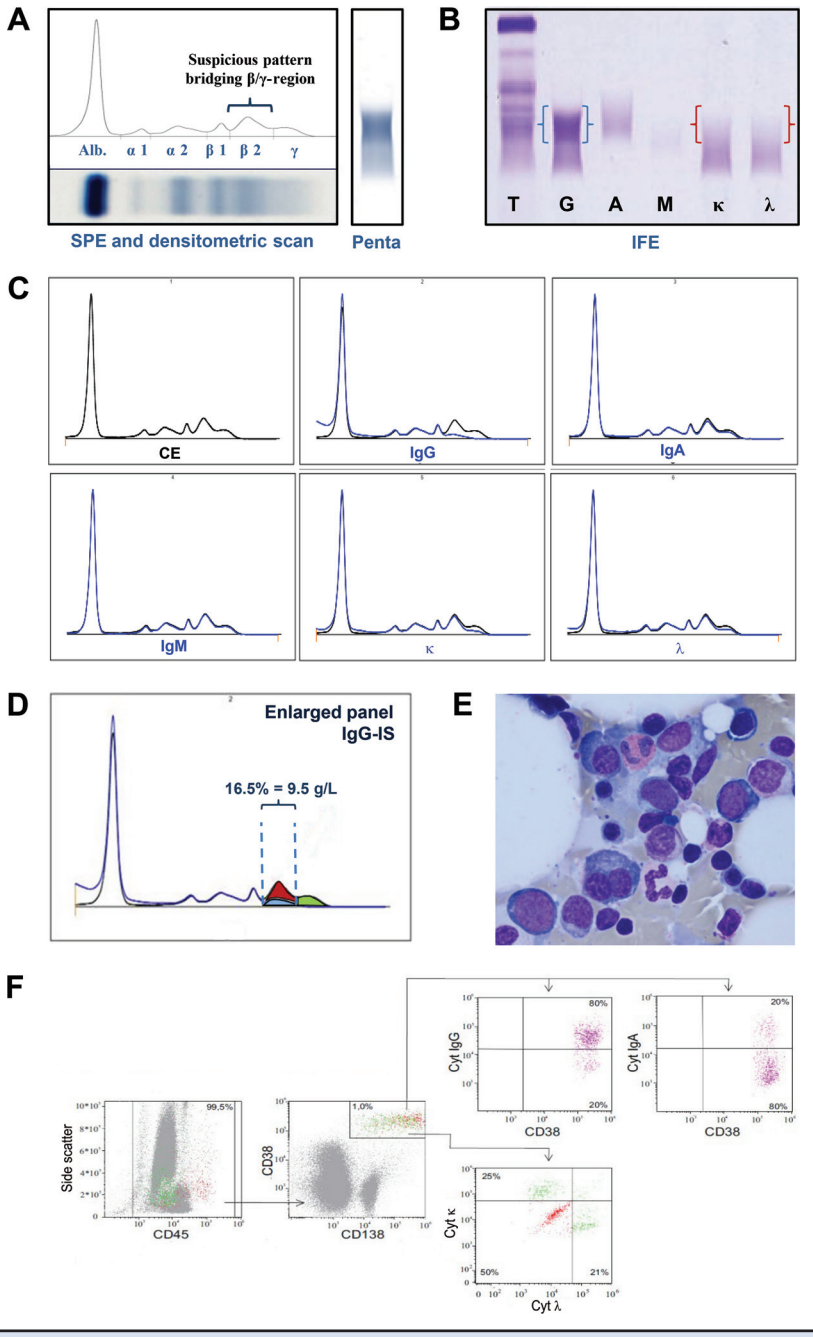

Fig. 1. Laboratory findings associated with γ-HC.

(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).

DISCUSSION

This patient was diagnosed with an asymptomatic γ-heavy chain disease (HCD) as an incidental finding during the workup of a bicytopenia. The γ-HCD was regarded as a monoclonal gammopathy of unknown significance (MGUS) that will be periodically monitored.

HCDs are rare B-cell proliferative disorders defined by the production of monoclonal heavy chains (α, γ, or μ) without associated light chain in serum and/or urine *(2)*. HCDs are caused by mutations in the constant-1 domain of the IgG heavy chain protein. This domain is responsible for binding to the light chains; therefore, mutations result in loss of binding of both light chains. Furthermore, the altered structure of the constant-1 domain cannot bind anymore to heat shock protein 78. This prevents heat shock protein 78-mediated degradation of the heavy chain, which leads to relatively high concentrations of the heavy chain protein *(3)*. There are 3 types of HCDs, and these are defined by the type of heavy chain: IgA (α-HCD), IgG (γ-HCD), and IgM (μ-HCD). All of these HCDs are associated with, or represent a variant of, a B-cell neoplasm. Clinical manifestations depend on the type of HCD and range from asymptomatic to very aggressive lymphomas *(4)*.

γ-HCD, also known as Franklin disease, is a very rare disorder, with approximately 130 cases described in literature since 1964 *(5)*. In 57% to 66% of patients, γ-HCD typically presents as a disseminated lymphoproliferative disease with lymphadenopathy and constitutional symptoms *(6, 7)*. Approximately 25% of patients present with lymphoma limited to the bone marrow or with localized extranodal disease most commonly involving the skin. Also, 9% to 11% of patients have no evident lymphoplasmacytic disease at the time of diagnosis. Most patients in this group do have an underlying autoimmune disease, which was also the case with the patient presented here *(4, 7)*.

The diagnosis of γ-HCD is established with serum electrophoretic methods and requires the detection of a monoclonal heavy chain without associated light chains. Interpretation of the electrophoretic data can be challenging for several reasons. First, a γ-HCD is rare and the laboratory specialist may not be familiar with the unique features of a heavy chain. Second, the γ-HC typically presents as a broad band, which could be suggestive of the presence of polyclonal protein *(1, 8)*. Third, the γ-HC often migrates as a low-concentration M protein in the β, and even α_2, region, where it could be partly masked by other serum proteins within the α_2/β region *(2, 8)*. Finally, IFE provides only indirect evidence in which the absence of light chains is indicative of free heavy chain. However, this requires that alternative explanations for the missing light chains be excluded because poor reactivity of reagents with light chains or the antigen-excess phenomenon may all lead to failure to detect light chains *(8, 9)*.

Table 1. EQA results of patient serum with γ-HC.

Reported M protein	Number of labs (%)
IgG heavy chain	29 (45%)
Abnormal pattern[a]	7 (11%)
IgG-κ M protein	1 (2%)
IgG-λ M protein	1 (2%)
No M protein reported	26 (41%)
Total	64 (100%)

[a] Mostly described as IgG M protein with unknown light chain.

Because of these analytical challenges, we advise confirming each heavy chain with at least 1 alternative analytical method to IFE that, if needed, is outsourced to a specialized laboratory. In this report, we confirmed the γ-HC using CE with IS *(10)*, the Hevylite immunoassays *(1)*, and multicolor flow cytometry on a bone marrow aspirate *(7)*. It is important to note that the clonal plasma cells could not be detected using immunohistochemistry. An additional approach is to confirm the presence of the heavy chain using the recently described mass spectrometry MASS-FIX method *(9)*.

Although γ-HCD is a rare disease and possibly difficult to recognize, we wondered whether clinical laboratories in fact might overlook or wrongly classify the diagnosis. To study this, serum of our patient was collected within a 1-month period and analyzed by 64 laboratories that participate in the Netherlands in the Dutch External Quality Assessment (EQA) program on M-protein diagnostics. Only 29 of 64 participants correctly reported the presence of the γ-HC (Table 1). Seven laboratories reported an abnormal pattern of IgG M protein with unknown or absent light chains. However, 41% of the laboratories did not detect any M protein, and 2 laboratories misclassified this M protein. The 29 laboratories that correctly identified the γ-HC reported a γ-HC concentration ranging from 1 to 21 g/L. The mean reported γ-HC concentration was 3.2 g/L, which is a strong underestimation of our established concentration of approximately 6 g/L. These data suggest that it is likely that HCDs are underdiagnosed in routine clinical practice, partly because laboratory specialists are not familiar with the unique electrophoretic features of a heavy chain. Moreover, quantification of the heavy chain protein is completely nonharmonized and extremely inaccurate. Whether participating laboratories in this EQA screened for an M protein using gel electrophoresis or CE made no significant difference with regard to the reported outcome of both detection rate and quantification of the heavy chain.

The consequence of missing the diagnosis of a γ-HCD is that such patients may be insufficiently screened for un-

POINTS TO REMEMBER

- HCDs are rare B-cell proliferative disorders, and diagnosis requires the detection of a monoclonal heavy chain without associated light chains.
- Recognition of the unique features of a heavy chain protein may be challenging, with all results suggesting a heavy chain protein requires confirmation using an alternative method (IFE combined with immunoselection, CE with IS, Hevylite testing, flow cytometry, and mass spectrometry).
- HCDs are most likely underdiagnosed in routine clinical practice. We observed that <50% of participants in the Dutch EQA program on M-protein diagnostics correctly identified a γ-HC (6 g/L). Quantification of a heavy chain protein is extremely nonharmonized.

derlying disseminated or localized lymphoproliferative disease and autoimmune disease. In addition, the finding of a γ-HC MGUS justifies more intensive monitoring than recommended for a low-risk MGUS. In general, prompt diagnosis of a symptomatic HCD is important because early disease may be cured with therapy based on the underlying clinicopathologic features. EQA programs may create awareness of the unique features of a heavy chain and thereby increase the detection rate of this rare type of M protein.

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Commentary

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This case study highlights a number of important considerations for external quality assessment (EQA) programs and laboratories. Those who interpret serum protein electrophoresis (SPE) and immunofixation electrophoresis (IFE) must be aware of rare diseases and how they present analytically. Once recognized, interpreters must understand how to evaluate and confirm these rare findings. For EQA programs, this case study exemplifies the benefits of using challenging samples to appraise the capability of laboratories.

γ-Heavy chain disease (γ-HCD) is demanding for interpreters for several reasons. Based on the very low prevalence of γ-HCD, even interpreters in high-volume laboratories may only see less than one case per decade. This may be compounded by the potential lack of available clinical information either as a reference testing laboratory or before the diagnosis is made. The authors aptly highlight several analytical challenges that may contribute to lack of recognition by interpreters, including the broad band appearance and α-2/β migration pattern.

The national proficiency testing (PT) survey results strongly support the need for education in this area. It would not be surprising to find similar results in other areas

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