Diagnostic Challenges in γ-Heavy Chain Disease: A Case of Overestimated IgG Concentration

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CASE DESCRIPTION

A 66-year-old male presented with a longstanding history of warm autoimmune hemolytic anemia (AIHA), requiring intermittent immunosuppressive therapy since 2011, complicated by splenomegaly and followed by splenectomy. Over time, he experienced multiple relapses refractory to rituximab, azathioprine, and danazol, with intermittent partial responses to corticosteroids and a transient hematologic improvement on a trial of parsaclisib, a PI3K-δ inhibitor, in 2019. Despite therapy, he remained prednisone dependent. In early 2025, he developed fatigue, a 25-pound unintentional weight loss, and small-volume abdominal lymphadenopathy and hepatomegaly on computed tomography scan.

Initial laboratory testing showed a serum total protein of 7.5 g/dL (Abbott Alinity, Biuret method; reference interval [RI], 6.0–8.0 g/dL) and an albumin level of 3.8 g/dL (Abbott Alinity, bromcresol green colorimetric method; RI, 3.5–5.0 g/dL), resulting in a γ gap of 3.7 g/dL. Quantitative immunoglobulin testing by immunoturbidimetry (Abbott Alinity) revealed markedly elevated IgG at 7383 mg/dL (RI, 700–1600 mg/dL), suppressed IgA at 48 mg/dL (RI, 60–400 mg/dL), and IgM <6 mg/dL (RI, 50–300 mg/dL). The reported IgG concentration appeared physiologically disproportionate relative to the measured total protein and γ gap, raising concern for analytical overestimation of IgG rather than falsely decreased total protein or albumin measurements, which were obtained using colorimetric methodologies less susceptible to paraprotein-related interference.

Serum free light chain analysis by immunoturbidimetry (Optilite®, The Binding Site) showed a markedly elevated free κ light chain concentration of 983.1 mg/L (RI, 3.3–19.4 mg/L) and a decreased free λ light chain concentration of 4.0 mg/L (RI, 5.7–26.3 mg/L), resulting in a markedly abnormal κ/λ ratio of 245.8 (RI, 0.26– 1.65). Although these findings initially suggested a clonal κ-restricted process, the degree of elevation was later recognized to be discordant with the remainder of the electrophoretic and mass spectrometric findings, raising concern for possible analytical interference.

Serum protein electrophoresis (SPEP; Hydragel B1-B2, Sebia) revealed 2 restricted bands in the α and β regions (M-spikes: 0.43 g/dL and 2.49 g/dL, respectively) with suppression of the γ fraction (Fig. 1B). Serum immunofixation electrophoresis (IFE; Hydragel 9IF, Sebia) showed 2 IgG heavy chain bands: a prominent band in the β region and a faint band in the α region, both without associated light chains (Fig. 1D). Urine protein electrophoresis

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(Hydragel B1-B2, Sebia) and immunofixation (Hydragel 9IF, Sebia), with antisera specific for both free and bound light chains, demonstrated similar patterns.

To exclude heterophile antibody interference as the cause of the overestimated IgG concentration, the patient's serum was serially diluted (1:25, 1:50, and 1:75), and immunoglobulin testing was repeated. The results were linear, with analyte recovery within 10%, making heterophile interference unlikely. The sample was then sent to Mayo Clinic Laboratories for immunonephelometry immunoglobulin quantitation (Siemens BNII), which also showed elevated IgG at 6920 mg/dL (RI, 767–1590 mg/dL), suppressed IgA at 38 mg/dL (RI, 61–356 mg/dL), and IgM <5 mg/ dL (RI, 37–286 mg/dL). Thus, the marked discordance between the reported IgG concentration and measured total protein persisted across different analytical platforms and methodologies, supporting a reproducible assay-related phenomenon rather than isolated instrument-specific error. It is important to note that other preanalytical and analytical factors, such as sample turbidity, particulate matter, and assay-specific methodological limitations, may contribute to spurious immunoglobulin results; however, these were not evident in the present case.

To further characterize the abnormal protein, the sample was analyzed by MASS-FIX, a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based immunotyping assay (1) (Fig. 2B). This approach combines nanobodies against IgG, IgA, IgM heavy chains, and κ and λ light chains in 5 different immunopurification wells and measures the accurate mass of the immunoglobulin light chain associated with each. Mass spectra were acquired over an *m/z* range of 4000 to 32 000 and reviewed in a composite. An isolated IgG heavy chain signal was identified without corresponding κ or λ light chain peaks. No monoclonal IgA or IgM species were detected. The IgG spectrum demonstrated multiple reproducible peaks in the approximately 13 to 14 kDa and 26 to 28 kDa regions, with an additional single broad peak between 18 and 19 kDa. These findings fall within a lower mass range than expected for intact IgG heavy chains and are consistent with truncated heavy chain species. The repeating pattern across charge states supports the presence of related molecular forms, potentially including monomeric and higher-order species. While reduction with tris(2-carboxyethyl)phosphine used in MASS-FIX is intended to dissociate immunoglobulin complexes, reduction may be incomplete in cases with high concentrations of abnormal immunoglobulin species. Under these conditions, residual multimeric forms can persist and contribute to the observed spectral pattern.

Bone marrow biopsy showed extensive lymphoplasmacytic infiltration, consistent with a small mature B-cell neoplasm with plasmacytic differentiation. Immunohistochemical studies confirmed IgG heavy chain expression. Most cells lacked light chain expression, with rare (<5%) κ-positive cells. The findings are consistent with γ-heavy chain disease (γ-HCD).

QUESTIONS TO CONSIDER	
1.	What mechanisms could explain the discordance between extremely elevated serum IgG and free κ light chains vs normal total protein levels in γ-HCD?
2.	How can analytical interference from truncated IgG heavy chains or immune complexes affect the accuracy of common immunoglobulin assays, including immunoturbidimetry, immunonephelometry, and mass spectrometry-based methods?
3.	What strategies or alternative methods are reliable for monitoring disease burden when standard IgG measurements are unreliable?

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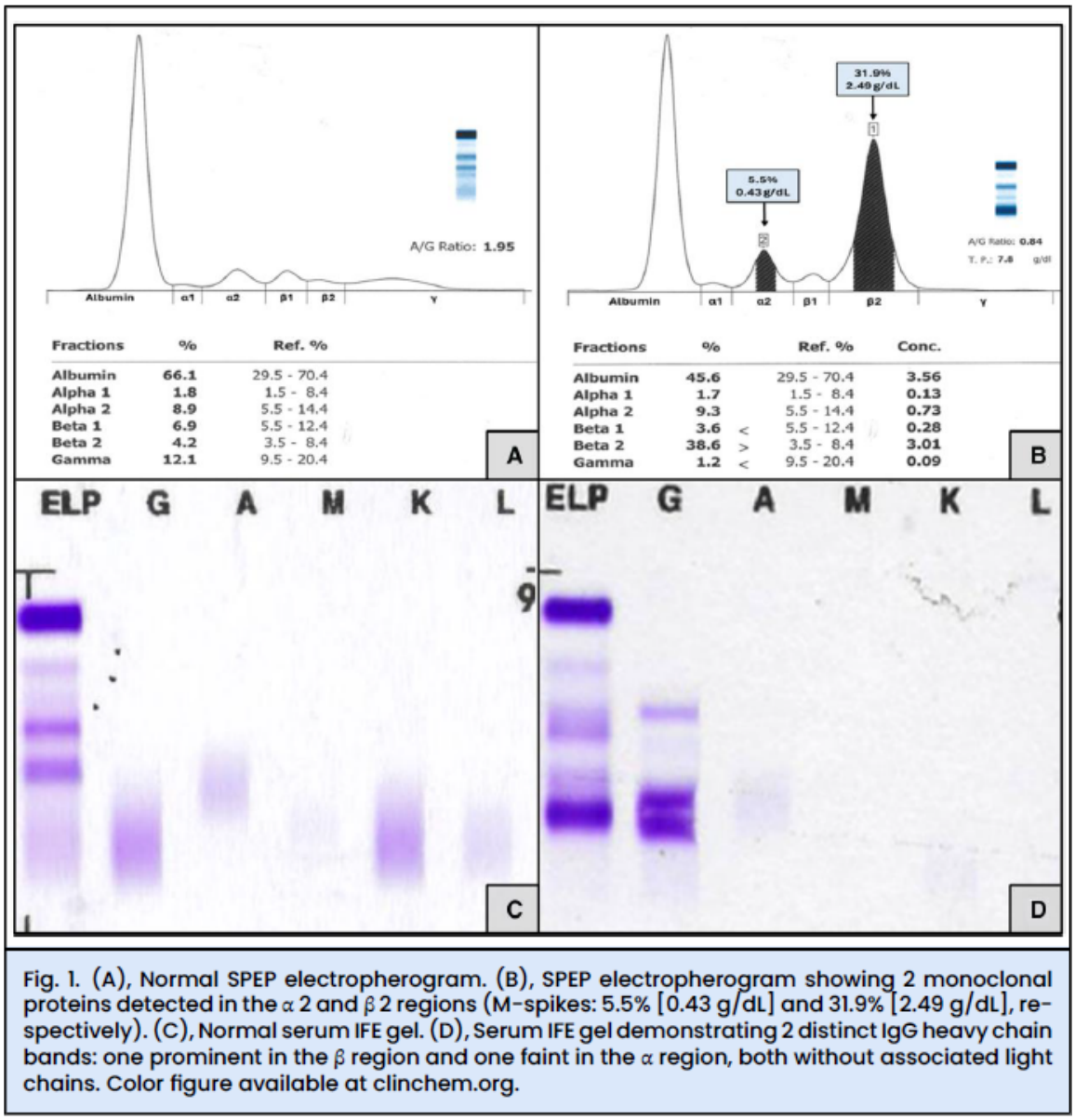

Fig. 1. (A), Normal SPEP electropherogram. (B), SPEP electropherogram showing 2 monoclonal proteins detected in the α 2 and β 2 regions (M-spikes: 5.5% [0.43 g/dL] and 31.9% [2.49 g/dL], respectively). (C), Normal serum IFE gel. (D), Serum IFE gel demonstrating 2 distinct IgG heavy chain bands: one prominent in the β region and one faint in the α region, both without associated light chains. Color figure available at clinchem.org.

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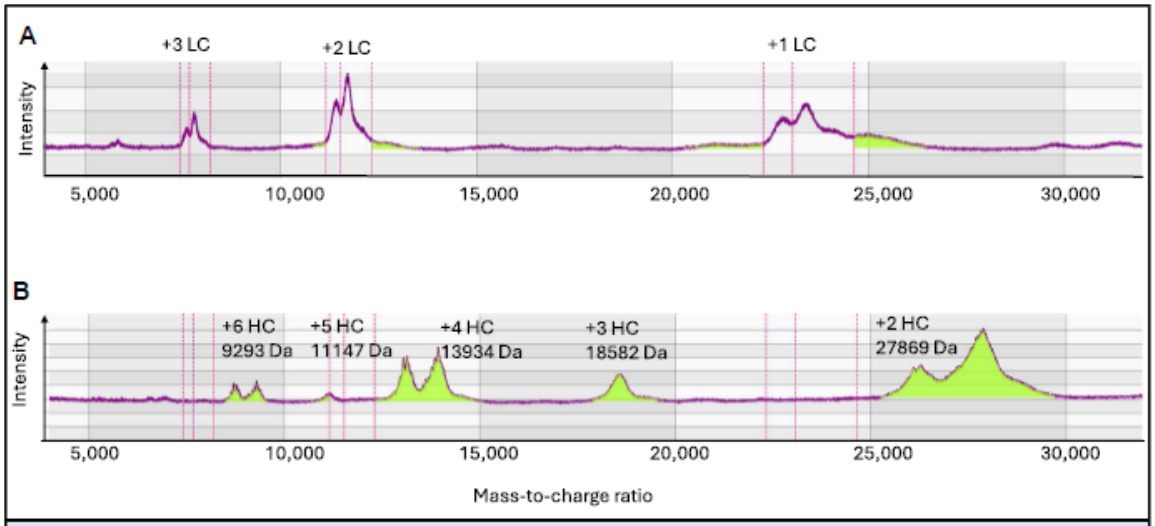

Fig. 2. (A), Normal IgG MASS-FIX immunopurification spectrum. (B), MASS-FIX immunotyping shows an isolated γ-heavy chain (HC) species captured with anti-IgG Fc nanobodies; no monoclonal proteins were detected with IgA, IgM, κ, or λ nanobodies. Spectra span 4000–32 000 Da (mass-to-charge, *m/z*). Magenta lines represent expected λ and κ light-chain (LC) mass range windows. Truncated IgG HC appeared as monomers or higher-order forms at +2 to +6 charge states. Intact heavy-chain mass is approximately 55 738 Da; MALDI-TOF accuracy is ±10 Da. Color figure available at clinchem.org.

REFERENCE

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Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the October 2026 issue of *Clinical Chemistry*. To view the case and comments online, go to https://academic.oup.com/clinchem/issue/72/10 and follow the link to the Clinical Case Study and Commentaries.

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