Clinical Biochemistry 53 (2018) 77–80

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Diagnostic implications of enumerating and reporting beta fraction(s) for the detection of beta-migrating monoclonal immunoglobulins in serum protein electrophoresis

Pak Cheung Chan[a,b,*], Bonny Lem-Ragosnig[a], Jialun Chen[b,1]

[a] Department of Clinical Pathology, Sunnybrook Health Sciences Centre, Ontario, University of Toronto, Ontario, Canada
[b] Department of Laboratory Medicine and Pathobiology, University of Toronto, Ontario, Canada

ARTICLE INFO

Keywords:
Serum protein electrophoresis
Monoclonal gammopathy
Beta-gamma bridge
Immunofixation electrophoresis

ABSTRACT

Background: Modern serum protein electrophoresis (SPE) resolves serum proteins in 6 major fractions, splitting beta (B) into beta1 (B1) and beta2 (B2). Beta-migrating monoclonal immunoglobulins (B-MC) commonly integrate into normal-looking peak shape(s) but may increase the fraction value, forming the basis for reflex testing. The objectives of this study were (1) to ascertain the value of beta fraction(s) reporting, and (2) to compare the diagnostic performance between different beta-flagging approaches, particularly ↑B versus ↑B1 and/or ↑B2.

Methods: We retrospectively studied 22,900 consecutive SPEs, and identified 3974 paired SPE and immunofixation electrophoresis (IFE) results obtained from the Sebia Capillarys™ 2 and Hydrasys™ electrophoresis systems respectively. ↑B, ↑B1 and ↑B2 were defined as fraction concentrations > 11, 6 and 5 g/L respectively, and their respective diagnostic metrics calculated using IFE as the reference standard.

Results: 32 beta-gamma bridges were B-MC negative and thus excluded. Of 3942 cases, 142, 18, 285 and 300 had ↑B, ↑B1, ↑B2 and ↑B1 and/or ↑B2 respectively, while their corresponding sensitivities for B-MC were 0.38, 0.09, 0.45 and 0.54 respectively. Comparing ↑B and ↑B1 and/or ↑B2, ↑B had significantly lower sensitivity but higher specificity (0.98 Vs 0.95) and positive predictive value (0.47 Vs 0.31). All 4 beta fraction increases had odds ratios ranged from 14 to 118.

Conclusion: Beta increases were associated with increased odds for B-MC, hence providing value and justification for their reporting and reflex testing. ↑B1 and/or ↑B2 detected more B-MC than ↑B, supporting separate reporting of B1 and B2.

1. Introduction

Serum protein electrophoresis (SPE) is a biochemical technique central to the investigation of Monoclonal Gammopathy, detecting and quantifying monoclonal immunoglobulin or its components (MC). As the technique evolved over the years, the resolution of serum proteins has also improved from 5 (single beta) to 6 (often referred to as "high-resolution" SPE) major protein fractions, namely albumin, alpha1, alpha2, beta1 (B1), beta2 (B2) and gamma (see Fig. 1). For detection of MC, high-resolution SPE has long been the recommended approach [1], although analytical systems that produce 5 fractions can still be found being used occasionally nowadays. The 6-fraction high-resolution systems improved the beta (B) resolution and thus increased the chance of revealing an MC not masked by normal B proteins. In the absence of a readily discernible MC, an increased B (↑B), ↑B1 and/or ↑B2 fractions may suggest the presence of an MC intermixed with background proteins. This is possible only if the fraction is enumerated and compared to a reference interval, a practice that is far from unanimous. The resultant ↑B, ↑B1 and/or ↑B2 fractions are often subjected to special commenting and further testing e.g. by immunofixation electrophoresis (IFE) and/or other methods. Indeed, a previous pan-Canadian report on SPE practice showed that 69% of the surveyed laboratories would comment the finding on patient reports and recommend or conduct further testing [2]. On the other hand, these increased B fractions can also be caused by increases in other B migrating proteins such as transferrin, beta-lipoprotein, hemopexin, complements, and polyclonal immunoglobulins. While the use of 5-fraction analytical systems is becoming obsolete, the reporting of 5 fractions, even though a 6-fraction

* Corresponding author at: Department of Clinical Pathology, Sunnybrook Health Science Centre, B204, 2075 Bayview Avenue, Toronto, ON M4N 3M5, Canada.
E-mail address: pc.chan@sunnybrook.ca (P.C. Chan).
[1] Recipient of a UROP award for summer research from University of Toronto.

https://doi.org/10.1016/j.clinbiochem.2018.01.021
Received 30 July 2017; Received in revised form 7 January 2018; Accepted 26 January 2018
Available online 31 January 2018
0009-9120/ © 2018 The Canadian Society of Clinical Chemists. Published by Elsevier Inc. All rights reserved.

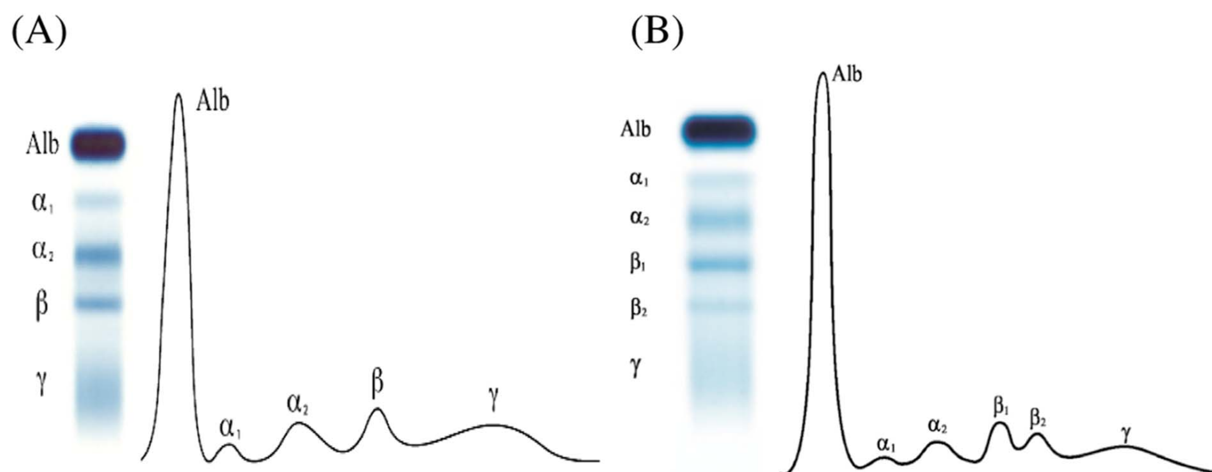

Fig. 1. SPE systems resolving serum proteins into 5 fractions ("low-resolution") (A) and 6 fractions ('high-resolution') (B).

electrophoretic system is being used, is surprisingly prevalent, partly because of historic reasons. The influence of external quality assurance (EQA) requirements for reporting 5 fractions in common schemes such as the College of American Pathologists (CAP) and Institute for Quality Management in Healthcare (IQMH) in Canada may, perhaps, be the most significant driving forces. Moreover, it is not clear how useful it is to report SPE fraction values, let alone the difference between reporting 5- and 6-fractions i.e. one versus 2 B sub-fractions in terms of MC detection. Therefore, the objectives of the current study were (1) to ascertain the value of beta fraction(s) reporting, and (2) to compare the diagnostic performance between reporting single B and its sub-fractions in a 6-fraction SPE.

2. Materials and method

We conducted a retrospective study covering 22, 900 consecutive SPE performed between January 2010 and June 2013 at our institution, a 1200-bed tertiary care facility with Oncology as one of the 6 focus areas. After the removal of repeat episodes, 3974 paired SPE and IFE results were available for analysis. Most of these paired SPE and IFE came from simultaneous requests from physicians. Details of this cohort have recently been described in another study examining hypogammaglobulinemia reporting practices [3]. SPE and IFE techniques were performed on the Sebia Capillarys™ 2 and Sebia Phoresis™ Electrophoresis Systems respectively. All SPE patterns and IFE were reviewed by two independent reviewers. In SPE, beta-gamma bridge is considered present when the nadir between beta2 and gamma fraction is much higher than that of the nadir between beta1 and beta2 according to the consensus judgement of the two reviewers.

Each protein fraction on SPE was integrated as the area under the curve all the way to the baseline i.e. x-axis and its protein concentration in g/L was computed as the percentage of the total area multiplied by the total serum protein concentration as per manufacturer's protocol. Serum protein concentration is determined using a Biuret method on the Roche Modular® P analytical system. The reference intervals of each SPE fraction represented roughly the central 95% limits (subject to rounding errors) of a local healthy population and were determined during the pre-implementation phase of the analytical technique more than a decade ago. The reference intervals for single B, B1 and B2 were 5–11, 3–6 and 2–5 g/L respectively, and increased beta (↑B), beta1 (↑B1) and beta2 (↑B2) were defined as the respective fraction concentrations being > 11, 6 and 5 g/L respectively. IFE was used to confirm the presence of MC in beta region and served as the reference test for the purpose of calculating diagnostic metrics such as sensitivity, specificity, positive and negative predictive values, and odds ratio using a 2 × 2 contingency table analysis (i.e. by proportions) conducted on the GraphPad Instat v3.0. Differences in proportions (sensitivity, specificity, positive and negative PP) were tested using the "N-1" Chi-squared test [7,8] and $p < 0.05$ was considered significant. The confidence intervals of the differences in proportions were calculated by the method of Altman [9]. The difference in sensitivity for detecting beta-migrating MC (B-MC) between ↑B and ↑B1 and/or ↑B2 (↑B1/↑B2) was further tested by the McNemar's test on all B-MC positive subjects as defined by IFE. A p-value < 0.05 is considered significant and the McNemar's test was conducted using the EP Evaluator® Release 11 from Data Innovations LLC.

3. Results

Of the 3974 paired SPE and IFE results, 1276 (32.1%) were IFE positive. There were 150 (3.8%) with ↑B, 19 (0.5%) ↑B1 and 305 (7.7%) ↑B2. Thirty two out of 3974 (0.8%) cases had beta-gamma bridges, with 3 IFE positives all in non-beta regions. Since the flagging of increased beta fractions was anticipated to affect the detection of only B-MC and that beta-gamma bridges were clearly not associated with any B-MC, all beta-gamma bridge cases which may cause beta increases, were thus excluded, leaving 3942 cases available for further analysis. After excluding these beta-gamma bridges, the IFE positive rates for ↑B (> 11 g/L), ↑B1 (> 6 g/L), ↑B2 (> 5 g/L) and ↑B1 and/or ↑B2 were 47.2% (67/142), 83.3% (15/18), 27.7% (79/285) and 31.0% (93/300) respectively. Results are summarized in Table 1.

Based on these 3942 paired SPE and IFE, diagnostic sensitivities of ↑B, ↑B1, ↑B2, and ↑B1 and/or ↑B2 were 0.38, 0.09, 0.45 and 0.54 respectively. Specificity, positive and negative predictive values, as well as odds ratios were also calculated (see Table 2 for details). Comparing to ↑B, ↑B1 and/or ↑B2 had a significantly higher sensitivity (McNemar's test and 'N-1' Chi-squared test, $p < 0.001$) and detected 93 B-MC as compared to only 67 in ↑B. ↑B1 and/or ↑B2, however, had a significantly lower specificity (0.95 Vs 0.98) and positive predictive value (0.31 Vs 0.47) ('N-1' Chi-squared test, $p < 0.001$), while the negative predictive value (0.98 Vs 0.97) was not significantly different ('N-1' Chi-squared test, $p > 0.05$). 95% confidence intervals (CI) estimated for each of these diagnostic parameters or metrics also confirmed these observations.

Every beta increase i.e. ↑B, ↑B1, ↑B2, and ↑B1 and/or ↑B2 was associated with increased odds for B-MCs, with odds ratios ranging from 14 in ↑B2 to 118 in ↑B1 (all had the lower 95% CI above 1). See Table 2 for details.

4. Discussion & Conclusion

The major beta (B1 and B2) migrating proteins in SPE include

Download English Version:

https://daneshyari.com/en/article/8317003

Download Persian Version:

https://daneshyari.com/article/8317003

Daneshyari.com