Peer Review File

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Reviewer Comments
Reviewer A

Comment 1
Thank you for asking me to review this article on approach to the serum protein abnormalities. The review is interesting and fills an important niche in the literature. However, there are some areas that need attention. In particular, I sense that the authors are laboratory experts, and their attention to this topic is fantastic, but there are a number of inaccuracies in the clinical application that need to be fixed.
Reply 1: Thank you, we will try and balance it better (indeed we are primarily laboratory based).

Comment 2: Major comment: hyper vs hypogammaglobulinemia not a useful distinction – polyclonal hypergammaglobulinemia vs monoclonal paraprotein, or monoclonal gammopathy, is what clinicians and laboratory scientists need to care about.
Response: Thank you for your valuable comments. We were trying to be simple first and then try to spread out to more specific examples but your point is, of course, accurate. Hypogammaglobinemia, was felt to be important to make sure no one misses the opportunity to diagnose immunodeficiency. Also, we felt clinically, there might be a lot of situations where immunoglobulins are measured in unwell patients with a range of symptomatology as an extended "screen" and so we are trying to make sure clinicians can then interpret the results in relation to the situation they find themselves in (even if the results are not what they were expected). We have added subheadings to make it clear that distinguishing polyclonal and monoclonal hypergammaglobulinaemias is very important.

Comment 3:
101 There are five classes of immunoglobulins, although only three are routinely
102 measured. The five classes are as follows, in descending concentration order:
103 immunoglobulin G (IgG), immunoglobulin A (IgA), immunoglobulin M (IgM),
104 Immunoglobulin D (IgD), Immunoglobulin E (IgE) (25).
Minor comment: it would be helpful to give example reference ranges here for each.
Response 3: example reference ranges have been included.

Comment 4:
140 the rest include
141 enzymes, carrier proteins, complement and more. Immunoglobulins are
142 synthesised by the plasma cells, the latter are synthesised by the liver (4). There is
143 poor consensus on reference range limits therefore are often local decided e.g.,

20-
144 39 g/L, or no lower limit (41). As a consequence, primary hypogammaglobulinemia
145 can take five to six years to diagnose despite a low calculated globulin fraction (41-
146 45). A few studies have suggested that a globulin less than 18 g/L (children ≥ten
147 years and not under haematology or oncology review) should indicate screening for
148 hypogammaglobulinemia (41, 42). There does not appear to be a well-defined cut149 off for adult patients.
Minor comment: This section is very confusing – please define globulin fraction. Why is it difficult to have a normal range?
Response 4: Thank you. We have reviewed this section and hope we have added some clarity to the point we were trying to make and have referred the readers to the figure, which might help.

Comment 5: 152 Immunoglobulin methods, 153 Minor comment: Change to " measurement methods for serum immunoglobulins"
Response 5: Thank you, changed.

Comment 6: Major comment: I am very surprised that mass spectrometry is not mentioned in this section. Mass spectrometry is increasingly important both for measurement of monoclonal paraproteins and IgG subclasses, and requires some discussion here (Zajec et al., 2020, van der Gugten et al., 2018, Murray et al., 2021)
Response: Apologies, from a UK hospital perspective we have not encountered anyone using mass spectrometry for this purpose and it is not available to us (mass spec primarily localized to reference laboratories mainly testing other analytes e.g. steroids, drugs). We are, though, trying to write an article that is useful internationally so thank you for letting us know that it is being used elsewhere. We will make the assumption that most laboratories across the world will be using older, assays on automated platforms but we have included a comment for those who do have access to staff and equipment to use mass spec for this role.

Comment 7: Box 2 – use the updated 2014 IMWG SLiM CRAB criteria (Rajkumar et al., 2014)
Response 7: thank you box 2 of figure 6 has been altered, and we have also referenced in the text.

Comment 8: Box 3 – age groups (50-59 years, etc). Also myeloma, B cell lymphoma most common
Response 8: I am so sorry but we don't understand this point? Do you mean this box should also include myeloma etc even if don't meet the CRAB criteria?

Comment 9: Table 5 and related text:

448 Hyperproteinaemia
449 Total protein concentration of 81 g/L and above are considered to be
450 hyperproteinaemic (1). Causes include dehydration, inflammation, autoimmune
451 disease and bone marrow dysfunction (table 5) (151).
The concepts of monoclonal gammopathy of renal significance (Leung et al., 2021) and monoclonal gammopathy of clinical significance (Schnitzler's, TEMPI, etc) (Marinkovic et al., 2022) should be mentioned and briefly discussed.
Response 9: Thank you, I have reiterated the renal damage and referenced Leung. I have also briefly expanded the section on end organ damage caused by protein deposition. Our focus is to present a diagnostic algorithm but we can see the relevance of the damage caused by hyperproteinaemia. The separate clinical entities have been added to table 5.

Comment 10: Minor comment: In Table 5, change "bone marrow dysfunction" to "plasma cell neoplasms and B cell lymphomas"
Response 10: Thank you, changed

Comment 11: Table 6: Major comment: be very careful here how you use the cutoff of a paraprotein > 30 g/L for myeloma vs < 30 g/L for mgus. This is a rule of thumb, but there are many, many exceptions:
1) IgA myeloma is often 15-20 g/L
2) Light chain myeloma (which accounts for 15 % of myeloma) may only demonstrate a trace band in the SPEP.
3) Patients with cryoglobulinemic myeloma may have a falsely decreased band when SPEPs are run below 37C as the paraprotein may partly precipitate out
4) Some patients even with classic IgG myeloma may present with a relatively low paraprotein - after all, 1% of myeloma is non-secretory.
Response 11: Thank you for these great points. Table 6 has been modified. Thanks again.

Comment 12: 527 Non-haematological malignancies that may have polyclonal hyperglobulinaemia
Major comment: the term "polyclonal hyperglobulinaemia" should be corrected to "polyclonal hypergammaglobulinaemia" throughout the manuscript.
Response 12: Thank you, I have made the changes.

Comment 13: IgG subtypes can be individually raised e.g. IgG4 in an fibroinflammatory disorder,
532 which may present with a raised total protein, calculated globulin fraction,
533 polyclonal IgG and symptoms such as lymphadenopathy, eosinophilia, etc. (161).
534 The existence of this condition is still highly debated and while some recognise this
535 as a unified disease other professionals do not. It is difficult to diagnose due to non536

specific symptoms and it remains a relatively rarely diagnosed disease (168).
Major comments: 1) Lymphadenopathy and eosinophilia are not symptoms, they are objective physical exam and laboratory findings, respectively
Response 13: excellent point, apologies for that inaccuracy, we have corrected.

Comment 14: 2) The existence of IgG4-RD is not debatable. One may as well say the existence of myeloma or lupus is debatable! Yes, it is a new entity, but it is a very well defined clinicopathological entity (Lanzillotta et al., 2020, Chen et al., 2019).
Response 14: thanks, the sentence has been removed

Comment 15: 3) Consider that there are ethnic differences – serum IgG4 in IgG4-RD tends to be higher in Asians than non-Asians (Harkness et al., 2020, Qi et al., 2018)
Response 15: Thank you, comment added

Reviewer B
Comment 1: Overall, this article is a thorough review of various causes and disorders associated with hyper- and hypo- proteinemias, albuminemias, and globulinemias. The authors provide detailed figures/tables and diagnostic algorithms for considerations for patients with abnormal protein measurements. Below are minor suggestions for enhancing readability for the readers and clarifications at certain points in the article. Some of the language appears to repeat through the text and some of it may be eliminated to reduce lengthiness.
Response 1: Thanks so much, we agree it is not as concise as it could be. We have gone through and tried to remove repetition and keep it as brief as possible.

Comment 2: Line 89 – recommend rewording to enhance readability and edit from "it is not normal to see a urinary albumin…" to something like "and in health, a urinary albumin creatinine ratio is less than 3mg/mmol".
Response 2: Thank you this is a better way of phrasing it, changes made.

Comment 3: Line 90 – recommend rewording slightly since only one function is listed in the text.
Response 3: Thank you, sentence amended

Comment 4: Line 101 – Please list the three that are routinely measured.
Response 4: done

Comment 5: Line 128 – Moderate to marked hemolysis and lipemia can also cause interference in total protein measurement and might be worth mentioning since these are common occurrences in the laboratory setting.
Response 5: A good point, thank you. Added

Comment 6: Line 142 – add B cells and plasma cells
Response 6: Thank you, amended

Comment 7: Line 171 – recommend rewording “small bands” to low concentrations of monoclonal proteins
Response 7: Thank you, amended.

Comment 8:Line 177 – define Bence jones proteins
Response 8: We have now included a definition
Comment 9: Line 179 – Did the authors find a sensitivity or negative predictive value associated with these tests? If so, can you provide these for the readers?
Response 9: added sensitivity and specificity rate from the referenced paper

Comment 10: Line 195 – Recommend adding that urine protein electrophoresis can characterize urinary proteins and is often utilized to detect Bence jones proteins.
Response 10: Amended

Comment 11: Line 254 – delete statement in parentheses. Can edit to 11g/L (i.e., 1.1g/dL) if needed.
Response 11: Deleted

Comment 12: Line 303 – Did the authors find any proposed articles for why albumin decreases with aging? Could it be related to underlying inflammation/inflammatory conditions that often occur in the elderly?
Response 12: Thank you, we have amended this sentence, so our intended statement is clearer. What we intended to say was how this age-related decline may be due to reduced nutrition in the older population or more likely a result of an underlying pathology

Comment 13: Line 308 – change “drop” to decrease
Response 13: Thank you, amended

Comment 14 Line 317-319 are similar to a previous statement used earlier
Response 14: Thank you, found, removed duplication

Comment 15: Line 320 – delete “the” between blood the draw
Response 15: Apologies – modified

Comment 16: Line 459 – did the author find any references that indicate if coritcosteroids can induce hyperalbuminemia?
Response 16: we put in the reference for hypogammaglobinaemia (a review paper which includes a few different references for hypogammaglobinaemia related to steroids) but I don’t think we said it caused hyperalbuminaemia so apologies if we have missed that somewhere. With a quick look I could not find that corticosteroids caused hyperalbuminaemia.

Comment 17: Additionally, did the author find any case series of hepatocellular carcinomas causing hyperalbuminemia?
Response 17: only in a dog so we did not include it in the article. We could find a few papers of hepatocellular carcinoma causing hypo but not hyper albuminaemia.

Comment 18: Line 537 – The reviewer does not agree with the terminology of bone marrow dysfunction. This would refer to a particular disorder that implies abnormal hematopoiesis in the marrow and shouldn't be used in the context of hyperglobulinemia.
Response 18: Thank you, I can see what you are saying. I have amended this to plasma cell neoplasms and B-cell lymphoma. I hope this is a better subtitle.

Comment 19: Table 2: Delete "8" in bromocresol purple dye factors section
Response 19: Apologies - deleted

Comment 20: Table 6 – The term "clonal" should be added when referring to the proportion of plasma cells.
Response 20: Thank you, amended