Peer Review File

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Reviewer 1

Comment 1: Line 22 – since both B cell and plasma cell disorders can produce a gammopathy, substituting 'lymphoproliferative disease' for 'plasma cell disorders' is recommended.

Reply 1: We agree with the reviewer that a gammopathy can be produced by other disorders apart from plasma cell disorders. However, our study was primarily focused on plasma cell disorders and therefore 'plasma cell disorders' is used in the manuscript.

Changes in the text: No changes in the text.

Comment 2: Line 62-63: All tests are expected to have a false-positive and false-negative rate. This sentence would be more useful if it provided the actual reported rates.

Reply 2: We agree with the reviewer. As we revisited reference 5 (Huang et al, J Clin Lab Anal 2014), the paper actually refers to false positive screening rates when using capillary zone electrophoresis (61.3%) and agarose gel electrophoresis (31.3%) based on the gold standard of immunofixation. On further considerations, we now feel this sentence is no longer relevant to our manuscript and it has now been removed.

Changes in the text: The text "Serum protein electrophoresis involves subjective interpretation and may be associated with false-positive results (5)" has been removed.

Comment 3: Line 46-49 and elsewhere – this conclusion overstates the data obtained in this study. The evaluated population excludes repeat testing in patients with lymphoid neoplasia so the data cannot be generalized to patients with lymphoid neoplasia, as this sentence would reasonably be interpreted. Additionally, most, ~ 68%, of the repeat

testing was performed within 6 months of the initial testing; the study cannot describe how many of these individuals would have been positive at 1 year without testing them at that time.

Reply 3: We agree that the current conclusion is an overstatement. We have narrowed it to patients without lymphoid neoplasia within our study, and changed the time period in our conclusion from one year to six months.

Changes in the text: In this pilot study, repeating serum protein electrophoresis within six months after a negative result generally returns a negative result in patients without lymphoproliferative disorder. A larger cohort with longer follow-up period may clarify if these are spurious observations or clinically progressive.

Comment 4: Line 82-84. Further description of the clinical indication for testing is recommended. Repeat testing because the same clinical indication was present later would be different than repeat testing because a second clinical indication developed. Likely the low sample size and large number of clinical indications will complicate this evaluation.

Reply 4: For 56% of the repeat requests the clinical indication for serum protein electrophoresis was the same as for the initial request. Following the reviewer's comment, we now feel that figure 1 is no longer useful and therefore it has been removed.

Changes in the text:
Under 'Methods':
The date and result of the serum protein electrophoresis test, patient demographics, clinical indication of the initial and repeat serum protein electrophoresis requests were extracted from the laboratory information system and electronic medical records. The clinical indications were broadly categorised into: abnormal serum globulin and/or total immunoglobulins, proteinuria and/or albuminuria and/or haematuria, abnormal peripheral blood smear, anaemia and/or leucopenia and/or thrombocytopenia, chronic kidney disease and/or acute kidney

injury, anaemia with chronic kidney disease and/or acute kidney injury, hypercalcaemia, previously abnormal serum protein electrophoresis and/or serum free light chains, osteoporosis and/or osteolytic bone lesions, skin changes, peripheral neuropathy, malignancy and/or autoimmune conditions.

Under 'Result'

For 56% of the repeat requests the clinical indication for serum protein electrophoresis was broadly the same as for the initial request.

Comment 5: Line 89 and 98 – The decision to exclude individuals with known lymphoid neoplasms should be reconsidered. As I understand the indications for this test in humans, this would exclude the most common indication and the most likely reason for development of a monoclonal gammopathy. If individuals were initially negative and developed a gammopathy, that would still be clinically relevant and should be investigated. If the authors would like to separate these groups, it seems reasonable to stratify the data by the presence of a known lymphoid neoplasm and/or treatment status but exclusion of this indication and then generalization to all indications seems inappropriate.

Reply 5: We thank the reviewer for the comments. However, the primary objective of this pilot study is to investigate the positive rate of repeat serum protein electrophoresis testing after a previous negative serum protein electrophoresis result, in patients without prior history of plasma cell and/or lymphoproliferative disorders – as this was an area with relative paucity of evidence. We have considered the comment by the reviewer and carefully avoided over-generalisation of our findings (kindly see above).

Changes in the text: No changes to the text.

Comment 6: Line 96 - The age of the patient population is summarized with a simple mean value of the entire patient population but the age distribution of the repeat testing group is not documented. Since the incidence of gammopathy increases with age, patient

age would reasonably be considered a variable when considering the need for testing. The age of the repeat testing group should be provided and the provided data should allow a better depiction of the age range, including min-max mean at a bare minimum. It would be clinically relevant to consider the relative risk of conversion to a positive test result in various age strata but the current study population is likely too small to be able to detect any of these differences with accuracy.

Reply 6: We agree with the reviewer on age-related risk of monoclonal gammopathy. We have now included the age of the repeat testing group: the minimum age was 18 years and the maximum age was 90 years.

Changes in the text: During the 23-month study period, a total of 4101 serum protein electrophoresis tests were performed in 2730 patients (median age, 69 years; range, 18-98 years).

Comment 7: Line 109 – while the data shows that 3 of the 160 converted to a positive result within the first year, this does not translate to 98% of the patients being negative at 1 year after initial testing. Most of the patients were not retested at 1 year. Please provide the conversion rate for each of the re-evaluation strata or provide some other means of accurately reporting the conversion rate. Additionally, the precision of the observed values (ie 95% CI) should be provided, as suggested in STROBE 16a. Given the low number of cases that are evaluated, I expect that this CI will be fairly wide.

Reply 7:

We agree with the reviewer's interpretation of the results. We have rephrased our sentence to mention that the precise conversion rate cannot be determined in this study owing to the small sample size and a lack of standardised retesting intervals. To further illustrate this point, we have constructed a new figure (Figure 1) depicting the cumulative retesting rate versus retesting intervals for serum protein electrophoresis.

Changes in the text: In this pilot study, only three of the 160 patients without prior history of plasma cell and/or lymphoproliferative disorders had positive repeat serum protein electrophoresis after an initial negative result. The precise conversion rate cannot be determined in this study owing to the small sample size and a lack of standardised retesting intervals. A larger prospective cohort with protocolised repeat testing and a longer follow-up period is required to document the clinical progression of this group of patients.

Comment 8: Line 112 – please confirm that this limit of detection has been evaluated in your lab or provide reference to an external (and non-manufacturer) source for this limit of detection. In my lab, we use similar equipment and have confirmed by spike and recovery the 0.3 g/dL limit of detection reported by Gwatherny et al 2015 EJIFCC.

Reply 8: The limit of 1 g/L for the quantitation of serum monoclonal protein is based on recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand by Tate and colleagues (Ann Clin Biochem 2012;49:242-56). We have now included a citation in the manuscript.

Changes in the text: The agarose gel electrophoresis system has a lower reporting limit of 1 g/L for serum monoclonal protein quantification as recommended by the Working Party on Standardised Reporting of Protein Electrophoresis of the Australasian Association for Clinical Biochemists (5).

Comment 9: 142-143 – Please clarify or reference support that a low M-protein concentration is of unclear clinical significance. The statement appears to be a simplification of the significance in human medicine, as it would be for veterinary medicine. The concentration of M-protein is only relevant in light of other clinical findings.

Reply 9: We agree that the significance of a low monoclonal protein concentration should be interpreted in light of other clinical findings.

Changes in the text: A larger prospective cohort with protocolised repeat testing and a longer follow-up period is required to document the clinical progression of this group of patients.

Comment 10: Line 149-157 – please better explain how this data corroborates and supports the prior published data. At best, we see 3 patients followed for ~ 16 months.

Reply 10: We agree the limited data in our pilot study are not strong basis for corroborating and supporting prior published data.

Changes in the text: The following text has been removed: "Our findings corroborated with prior studies where it is estimated that 56% of women 70 years of age diagnosed as having monoclonal gammopathy of undetermined significance have had the condition for more than 10 years, including 28% for more than 20 years (6). Corresponding values for men are 55% and 31%, respectively."

Reviewer 2

Comment 1:

Figure 1:

-Consider labeling the x axis with units. It is presumed to be percent but not explicitly defined.

-I believe "leucopenia" is more commonly spelled as "leukopenia".

Reply 1: Following the reviewer's comment, we now feel that figure 1 is no longer useful and therefore it has now been removed. We agree that "leukopenia" is more appropriate.

Changes in the text: The clinical indications were arbitrarily and broadly categorised into: abnormal serum globulin and/or total immunoglobulins, proteinuria and/or albuminuria and/or haematuria, abnormal peripheral blood smear, anaemia and/or leukopaenia and/or thrombocytopaenia, chronic kidney disease and/or acute kidney injury, anaemia with chronic kidney disease and/or acute kidney injury, hypercalcaemia, previously abnormal serum

protein electrophoresis and/or serum free light chains, osteoporosis and/or osteolytic bone lesions, skin changes, peripheral neuropathy, malignancy and/or autoimmune conditions.

Comment 2:

Discussion:

-The authors may wish to address whether their findings would support a utilization management initiative to reduce repeat SPE testing in the case of recent negative results. SPE is a costly laboratory test involving significant manual and professional labor. Would cancellation of repeat tests or incorporating an approval process for repeat test requests be appropriate? For example, others have incorporated order strategies for monoclonal test request workups to address over utilization (https:// pubmed.ncbi.nlm.nih.gov/31776551/).

Reply 2: We thank the reviewer for the comments. Incorporating an approval process for repeat test requests within a time period would be reasonable to consider, in conjunction with the hospital physicians. However, as noted by reviewer 1, this pilot study is not powered enough to make conclusive statement regarding the utility of repeat SPE. We would like to keep be more conservative and avoid direct recommendation/ common on a utilization management initiative to reduce SPE testing in the case of recent negative results.

Changes in the text: No changes in the text.

Comment 3: Based on the authors review of repeat test requests and clinical indication, the authors may wish to comment if they have hypotheses regarding why repeat testing is requested in their practice setting. For example, the initial result may not be available to the provider ordering repeat testing, the clinical picture has changed for the patient suggesting repeat testing may be warranted, the provider does not trust the findings of the original result. Strategies for addressing excess test utilization may differ depending on the trigger for repeat test requests.

Reply 3: We thank the reviewer for the suggestions. At least a third of repeat test requests

were ordered within 12 weeks of the initial negative test. Possible reasons include the requestor for the repeat test may not be aware of the initial result, the provider does not trust the findings of the original result. For the tests that were requested after one year of the initial negative test, a possible reason is that the clinical picture may have changed for the patient suggesting repeat testing may be warranted. However, these causes were not directly solicited nor confirmed with the clinicians, we wish to avoid speculating the causes of repeat testing.

Changes in the text: No changes in the text.