The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

A case of clinical confusion due to erroneous M-protein quantifications: to splice or skim?

Pak Cheung Chan[1,2], Amir Karin[3,4], Signy Chow[5,6]

[1] *Division of Clinical Biochemistry, Department of Laboratory Medicine and Molecular Diagnostics, Sunnybrook Health Sciences Centre, Toronto, Canada*
[2] *Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada*
[3] *Division of Clinical Chemistry, Department of Pathology and Laboratory Medicine, Vancouver Coastal Health, Vancouver, Canada*
[4] *Department of Pathology and Laboratory Medicine, The University of British Columbia, Vancouver, Canada*
[5] *Division of Hematology, Department of Medicine, Sunnybrook Health Sciences Centre, Toronto, Canada*
[6] *Department of Medicine, University of Toronto, Toronto, Canada*

ARTICLE INFO

Corresponding author:
Pak Cheung Chan
Division of Clinical Biochemistry
Department of Laboratory Medicine
and Molecular Diagnostics (DLMMD)
Sunnybrook Health Sciences Centre (CG76A)
2075 Bayview Ave
Ontario M4N3M5
Canada
E-mail: pc.chan@utoronto.ca

Key words:
serum protein electrophoresis, M-protein quantification, perpendicular drop, tangential skim, monoclonal gammopathy

ABSTRACT

An M-protein identified on electrophoresis is conventionally quantified by integrating the M-spike from baseline (PD), invariably including some irrelevant/background proteins. The use of an alternative approach that skims the M-spike tangentially thereby excluding the background proteins (TS), however, has been scanty. We report herein a case in which PD overestimated the M-proteins inconsistently, leading to confusion over relapse in a multiple myeloma patient.

At diagnosis, a 65-year old male had an IgG kappa M-spike of 44 g/L which decreased to 6 g/L (PD) following chemotherapy. Six weeks after autologous stem cell transplantation (ASCT), two M-spikes measuring respectively 10 and 5 g/L emerged. Together with decreases in hemoglobin and blood cell counts, a relapse was suspected. Bone marrow examinations, however, did not reveal any significant plasmacytosis or clonal restriction. Re-analyses by TS reduced the

original M-protein estimations by 12% and 88% pre- and post-ASCT respectively, and corroborated the disease activity/status consistently.

❖❖❖❖❖

Abbreviations

M-spike: monoclonal spike; PD: perpendicular drop (M-spike integration from baseline); TS: tangential skim (M-spike integration from a tangential line drawn between two inflection points); SPE: serum protein electrophoresis; CZE: capillary zone electrophoresis; AGE: agarose gel electrophoresis; MM: multiple myeloma; BM: bone marrow; ASCT: autologous stem cell transplant; ISS: International Staging System; RSV: Respiratory Syncytial Virus; Hb: hemoglobin.

❖❖❖❖❖

INTRODUCTION

Quantification of monoclonal immunoglobulins (M-proteins) by serum protein electrophoresis (SPE) plays an important role in the diagnosis, prognosis and management of monoclonal gammopathies such as monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma (MM), and Waldenström's macroglobulinemia [1-3]. It is conventionally performed by integrating the areas under the demarcated M-protein peak (M-spike) on the electrophoretogram all the way to baseline perpendicularly, hence referred to as the perpendicular-drop (PD) method. This approach invariably includes both the M-protein and uninvolved background proteins above baseline, leading to a variable degree of overestimation depending on the amount of background proteins present. While an alternative tangential-skim (TS) method that excludes most of the background proteins has been described and shown to be more reliable, especially at low M-protein concentrations [4-7], its adoption in clinical practice has remained surprisingly scanty [9-11]. We, herein, describe a case of clinical confusion over relapse and stem cell transplant failure in a multiple myeloma patient whose M-protein was monitored by PD. This case also exemplifies the susceptibility of PD to errors due to background proteins (mostly uninvolved polyclonal immunoglobulins), particularly when they change over the course of a monoclonal gammopathy.

CLINICAL-DIAGNOSTIC CASE

A 65-year-old male was referred to Hematology with a one-year history of bony pain, particularly in the left femur. An X-ray demonstrated osteolytic lesions and SPE demonstrated an IgG kappa M-protein of 46 g/L. His medical history also included thalassemia minor, gout, hypertension and hernia. Myeloma survey confirmed osteolytic/sclerotic lesions on left femur and evidence of involvement of other areas including the left tibia, right clavicle, right femur head, left T1 vertebral body and right proximal tibia. MRI provided further evidence of myelomatous infiltrations within the marrow with additional focal lesions at T8, T10 and T11. Initial blood work at our centre showed significant anemia (Hb 109 g/L), an IgG kappa M-spike of 44 g/L (PD integration on the Sebia Capillarys II Electrophoretic System), reduced IgA and IgM, elevated beta2-microglobulin at 3 mg/L and a grossly elevated free kappa to free lambda ratio of >100 (The BindingSite). Bone marrow examinations reported 10% clonally-restricted plasma cells and cytogenetic studies detected translocations between chromosomes 11 and 14, t(11, 14). A diagnosis of stage 1 (ISS) MM was formally made. He received prophylactic intramedullary nailing of the femur, followed by radiation therapy. He was treated with 4 cycles of chemotherapy comprising of Cyclophosphamide, Bortezomib and Dexamethasone (CyBorD) with a plan for autologous stem cell transplant (ASCT). He achieved a partial response with the M-spike decreased to 6 g/L and a normal free light chain

ratio of 1.64 (0.26-1.65). ASCT was then arranged at a regional referral centre. He unfortunately developed deep vein thrombosis requiring anticoagulation and his ASCT was delayed by one month. He received an additional cycle of CyBorD prior to transplant.

Four weeks after ASCT, he developed autoimmune hemolytic anemia, which was thought to be related either to medication (colchicine) or a viral infection (RSV). He also developed two flares of gout post-transplant. Six weeks after ASCT, routine lab monitoring at our centre showed a large increase in total IgG to 30 g/L (by immunoturbidimetry), and two M-spikes (both of the same original IgG kappa isotype) measured 10 and 5 g/L, respectively (PD), had emerged on SPE. The free light chain ratio increased to 5.64 while both RBC and platelet counts were decreased. This was initially attributed to a brisk immune reconstitution. As he was also monitored by the transplant centre at ten weeks post-transplant, the two increased M-spikes were confirmed at their laboratory using a gel-based electrophoresis system (Sebia Hydrasys Electrophoresis System); both demonstrated the same IgG kappa isotype as his original disease and measured 9.6 and 8.6 g/L (PD), respectively. By then, Hb had dropped to 67 g/L and platelet numbers to $72x10^9$/L. The clinical picture was somewhat confounded by another gout flare occurring at the same time as well as a new onset of scrotal bleeding. However, given the confirmation of M-protein increases at two institutions more than 4 weeks apart, a relapse post-transplant was suspected. He was started on second line treatment and a bone marrow aspirate and biopsy was performed a week later to confirm the suspected myeloma progression.

The bone marrow examination surprisingly revealed no significant plasmacytosis (1%) or clonal restriction. At the same time, SPE once again demonstrated two M-spikes at 9.5 and 8.3 g/L, respectively (gel-based, PD). Attempts to reconcile the conflicting findings led to discussions with the Clinical Biochemist overseeing electrophoresis, and resulted in re-analyses of all M-spikes originally quantified by PD since diagnosis using the alternative TS method. TS yielded much lower M-protein concentrations than PD; an average reduction of 12% and 88% pre- and post-ASCT, respectively. TS eliminated the M-spike overestimations due to background polyclonal immunoglobulins and gave consistent results that corroborated the disease status and other laboratory findings. See Table 1 for a summary of general laboratory findings. PD overestimated the M-protein concentrations inconsistently over the course of the disease as the background polyclonal immunoglobulin concentrations changed, particularly after ASCT. Selected electrophoretograms at different time points and the trending of M-protein concentrations are shown in Figure 1 and 2, respectively.

DISCUSSION

M-protein quantification plays an important role not only in diagnosing but also in prognosticating

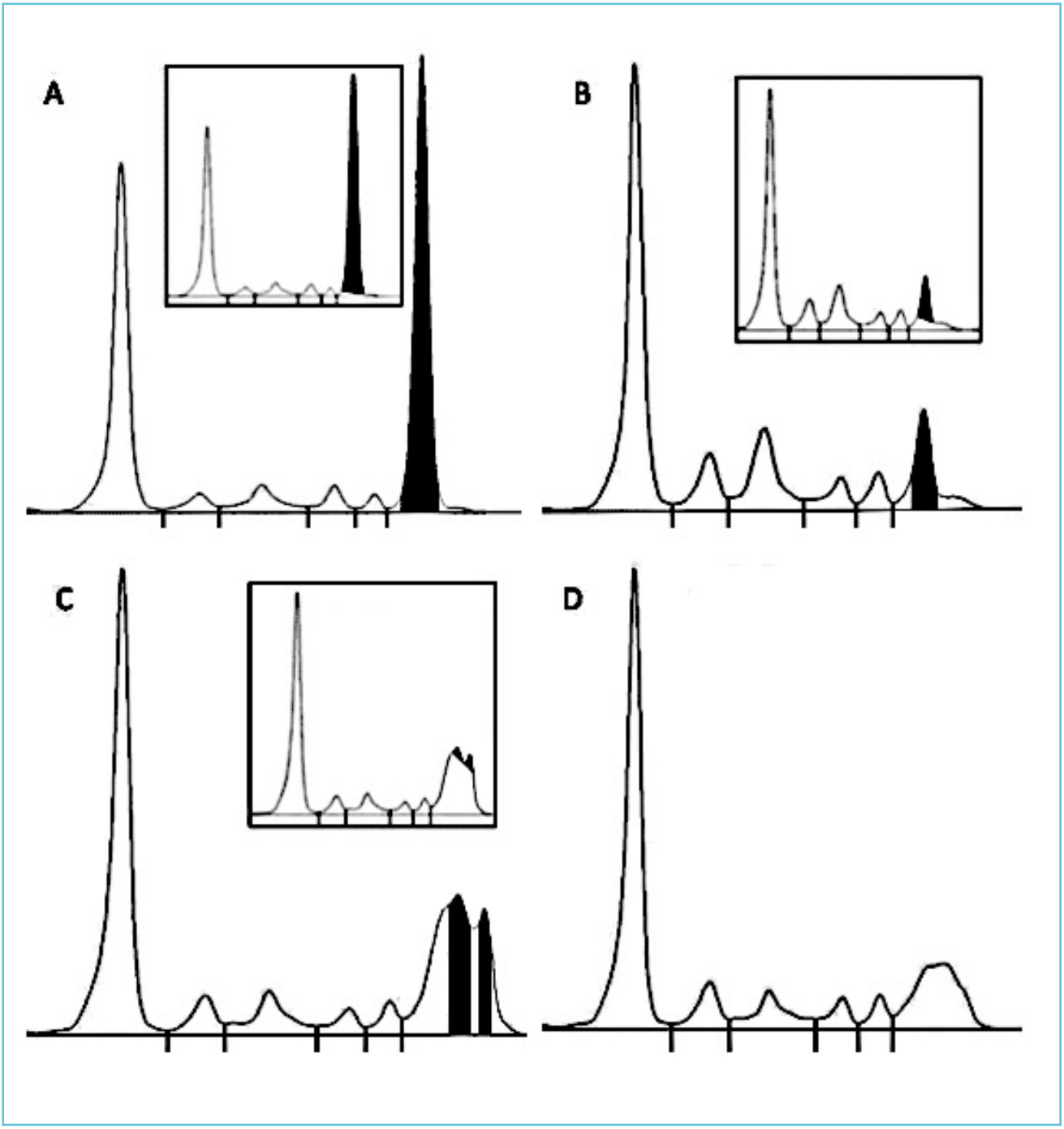
Table 1 Laboratory findings at selected time points following the diagnosis of multiple myeloma

Tests	At Diagnosis*	At ASCT	6 weeks post-ASCT	10 weeks post-ASCT	11 weeks post-ASCT# (BM exam)	Reference interval
Total Proteins, g/L	100	60	80	83	82	64-83

Albumin, g/L	36	36	37	34	34	35-50
Creatinine, µmol/L	98	64	74	72	74	44-106
LDH, IU/L	121	203	370	296	425	100-250
Hb, g/L	109	79	83	67	91	130-180
RBC, 10^{12}/L	5.18	3.75	3.59	2.85	3.77	4-6
WBC, 10^{9}/L	4.6	8.2	5.9	3.8	3	4-11
Platelet, 10^{9}/L	152	214	105	72	94	150-400
IgG, g/L	57.9	8.99	30.22	-	-	6.00-16.00
IgA, g/L	<0.2	0.23	0.4	-	-	0.70-4.00
IgM, g/L	0.14	0.21	0.4	-	-	0.40-2.50
sFLC						
FK, mg/L	212.5	10.5	98.2	-	-	3.3-19.4
FL, mg/L	<2.0	6.4	17.4	-	-	5.7-26.3
FK/FL ratio	>100	1.64	5.64	-	-	0.26-1.65
M-protein, g/L						
CZE (PD)	44.3	6	10.3, 5.4	-	-	ND
AGE (PD)	-	4.4	-	9.6, 8.6	9.5, 8.3	ND
M-protein, g/L (Re-analysis)						
CZE (TS)	43.4	4.9	0.8, 0.9	-	-	ND
AGE (TS)	-	3.6	-	1, 1	0.9, 1	ND

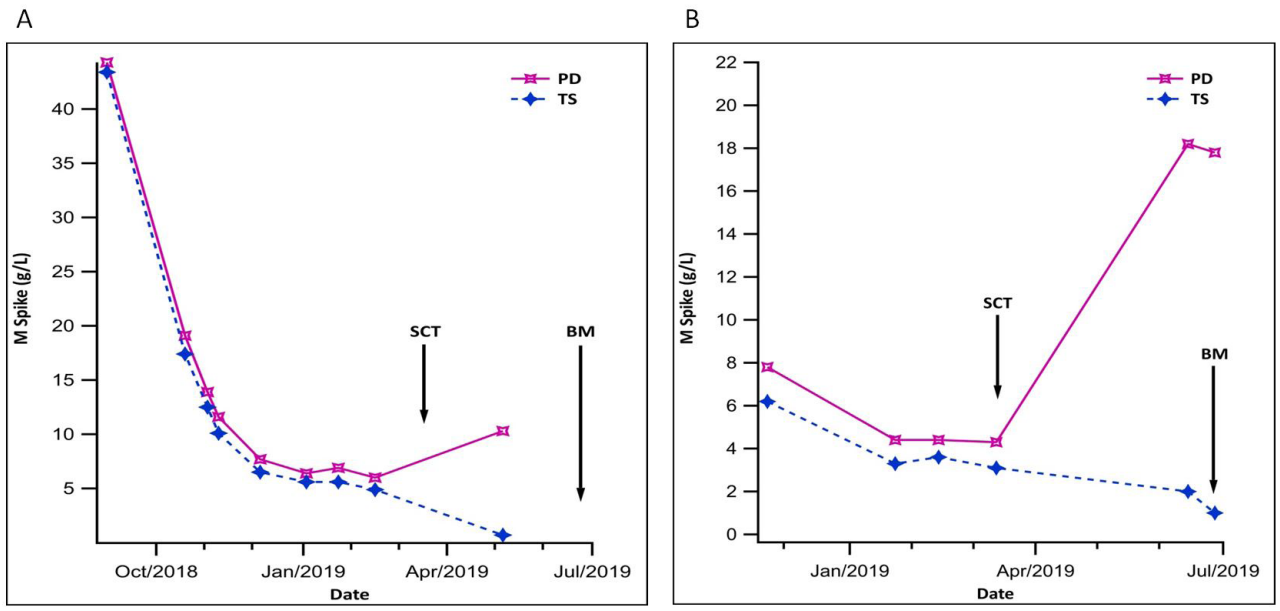
**Diagnosis confirmed with BM examination, followed by 5 cycles of Cyclophosphamide, Bortezomib and Dexamethasone; # Initiation of second line chemotherapy with Daratumumab, Lenalidomide and Dexamethasone; ASCT: autologous stem cell transplant; LDH: lactate dehydrogenase; Hb: hemoglobin; sFLC: serum free light chain assays; FK: free kappa light chain; FL: free lambda light chain; CZE: capillary electrophoresis; AGE: agarose gel electrophoresis; PD: perpendicular drop (the default method); TS: tangential skim; ND: not detected.*

Figure 1 **Electrophoretograms showing M-spike quantifications using both PD and TS methods at selected time points during the course of disease**



M-protein concentrations were estimated by demarcating the corresponding M-spikes (highlighted) using the perpendicular drop (PD) and subsequently the tangential skim (TS) (inset) methods at different time points during the course of treatment: (A) when a diagnosis of multiple myeloma was first established, (B) At autologous stem cell transplant (ASCT), (C) 11 weeks post-ASCT when a bone marrow (BM) examination was performed for the suspicion of disease progression, and (D) 15 weeks post-ASCT when the M-spikes were barely discernible. Major serum protein fractions from left to right were albumin, alpha1, alpha2, beta1, beta2 and gamma.

Figure 2 **Trending of M-protein concentrations as determined by PD and TS on two different electrophoretic systems**



(A) Trending of M-protein concentrations as determined by both PD and TS methods on a capillary electrophoresis platform at the home institution since diagnosis; (B) Trending of M-protein concentrations by both PD and TS methods using agarose gel electrophoresis at the referral centre where stem cell transplant (SCT) was performed. BM: bone marrow examination.

and monitoring monoclonal gammopathies. For instances, M-protein greater than 15 g/L is a predictor for disease progression in MGUS [12] while < 30 g/L in the absence of end organ damage defines MGUS and ≥ 30 g/L is considered smoldering or active MM, depending on the presence of end organ damage [1,2]. In patients with MM undergoing treatment, a 25 % decrease in M-protein concentration is considered as a minimal response, while a 50 % decrease as partial, and a decrease of 90 % or more as a very good partial response [2]. A measurable disease is defined as having a serum M-protein ≥ 10 g/L and/or an M-protein excretion in urine of ≥ 200 mg/24 h, and that it should be tracked by changes in the M-protein concentration [13].

While SPE is generally preferred over total immunoglobulin determinations for estimating the M-protein concentrations, most clinical guidelines do not explicitly specify which SPE integration method should be used [1-3]. Yet, drastic differences between PD & TS have been reported based on observations from patient studies [5, 12] as well as spiked sample analyses [6, 8]. These studies have highlighted the differential effects of background proteins (mostly polyclonal immunoglobulins in the gamma fraction of SPE) on M-protein estimations by PD and TS, with the former being most adversely affected when the background is high.

In disease diagnosis and monitoring, when the M-protein concentration is relatively high and/or the background protein concentration is low and stable, there is little clinical impact as the difference between PD and TS is minimal. However, when the background protein concentrations change during the disease course, the accuracy of the M-protein determination can be

jeopardized leading to confusion. In the current case, the patient had suppressed polyclonal immunoglobulins due to immune paresis at diagnosis but the uninvolved immunoglobulin concentrations increased, likely due to a combination of the successful ASCT and the inter-current conditions including, but not necessarily limited to, the gout flare-ups and the autoimmune hemolytic anemia. Moreover, changes in the concentrations of these uninvolved immunoglobulins during the course of a monoclonal gammopathy may occur more often than what we anticipate, and the causes are plenty: infections, autoimmune diseases, inflammatory conditions, SCT, as well as immune paresis caused by expanding tumors and/or chemotherapy. Unfortunately, as demonstrated in this case, these uninvolved immunoglobulins may affect the M-protein determination by PD to such an extent that the care providers can be misled into performing additional invasive investigations. TS is a simple but robust alternative that would obviate such scenarios without introducing extra cost in laboratory instrumentation or analysis. However, PD continues to be recommended by some authorities for routine M-protein determinations on the sole basis of a marginally improved reproducibility [13, 14]. This factor alone is unlikely to translate to clinical benefit, especially if the patient is monitored at the same institution.

In summary, we reported a case in which the PD method of M-protein determination produced erroneous results that led to clinical confusion, unwarranted investigations and an unnecessary change in the care path. The alternative TS approach was shown to produce results that were in line with the patient's clinical development and other laboratory findings such as the bone marrow examination results. While our case provided one particular scenario in which the use of TS could have prevented the confusion over relapse, the resistance of TS to the interferences of background proteins in general would provide more consistent and reliable M-protein estimations especially for disease monitoring, and should, thus, merit a wider acceptance into routine practice.

LEARNING POINTS/ TAKE-HOME MESSAGES

- Changes in concentrations of polyclonal immunoglobulins affect adversely the accuracy of the conventional M-protein quantification by PD in electrophoresis.
- Polyclonal immunoglobulin concentrations do change over the course of a monoclonal gammopathy, probably more often than we expect, and can cause erroneous M-protein quantifications, leading to confusion over disease activity and treatment response.
- TS requires no extra hardware or software for implementation, and remains one of the most reliable and accessible approaches for minimizing the effect of polyclonal immunoglobulins on M-protein quantifications.

❖❖❖❖❖

Disclosures & authors' contributions

Disclosures: No conflicts of interests declared by all authors.

Contributions:

Chan: Inception, data analysis, manuscript drafting & review; Amir: Data collection and analysis, manuscript review; Chow: Case discovery, manuscript review.

❖❖❖❖❖

REFERENCES

1. Bergstrom DJ, Kotb R, Louzada ML, Sutherland HJ, Tavoularis S, Venner CP. Consensus Guidelines on the Diagnosis of Multiple Myeloma and Related Disorders: Recommendations of the Myeloma Canada Research

Network Consensus Guideline Consortium. Clinical Lymphoma, Myeloma & Leukemia. 2020;20 (7):e352-67.

2. Lakshman A, Rajkumar SV, Buadi FK, et al. Risk stratification of smoldering multiple myeloma incorporating revised IMWG diagnostic criteria. Blood Cancer J. 2018;8:59

3. Kastritis E, Leblond V, Dimopoulos MA, et al. Waldenström's macroglobulinaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology. 2018;29 (Supplement 4): iv41–iv50

4. Keren DF, Schroeder L. Challenges of measuring monoclonal proteins in serum. Clin Chem Lab Med. 2016; 54(6):947–961.

5. Schild C, Wermuth B, Trapp-Chiappini D, Egger F, Nuoffer JM. Reliability of M protein quantification: comparison of two peak integration methods on Capillarys 2. Clin Chem Lab Med. 2008;46:876-877.

6. Turner KA, Frinack JL, Ettore MW, et al. An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part I: factors impacting limit of quantitation of serum protein electrophoresis. Clin Chem Lab Med. 2020;58:533–546.

7. Cárdenas Fernández MC, Pérez Surribas D, Pérez Garay R, et al. Vertical cutoff methods in serum protein electrophoresis for the measurement of monoclonal protein concentrations: Which is best?. Clin Chim Acta. 2020; 510:573-580.

8. Jacobs JFM, Turnera KA, Graziani MS, et al. An international multi-center serum protein electrophoresis accuracy and M-protein isotyping study. Part II: limit of detection and follow-up of patients with small M-proteins. Clin Chem Lab Med. 2020;58(4):547–559.

9. Genzen JR, Murray DL, Abel G, et al. Screening and Diagnosis of Monoclonal Gammopathies: An International Survey of Laboratory Practice. Arch Pathol Lab Med. 2018; 142:507-515.

10. Tate J, Graziani MS, Willrich M, Jacobs H, Moss M. Report of Survey conducted by IFCC WG Harmonisation of Interpretive Commenting EQA (WG-ICQA) subgroup: Results of an international survey of the reporting of protein electrophoresis and serum free light chains, and quantification of small monoclonal proteins. IFCC 2017

11. Wijeratne N, Tate JR, Wienholt L, Mollee P. Report of the Survey Conducted by RCPAQAP on Current Practice for Paraprotein and Serum Free Light Chain Measurement and Reporting: a Need for Harmonisation. Clin Biochem Rev. 2019;40:31-42.

12. Miller JJ, Taher J, Kulasingam V, Chan PC. To skim or splice? Comparing the quantification of M-proteins using two peak-integration protocols across multiple electrophoresis platforms. Clin Biochem. 2022:102:44-49

13. Dejoie T, Lakomy D, Caillon H, Pegourié B, Decaux O. IFM (Intergroupe francophone du myélome) recommendations for uniform interpretation of serum and urine protein electrophoresis in multiple myeloma diagnosis and follow-up. Ann Biol Clin (Paris). 2016;74:429-41.

14. Tate JR, Smith JD, Wijeratne N, Mollee P. Proposed Addendum to 2012 Recommendations for Standardised Reporting of Protein Electrophoresis in Australia and New Zealand. Clin Biochem Rev. 2019;40 (1):23-30.