MALDI-TOF-MS Application in M-Protein Detection and Its Associations with Clinical Laboratory Findings in Geriatrics

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ABSTRACT

Currently, the methods used to detect M-protein such as SPEP (serum M-protein electrophoresis), IFE (immunofixation electrophoresis), and sFLC (serum free light chain) assay are hyposensitive, laborious, time-consuming, and expensive. In this study, the authors assessed the performance of MALDI-TOF-MS in the detection of M-protein in geriatrics. With IFE as the gold standard, the specificity, sensitivity, and consistency of MALDI-TOF-MS were 92.30, 89.68, and 90.06%, respectively. MALDI-TOF-MS achieved an agreement of 100.00, 100.00, 89.68, 88.89, and 87.50% in the detection of IgA, biclonal, negative, IgM, and IgG isotypes of M-protein, respectively. In addition, the results showed that the glycosylated haemoglobin level was increased in the MS-IFE+ group as compared to the MS+IFE- group. This study supports that the MALDI-TOF-MS is an alternative method for M-protein detection in aged subjects. The difference in glycosylated haemoglobin level may provide an explanation for the different detection results for M-protein between IFE and MALDI-TOF-MS.

Key Words: *M-protein, MALDI-TOF-MS, Immunofixation electrophoresis, Clinical laboratory findings, The aged.*

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INTRODUCTION

Currently, serum M-protein electrophoresis (SPEP) combined with immunofixation electrophoresis (IFE), which is used to determine M-protein isotype, is the standard method for M-protein detection. Although serum free light chain (sFLC) assay being developed with increased sensitivity (SPEP, 100 mg/dL; IFE, 10 mg/dL; sFLC, 0.15 mg/dL),[1] combination of these methods is hyposensitive, laborious, time-consuming, and expensive.[2] Matrix-assisted laser desorption / ionisation-time-of-flight mass spectrometry (MALDI-TOF-MS) is a recently developed technology, for which the sample is prepared as a dried mixture, with an automated sample acquisition available of seconds.[3] Several studies have confirmed the feasibility of MALDI-TOF-MS in M-protein detection in both the serum and urine.[4,5] However, its application in identifying M-protein dysregulation of elderly population remains unclear.

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In the current study, the authors assessed the performance (sensitivity, specificity, and consistency) of MALDI-TOF-MS in M-protein detection in elderly. Also, these analysed the relationship between M-protein dysregulation detected by MALDI-TOF-MS and/or IFE and other clinical laboratory findings to explore the influencing factors of the different detection results by the two methods.

TECHNIQUE

The peripheral blood samples collected from 61 IFE positive (IFE+) and 95 IFE negative (IFE-) serum/blood samples were used to determine the cut-off value of κ: l detected by MALDI-TOF-MS. Additional 181 serum/blood samples were collected from 167 individuals who underwent a medical examination in the department of geriatrics to verify the performance of MALDI-TOF-MS in the detection of M-protein. The blood samples were centrifuged for 5 minutes at a speed of 2000 rpm and the supernatant (serum) was collected for M-protein detection. For the MALDI-TOF-MS assay, all spectra were acquired on QuanTOF (Intelligene Biosystems, China) with the following settings: Source voltage 19 kV, laser frequency 5 kHz, laser energy 8 mJ, scanning speed 2 mm/s, mass range 0-200,000 m/z, and 10-rows scan per spot. The mass spectrometric analysis speed by QuanTOF was about 15s per sample spot with the above settings. With the IFE results as a gold standard, the optimal cut-off for κ-type M-protein was 2.03, while it was 1.011 for the l-type M-protein. The specificity, sensitivity, and consistency of MALDI-TOF-MS in the detection of M-protein were 92.30, 89.68,

and 90.06%, respectively (Table I). It achieved an agreement of 100.00, 100.00, 89.68, 88.89, and 87.50% in the detection of IgA, biclonal, negative, IgM, and IgG isotypes of M-protein, respectively. Figure 1A demonstrated the significant difference in clinical laboratory findings between the groups, including anti-histone antibodies, glycosylated haemoglobin, lactate dehydrogenase (LDH), and fibrinogen degradation products (FDP). Throughly, the FDP level was increased and the red blood cell (RBC) level was decreased in the MS+IFE+ group compared to the MS-IFE- group. The glycosylated haemoglobin was significantly increased in the MS-IFE+ group compared to the MS+IFE- group (Figure 1B).

DISCUSSION

A change in the ratio of immunoglobulin κ: λ in the serum has been identified to be significantly associated with various disease statuses.[6] Detection of the ratio of κ: λ by MS in used to diagnose and monitor M-proteins and has been demonstrated to be a more sensitive biomarker of secretory neoplasia than IFE, allowing detection of the formerly deemed non-secretory cases.[7] Kohlhagen *et al*. demonstrated that the sensitivity and specificity of the MALDI-TOF-MS assay in M-protein detection were 95.8% with IFE results as references.[5] Mehra *et al*. reported the sensitivity and specificity of MALDI-TOF-MS in M-protein identification were 98.3 and 52.2%, respectively, in patients with plasma cell disease.[8] Herein, with IFE results as a gold standard, the specificity, sensitivity, and consistency of MALDI-TOF-MS in M-protein detection in geriatrics were 92.30, 89.68, and 90.06%, respectively. A prevalence of abnormal M-proteins in 5% of the population aged >50 as detected by MS had been reported,[9,10] while it was 19.16% (32/167) in the present cohort. Differences in age (mostly higher than 60 years) and disease status (most of the individuals were inpatient with some diseases) were the main reasons accounted for the difference, as well as the various sensitivity. The average analytical sensitivity of MS to detect and isotype a series of 27 M-protein positive patient samples demonstrated that Mass-Fix detected 60% of samples diluted in normal human serum while the IFE method detected 30%.[11]

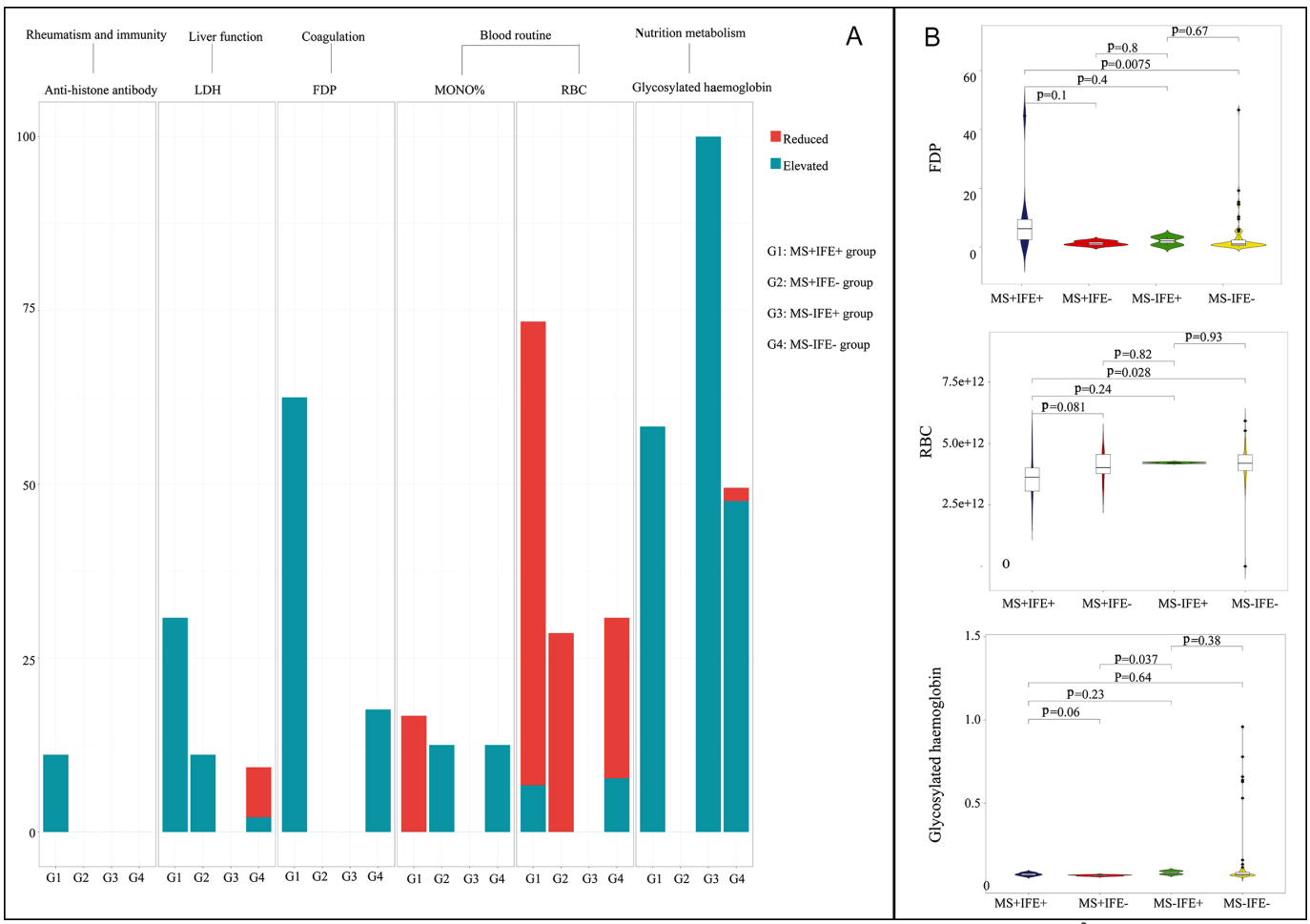

Figure 1: Factors associated with the M-protein detection results of MALDI-TOF-MS and IFE. (A) Chi-square (χ^2) / Fisher's exact tests demonstrated that the dysregulation proportions of glycosylated haemoglobin, FDP, RBC, LDH, and glycosylated haemoglobin showed significant difference between groups. (B) Mann-Whitney U test demonstrated the levels of FDP, RBC, and glycosylated haemoglobin showed significant difference between groups.

Table I: The specificity, sensitivity, and agreement of MALDI-TOF-MS and IFE in detection of M-protein.

Test method	SPEP+/IFE+	SPEP-/IFE+	SPEP-/IFE-
MALDI-TOF-MS+	15	9	16
MALDI-TOF-MS-	0	2	139
Sensitivity	92.30%		
Specificity	89.68%		
Agreement	90.06%		

Moreover, the current study clarified the relationship between M-protein dysregulation and other clinical laboratory findings to explore the influencing factors of the different detection results by the two methods. Results of this part demonstrated that the glycosylated haemoglobin level significantly increased in the MS-IFE+ group as compared with the MS+IFE- group. Glycosylated haemoglobin is a regular assessment index for long-term glycaemic management. The content of glycosylated haemoglobin is unusually accumulated in people with chronic hyperglycaemia, and it is positively associated with metabolic control.[12] The findings indicate that glycosylated haemoglobin level was related to the different detection results of MALDI-TOF-MS and IFE, although the underlying mechanism remains largely unknown.

CONCLUSION

This study reveals that the MALDI-TOF-MS is an alternative method for M-protein detection in geriatrics. In addition, the authors demonstrated that M-protein deregulation was associated with other clinical laboratory findings, especially of the glycosylated haemoglobin, which may provide an explanation for the different results for M-protein between IFE and MALDI-TOF-MS.

ETHICAL APPROVAL:

This study protocol was reviewed and approved by the Ethics Committee of Shanghai Ninth People's Hospital, Shanghai, China (Approval No: SH9H-2022-T16-2).

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

JC, YZ, MC, HW: Study conception and design.
YS, XL: Material preparation, data collection, and analysis.
All authors approved the final version of the manuscript to be published.

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