

Detection of free light chain monoclonal proteins co-migrating with intact monoclonal proteins in patients with monoclonal gammopathy

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Abstract

Background: In a small, but potentially significant proportion of patients with a monoclonal gammopathy, patients show the existence of an intact monoclonal (M-) protein co-migrating with a free light chain (FLC) M-protein. Using traditional methods for detection of monoclonal immunoglobulins, only the intact M-protein may be detectable, and hence the FLC M-proteins may be missed.

Methods: Immunofixation electrophoresis (IFE) using two different sets of antisera were compared (one detecting both free and bound FLC epitopes, and one detecting only the free FLC epitopes), alongside urine protein electrophoresis and the Freelite assay in order to ascertain the best methods of detecting both types of M-proteins in this subset of patients.

Results: A total of 2% of the patient population tested were shown to have a FLC M-protein migrating coincidentally with an intact M-protein. These were not detected by IFE using the widely utilised antisera to both free and bound FLC epitopes, and hence may have been missed during routine testing, but were detectable using the other methods.

Conclusions: This study highlights the important finding that in some patients with both an intact and a FLC M-protein, the FLC M-protein may be missed during routine testing. In incidences where no corresponding urine sample is sent to the laboratory alongside the serum sample, we would suggest testing for the presence of FLC M-proteins in this subset of patients using the Freelite assay, if no urine sample can be obtained, to ensure all FLC M-proteins are appropriately detected.

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Introduction

Monoclonal gammopathies encompass a range of conditions in which excessive amounts of immunologically homogeneous immunoglobulin are produced from a clone of abnormal plasma cells. These diseases include multiple myeloma, Waldenström's macroglobulinemia, light chain multiple myeloma, AL amyloidosis, non-secretory myeloma, light chain deposition disease and monoclonal gammopathy of undetermined significance. The secreted immunoglobulin (referred to as monoclonal [M-] protein or paraprotein) can be an intact protein, free light chain (FLC) only, or more rarely, the heavy immunoglobulin chain only.¹

Laboratory investigations for patients with a suspected monoclonal gammopathy include serum protein electrophoresis or capillary zone electrophoresis as screening tools, followed by immunofixation electrophoresis (IFE) for typing the monoclonal protein as described by Attalman and Levinson.¹ The evaluation of patient urine should also be performed by urine protein electrophoresis (UPE) and immunofixation in every patient

suspected of having a monoclonal gammopathy. In addition, the more recent FLC assay (Freelite), developed by Bradwell *et al.*, allows quantification of kappa (κ) and lambda (λ) FLCs in serum.^{2–3}

The purpose of this study was to determine the frequency at which FLC M-proteins co-migrate with an intact M-protein. In such incidences, the FLC band may be difficult to distinguish from the intact M-protein, and hence may be missed during routine testing. Methods of detecting FLC M-protein in these patients were then assessed.

Methods

One hundred and fifty serum samples randomly selected from different patients with a suspected monoclonal gammopathy were analysed for detection of both intact and FLC M-proteins by IFE using antisera with reactivity to IgA, IgM and IgG heavy chains, as well as two different types of antisera to detect FLCs: one that detected both free and bound kappa or lambda light chains (F + B κ

and F + B λ) and one that detected only the free kappa or lambda light chains (F κ or F λ). Any sample with a FLC M-protein without a corresponding IgA, IgM or IgG heavy chain was also tested for IgD and IgE reactivity with specific antisera. In addition, all 150 serum samples had κ and λ FLC concentrations measured using the Freelite assay. Of the 150 samples, 119 had a corresponding urine sample and had UPE performed previously.

Immunofixation electrophoresis

This was performed on the Sebia Hydrasys using the different antisera as described above, according to the manufacturer recommendations. All reagents were supplied by Sebia (Issy-les-Moulineaux, France).

Serum FLC quantification

Both κ and λ FLC concentrations were measured on the Roche Integra 400 Plus analyser (West Sussex, UK) using Freelite reagents (Binding Site, Birmingham, UK). All samples were analysed neat and also at a dilution of 1/100 in order to check for antigen excess. The normal reference ranges developed by Katzmman *et al.*⁴ were used for κ and λ concentrations and also the calculated κ/λ ratio.

Results

Of the 150 serum samples tested, 12 had detectable FLC M-proteins by IFE using the F + B κ or F + B λ antisera (with no corresponding heavy chain reactivity), while only seven had detectable FLC M-protein by IFE using the F κ or F λ antisera. Three of the 150 patient samples were found to have an intact M-protein band with a co-migrating FLC band, representing 2% of the total patient population tested (Table 1).

Of the 150 serum samples, 43 had an elevated κ or λ concentration plus an abnormal κ/λ ratio using the Freelite assay on the Roche Integra 400. Of the 119 samples with a paired urine sample, 10 had detectable FLC M-protein (Bence Jones protein [BJP]) by UPE.

Discussion

This study aimed to assess the frequency at which patients have an FLC M-protein migrating coincidental with an intact M-protein, and the best method of detecting both the intact and free M-protein in this subset of patients.

A total of three out of the 150 different patient serum samples analysed (2%) were shown to have an intact M-protein plus a co-migrating FLC M-protein. In these patients, the FLC M-protein was only detectable using the antisera with reactivity to the 'hidden' FLC epitopes (F κ or F λ) and not the antisera detecting the intact and free protein collectively (F + B κ or F + B λ). In each of these three cases, an intact M-protein was detectable using the heavy chain antisera plus the F + B κ or F + B λ antisera. However, no additional FLC-only band was distinguishable from the intact band using this set of antisera, and hence

Table 1 Comparison of kappa and lambda free light chain detection by immunofixation electrophoresis using two different antisera reactivities (free + bound [F + B] and free-only [F-only]), urine protein electrophoresis (UPE) and the Freelite assay

	F + B	F-only	UPE	Freelite
κ FLC	4	3	7	27
λ FLC	8	4	3	16
Total	12	7	10	43

would have been missed during routine testing. A BJP band was observed in all three patients' urine by UPE and all had a corresponding raised κ or λ concentration plus an abnormal κ/λ ratio using the Freelite assay. Therefore, it is likely that the FLC bands picked up by the F κ or F λ antisera represented true FLC M-proteins. In comparison to either IFE or UPE, the Freelite assay did identify significantly more FLCs overall; however, the majority of these patients only had a slight increase in either κ or λ concentration plus an abnormal κ/λ ratio, or had a M-protein band plus renal function impairment.

This study highlights the potential that some patients with FLC M-proteins are being 'missed' using antisera detecting both free and bound FLC epitopes when they also have an intact migrating M-protein. It is important to note that the F κ or F λ antisera only detected seven of the total twelve FLCs detected by the F + B κ or F + B λ antisera, suggesting it may be less sensitive, and hence the 2% of samples shown here to have a co-migrating FLC may in fact be an under-representation.

The lack of detection of FLC M-protein would be particularly apparent in those patients lacking a corresponding urine sample (representing 20% of samples in this study). Therefore, in this subset of patients, we would recommend testing for the presence of FLC M-proteins as part of follow-on testing if no urine sample can be obtained. We suggest the Freelite assay as the most suitable method of doing this, as IFE using the antisera detecting only the free epitope failed to pick-up all FLC M-proteins.

DECLARATIONS

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Contributorship: KW was involved in performing the majority of the experimental work and analysing results, and also wrote the first draft of the manuscript under the supervision of AR. RS performed some experimental work and was involved in sample collection. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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