

Paraprotein estimation: a comparison of immunochemical and densitometric techniques

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SUMMARY Paraproteins have been estimated by both immunochemical methods and densitometric analysis of electrophoretic strips. Correlation between the results obtained by these two methods, although generally good for assessing trends, varied between patients.

Immunochemical methods are routinely used to estimate immunoglobulins. However, since monoclonal immunoglobulins often differ considerably in antigenic expression by comparison with normal immunoglobulins of the same class, electrophoresis combined with densitometry may offer advantages in measuring such proteins (Hobbs *et al.*, 1966). It is common experience that individual values estimated by these two methods may differ considerably. In extreme instances the 'immunochemical value' for the immunoglobulin class to which the monoclonal protein belongs may exceed the total serum proteins as estimated by the Biuret technique. What is important is the consistency of measurement by either method and the manner in which measurements reflect the changes in the clinical state of the patient. Diagnosis itself does not depend on quantitation, although the absolute value of the paraprotein may contribute towards a decision as to the likelihood of malignancy. This paper presents the results of a comparison of the two methods.

Patients and sera

A total of 186 serum samples were analysed including serial specimens (116 in all) from 11 patients with clinical myelomatosis or primary macroglobulinaemia and one with a monoclonal gammopathy. An additional 70 samples were obtained from a further 40 patients, the majority of whom had established myelomatosis, although serial serum samples were not available. All patients had monoclonal proteins in one of the three major immunoglobulin classes (IgG, IgA, IgM).

Electrophoresis of sera was performed in agarose (Corning ACI System) with densitometric estima-

tion of the paraprotein band (Varian MPS spectrophotometer) after staining with amido black. Total protein estimations were made using the Biuret method of Doumas (1975). The coefficient of variation of the densitometric analysis was 5.9%.

Immunochemical estimation of the relevant immunoglobulin was performed using single radial immunodiffusion (Mancini *et al.*, 1965) with a coefficient of variation in this department of 7.3%. The antisera used were obtained from Dr D. Catts, Department of Experimental Pathology, Birmingham University, having been raised against normal human γ chain, or pooled monoclonal α or μ chains, and absorbed to render them specific. Three large batches of IgG and IgA antisera and a single batch of anti IgM were used in the period of study, although no significant differences were found in between-batch estimations of normal and pathological sera.

Initial typing of the paraprotein was by immunoelectrophoresis of serum against monospecific antisera.

Results

Figure 1 (a, b, c) shows the comparison between the two methods for all 186 samples. There is a highly significant overall correlation ($r = 0.83$; $p = 0.001$) between the two methods and this is also the case with respect to individual Ig classes (Table 1). On plotting the regression equation for each Ig class the slope for IgG is 0.88, indicating that both methods give comparable results for IgG. However, for IgA and IgM, the slopes are 0.62 and 0.76 respectively, showing a slight tendency for higher values by radial diffusion. This may be a reflection of the difference in standards for the methods employed; or since the anti-IgA and anti-IgM antisera used in

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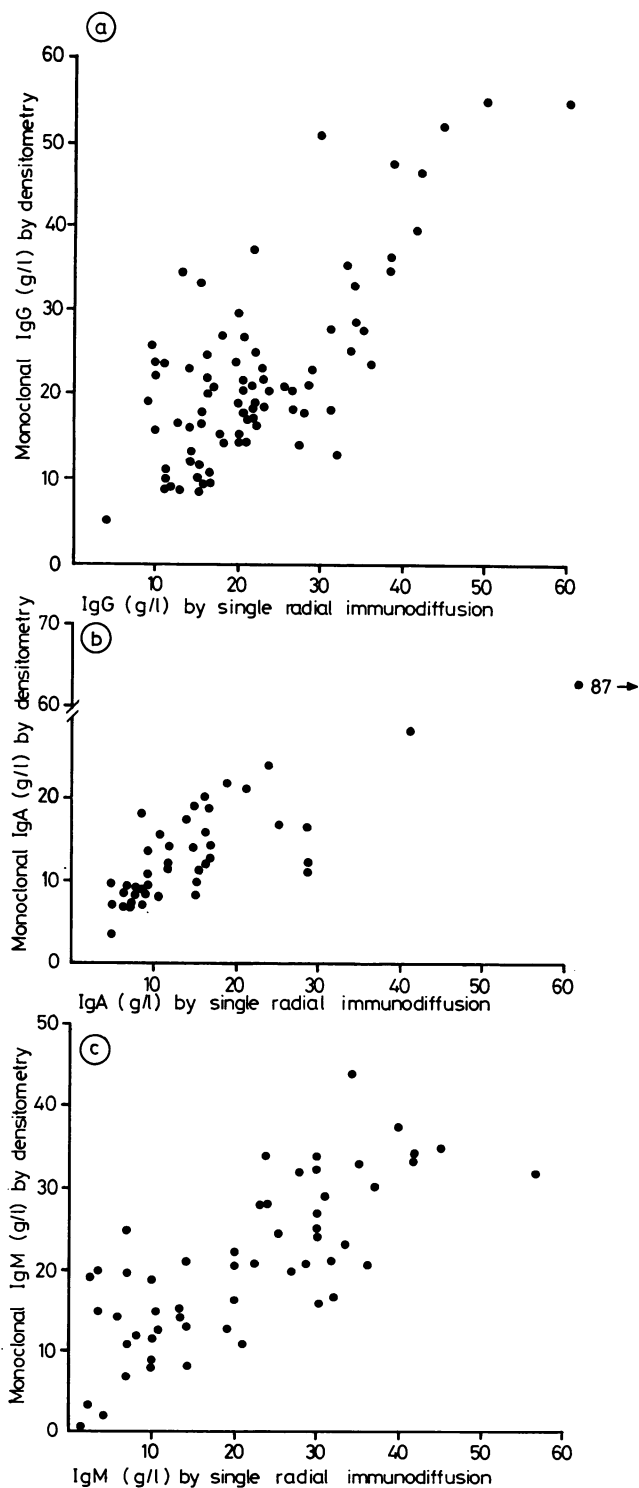


Fig. 1 Comparison between immunochemical and densitometric measurements of monoclonal proteins in the classes IgG (a), IgA (b), and IgM (c).

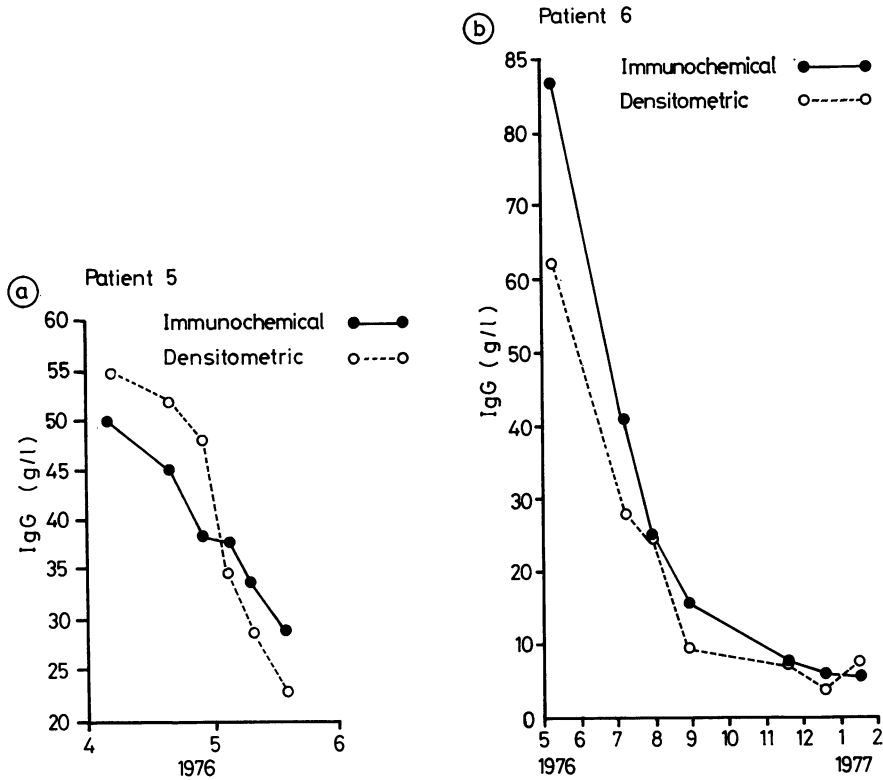


Fig. 2 Sequential changes in the monoclonal protein levels of patients 5 and 6 (Table 2).

Table 1 Correlation between immunochemical and densitometric analysis of monoclonal proteins

Ig class of monoclonal proteins	Number of estimations	Correlation coefficient	P value
G	87	0.85	<0.001
A	43	0.90	<0.001
M	56	0.76	<0.001
Total	186	0.83	<0.001

the immunochemical method are raised against monoclonal antigens, it may be due to the antigenic differences between the monoclonal proteins and the polyclonal standard.

On separately analysing samples from different patients (Table 2) it appears that the values by the two methods are well correlated in some patients (5 and 6) but not in others (7, 9, 10). Figure 2 (a, b) shows the changes in paraprotein level with time, estimated by both techniques, for two patients (5 and 6). Both methods reflect the same trend in paraprotein levels, and either method could have been used in the assessment of the response to treatment.

The same cannot be said of other patients in

Table 2 Correlation between the two methods in serial samples from patients

Ig class	Patient number	Number of estimations	Correlation coefficient	P value
G	1	11	0.73	<0.01
G	2	7	0.51	NS
G	3	18	0.57	<0.05
G	4	5	0.78	<0.05
G	5	6	0.93	<0.01
A	6	7	0.98	<0.001
A	7	8	0.05	NS
A	8	21	0.63	<0.01
M	9	9	0.43	NS
M	10	6	0.03	NS
M	11	11	0.68	>0.02
M	12	7	0.83	>0.01

whom individual values showed no significant correlation. Patient 2 (Fig. 3) had a monoclonal IgG, slight Bence-Jones proteinuria, and a very slight plasmacytosis of the marrow, and her clinicians decided not to treat her with cytotoxic drugs. She also had dermatological and bowel symptoms, but her haematological state remained constant over the period represented in the figure. The densitometric

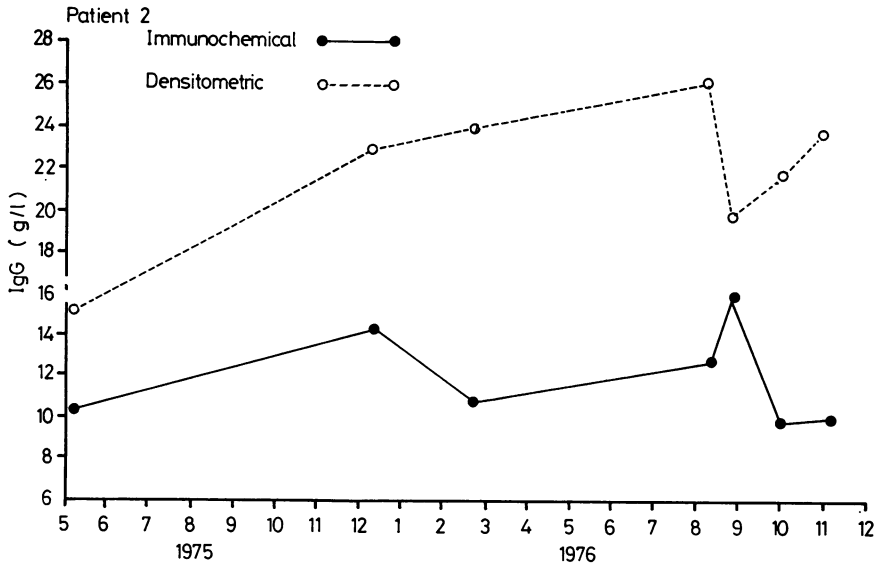


Fig. 3 Sequential changes in the monoclonal protein level of patient 2 (Table 2).

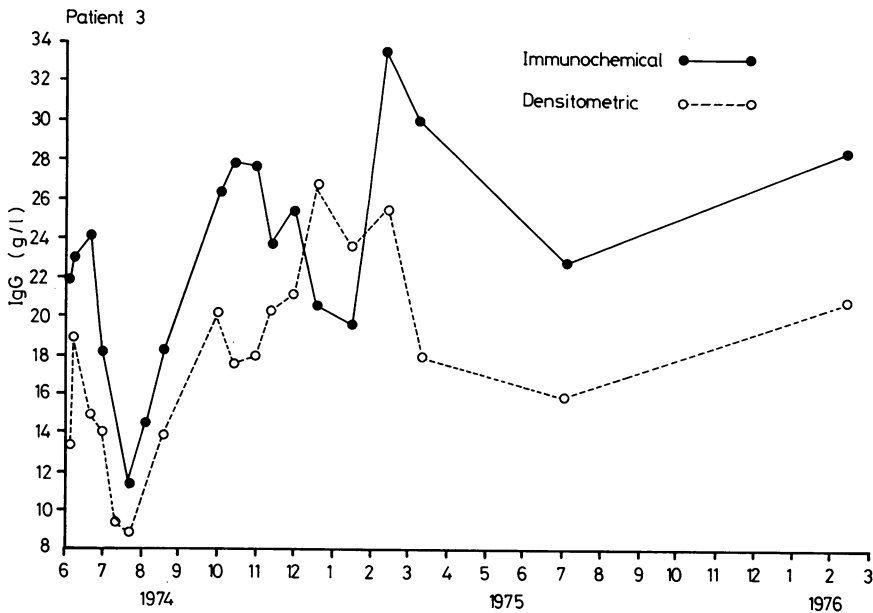


Fig. 4 Sequential changes in the monoclonal protein level of patient 3 (Table 2).

estimations indicated a gradual increase in the monoclonal protein over a period of two years, while the immunochemical technique showed little overall change.

Patient 3 (Fig. 4) had an IgG myeloma and was

started on treatment in June 1974, after which there was a sharp fall in the levels of monoclonal protein and IgG with a subsequent rise and variation over the next two years. Although broad trends over several months are in the same direction, interpretation of

individual values could be different, depending on the method used.

Discussion

While there is a clear overall correlation between the immunochemical estimation of immunoglobulin classes containing a monoclonal protein and the densitometric quantitation of the monoclonal protein, there appears to be greater concordance between the two methods in some patients than in others. This is at variance with some authors, who suggest that results by both methods are always comparable (Dugue *et al.*, 1971). Moreover, in those patients reported here, in whom the correlation between the two methods was poor, there was no consistency as to whether either method corresponded to the clinical progress of the patient. Thus, although monitoring of the myeloma protein level can be helpful in the management of the clinical case, and densitometry has been advocated as the method of choice, neither method is clearly superior for this purpose. The ideal method perhaps would be to use an anti-'idiotype' antiserum, raised against the specific antigenic determinants of each myeloma protein (Hopper and Nisonoff, 1971). Such sera do not react with normal immunoglobulins. However, this is not a practical solution in routine clinical practice, and it may be wise to employ both methods initially until the pattern of response to treatment emerges.

Caution must be observed in the use of immunochemical techniques for this purpose. Because of the problem of non-parallel dilution curves in the single radial diffusion test between myeloma and normal

immunoglobulins, it is necessary to measure the serum level from a given patient using the same dilution on each occasion. Moreover, if different batches of antisera are used at different times, then it is preferable in analyses of serial specimens for any specimen to be estimated at the same time as a stored aliquot of the previous specimen from the same patient.

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