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Paraproteins: A Regional South Australian Experience

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SUMMARY We have performed a systematic review of all new serum and urinary paraproteins detected over a six year period in an immunodiagnostic laboratory serving a population of 400,000 people. Clinical diagnoses and associated laboratory features were ascertained from a computerized laboratory database or from clinical notes. Over the period of study, serum or urine paraproteins were detected in 613 new patients. These consisted of 568 patients with serum paraproteins and 45 patients with urinary monoclonal free light chain (in the absence of a serum paraprotein). These paraproteins occurred more commonly in males and the frequency increased with age. Approximately 30% of the serum paraproteins and 60% of urinary monoclonal free light chain were associated with B cell lymphoproliferative disorders (multiple myeloma, plasmacytoma, Waldenstrom's macroglobulinemia, non-Hodgkins lymphoma, chronic lymphocytic leukemia, etc) with the remainder being labeled as monoclonal gammopathies of uncertain significance (MGUS). At clinical presentation, patients with lymphoproliferative disorders tended to have higher levels of paraprotein, B2 microglobulin, the presence of free urinary light chain and demonstrated molecular size heterogeneity of the paraprotein but there was considerable overlap. A good correlation was noted between paraprotein concentration and viscosity in most patients. In conclusion paraproteins were most frequently encountered in the context of a gammopathy of uncertain significance. Features which suggested lymphoproliferative disorders included higher levels of serum paraprotein (>15g/l), elevated levels of B2-microglobulin and the presence of urinary free high chain. However, as much overlap was seen with patients with MGUS, regular monitoring of paraprotein level is considered mandatory in the management of these patients.

Paraproteins are defined as monoclonal immunoglobulins and are produced by the same single clone of B cells and are readily visible on the serum and/or urine protein electrophoretogram as sharp M bands in the βor γ region. Paraproteins, being immunoglobulins, are generally classified as belonging to one of the 5 immunoglobulin classes (IgG, IgA, IgM, IgD, IgE) or light chain types, i.e. κ or λ.[1] The presence of a paraprotein may reflect a progressive B cell lymphoproliferative disorder or, more commonly, a monoclonal gammopathy of uncertain significance (MGUS).[2] It is important to distinguish between these disorders as the former may have grave long term consequences requiring specific management whilst the latter may need no more than regular paraprotein surveillance. Furthermore, there are also a number of other clinical disorders that have been clearly linked with the presence of a serum or urinary paraprotein and thus the identification of this serum marker may alert the clinician to the presence of these disorders.[1]

In this current paper we have reviewed the frequency and characteristics of paraproteins detected in a regional South Australian immunopathology laboratory over a six year period. Specific disease associations have been sought together with other laboratory features that may be of assistance in distinguishing those patients with lymphoproliferative disorders. In a previous paper we have described our local experiences with IgM paraproteinemia.[3]

MATERIALS AND METHODS

Patient sera and clinical information

All new serum and urinary paraproteins identified in the immunodiagnostic laboratory over a six year period (July 1996-July 2001) were reviewed. These paraproteins were identified from the AUSLAB electronic laboratory database for this time period. The clinical diagnosis in each patient with the paraprotein was established from a systematic review of the clinical data held on the database. Information was sought on the clinical diagnosis made by the

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tive factor. Anti-epileptic therapy was needed during the active period, which could be discontinued without major sequelae. Most CNS symptoms of NP manifestation responded well to high dose corticosteroids.

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attending physician(s), bone marrow and lymph node findings and lymphocyte phenotyping and, when necessary, from a review of individual patient's case notes. Other relevant laboratory findings were also noted during the review. The majority of patients categorized as MGUS have been followed up for at least 2 years but only a small proportion have undergone bone marrow or lymph node examination or had blood lymphocyte phenotyping performed.

Serum and urine electrophoresis and immunofixation

Serum and urine electrophoresis and immunofixation was performed in 1% agarose and stained with Coomassie brilliant blue R according to standard techniques previously published by our laboratory.[4] Quality of performance of this and other laboratory techniques is regularly assessed by participation in the Quality Assurance Program of the Royal College of Pathologists of Australasia.

Quantities of M band

Densitometry of the paraprotein was performed using the Beckman Appraise Densitometer as previously described.[4] The total protein of the serum sample was measured by the biuret technique. Quantitation of the paraprotein was determined by calculating the % area subtended by the paraprotein and multiple by the serum total protein. Immunoglobulins IgG, IgA and IgM were measured by rate nephelometry.

Beta-2 microglobulin

Serum Beta-2 microglobulin (Beta-2 M) was measured by an RIA technique using the commercial Pharmacia Kit.[4] The normal reference range in our laboratory is < 2.5 mg/l for ages 40-59 years and <3.0 mg/l for ages 60-80 years.

Serum viscosity

Serum viscosity was measured at 37°C using an Oswald viscometer, as previously described,[4] and the results expressed relative to distilled water. The normal reference range in our laboratory is 1.4 - 2.0.

Assessment of molecular size of immunoglobulins

This was performed by immunoblot analysis.[5] Briefly, serum proteins were separated on SDS 2.5% or 3.6% polyacrylamide gel slabs and the separated proteins transferred to nitrocellulose as previously described. The immunoglobulin bands were developed using a biotin-avidin anti-Fc (or anti-μ) heavy chain specific conjugate (Dako Immunoglobulins, Glostrup, Denmark). Normal serum and serum containing both low and high molecular weight immunoglobulin species were included in each electrophoretic run and these previously characterized proteins were used as internal markers for molecular weight determination as described elsewhere.[5] For some experiments immunoblotting was performed using developing anti-sera to immunoglobulins on 1/3 of the gel, anti-sera to albumin (Beckman Instruments, Fullerton, CA, USA) on the middle third and anti-sera to $alpha_1$, anti-trypsin (Beckman Instruments) on the remaining third. Specificity was controlled using an irrelevant developing antiserum to CRP in addition to using multiple anti-sera on the same blot as described above. Following immunoblotting, densitometry on the bands were performed using a Camag electrophoresis scanner. The profile of the bands representing the immunoglobulin species was traced onto exposed x-ray film and the proportions that each band constituted determinated by the method of planimetry.

RESULTS

Paraprotein frequency

Over the six year study period our laboratory detected paraproteins in 613 new patients (Table 1). As our laboratory provides an immunodiagnostic service to a population of ~ 400,000 people in the southern sector of Adelaide, this equates to ~2 new paraproteins detected each week or an annual incidence of paraproteinemia of ~1:4000 of the population. The new paraproteins consisted of 568 serum paraproteins and 45 urinary monoclonal free light chain (in the absence of a serum paraprotein).

The frequency of paraproteins occurred more commonly in males (M:F, 350:263) and increased with age (Fig. 1). The κ:λ ratio was 2.4:1.

Table 1 Paraproteins[1]

	κ	λ
IgG	227	97
IgA	44	19
IgM	91	28
Free light chain	30	15
Biclonal etc.[2]	62	

[1]new patients diagnosed with a paraprotein over the interval 1996-2001.
[2]patients with 2 or more serum paraproteins

Disease association

Serum and urinary paraproteins were observed in patients with a number of different clinical disorders as listed in Table 2. The clinical distinction between multiple myeloma (MM-135 patients), Waldenstrom's macroglobulinemia (WM - 22 patients) and non-Hodgkins lymphoma with paraproteinemia (NHL - 16 patients) was made by the attending hematologists and generally was dependent on marrow infiltration with plasma or lymphocytoplasmoid cells whilst NHL had more prominent lymph node involvement. Patients with MGUS had a variety of medical, surgical and psychiatric conditions including some with a proven solid tissue malignancy.

Other laboratory features

In an attempt to differentiate between the various malignant and benign disorders, a number of laboratory features noted at clinical presentation have been tabled for many of the patients. Patients with MM and WM generally had higher presenting levels of paraproteins (Table 3), higher frequencies of urinary free light chain (Table 4) and some degree of immunoparesis (Fig. 2) than patients with MGUS.

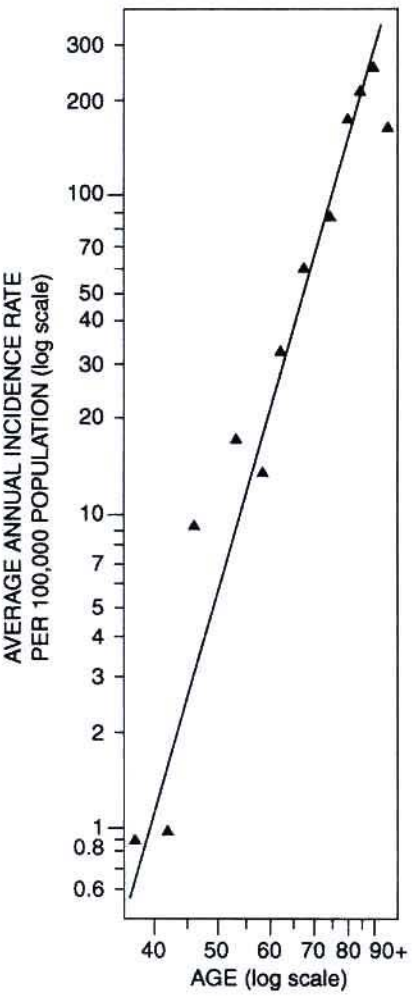

Fig. 1 The estimated average annual incidence rate per 10^5 population of paraproteinemia according to age at presentation.

Table 2 Paraproteins: disease associations

	Light chain[1]	IgG	IgA	IgM	Mixed[2]
Multiple Myeloma	21	75	25	0	16
Plasmacytoma	2	0	1	0	0
Amyloid	4	1	3	1	0
Waldenstrom's Macroglobulinemia	0	0	0	21	1
Non Hodgkins Lymphoma	1	8	1	4	4
Cold Agglutinin Disease	0	0	0	1	1
Chronic Lymphocytic Leukemia	2	1	0	4	3
Mixed Cryoglobulinemia	0	0	0	2	0
MGUS	15	239	33	87	37
TOTAL	45	324	63	119	62

[1]Urinary monoclonal light chain only without serum paraprotein
[2]Two or more serum paraproteins

Similarly, levels of B2M tended to be higher in patients with lymphoproliferative disorders (Fig. 3) but in all these laboratory measurements, there was some overlap between the various diagnostic categories.

Serum viscosity

The serum viscosity was measured in 43 patients. In general a curvilinear regression was observed between paraprotein concentration and viscosity with IgM paraproteins showing the highest viscosities for the same paraprotein concentration (Fig. 4). However, there was some variation between paraproteins of the same class suggesting that paraproteins may vary with regards their intrinsic viscosity or in their interactions with other serum proteins. To investigate this further, we investigated the molecular size of individual paraproteins.

Table 3 Levels of paraproteins

	Paraprotein concentration[1] (g/l)
IgG MM	28.3 (1-62)
IgG MGUS	8.1 (1-27)
IgA MM	30.3 (1-71)
IgA MGUS	9.8 (1-35)
IgM WM	23.8 (5-44)
IgM MGUS	7.1 (1-33)

[1]Mean (range)

Table 4 Frequency of monoclonal free light chain in myeloma, macroglobulinemia and MGUS

	% monoclonal light chain in urine
IgG MM	45%
IgG MGUS	16.2%
IgA MM	27%
IgA MGUS	5.5%
IgM WM	89%
IgM MGUS	40%

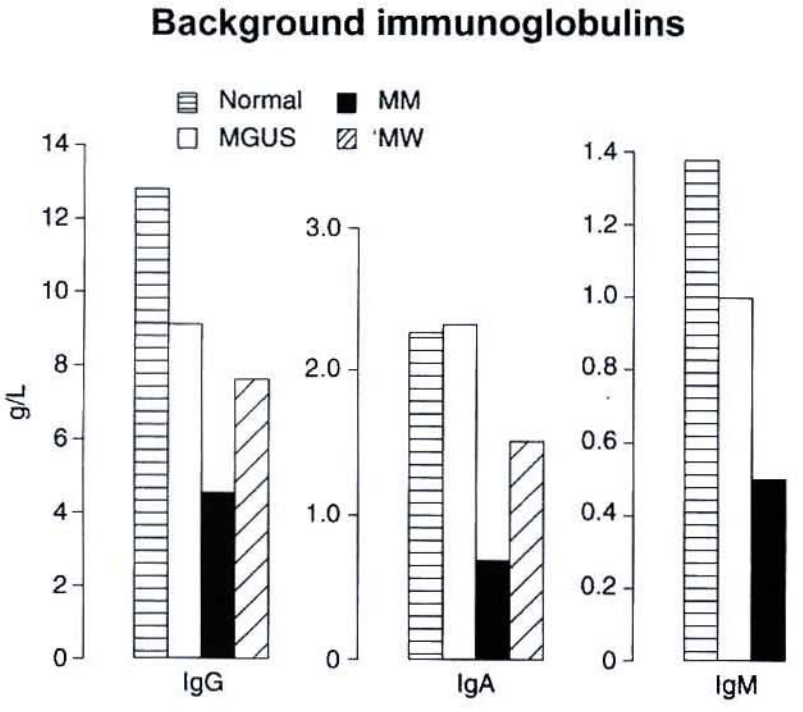

Fig. 2 Mean levels of "background" IgG, IgA and IgM in multiple myeloma (MM), Waldenstrom's macroglobulinemia (WM), monoclonal gammopathy of uncertain significance (MGUS) and in healthy adults (normal).

Molecular size heterogeneity of paraproteins

Considerable molecular size heterogeneity was observed between paraproteins and within paraproteins of the same class (Fig. 5). IgM paraproteins demonstrated a polymeric series with the pentameric species (M.W. ~ 10^6 daltons) being predominant in all paraproteins studied. However, decamers were observed in 80% of the sera and constituted up to 20% of the paraprotein. Monomeric IgM was also observed in the majority of the sera consituting up to 37% of the paraprotein whilst dimers, trimers, quadramers were also apparent in many of the sera. In general, molecular size heterogeneity of IgM paraproteins was most evident in those sera obtained from patients with malignant B cell lymphoproliferative disorders (WM, NHL) but heterogeneity was not unique to this subset and was observed in some patients with IgM MGUS.

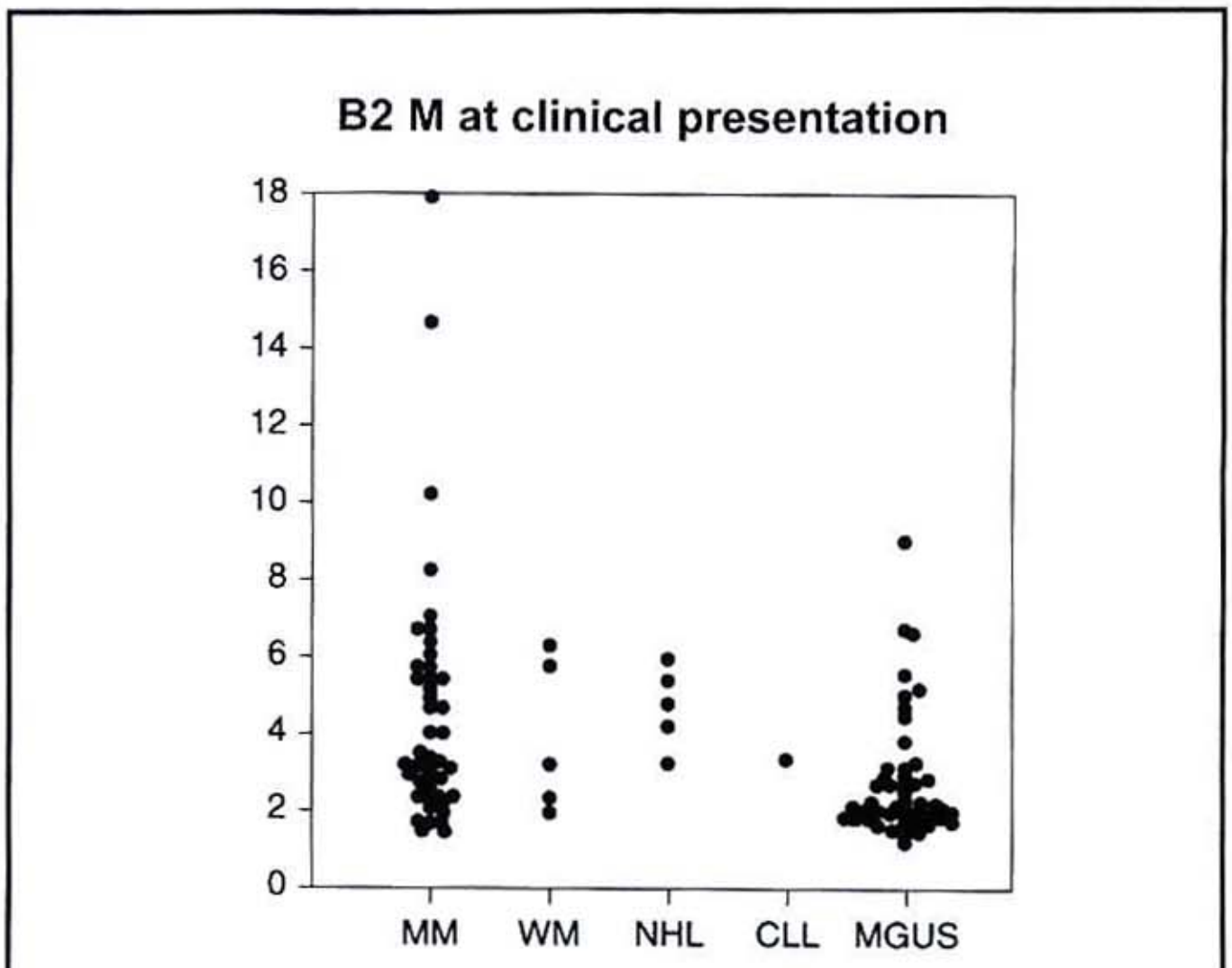

Fig. 3 Levels of β2-microglobulin at clinical presentation for patients with multiple myeloma (MM), Waldenstrom's macroglobulinemia (WM), non-Hodgkins lymphoma (NHL), chronic lymphocytic leukemia (CLL) and in monoclonal gammopathy of uncertain significance (MGUS). The normal adult level is less than 3 mg/l.

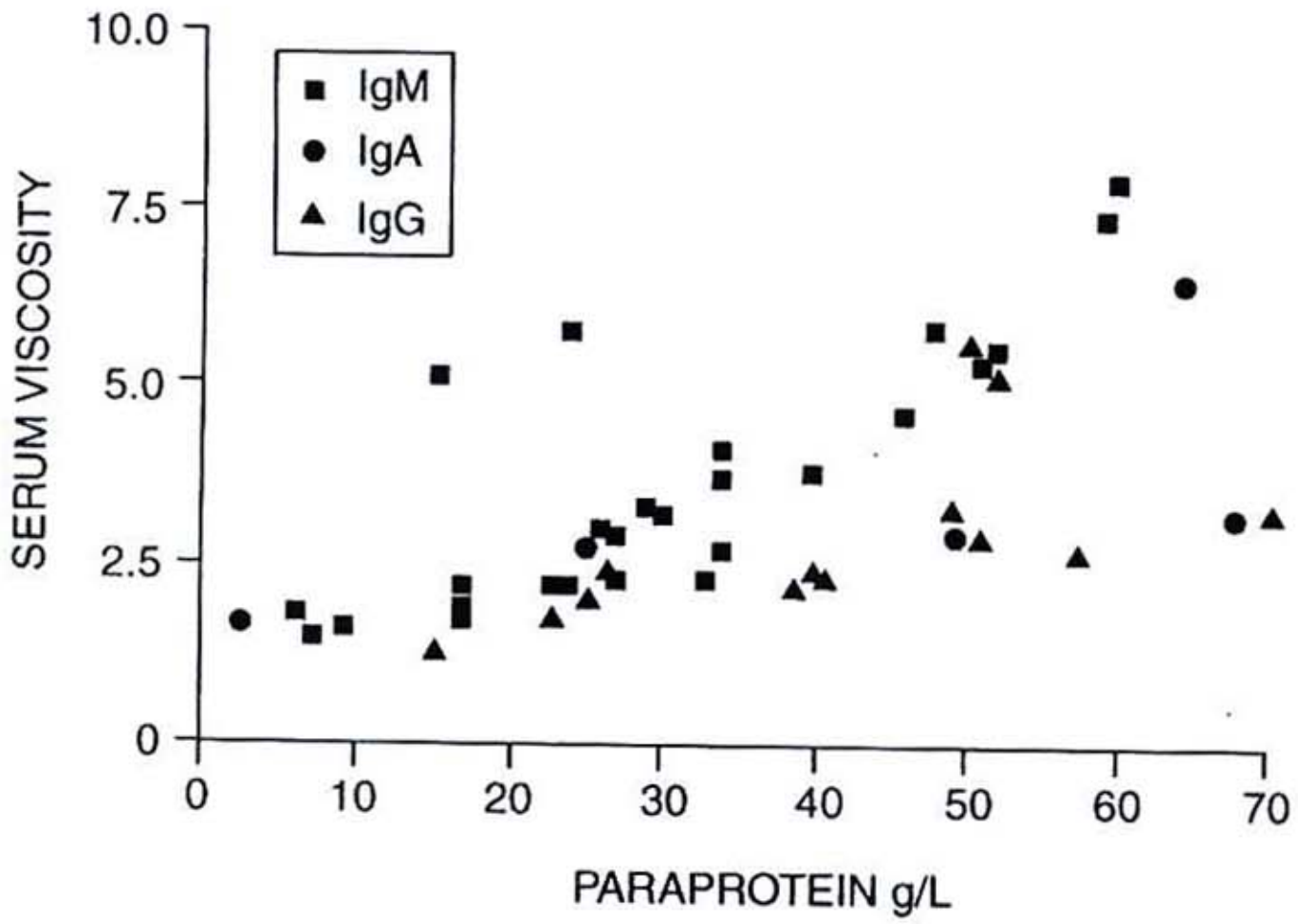

Fig. 4 Serum viscosity plotted against paraprotein concentration. In general the hyperviscosity syndrome is seen when viscosity is > 5.

IgA paraproteins also demonstrated molecular size heterogeneity. In MM the predominant IgA molecular species was either the monomer or the dimers with trimers and heavier oligomers also apparent. In IgA MGUS the monomer was the predominant species in all except one patient with smaller quantities of dimers and oligomers being present.

The proportion of the various molecular species for both IgM and IgA paraproteins were stable over time and there was no relationship between paraprotein concentration and the proportion of the various molecular species.

Intermediate complexes of IgM and IgA paraprotein species covalently bound to molecules of albumin or α1-anti-trypsin was observed in many of the sera (Fig. 6).

IgG paraproteins were in the monomeric state for paraproteins of subclasses 1, 2 and 4, but some polymerization was evident for paraproteins of the IgG_3 subclass. Albumin and α1-anti-trypsin complexes were not observed with IgG paraproteins.

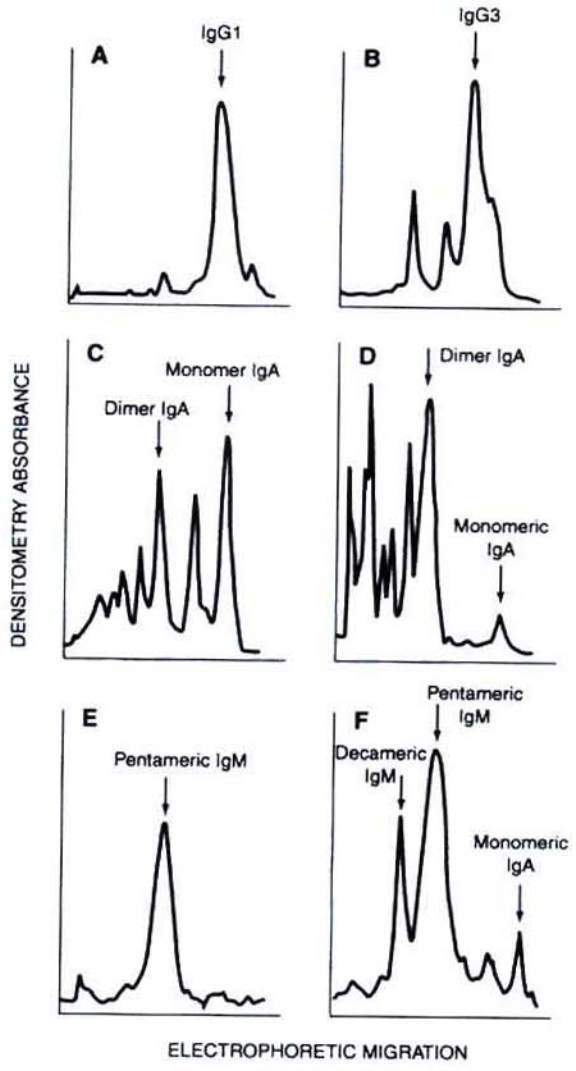

Fig. 5 Densitometric profiles of molecular sizes of serum paraproteins. Panel A - patient with IgG_1 myeloma. Panel B - patient with IgG_3 myeloma (note presence of small quantities of dimers). Panel C - patient with IgA myeloma of the oligomeric variety. Panel D - patient with IgA myeloma of the polymeric variety. Panel E - patient with IgM MGUS (2.5% gel). Panel F - patient with Waldenstrom's macroglobulinemia (2.5% gel) and note the presence of decamers, pentameric and oligomers.

Other observations

Single component cryoglobulins were noted in 4 patients (three with IgM and one with IgG paraproteins) whilst 2 patients had mixed cryoglobulins (both with monoclonal IgM rheumatoid factors reacting with polyclonal IgG). Antibody activities noted amongst the IgM paraproteins were rheumatoid factors in 3 patients, anti-I (cold agglutinins) in 2 patients, an anti-Sepharose (? anti-carbohydrate) activity in one and a final patient's paraprotein appeared to interfere with nephelometric protein estimations.

Unexplained peripheral neuropathies were observed in 6 patients, 4 with IgM paraproteins, 1 with IgA and 1 with free lambda.

DISCUSSION

Paraproteins are frequently detected in the immunopathology laboratory and their presence and disease association may have important implications for the patient. In our own experience we detected about 2 new paraproteins each week with the ratio between G, A, M and free light chain paraprotein being similar compared with other studies[6, 7] and most likely reflects the basic biological ratios between the synthetic secretory rates of the differing immunoglobulin class – specific plasma cell population.[1] In addition the gender distribution fa-

voring males and the increased κ:λ ratio is also similar with other published figures. The absolute incidence of our paraproteins (i.e. number new cases/year) seemed in the high range[8, 9] but it is most likely explicable on our patient referral patterns and on our use of an in house moderate/high sensitive serum electrophoresis technique which uses high grade 1% agarose and Coomassie brilliant blue R as the protein stain. Furthermore, monoclonal bands are identified by visual inspection of the gels and confirmed by a sensitive immunofixation technique. Participation in the Quality Assurance Program of the RCPA indicates that our electrophoretic technique is capable of detecting minor bands missed by some other laboratories using a variety of other techniques (e.g. cellular acetate, amido block stains, etc).[10]

B cell lymphoproliferative disorder (MM, WM, NHL, CLL) accounted for 30% of cases in which paraproteins were observed. In other words, in our patient population, paraproteins were most commonly observed in the clinical setting of a MGUS. In an attempt to distinguish these two groups we sought further serological features at the time of the initial clinical presentation. In general, our patients with B cell lymphoproliferative disorders (with paraproteins) had higher mean levels of paraproteins at clinical presentation associated with the presence of urinary free light chain and elevated B2M but there was some overlap with the more commonly encountered patients with MGUS. As it is important to distinguish these two diagnostic categories and furthermore, knowing that 20-30% of patients with MGUS will evolve over 10 years to the malignant variety,[1] it seems to us that it is mandatory that patients with paraproteins are carefully monitored over time. This monitoring would include regular (6-12 monthly) assessment of circulating levels of the paraprotein with possibly less frequent (2 yearly) clinical and more detailed laboratory assessment. This would allow one to detect early those patients who are developing MM, WM or a similar B cell lymphoproliferative disorder.

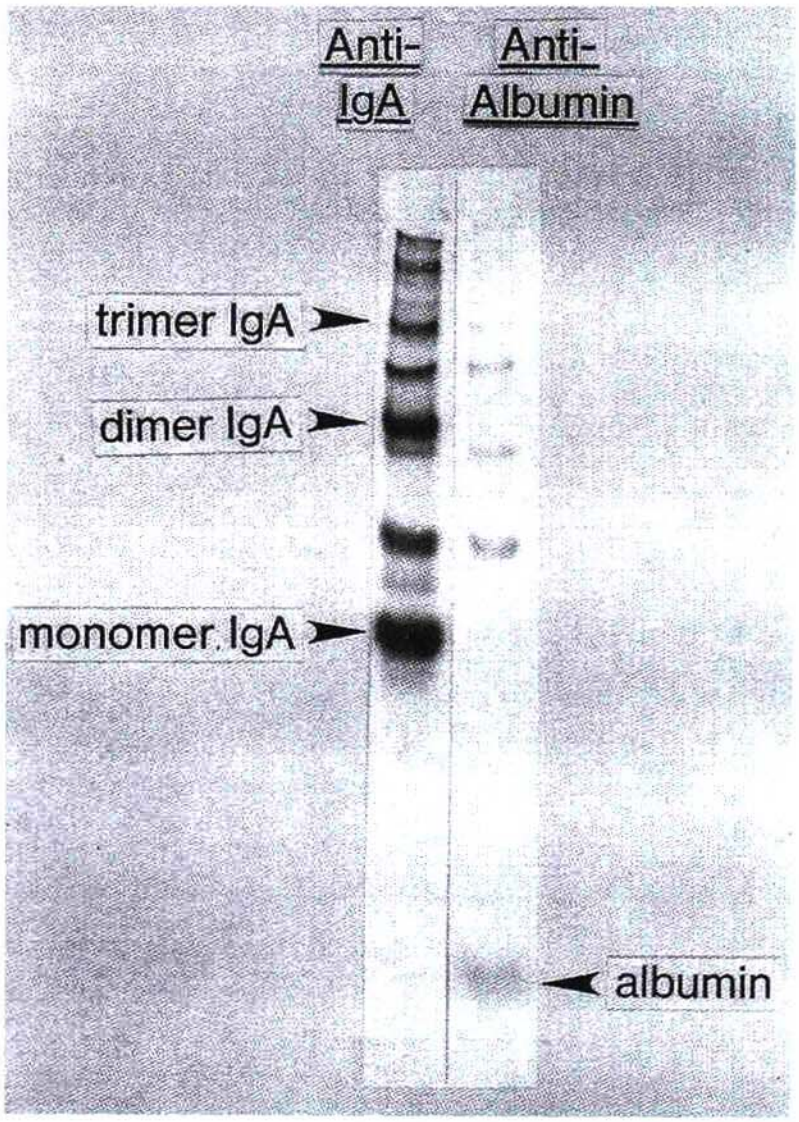

Fig. 6 Immunoblotting of serum with IgA paraprotein-stained for α heavy chain and albumin. The positive high molecular staining of albumin coincidence with α chain staining suggesting an IgA - albumin covalently bound complex. Similar appearances were obtained for experiments using anti-α1-antitrypsin in place of anti-albumin.

The detection of monoclonal urinary free light chain has been previously considered as a suggestive feature of a B cell lymphoproliferative disorder.[1] In our own series the finding of free light chain in the absence of a serum paraprotein was associated with a malignant B cell disorder in 60% of cases but this figure declined in the

presence of a serum paraprotein. Indeed, in patients with serum IgM MGUS monoclonal light chain proteinuria was noted in 40% of cases. However, the finding of large quantities of monoclonal light chain proteinuria should alert the physician to be most thorough in attempting to confirm the presence of a B cell lymphoproliferative disorder.

Serum viscosity was frequently elevated in our patients and we observed a non-linear correlation between levels of paraprotein and viscosity. Symptoms of the hyperviscosity syndrome (fatigue, weakness, bleeding, drowsiness, breathlessness and visual disturbance) are observed in ~10% of patients with WM and 5% of patients with MM and generally when the paraprotein level is > 50g/l or the serum viscosity > 5.[12] However, the symptomatic viscosity threshold varies between patients and there are some paraproteins who seem to have greater intrinsic viscosity compared with others (for the same paraprotein concentration). One possible explanation for this variation might relate to molecular size heterogeneity between individual paraproteins.

We observed that paraproteins frequently existed in multiple molecular forms.[13] With IgM paraproteins the pentamer form was the dominant species in all patients but decamers were also observed in most patients, particularly those with high levels of IgM. The proportion of decamers appeared stable over time. Monomeric and oligomeric IgM were also evident, notably in those patients having a malignant form of lymphoproliferative disorder. In some patients, these lower molecular sized species made up to 37% of the total circulating IgM paraprotein level.[14] Again, in addition to the presence of monomeric and oligomeric IgM, intermediate species migrating between these were seen in some sera and immunoblotting analysis revealed that they resulted as a consequence of the interaction of monomeric or dimeric IgM with one or two molecules of albumin or α1-anti-trypsin. No significant relation was observed between the quantity of the circulating IgM paraprotein in patients with malignant forms of the disorder and the levels of unpolymerized form or low molecular weight IgM in agreement with other earlier reports.[15,16] IgA paraproteins also showed molecular size heterogeneity with the paraprotein forming a polymeric series. In IgA MGUS the predominant IgA molecular species was the monomer whilst in IgA MM higher molecular weight polymers were apparent in many patients, with the dimers frequently being predominant. We have previously subdivided IgA paraproteins in MM into monomeric or polymeric (depending on the proportion of higher polymers) and it appears that those patients with the polymeric variety are at greater risk for the development of the hyperviscosity syndrome (for the same concentration of paraprotein).[17] As with IgM, paraprotein complexes with albumin or α1-anti-trypsin were also observed.

IgG3 paraproteins have been previously noted to have a propensity to polymerize and we observed IgG3 dimers in several of our patients. This is perhaps partly the explanation whilst this IgG subclass seems more prone to develop the hyperviscosity syndrome than other IgG paraprotein subclasses (for similar levels of paraprotein).[1]

As with other studies[18] we observed interesting antibody/functional activities in some of our IgM paraproteins. These included paraproteins having cryoglobulin properties, rheumatoid factor and anti-I binding. There were some reports which suggest that single component cryoglobulinemia is an adverse prognostic factor in WM.[19] IgM paraproteins with rheumatoid factor are a characteristic serological feature in mixed essential cryoglobulinemia and we encountered two such examples in our own series. It is of interest that these patients frequently have high levels of monomeric IgM.[20] Paraproteins with anti-I activity are the serological feature of Cold Agglutinin Disease[18] characterized by cold induced hemolysis and 2 patients were diagnosed with this disorder in the current series. Paraproteins with other binding activities were also noted including one which caused interference with the nephelometric CRP assay.

Paraproteinemia is clearly associated with the development of peripheral neuropathy with some of the IgM paraproteins being shown to have myelin associated glycoprotein binding activity.[21] Four examples of IgM paraproteinemia with associated peripheral neuropathy were encountered in the current series.

In conclusion, the detection of a new paraprotein was commonly encountered in our regional immunodiagnostic laboratory. The majority of these patients had a MGUS. Whilst trends were evident, there was no absolute serological method to distinguish these patients on clinical presentation from those with B cell lymphoproliferative disorders. We believe it is important that patients with MGUS are regularly monitored to detect any progression to the malignant state (which has been previously estimated to proceed at 20-30% over 10 years and 50-65% over 25 years[2,12,22] or about 1 percent per year).

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