Multiple Myeloma

PART I. CLINICAL, BIOCHEMICAL AND IMMUNOCHEMICAL ASPECTS

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SUMMARY

The clinical, biochemical and immunochemical features of 52 patients suffering from multiple myelomatosis are reported. Three major criteria were used to establish the diagnosis: serum and urine protein chemistry, bone marrow morphology and the radiological appearances of bone. The fact that abnormalities of gamma globulin synthesis were present in 49 of the 52 patients indicates the importance of adequate examination of urine by electrophoresis and immunochemical techniques in the early diagnosis of multiple myeloma. Bradshaw's test, which is a simple screening test for Bence-Jones proteinuria, was positive in 63% of patients.

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The demonstration of disordered gamma globulin synthesis is an important criterion in the diagnosis of multiple myelomatosis.[1] Presently available methods permit the identification of different immunochemical classes of gamma globulins. The immunochemical class of a paraprotein ('M'-component) may have an important bearing on the prognosis and management of patients with multiple myelomatosis.[2] Moreover, accurate quantitation of paraproteinaemia and Bence-Jones proteinuria is extremely helpful in assessing a patient's response to therapy.[3-6]

In this report the clinical, biochemical and immunochemical features of 52 patients suffering from multiple myelomatosis are reviewed. Special reference is made to the use of currently available techniques for identifying and quantitating abnormal protein synthesis, and to the interpretation of screening tests which can be used at the bedside to detect Bence-Jones proteinuria.

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MATERIALS AND METHODS

The diagnosis of multiple myelomatosis was based upon the finding of radiological abnormalities of bone, on the morphological features of a bone marrow aspirate, and on the results of serum and urine protein studies.

Radiological Criteria

Radiographs of any tender bones were studied. In the absence of bony tenderness, a limited radiological skeletal survey was done.

Bone Marrow Examination

The characteristic bone marrow abnormality in myelomatosis is the presence of 'myeloma cells', which are atypical plasma cells with punched-out nucleoli. The presence of such cells, which usually accounted for more than 10% of the nucleated cells in the aspirate, was regarded as diagnostic.

Serum and Urine Protein Studies

Serum and early morning urine samples preserved with sodium azide (1 mg/ml) were examined. Sera were stored at 4 - 6°C until the investigations were completed, and then frozen in 0,5-ml aliquots at −20°C. Repeat analyses were performed on a thawed aliquot which was then discarded.

The salicylsulphonic acid test and Bradshaw's test[7] were used as screening tests for proteinuria. The former detects albumin and globulins, and is performed by a dropwise addition of 10% salicylsulphonic acid to urine. Bradshaw's test detects only the presence of globulin and is performed by layering clear, filtered urine on concentrated hydrochloric acid so as to form two unmixed layers. A positive result for the presence of globulins is the formation of a precipitate of protein at the urine-acid interface (Fig. 1).

A further aliquot of urine was then prepared for electrophoresis by concentrating it, using a Sartorius concentration apparatus, until the protein concentration was approximately 0,5 - 1 g/100 ml.

The total protein of both serum and urine was estimated by a biuret technique.[8] Serum protein fractions were quantitatively measured using a standard cellulose acetate microzone electrophoretic technique (Beckman Microzone

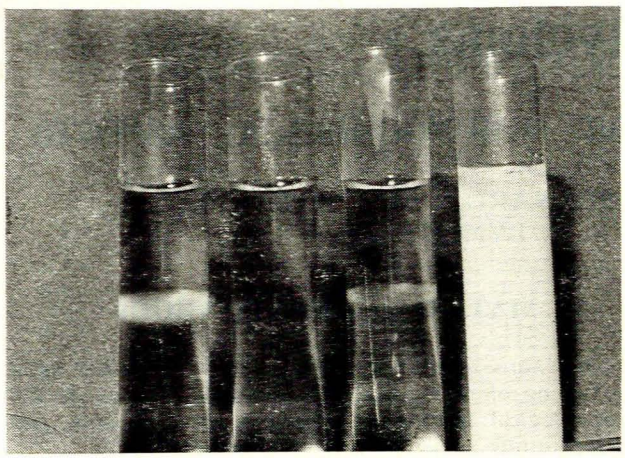

Fig. 1. Screening tests for the presence of globulin. Extreme left: **Bradshaw's test is positive in urine showing a Bence-Jones band on electrophoresis. Urine total protein was 120 mg/100 ml.** Left: **Negative Bradshaw's test.** Right: **Weakly positive Bradshaw's with urine showing non-selective proteinuria on electrophoresis.** Extreme right: **The same urine as in** (right) **tested with 10% salicylsulphonic acid.**

Apparatus). Cognisance was taken of the limitations of the protein binding of the dye, Ponceau S, used as the protein stain.[9] The linearity of the relationship between the optical density and the concentration of paraprotein bands up to 6,0 g/100 ml was verified.

Electrophoresis of serum and concentrated urine was performed simultaneously on 5-cm cellulose acetate strips (Beckman) using application points at the same horizontal origin (Fig. 2). With the aid of a Pasteur micropipette, 5 μl of each was applied over a width of 1,5 - 2 cm.

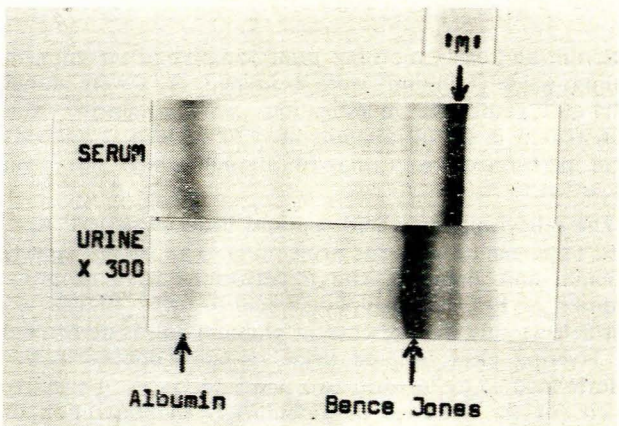

Fig. 2. Electrophoretic preparation of serum and urine performed coincidentally. Both samples were applied at the same transverse level.

The strips were run in a barbitone buffer, pH 8,6 for 1 hour at 200 V in a Shandon electrophoresis tank. The strips were stained with 0,25% naphthalene black 10 B in absolute methanol containing 3% trichloracetic acid. After 10 minutes the strips were destained by washing twice in 10% acetic acid in methanol for 10 minutes, and finally twice with pure methanol for 10 minutes. The electrophoretic appearance of the sample of concentrated urine was used as conclusive proof of the presence of Bence-Jones protein, and a visual quantitative estimate of the Bence-Jones fraction was made. Immuno-electrophoresis was performed by running the samples in 1% agar gel in electrophoresis buffer on microscope slides in a Shandon electrophoresis tank. Appropriate antisera (Hyland, Behringworke AG) were used and the results of the light chain typing were read between 2 and 4 hours after the antiserum had been applied (Fig. 3). The results of heavy

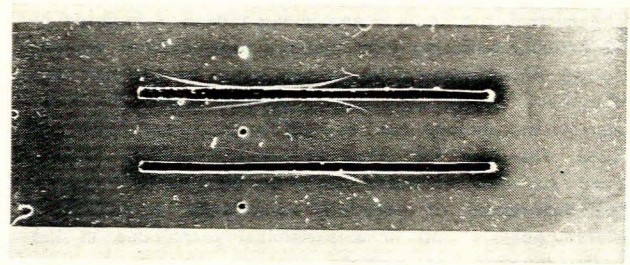

Fig. 3. Two different type kappa immuno-electrophoretic arcs due to Bence-Jones protein (upper trough) and one type lambda Bence-Jones protein (lower trough). The urine samples were applied to the wells.

chain typing were read after approximately 20 hours, although in many instances the results were positive from about 4 hours (Fig. 4). It was found that the results of urine electrophoresis were best read 4 hours after applying the antiserum, since precipitin arcs occasionally disappeared after overnight incubation, yielding a false negative result.

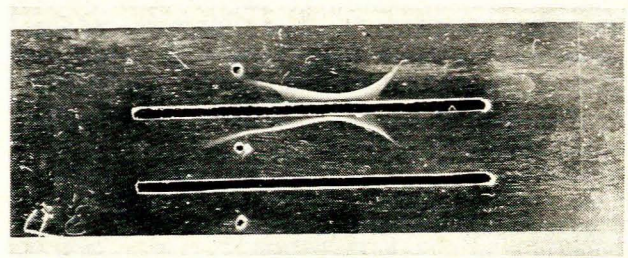

Fig. 4. Immuno-electrophoretic appearances of two IgG paraproteins 20 hours after application of antiserum (upper trough). Marked diminution in serum IgA in both patients caused failure of development of reaction to IgA antiserum (lower trough). The serum samples were applied to the wells. Antisera were applied to the troughs.

In every case serum immunoglobulins other than the paraprotein were determined by radial immunodiffusion.[10] Results were expressed in international units per ml (IU/ml) using a pooled serum standard which had been calibrated against the World Health Organisation International Reference Standard 67/97.[11] Paraproteins were not measured by immunodiffusion, since their idiotypic behaviour in different patients led to erroneous results being obtained.

RESULTS

Clinical Features

Fifty-two patients have been treated at the Haematology Clinic of the Johannesburg General Hospital since 1965. Of these, 22 were males (mean age 58,7 years, range 38-84 years) and 30 were females (mean age 64,6 years, range 44 - 86 years); and 71% were over 60 years of age.

The most common presenting symptoms were bone pain 65% and those associated with anaemia 40%. Others were pathological fractures 21%, recurrent infections 19%, fever 6%, weight loss 13% and miscellaneous 15%. Symptoms had been present for more than 3 months in 38% of patients.

Physical examination demonstrated bone tenderness in 16 patients (30%), splenomegaly in 9 and hepatomegaly in 6, while 22 patients were entirely normal.

Thirty-three patients (63%) were anaemic, with haemoglobin concentrations less than 11,5 g/100 ml, and the erythrocyte sedimentation rate was raised (>20 mm/first hour Westergren) in 47. Hyperuricaemia (serum uric acid >6,5 mg/100 ml) was present in 25 patients, and renal insufficiency (urea >40 mg/100 ml, serum creatinine >1,5 mg/100 ml, creatinine clearance <80 ml/min) in 18. A serum calcium greater than 5,5 mEq/litre was present in 6 patients.

Diagnostic Criteria

Eighty-three per cent of the patients showed radiological evidence of myeloma deposits in bone. The most frequently involved bones were the skull (40%) and the spine (34%). Bone marrow examination showed evidence of infiltration with myeloma cells in 40 of 43 patients in whom it was done. Abnormal globulins were demonstrated in 49 patients. At least 2 of these 3 major criteria of multiple myelomatosis were present in all patients.

Twenty-three patients presented with an abnormal elevation in the serum total protein, due mainly to paraprotein ('M'-component). In 14 patients there was hypoalbuminaemia (serum albumin <3,0 g/100 ml), and in one patient hypoproteinaemia (serum total protein <6,5 g/100 ml) in association with a daily Bence-Jones protein excretion of more than 30 g. The 'M'-component was greater than 1,0 g/100 ml in 46 patients at the time of diagnosis, concentrations ranging between 1,0 and 7,4 g/100 ml (mean 3,8 g/100 ml). No serum 'M'-component was identified in 6 patients, but in 3 of them Bence-Jones protein was detected in the urine in a concentration greater than 100 mg/100 ml.

IgG myelomatosis occurred in 36 patients, IgA in 9 and Bence-Jones myelomatosis in 3. One case of IgD myelomatosis was found. In 3 patients in whom the diagnosis was made on marrow morphology and the radiographic appearances of bone, no serum paraprotein was detected and urine was not examined. A marked reduction in the concentration of serum immunoglobulins was found in 43 patients. This applied particularly to the group with IgG paraproteins. Patients with IgA or Bence-Jones myelomatosis showed only slight or moderate immune paresis.

Electrophoresis of concentrated urine showed a Bence-Jones band in 34 patients. Forty-two per cent were type kappa and 58% type lambda. The total protein concentration in the urine was greater than 20 mg/100 ml in 88% of them. Analysis of the protein showed the additional presence of albumin in 67%, transferrin in 50% and the paraprotein in 25%. Urine examination for protein in the remaining patients tested was negative. The salicylsulphonic acid test was positive in all patients with albuminuria, but in only 75% of patients with Bence-Jones proteinuria. Bradshaw's test, which was used as a screening test for Bence-Jones protein and does not detect the presence of albumin, was positive in 63% of the patients in whom a Bence-Jones protein band was identified on electrophoresis. Electrophoresis of some patients' urine specimens showed a band simulating a Bence-Jones protein band in the late beta region of the electrophoretic strip, which failed to react with light chain or Bence-Jones protein antisera. These cases gave a positive reaction with antiserum to transferrin.

DISCUSSION

Multiple myeloma has in the past usually been reported to occur more commonly in men. In the present series 58% of patients were women, the female predominance being particularly striking in the older patients. This finding supports the suggestion that a change in the sex distribution has occurred recently.[12] The explanation for the apparent change is unknown. Our clinical findings were similar to those reported from other centres.[13] It is important to note the absence of any physical abnormality in 42% of patients despite the frequent presence of advanced disease.

The early diagnosis of multiple myeloma usually depends upon the detection of abnormalities of globulin synthesis. In a mouse plasmacytoma model it was shown that the level of serum paraprotein reflects the size of the tumour;[14,15] the number of tumour cells and the amount of paraprotein increase exponentially and are correlated with each other. Similar conclusions were reached in patients in whom the diagnosis was subsequently established.[17] It has been estimated on the basis of median tumour cell doubling times that a serum IgG paraprotein may first be detected when the immunocytoma has a mass as small as 20 g.[18] Bence-Jones protein can be detected with certainty at a level as low as 15 mg/24 hours if the urine is concentrated 300 times.[17] This quantity of Bence-Jones protein could be produced by as little as 3 g of immunocytoma.[18] Since the tumour mass at the time of clinical presentation is probably about 1 kg it is estimated that detectable biochemical abnormalities may precede symptoms by as long as 5 years.[18] The need for careful immunochemical examination of serum and concentrated urine specimens is therefore readily apparent. Abnormal globulin synthesis was present in 49 of the 52 patients in this investigation.

Screening tests for Bence-Jones protein may be misleading. Bradshaw's test has been found to be unsurpassed as a screening test,[19] being positive in 95% of urine specimens containing Bence-Jones protein in concentrations

greater than 5 mg/100 ml. A positive result was obtained in only 63% of patients with Bence-Jones proteinuria in the present investigation. Moreover, other globulins which were characterised by means of electrophoresis and immuno-electrophoresis, also sometimes led to a positive screening result in the urine. Albustix (Ames Co., Miles Laboratories Inc., USA) cannot be used to detect Bence-Jones protein, and salicylsulphonic acid, although sensitive, lacks specificity and gives a positive result for most proteins in the urine.

It is noteworthy that in the present investigation one quarter of patients with Bence-Jones proteinuria would have been missed if adequately concentrated urine had not been subjected to electrophoresis. The urine of any person suspected of having multiple myelomatosis should therefore be concentrated and submitted to electrophoresis. If a band is found on electrophoresis, immuno-electrophoresis must then be done in order to characterise the immunochemical specificity of the Bence-Jones band.

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