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REVIEW ARTICLE

Old but Still Relevant: High Resolution Electrophoresis and Immunofixation in Multiple Myeloma

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Abstract Introduction: High resolution electrophoresis (HRE) and immunofixation (IFX) of serum and urine are integral to the diagnostic work-up of multiple myeloma. Unusual electrophoresis patterns are common and may be misinterpreted. Though primarily the responsibility of the hematopathologist, clinicians who are responsible for managing myelomas may benefit from knowledge of these. In this review article we intend to discuss the patterns and importance of electrophoresis in present day scenario. Methods: Patterns of HRE and IFX seen in our laboratory over the past 15 years were studied. Results: Monoclonal proteins are seen on HRE as sharply defined bands, sometimes two, lying from γ- to α-globulin regions on a background of normal, increased or decreased polyclonal γ-globulins, showing HRE to be a rapid and dependable method of detecting M-protein in serum or urine. Immunofixation complements HRE and due to its greater sensitivity, is able to pick up small or light chain bands, not apparent on electrophoresis, including biclonal disease even when electrophoresis shows only one M-band. Special features liable to misinterpretation are discussed. Familiarity with the interpretation of the varied patterns seen in health and disease is essential for providing dependable laboratory support in the management of multiple myeloma.

Keywords High resolution electrophoresis · Immunofixation · Myeloma

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Introduction

With the advent of the highly sensitive and specific, serum free light chain assay, what tests from amongst high resolution electrophoresis (HRE) and immunofixation (IFX) of serum and urine, to use in screening for monoclonal gammopathies, has been a subject of debate and continued evaluation [1–3]. Even though serum free light chain assay has impacted the management of myeloma in a major way, HRE and IFX continue to be integral to the diagnosis of myeloma. Uncommon and atypical patterns, may, however, lead to misinterpretation. In this review that builds on our earlier published report on low cost options in setting up a myeloma laboratory [4], we present a few sample patterns of HRE and IFX that we have observed in the myeloma laboratory of our tertiary care cancer center. These provide a range of findings that should be informative to the clinician and also help the hematopathologist in correct interpretation.

Methods

High resolution electrophoresis and IFX on serum and urine using agarose as support medium was carried out according to standard procedures [4–7]. Briefly, samples are applied on a 0.5–1.5 % agarose (medium electro-endosmosis; EEO 0.13–0.19) gel and then electrophoresed using barbital buffer, pH 8.6. Urine is concentrated 50–200X before being run for electrophoresis or IFX [4]. Procedure for IFX is essentially the same, except that a plastic, rather than glass support is used for the agarose gel. The first lane is used as a reference electrophoresis lane for comparison with the remaining, that are assessed for reactivity to antibodies to different heavy and light chains.

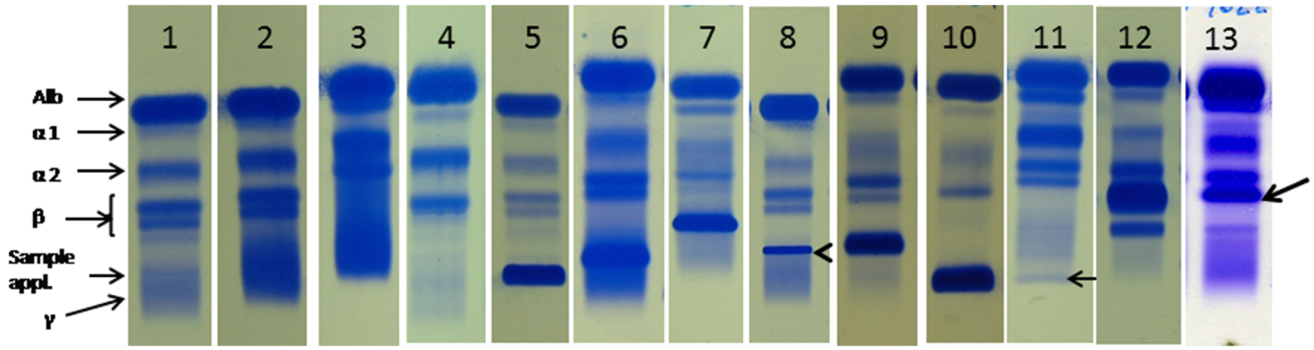

Fig. 1 High resolution electrophoretic patterns on agarose gel (Brilliant Blue R-250 stained gels). *Lane 1* Normal human serum. *Lane 2* Polyclonal hypergammaglobulinemia. *Lane 3* Polyclonal hypergammaglobulinemia with β–γ bridging. *Lane 4* Hypogammaglobulinemia. *Lane 5* Thick M-band in mid γ-globulin region on reduced polyclonal γ-globulin background. *Lane 6* Thick M-band in mid-γ globulin region on increased polyclonal γ globulin background. *Lane 7* Thick M-band in fast-γ globulin region on normal polyclonal γ globulin background. *Lane 8* Sharp narrow M-band in fast γ-globulin region (Waldestrom's macroglobulinemia). *Lane 9* Thick M-band in fast-γ globulin region on reduced polyclonal γ-globulin background. *Lane 10* M-band in slow γ globulin region at extreme cathodal region. *Lane 11* A faintly stained M-band in slow γ globulin region at extreme cathodal region (on treatment). *Lane 12* Two bands with different electrophoretic mobilities in γ globulin and β–γ interzone (partially). *Lane 13* M-band in the β region

An M-protein appears as a sharp, well-defined band reactive with a single heavy chain class antiserum and a similar band with either kappa or lambda light chain antisera [4–7]. Standard dilutions for different heavy and light chains have been recommended [5, 8] and are very helpful. Electrophoresis plates are read in the direction of the run, cathode to anode, to identify different bands, and also across, so that different samples can be compared with each other [5, 7]. In addition the γ-globulin zone is checked for alteration in its staining intensity.

Results

Electrophoretic patterns in serum and urine are illustrated in Figs. 1 and 2 and IFX results in Fig. 3. Table 1 gives an idea of the annual service load of our myeloma laboratory.

High Resolution Electrophoresis: Normal and Polyclonal Hypergammagblobulinemia

Normal

Figure 1, lane 1, illustrates the protein bands seen in normal serum. Albumin (abbreviated as alb, in the figure), a sharply defined band is the most anodal (top-most part of the run), followed by the globulins: α1-, α2-, β- and γ-globulin. The β-globulin band is composed of the anodal transferrin and the cathodal complement. γ-Globulin produces the diffusely stained zone area behind (cathodal to) the sample application line (abbreviated 'sample appl.' in the figure). An immunoglobulin population that moves more strongly toward the anode (the positive pole; top part of sample lanes in the figures), that is, in the direction of the electrophoretic run, is referred to as having 'fast' electrophoretic mobility; conversely, immunoglobulin molecules that remain close to the cathode are said to be 'slow'. M-bands in the γ-region may be anywhere between the extreme cathodal (fast γ) region and the sample application site (slow γ) region. The clear zone between the sample application site and the β-globulin is often referred to as the β–γ interzone.

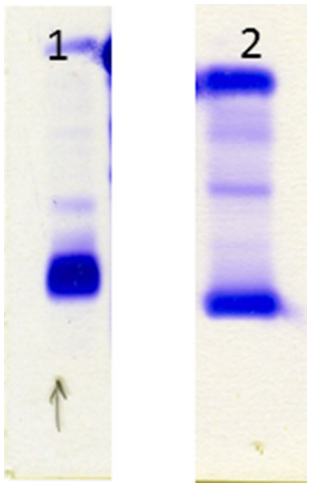

Fig. 2 High resolution urine electrophoresis (Brilliant Blue R-250 stained gels). *Lane 1* Broad, dense urinary M-band in γ globulin region; albumin and other bands due to proteinuria. *Lane 2* Thick M band in γ globulin region; albumin and other bands due to proteinuria

Hypergammaglobulinemia

Figure 1, lane 2, is an example of increased density of the γ-globulin region, signifying polyclonal hypergammaglobulinemia.

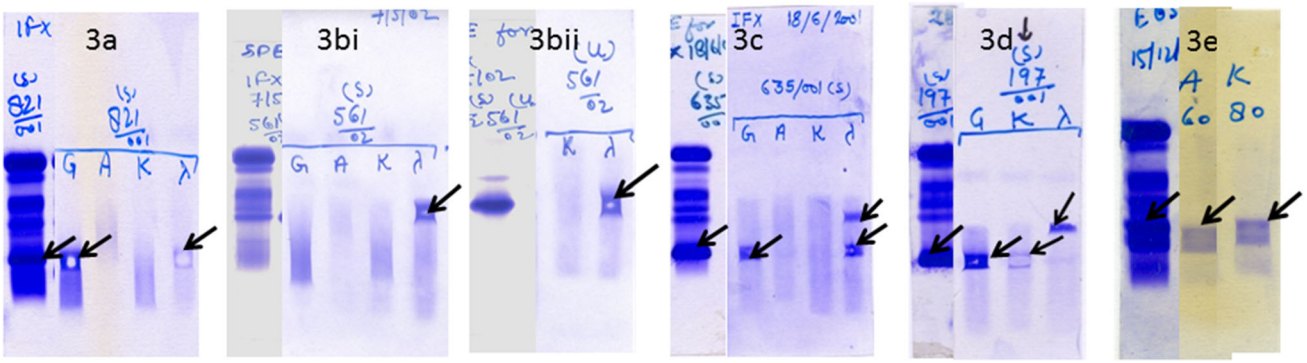

Fig. 3 Immunofixation. First lane in each figure is reference electrophoresis (Brilliant Blue R-250 stained gels). **a** Serum M-band reacting with γ- and λ-but not with α- or κ-antibodies (*arrows*). *IgG λ myeloma.* **b** Serum (**bi**) and urine (**bii**) M bands reacting with λ but not with γ-, α- or k-antibodies (*arrows*). The diffuse staining in the negative lanes is due to the polyclonal serum or urine antibodies. *Ig λ myeloma.* **c** M band in γ globulin reacting with γ- and λ-antibodies (*arrows*). The slower band has the same mobility as the obvious M band. The more anodal band represents free λ-chain. *IgG λ myeloma with free λ-light chains*. **d** Biclonal multiple myeloma (*arrows*). *IgG* κ*-myeloma with free λ-light chains.* **e** Two M-bands, both IgA κ Myeloma

Table 1 Serum and urine electrophoreses run annually

Sample	Total no. of cases including follow-up	Total no. of positive cases including follow-up	Total no. of new cases for screening	No. of positive new cases (% new cases)	No. of cases in which immunofixation was performed
Serum	2108	966	327	206 (62.9 %)	223
Urine	254	93	230	73 (31.7 %) (including 56 cases positive for both urine and serum; 17 positive for light chain only)	73 (both serum and urine immunofixation was done)

β–γ Bridging

In Fig. 1, lane 3, the polyclonal hypergammaglobulinemia extends into the β region, producing the appearance known as β–γ bridging.

Hypogammaglobulinemia

Figure 1, lane 4, is example of decreased density of the γ-globulin region, signifying hypogammaglobulinemia.

High Resolution Electrophoresis: Myeloma

High resolution electrophoresis showed monoclonal bands of varying thicknesses on a background of normal, increased or decreased polyclonal γ-globulins.

Figure 1, lane 5, shows thick M-band in mid-γ globulin region on a background of reduced polyclonal γ-globulin, while Fig. 1, lane 6, shows a similar band on an increased polyclonal background.

Figure 1, lane 7 and lane 8, show M-band in fast γ-globulin region overlying the sample application site, on a normal polyclonal γ-globulin background. The band in lane 7 is thicker and extends into the β–γ inter-zone. Figure 1, lane 9, shows similar M-band, but on a reduced polyclonal background.

Figure 1, lane 10, shows a thick M-band in slow γ-globulin region almost at extreme cathodal region. Figure 1, lane 11, taken from a patient of myeloma on treatment, shows M-band in the same position (arrow). Polyclonal γ-globulin is barely visualized in lane 10 and is reduced in lane 11.

Figure 1, lane 12, shows two bands of unequal thickness. The slower band is at the fast γ-region. The faster thicker band is in the β–γ interzone.

Figure 1, lane 13, shows a narrow M-band in β-globulin zone overlying the complement band (arrow).

Figure 2, lanes 1 and 2 show urine electrophoresis showing M-band in γ-globulin region. Albumin as well as other globulin bands are also present, signifying global proteinuria.

Immunofixation

Representative IFX patterns are illustrated in Fig. 3.

In Fig. 3a, serum electrophoresis shows M-band that on IFX is seen to be reacting with γ- and λ-(lanes G and λ) but not with α- or κ-antibodies (lanes A and K), signifying IgGλ myeloma. Diffuse staining in the remaining lane G is due to polyclonal IgG present in this patient's serum, in addition to the monoclonal IgG protein. A much lighter,

diffusely stained area in a more anodal position, is seen in lane A (reacted with anti-α antiserum) clearly showing the faster migration of IgA. The diffuse staining in lane K is similarly due to the κ chain present in the normal polyclonal antibodies.

The serum (Fig. 3bi) and urine electrophoresis (Fig. 3bii) show M-band in β-globulin region which is further confirmed by IFX as λ light chain (no reaction with heavy chain antisera), signifying a case of λ light chain myeloma. Diffuse staining in the negative lanes is due to the polyclonal serum or urine immunoglobulins.

Figure 3c shows M-band in γ-globulin region reacting with γ- and λ-antibodies. There are, however, two bands in the λ lane. The slower band has the same mobility as the obvious M-band in the γ-globulin region and hence represents γ chains that are part of the complete IgG molecule. The more anodal band represents free λ-chain. These findings indicate a case of IgGλ myeloma with free λ light chains.

In Fig. 3d, the thick band at sample application site in fast γ-globulin region on serum electrophoresis is seen to be IgGκ. There is an additional band in the λ lane, signifying free λ chain in addition to the IgGκ M-band, proving a diagnosis of biclonal multiple myeloma (IgGκ plus λ chain myeloma).

In Fig. 3e, the two bands in the β–γ interzone on serum electrophoresis are both seen to be IgA κ multiple myeloma.

Figure 3d and a, shows the artefact introduced due to antigen excess (prozone effect). In Fig. 3a this is seen as a small clear zone that interrupts the anti-γ positive band (lane G). In Fig. 3d this effect is much more pronounced and has split the anti-κ positive band (lane K) to make it appear as two bands, fainter than the corresponding anti-γ positive band (lane G) band. The anti-λ positive band (lane λ) does not have this artefact.

Annual Service Load of Myeloma Laboratory

Aside from our own center, we get referrals for myeloma screening from departments of nephrology, orthopaedics, medicine, haematology and to a lesser extent from neurology, cardiology and dermatology. To keep running costs low, unless the clinical features strongly indicate the need for urine electrophoresis upfront, we do serum electrophoresis in all cases, and generally perform urine examination only if the serum shows an M-band or hypogammaglobulinemia. Data for 1 year is presented in Tables 1 and 2.

Table 1 shows that of the 327 new cases screened for myeloma, 206 (63 %) had M-band in the serum, including 56 patients who had both complete immunoglobulin molecule as well as free unbound light chains. Seventeen of these 327 patients (5.2 %) had hypogammaglobulinemia due to light chain myeloma. Two hundred and thirty cases were screened for urine M-band, of which 73(31.7 %) were positive, including 17 patients of light chain myeloma who had only urine M-band. The 223 cases in which IFX was done, included those in whom serum electrophoresis showed either an abnormal band (n = 206) or hypogammaglobulinemia (n = 17).

Table 2 Relative distribution of types of monoclonal proteins in new cases

Type of M-protein	No. of cases	Percentage (%)
Serum (n = 223)		
IgGκ	81	36.2
IgGλ	45	20.2
IgGκ + κ	18	8.1
IgGλ + λ	38	17.0
IgAκ	8	3.6
IgAλ	13	5.8
IgMκ	3	1.3
Only κ light chain	8	3.6
Only λ light chain	9	4.0
Urine (n = 73)		
Only κ light chain	28	38.3
Only λ light chain	45	61.4

Table 2 shows that IgG myeloma constituted 81.6 % of all cases examined and light chain myeloma was 7.6 % of the total. Urinary κ and λ light chains were present in 28 (38.3 %) and 45 (61.4 %) cases, respectively. This included patients of light chain myeloma as well as patients having monoclonal serum free light chains in addition to complete immunoglobulin molecules.

Discussion

When coined, in 1889, the term 'myeloma' implied a tumour arising from the bone marrow. The belief that it could arise from any marrow cell led to the use of terms such as erythroblast or megakaryoblastic, and later, the tautological term, plasma cell myeloma [9]. Plasma cells that make up myeloma are monoclonal, a feature that is reflected in the homogeneity of immunoglobulin molecules that are secreted by them. For this reason these immunoglobulin molecules are referred to as M (monoclonal) protein or paraprotein. The term 'gammopathy' was introduced in the erroneous belief that that paraproteins migrated only to the γ region on electrophoresis; diseases that produced them were referred to as monoclonal gammopathies [9].

Monoclonal gammopathies are most simply investigated by electrophoresis supplemented by IFX. Electrophoresis

separates proteins based on differences in their charge/-mass ratio. The currently used HRE is an example of zone electrophoresis. In contrast to its forerunner, the moving boundary electrophoresis, introduced by Tesileus in 1937, zone electrophoresis uses a supporting medium. This ensures that the separated proteins do not re-mingle after the current is switched off and hence can be fixed and detected by staining with protein binding dyes.

Filter paper, the earliest support medium, to be used, allowed visualization of five, rather fuzzily delineated bands, the fastest (most anodal) being albumin followed by the globulins. Though cellulose acetate membrane and the currently recommended agarose support media that later supplanted filter paper, resolve serum proteins into a greater number of bands, and hence are high resolution, serum protein electrophoresis is still reported in the conventional 5-band format: albumin, α_1, α_2, β and γ globulins.

The designations α_1, α_2, β and γ globulins merely indicate the speeds of migration of the heterogeneous collection of proteins that make up serum globulins. Examples of specific proteins at these locations include α1-antitrypsin at the α_1, haptoglobin and α_2-macroglobulin at α_2, transferrin and complement at β, and immunoglobulins and C-reactive protein at the γ region. Electrophoresis plates are read in the direction of the run, cathode to anode, to identify different bands, and also across, so that different samples can be compared with each other [10]. Even in the presence of M band it is important to observe the background polyclonal γ-globulin whether normal, increased or decreased.

Immunofixation identifies the heavy and light chain type of the monoclonal protein; these are relevant to prognosis and renal involvement. International Myeloma Working Group (IMWG) strongly recommends IFX on serum and urine, preferably a 24-h sample, in all patients of multiple myeloma at diagnosis [11], in addition to the more recently introduced serum free light-chains [3]. Typical sensitivity levels of these assays are 1–2 g/L for serum HRE and 150–500 mg/L for IFX. [12] Serum free light chain assays have a sensitivity of less than 1 mg/L. IMWG has also laid down criteria for relapse and disease progression based on the findings of HRE, IFX and serum free light chain assay [13]. IFX testing is relevant on follow-up as it confirms complete response at baseline in serum and in urine in those with proteinuria [14].

For doing HRE and IFX one may use in-house procedures or opt for commercial methods. Our analysis was based on our manual in-house method [4]. Properly done, it gives excellent and clear-cut separation of bands. The destaining solution removes background stain, rendering the background transparent and colorless; after prolonged storage, however, the dried agarose does acquire some color. In the manual method one can make the runs slightly longer than we have done. Through a series of steps, including regenerating with activated charcoal, rather than discarding the used destaining solution, our procedure for HRE of serum costs only approx. Rs. 25.00 per patient. Results of equal clarity can be achieved at substantially higher cost and some amount of ease by commercial semi-automated gel electrophoresis machines. They have the advantage of processing hundreds of HRE and IFX samples in a day and hence may be the ideal choice for a very busy referral commercial or even an academic lab with an exceptionally high load of myeloma testing. High-performance capillary electrophoresis is an automated technique in which proteins in a buffer can be separated in a narrow capillary. Though the procedure uses a liquid medium, it has exquisite resolution compared to Tiselius' procedure [15]. Capillary electrophoresis may be able to detect paraprotein peaks as well as, or even slightly better than HRE [16].

Polyclonal hypergammaglobulinemia is seen as diffuse, rather than sharply defined, increase in intensity of staining of the γ-globulin region that sometimes extends into the β-region. The latter appearance, that looks as if the electrophoresis run was faulty, is known as β–γ bridging (Fig. 1, lane 3) and is seen in liver cirrhosis and rheumatoid arthritis [5].

In contrast to benign hypergammaglobulinemias, a large variety of appearances are seen in paraproteinemias. The patterns we have illustrated show M-bands of different widths and densities at slow γ, that is, extreme cathodal end (Fig. 1, lanes 10 and 11), mid-γ (Fig. 1, lanes 5 and 6), fast-γ including the sample application site (Fig. 1, lanes 7, 8 and 9), β–γ interzone (Fig. 1, lane 12, the more anodal thick band) and β regions (Fig. 1a, lane 13), in accord with the fact that M-bands are present not just in the γ-globulin region, but may be seen all the way up even to the α region. While M-band on a normal (Fig. 1, lane 7) or increased (Fig. 1, lane 6) polyclonal background implies presence of normal polyclonal immunoglobulins apart from the monoclonal immunoglobulin, M-band on a reduced polyclonal background (Fig. 1, lanes 5 and 9) indicates a disease state, as happens often in myeloma, where normal polyclonal immunoglobulins are reduced, making the patient prone to infection [5].

Special situations relevant to what we have presented and potential pitfalls that can impact diagnosis and management, include bands at the sample application site and in regions outside of γ, false negatives and positives, myelomas of the light chain, IgD and IgE variety and recognition of biclonal myeloma.

Bands at the sample application site may be IgG, IgM (Fig. 1, lane 8; arrow), IgD and cryoglobulins. Cryoglobulins have a lot of complexed material—polymerized IgA

and IgG3 or large aggregated IgM paraprotein that precipitate and produce artefactual small bands at sample application site [5, 17, 18]. Immunofixation does not help as bands are seen in all lanes irrespective of the antibody applied. Correct diagnosis requires mild sulphydryl reduction of the serum using dithiothreitol prior to IFX. This reduces the size of the complexes without destroying the antigenicity [18, 19].

M-bands in the β-(Fig. 1, lane 13) or α-region constitute a special case as they overlie the normal serum protein bands in these regions and hence are liable to be missed or misinterpreted. They often need IFX in order to be demonstrated with certainty. Their quantitation through densitometry is inexact for the same reason [3, 5, 18].

False positives, that is, bands not due to myeloma but mistaken for M-band, include fibrinogen seen in β–γ interzone in incompletely clotted samples, transferrin in iron deficiency anemia in β-region, C-reactive protein in mid-γ region and hemoglobin–haptoglobin complex seen in patients with hemolysis (α2-region) [18].

In the absence of any suspicious band, false negatives have to be excluded by ruling out light chain myeloma (most common) and IgD and IgE myeloma. In this respect, the diagnostic workup of light chain myeloma merges with that of IgD and IgE.

Reduced staining intensity in the γ-globulin region, that is, hypogammaglobulinemia without an M-band (Fig. 1, lane 4) indicates, in the appropriate age group, light chain myeloma (approx. 8 % of all our cases), which has only monoclonal light, and not heavy chains. In the setting of this finding urine electrophoresis is the next test that is required to be done to confirm presence of Bence Jones proteinuria Owing to their small size light chains escape into, and are easily demonstrable in the urine by HRE and IFX. In the unusual event of their being present in the serum, the abnormal band is faint or subtle, except when the light chains have polymerized or there is renal impairment [7, 20]. The ability of IFX to pick up light chain band in the serum even when it is not evident on HRE, can serve to diagnose light chain myeloma when urine electrophoresis is not possible for any reason [21]; this has also been exploited as a means of response assessment in myeloma [3].

IgD and IgE myeloma is characterized by a small (nearly always <2 g/dl; γ, β and β–γ regions), or absent (40 %) M-protein spike on serum electrophoresis and light chain proteinuria (>90 %) [22–24]. Because of the latter feature, especially when there is no abnormal band in the serum, IgD and IgE myeloma resemble light chain myeloma. The standard teaching is that if only monoclonal light chain is detected in the serum or urine, that is, the sample tests negative for IgG, IgA and IgM, the patient must be screened for the presence of IgD and IgE monoclonal protein in order to avoid false negatives [24]. Though reduced polyclonal gammaglobulinemia on serum electrophoresis without a serum spike makes the more common light chain myeloma more likely, the ideal practice still is to rule out IgD and IgE myeloma, so that a small IgD or IgE band is not missed [24, 25]; Ouchterlony immunodiffusion with IgD and IgE antisera can be used for this purpose (Robert A Kyle, personal communication). Thus, such labs in resource-poor settings, which test only for light chain proteinuria and not for the much rarer IgD and IgE in the work-up of a patients having hypogammaglobulinemia as the sole finding, may be missing some cases of IgD and IgE myeloma. Testing urine for proteins is a simple way of looking for light chains; sulfosalicylic acid test rather than the dipstick is recommended because the latter often misses light chains; a negative test result makes the likelihood of Bence Jones proteinuria extremely small ([14, 19]; Robert A Kyle, personal communication). Clinical features of IgD and IgE myeloma are similar to that of other myelomas but Bence-Jones proteinuria, extramedullary involvement, lytic lesions and amyloidosis have been reported to be more frequent in IgD [26]; plasma cell leukemic presentation is common in IgE myeloma [27].

An interpretation of biclonality requires that there be two bands with different light or heavy chains. As our case (Fig. 3d) shows, this can happen even if there is only one band on HRE. Here, IFX unexpectedly showed a second light chain band of different isotype from the first. Cases with two thick bands on HRE (Fig. 1, lane 12), approximately 0.2 % in our experience, in contrast, have all turned out, on IFX, to be monoclonal, generally IgG or IgA (Fig. 3e), though IgM is also known to occur. Two bands in such a situation represent, as has been demonstrated for urine light chains as well, monomers and polymers of the same monoclonal protein [28, 29]. Less frequently, two or more faint bands may be seen on a polyclonal background due to oligoclonal expansion in infectious or autoimmune disease [5, 7].

Urinary bands in monoclonal gammopathies may be monoclonal, or may be serum proteins that have leaked into the urine due to proteinuria. Urine electrophoresis normally does not show bands. Urine findings in myeloma depend on whether the malignant plasma cells produce monoclonal free (unbound to heavy chains) light chains or not, and whether there is renal damage. Electrophoresis shows monoclonal urinary free light chains in light chain myeloma, and those intact immunoglobulin myelomas that produce free light chains in addition to the complete immunoglobulin molecule (Fig. 3c) [5]. Renal damage causes one or more serum proteins to appear in the urine, independent of monoclonal proteinuria, and may also allow complete monoclonal immunoglobulin molecules (IFX positivity with both heavy and light chain) to enter the

urine. In a large series, 10 % of patients had proteinuria consisting mainly of albumin but no monoclonal proteins [30]. The two cases we have presented show bands due to proteinuria in addition to an M-band. Serum and urine paraproteins in an individual patient may have different relative mobilities [7].

High resolution electrophoresis and the more sensitive IFX are complementary. While interpreting IFX results it helps to remember that sometimes the dilutions of the serum or urine (hence antigen) and antibody are not matched, and there may be, because of antigen excess, a clearing in the center of the immunoprecipitate, the prozone effect (Fig. 3a). In most cases there should be no need to repeat the test because the interpretation is clear. Sometimes, if this is excessive, the immunoprecipitate band may be faint and appear split, as has almost happened in Fig. 3d, or may even be barely seen. In such a situation one may consider repeating the test at a greater antigen dilution [8].

Immunofixation proves the monoclonality of an abnormal band on HRE and identifies the type of myeloma with respect to its constituent heavy and light chain. In immunofixation, the first, reference HRE lane, is cut away at the end of the electrophoresis run and processed separately from the rest, which are tested for their immunoreactivity with heavy and light chain antisera. For interpreting the IFX result the reference electrophoresis lane is aligned with the rest; in case the heavy and light chain immunoglobulins identified by IFX have the same mobility as the abnormal band on seen HRE, it means they are part of the same complete immunoglobulin molecule. Figure 3a, b, e are examples of this. Figure 3d and e, however, each show an additional free light chain band. Due to their smaller size they are expectedly faster and hence anodal to the complete immunoglobulin band. In the former (Fig. 3c) the light chain type is λ in both, indicating IgGλ myeloma with free λ light chains; in the latter the additional free light chain is of different isotype, hence a case of biclonal myeloma with one clone making IgGκ and the other λ chain. Immunofixation is thus essential for diagnosing biclonal myeloma; it was because of immunofixation that we were able to diagnose biclonal disease when only one band was present on HRE and could refute it when two bands were present (Fig. 3e). Because it picks up monoclonal light chains not apparent on electrophoresis IFX will identify light chains in the setting of hypogammaglobulinemia [21].

In the initial diagnostic work-up IFX on serum has been recommended in two kinds of settings: first, where HRE shows an abnormal band and second, where an abnormal band is not obvious. In the first situation, IFX confirms the monoclonal nature of the band and more importantly, is vital in the diagnosis of those cases of myeloma which have bands of α- or β-mobility overlying the normal serum protein bands. In the second situation where an abnormal band is not obvious, one is guided by both the electrophoresis findings and also how strong the clinical suspicion of myeloma is. Hypogammaglobulinemia is an indication for IFX in urine or serum to confirm the diagnosis of light chain myeloma [5]. Also, if the clinical suspicion of a monoclonal gammopathy is strong, then even in the setting of negative electrophoresis, IFX with κ- and λ-light chain antisera may show small M-proteins [10] and may establish the diagnosis of heavy chain disease and IgD and IgE myeloma [23, 24]. During the course of treatment of myeloma IFX on serum is useful in assessing myeloma patients on therapy [3].

In conclusion HRE of serum supplemented with the very sensitive IFX electrophoresis are simple tests that are vital to the diagnosis and fairly detailed characterization of multiple myeloma. Immunofixation, in addition, is also an integral part of disease monitoring and response evaluation. Thorough familiarity with the many patterns seen in these tests is important for any laboratory providing this service.

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