Prognosis in monoclonal proteinaemia

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Citation

Schaar, C. G. (2006, November 9). *Prognosis in monoclonal proteinaemia*. Retrieved from https://hdl.handle.net/1887/4983

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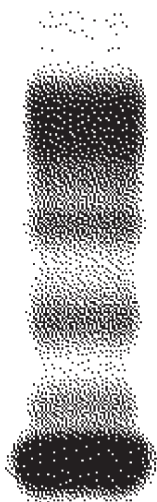
1 Introduction

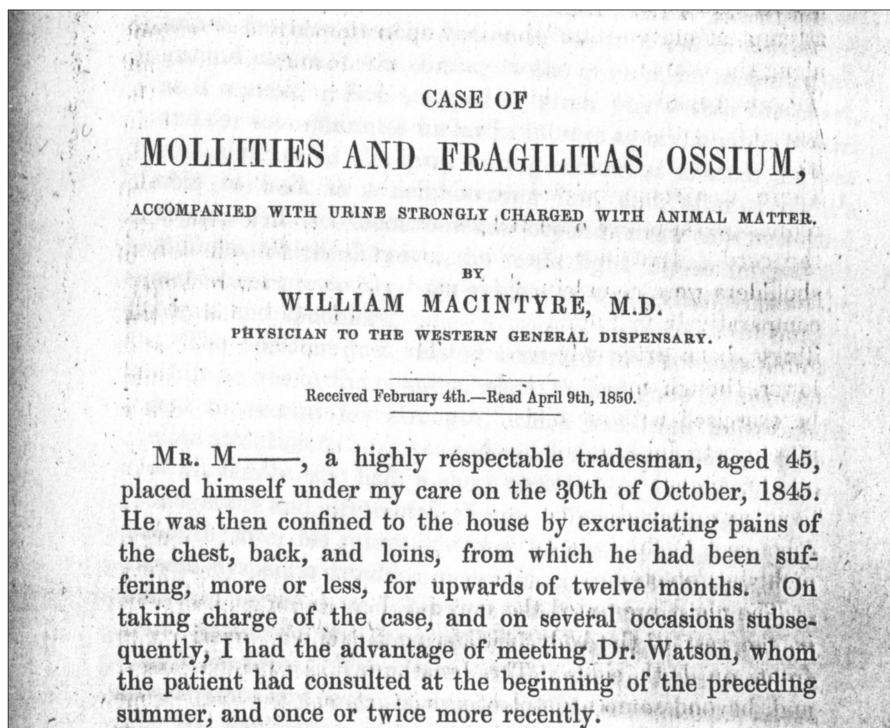


Monoclonal proteinaemia (M-proteinaemia) is a common finding in the blood of people aged 50 years and above. It is usually associated with multiple myeloma, non-Hodgkin's lymphoma, or other haematological diseases, respectively. However, in the majority of cases no related disease is present. The unravelling of a connection between the clinical finding of M-proteinaemia and the underlying disorder has started more than 150 years ago and albeit huge advances since, some features are still ill-defined.

History of monoclonal proteinaemia

First description of a monoclonal protein

In September 1844 a wealthy London grocer developed chest pains for which he visited Dr Thomas Watson, a leading general practitioner of London. Initially, a plaster cast and steel and quinine helped but after several months he developed severe pains in the chest and back with oedema. Dr William Macintyre, physician to the



CASE OF

MOLLITIES AND FRAGILITAS OSSIUM,

ACCOMPANIED WITH URINE STRONGLY CHARGED WITH ANIMAL MATTER.

BY

WILLIAM MACINTYRE, M.D.

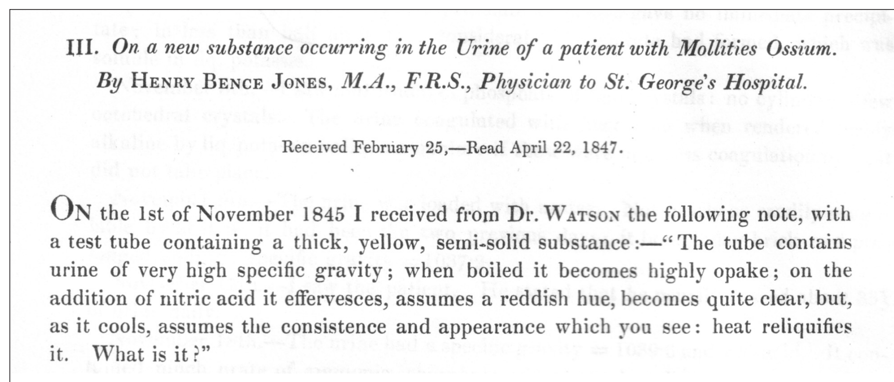
PHYSICIAN TO THE WESTERN GENERAL DISPENSARY.

Received February 4th.—Read April 9th, 1850.

MR. M——, a highly respectable tradesman, aged 45, placed himself under my care on the 30th of October, 1845. He was then confined to the house by excruciating pains of the chest, back, and loins, from which he had been suffering, more or less, for upwards of twelve months. On taking charge of the case, and on several occasions subsequently, I had the advantage of meeting Dr. Watson, whom the patient had consulted at the beginning of the preceding summer, and once or twice more recently.

Figure 1. **Detail of the first page of the paper by Dr William Macintyre.**

Metropolitan Convalescent Institution and the Western General Dispensary in St. Marylebone was called in[1].
Because of the oedema Dr Macintyre examined the patient's urine and noted the peculiar reaction of the urine when it was heated, cooled and reheated[1]. Both physicians independently sent a urine sample for analysis to Dr Henry Bence Jones, a 31-year old chemical pathologist at St George's hospital. The specimen sent by Dr Watson was accompanied with the following note:



III. *On a new substance occurring in the Urine of a patient with Mollities Ossium.*
By Henry Bence Jones, *M.A., F.R.S., Physician to St. George's Hospital.*

Received February 25,—Read April 22, 1847.

On the 1st of November 1845 I received from Dr. Watson the following note, with a test tube containing a thick, yellow, semi-solid substance:—"The tube contains urine of very high specific gravity; when boiled it becomes highly opake; on the addition of nitric acid it effervesces, assumes a reddish hue, becomes quite clear, but, as it cools, assumes the consistence and appearance which you see: heat reliquifies it. What is it?"

Figure 2. **Detail of the first page of the paper by Henry Bence Jones.**

Bence Jones carried out extensive chemical analyses on this unusual heat-precipitable substance and concluded that it was the 'the hydrated deutoxide of albumen'[2]. According to his estimate, the enormous quantities of this particular albuminous substance voided in the urine were in the same concentration as albumen in the serum. No amount of food could compensate for such a loss.
The patient died on January 2, 1846*. At autopsy the sternum, cervical, thoracic and lumbar vertebrae were soft, fragile and easily breakable and could be cut with a knife. This abnormal softness of the bones was named 'mollities ossium'. Histological examination of the affected bones was made by John Dalrymple, surgeon to the Royal Ophthalmic Hospital and a member of the Microscopical Society. He described great numbers of nucleated cells, of variable size and shape, and often larger than an erythrocyte. They contained frequently two or three nuclei. These descriptions, though

*) The identity of the patient in this first recorded case of multiple myeloma remained unknown for more than a century. Macintyre referred to him only as Mr M., and Bence Jones never identified him by name. In 1967 by careful and meticulous research of the Register of Deaths for the London area for the first quarter of 1846, and by process of elimination based on the known information of age, date of death, occupation and cause of death the correct death certificate was found[3]. The patient was identified as Thomas Alexander McBean and the cause of death was given as atrophy from albuminuria. At that time the term 'albuminuria' was equivocal to proteinuria.

incomplete, are not inconsistent with malignant plasma cells[4]. He also noticed the high degree of vascularity in the diseased bone:

While this destructive process goes on, we have evidence of undue turgescence of the vascular system. I am not aware whether any injection, or at least any *microscopically successful* injection, has ever been made of bone in this disease, but its greatly increased redness, even the extravasation of the so numerous blood disks, are proofs of the fact. Blood long effused undergoes many changes, microscopically considered; the cells become shrivelled, irregular, crenated, &c.; here, however, the disks are, for the most part, wholly normal.

Presuming, therefore, either a high degree of vascular congestion or of vascular activity to be present, the proper functions are perverted. Original structure is removed, soft as well as hard elements are carried out of the part by its own vascular system, and out of the body by the excretory glands, the still healthy kidneys. The particular case now under observation

Figure 3. **First description of bone marrow hypervascularity by Dr John Dalrymple.**

All three physicians Dalrymple, Bence Jones and Macintyre believed this disorder to be a malignant bone disease.

With the following statement Dr Bence Jones emphasized the role of 'albumenosuria' in the diagnosis of multiple myeloma: 'This substance must again be looked for in acute cases of mollities ossium'. Hereby the first monoclonal protein, and the first tumour marker, the 'Bence Jones protein' had been described[2].

From 'mollities ossium' to multiple myeloma

Two years before the death of Mr M., another and probably the first description of possible multiple myeloma was given in 1884 by Solly as 'mollities ossium'[5]. The patient he described was a 39-year-old woman who died four years after an illness that caused progressive bone pains, kyphoscoliosis and eventually spontaneous fractures of both clavicles, the right arm and both thighs. Her appetite was failing despite an allowance of wine, arrow-root, a mutton chop, a pint of porter and an infusion of orange peel, a rhubarb pill and (luckily) an opiate at night. On autopsy multiple fractures were present with fragile bones with 'an unnatural degree of vascularity'. Microscopic examination revealed numerous oval cells with one, rarely two, bright central nuclei[5]. In 1873 von Rustizky described the particular pathology of this condition with multiple tumours of the bone marrow and named it 'multiple myeloma'[6].

Conclusions.

1st. The ultimate analysis of this substance may be represented by C_{48}, H_{38}, N_6, O_{18}, or by C_{40}, H_{31}, N_5, O_{15}; according as protein is $=C_{48}$, H_{37}, N_6, O_{15}, or C_{40}, H_{30}, N_5, O_{12}.

	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	C_{48}, H_{38}, N_6, O_{18}; Reckoned.	or C_{40}, H_{31}, N_5, O_{15}. Reckoned.
C	51·68	51·50	52·25	52·29	52·16	52·19	52·25	52·10	52·00
H	7·15	7·09	6·97	7·17	7·16		6·97	6·70	6·85
N	15·24			14·81				15·17	15·15
O								26·00	25·99

The sulphur and phosphorus in this substance, per cent., gave

Sulphur	=1·36	1·09	1·03 per cent.
Phosphorus	=	·20	·19 per cent.

Hence it is an oxide of albumen, and from the ultimate analysis, it is the hydrated deutoxide of albumen.

2nd. In the above case of mollities ossium 66·97 parts of this hydrated deutoxide of albumen were passing out of the body in every 1000 parts of the urine. Hence, therefore, there was as much of this peculiar albuminous substance in the urine as there is ordinary albumen in healthy blood. So far, then, as the albumen alone is concerned, each ounce of urine passed was equivalent to an ounce of blood lost.

3rd. The peculiar characteristic of this hydrated deutoxide of albumen was its solubility in boiling water, and the precipitate with nitric acid being dissolved by heat and reformed when cold. By this reaction a similar substance in small quantity may be detected in pus and in the secretion from the vesiculæ seminales.

4th. This substance must again be looked for in acute cases of mollities ossium. The reddening of the urine on the addition of nitric acid might perhaps lead to the rediscovery of it; when found, the presence of chlorine in the urine, of which there was a suspicion in the above case, should be a special subject of investigation, as it may lead not only to the explanation of the formation of this substance, but to the comprehension of the nature of the disease which affects the bones.

Lastly, I am much indebted to Dr. Watson and Dr. MacIntyre for enabling me to follow out this case, and also to Professor Fownes for the use of his laboratory at University College.

Figure 4. **Chemical analysis and description of the urinary light chain protein by Henry Bence Jones.**

Another famous case of multiple myeloma (MM) was described in 1889 by Dr Otto Kahler. A 46-year-old physician named Dr Loos suffered from severe pains in the ribs, spine, left shoulder and right clavicle. Albuminuria was first noted after two years, after which anaemia, severe kyphosis, recurrent bronchial infections and loss of height occurred. On autopsy masses containing large round cells in ribs and thoracic vertebrae were seen. Kahler recognized that the urinary protein had the same characteristics as those described by Bence Jones[7] and the urine was described in detail by Huppert[8]. In 1900 Wright was the first to identify the plasma cells as tumour cells MM[9].

The name multiple myeloma stuck until today, though this disease is still often referred to as 'Kahler's disease' in The Netherlands and 'Rustizky's disease' in Russia.

Aside from diagnostic advances in serum protein examinations ante mortem recognition of MM was greatly enhanced by utilizing X-rays[10] and bone marrow examination[11]. No effective treatment was found until 1947 when urethane was discovered and followed 15 years later by melphalan (L-phenylalanine mustard)[12], which remained the cornerstone of MM-therapy up till the last decade of the last century. As the main scope of this historic introduction is on monoclonal proteinaemia I will not further elaborate on the history of the diagnostic and therapeutic advances in MM.

Bence Jones protein: source and kinetics

In 1846, J.F. Heller described a protein in the urine that precipitated when warmed a little above 50 °C (122 °F) and disappeared on further heating. Although Heller did not recognize the precipitation of the protein when the urine was cooled, it is almost certain that this was Bence Jones protein. He distinguished this new protein from albumin and casein[13]. Dr R. Fleisher, a clinical physicist who investigated normal and pathological bone marrow (knochenmark) was the first to use the term 'Bence Jones protein'[14].
Bradshaw found that meals had little or no influence on the amount of Bence Jones proteinuria. There was no nocturnal variation and the excretion rate was believed to be fairly constant during the day[15]. Walters made a study of three patients and confirmed that the quantity of Bence Jones proteinuria was independent of the protein intake. Furthermore, no diurnal variation was found. Bence Jones protein was demonstrated in the blood of one patient and in the bronchial secretions of another. Walters concluded that Bence Jones protein was of endogenous origin and was probably derived from blood proteins through the action of abnormal cells in the bone marrow[16]. In this era before the rise of medical ethical committees one patient was even given an intravenous injection of Bence Jones protein which appeared to increase the amount of Bence Jones proteinuria. This first intra-venous injection of a MOAB (MOnoclonal AntiBody) ever reported was not surprisingly, however, accompanied by cold chills and shivering for up to two hours[16]!
Two distinct groups of Bence Jones proteins were recognized by Bayne-Jones and Wilson in 1922. They demonstrated that Bence Jones proteins consisted of a group of similar, but not identical, proteins. They made 12 preparations of Bence Jones proteins from five patients and immunized rabbits by intravenous injection of the Bence Jones protein and next precipitin tests were performed with the Bence Jones preparations. Two distinct groups were found and designated group I and group II[17].
In 1956 Korngold and Lippari identified three different Bence Jones proteins using the Ouchterlony test, but two were always found together in the urine of the same patient. They showed that anti-sera to Bence Jones protein also reacted with myeloma proteins[18]. Later, as a tribute to Korngold and Lippari, the two classes of Bence Jones proteins have been designated *kappa and lambda* (κ and λ)[18].

Using ^{13}C-labelled glycine, Putnam and Hardy demonstrated in 1955 that synthesis of the abnormal serum globulin and that of Bence Jones protein were independent processes. Bence Jones protein was found to be rapidly excreted and was thought to be derived from the nitrogen pool rather than from the plasma or a tissue protein precursor[19].
In 1962, more than a century after the description of the unique heat properties, Edelman and Gally showed that the light chains prepared from an IgG monoclonal protein and the Bence Jones protein from the same patient's urine had an identical amino acid composition, similar spectrofluorometric behaviour, identical appearance on chromatography on carboxymethylcellulose and on starch gel electrophoresis after reduction and alkylation, the same ultracentrifugal pattern, identical thermal solubility and the same molecular weight. These chains precipitated when heated to between 40 °C (104 °F) and 60 °C (140 °F), dissolved on boiling and re-precipitated with cooling to between 40 °C and 60 °C, which is identical with the heat properties of the Bence Jones proteins[20].

Serum gammaglobulins and monoclonal proteinaemia

A specific substance with neutralizing activity (antibody) was described in 1890 in the blood of animals immunized with diphtheria and tetanus toxin[21]. Tiselius used the moving boundary method of electrophoresis in his doctoral dissertation in 1930 to demonstrate the homogeneity of serum globulins. His manuscript describing the apparatus for electrophoresis was not accepted by the Biochemical Journal, because it was considered too physical. Next it was published in the Transactions of the Faraday Society[22], eventually this article led to the Nobel Prize and the presidency of the Nobel foundation. Later that same year, Tiselius described the separation of serum globulins into three components, which he designated α, β, and γ[23]. Two years later, Tiselius located antibody activity in the gammaglobulin fraction of plasma proteins. They noted that antibodies to Pneumococcus type I were found in the area of γ mobility in rabbit serum and that antibodies to pneumococcal organisms migrated between β and γ in horse serum[24]. Hyperproteinaemia as a feature of MM was recognized by Perlzweig in 1927 before the discovery of protein electrophoresis. He demonstrated Bence Jones proteins in both urine and blood in the same patient[25]. Longsworth *et al* applied electrophoresis to the study of multiple myeloma and demonstrated the tall narrow-based church spire peak[26]. As Tiselius apparatus was cumbersome to use paper electrophoresis became more popular and in turn was replaced by cellulose acetate. Currently, high-resolution electrophoresis on agarose gel is employed in most laboratories.
Grabar and Williams described immunoelectrophoresis, which facilitated the diagnosis of multiple myeloma[27]. Immunofixation or direct immunoelectrophoresis was described by Wilson when he applied the antisera on the surface of the agar directly after the completion of electrophoresis[28]. Immunofixation is useful in the recognition

of small monoclonal light chains when none are found in immunoelectrophoresis[29]. When combined with immunofixation, high resolution agarose gel electrophoresis is more sensitive than immunoelectrophoresis in detecting small monoclonal proteins[30]. Kunkel believed that monoclonal proteins were the equivalent of normal antibodies produced by normal plasma cells. He showed that each heavy chain subclass and light chain type in monoclonal proteins had its counterpart among normal immunoglobulins and also among antibodies. After the discovery of the two types of light chains, κ and λ, in the monoclonal proteins of a ratio of approximately 2:1, the same ratio was detected among normal immunoglobulins. Similarly like IgG, IgA[31] and IgD[32] isotypes were discovered among myeloma proteins and were then found as normal serum components[33]. It was recognized that some antibodies migrate in the fast γ region and that some sediment in the ultracentrifuge at 7S and others at 19S. Strangely, the concept of a family of proteins with antibody activity was not proposed until the late 1950's by Heremans[34]. The term gammaglobulin was used for any protein that migrated in the γ region during electrophoresis, later these were divided as immunoglobulins IgG, IgA, IgM, IgD and IgE.

The concept of polyclonal versus monoclonal gammopathies was lucidly presented by Waldenström in the Harvey lectures[35]. In that same year he already had described a series of patients with a heavy M-protein; 'macroglobulin', and a clinical picture different from MM often presenting with lymph-node enlargement and hepatosplenomegaly[36]. Rather quick this syndrome was referred to as Waldenström's macroglobulinaemia. He then clearly described patients with a narrow band of hypergammaglobulinaemia as having a paraprotein (monoclonal protein). Although many of these patients had multiple myeloma or macroglobulinaemia others had no evidence of malignancy and were considered as having 'essential hypergammaglobulinaemia' or 'benign monoclonal gammopathy'[35]. This distinction is very important as a monoclonal gammopathy can indicate a malignant process whereas patients with a polyclonal gammopathy usually have a reactive or inflammatory cause[35].

Since the 1950's, with increasing deployment of serum protein-electrophoresis an increasing number of (case) reports of monoclonal gammopathies in the absence of MM or macroglobulinaemia were reported. Many names have been given for this condition (Table 1). The frequency of M-proteinaemia not apparently related to a malignant disease turned out to be relatively high and increased during advanced age with a prevalence of 1.5-3% in persons of 70 years and older[37-40]. The need was felt for both physicians and their patients for easily obtainable screening clues whether the M-protein would remain stable or whether it would progress to MM, macroglobulinaemia or another haematological malignancy. That this was a real risk was demonstrated by Kyle by close monitoring of 241 persons with 'benign' M-proteinaemia. In a relatively short five year follow-up 11% developed an M-proteinaemia related malignancy[41]. Hence this condition could not be termed 'benign' and thus the term Monoclonal Gammopathy of Unknown Significance 'MGUS' was coined. During

longer follow-up of median 22 (20-35) years of the same group of persons with MGUS 24% ultimately developed a related haematological malignancy[42]. In the largest retrospective follow-up study on MGUS thus far (1384 patients) the yearly risk of progression to MM or a related disorder was demonstrated to be 1%[43].

Monoclonal protein detection in 2005

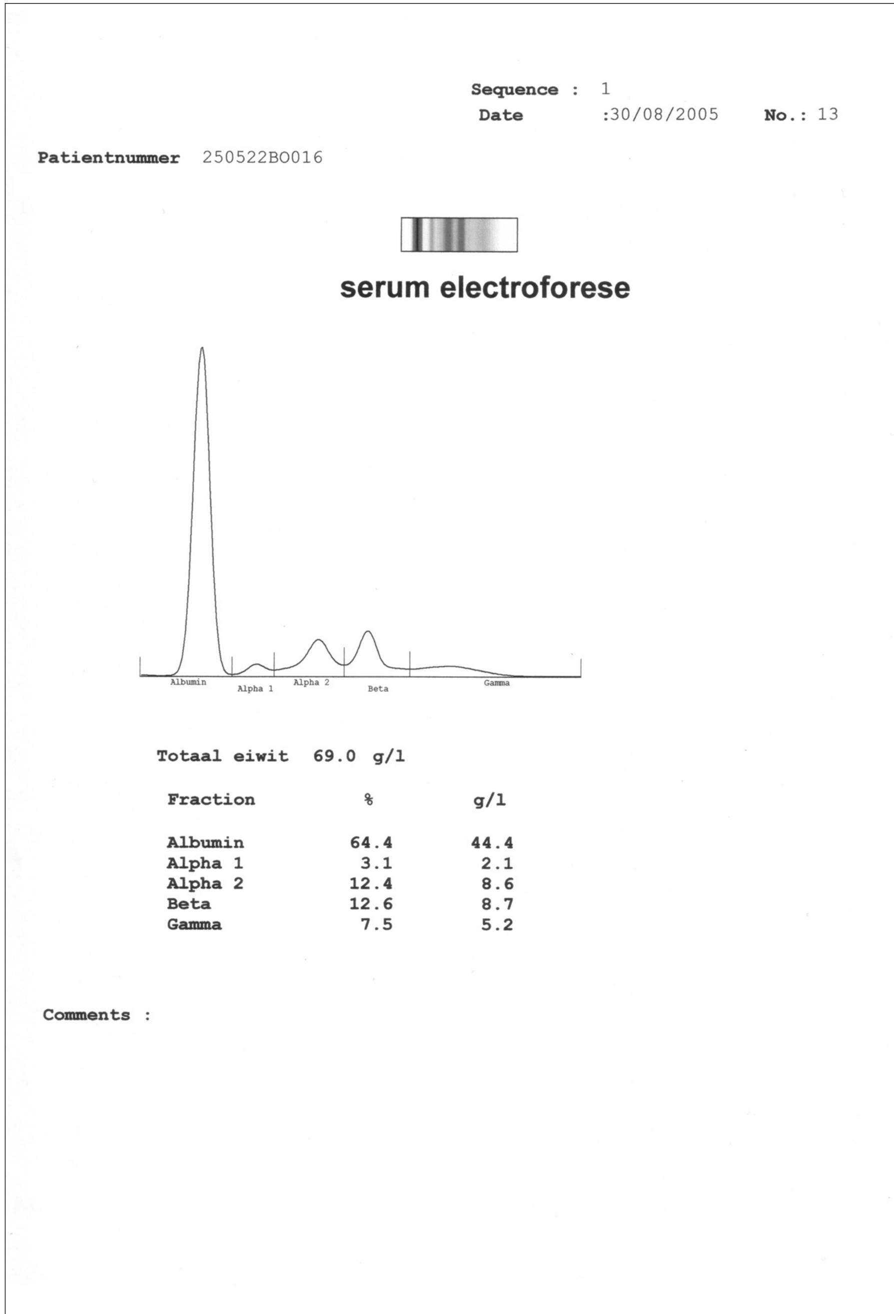
There are several methodologies available for the detection of an M-protein in serum or urine. The tests are described in logical order (e.g. screening and then elaborate determination of the heavy class and light chain type) and divided in serum and urine tests.

Serum analysis

Analysis of serum for the presence of M-proteins is usually performed after clinical suspicion on the presence of an M-protein related disorder has risen (e.g. elevation of the erythrocyte sedimentation rate or serum viscosity, anaemia, back pain, weakness or fatigue, osteopenia, osteolytic lesions, or spontaneous fractures, renal insufficiency with a bland urine sediment, heavy proteinuria in a patient over age 50, hypercalcaemia, hypergammaglobulinaemia, immunoglobulin deficiency, Bence Jones proteinuria, or recurrent infections). Initially electrophoretic techniques are used, supplemented with additional tests for protein quantification and methodologies to determine whether the protein is indeed monoclonal (arises from a single clone of plasma cells).

Serum protein electrophoresis: Serum protein electrophoresis (SPE) is an inexpensive and easy to perform screening procedure. Agarose gel electrophoresis is the recommended method for the detection of an M-protein. In the electrophoretic methodologies, proteins are classified by their final position after electrophoresis is complete into five general regions: albumin, α-1, α-2, β and γ (Figure 5).

These regions, which also use a Greek lettering system, do not refer to the immunoglobulin class to which an M-protein may belong, and refer only to mobility through the support medium. The various immunoglobulin classes (IgG, IgA, IgM, IgD, and IgE) are usually of γ mobility and make up most of the γ region, but they may also be found in the β-γ and β regions, and may occasionally extend into the α-2 globulin area. SPE should always be performed in combination with immunofixation in order to determine clonality. An M-protein usually presents as a single narrow peak, like a church spire, in the γ, β, or α-2 region of the densitometer tracing or as a dense, discrete band on the agarose gel. This is in contrast to polyclonal immunoglobulins which present as a diffuse broad band on the SPE in the γ region.



Sequence : 1
Date :30/08/2005 No.: 13

Patientnummer 250522BO016

serum electroforese

Albumin Alpha 1 Alpha 2 Beta Gamma

Totaal eiwit 69.0 g/l

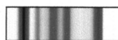
Fraction	%	g/l
Albumin	64.4	44.4
Alpha 1	3.1	2.1
Alpha 2	12.4	8.6
Beta	12.6	8.7
Gamma	7.5	5.2

Comments :

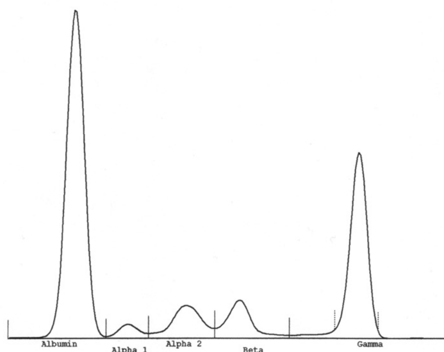
Figure 5a. **Normal serum protein electrophoresis and densitometry.**

Sequence : 1
Date :30/08/2005 **No.:** 17

Patientnummer 081125WO602



serum electroforese



Totaal eiwit **79.0 g/l**

Fraction	%	g/l
Albumin	52.0	41.1
Alpha 1	2.5	2.0
Alpha 2	8.6	6.8
Beta	8.0	6.3
Gamma	28.9	22.8
Peak 1	27.5	21.7

Comments : ENV / IggK / FmP=21,7

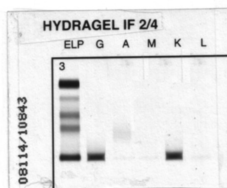


Figure 5b. **Serum protein electrophoresis with subsequent densitometry and immunofixation revealing the presence of an IgG-κ M-protein of 21.7 g/l.**

Immunofixation

Immunofixation is performed in order to confirm the presence of an M-protein and to determine its type. In immunofixation, the patient's serum is electrophoresed into at least five separate lanes. Following electrophoretic separation of the serum proteins, each sample is overlaid with a different monospecific antibody, usually three for the heavy chain component and two for the light chain component (e.g., anti-γ, anti-μ, anti-α, anti-κ, and anti-λ, respectively). Precipitation of proteins (i.e., the antigen-antibody complex) is allowed to occur, followed by washing (nonprecipitated proteins wash out) and staining of the remaining immunoprecipitates. An M-protein is characterized on immunofixation by the combined presence of a sharp, well-defined band associated with a single heavy-chain class and a sharp and well-defined band with similar mobility characteristics which reacts with either κ or λ light chain antisera, but not both (Figure 6).
Other reasons for immunofixation can be: detection of a small amount of M-protein in the presence of normal or increased background immunoglobulins, recognition and distinction of biclonal or triclonal gammopathies. Furthermore, the possibility of IgD and IgE monoclonal proteins must be excluded by immunofixation using IgD and IgE antisera in all patients with a monoclonal light chain in the serum or urine but no reactivity to anti-G, anti-M, or anti-A.

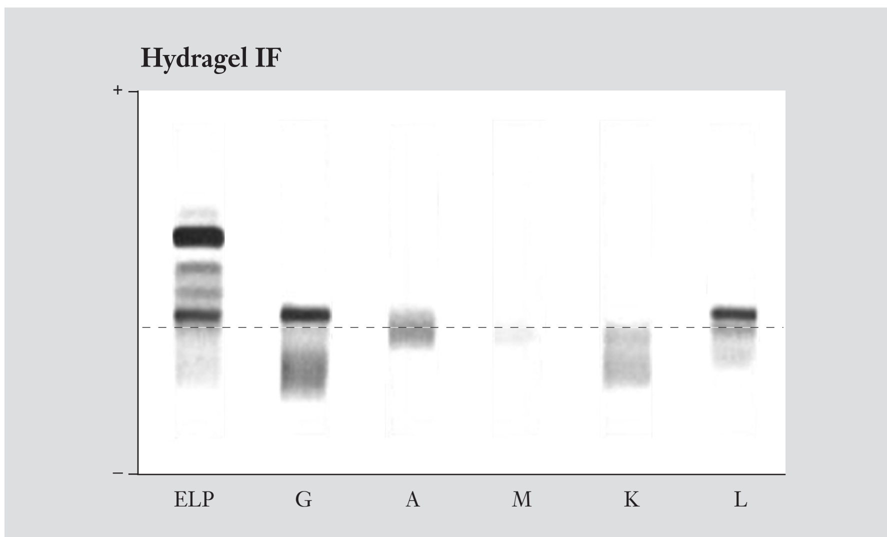


Figure 6. **Immunofixation demonstrating the presence of an IgG-λ M-protein.**

Immunoelectrophoresis: Immunoelectrophoresis differs from immunofixation in that the end-point is a precipitin arc rather than a distinct band; most laboratories rely on immunofixation techniques.
Quantization of immunoglobulins: Quantization of immunoglobulins is the most useful technique for the detection of hypogammaglobulinaemia. The use of a rate nephelometer is a good method for this purpose. The degree of turbidity produced by antigen-antibody interaction is measured by nephelometry in the near ultraviolet regions. Because the method is not affected by the molecular size of the antigen, the nephelometric technique accurately measures IgM, polymers of IgA, or aggregates of IgG.
Capillary zone electrophoresis: Capillary zone electrophoresis measures protein on-line via light absorbance techniques; protein stains are not necessary and no point of application is seen. The electrophoretograms are similar to those seen with high resolution agarose gel serum protein electrophoresis. Following capillary electrophoresis, immunotyping can be performed by an immunosubtraction procedure in which the serum sample is incubated with sepharose beads coupled with anti-γ, -α, -μ, -κ, and -λ antisera. After incubation with each of the heavy and light chain antisera, the supernatants are reanalyzed to determine which reagent(s) removed the electrophoretic abnormality. Capillary electrophoresis appears to be slightly more sensitive than agarose gel electrophoresis. The immunosubtraction procedure is technically less demanding, is automated, and is therefore a useful procedure for immunotyping M-proteins.
Free light chains in serum: Immunoassays are now available for detection of low concentrations of monoclonal free light chains in serum[44]. Using this assay Drayson *et al* reported that 68% of patients previously diagnosed as having nonsecretory myeloma were reclassified as light chain myeloma[45]. Measurement of free light chains may be useful in diagnosis and monitoring progress of patients with light chain myeloma, primary systemic amyloidosis, and in patients after high dose chemotherapy for MM[44].

Analysis of urine

Dipstick testing: Dipsticks are used in many laboratories to screen for the presence of protein in the urine. The dipstick is impregnated with a buffered indicator dye that binds to protein and produces a colour change proportional to the amount of protein bound to it. However, dipsticks are insensitive to the presence of Bence Jones protein (free κ or λ light chains) and should not be used for this purpose.
Sulfosalicylic acid test: The SSA test is performed by mixing one part urine supernatant (e.g., 2.5 ml) with three parts 3 percent sulfosalicylic acid, and semiquantative grading of the resultant turbidity. When proteinuria is present but the dipstick test is negative this should heighten the suspicion of Bence Jones proteinuria. Immunofixation of an adequately concentrated urine specimen is the following recommended test.

24-hour urine collection: Patients with a serum M-protein concentration >1.5 g/dl or those with a diagnosis or clinical suspicion of a plasma cell dyscrasia should have electrophoresis and immunofixation of an aliquot from a 24-hour urine collection. A 24-hour urine collection is necessary for determination of the total amount of protein excreted in the urine per day. The quantity of M-protein excreted is determined by measuring the size (percent) of the M-spike in the densitometer tracing and multiplying it by the total 24-hour urinary protein excretion. The amount of protein can be expressed as mg/dl or mg/l but it is much more useful to report the M-protein in g/24 hours because of wide variability in the daily urinary volume. The 24-hour urine specimen requires no preservative and may be kept at room temperature during collection. Generally, the amount of urinary monoclonal protein correlates directly with the size of the plasma cell burden, as long as renal function is relatively normal. Consequently, urinary M-protein excretion is useful in determining the response to chemotherapy or progression of disease.
Immunofixation: Immunofixation is the logical next method for identification of a monoclonal protein in the urine. Immunofixation can be performed even if the routine urine analysis is negative for protein, 24-hour urine protein concentration is within normal limits, and electrophoresis of a concentrated urine specimen shows no globulin peak. Immunofixation is sufficiently sensitive to detect a urine M-protein of 0.04 g/l.

Current classification system on monoclonal proteinaemia

Recently a new and classification system was developed for the monoclonal gammopathies by The International Myeloma Working Group[46]. The rationale was to use simple and easily obtainable criteria based on routinely available laboratory tests rather than attempting to cover all diagnostic situations[46]. This will result in definitions that will be easy to accept and to use in everyday practice and will facilitate the comparison of data of diverse investigations and therapeutic trail data[46]. These guidelines are found in Table 2. For comparison, older classification systems by Durie and Salmon[47], Kyle and Greipp[48], and the British Columbia Cancer Agency (BCCA)[49] are shown in Table 3.

The CCCW-paraprotein database

Considering the historic development in the detection of MGUS a ‘Paraprotein Task Force’ was initiated by the Comprehensive Cancer Centre West in the Western part of The Netherlands. The final goals were to develop guidelines for physicians on

MGUS and furthermore to develop a population-based M-protein registry in which patients with MGUS could be followed prospectively.
From 1991 till 1993 a population-based registry on M-proteinaemia was carried out in the region of the Comprehensive Cancer Centre West (CCCW), a geographical area with 1.6 million inhabitants. Clinical chemists, internists, haematologists, pathologists and other physicians reported all patients with newly diagnosed M-proteinaemia or multiple myeloma in the CCCW-area. Information on patient characteristics, laboratory test results, and results of bone marrow examination and skeletal x-rays were documented. The M-protein-related diagnosis, comorbidity and therapy were recorded and a serum sample was frozen at -80 °C. Follow-up was done annually. At follow-up, clinical data, any evolution into MM or other haematological malignancy, as well as comorbidity were recorded. In total, 1464 patients have been registered. This registry has already resulted in one thesis[50] and has been described in detail before[50].

Aims of this thesis

In the present thesis we first examine in **Chapters Two and Three** the discriminatory value of serum interleukin-6 and serum syndecan-1 in patients with newly diagnosed MGUS as both are important in MM-bone marrow interactions and have been reported to be of prognostic value in MM. We focus in **Chapter Four** on the probable association between M-proteinaemia and non-haematological malignancies ('solid tumours') as a relation is suggested in the older literature. Long term prognosis in MGUS is discussed in **Chapter Five** with a prospective follow-up of the whole CCCW-cohort. Finally, in **Chapters Six and Seven** the focus is more on MM therapy. In **Chapter Six** we examine the relationship between response to treatment as determined by the M-protein decrement found during melphalan-prednisone (MP) therapy during the HOVON-16 trial. In **Chapter Seven** the role of interferon-α as maintenance therapy after MP-induction therapy is discussed with an emphasis on quality of life.

Table 1. **Synonyms for MGUS.**

Synonym	Year	Author
Essential hyperglobulinaemia	1952	Waldenström[51]
Benign proteinaemia	1955	Olhagen-Liljestrand[52]
Nonmyelomatous paraproteinaemia	1957	Smith[53]
Dysgammaglobulinemic syndrome	1959	Hammack *et al*[54]
Atypical dysproteinaemia	1959	Creyssel *et al*[55]
Symptomless myelomatosis	1959	Baker-Martin[56]
γ_1-syndrome	1960	Schettler[57]
Essential, monoclonal benign hypergamma-globulinaemia	1961	Waldenström[35]
Cryptogenic transitory paraproteinaemia	1961	Schobel[58]
Essential hyperdysglobulinaemia	1961	Olmer et al[59]
Facultative paraproteinaemia	1961	Spengler[60]
Monoclonal gammopathy of unknown etiology	1963	Osserman[61]
Rudimentary paraproteinaemia	1963	Märki[62]
Benign, essential monoclonal non-macro-molecular hypergammaglobulinaemia	1964	Waldenström[63]
Begleitparaproteinämie	1964	Riva[64]
Secondary paraproteinaemia	1964	Videbaek[65]
Idiopathic paraproteinaemia	1964	Rádl[66]
Lanthanic proteinaemia	1967	Zawadski[67]
Asymptomatic paraimmunoglobulinaemia	1969	Engle[68]
Asymptomatic paraproteinaemia	1972	Meijers[69]
Nonmyelomatous monoclonal immuno-globulinaemia	1972	Zawadski[70]
Accompanying paraproteinaemia	1972	Siebner[71]
Asymptomatic or occult plasma cell dyscrasias	1972	Isobe[72]
MGUS (Monoclonal Gammopathy of - Undetermined Significance)	1978	Kyle[73]

Table 2. **Diagnostic criteria in monoclonal proteinaemia according to The International Myeloma Working Group.**

Monoclonal gammopathy of unknown significance
M-protein in serum <30 g/l
Bone marrow plasma cells <10% and low level of plasma cell infiltration in a trephine biopsy (if done)
No evidence of B-cell proliferative disorders
No related organ or tissue impairment (no end organ damage, including bone lesions)

Asymptomatic myeloma (smouldering myeloma)
M-protein in serum ≥30 g/l
and/or
Bone marrow plasma cells ≥10%
No related organ or tissue impairment (no end organ damage, including bone lesions) or symptoms

Symptomatic multiple myeloma
M-protein in serum and/or urine
Bone marrow (clonal) plasma cells[a] or plasmocytoma
Related organ or tissue impairment (no end organ damage, including bone lesions) or symptoms

[a]) If flowcytometry is performed most plasma cells (>90%) will show a 'neoplastic' phenotype. Some patients may have no symptoms but have related organ or tissue impairment.

Non-secretory myeloma
No M-protein in serum and/or urine with immunofixation
Bone marrow clonal plasmacytosis ≥10% or plasmocytoma
Related organ or tissue impairment (no end organ damage, including bone lesions)

Solitary plasmacytoma of bone
No M-protein in serum and/or urine with immunofixation[c]
Single area of bone destruction due to clonal plasma cells
Bone marrow not consistent with multiple myeloma
Normal skeletal survey (and MRI of spine and pelvis if done)
No related organ or tissue impairment (no end organ damage other than solitary bone lesion) or symptoms

(Table 2)

Extramedullary plasmacytoma
No M-protein in serum and/or urine with immunofixation[b]
Extramedullary tumour of plasma cells
Normal bone marrow
Normal skeletal survey
No related organ or tissue impairment (no end organ damage, including bone lesions) or symptoms

Multiple solitary plasmacytomas (± recurrent)
No M-protein in serum and/or urine with immunofixation[b]
More than one localized area of bone destruction or extramedullary tumour of clonal plasma cells which may be recurrent
Normal bone marrow
Normal skeletal survey (and MRI of spine and pelvis if done)
No related organ or tissue impairment (no end organ damage the localized bone lesions)
[b]) A small M-component may sometimes be present

Myeloma-related organ or tissue impairment (end organ damage) (ROTI) due to the plasma cell proliferative process
Calcium levels increased: serum calcium >0.25 mmol/l above the upper limit of normal or >2.75 mmol/l
Renal insufficiency: creatinine >173 mmol/l
Anaemia: Haemoglobin 2 g/dl below the lower limit of normal or haemoglobin <10 g/dl
Bone lesions: lytic lesions or osteoporosis with compression fractures (MRI or CT may clarify)
Other: symptomatic hyperviscosity, amyloidosis, recurrent bacterial infections (>2 episodes in 12 months)
CRAB (calcium, renal insufficiency, and anaemia or bone lesions)

Table 3. **Additional monoclonal protein classification systems.**

A. Diagnostic criteria according to Durie and Salmon

Multiple myeloma (MM)

Major criteria:

1. Plasmocytoma on tissue biopsy
2. Bone marrow plasmacytosis with ≥30% plasma cells
3. Monoclonal protein serum electrophoresis and immunofixation: IgG >35 g/l, IgA >20 g/l, light chain excretion on urine electrophoresis ≥1 g/24 hours in the absence of amyloidosis

Minor criteria:

a. Bone marrow plasmacytosis with 10 to 30% plasma cells
b. Monoclonal protein serum present, but less than levels defined above
c. Lytic bone lesions
d. Normal IgM < 500 mg/l, IgA < 1 g/l or IgG < 6 g/l

The diagnosis of multiple myeloma requires a minimum of one major and one minor criterion (1 + a not sufficient) or 3 minor criteria that must include a + b.

Indolent myeloma (IMM)

Criteria as for myeloma with the following limitations:

a. Absent or only limited bone lesions (≤3 lytic lesions), no compression fractures
b. Monoclonal protein serum levels IgG <70 g/l, IgA < 50 g/l
c. No symptoms or associated disease features: Karnofsky performance status >70%, haemoglobin >6.8 mmol/l, serum calcium normal, serum creatinin <177 μmol/l (3.0 mg/dl), no infections

Smouldering multiple myeloma (SMM)

Criteria as for indolent myeloma with additional constraints:

a. There must be no demonstrable bone lesions
b. Bone marrow plasma cells 10-30%

Monoclonal gammopathy of undetermined significance (MGUS)

1. Monoclonal protein levels IgG ≤35 g/l, IgA ≤20 g/l, Bence Jones protein ≤1.0 g/24 hours
2. Bone marrow plasma cells <10%
3. No bone lesions
4. No symptoms

(Table 3)

B. Diagnostic criteria according to Kyle and Greipp

Multiple myeloma (MM)

1. M-protein present in serum or urine
2. ≥ 10% bone marrow plasma cells, or aggregates on biopsy
3. One or more ancillary findings, must not be attributable to another cause:
   a. anaemia
   b. lytic bone lesions, or osteoporosis and ≥30% plasma cells in bone marrow
   c. bone marrow plasma cell labelling index >1%
   d. renal insufficiency (adult Fanconi syndrome or light chain deposition disease not sufficient)
   e. hypercalcaemia

Smouldering multiple myeloma (SMM)

1. Serum monoclonal protein (usually >30 g/l) and 10% or more bone marrow plasma cells or aggregates on biopsy
2. No anaemia, renal failure or hypercalcaemia attributable to myeloma
3. Other ancillary tests negative:
   a. bone lesions absent on radiographic bone survey
   b. bone marrow plasma cell labelling index <1%
   c. plasmablasts absent
   d. normal β-2 microglobulin level in the absence of renal insufficiency, absence of circulating isotype specific plasma cells, peripheral blood B-cell labelling index <0.5%, absence of light chain isotype suppression, urinary light chain <0.5 g/24 hours, stable monoclonal protein in serum or urine during follow-up.

Monoclonal gammopathy of undetermined significance (MGUS)

1. Serum monoclonal protein (usually l<30 g/l)
2. No anaemia, renal failure or hypercalcaemia
3. <10% bone marrow plasma cells without aggregates on biopsy
4. Ancillary tests negative (as above)

Solitary plasmacytoma

1. Single plasma cell tumour
2. No diagnostic criteria for systemic multiple myeloma
3. Little or no monoclonal protein after radiotherapy
4. <10% bone marrow plasma cells

(Table 3)

C. Diagnostic criteria according to the British Columbia Cancer Agency (BCCA)
Multiple myeloma (MM) *At least two of the following:* 1. Monoclonal protein present in serum or urine 2. Lytic bone lesions 3. ≥10% bone marrow plasma cells
Indolent multiple myeloma (IMM) *Criteria as for multiple myeloma with the following additional criteria:* 1. No symptoms 2. Satisfactory peripheral blood counts 3. No monoclonal protein in the urine 4. Normal serum calcium 5. Stable monoclonal protein level 6. No lytic bone lesions 7. No renal or neurological disease due to myeloma
Monoclonal gammopathy of undetermined significance (MGUS) *All of these must be present:* 1. Serum monoclonal protein 2. No lytic bone lesions 3. Bone marrow plasmacytosis <10%
Solitary plasmacytoma *All these criteria must be present:* 1. Biopsy proof of a plasma cell tumour 2. No lytic bone lesions except the tumour itself 3. Bone marrow plasmacytosis <10% (A serum and/or urine monoclonal protein may be present)

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