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Prognosis in monoclonal proteinaemia

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PROGNOSIS IN MONOCLONAL PROTEINAEMIA

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The most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' (I found it!) but 'That's funny ...'.

Isaac Asimov

Aan Yvonne, Lisette, Céline en Florence

Aan mijn moeder

In liefdevolle herinnering aan mijn vader

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

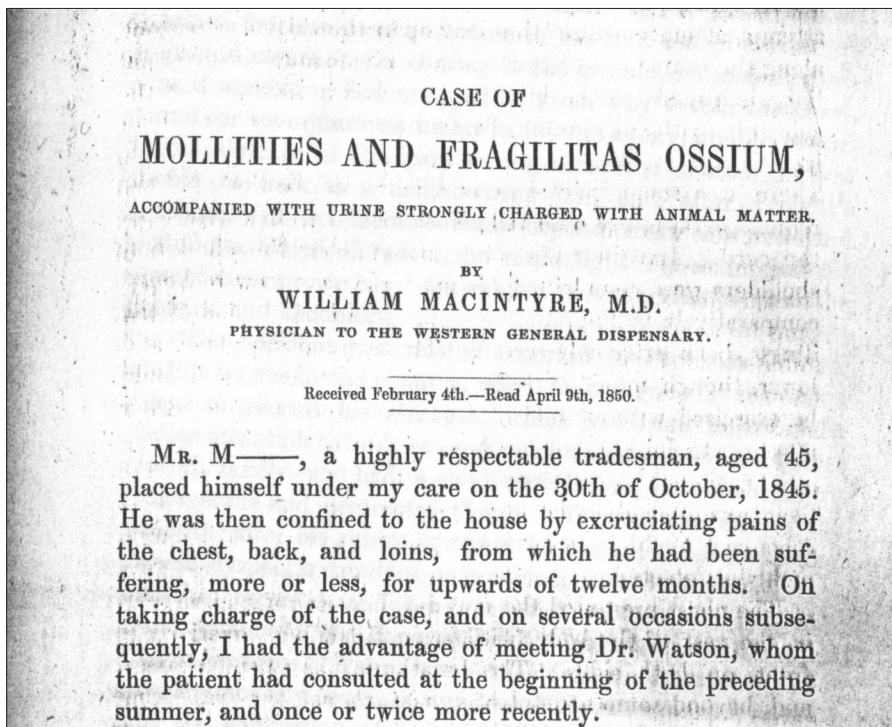


Figure 1. Detail of the first page of the paper by Dr William Macintyre.

Metropolitan Convalescent Institution and the Western General Dispensary in St. Marylebone was called in¹.

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 reheated¹. 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:

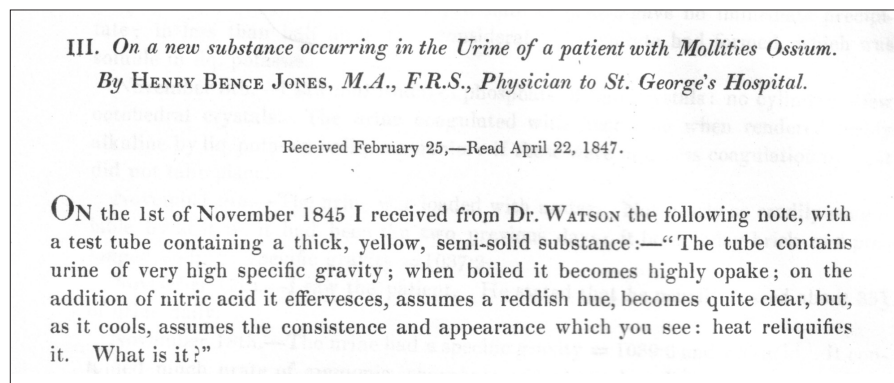


Figure 2. Detail of the first page of the paper by Henry Bence Jones.

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 found³. 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 cells⁴. 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 ‘albumenuria’ 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 described².

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 nuclei⁵. 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 $C_{48}H_{38}N_6O_{18}$, or by $C_{40}H_{31}N_5O_{15}$; according as protein is $=C_{48}H_{37}N_6O_{15}$, or $C_{40}H_{30}N_5O_{12}$.

	1st.	2nd.	3rd.	4th.	5th.	6th.	7th.	$C_{48}H_{38}N_6O_{18}$; Reckoned.	$C_{40}H_{31}N_5O_{15}$; 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 Jones⁷ and the urine was described in detail by Huppert⁸. In 1900 Wright was the first to identify the plasma cells as tumour cells MM⁹.

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-rays¹⁰ and bone marrow examination¹¹. 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 casein¹³. 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 day¹⁵. 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 marrow¹⁶. 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 (MOncloclonal AntiBody) ever reported was not surprisingly, however, accompanied by cold chills and shivering for up to two hours¹⁶!

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 II¹⁷. 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 proteins¹⁸. 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 precursor¹⁹.

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 proteins²⁰.

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 toxin²¹. 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 serum²⁴. 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 patient²⁵. Longsworth *et al* applied electrophoresis to the study of multiple myeloma and demonstrated the tall narrow-based church spire peak²⁶. 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 myeloma²⁷. Immunofixation or direct immunoelectrophoresis was described by Wilson when he applied the antisera on the surface of the agar directly after the completion of electrophoresis²⁸. Immunofixation is useful in the recognition

of small monoclonal light chains when none are found in immunoelectrophoresis²⁹. When combined with immunofixation, high resolution agarose gel electrophoresis is more sensitive than immunoelectrophoresis in detecting small monoclonal proteins³⁰. 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, IgA³¹ and IgD³² isotypes were discovered among myeloma proteins and were then found as normal serum components³³. 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 Heremans³⁴. 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 lectures³⁵. 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 hepatosplenomegaly³⁶. 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 cause³⁵.

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 older³⁷⁻⁴⁰. 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 malignancy⁴¹. 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 malignancy⁴². 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.

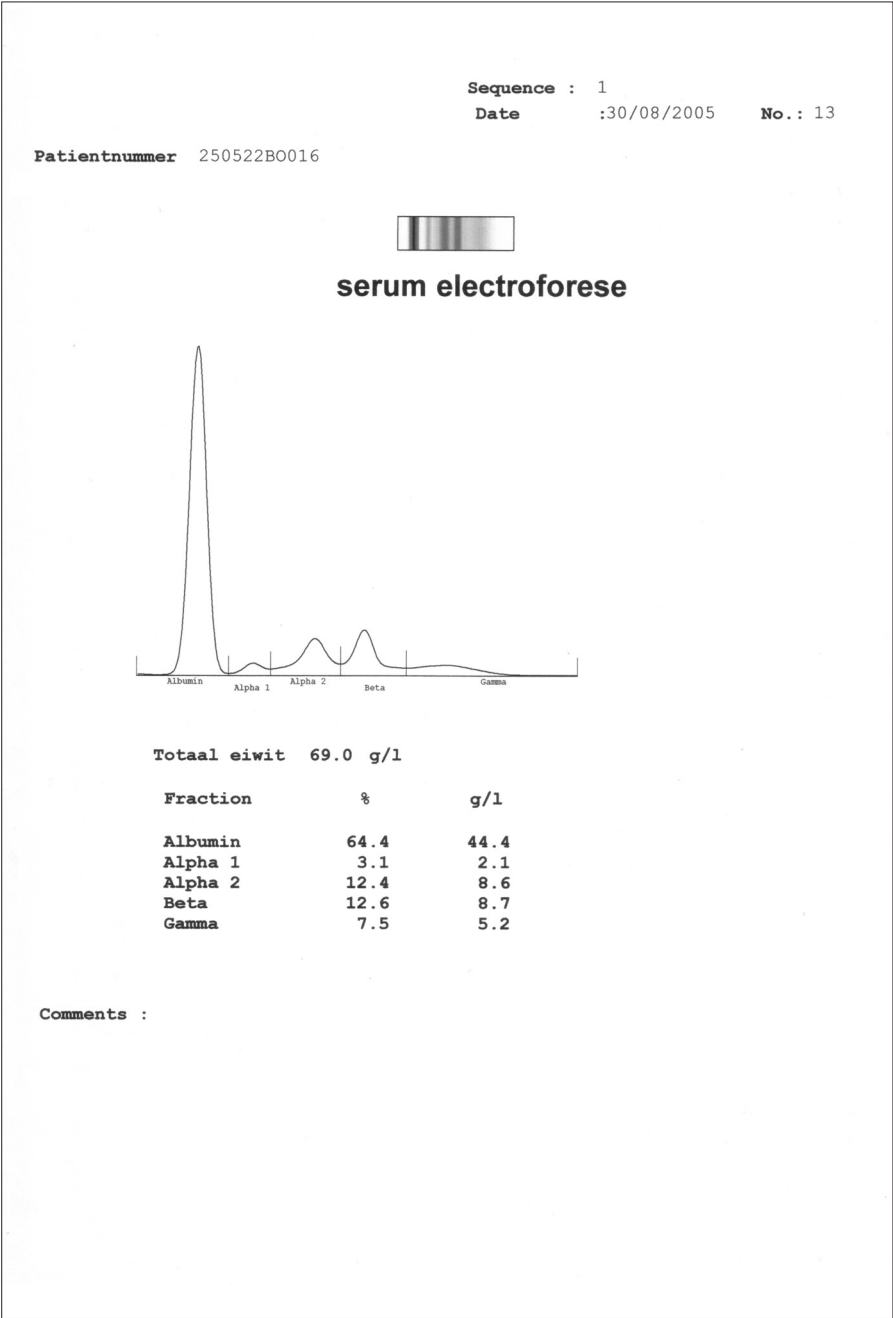


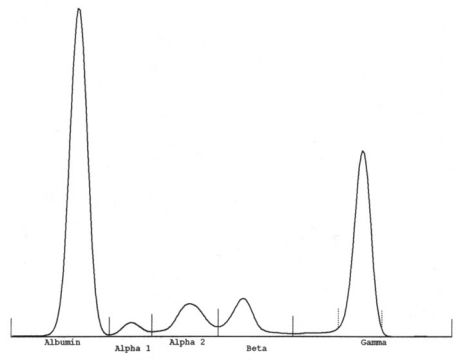
Figure 5a. Normal serum protein electrophoresis and densitometry.

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 / IgGκ / FmP=21.7

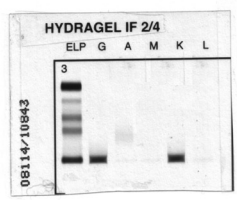


Figure 5b. Serum protein electrophoresis with subsequent densitometry and immunofixation revealing the presence of an IgG-κ M-protein of 21.7 g/l.

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.

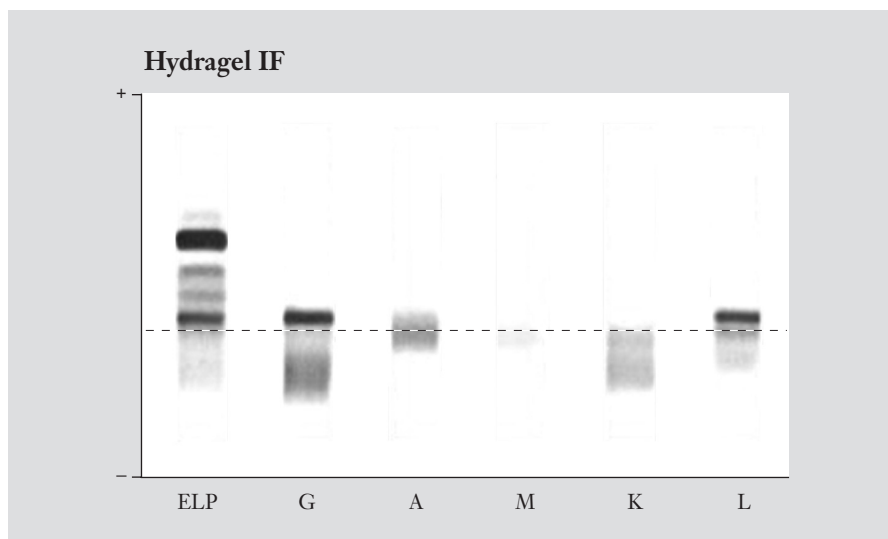


Figure 6. Immunofixation demonstrating the presence of an IgG- λ M-protein.

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 serum⁴⁴. Using this assay Drayson *et al* reported that 68% of patients previously diagnosed as having nonsecretory myeloma were reclassified as light chain myeloma⁴⁵. 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 MM⁴⁴.

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 proteinemia

Recently a new and classification system was developed for the monoclonal gammopathies by The International Myeloma Working Group⁴⁶. The rationale was to use simple and easily obtainable criteria based on routinely available laboratory tests rather than attempting to cover all diagnostic situations⁴⁶. 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 trial data⁴⁶. These guidelines are found in Table 2. For comparison, older classification systems by Durie and Salmon⁴⁷, Kyle and Greipp⁴⁸, 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 thesis⁵⁰ and has been described in detail before⁵⁰.

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 <i>et al</i> ⁵⁴
Atypical dysproteinaemia	1959	Creysse <i>et al</i> 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 <i>et al</i> ⁵⁹
Facultative paraproteinaemia	1961	Spengler ⁶⁰
Monoclonal gammopathy of unknown etiology	1963	Osserman ⁶¹
Rudimentary paraproteinaemia	1963	Märki ⁶²
Benign, essential monoclonal non-macromolecular 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 immunoglobulinaemia	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 proteinemia 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 plasmacytoma 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 plasmacytoma 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 $\geq 30\%$ 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 μ mol/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. $\geq 10\%$ 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 $\geq 30\%$ 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 <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) <i>At least two of the following:</i> 1. Monoclonal protein present in serum or urine 2. Lytic bone lesions 3. ≥10% bone marrow plasma cells
Indolent multiple myeloma (IMM) <i>Criteria as for multiple myeloma with the following additional criteria:</i> 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) <i>All of these must be present:</i> 1. Serum monoclonal protein 2. No lytic bone lesions 3. Bone marrow plasmacytosis <10%
Solitary plasmacytoma <i>All these criteria must be present:</i> 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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2 Serum interleukin-6 has no discriminatory role in paraproteinaemia nor a prognostic role in multiple myeloma

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Abstract

We determined interleukin-6 (IL-6) in serum of 212 well-defined patients with newly diagnosed paraproteinaemia and evaluated its discriminatory value and its prognostic role in multiple myeloma (MM). Results were compared with serum neural cell adhesion molecule and β -2-microglobulin, both established prognostic MM markers. Paraproteinaemia related diagnoses were: MM (60), other haematological diseases (46), solid tumours (35), auto-immune diseases (17) and monoclonal gammopathy of unknown significance (MGUS) (54). IL-6 ranges in all diagnostic groups overlapped widely and did not serve as a discriminatory marker in newly diagnosed paraproteinaemia even when patients with infection or fever (42) were excluded. In MM high IL-6 levels (≥ 50 pg/ml) were not associated with a shorter survival ($p=0.24$). We compared our results with 20 published studies on serum IL-6 in paraproteinaemia and/or MM. IL-6 data have to be related to the assay used (bio- or immunoassay) and to the status of MM (newly diagnosed, during therapy, progressive disease). We conclude that serum IL-6 is not specific for paraproteinaemia-related diseases and will not serve as a reliable discriminatory or prognostic marker in paraproteinaemia and MM.

Introduction

Monoclonal gammopathy or paraproteinaemia is not synonymous with multiple myeloma (MM). At first presentation a diagnosis of MM, plasmacytoma, amyloidosis or other haematological malignancy is made in only 37% of these patients¹. The majority of patients with a newly diagnosed paraprotein, does not fulfil the diagnostic criteria for MM and hence are classified as having monoclonal gammopathy of undetermined significance (MGUS). Follow-up of MGUS patients for more than twenty years has shown that MM, amyloidosis or other haematological malignancies arise in 24%, demonstrating that lifelong follow-up is mandatory¹.

For newly diagnosed patients with paraproteinaemia the need is felt for an easy (serum) parameter to distinguish MM from other causes of paraproteinaemia. Interleukin-6 (IL-6) is a potent *in vitro* and *in vivo* growth factor for human myeloma cells and has therefore received much attention as a possible tumour-, disease activity-, and prognostic marker in MM and other causes of paraproteinaemia². Recently, the diagnostic value of serum neural cell adhesion molecule (NCAM) in newly diagnosed patients with MM and other well-defined monoclonal gammopathies of different causes was demonstrated. The specificity was excellent (97%) but the sensitivity was low (52%)³. We decided to test the diagnostic value of serum IL-6 in paraproteinaemia and its prognostic value in MM in the same group of patients. Moreover we included the results with NCAM, C-reactive protein (CRP) and β -2-microglobulin (β 2M) in the analysis of these patients. In addition, we compared our IL-6 data with all published series available.

Patients and methods

Patients: From 1991 till 1993 a population-based registry on paraproteinaemia was carried out in the region of the Comprehensive Cancer Center West (CCCW). All patients with newly diagnosed paraproteinaemia and/or multiple myeloma in the Midwestern part of The Netherlands (1,6 million inhabitants and 15 hospitals in 1992) were reported by clinical chemists, internists, haematologists, pathologists and other physicians. Information on patient characteristics (age, sex, performance status), laboratory tests results (haemoglobin, leukocyte- and thrombocyte counts, creatinin, calcium, albumen, total protein, paraprotein type and concentration in serum and urine), and results of bone marrow and skeletal x-rays were obtained. The paraprotein-related diagnosis, comorbidity and therapy were recorded. A serum sample obtained at registration was centrally stored at -80 °C. Follow-up was done at least once yearly. In total, 1464 patients have been registered. The setup and contents of this registry have been described⁴. Paraproteins were either detected by agarose or cellulose-acetate electrophoresis depending on the method used in each hospital where

the patient was first seen. For inclusion in the registry each paraprotein had to be confirmed by immunotyping (immunofixation). Serum was available from 867 patients. Out of this series, 212 sera were selected in whom all diagnostic tests had been performed and a paraprotein-related diagnosis was available³.

Multiple myeloma was diagnosed in 60 patients, other haematological diseases in 46 patients, solid tumour in 35, auto-immune disease in 17 and MGUS in 54 patients. Different stages among the MM patients were: indolent myeloma 9 cases, stage I 13 cases, stage II 4 cases and stage III 34 cases. Other haematological malignancies consisted of lymphoproliferative diseases: 39 (15 immunocytoma, 18 other B-cell non-Hodgkin's lymphoma, 4 B-cell chronic lymphocytic leukaemia, 2 Hodgkin's disease) and myeloproliferative diseases: 7 (6 myelodysplastic syndrome, 1 promyelocytic leukaemia). According to the criteria of the British Columbia Cancer Agency (BCCA) the diagnosis of MM was made when at least two of the following criteria were present: paraprotein in serum and/or urine, lytic bone lesion(s), or more than 10% plasma cells in bone marrow cytology^{5,6}. A paraprotein in serum (but not in urine), with more than 10% plasma cells in cytology, in the absence of symptoms, anaemia, leucocytopenia, thrombocytopenia, hypercalcaemia, renal failure due to myeloma or lytic bone lesions was diagnosed as indolent MM^{3,5}. Any paraprotein without indication of MM or other (haematological) malignancies, or auto-immune disease with less than 10% plasma cells in bone marrow cytology was termed MGUS³.

Laboratory Methods: Serum samples stored at -80 °C were thawed and shipped on ice to the Marburg University (Germany) where NCAM and IL-6 determinations were done. For IL-6 determinations an enzyme linked immunoabsorbent assay (LD Zytokit IL-6 ELISA, LD Labor Diagnostika Heiden, Germany) was used with a sensitivity of 1 pg/ml. The analyses of β 2M, CRP and NCAM have been described before³. Interassay coefficients of variation for IL-6 were less than 8%, for NCAM 2-8%, for β 2M 7-10% and for CRP less than 8%. Upper normal serum values were 20 U/ml for NCAM, 3 mg/l for β 2M and less than 10 mg/l for CRP. As median values and ranges for serum IL-6 differ among normal control groups no reference values were available (see also Table 3). IL-6, NCAM, CRP and β 2M were determined in blinded samples. Other laboratory results were extracted from the registry.

Statistics: The comparison of median values of laboratory parameters for different diagnostic categories was done using Mann-Whitney's test (2 groups) or Kruskal-Wallis test (more than 2 groups). Survival was calculated from diagnosis to death (event) or to being alive at last follow-up (censoring). Survival curves were made using the Kaplan Meier methods and compared with the log-rank test. Analyses were performed using SPSS/PC+; data were entered in a database using SPSS Data Entry II (both SPSS Inc, Chicago, IL).

Literature search: For the period January 1966 to May 1999 we searched the medical literature in Medline using the items: 'paraproteinaemia', 'monoclonal gammopathy', 'interleukin-6' and 'multiple myeloma'. All studies on serum IL-6 in patients with paraproteinaemia and/or MM were reviewed.

Results

Interleukin-6: Serum IL-6 was measurable in all 212 patients studied. The median values and ranges for IL-6, NCAM, CRP and β 2M are shown in Table 1. Figure 1 is a graphic display of the individual IL-6 values grouped by diagnostic category. The median levels of IL-6 showed significant differences between the diagnostic categories (Kruskall Wallis test, $p < 0.001$), but overlapped widely and none had a characteristic range. IL-6 values correlated with CRP ($r = 0.42$; $p < 0.0001$), but not with NCAM, osteolysis and β 2M. At presentation 42 patients (lymphoproliferative (9), solid tumour (6), auto-immune disease (2), MGUS (15), MM I (6), MM III (4)) had either a documented infection or fever without a known cause. Excluding these from analysis did not make any difference (Table 1, $p = 0.005$).

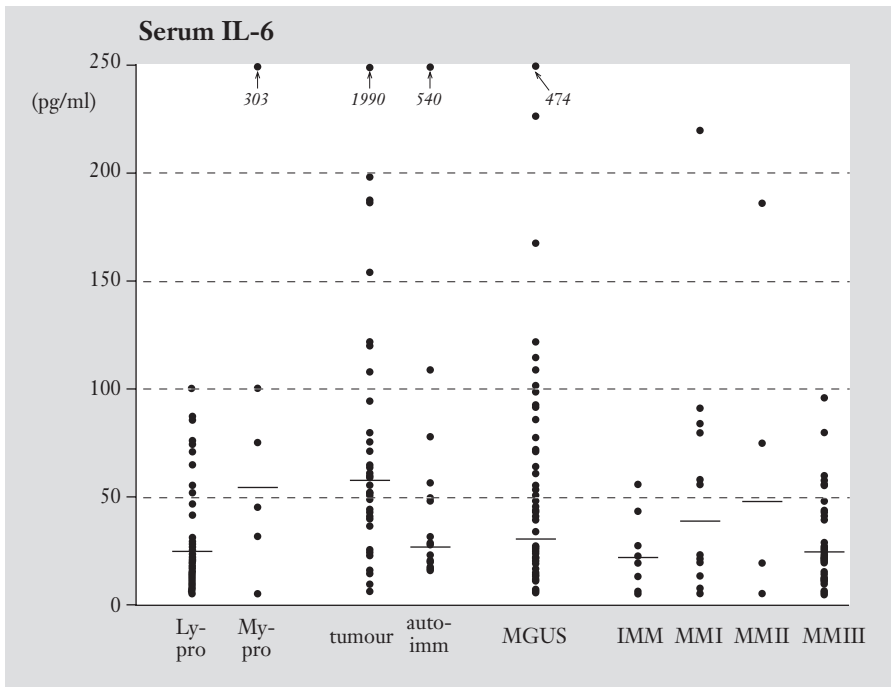


Figure 1. Range of serum IL-6 in all diagnostic categories. Median levels of serum IL-6 are depicted as bars. Outliers are represented by an arrow and accompanied by the actual value.

Ly-pro: lymphoproliferative disease;
 My-pro: myeloproliferative disease;
 tumour: solid tumour;
 auto-imm: auto-immune disease;
 IMM: indolent MM.

Table 1. Median serum value (ranges) of IL-6, NCAM, CRP, β 2M.

Category	N	NCAM (U/ml)	CRP (mg/l)	β 2M (mg/l)	Serum IL-6 (pg/ml) unselected	no infection/fever	N
Lymphoproliferative	39	9.2 (1.5-30.2)	6 (2-236)	3.4 (1.5-14.7)	25 (6-100)	22 (7-100)	30
Myeloproliferative	7	4.1 (2.7-12.7)	33 (4-236)	7.4 (2.4-22.2)	55 (6-303)	55 (6-303)	7
solid tumour	35	5.1 (1.0-17.4)	50 (2-335)	3.2 (2.0-16.0)	58 (7-1,990)	58 (7-1,990)	29
auto-immune	17	5.1 (1.7-24.0)	6 (2-158)	2.5 (1.6-16.1)	29 (18-540)	27 (18-540)	14
MGUS	54	7.1 (2.4-19.2)	25 (2-155)	3.2 (1.1-9.3)	31 (7-474)	28 (7-474)	39
IMM	9	16.4 (5.7-110.4)	4 (2-163)	2.5 (1.3-5.7)	22 (6-56)	22 (6-56)	9
MMI	13	10.3 (5.5-181.3)	8 (2-334)	3.9 (2.0-9.4)	39 (6-220)	22 (6-58)	7
MMII	4	31.3 (3.0-85.2)	16 (5-97)	5.8 (3.4-7.4)	48 (6-186)	48 (6-186)	4
MMIII	34	24.3 (2.4-219.7)	4 (2-116)	5.9 (2.0-48.6)	25 (6-96)	25 (6-96)	30

For practical clinical purposes all diagnostic categories were combined to three major groups; non-MM (lymphoproliferative, myeloproliferative, solid tumour, and autoimmune disease), MGUS and MM (IMM, MM I, MM II, MM III). Median serum IL-6 values in non-MM and MGUS were significantly higher than in patients with MM (Table 2). IL-6 values in MGUS were higher than in MM though this difference disappeared after excluding all patients with a documented infection or fever at diagnosis. β 2M and NCAM levels were significantly higher in MM compared to non-MM and MGUS whereas CRP was higher in the latter two (Table 2).

Serum IL-6 and survival in patients with MM: We investigated whether high IL-6 serum levels at diagnosis were of prognostic significance for survival in patients with MM. Median time of follow-up was 44 months (range 0-74 months). Patients with MM and initial high values of IL-6 (≥ 50 pg/ml) showed a median survival of 23 months compared to 33 months in MM-patients with lower IL-6 values (< 50 pg/ml) though this was not statistically significant ($p=0.24$; Figure 2). Even at several other cut-off values (25, 75, and 100 pg/ml) no significant correlation of serum IL-6 at diagnosis with survival was found. Not surprisingly an elevated CRP (≥ 10 mg/l) was also not associated with shorter survival. In contrast, elevated values of the established prognostic serum marker β 2M (> 3 mg/l) were significantly ($p=0.013$) associated with a worse prognosis. Median survival time in patients with elevated β 2M was less than half the median survival time in patients with normal serum values (22 months vs. 47 months; Figure 2). Serum NCAM proved to be a relevant prognostic factor as well. Patients with MM and a high NCAM level (≥ 20 U/ml) at diagnosis had a median survival time of 22 months whereas normal levels were associated with a median survival of 54 months ($p=0.04$; Figure 2).

Table 2. Patients grouped as non-MM, MGUS and MM, comparison of median serum values of IL-6, NCAM, CRP, and β 2M.

Category	N	NCAM (U/ml)	CRP (mg/l)	β 2M (mg/l)	Serum IL-6 (pg/ml)		N
					unselected	no infection/fever	
non-MM	98	6.7 (1.0-30.2)	17 (2-335)	3.4 (1.5-22.2)	43 (6-1,990)	37 (6-1,990)	81
MGUS	54	7.1 (2.4-19.2)	25 (2-155)	3.2 (1.1-9.3)	31 (7-474)	28 (7-474)	39
MM	60	20.0 (2.4-219.7)	5 (2-334)	4.4 (1.3-48.6)	24 (6-220)	23 (6-186)	50
Mann-Whitney							
non-MM vs MGUS		p=0.8	p=0.7	p=0.2	p=0.9	p=0.5	
non-MM vs MM		p=0.00001*	p=0.003*	p=0.02*	p=0.01*	p=0.01*	
MGUS vs MM		p=0.00001*	p=0.04*	p=0.002*	p=0.03*	p=0.1	

* = significant

Legend to Figure 2 (p.39):
IL-6 (<50 pg/ml):
 at start n=45;
 at 36 months n=20
IL-6 ($\geq$ 50 pg/ml):
 at start n=15;
 at 36 months n=5
 β 2M (<3 mg/l):
 at start n=15;
 at 36 months n=10
 β 2M ($\geq$ 3 mg/l):
 at start n=45;
 at 36 months n=15
NCAM (<20 U/ml):
 at start n=29;
 at 36 months n=17
NCAM ($\geq$ 20 U/ml):
 at start n=31;
 at 36 months n=8

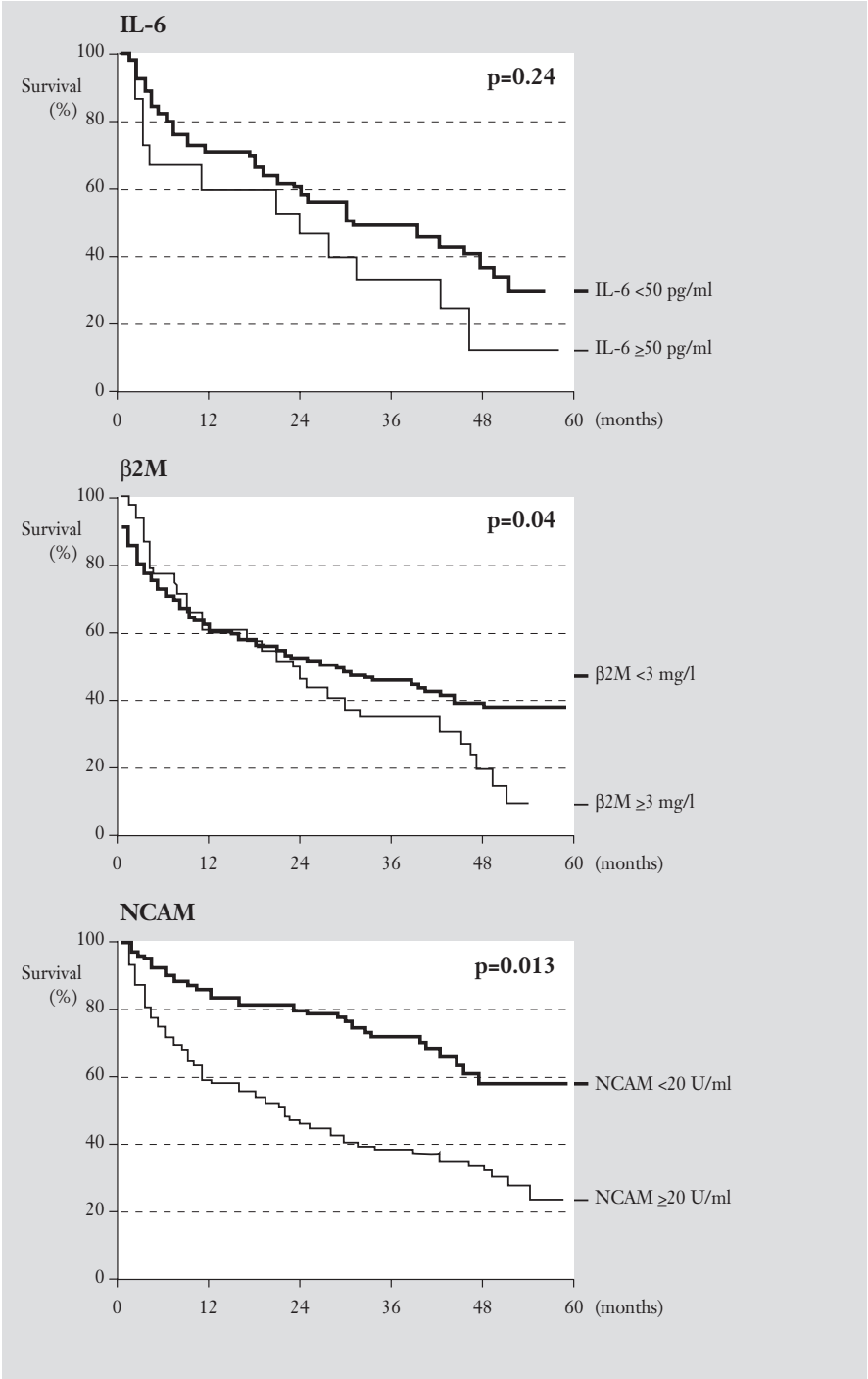


Figure 2. Survival curves of patients with MM according to high or low values in respectively IL-6, β2M and NCAM.

Discussion

In this group of well-defined patients with newly diagnosed paraproteinaemia serum IL-6 did not discriminate between the different diagnostic groups. Median serum IL-6 levels were higher in MGUS compared to MM although this difference disappeared when all patients with a documented infection or fever were excluded. As far as the prognostic role of serum IL-6 in MM is concerned, we are in agreement with Kyrtsolis⁷ and could not demonstrate the usefulness of serum IL-6 as a prognostic factor which is in contrast with others⁸⁻¹⁰. The established prognostic serum parameters β 2M¹¹ and NCAM^{12;13} were both prognostic factors in the CCCW multiple myeloma patients. This underlines that we selected a representative group of patients for this serum IL-6 study.

Studies on serum IL-6 in paraproteinaemia differ in many aspects hampering comparisons with our own IL-6 data (Table 3). Firstly, uniformity in the patient-populations studied is lacking. The patients were either treatment-naïve, had received treatment before or were undergoing treatment when serum IL-6 was measured.

Table 3. Overview of published serum IL-6 investigations in patients with paraproteinaemia.

Author	Patients (N)*	Time#	Serum IL-6 upper reference limit (URL) and range in pg/ml, differences in (median) serum IL-6 levels among groups
Bioassay			
Bataille <i>et al</i> , 1989	MGUS (22), SMM (130), MM (85), PCL (11)	AD/DT	URL: 5, (0-700). Elevated IL-6 in 100% of PCL, 35% of MM and 3% of patients with MGUS or SMM. Elevated levels in 60% of patients with progressive MM or at relapse.
Reibnegger <i>et al</i> , 1991	MM: I (13), II (12), III (16)	AD	URL: not given, (1.0-12.5). Rise of IL-6 in advanced disease.
Ludwig <i>et al</i> , 1991	MGUS (5), MM: I (13), II (12), III (16), PCL (1)	AD	URL: 7, (0-30). Rise of IL-6 in advanced disease. Elevated levels associated with poor survival.
Nachbaur <i>et al</i> , 1991	MGUS (24), MM/III (23), MPS (8), NHL (25), normal (10)	AD/DT	URL: 5, (1-33). Elevated IL-6 in 42% of MM and 13% of MGUS vs normal controls. Rise of IL-6 in advanced and progressive disease. No difference in IL-6 between MGUS and MM I.
ELISA and Bioassay			
Kiss <i>et al</i> , 1994	MM (63), MGUS (8), normal (25)	AD/DT	URL: 5 (ELISA), (0-107 ELISA; 0-823 bioassay). IL-6 in MM equivalent to normal controls (ELISA). Discrepancy of bioassay vs ELISA, 15 negative by ELISA tested positive by bio-assay.
Thaler <i>et al</i> , 1994	MGUS (57), SMM (7), MM: I (39), II/III (25); normal (40)	AD/DT	URL: not given, (<3-35). No difference in IL-6 between MM I and MGUS. Higher IL-6 in MGUS vs normal, and in advanced or progressive MM.

(Table 3)

ELISA	MM (34), normal (8)	AD/DT	URL: not given, (200-8200). No difference in IL-6 between MM (all disease stages) and normal controls.
Brown <i>et al</i> , 1991	MM (60)	AD/DT	URL: not given, (80-18,800). IL-6 detectable only in 6/60 patients with MM.
Ballester <i>et al</i> , 1992	MGUS (45), MM (51), solid tumour (8 colon cancer, 8 melanoma), infections (20), normal (30)	not given	URL: not given, (not detectable-164). Wide and overlapping ranges of IL-6 in all groups. No discriminatory value.
Merico <i>et al</i> , 1993	MGUS (6), MM: I (12), II (3), III (15); normal (24)	AD/AR	URL: 100, (120, 4 patients). Elevated IL-6 in 4/30 patients with MM.
Tienhaara <i>et al</i> , 1994	MM: I (5), II (13), III (12)	AD	URL: 3300, (<400-43,900); elevated IL-6 in 30%, association with poor survival (only in univariate analysis).
Ballester <i>et al</i> , 1994	MM (30), normal (20)	AD/DT	URL: not given, (0-3,360). IL-6 detectable in only 3/27 patients with MM.
Greco <i>et al</i> , 1994	MM (39), MGUS+solid tumour (76), MGUS-solid tumour (22)	not given (MGUS-tumour)	URL: not given, (0.1-397). IL-6 in MM > MGUS without a solid tumour
Pelliniemie <i>et al</i> , 1995	MM: I (57), II (100), III (52)	AD	No difference in IL-6 between MM and all MGUS (with and without solid tumour).
Fillela <i>et al</i> , 1996	MGUS (22),SMM (5), MM (46), normal (30)	AD/DT	URL: 3.2, (0.4-107). Rise of IL-6 in advanced disease and associated with poor survival.
			URL: 5, (<2-210). Elevated IL-6 in 3% of normal controls, 14% of MGUS, 20% of SMM, 22% of MM at diagnosis, 12% of MM at plateau phase and 92% of MM with progressive disease.

(Table 3)

Kyrtonis <i>et al</i> , 1996	MM: I (3), II (34), III (17); normal (25)	AD	URL: not given, (0-800). Elevated IL-6 in MM vs normal controls. Rise of IL-6 in advanced disease or at relapse and decrease on response to therapy. No correlation with survival.
Cohen <i>et al</i> , 1998	Monoclonal gammopathy (106), Control (1626)	AD	URL: not given. Population based study, no difference in IL-6 between monoclonal gammopathy and controls.
Schaar <i>et al</i> , 1998	IMM (9), MM: I (13), II (4), III (34); MGUS (54), lymphoprol (39), myeloprol (9), solid tumour (35), auto-immune (15)	AD	URL: not given, (6-1,990). No discriminatory role in paraproteinaemia nor a prognostic role in MM.
RIA			
Petterson <i>et al</i> , 1992	MM (16), MGUS (12), CLL (9), prim. Sjögren's syndrome (22), normal (32)	AD	URL: 20, (0-750). Higher IL-6 in MGUS and primary Sjögren's syndrome vs MM, CLL and normal controls.
Solary <i>et al</i> , 1992	MGUS (28), MM (55), WM (19), AML (13), ALL (5), HL (8), NHL (24), CD (2), normal (66)	AD	URL: not given, (113-8,887). Low IL-6 in CD and normals. Higher IL-6 in all other categories, no difference in IL-6 between MM, MW and MGUS. No discriminatory value.
DuVillard <i>et al</i> , 1994	MGUS (128), MM (66), WM (27), NHL (11), CLL (7)	AD	URL: 335, (53 - 2464). Elevated IL-6 in 45% of NHL, 35% of MM and 15% of MGUS patients, no predictive value of IL-6 in paraproteinaemia.

*) Patients; abbreviations used: SMM: smouldering MM; PCL: plasma cell leukaemia; NHL: non Hodgkin's lymphoma; HL: Hodgkin's lymphoma; CD: Castleman's disease; CLL: chronic lymphocytic leukaemia; WM: Waldenström's macroglobulinaemia; AML: acute myeloid leukaemia; ALL: acute lymphatic leukaemia. # Time: Time of IL-6 determinations; abbreviations used: AD: at diagnosis; DT: during therapy; AR: at relapse.

Secondly, cut-off values for the upper normal IL-6 level in serum were either not given^{7;14-17} or ranged widely from 3.2 pg/ml¹⁰ to 3,300 pg/ml⁹. Each study reported a different range of values, even for normal controls (Table 3).

Thirdly, and perhaps this is the most important, serum IL-6 was either determined by bio-assay or immuno-assay (i.e. radio-immunoassay (RIA) or enzyme-linked-immunoassay (ELISA)). Studies using a bioassay^{8;18-20} all found a correlation between serum IL-6 and disease stage in MM. High values were found in MM compared to normal controls or patients with MGUS, and levels were higher in advanced disease. As the final result measured in bioassays is the interaction of several biological activators and inhibitors these tests could be lacking in specificity. Immunoassay methods (ELISA, RIA) were therefore developed to measure more specifically the amount of immunoreactive IL-6. Two studies used both a bioassay as well as an ELISA^{16;21}. Thaler *et al* confirmed a good correlation between both assays though Kiss *et al* reported 15 samples being positive by bioassay which were all negative by ELISA. Subsequent studies using only immunoassay methods reported conflicting results. Serum IL-6 levels in MM varied from undetectable to clearly related with advanced disease, and comparisons with normal controls, patients with MGUS or primary Sjögren's syndrome also yielded discrepancies not easily explained^{7;9;10;14;22-29}. Epitopes on circulating IL-6 can be protected from binding to monoclonal antibodies by binding to α -2 macroglobulin or the soluble IL-6 receptor (sIL-6R) (Brown *et al*, 1991). This 'immunoassay-shielded IL-6' could well retain its biological activity and be measured in a bioassay though not in an immunoassay. Recently, knowledge on the role of sIL-6R in MM has grown considerably giving a diverse though complicated picture³⁰. The biological effect of IL-6 is influenced by sIL-6R and the IL-6/sIL-6R complex could amplify the effect of serum IL-6. Serum IL-6R levels are higher in patients with MM compared to MGUS making it a promising discriminatory marker³¹.

Fourthly, IL-6 has other biologic activities such as induction of acute phase proteins³². Thus, IL-6 being a pleiotropic cytokine, is also elevated during inflammation and/or infection making it important to exclude all patients with any diagnosis that influences the acute phase response when comparing patients with MM or MGUS to patients in other diagnostic categories. We observed high serum IL-6 levels in patients with MGUS. It is important to look at the definition 'MGUS' which is widely used in the literature⁴. In most published studies on paraproteinaemia the absence of clinical evidence for a haematological malignancy has been accepted as a definition of MGUS. Greco¹⁵ already demonstrated the importance of clearly defining patients with MGUS because the presence of a solid tumour markedly influenced the serum levels of IL-6, CRP and β 2M in patients with paraproteinaemia diagnosed as 'MGUS', which could also be demonstrated by us.

In conclusion, although IL-6 is an important cytokine as far as the pathogenesis of MM is concerned, the IL-6 serum value is a poor diagnostic marker in patients with paraproteinaemia and an unreliable prognostic marker in MM.

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3 Serum syndecan-1 in newly diagnosed monoclonal proteinaemia

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Abstract

Serum syndecan-1 was investigated in 189 patients with newly diagnosed monoclonal proteinemia (MM (66), MGUS (54), provisional MGUS (no bone marrow examination performed; 69)) and 36 controls. Syndecan-1 levels ranged widely between all diagnostic categories and were of limited discriminatory value (sensitivity 68%, specificity 78%) in newly diagnosed monoclonal proteinemia.

In the large majority of patients with newly diagnosed monoclonal proteinaemia (M-proteinaemia) there is no evidence for the presence of multiple myeloma (MM), plasmacytoma, amyloidosis, macroglobulinaemia or other haematological malignancy. Therefore, the need for an easily obtainable serum discriminatory marker is felt. The best-known serum marker to distinguish between these categories is the M-protein concentration itself, but much overlap exists¹. Serum syndecan-1 (CD-138) is an independent prognostic marker in MM and is expressed on pre-B-cells, lost during differentiation and re-expressed on normal and malignant plasma cells^{2,3}.

We investigated the discriminatory value of serum syndecan-1 in 189 patients with newly diagnosed M-proteinaemia registered prospectively in a population-based registry. During a three-year period 1464 patients with newly diagnosed M-proteinaemia or MM were entered. Information on patient characteristics, laboratory tests results, M-protein-related diagnosis, comorbidity, results of bone marrow examination and skeletal x-rays, and therapy were documented annually. A serum sample at first diagnosis was frozen at -80 °C. The set-up and contents of this registry have been described previously⁴. From 867 patients with serum available, 189 were evaluable for the present study. The other 678 sera were excluded for the following reasons: other haematological malignancy present, insufficient clinical data concerning the stage of disease, the serum was not taken at diagnosis or an insufficient amount was left for the syndecan-1 determination. The diagnoses of MM and MGUS were made according to the criteria by Durie and Salmon⁵. In the absence of clinical evidence of MM or other haematological malignancy and a low M-protein concentration (<20 g/l) the patient is often diagnosed with MGUS and a bone marrow examination is not considered to be necessary⁶. For precise definition therefore, MGUS was divided in two categories: 'definite' MGUS (confirmed by bone marrow examination) and 'provisional' MGUS (no bone marrow examination performed). Control sera were used from patients without M-proteinaemia, confirmed by protein electrophoresis.

Syndecan-1 was determined using an enzyme-linked immunosorbent assay (Diacclone Research, Besançon, France). Median values of laboratory parameters for different diagnostic categories were compared using Mann-Whitney's test or Kruskal-Wallis test when appropriate. Survival curves were made using the Kaplan Meier method and compared with the log-rank test. Analyses were performed using SPSS 12.0 for Windows.

Serum syndecan-1 levels of all diagnostic categories with newly diagnosed M-proteinaemia are shown in Figure 1 and Table 1. The median levels were highest in MM with significant differences between the diagnostic categories ($p < 0.0001$), however, a wide overlap exists. These results are in accordance with two studies on smaller series comparing serum syndecan in MGUS and MM^{7,8}. In contrast to Seidel but in accordance with Maisnar, we could not demonstrate a relation between serum syndecan-1 levels and MM stages (Table 1; $p = 0.06$)^{2,7}. Using a cut-off value of 166 ng/ml (e.g. the mean level + 2SD of normal human sera; information on kit leaflet) sensi-

tivity and specificity were respectively 68% and 78%. Like Seidel et al we confirmed the prognostic significance of high serum syndecan levels at diagnosis in patients with MM². Median time of follow-up for patients with MM still alive was 8.1 (0.9-10.2) years. Patients with MM and high serum syndecan-1 levels (≥ 166 g/l; n=45) showed a median survival of 1.3 years compared to 4.7 years in 21 MM-patients with lower serum syndecan-1 levels (p=0.0018). In a Cox regression analysis corrected for M-protein isotype and Salmon and Durie stage, elevated serum syndecan remained of prognostic importance with a hazard ratio of 3.6 (95% CI 1.7-7.6).

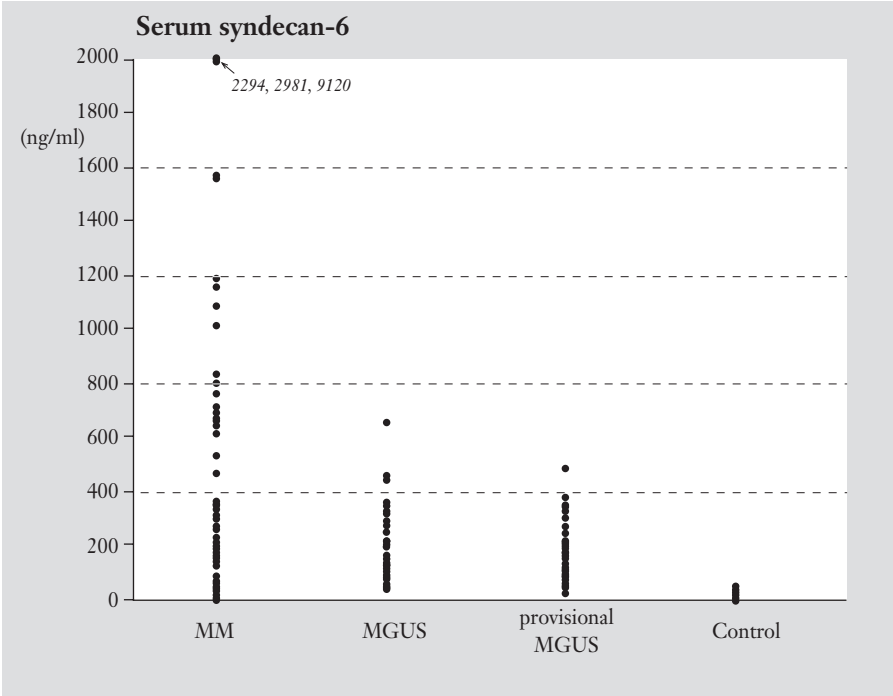


Figure 1. Serum syndecan levels in 226 patients with newly diagnosed M-proteinaemia.

Abbreviations: MM: Multiple myeloma,
MGUS: definite MGUS,
prov MGUS: provisional MGUS.

Median serum syndecan-1 levels in ng/ml (range): Multiple Myeloma 226 (3-9120);
MGUS: 128 (50-656); Provisional MGUS 91 (22-494), Controls 5 (0-52).

Table 1. Median serum values (ranges) of syndecan-1 in newly diagnosed patients with M-proteinaemia.

Category	N	Syndecan-1 (ng/ml)
Multiple myoma (all)	66	226 (3-9120)
• MM stage I	24	194 (3-667)
• MM stage II	5	290 (160-1019)
• MM stage III	37	238 (30-9120)
MGUS	54	128 (50-656)
Provisional MGUS*	69	91 (22-494)
Control Patients	36	5 (0-52)

*) MGUS without confirmatory bone marrow examination

During an 8.8 (4.3-10.7) year follow-up of all patients alive with definite MGUS or provisional MGUS (n=123) MM developed in only two patients at respectively 6 and 12 months. One patient had a low (83 ng/ml) and one a high (176 ng/ml) level of serum syndecan.

In conclusion, serum syndecan-1 is an important prognosticator for patients with MM, but this marker is of no discriminatory value in patients with newly diagnosed M-proteinaemia.

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4 Monoclonal proteinaemia and solid tumours

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Abstract

A higher prevalence of solid tumours in patients with M(onoclonal) proteinaemia without a co-existing haematological malignancy has been reported. We investigated this association by linking a population-based registry of patients with newly diagnosed M-proteinaemia (n=1464) with the Regional Cancer Registry. Patients were followed for a median of 7.4 years for those alive. In total 167 (11%) patients with 173 solid tumours were compared to 861 patients with 'M-proteinaemia only' (without a haematological malignancy). The M-protein isotype or level or clinical parameters did not differ between the groups. M-protein isotype was not associated with a specific tumour type. Standardized morbidity ratios (SMR) for nearly all solid tumours were elevated in the year of M-protein discovery, but the excess risk disappeared during follow-up suggesting selection through diagnostic investigations rather than a causal role. In this large series of patients with both newly diagnosed M-proteinaemia and a solid tumour no relationship could be established.

Introduction

For many decades, it has been assumed that in patients with monoclonal (M)-proteinaemia and without any evidence of a co-existing haematological malignancy, the prevalence of solid tumours is increased suggestive of a causal relationship¹⁻⁸. Further analysis of this relationship could determine more exactly the incidence of this phenomenon in patients with a solid tumour and vice versa, thereby establishing the relevance of screening in cases of M-proteinaemia. To this end we linked a population-based M-protein database^{9;10} to the regional cancer registry and compared solid tumour M-protein patients with other groups in order to try to establish a time relationship between both diagnoses.

Patients and methods

Patient population

From 1991 until 1993, a population-based registry on M-proteinaemia was set up 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 tests results, and results of bone marrow examination and skeletal x-rays were documented. The M-protein-related diagnosis, comorbidity and therapy were recorded. Follow-up was done annually. At follow-up, clinical data, any evolution into a haematological malignancy, appearance of any solid tumour, M-protein levels and other relevant laboratory tests were collected from the patients' hospital charts or from the general physician. In total, 1464 patients have been registered. The set-up and contents of this registry have been described previously^{9;10}.

Diagnostic criteria for monoclonal proteinaemia and solid tumours

M-proteins were either detected by agarose or by cellulose acetate electrophoresis depending on the method used in the various hospitals involved. For inclusion in the registry, each M-protein had to be confirmed by immunotyping (immunofixation). The presence of a solid tumour preferably had to be confirmed by histology, otherwise a clinical diagnosis had to be based on at least radiological evidence of a tumour. Cancer sites were grouped according to the World Health Organization's International Classification of Diseases for Oncology (ICD-O)¹¹.

Linkage to the Regional Cancer Registry

For verification and to ensure the completeness of our data on solid tumours, the database was linked to the regional database of the Netherlands Regional Cancer Registry¹². In this cancer registry, all patients with newly diagnosed malignancies living in the CCCW region reported by the pathology laboratories are entered. The date of the cytological or histological confirmation constitutes the date of diagnosis. In addition, all hospitals employ a separate registry of the discharge diagnoses. For the present study, patient data were linked if name, sex and date of birth were identical in both databases to exclude the probability of false-positive or false negative linkages.

Solid tumour prevalence and incidence analysis

Follow-up started at registration (between 1991 and 1993) and is still ongoing. For the solid cancer linkage study, complete coverage with the Regional Cancer Registry was guaranteed until January 1st 1998. Endpoints were the development of a (haematological) malignancy or death, and patients still alive were censored for all other events on January 1st 2002. First the prevalence of a solid tumour at first diagnosis was calculated. Patients were diagnosed with an M-protein-related solid tumour if the tumour was diagnosed within the time frame of two years, one preceding or following the discovery of the M-protein. Thus, all malignancies that could be associated with the M-protein, but were not present anymore due to treatment were included, as well as any asymptomatic multiple myeloma (MM), other haematological malignancy or solid tumours that developed later on. In case of a simultaneous haematological malignancy or solid tumour during this period, the M-protein was considered to be associated with the former and not with the solid tumour.

Secondly, Standardized morbidity ratios (SMR) for the most prevalent tumours were determined for the period between registration (1991-1993) until January 1, 1998. Patients with newly diagnosed M-proteinaemia were at risk until the diagnosis of a solid tumour, MM, other haematological malignancy was made or until they died. Multiplication of person-years under observation by the age-, sex-, and period-specific incidence rates yielded the number of solid tumours expected in the M-protein cohort if they experienced the same risk as was prevalent in the region of the CCCW. With this method, standardized incidence rates between patient and reference group were compared (indirect standardization) and expressed as the ratio of the incidence rates (SMR), which may be viewed as a relative risk. Confidence limits for the SMR were based on a Poisson distribution for the observed number of deaths¹³.

Statistical methods

Statistical methods to compare the 'M-proteinaemia Only' versus 'Solid Tumour group' included Mann Whitney's test and in the case of case-control design, chi-square test when appropriate. Analyses were performed using SPSS version 10. Data were entered in the database using SPSS Data Entry version 2 (both SPSS Inc. Chicago, Il., USA).

Results

Prevalence of solid tumours at first diagnosis of M-proteinaemia

The database consisted of 1464 patients with an initial diagnosis of M-proteinaemia. The frequency of newly discovered cases was 31/100,000 inhabitants and 189/100,000 for people above 70 years of age⁹. In 271 patients, a diagnosis of MM was made, in 164 another haematological malignancy was diagnosed, but in the large majority no explanation was found (provisional⁹ or definite monoclonal gammopathy of unknown significance, MGUS) (Table 1).

In total, 173 solid tumours without any evidence of MM or other haematological malignancy were diagnosed in 167 (11%) patients. The types of tumour found are depicted in Figure 1. Nearly all tumours (n=167; 96%) were (adeno)carcinomas, the other six malignancies consisted of melanomas (n=4), sarcoma (n=1) and leiomyosarcoma (n=1).

Table 1. Clinical characteristics in all diagnostic groups with M-proteinaemia.

Monoclonal proteinaemia only		Solid tumour	P	Multiple myeloma ^a	Other hematological malignancies ^b
Number	861 (59)	167 (11)		271 (19)	164 (11)
Sex					
M (%)	423 (49)	103 (62)	0.004	138 (51)	96 (58)
F (%)	438 (51)	64 (38)		133 (49)	69 (42)
Median age (range, years)					
M	73 (17-103)	75 (37-95)	0.05	71 (28-93)	72 (21-94)
F	72 (20-103)	75 (37-95)	0.03		
	75 (17-98)	76 (47-92)	0.91		
M-protein type & level (g/l)					
IgG (%)	618 (72)	29 (76)	0.45	155 (57)	75 (46)
Median (range)	10 (1-30)	10.5 (<1-85)		32.5 (6-117)	12.3 (<1-34)
IgA (%)	80 (9)	10 (6)	0.71	75 (28)	5 (3)
Median (range)	8.4 (4-31)	10 (2-47)		28.1 (5-81)	15.6 (2-25)
IgM (%)	159 (19)	30 (18)	0.19	4 (2)	81 (49)
Median (range)	7 (1-21)	10 (<1-30)		22.1 (2-57)	13.8 (2-110)

In the patient with prostate carcinoma, two haematological malignancies were observed: chronic lymphocytic leukaemia (CLL) and Hodgkin's lymphoma. M, male; F, female; MGUS, monoclonal gammopathy of unknown significance.

^{a)} In five patients, a solid tumour was present (carcinoma of lung, breast, thyroid, ovary, and unknown primary site).

^{b)} In eight patients, a solid tumour was present (melanoma, carcinoma of unknown primary site, bladder, prostate, colon, larynx, skin, and liver).

Men (n=103)**Head & Neck: 4 (4%)**

Meninges
Hypopharynx
Nasopharynx
Larynx

Respiratory tract: 36 (35%)

Lung: 35 (34%)
Pleura

Gastrointestinal tract: 25 (24%)

Oesophagus
Stomach: 5 (5%)
Liver
Biliary system: 2 (2%)
Pancreas: 4 (4%)
Colon & rectosigmoid: 12 (12%)

Urogenital tract: 24 (23%)

Kidney: 3 (3%)
Urether & bladder: 8 (8%)
Prostate: 13 (13%)

Skin: 3 (3%)**Melanoma: 4 (4%)****Unknown primary: 8 (8%)****Women (n=64)****Head & Neck: 2 (3%)**

Gingiva
Thyroid

Respiratory tract: 5 (7%)

Lung: 5 (7%)

Breast: 19 (28%)**Gastrointestinal tract: 21 (31%)**

Oesophagus
Stomach: 3 (4%)
Biliary system: 2 (3%)
Pancreas: 6 (9%)
Colon & rectosigmoid: 10 (15%)

Urogenital tract: 12 (17%)

Kidney: 2 (3%)
Ovary: 5 (7%)
Urether & bladder: 2 (3%)
Uterus
Cervix
Vulva

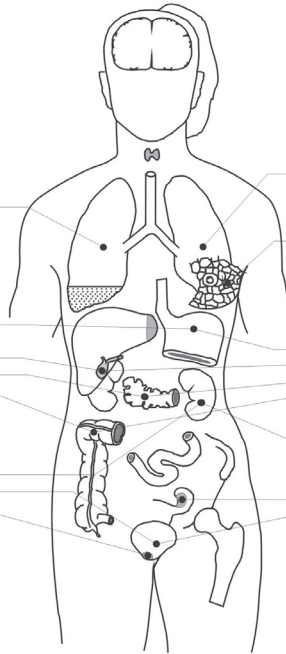
Skin: 2 (3%)**Unknown primary: 7 (10%)**

Figure 1. Distribution of solid tumours and M-proteinaemia. Two tumours were diagnosed in six patients: bilateral breast (2), breast + pancreas, breast carcinoma + colon leiomyosarcoma, squamous skin + melanoma, pancreas + gingiva. No percentage between brackets means only one tumour found. Modified from Goffinet and colleagues¹⁹.

Solid tumour and M-protein isotype

To study whether specific tumour types were related to specific M-protein isotypes, we selected patients with one of the six most frequently occurring solid tumours, lung (n=40), colon (n=21), breast (n=15), prostate (n=13), bladder (n=10) and pancreas (n=9). In all, the IgG isotype predominated (60-85%), followed by IgM (7-30%) and IgA (0-10%). No clear preferential M-protein types were seen within specific tumour groups.

'Solid Tumour Group' versus 'M-proteinaemia Only Group/MGUS'

To study whether patients with M-proteinaemia and a solid tumour (i.e. 'Solid Tumour Group') had specific characteristics in demographics or M-protein isotype and levels, we compared this group with the patients without any malignancy (Table 1). In the tumour group, male gender predominated. In addition, small, but significant, differences in age were seen. The distribution of M-protein isotypes and levels was identical with the majority of patients expressing a median of 10 g/l IgG monoclonal proteinaemia.

Response of the M-protein on cancer treatment

When M-proteins are causally related to a solid tumour, one would expect to find an increase of the M-protein levels during tumour progression and a decrease after tumour disappearance. In the Solid Tumour group, 64 out of 167 patients died within one year after the detection of the M-protein leaving 103 patients with follow-up data. In 25 patients (median follow-up 37 months, range 3-95 months) the M-protein was measured at least once after the first detection and therapy (if any) of the solid tumour. Since in four patients a haematological malignancy developed (see 'Follow-up since entry in the M-protein database'), a relationship (rising or lowering of the M-protein in correspondence with the progression or decrease of the tumour) could be studied in only 21 patients. In this small group of patients, no convincing relationship was seen between the behaviour of the solid tumour and the M-protein levels (data not shown).

Follow-up since entry in the M-protein database

During follow-up (last analysis 1-1-2002) a new solid tumour was detected in 23 patients in addition to those already diagnosed in the 167 patients. All of these tumours occurred in the period 1992-1998 (see Table 2). In the years thereafter, no additional tumours were found. Out of the 167 patients in whom a solid tumour was diagnosed simultaneously with the M-protein, three patients developed MM (14, 56 and 65 months after the detection of the M-protein) and one patient developed a non-Hodgkin's lymphoma (NHL) (28 months after the detection of the M-protein). For comparison, in the Monoclonal proteinaemia only /MGUS group, 28 developed MM, and another 17 developed haematological malignancies, consisting of NHL (n=12), myelodysplastic syndrome or acute myeloid leukaemia (n=3) or myeloproliferative disease (n=2).

Table 2. Standardized morbidity ratios (SMR) for the most prevalent solid tumors in the year of discovery of the M-protein and the years after.

Solid tumour (all (adeno)carcinoma)	Year of M-protein discovery (1991,1992, 1993)				Follow-up (1992-1997)			
	Observed number	Expected number	SMR	95% conf. interval	Observed number	Expected number	SMR	95% conf. interval
Men								
Lung	19	0.9	21.1	12.5-31.9	5	4.1	1.2	0.4-2.6
Colon & rectosigmoid	4	0.4	10	2.5-22.5	1	1.7	0.6	0-2.4
Prostate	5	0.9	5.6	1.7-11.6	3	4.5	0.7	0.2-1.7
Unknown primary	5	0.2	25	7.6-52.4	2	0.8	2.5	0.2-7.3
Pancreas	1	0.1	10	0-40	0	0.25	0	0-4
Stomach	2	0.3	6.7	0.6-19.4	0	0.8	0	0-1.3
Bladder	3	0.2	15	2.7-37.3	1	1.0	1	0-4.0
Women								
Lung	3	0.1	30	5.4-74.6	0	0.6	0	0-1.7
Colon & rectosigmoid	4	0.4	10	2.5-22.5	5	1.6	3.1	1.0-6.5
Breast	3	0.6	5	0.9-12.4	3	2.9	1.0	0.2-2.6
Unknown primary	5	0.1	50	15.2-104.7	2	0.6	3.3	0.3-9.7
Pancreas	4	0.1	40	10-90	2	0.4	5.0	0.4-14.6
Stomach	2	0.1	20	1.7-58.3	0	0.7	0	0-1.4
Bladder	0	0.1	0	0-40	1	0.29	3.5	0-13.8

Standardized Morbidity Ratio

Cumulative follow-up of all 1464 patients during this selected period (1991-1998) was 3060 person-years with a median follow-up of 1.3 years (range 0-7 years) and a median follow-up of 7.4 years (range 10 months-7 years) for those still alive. Cumulative follow-up (measuring the time interval between the date of diagnosis of the M-proteinaemia and the date of diagnosis of the solid tumour) for the Solid Tumour group was 24 person-years (median less than 1 day, range 0-2.8 years). In conclusion, most solid tumours were diagnosed simultaneously with the detection of the M-protein (median follow-up less than one day, see above). In the first year after the detection of the M-protein, SMR for nearly all solid tumours showed an increased risk (range 0-50). However, all declined sharply or normalised during subsequent follow-up years (Table 2).

Discussion

In this population-based registry on patients with newly diagnosed M-proteinaemia we describe the largest series collected thus far of patients with both a solid tumour and a M-protein but without any evidence of a co-existing haematological malignancy. Since 1928, investigators reported an increased prevalence of solid tumours in patients with M-proteinaemia suggesting a paraneoplastic phenomenon. For comparison with our cohort, we selected only studies with more than 100 patients, with a description of the related malignancy including concise histopathology and information on the determination of the M-protein, and were left with 8¹⁻⁸. Identical to our series, nearly all solid tumours described were (adeno)carcinomas. M-proteins were mostly of the IgG isotype and levels (if investigated) were generally lower than 30 g/l.

The co-existing tumours in this M-protein database were manifest at the diagnosis of the M-protein in the large majority of patients. During follow-up, only a small additional number of solid tumours were detected. Kyle and colleagues *et al* observed the development of a second tumour in 15 of 241 MGUS-patients during a 20-35 year follow-up¹⁴ and Pasqualetti and colleagues reported 31 out of 263 similar patients who died due to a solid tumour during a median follow-up of 11.5 years¹⁵. In contrast, in the only prospective study investigating the incidence of haematological and solid malignancies in patients with M-proteinaemia, Gregersen and colleagues did not observe an increased risk of solid tumours in 1229 patients during follow-up (mean 4.8 years, range 0-15.7 years)¹⁶. Similarly to our findings, the risk for developing a solid tumour was increased in the first year of follow-up though this risk diminished thereafter¹⁶. Approaching the probable relationship between M-proteinaemia and cancer the other way around yielded no association either: in two cross-sectional stud-

ies the prevalence of M-proteinaemia in patients with non-haematological tumours was not increased when compared to the prevalence in the general population^{17;18}.

In conclusion, we did not observe differences in clinical characteristics between patients with 'M-proteinaemia only/MGUS' and patients with 'Solid tumour and M-proteinaemia'. There was no relation between specific solid tumours and M-protein isotype nor did the serum level of the M-protein change after anti-tumour therapy (although the number of patients was small in this analysis). Although risks for nearly all solid tumours found were initially elevated in patients with newly diagnosed M-proteinaemia, these decreased in the year after suggesting a diagnostic selection of patients rather than a causal role.

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5 Long term follow-up of a population based cohort with monoclonal proteinaemia

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Abstract

Background: Prospective studies on the risk of malignant transformation in patients with monoclonal proteinemia of undetermined significance (MGUS) and specifically factors predictive of survival are lacking.

Objective: To determine factors predictive of malignant transformation and survival in patients with MGUS.

Design: Prospective hospital-based cohort study.

Setting: Dutch Comprehensive Cancer Centre West comprising 1.6 million inhabitants and 15 hospitals.

Patients: Patients with newly diagnosed M-proteinemia (n=1464) with a median age of 73 (17-103) years, included from January 1991 through December 1993 and followed yearly.

Measurements: M-protein related diagnoses, patients' characteristics, laboratory investigations, bone marrow examinations and skeletal X-rays were registered. Main end-points were death, or new diagnoses of multiple myeloma and non-Hodgkin's lymphoma. Kaplan-Meier survival curves were compared with age and gender matched survival data from the total Dutch population. Cumulative malignant transformation was corrected for death using a competing risk model. Risk factors for transformation or death were analyzed by univariate and multivariate analyses.

Results: M-protein-related diagnoses were: multiple myeloma 294 (20%), other haematological diseases 163 (11%) and MGUS 1007 (59%) consisting of definite MGUS (bone marrow confirmed) 213 (15%) and provisional MGUS (without bone marrow confirmation) 794 (54%). Malignant transformation in all MGUS patients was associated with rising M-protein levels, the IgA and IgM isotype and occurred at a yearly rate of 0.4%. All MGUS patients survived less than a matched cohort of the Dutch population, even in the absence of M-protein-associated comorbidity. Serum albumin levels at entry appeared highly predictive for survival.

Conclusions: M-proteinemia is not an innocent symptom. Although malignant transformation occurs rarely, survival is shortened irrespective of comorbidity.

Monoclonal gammopathy of undetermined significance (MGUS) is characterized by the presence of a monoclonal protein (M-protein) in the serum and/or urine without the evidence of multiple myeloma, amyloidosis or another haematological malignancy being present¹. Other M-protein associated conditions are infections, autoimmune diseases, organ and stem cell transplantation². In persons of seventy years or older the prevalence rises up to 3% making it the most commonly found immunoglobulin disorder in this age group³⁻⁵. Patients with MGUS have been reported to run an increased yearly 1-2% risk of eventually developing a malignant M-protein related haematological malignancy, most often multiple myeloma or lymphoplasmacytic lymphoma⁶⁻¹⁷. However, nearly all studies on this subject are retrospective and often single centre based. The only prospective (single centre) study thus far reported a lower incidence of malignant transformation, but maximum follow-up was only 26 months¹⁵. In 1991 a Dutch multi-centre population-based registry was initiated that prospectively collected all patients with newly diagnosed M-proteinaemia and/or multiple myeloma during a three-year period. From this unique cohort clinical and serological data offered much insight in the occurrence and prognostic factors on multiple myeloma and (probably more interesting) non-myeloma patients with monoclonal proteinaemia in the Netherlands¹⁸⁻²⁴. For the first time we report on the long term follow-up (median 9 years for those alive) of all these 1464 patients of whom 1007 (69%) were initially diagnosed with MGUS.

Patients and methods

Patients

A prospective registry on M-proteinaemia was initiated in the region of the Comprehensive Cancer Centre West from 1991 till 1993. All patients with newly diagnosed M-proteinaemia and/or multiple myeloma in the Midwestern part of The Netherlands (1.6 million inhabitants and 15 hospitals in 1992) were reported by clinical chemists, internists, haematologists, pathologists and other physicians. Information on patient characteristics (age, gender, performance status), laboratory test results (haemoglobin, leukocyte and thrombocyte counts, creatinin, LDH, alkaline phosphatase, calcium, albumin, total protein, M-protein isotype and concentration in serum and urine), and results of bone marrow and skeletal X-rays were obtained. Non-involved immunoglobulins were considered reduced if at least one of the other immunoglobulin isotypes was reduced, respectively polyclonal IgG <6 g/l, IgA <1.0 g/l, IgM <0.5 g/l. The M-protein-related diagnosis, comorbidity and therapy were recorded. A serum sample obtained at registration was centrally stored at -80 °C. Follow-up was done at least once yearly until May 1st 2004. In total, 1464 patients have been registered. M-proteins were either detected by agarose- or cellulose-acetate

electrophoresis depending on the method used in each hospital where the patient was first seen. For inclusion in the registry each M-protein had to be confirmed by immunotyping (usually immunofixation). The setup and contents of this registry have been described²².

Monoclonal protein related diagnoses

Four M-protein related diagnoses were defined: Multiple Myeloma, other Haematological Malignancies, ‘*definite* MGUS’ and ‘*provisional* MGUS’. We followed the criteria for classification of M-protein related diagnoses made by the International Myeloma Working Group (IMWG)²⁵ and recently reclassified all cases. Similarly, the diagnosis of lymphoplasmacytic lymphoma/Waldenström’s macroglobulinaemia was made when the IgM-M-protein serum level was more than 30 g/l and/or bone marrow lymphocytosis of at least 10% was present. *Definite* MGUS was diagnosed if the M-protein level was less than 30 g/l and the percentage of bone marrow plasma cells (lymphocytes) was less than 10% in the absence of evidence of other B-cell proliferative disorders and organ or tissue impairment²⁵. This means that according to this classification a bone marrow examination (mandatory) and skeletal X-rays (optimal) had to be performed²⁵. Patients with serum M-protein levels of less than 30 g/l who were classified as having MGUS by the treating physician, but in whom no confirmatory bone marrow examination had been performed were included in a separate group labeled *provisional* MGUS²².

End points and statistics

The primary endpoints in this study were survival and malignant transformation defined as the development of multiple myeloma or B-cell non-Hodgkin’s lymphoma. Survival curves were calculated with the Kaplan-Meier estimate, and subgroups were compared using the log-rank test. Hazard rates were calculated for each potential prognostic factor in a Cox proportional hazards model. To study which variables had an independent influence on survival, all variables with an univariate association ($p < 0.10$) were entered simultaneously in a Cox proportional hazards model. As the median age of patients with M-proteinaemia was high, survival curves of the four diagnostic groups with M-proteinaemia were compared to the survival of an age and sex matched cohort of the total Dutch population (Statline, Statistics Netherlands 2004). Cumulative incidence of malignant progression was calculated using a competing risk model in which death without progression was the competing event²⁶. Potential risk factors were examined univariately in a Cox-proportional-hazards model. All data were calculated using SPSS version 12 (Statistical Package for the Social Sciences, Chicago, Ill, USA), S-Plus version 6.0 (Insightful, Seattle, WA) or R (Version 1.9.1).

Results

Characteristics of all patients

In total 1464 patients were included; 760 (52%) were men, 704 (48%) were women, median age was 73 (17-103) years. Multiple myeloma was diagnosed in 294 patients (20%). Other haematological diseases were diagnosed in 163 (11%) patients and consisted of: myelodysplastic syndrome (17), lymphoplasmacytic lymphoma/Waldenström's macroglobulinaemia (55), other B-cell non-Hodgkin lymphoma (54), chronic lymphocytic leukaemia (21), cryoglobulinaemia (2), Hodgkin's lymphoma (4), chronic myeloid leukaemia (2), acute myeloid leukaemia (4), plasma cell leukaemia (1), haemolytic anaemia (3). MGUS was diagnosed in 1007 (69%) patients; *definite* MGUS with confirmatory bone marrow examination in 213 (21%) patients and *provisional* MGUS without confirmatory bone marrow examination in 794 (79%) patients (Table 1). The provisional MGUS group was further subdivided in the following categories related to the most probable M-protein-related internal diagnosis made by the treating physician: 1. Paraneoplastic (solid tumour present), 2. Autoimmune disease, 3. Para-infectious and, 4. Other, e.g. those patients in whom no internal diagnosis was made (Table 2).

Characteristics of MGUS patients

The baseline characteristics for all MGUS patients and -where appropriate- separately for definite MGUS and provisional MGUS are reported in Table 2. There were no significant differences between both MGUS groups but for lower M-protein levels (<10 g/l) in provisional MGUS than in definite MGUS (Figure 1). The M-protein isotype most often found was IgG (72.6%), followed by IgM (18.6%) and IgA (8.5%). A biclonal M-protein was found in 107 patients (10.6%). The light chain was κ in 64% and λ in 36%. Anaemia, defined as a haemoglobin level of less than 7.4 mmol/l (12 mg/dl) occurred in 32% and was always due to non-M-protein related causes as was renal insufficiency (Table 2). Urine examination was performed in 533 (53%) of all MGUS patients and in 69 (7%) a urinary protein electrophoresis was performed. Initial M-protein serum values ranged from unquantifiable (small band on electrophoresis but not quantifiable by densitometry) to 29.6 g/l. The unquantifiable group was categorized into the <5 g/l cohort (Figure 1). In provisional MGUS M-protein related internal diagnoses were given as well in Table 2.

Table 1. Baseline characteristics of all patients with M-proteinaemia.

	Multiple myeloma (294)	Other haemato- logical diseases (163)	All MGUS (1007)
Men/Women (%)	50.3/49.7	58.3/41.7	51.3/48.7
Median age in years (range)	71 (28-93)	72 (21-94)	73 (17-103)
<40 years %	1.4	6.1	3.8
40-70 years %	46.6	34.4	35.4
70 years %	52	59.5	60.8
M-protein isotype (%)			
IgG	60.3	42.3	72.6
IgA	26.9	3.1	8.5
IgD	0.3	–	0.1
IgM	0.3	52.8	18.6
light chains	9.5	1.8	0.2
non-secreting	2.4	–	–
unknown (%)	0.3	–	–
κ/λ (%)	66/34	75/25	64/36
Reduced immunoglobulins (%)	67	43	25
Bone marrow plasma cells % (range)	28 (1-100)	5 (1-16)	5 (0-9)
Haemoglobin			
mmol/l (range)	7.1 (1.6-10.4)	7.1 (2.6-10.1)	8.0 (2.5-11.4)
g/dl (range)	11.4 (2.6-16.8)	11.5 (4.2-16.3)	12.9 (4.0-18.4)
Serum creatinine			
μmol/l (range)	106 (44-2016)	88 (53-663)	88 (27-1759)
mg/dl (range)	1.2 (0.5-22.8)	1.0 (0.6-7.5)	1.0 (0.3-19.9)
% ≥177 μmol/l (2.0 mg/dl)	14	6	7

Table 2. Baseline characteristics MGUS patients.

	Definite MGUS (213)	Provisional MGUS (794) All (794)	Related internal diagnoses			
			Paraneoplastic (99)	Auto-immune disease (34)	Para-infectious (55)	None (606)
Men/Women %	48/52	52/48	66/34	24/76	56/44	51/49
Median age in years (range)	72 (32-91)	74 (17-103)	76 (38-93)	72 (23-92)	66 (20-93)	74 (17-103)
<40 years %	2	4	1	6	16	4
40-69 years %	41	34	31	32	40	34
≥70 years %	57	62	68	62	44	62
M-protein isotype (%)						
IgG	76.5	71.5	73.7	60.6	74.5	71.5
IgM	16.4	19.1	22.3	27.3	20	18.2
IgA	7.1	8.9	4.0	12.1	5.5	9.9
IgD	–	0.1	–	–	–	0.2
light chains	–	0.2	–	–	–	0.2
κ/λ %	71/29	62/38	71/29	68/32	60/40	60/40
Haemoglobin						
mmol/l (range)	7.6 (2.7-11.4)	8.1 (2.5-11.4)	7.9 (4.7-10.1)	7.9 (4.4-10.6)	8.1 (5.4-10.8)	8.2 (2.5-11.4)
g/dl (range)	12.2 (4.4-18.4)	13.1 (4.0-18.4)	12.8 (7.6-16.3)	12.7 (7.1-17.1)	13.1 (8.7-17.4)	13.2 (4.0-18.4)
Serum creatinine						
μmol/l (range)	87 (44-1759)	88 (27- 1158)	88 (53-406)	80 (35-937)	80 (35- 274)	88 (27- 1158)
mg/dl (range)	0.98 (0.5-19.9)	1.0 (0.3-13.1)	1.0 (0.6-4.6)	0.9 (0.4-10.6)	0.9 (0.4-3.1)	1.0 (0.3-13.1)
% >177 μmol/l (2.0 mg/dl)	9	6	3	3	4	6

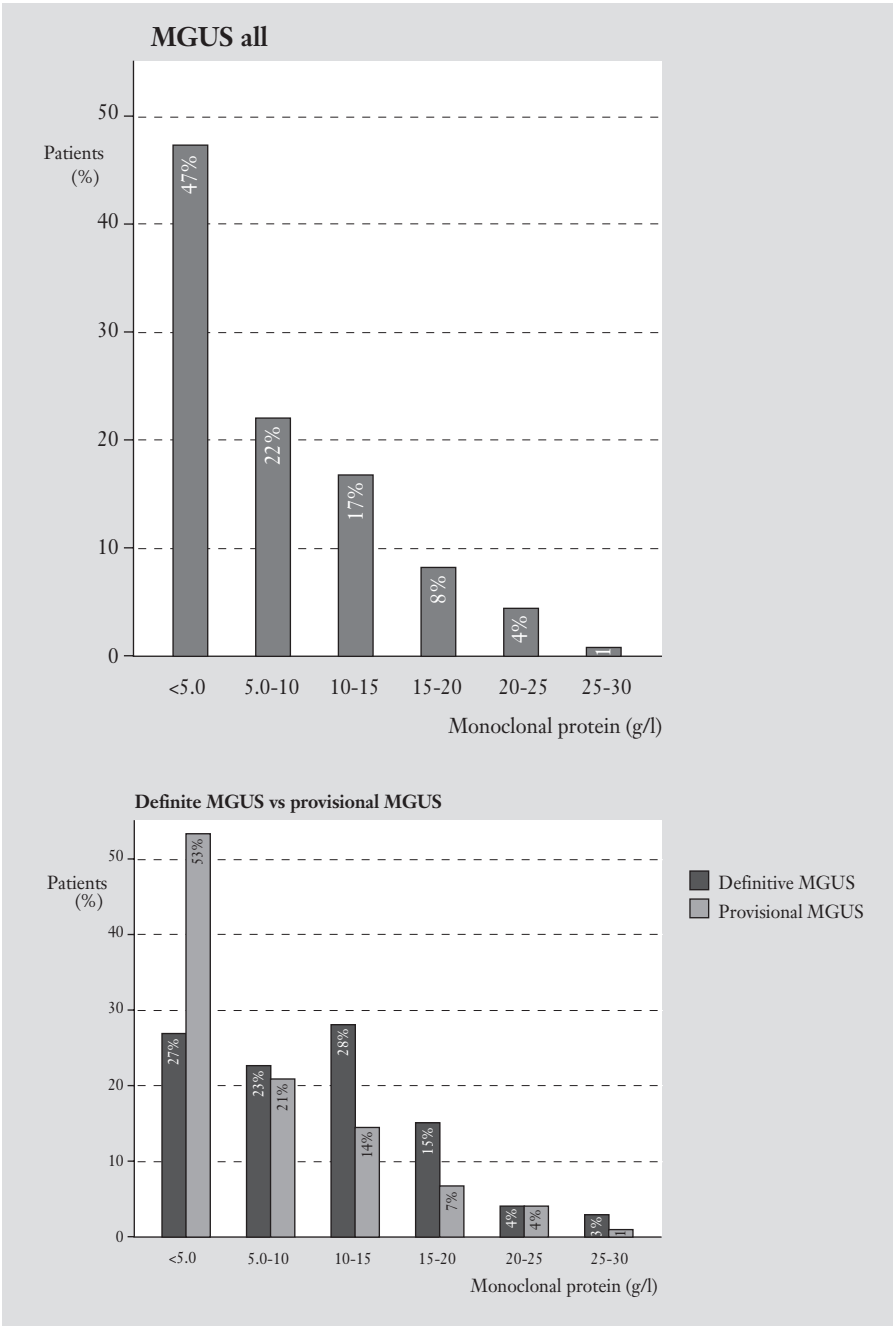


Figure 1. Monoclonal protein levels in MGUS. Upper part concerns all patients with MGUS. Lower part patients with definite MGUS and with provisional MGUS.

Outcome

Follow-up time

All 1464 patients were followed for 6543 person-years (median 3.9, range 0-12.4 years) during which 1007 (69%) died. Median follow-up was 8.9 (0.4-12.0) years for those alive and 2.0 (0-11.4) years for those who died. Total follow-up for all MGUS-patients was 4782 person-years with a median of 4.5 (range 0-12.0) years; median follow-up was 9.0 (0.4-12.0) years for those alive and 2.0 (0-11.4) years for those who died. Median follow-up for those alive with definite MGUS and provisional MGUS was not different: 8.8 (0.8-11.9) years and 9.0 (0.4-12.0) years respectively. In total 19 patients were lost to follow-up.

Survival, strong prognostic role of albumin in MGUS

The 1464 patients experienced a median survival of 4 (95% CI 3.6-4.4) years. Median survival in multiple myeloma was 2.7 (95% CI 2.0-3.4) years and median survival was 3.6 (95% CI 2.4-4.8) years in patients with haematological diseases (Figure 2). Patients with definite MGUS survived longer for a median of 6.7 (95% CI 4.7-8.7) years compared to 4.3 (95% CI 3.6-4.9) years in those with provisional MGUS ($p=0.0003$). All 1007 MGUS patients experienced a median survival of 4.6 (95% CI 4.0-5.3) years which was far below the survival in an age-matched cohort of the Dutch population (Figure 2). In patients with provisional MGUS survival was also influenced by the M-protein related internal diagnosis (Figure 3). Survival was worst in patients with M-proteinaemia associated with a solid tumour (median 0.4 (95% CI 0.2-0.5) years) compared to the patients with auto-immune disease (median 6.1 years, 95% CI (4.0-not attained), parainfectious (median 6.1 years (95%CI 2.5-9.7) and the patients without an internal diagnosis (median 4.9 years (95% CI 4.2-5.6). In a univariate analysis, M-protein isotype or serum level or light chain isotype did not influence survival in MGUS patients. However, age, gender and especially *serum albumin* were important factors (Table 3, Figure 4), even when patients with solid tumour related M-proteinaemia were excluded from the analysis (data not shown). For instance, patients with a serum albumin of less than 30 g/l ($n=208$) survived for a median of 1.9 (95% CI 1.2-2.6) years compared to patients with a serum albumin of 40 g/l or more surviving for a mean of 8.5 (95% CI 8.0-9.0) years. The variables that were univariately

Legend to Figure 1 (p.72):

M-protein levels are divided in six categories:

- <5 g/l = M-protein level less than 5 g/l;
- 5-10 g/l = M-protein level 5 till 10 g/l;
- 10-15 g/l = M-protein level 10 till 15 g/l;
- 15-20 g/l = M-protein level 15 till 20 g/l;
- 20-25 g/l = M-protein level 20 till 25 g/l;
- 25-30 g/l = M-protein level of 25 g/l or more.

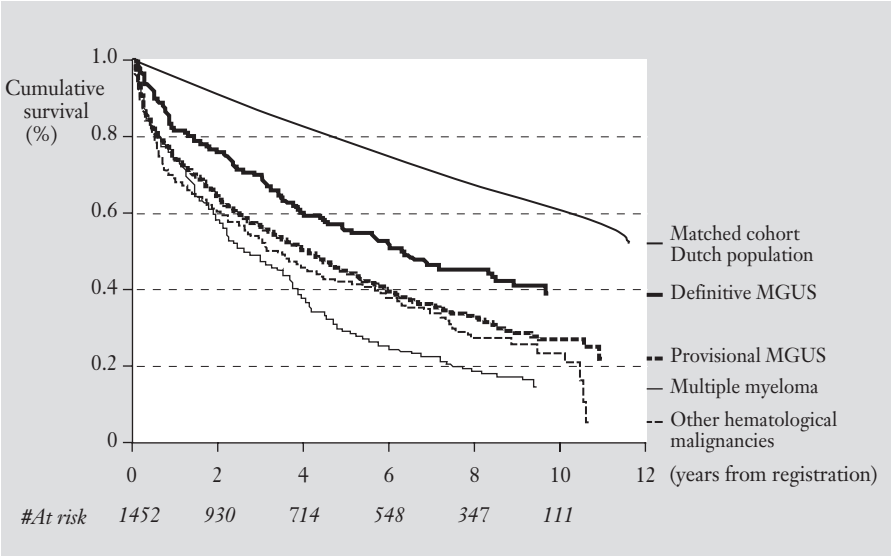


Figure 2. Survival in multiple myeloma, other hematological malignancies, definite MGUS and provisional MGUS compared to an age and gender matched cohort of the Dutch population.

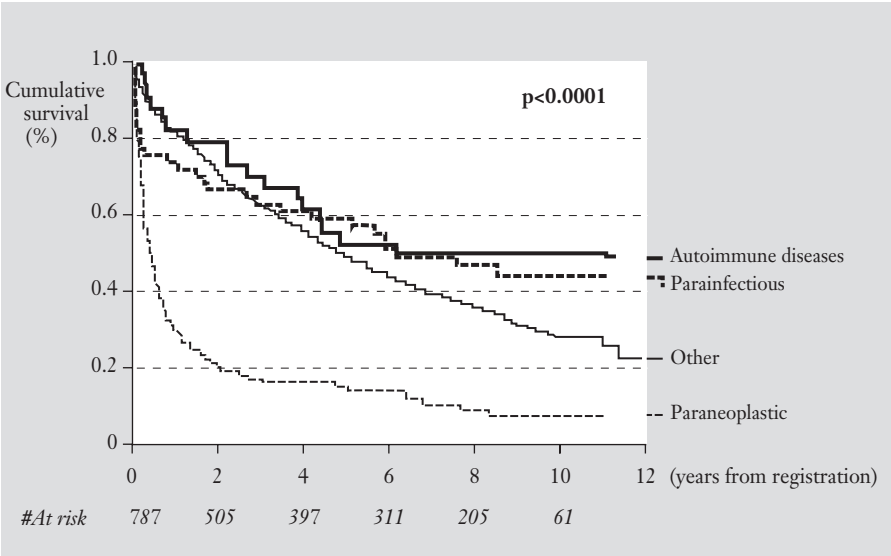


Figure 3. Survival in provisional MGUS patients divided by related internal diagnoses.

related to survival ($p < 0.10$), e.g. M-protein-related diagnosis, IMWG-status (definite MGUS versus provisional MGUS), gender, age and serum albumin, all remained significant in the multivariate analysis (Table 3).

Table 3. Univariate and multivariate analyses on risk factors for survival in MGUS.

Factor	Univariate			Multivariate		
	HR	95% CI	P	HR	95% CI	P
Men versus women	1.24	1.06-1.44	0.007	1.34	1.15-1.57	0.000
Age (years)			<0.001			<0.001
<60	1			1		
60-70	2.48	1.74-3.52		2.23	1.56-3.19	
70-80	4.83	3.52-6.64		4.02	2.90-5.55	
>80	8.57	6.22-11.81		7.21	5.18-10.04	
Albumin (g/l)			<0.001			<0.001
<30	4.29	3.35-5.49		3.12	2.41- 4.03	
30-35	2.71	2.14-3.44		2.14	1.68-2.73	
35-40	2.16	1.73-2.70		1.67	1.33-2.09	
>40	1					
missing	1.85	1.38-2.48		1.42	1.06-1.91	
Provisional MGUS versus definite MGUS	1.44	1.18-1.76	<0.001	1.43	1.16-1.76	0.001
Internal diagnosis			<0.001			<0.001
paraneoplastic	3.70	2.96-4.61		3.12	2.48-3.93	
auto-immune	0.69	0.43-1.06		0.69	0.44-1.09	
para-infectious	0.80	0.56-1.16		0.82	0.56-1.20	
other	1			1		
Serum level of M-protein g/l						
All	0.99	0.98-1.00	0.44			
separate for IgG	0.99	0.98-1.02	0.94			
separate for IgM	0.98	0.95-1.01	0.22			
separate for IgA	0.99	0.95-1.04	0.77			
M-protein isotype						
IgG	1		0.63			
IgM	1.10	0.90-1.34	0.36			
IgA	0.98	0.74-1.29	0.88			
Light chain						
κ/λ	1.03	0.88-1.21	0.69			

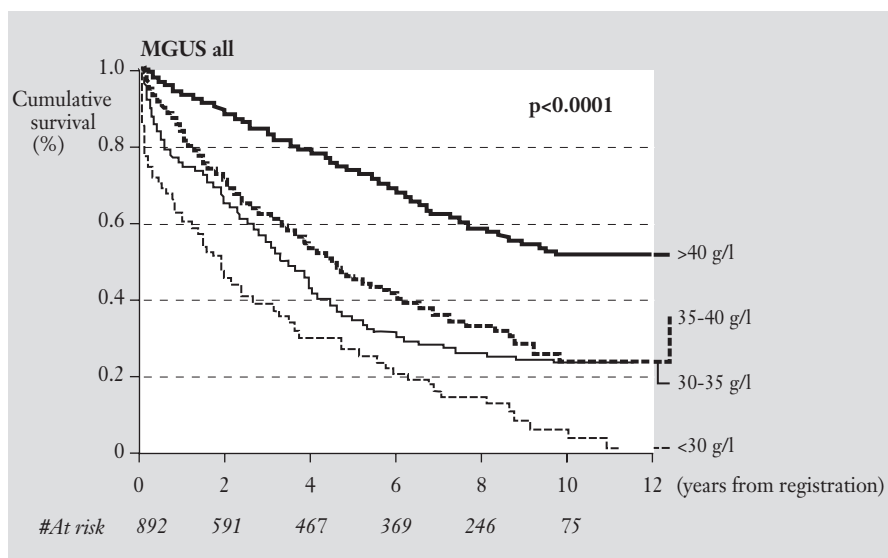


Figure 4. The significance of survival in MGUS in relation to presenting albumin levels: <30 g/l: serum albumin less than 30 g/l; 30-35 g/l: serum albumin 30 till 35 g/l; 35-40 g/l: serum albumin 35 till 40 g/l; >40 g/l: serum albumin of 40 g/l or more.

Malignant transformation

During follow-up of all MGUS-patients, multiple myeloma developed in 26 patients, of whom only 6 had been initially classified in the definite MGUS group. The transformation occurred at a median interval of 2.3 (0.1-6.4) years for all MGUS patients; 1.4 and 2.3 years in definite MGUS and provisional MGUS respectively. Other haematological malignancies (lymphoplasmacytic lymphoma (8) and other B-non-Hodgkin's lymphoma (4)) developed in 12 patients of whom 3 in the definite MGUS group. The probability for MGUS-patients of developing multiple myeloma or B cell lymphoma was 3.1% at five years and seems to plateau thereafter to 3.9% at ten years (Figure 5). Risk of progression was not significantly different in definite MGUS compared to provisional MGUS (log rank $p=0.69$).

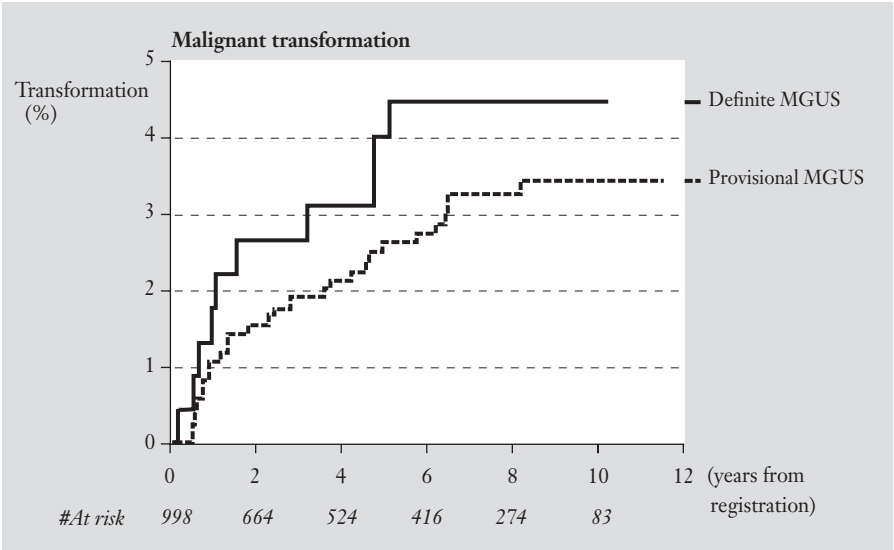


Figure 5. Risk of malignant transformation into multiple myeloma or non-Hodgkin's lymphoma in all MGUS patients, using a competing risk model with death without transformation as competing event.

Risk factors for malignant transformation

The univariate influence of several baseline factors on transformation was examined using Cox regression. The only significant factors for malignant transformation were the serum M-protein level and M-protein isotype. The estimated hazard ratio for M-protein level was 1.148, indicating that an increment of the serum M-protein of 1 g/l increased the risk of transformation 1.16 times. The IgA isotype had the highest risk on malignant transformation, the IgG isotype the lowest. Gender and albumin were not significantly related to the risk of transformation (Table 4).

Table 4. Univariate analysis on risk factors for malignant transformation in MGUS.

Factor	HR	95% CI	P
Men versus women	1.44	0.75-2.73	0.27
Age (years)			
<60	1.00		
60-70	2.39	0.91-6.29	0.33
70-80	1.78	0.68-4.70	
>80	1.41	0.45-4.41	
Albumin (g/l)			
<30	0.74	0.22-2.50	
30-35	0.55	0.19-1.62	0.61
35-40	0.80	0.37-1.72	
>40	1.00		
missing	0.37	0.09-1.59	
Provisional MGUS versus definite MGUS	1.16	0.56-2.39	0.69
Serum level of M-protein g/l			
All	1.16	1.10-1.22	0.000
separate for IgG	1.11	1.04-1.19	0.004
separate for IgM	1.19	1.08-1.30	0.000
separate for IgA	1.27	1.13-1.43	0.000
M-protein isotype			
IgG	1.00		
IgM	2.37	1.09-5.12	0.001
IgA	4.45	2.00-9.90	
Light chain			
κ/λ	1.37	0.68-2.77	0.39

Discussion

Fifteen years ago, we initiated a registry for all newly diagnosed patients with M-proteinaemia. We wondered whether we could predict if and when these patients would progress into multiple myeloma or B cell lymphoma. Next we documented M-protein associated co-morbidity and measured overall survival. In many of these patients, the M-protein was found accidentally. Some laboratories routinely ran a protein electrophoresis when discrepancies between total serum protein and serum albumin were found. Therefore, many patients, mostly above the age of 70, were not referred to an

internist/haematologist, neither were further tests such as a bone marrow examination performed. These patients were mostly classified as MGUS, even if they had not undergone a bone marrow examination mandatory to fulfill the criteria of the International Myeloma Working Group. Because we wanted to know whether the risk of transformation and survival would differ between MGUS patients who fulfilled all criteria versus the patients who probably were less well investigated, we decided to subclassify the MGUS category into *provisional* MGUS (no confirmatory bone marrow performed) and *definite* MGUS (all criteria fulfilled). All these patients were registered in our database, thereby well reflecting the healthcare policy in The Netherlands.

M-proteinaemia and survival

Using this large prospective study, we could demonstrate that M-proteinaemia is associated with a shortened survival compared to an age- and gender-matched cohort of the total Dutch population, irrespectively of the associated diagnosis. Not surprisingly, survival was influenced by the presence of a solid tumor in the provisional MGUS group. However, survival was also decreased in the absence of M-protein associated co-morbidity as can be seen in Figures 3 and 4.

Four studies addressed survival in MGUS,^{1;7;8;27} of which three observed a decreased life expectancy^{1;8;27}. Gregersen *et al* demonstrated an excess of death due to infections, heart and lung disease for which no explanation was found²⁷.

The fact that more than half of all patients have died since the start of the study, enabled us to study risk factors for survival in the presence of M-proteinaemia. Remarkably, serum albumin levels at presentation appeared highly predictive for survival. In a multivariate analysis incorporating age, gender, and MGUS versus provisional MGUS, serum albumin remained a strong factor, even when obviously ill patients such as those with solid tumors were omitted from the analysis. It was possible to divide the MGUS patients by serum albumin strata, which resulted in impressive survival differences, ranging from a median survival of less than 2 years for those with serum albumin <30 g/l to more 8.5 years for those with a serum albumin >40 g/l (Figure 4). Whereas albumin in our study was studied in MGUS patients, it is important to realize that Greipp *et al* showed that serum albumin, dichotomized as above or below 35 g/l, appeared also an important prognosticator in patients with established multiple myeloma²⁸.

Malignant transformation

We showed that the cumulative probability of malignant transformation was only 3.9% in 10 years (0.4% per year) when using a competing risk analysis. Given the high incidence of death (50% and 67% at 5 and 10 years respectively), the risk of transformation would have appeared higher (namely 6.6% at 10 years) if this competing-

risk model had not been applied. The reasons for this observed low risk of transformation could be dual: in most older studies, the modern statistics incorporating competing risk strategies had not been applied, resulting in erroneously elevated risk estimates. Secondly, most studies were retrospective in nature and most often single centre based, whereas our cohort was prospective and consisted of unselected patients. In these retrospective studies with at least 100 patients, cumulative risks of malignant transformation varied from 11% to even 51% at 15 years^{6-10;14;16;17;29;30}. In the largest retrospective study, Kyle observed a yearly transformation incidence of 1.2%¹⁷. The three prospective studies published thus far, concerned only low numbers of MGUS-patients (<70), which makes it impossible to draw firm conclusions^{12;15;31}.

Factors associated with malignant transformation

We could confirm that the serum M-protein level, and especially the IgA and IgM isotype were the main factors most consistently associated with the risk of malignant transformation^{8;9;14;16;17;30}. We calculated that for each gram M-protein per liter increase, the risk of malignant transformation rose with 1.16.

Less often, other factors have been reported to be of prognostic importance: κ light chain¹³, Bence Jones proteinuria^{9;14;16;29;30}, raised ESR^{16;29;30}, female sex¹⁴, age above 70 years¹⁴, bone marrow plasma cell percentage^{9;16} and hypogammaglobulinaemia^{9;14;16}. None of these factors were significant predictors in our study, although we observed a slightly higher (HR=1.37 (95% CI 0.68-2.77)) risk on transformation for κ versus λ M-proteinaemia. Recently, an abnormal serum free light chain ratio (κ/λ ratio <0.26 or >1.65) was reported to be associated with malignant transformation in MGUS³², though this novel technique is not routinely available at most laboratories.

In our study, the risk of malignant transformation did not depend on whether a bone marrow examination was performed, because we observed similar results in both MGUS groups. The M-protein isotype and the level of M-protein at initial diagnosis appeared the most important prognostic factors for transformation. Even then, the risk remained low, justifying the general health policy that in asymptomatic patients, and in patients with solid tumors or autoimmune diseases, who present with low levels of M-proteinaemia (<10 g/l), a bone marrow examination can be postponed until a significant increase of the M-proteinaemia is observed. This policy has been supported by others¹⁷, although one has to be aware of the fact that an asymptomatic myeloma could be missed. In a previous study, using our database that also included a large number of patients with multiple myeloma, we devised a Myeloma Risk Score using the M-protein serum level and isotype, which identified patients with newly diagnosed M-proteinaemia in whom the likelihood of multiple myeloma being present was sufficiently high warranting the need for a bone marrow examination²¹.

Obviously, as MGUS is associated with diseases other than haematological diseases such as multiple myeloma and B cell lymphoma², the clinical context in which MGUS is seen determines the prognosis of the patient. Other associated conditions have to be ruled out as this is of prognostic importance.

In conclusion, in our study in which the largest cohort of patients with MGUS published thus far was prospectively followed, the risk of malignant transformation was much lower than reported in retrospective studies. Despite this low risk of transformation, we feel that establishing the diagnosis of MGUS is not anymore of 'undetermined significance', but an important signal as it is synonymous with a shortened survival especially when the serum albumin level is low.

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6 Early response to therapy and survival in multiple myeloma

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Abstract

Whether response upon chemotherapy is a prognosticator in multiple myeloma (MM) is still not known. Therefore, the relation between survival and the rate of monoclonal protein (M-protein) decrement during the first cycles of therapy was prospectively assessed in 262 patients with newly diagnosed MM, treated in a phase III trial (HOVON-16). During melphalan-prednisone therapy (MP: melphalan 0.25 mg/kg & prednisone 1.0 mg/kg orally for 5 days every 4 weeks) M-proteins were collected monthly. Excluded were patients with light chain disease (n=18), IgM-MM (n=1) and no immunotyping (n=1). Out of the 242 patients studied, 75% had IgG M-protein and 25% IgA; MM stages: I: 1%, II: 35% and III: 64%. The median M-protein decrement after the first cycle of MP was 21% for IgG and 27% for IgA, and declined to <5% after four cycles. An obvious survival advantage was seen for patients reaching an M-protein decrement of at least 30% after the first MP-cycle, which became significant when a M-protein decrement of 40% or more was reached. As established prognostic parameters (Salmon & Durie stage, serum creatinin, and haemoglobin) remained prognostically significant as well we conclude that early response to melphalan and prednisone predicts for survival in MM.

Introduction

Prognosis in patients with multiple myeloma (MM) is defined by β -2microglobulin (β 2M)¹, serum neural cell adhesion molecule (NCAM)², myeloma cell mass and organ dysfunction (Salmon & Durie stages)³ and speed of proliferation (plasma cell labelling index)⁴. In most MM patients the serum M-protein level reliably reflects the total myeloma cell mass⁵ and response to anti-myeloma therapy is usually estimated by serial measurements of the serum M-protein. Another favourable prognostic factor for survival could be an early response to instituted therapy similarly to aggressive NHL^{6,7} and ALL⁸. This has been investigated by several groups yielding contradicting results. However, these generally retrospective studies mostly studied non-uniformly treated patients, and patient numbers were small. Therefore, we investigated the relation between M-protein *decrement-rate* in relation to survival in 242 MM patients treated with standard melphalan-prednisone (MP) therapy by prospectively measuring M-protein levels during each cycle.

Patients and methods

In 1991 a prospective multi-centre phase III study (HOVON-16) was initiated by the HOVON (Dutch Haemato-Oncology Cooperative Group HOVON) investigating the effect of low dose interferon α -2b on progression free and overall survival as well as the quality of life after remission-induction treatment with MP in newly diagnosed patients with MM⁹. A total of 262 patients with newly diagnosed MM were entered by 35 participating hospitals in the Netherlands and were staged according to the criteria of Durie & Salmon³. For the present analysis 20 patients were excluded: 18 patients with light chain disease, one with IgM-myeloma and one patient in whom the M-protein could not be immunotyped. Further patient characteristics of the remaining evaluable 242 patients are listed in Table 1. For this analysis the data from the first eight months during MP remission induction therapy were used.

Treatment: According to the HOVON-16 protocol all patients were treated with melphalan 0.25 mg/kg and prednisone 1.0 mg/kg orally during 5 days repeated every 4 weeks. Response to treatment was measured by serial serum M-protein determinations (or Bence Jones proteinuria) and MP-therapy was continued for at least 10 months even if a plateau-phase was reached earlier. If further reduction (regression) of the M-protein was achieved MP was given for more than ten months and continued until a plateau phase was reached. Next, patients were randomised to either maintenance treatment with interferon- α (n=46) or no maintenance (n=44). Due to various reasons (refusal (18), concomitant disease (9), protocol violation (6), WHO performance status >2 (4), allotransplantation (1)) 38 (30%) patients were not randomised. The median survival of the patients in both randomisation arms was identical. Therefore, survival data from all patients could be used for our study⁹.

Table 1. Patient characteristics at baseline.

	All patients (n=242)	IgG (n=182)	IgA (n=60)
Male (%)	133 (55)	96 (53)	37 (62)
Female (%)	109 (45)	86 (47)	23 (38)
Age (yrs), median (range)	70 (34-91)	69 (34-91)	72 (38-84)
Median M-protein (g/l, range) at baseline	45 (7-118)	46 (9-118)	39 (7-118)
Duric & Salmon stage (%)			
IB	2 (1)	1 (1)	1 (2)
IIA	82 (34)	66 (36)	15 (25)
IIB	4 (2)	2 (1)	2 (3)
IIIA	129 (53)	96 (53)	33 (55)
IIIB	26 (10)	17 (9)	9 (15)
Creatinin			
≤177 mmol/l	208 (86)	160 (88)	48 (80)
>177 mmol/l	34 (14)	22 (12)	12 (20)
Haemoglobin			
<6.2 mmol/l	80 (33)	59 (32)	21 (35)
≥6.2 mmol/l	162 (67)	123 (68)	39 (65)

Statistical methods: Repeated measurement models with the serum M-protein value as a dependent variable were used to describe the changes for both IgG and IgA M-protein levels over time. To this end, SAS software (Proc. mixed procedure, version 8.0, SAS institute, Cary, N.C.) was used. Survival curves were generated using the life table methods of Kaplan & Meier and compared using the log-rank test. The single factor and multifactor statistical analyses of potential prognostic factors were carried out using the Cox proportional hazards model. All survival analyses were performed using SPSS version 9 (Statistical Package for the Social Sciences, Chicago, Ill, USA).

Results

Patterns of M-protein decrement: M-protein serum levels were serially determined at the beginning and every four weeks just before the start of each cycle of MP. Per cycle, data were available from a median (range) of 81% (77-90%) of the patients at risk. M-protein decrements (compared to the initial M-protein serum level) were most promi-

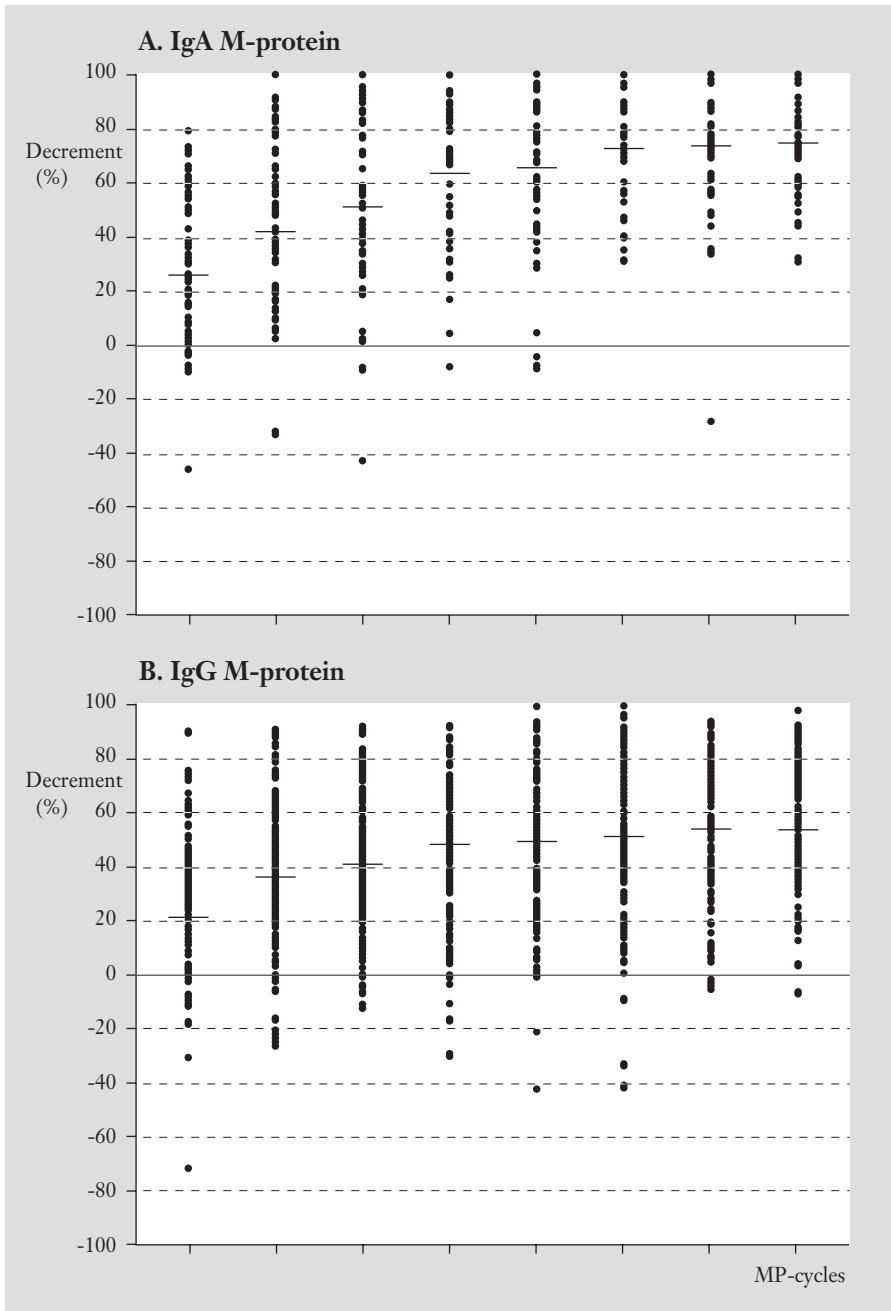


Figure 1. M-protein decrements during MP therapy.

Figure 1A: Median values (and ranges) of IgA M-protein decrements during respectively 1-8 MP cycles: 27% (-333-80); 43% (-121-100); 52% (-40-100); 64% (-7-100); 66% (-7-100); 73% (33-100); 74% (-26-100); 75% (-158-100).

Figure 1B: Median values (and ranges) of IgG M-protein decrements during respectively 1-8 MP cycles: 21% (-71-90); 36% (-26-90); 41% (-12-90); 49% (-29-92); 50% (-42-100); 52% (-40-100); 55% (-40-94); 55% (-5-100).

nent after the first cycle of MP both for IgG (27% decrement) and IgA (21% decrement); an additional decrement of 15% and 16% respectively was seen after the second cycle of MP and declined generally to less than 5% after 4 cycles (Figure 1).

Survival: In this generally elderly patient group (median age 70 years, range 35-91 years) the median survival from the time of start of MP-therapy was 31 months (range 0-88 months) for all patients (n=242); at 60 months 166 patients had died. A 50% decrease of the M-protein level was reached by 14% of patients already after the first MP-cycle. They subsequently survived for a median of 50 months compared to 29 months for those patients who experienced a lesser M-protein decrement (log-rank analysis $p=0.02$; see Figure 2). To study whether early responders would benefit compared to slow/late responders, median survival was compared for various degrees of M-protein reached after each subsequent MP-cycle. Given the data presented in Figure 1 we focussed on the M-protein decrements reached after the first cycle of MP. An obvious survival advantage was seen for patients reaching an M-protein decrement of at least 30% after the first MP cycle, which became significant when an M-protein decrement of 40% or more was reached (Figure 2). The number of patients obtaining decrements more than 60% (n=22), 70% (n=10) and 80% (n=1) was too low for reliable analyses. The same survival analysis for patients with either an IgG or an IgA M-protein yielded an identical pattern although numbers for the IgA subset were rather low (Figure 3). As some investigators have reported that it takes three months for MP to show an effect¹⁰, we repeated the same survival analysis using the 225 patients who survived for more than three months since the start of MP-therapy. These 225 patients experienced a median survival that was not different when compared to the median survival of all 242 patients (33 months vs. 31 months; n.s.). However, a survival advantage was seen again for patients who reached a 50% M-protein decrement after three cycles of MP (median survival in responders 50 months versus 31 months). Established factors like Salmon and Durie stage, serum haemoglobin, serum creatinin, and M-protein isotype were all prognosticators in these patients underlining that we selected a representative group of myeloma patients for this study (Table 2).

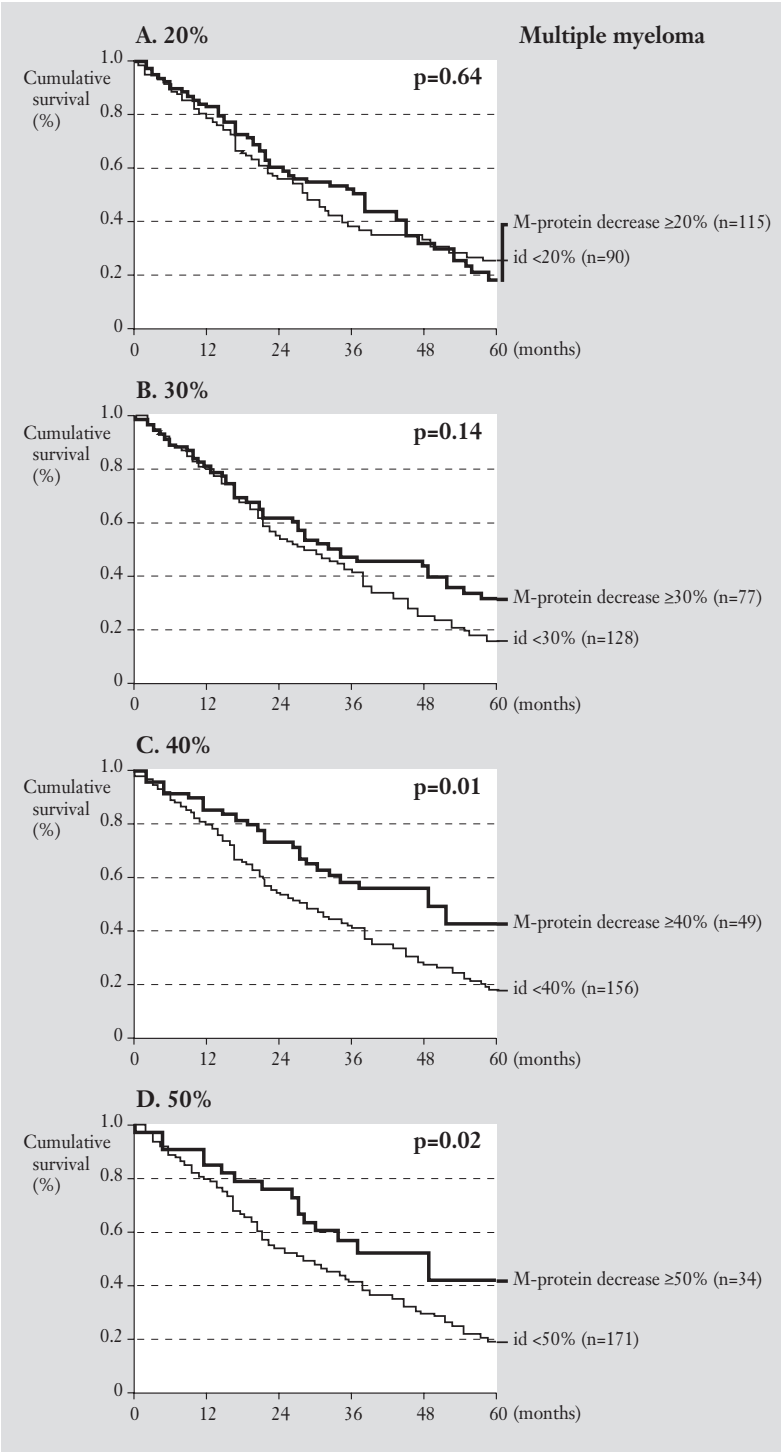


Figure 2. Survival advantage in patients with MM with increasing M-protein decrement after one cycle of MP.

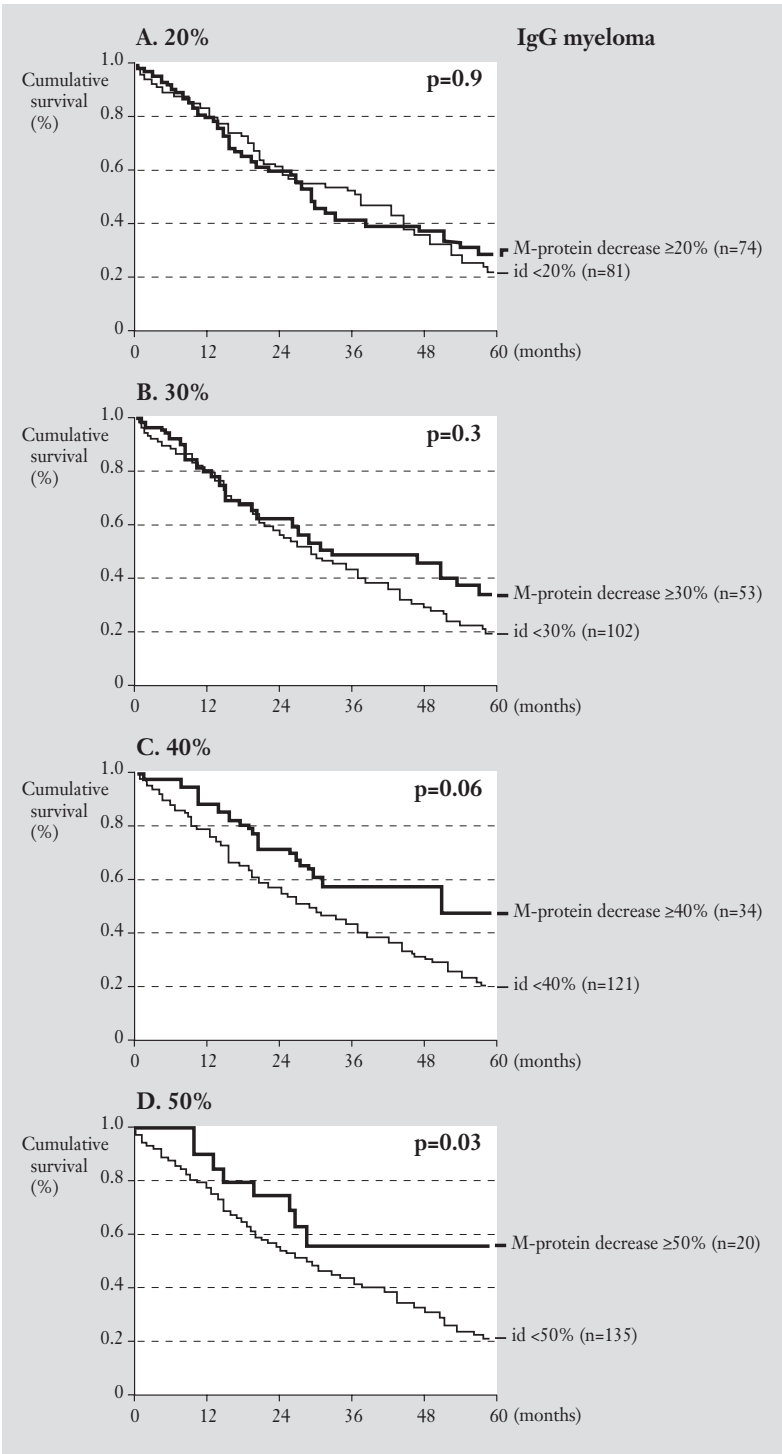
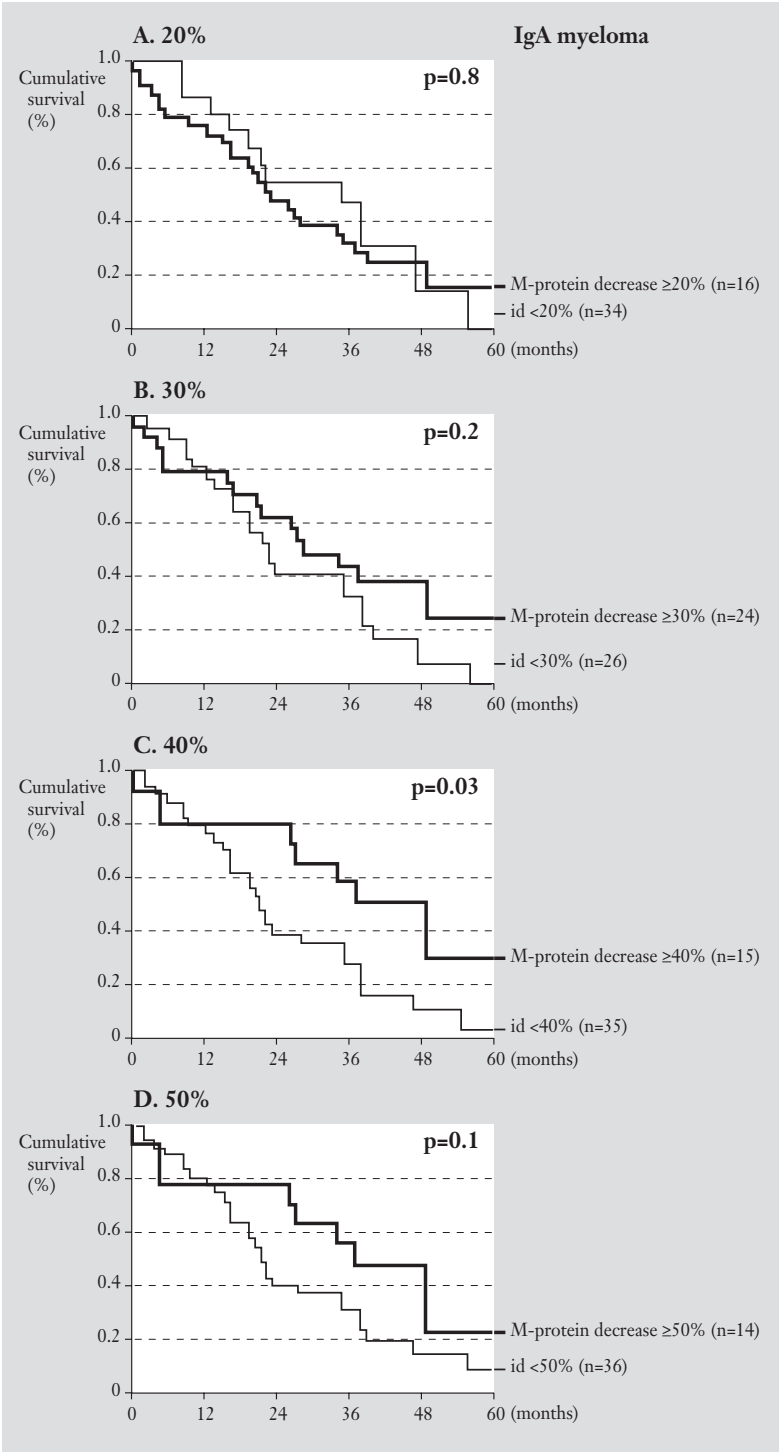


Figure 3. Survival in patients with MM with increasing M-protein decrement after the first cycle of MP,



related to the subtype of M-protein: left IgG myeloma;
right IgA myeloma.

Table 2. Established prognosticators.

Prognostic marker	N (%)	Median survival (months)	Log rank
Haemoglobin			
≤6.2 mmol/l	80 (33)	20	P= 0.0005
>6.2 mmol/l	162 (67)	35	
Creatinin			
≤177 µmol/l	208 (82)	35	P< 0.0001
>177 µmol/l	34 (14)	13	
M-protein isotype			
IgG	182 (75)	33	P=0.01
IgA	60 (25)	22	
Salmon & Durie Stage			
II	85 (35)	38	P=0.02
III	155 (64)	23	

Discussion

In patients with MM we demonstrated a relation between survival and early response to therapy as defined by the rate of M-protein reduction. Therapy consisted of the classical melphalan/prednisone cycles and the patients in this trial belonged to the elderly age group which is representative of the average age of the newly diagnosed MM-patient.

Studies on the subject of early response to therapy and survival in MM have yielded miscellaneous results, probably because of mostly retrospective analyses. Studies that reported a survival disadvantage for early responding patients compared to slowly responding patients¹⁰⁻¹³ differ in many ways from ours. They were all retrospective, response criteria as well as time to response were different and melphalan/prednisone dose and dosing schedules varied. No survival difference between early and slowly responding MM-patients has also been reported^{14;15}. Boccadoro *et al*, however, managed to separate the early responding patients in two subgroups on the basis of a low (<2%) or high (≥ 2%) plasma cell labelling index with the latter group having a worse prognosis¹⁴. In the study by Blade *et al* only response to therapy (but not the rate or degree) was associated with an improved survival¹⁵. Finally, two studies are in agreement with our study^{16;17}. McLaughlin *et al* retrospectively analyzed the data of only 43 out of 525 MM-patients (8%) for whom serum and urine M-protein data were complete during the first six months of therapy¹⁶. In the only prospective study thus far, Powles *et al* observed a survival advantage for patients who obtained at least a 50% M-protein decrement on infusional chemotherapy in a relatively young age

group (median age 52 years; range 30-74)¹⁶, although this response was no longer predictive if induction was followed by high dose chemotherapy¹⁸. These data strongly support ours, where we prospectively studied the response to classical melphalan/prednisone chemotherapy in a group of elderly MM-patients representative of everyday practice.

It has been suggested that MP-therapy takes three months to take an effect¹⁰. We could not demonstrate any evidence for this, rather, our results indicate that response to MP-chemotherapy is already observed after the first cycle (month) of MP-therapy. The median M-protein decrement achieved after the first cycle of MP-therapy was higher in patients with an IgA-M-protein compared to an IgG M-protein (27% respectively 21%). This might be due to the shorter half-life of IgA compared to that of IgG, 5.8 versus 23 days¹⁹, but this did not translate into a survival advantage for patients with IgA-MM.

In conclusion, in this group of homogeneously treated elderly patients with MM, an early response defined by an M-protein decrement of more than 40% is highly predictive for a longer survival.

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7 Interferon- α as maintenance therapy in patients with multiple myeloma

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Abstract

Background: The effect of interferon- α 2b (IFN- α) on progression free and overall survival as well as quality of life was studied in mainly elderly patients with multiple myeloma (MM), who reached a plateau phase after melphalan/prednisone induction.

Patients and methods: In an open phase III trial 262 patients, median age 69 (range 34-91) years, received at least 10 monthly courses of melphalan/prednisone followed by response evaluation. Plateau phase was reached by 128 patients. Next, 90 patients were randomized between IFN- α 2b and no maintenance therapy. Reasons for non-randomization were: refusal (18), concomitant disease (9), protocol violation (6), WHO performance status ≥ 2 (4), allogeneic transplantation (1).

Results: At a median follow-up from diagnosis of 97 (0-140) months for those patients alive, IFN- α 2b therapy was associated with improved progression free survival (median 13.5 months versus 8.4 months from randomization) though this did not translate in a better overall survival (41.0 versus 38.4 months). A third of patients discontinued IFN- α due to toxicity. No differences were observed between patient groups in quality of life.

Conclusions: IFN-maintenance therapy in MM prolongs progression free survival and, provided that the burden of toxicity is not too high, does not adversely affect quality of life.

Introduction

In the last years the treatment-modalities of multiple myeloma (MM) have extended enormously. High dose melphalan supported by autologous stem cell transplantation has become standard therapy for patients up to the age of 70 years. Allogeneic and especially non-myeloablative transplantations are being explored in the same age group. New anti-myeloma agents include immunomodulatory drugs (thalidomide, CC15013) and the proteasome inhibitor bortezomib¹. All these treatment options have to be incorporated into studies to determine their value and place in the treatment of MM. Because stem cell transplantation is often not a viable option for patients older than 70 years with MM, melphalan and prednisone (MP) remains the standard therapy for this age-group¹. Prior to the recent development in therapies mentioned interferon- α (IFN- α) was investigated in the treatment of MM. Two methodologically different meta-analyses on this subject, one by using individual patient data² and the other by using the results of published studies³ reported increases in both progression free survival and overall survival when IFN- α was either used during induction or maintenance therapy for MM^{2;3}. As IFN- α might still be of value in the treatment of MM when transplantation is not an option, we investigated the data of the HOVON-16 phase III trial in which IFN- α 2b maintenance therapy was compared with no maintenance therapy after plateau phase was reached with MP in mainly elderly patients with MM. With a long follow-up of 8 years for those alive, mature data were obtained. The primary endpoints were the Progression Free Survival (PFS) and Overall survival (OS); the secondary endpoint was quality of life (QoL).

Patients and methods

Patients

Between September 1991 and September 1997 31 HOVON centres entered patients with newly diagnosed MM (stage IB, IIA, II B, IIIA and IIIB) and a measurable M-protein in serum or urine. The diagnostic and staging criteria according to Salmon and Durie were used⁴. No previous treatment with either chemotherapy or IFN- α was allowed.

Study design

All patients received induction with MP. Melphalan was given at a dose of 0.25 mg/kg for 5 days and prednisone 1.0 mg/kg for 5 days every four weeks. The minimal time of treatment with MP was 10 months even if a plateau phase was reached earlier. If after 10 months of treatment a further response was seen, treatment with MP had to

be given till a plateau phase was reached. Patients in plateau phase were candidate for randomization between IFN- α 2b or no further maintenance therapy. Patients who showed no response or who showed a relapse of their disease within 10 months after an initial response went off study.

IFN- α 2b was started at a fixed dose of 3 million units three times per week and was given till disease progression was observed. When WHO haematological grade 3 toxicity was encountered dose reduction to 50% was mandatory and in case of grade 4 toxicity IFN had to be stopped.

Definition of disease progression and response to treatment

Response to treatment was defined as more than 25% regression of the M-protein. A plateau phase was reached when the mean M-protein level of the last two months was at least 15% lower than the mean M-protein level of the preceding two months. Disease progression was defined as an increase of more than 25% of the mean M-protein level of the last two months compared to the mean M-protein level of the preceding two months, or progression of osteolytic lesions, hypercalcaemia and or (progression of) transfusion requirement. In case of disease progression or relapse, further therapy was given according to the discretion of the responsible physician.

Quality of life

For the QoL analysis the Rotterdam Symptom Checklist (RSCL) was used⁵. The RSCL measures psychological and physical distress as experienced by cancer patients and scores for the most important three items namely 1) physical and 2) emotional capability and 3) performance of normal daily activities. This questionnaire was completed every three months by patients who were randomized after they reached plateau phase and enabled us to score the quality of life of the three items in time. All the individual scores were calculated on a scale from 0 to 100, such that a lower score implies a better functioning or well-being.

Statistical methods

This report contains all the data as they were available on May 2004. Response data are presented on the basis of 'intention to treat' (n= 262) or on the basis of those patients who actually received any therapy (n=254). All the survival analyses were performed using Kaplan-Meier plots and log-rank tests. Median survival estimates were calculated with 95% confidence intervals. In the calculation of progression free survival, patients who died without progression were censored. Response rates were compared with the Chi square tests. The mean QoL values at different time points were estimated using a linear mixed model and the QoL scores were compared by test-

ing the significance of the interaction between treatment arm and time. In this way account was taken of missing measurements due to the drop out of patients. The sample size was calculated as follows: a mean PFS from randomization of 9 months (SD (3 months) was expected. An increase of 50% of this PFS was considered as clinical useful. To show such a difference with α 0.05 and a power of 0.90 a sample size of 40 patients per treatment arm was considered sufficient.

Results

Patients characteristics

During September 1991 - September 1997 282 patients from 31 HOVON centres were entered in the study. Not eligible were 20 patients mainly due to the absence of a good disease parameter: stage I A (6 patients), non-secretory MM (12), insufficient data (2). The median follow-up of all patients from start therapy was 30 months (range 0-140 months) and 97 months (0-140) for those alive. The median follow-up from randomization was 41 months for all patients (range 0-131 months), and 78 months for those alive (33-131). All data presented here are based upon the 262 evaluable patients (intention to treat) although eight patients actually did not receive any therapy (six died before receiving any therapy, one patient refused therapy after registration and one patient was lost to follow up shortly after the first course of therapy) and ten were not evaluable for response (mainly insufficient data available). Patient characteristics are given in Table 1.

Response rate and randomization

Response to MP was achieved in 192 (73%) patients, and 16 additional patients had some regression of their M-protein (>15% but <25%). The overall response rate was 79%. No response or progression under MP was seen in respectively 23 (9%) and 11 (4%), whereas 56 (21%) showed a response but progressed again during the first 10 months of MP therapy. Out of the 262 patients, 128 (49%) reached a plateau phase, and of those only 90 patients could be randomized. Reasons for non-randomization of these 38 patients were: refusal (18), concomitant disease (9), protocol violation (6), WHO performance status >2 (4), allogeneic transplantation (1). In total 46 patients received IFN- α 2b and 44 patients no maintenance therapy; both groups were similar in terms of sex, age and disease stage.

Table 1. Patients' characteristics.

	All patients (n=262)	IF-alpha (n=46)	No IF-alpha (n=44)
Male/female (%)	54/46	57/43	48/52
Median age (range) years	69 (34-91)	68 (44-84)	67 (46-84)
M-protein isotype (%)			
IgG/IgA/BJ/IgM/unknown	69/23/7/1/1	80/13/5/2/0	73/22/5/0/0
Stage (%)			
I	1	2	2
IIA/B	34	48	34
IIIA/B	65	50	64
WHO performance (%)			
0	25*	46**	36**
I	50	46	57
II	19	4	4
III	4	2	
IV	1		
Unknown	1	2	2
Skeletal involvement (%)			
0 lesions	25	24	27
1-5 lesions	35	41	37
>5 lesions	40	35	36

* WHO performance at entry study

** WHO performance at randomisation

Response duration

The median PFS from randomization for patients randomized to IFN- α 2b was 13.5 months whereas those patients who did not receive any maintenance therapy showed a median PFS of 8.4 months respectively ($p=0.04$) (Figure 1). The PFS at three years was 25% in the IFN- α 2b group and only 4% in the control group. The median PFS from diagnosis for patients randomized was 27.3 months and 20 months respectively ($p=0.01$). At three years after diagnosis, the PFS was 36% in the IFN- α 2b group versus 21% in the control group.

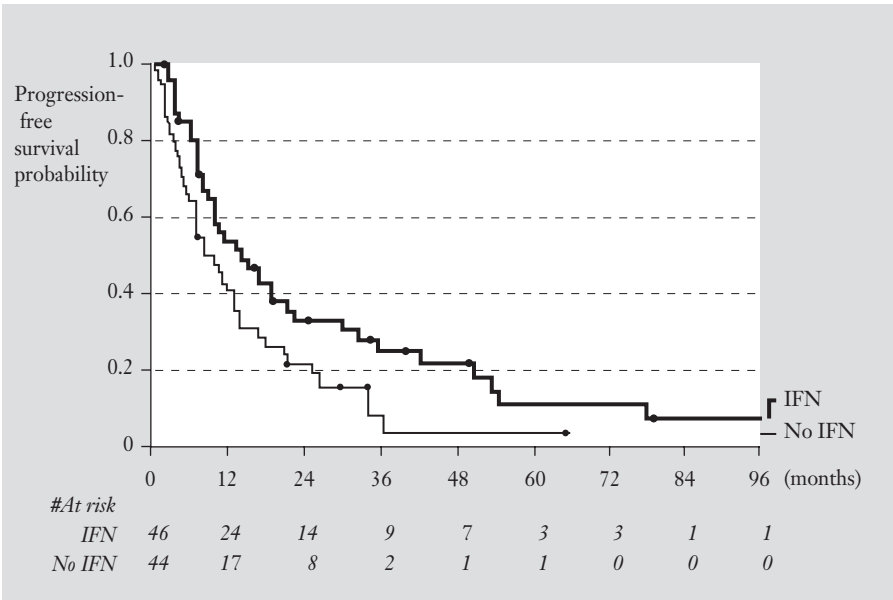


Figure 1. Progression free survival since randomization. The solid curve represents the patients with IFN maintenance, the thin curve those patients without maintenance therapy.

Survival

At the moment of evaluation (August 2004), 21 patients were still alive of whom one was still in the study. In total 241 patients died of whom 169 of MM, 70 due to other causes, one patient due to MP-related toxicity, and from one patient the cause of death was unknown. The median OS of the whole patient group (n=262) was 35.1 months (95% CI 28.9-41.4 months) from the date of diagnosis (Figure 2), and 29.9 months (95% CI 23.4-36.4 months) from start of MP therapy. For patients with IgG, IgA and Bence Jones M-protein the median survival from start of therapy was 33.4, 22.9 and 20.2 months, respectively. Survival from randomization showed an advantage for patients treated with IFN- α during the first years but later survival seemed to be similar to those patients who did not receive IFN- α (Figure 2). Median survival from randomization was equal being 41.0 months and 38.4 months respectively, which demonstrates that the better PFS did not translate in a better overall survival. The median OS from diagnosis of those patients who reached plateau phase but could not be randomized was 42.8 months (95% CI 34.5-51.1 months), versus 53.5 months (95% CI 43.1-64.0) for patients treated with IFN- α and 50.2 months (95% CI 30.9-69.5) for the control group.

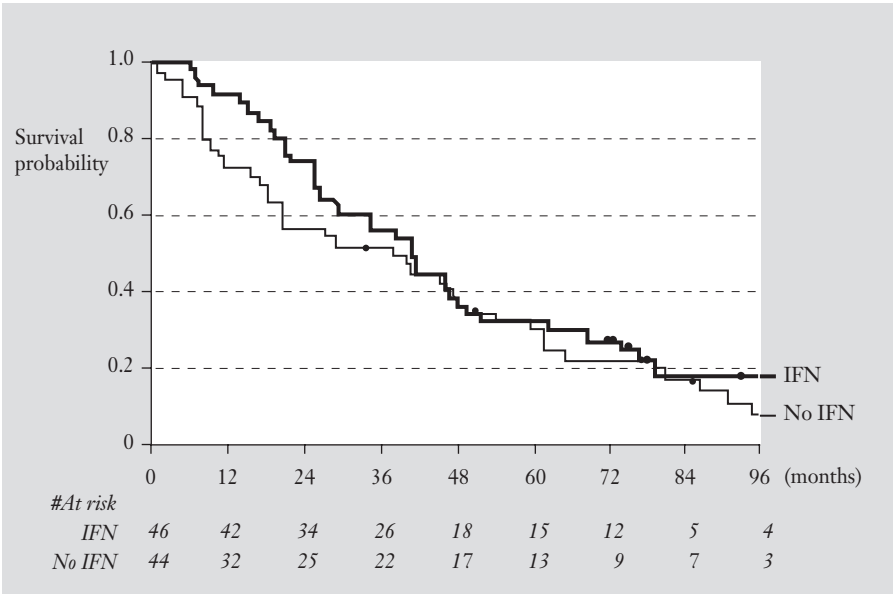


Figure 2. **Survival since randomization.** The solid curve represents the patients with IFN maintenance, the thin curve those patients without maintenance therapy.

Toxicity

Toxicity was evaluated in the 254 patients who actually started induction therapy, of whom 17 (7%) had to end MP-therapy because of toxicity. Myelotoxicity leading to dose modification and/or treatment delay in the first 5 MP-cycles was observed in 155 patients (61%). One patient died (0.3%) due to neutropenic sepsis. Treatment with IFN- α was experienced as toxic, because 17 (37%) patients had to stop because of adverse events. Reasons why patients went off study are shown in Table 2.

Table 2. **Reasons for going off study.**

Reason	Patients
Progression/ no response during MP	68
Toxicity MP	17
Progression after randomization	60
Toxicity IFN- α 2b	17
Protocol violation	22
Refusal to be randomized	18
Death due to comorbidity	23
Others, including 3 ineligible patients	36
Still in study	1

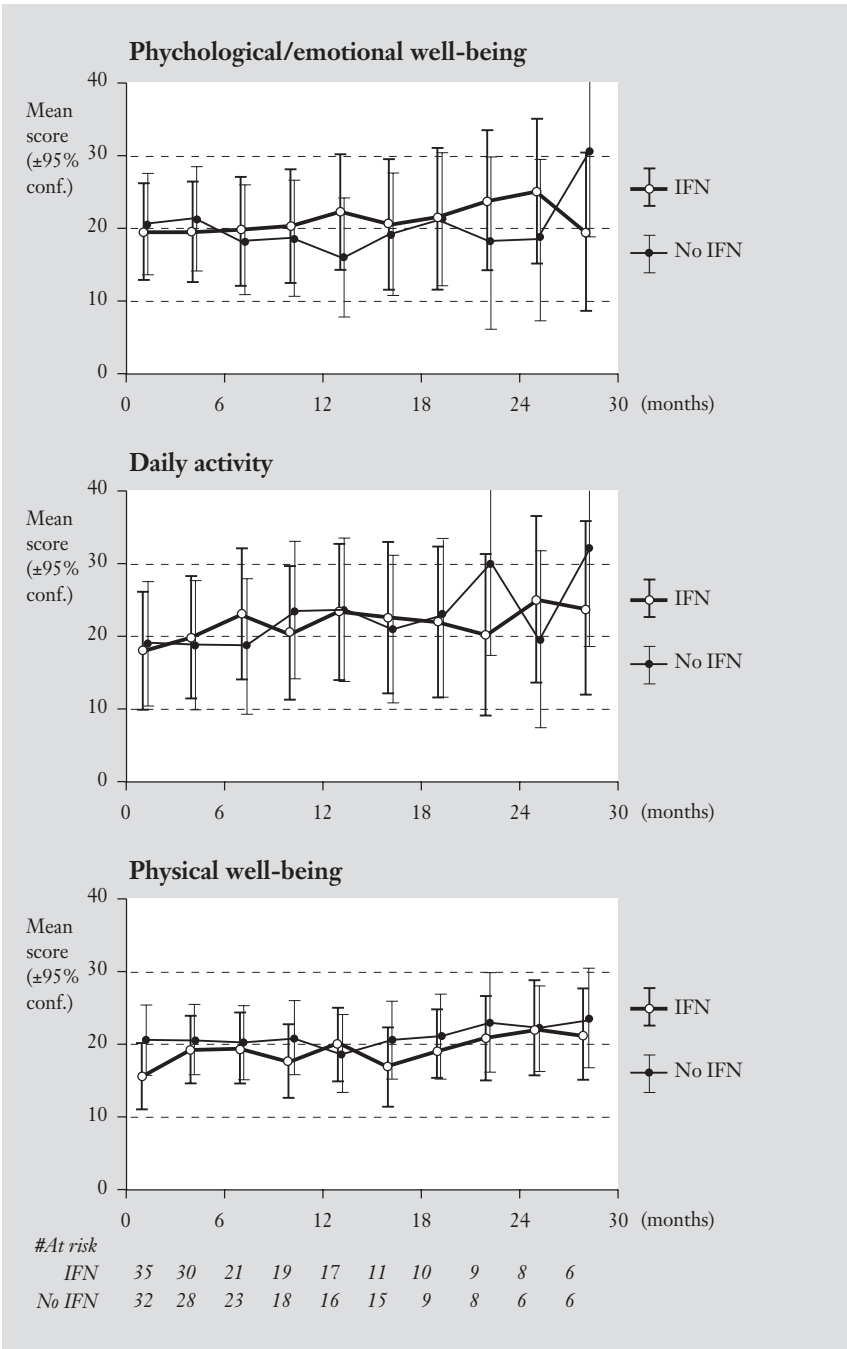


Figure 3. Estimated mean quality of life scores with 95% confidence interval. The solid curve represents the patients with IFN maintenance, the thin curve those patients without maintenance therapy. All scales are transformed into 0-100, in such a way that a lower score implies better functioning or well-being.

Quality of life

The QoL score could be calculated up to 3 years after randomization. Data are visualized in Figure 3. The score for physical well being started at 20 points (out of a range from 0-100, a score of 0 meaning no complaints at all) and although there was a slight increase in time there was no difference between the two groups ($p=0.32$). The score for psychological / emotional well- being started also at a score of 20 (out of a range from 0-100, a score of 0 meaning no complaints at all), showed some fluctuations in time, but there was no difference between the two groups ($p=0.71$). There was hardly any fluctuation in the score for daily activity. Both groups started at a score of 20 (out of a range from 0-100, a score of 0 reflecting complete normal functioning) and ended after 3 years at almost the same score without any significant difference ($p=0.77$).

Discussion

In this study we demonstrated that only one third of typical elderly MM-patients were candidate for IFN- α as maintenance therapy. Of these patients more than one third had to stop this therapy due to the toxicity. This means that only a minority could receive this kind of maintenance treatment for a longer period. It is thus remarkable that in the small groups studied, a significant PFS was seen in favor of those patients who received IFN- α . This did not translate in a better OS most likely because the groups were too small to detect minor differences.

IFN- α maintenance therapy in MM has yielded conflicting results as was recently addressed in a review on therapy in MM⁶. Two methodologically different meta-analyses on the subject of IFN- α treatment in MM have been performed investigating nearly the same data of all randomized trials on this subject⁶. The meta-analysis of the Myeloma Trialists' Collaborative Group used individual patient data and observed an almost similar PFS as we did. In this meta-analysis the OS was better in patients treated with IFN- α maintenance therapy (7 months)². However, the survival from progression was worse in the IFN- α treated patients, which may also be the explanation that the better PFS of our patients did not lead to a better OS. The meta-analysis by Fritz and Ludwig used published data instead of individual patient data and also reported a benefit for the IFN- α treatment arms³. Both meta-analyses, demonstrated a benefit of IFN- α whether it was used during induction or maintenance therapy. The greatest benefit was seen in IFN- α maintenance therapy with an OS of 7² and 3.1³ months versus a prolongation of the OS of 2.4³ and 2² months when IFN was used during induction therapy. For all patients (IFN-induction and IFN maintenance therapy combined) the PFS was prolonged by respectively 6 and 4.6 months² and OS by 4² and 3.7 months³.

Interferon as maintenance therapy is associated with a wide range of possible serious side effects probably especially in elderly patients. In the Nordic Myeloma Study with patients between 55 and 81 years, more than 50% had to reduce the dose of interferon or had to stop this treatment completely⁷. Also in our study a substantial number of patients stopped the IFN- α therapy. Ludwig *et al* have shown in a cohort of 355 US myeloma patients, that 58% of them were willing to undergo this kind of potential toxic therapy if a 6 months gain of PFS or OS could be expected⁸. In the Canadian study on the role of interferon maintenance therapy, patients treated with interferon experienced substantial toxicity, but also in this study patients indicated that the clinical benefits of interferon outweighed these negative effects⁹. On the other hand, in the Nordic QoL study in patients who received chemotherapy in combination with IFN- α , the reduction of the QoL was not considered by patients to justify the positive effect of IFN- α ¹⁰.

We conclude therefore that although improvement of the PFS and sometimes also OS can be observed when patients with MM receive IFN- α as maintenance therapy after standard MP treatment, many will never be eligible for this kind of immunotherapy. However, those patients that tolerate IFN- α maintenance therapy can derive benefit in terms of substantial prolongation of both PFS and OS. Evidently, one must conclude that IFN- α does play a role in the treatment of these patients and should be investigated further alongside with the most recent therapeutic advances in the treatment for multiple myeloma.

List of participating centres

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8 Summary and general discussion



Monoclonal proteinaemia (M-proteinaemia, formerly referred to as paraproteinaemia) is usually associated with the presence of multiple myeloma (MM) or other haematological malignancies such as chronic lymphocytic leukaemia (CLL), lymphoplasmacytic lymphoma or other B-cell non-Hodgkin's lymphomas (B-NHL). However, in patients with newly diagnosed M-proteinaemia these diagnoses are established in only 29-37%^{1,2}.

In the absence of MM or other haematological malignancies, the M-protein is often referred to as MGUS (Monoclonal Gammopathy of Undetermined Significance). This definition of MGUS is loosely used to incorporate other M-protein-associated non-haematological internal disorders like infections, autoimmune diseases and polyneuropathy as well³.

M-proteinaemia is frequently encountered in persons older than 50 years⁴⁻¹⁰. The incidence increases as age advances rising from 1% in the fifth to 3% and 5.7% in the seventh⁴ and eighth⁵ decade, respectively, and has even been reported to rise to 19% in the ninth decade¹¹. As general life expectancy rises in the developed world and the population ages, the chance to encounter a person with M-proteinaemia rises also making the continuing evaluation of the diagnostic approach relevant. Incidence, prevalence as well as follow-up of M-proteinaemia have been investigated in relatively few, mostly retrospective, studies. The M-protein classification systems of Durie and Salmon¹², the British Columbia Cancer Agency¹³ and of the International Myeloma Working Group (IMWG)¹⁴ require a bone marrow examination for the confirmation of MGUS, in every day clinical practice this is often not performed when the M-protein level is low (e.g. less than 20 g/l)¹⁵.

In 1990 the Comprehensive Cancer Centre West (CCCW) decided to initiate a Paraprotein Task Force in order to establish a population-based registration on M-proteinaemia. The most important aims 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. Permission for this registry was obtained from the management and medical ethics committees of all hospitals^{2,16}. From 1991 till 1993 this population-based registry on newly diagnosed M-proteinaemia was carried out within this CCCW region that serves a geographical area around Leiden in the mid-western part of The Netherlands with 1.6 million inhabitants. All patients with newly diagnosed M-proteinaemia or MM were reported by clinical chemists, internists, haematologists, pathologists, and other physicians. M-proteins were either detected by agarose or by cellulose acetate electrophoresis depending on the method used in the various hospitals. For inclusion in the registry, each M-protein had to be confirmed by immunotyping (immunofixation). Information on patient characteristics (age, sex, performance status), laboratory tests results (haemoglobin, leukocyte and platelet counts, creatinin, calcium, albumin, total protein, M-protein type and level in serum and urine), and results of bone marrow examination and skeletal X-rays were obtained. The M-protein-related diagnosis and therapy were

recorded. Furthermore, a distinction was made between patients whose diagnosis of MGUS was confirmed by a bone marrow examination versus those in whom no bone marrow examination had been performed by the treating physician. The first group was termed 'definite MGUS' and the latter 'provisional MGUS'. A serum sample obtained at registration was centrally stored at -80°C . Follow-up was performed annually. In total 1464 patients have been registered during 1991-1993. The development of the CCCW database and the initial findings at registration including the development of a Myeloma Risk Score have been published in a thesis¹⁷. This thesis elaborates on discriminatory serum parameters (interleukin-6 and syndecan), the association between M-proteinaemia and non-haematological malignancies as well as the final follow-up data of the whole cohort. In addition, data from the HOVON-16 myeloma study could be used to receive more insight in the kinetics of M-protein levels. Using data of this study, the prognostic role of the M-protein decrement during anti-myeloma therapy was investigated, as well as the value of interferon-alfa during maintenance therapy.

Discriminatory serum markers in M-proteinaemia

Bone marrow examination is currently the most informative, yet time consuming and patient unfriendly, discriminatory marker in M-proteinaemia¹⁴. Evidently, it would be very helpful to have a serum marker that facilitates the distinction between a true malignant plasma cell proliferation and other more benign conditions. The best-known serum marker to differentiate between these categories is the M-protein concentration itself, but much overlap among these disorders exists¹⁶. Several other tumour markers have been reported in the literature, however with ambiguous results such as $\beta 2$ -microglobulin¹⁸, serum NCAM¹⁹ and C-reactive protein²⁰.

In **Chapter 2** we investigated whether serum interleukin-6 (IL-6) could serve as discriminatory marker in newly diagnosed M-proteinaemia. Serum IL-6 is a pleiotropic cytokine. It induces not only the formation of acute phase proteins²¹ but serves as an autocrine and paracrine growth factor for (malignant) plasma cells as well^{21;22}. Serum IL-6 levels have reported to be high in patients with MM, rising during progression: at diagnosis high serum IL-6 levels are associated with an adverse prognosis²³⁻²⁹. We determined serum IL-6 levels by using an enzyme linked immunoabsorbent assay (ELISA) in 212 well defined patients with M-proteinaemia: MM (60), other haematological diseases (46), solid tumours (35), autoimmune diseases (17) and MGUS (54). Serum IL-6 ranges in all diagnostic groups analyzed overlapped widely, and therefore cannot serve as a discriminatory marker in newly diagnosed M-proteinaemia, even when patients with infection or fever (42) are excluded. In MM high IL-6 levels (≥ 50 pg/ml) were not associated with shorter survival ($p=0.24$), although elevated levels of established prognostic markers like $\beta 2\text{M}$ and NCAM were, signifying that we did

choose a representative patient population. We compared our results with 20 published studies on serum IL-6 in M-proteinaemia and/or MM and concluded that IL-6 data have to be related to the assay used (bio- or immunoassay) and to the status of MM (newly diagnosed, during therapy, progressive disease). Since the publication of this paper no further studies on serum IL-6 in MM have been published. We therefore conclude that serum IL-6 is not specific for M-proteinaemia-related diseases and can not serve as a reliable discriminatory or prognostic marker in M-proteinaemia and MM.

The search for the ‘Discriminatory Serum Grail’ in M-proteinaemia continues in **Chapter 3** with the evaluation of serum syndecan-1 as a serum marker. Soluble syndecan-1 (CD138) is an independent prognostic marker in MM and is expressed on pre-B-cells, lost during differentiation and re-expressed on normal and malignant plasma cells^{30;31}. Serum samples from a subset of 226 patients of the CCCW cohort with newly diagnosed M-proteinaemia were analyzed. M-protein related diagnoses were: MM (n=66), other haematological malignancies (n=37), definite MGUS (n=54), provisional MGUS (no bone marrow examination performed; n=69) and 36 controls. Median serum syndecan-1 levels were higher in MM (226 ng/ml) than all other diagnostic groups (haematological malignancies 79 ng/ml, definite MGUS 128 ng/ml, provisional MGUS 128 ng/ml, controls 5 ng/ml; $p < 0.0001$). However, ranges overlapped widely between all categories. High syndecan-1 levels (>166 ng/ml) did not predict progression of MGUS into MM, but were on the other hand associated with worse survival in MM. The prognostic value in MM was thus reconfirmed. However, with a sensitivity of 68% and specificity of 78%, it is of limited discriminatory value in patients with newly diagnosed M-proteinaemia.

Up to date still no definitive discriminatory marker, other than the M-protein level itself, in M-proteinaemia has been found.

Solid tumours and M-proteinaemia

Previous investigators reported an increased prevalence of solid tumours of up to 43% in patients with M-proteinaemia suggesting a paraneoplastic phenomenon³²⁻⁷⁴. We investigated in **Chapter 4** this possible association by determining the total number and types of non-haematological malignancies in the CCCW cohort. The registration of all patients in the CCCW database was extensive, the presence of non-haematological malignancies could easily be determined. As patient data have been subjected to additional databases, the non-haematological malignancy of patients in this study could readily be confirmed by linkage to the Regional Cancer Registry. Moreover, additional information on that tumour (histology, therapy) had been collected and even tumours not registered in the CCCW database were added, providing a more complete picture. This joint study represents a unique example demon-

strating the importance of national registration in databases. Out of 1464 patients in the CCCW database 167 (11%) were diagnosed with 173 solid tumours without any evidence of MM or other haematological malignancy being present. The types of tumour found are depicted in Figure 1, Chapter 4. Nearly all tumours (n=168; 96%) were (adeno)carcinomas, the other five malignancies consisted of melanomas (4) and leiomyosarcoma (1). We found no relation between tumour type/histology and M-protein isotype. For comparison with our cohort, we selected only studies with more than 100 patients, with description of the related malignancy including concise histopathology and information on the determination of the M-protein, and were left with eight studies^{38;46;53;56;66;67;70;72} and found similar findings on tumour type, histology, M-protein isotype etc.

The question still remains whether the co-existence of a solid tumour and a M-proteinaemia is accidental or relational. Several hypotheses have been suggested to explain a relation between M-proteinaemia and solid tumours:

1. The M-protein is a part of the humoral immune response against solid tumours and could exert an anti-tumour effect. There have been indeed reports of plasma cell infiltrates surrounding solid tumours^{61;63;73}. Long ago, five patients were described with M-proteinaemia and carcinomas surrounded by monoclonal plasma cells that contained the secreted M-protein, although no reactivity between the serum-M-components and tumour antigens could be demonstrated⁶³. A causal relationship would require a decrease of the M-protein during successful therapy of the solid tumour. However, data on follow-up of the M-protein after anti-tumour therapy are scarce and do not show a change in the serum level^{39;55}. Our results on 21 patients confirm this lack of relationship. In 21 patients the M-protein was present at least once during follow-up without an accompanying haematological malignancy. In this largest series reported thus far no clear temporal relation between tumour status (therapy) and the level of the M-protein was seen.
2. MGUS can be seen as a marker of a dysfunctioning immune system with less immunosurveillance and hence a greater tendency to develop malignancies. If that were the case, one would expect an increased incidence of solid tumours during follow-up of patients with MGUS. Kyle *et al* observed the development of a second tumour in 15 of 241 MGUS-patients during a 20-35 year follow-up¹. Pasqualetti *et al* reported 31 out of 263 MGUS-patients (11.8%) who died due to a solid tumour during a median follow-up of 11.5 years⁷⁵. In contrast Gregersen *et al* did not observe an increased risk of solid tumours during follow-up (mean 4.8 years, range 0-15.7 years) in 1229 MGUS patients. In their first years of follow-up the risk for developing a solid tumour was increased, although this risk diminished thereafter⁷⁶, which gives arguments to the third hypothesis:
3. Diagnostic selection: Standardized mortality ratios were elevated in the first year for nearly all tumour types but declined thereafter to normal values. The time-dependent decrease in the risk of solid tumours in Gregersen's study and ours can

be explained by selection of patients through diagnostic investigations. It is likely that during the diagnostic workup for a suspected malignancy, especially in the presence of lytic bone lesions, protein-electrophoresis is readily performed. In two cross-sectional studies the prevalence of M-proteinaemia in patients with non-haematological malignancies was not increased when compared to the prevalence in the general population^{58;77}.

4. Age: during advancing age both the prevalence of solid tumours and of M-proteinaemia increase⁵. This could be an explanation for the assumed relation between M-proteinaemia and solid tumours.

In conclusion, we observed 167 patients (11.4%) with a coexistent solid tumour using a well-defined population-based registry on M-proteinaemia. In a case-control analysis we could not find any difference in clinical characteristics between patients with 'M-proteinaemia only' and patients with 'Solid tumour and M-proteinaemia'. There was no relation between specific solid tumours and M-protein isotype and during follow-up on the M-protein no relation with the tumour could be established either. Although risks for the most prevalent solid tumours found were elevated initially, they decreased in the year, after suggesting a diagnostic selection of patients rather than a causal role. This was one of the largest series of patients with M-proteinaemia in which a true estimate of incidence and prevalence of solid tumours has been described.

Follow-up on the total M-proteinaemia cohort

In **Chapter 5** the final aim of the CCCW registry is addressed; long-term follow-up of the whole cohort patients with M-proteinaemia registered between 1991 and 1993. Patients with MGUS have been reported to run an increased yearly 1-2% risk of eventually developing a malignant M-protein-related haematological malignancy, most often multiple myeloma or lymphoplasmacytic lymphoma^{15;75;78-87}. However, nearly all studies on this subject were either small or retrospective and often single centre based. All 1464 patients of the CCCW cohort were followed prospectively for 6543 person-years (median 3.9, range 0-12.4 years) during which 1007 (69%) died. Median follow-up was 8.9 (0.4-12.0) years for those alive and 2.0 (0-11.4) years for those who died. Total follow-up for all MGUS-patients was 4782 person-years with a median of 4.5 (range 0-12.0) years; median follow-up was 9.0 (0.4-12.0) years for those alive and 2.0 (0-11.4) years for those who died with no differences between patients with definite MGUS and provisional MGUS. The 1464 patients experienced a median survival of 4 (95% CI 3.6- 4.4) years. Patients with definite MGUS survived longer for a median of 6.7 (95% CI 4.7-8.7) years compared to 4.3 (95% CI 3.6-4.9) years in those with provisional MGUS ($p=0.0003$). All 1007 MGUS patients experienced a median survival of 4.6 (95% CI, 4.0-5.3) years which was far below the

survival in an age-matched cohort of the Dutch population. Not surprisingly, survival was also influenced by the M-protein related internal diagnosis especially MGUS associated with a solid tumour (median 0.4 (95% CI 0.2-0.5) years) compared to patients without an internal diagnosis (median 4.9 years (95% CI 4.2-5.6)). In a univariate analysis, M-protein isotype or serum level or light chain isotype did not influence survival in MGUS patients. However, age, gender and especially *serum albumin* were important factors. For instance, patients with a serum albumin of less than 30 g/l (n=208) survived for a median of 1.9 (95% CI 1.2-2.6) years compared to patients with a serum albumin of 40 g/l or more, who survived for a mean of 8.5 (95% CI 8.0-9.0) years. The variables that were univariately related to survival ($p < 0.10$), e.g. M-protein-related diagnosis, IMWG-status (definite MGUS versus provisional MGUS), gender, age and serum albumin, all remained significant in the multivariate analysis. The probability for MGUS-patients of developing multiple lymphoma or B cell lymphoma was 3.1% at five years and seemed to plateau thereafter to 3.9% at ten years. Risk of progression was not significantly different in definite MGUS compared to provisional MGUS (log rank $p = 0.69$). The only significant factors for malignant transformation were the serum M-protein level and M-protein isotype. The estimated hazard ratio for M-protein level was 1.148, indicating that an increment of the serum M-protein of 1 g/l increased the risk of transformation 1.148 times. The IgA isotype had the highest risk on malignant transformation, the IgG isotype the lowest. Using this large prospective study, we could demonstrate that MGUS is associated with a shortened survival compared to an age- and gender-matched cohort of the normal Dutch population, irrespectively of the M-protein associated diagnosis. The diagnosis of MGUS is not anymore of 'undetermined significance', but an important signal as it is synonymous with a shortened survival especially when the serum albumin level is low.

Prognostic significance of M-protein decrement rate in anti myeloma therapy

As nearly all malignant plasma cells secrete an intact or part of an immunoglobulin, serial serum M-protein evaluation plays a major role in the evaluation of anti-myeloma therapy. The M-protein isotype and level are part of the diagnostic criteria of MM and the level of M-protein generally reflects the amount of malignant plasma cells. Response to instituted anti-myeloma therapy is therefore usually measured by the change of the M-protein serum level. A rise in the serum M-protein level corresponding with progression, no change with no response or plateau phase and a decrement obviously with response to therapy. Finally, one of the criteria of so called complete response of MM after therapy requires the total absence of measurable amount of M-protein.

In **Chapter 6** we thus investigated in MM-patients who were treated with melphalan-prednisone therapy whether the *rate of serum M-protein decrement* was of prognostic value.

Studies on the role of an early response to therapy and survival in MM have yielded miscellaneous results, with most studies reporting a survival advantage for slow responders⁸⁸⁻⁹¹, and in contrast some others for quick responders^{92;93}.

Data of the HOVON-16 phase III trial were used in which 262 patients with MM received classical melphalan-prednisone therapy (MP: melphalan 0.25 mg/kg & prednisone 1.0 mg/kg orally for 5 days every 4 weeks). In this protocol, M-protein serum levels were serially determined at the beginning of each cycle of MP. After reaching plateau phase, patients were randomized to interferon- α maintenance therapy or no maintenance therapy at all. Exclusion criteria were the presence of light chain disease (n=18), IgM-MM (n=1) or if no immunotyping was performed (n=1). Out of the 242 patients studied, 75% had IgG M-protein and 25% IgA; MM stages were: I: 1%, II: 35% and III: 64%. The median M-protein decrement after the first cycle of MP was 21% for IgG and 27% for IgA, and declined to <5% after four cycles. An obvious survival advantage was seen for patients reaching an M-protein decrement of at least 30% already after the first MP-cycle, which became significant when an M-protein decrement of 40% or more was reached. As established prognostic parameters (Salmon & Durie stage, serum creatinin, and haemoglobin) remained significant as well, we conclude that early response to melphalan and prednisone predicts for survival in MM. It has been suggested that MP-therapy takes three months to take an effect⁹⁰. We could not demonstrate any evidence for this, rather, our results indicate that response to MP-chemotherapy is already observed after the first cycle (month) of MP-therapy. A similar relationship has been investigated and demonstrated for VAD-like chemotherapy⁹³, but data on dexamethasone-thalidomide are lacking.

Maintenance therapy with interferon- α in multiple myeloma

In **Chapter 7** we further investigated the effect on progression free survival (PFS) and overall survival (OS) of interferon- α (IFN- α) in patients that attained plateau phase after therapy with melphalan and prednisone. This was the primary endpoint of the HOVON-16 study; the secondary endpoint was quality of life (QoL). We demonstrated that only one third of typical elderly MM-patients were candidate for IFN- α as maintenance therapy. Of these patients more than one third had to stop this ther-

apy due to the toxicity. This means that only a minority could receive this kind of maintenance treatment for a longer period. It is thus remarkable that in the small groups studied, a significant longer PFS was seen in favour of those patients who received IFN- α . This did not translate in a better OS, most likely because the groups were too small to detect minor differences. QoL was not affected by IFN- α . Evidently, one must conclude that IFN- α does play a role in the treatment of these patients and should be investigated further alongside with the most recent therapeutic advances in the treatment for multiple myeloma.

The power of the CCCW database

A series of recent studies reflect the success and power of the CCCW Paraprotein database^{2;16;19;94-104}. A population-based database is hard to establish, but indispensable for correct evaluation of the hypotheses made on a population level and corresponding questions asked. Also, linking of this database with that of the Regional Cancer Registration proved not only to be feasible but generated additional information on the prevalence of and subsequent occurrence of solid tumours during follow-up in patients with MGUS.

Investigational approaches to newly diagnosed M-proteinaemia varied widely between physicians and hospitals¹⁷. Initial serum M-protein level and isotype were predictive in the appraisal of the chance of a future malignant transformation, unexpectedly a bone marrow examination at serum M-protein diagnosis was not. These results support the current practice in The Netherlands in which a bone marrow examination is generally not advised at M-protein levels of 10 g/l or less (CBO consensus 2001¹⁰⁵). Patients with provisional MGUS showed an inferior overall survival probably reflecting a lesser general physical condition in which the treating physician withheld further investigation on the cause of M-proteinaemia as it would have no consequences. In a multivariate analysis serum albumin level was highly significant in survival of MGUS-patients. Serum albumin is established marker of disease progression in multiple myeloma^{106;107}, reflects general nutritional status and is inversely correlated with serum IL-6 levels¹⁰⁷. As serum albumin is prognostically significant in both MGUS and MM (ISS scoring system) further elaboration of this association seems most interesting. In conclusion, this database gives an accurate documentation on the diagnostic strategies concerning newly diagnosed M-proteinaemia during 1991-1993 in the CCCW region. In addition, this is the first cohort study with a truly prospective follow-up on newly diagnosed M-proteinaemia and especially MGUS.

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Samenvatting en discussie



Inleiding

Monoclonale proteïnemie, MGUS en multipel myeloom

Alle bloedaanmaak begint bij de stamcel die tot diverse soorten bloedcellen kan uitrijpen, rode of witte bloedcellen of bloedplaatjes. Er bestaan diverse soorten witte bloedcellen die samen voor de afweer zorgen. Eén van deze soorten witte bloedcellen zijn plasmacellen die ontstaan zijn na rijping van voorloper B-cellen tot B-lymfocyten en uiteindelijk tot plasmacellen. Plasmacellen vormen afweerstoffen (immunoglobulinen) welke in het bloed terechtkomen en dienen voor de afweer tegen bacteriën en virussen. Elke plasmacel produceert één specifiek (monoclaal) immunoglobuline. Normaal worden er miljoenen soorten afweerstoffen gevormd maar als één plasmacel gaat woekeren, leidt dat tot ongeremde aanmaak van de bij die plasmacel horende immunoglobuline; het zogenaamde M-proteïne (Monoclaal proteïne). Een immunoglobuline is een complex eiwit dat bestaat uit een combinatie van 2 dezelfde zware en 2 dezelfde lichte eiwit ketens. Er zijn 5 soorten (isotypen) zware ketens: IgG, IgM, IgA, IgD en IgE en twee soorten lichte ketens: κ en λ . Het M-proteïne wordt benoemd naar de zware en lichte ketens waaruit het is opgebouwd maar kan ook bestaan uit een gedeelte van een immunoglobuline, bijvoorbeeld uit alleen zware of alleen lichte ketens. Lichte ketens kunnen ook in de urine gevonden worden en dan spreekt men ook wel van Bence Jones eiwitten.

Multipel myeloom is de benaming van de kwaadaardige woekering van plasmacellen in het beenmerg, in Nederland ook wel bekend als de ziekte van Kahler (genoemd naar de Oostenrijkse prof. dr. O. Kahler, een van de eerste Europese artsen die deze ziekte heeft beschreven). De belangrijkste complicaties van multipel myeloom zijn:

- Verdringing van het gezonde beenmerg door de plasmacel woekering. Gevolgen kunnen zijn het optreden van bloedarmoede door onvoldoende aanmaak van rode bloedcellen, het optreden van een bleedingsneiging door een tekort aan bloedplaatjes en een toegenomen vatbaarheid voor infecties door verminderde aanmaak van witte bloedcellen en de overige normale immunoglobulinen (afweerstoffen).
- Botaantasting door de maligne plasmacellen geeft botpijnen en een verhoogde kans op optreden van 'spontane' botbreuken. Ook kan het kalkgehalte in het bloed verhoogd raken (hypercalciëmie) hetgeen weer aanleiding kan geven tot obstipatie, dorst, uitdroging en een verlaagd bewustzijn.
- Verminderde nierfunctie door neerslag van de Bence Jones eiwitten in de nier, uitdroging bij hypercalciëmie of een combinatie van beide.

Therapie voor het multipel myeloom bestaat voornamelijk uit diverse vormen van chemotherapie, hooggedoseerde chemotherapie met perifere stamcel ondersteuning voor patiënten tot en met 65 jaar en minder intensieve chemotherapie voor diegenen die ouder dan 65 jaar zijn. Verder behoren ook de immunomodulantia zoals thalidomide, lenalidomide en interferon-alfa tot het therapeutisch arsenaal. Tot de onder-

steunende therapie behoren de bisfosfonaten (om botafbraak te remmen) en bestraling op ernstig aangetaste botdelen als direct effect nodig is. Een uitgebreidere bespreking valt echter buiten het bestek van deze inleiding.

Het vinden van een M-proteïne wordt vaak gezien als een bewijs voor de aanwezigheid van een multipel myeloom, een andere kwaadaardige plasmacelaandoening zoals de ziekte van Waldenström of lymfeklier kanker (non-Hodgkin lymfoom). Echter, in zeker tweederde van de personen bij wie een M-proteïne wordt gevonden is er geen sprake van een hematologische maligniteit, maar lijkt het gevonden M-proteïne eerder het gevolg van een afweerrespons die enigszins uit balans is. M-proteïnemie wordt ook gerelateerd aan auto-immuunziekten, infecties, na beenmergtransplantatie en bij andere vormen van niet-hematologische maligniteiten.

Een M-proteïne in het bloed (M-proteïnemie) wordt relatief vaak gevonden op hogere leeftijd, de prevalentie van M-proteïnemie bij personen van 70 jaar en ouder bedraagt 3% en stijgt zelfs naar 19% in de tiende decade. Studies over langdurige (retrospectieve) follow-up bij M-proteïnemie laten zien dat in de loop der jaren het risico op een kwaadaardige ontwikkeling van de M-proteïnemie steeds stijgt. Zo toonde een recent gepubliceerde studie van Kyle uit de Mayo Clinics een jaarlijks risico op maligne ontanding van circa 1% per jaar aan en was het cumulatieve risico op maligne ontanding 30% na 25 jaar follow-up. Daarom moeten patiënten met M-proteïnemie levenslang vervolgd worden. In dit licht is de benaming die Kyle gaf aan een M-proteïne zonder aanwijzingen voor een hematologische maligniteit begrijpelijk: **MGUS** (Monoclonal Gammopathy of Undetermined Significance).

IKW cohort

In 1991 werd door het Integraal Kankercentrum West (IKW) de Paraproteïne Stuurgroep in het leven geroepen om een onderzoek naar M-proteïnemie in de regio van het IKW te initiëren en begeleiden (de oude naam voor M-proteïne was paraproteïne). Dit gebied omvat een geografisch gebied rondom Leiden met destijds in totaal 1.6 miljoen inwoners. Doel was om inzicht te krijgen in de incidentie, prevalentie en bij detectie gestelde M-proteïne-geassocieerde diagnosen. Tevens was men geïnteresseerd in het beleid van de artsen bij nieuw gediagnosticeerde M-proteïnemie, toepassing en ontwerp van klinische beslisseregels en vooral ook in de uitkomst op lange termijn bij alle patiënten met een MGUS.

Alle nieuw gediagnosticeerde patiënten met een M-proteïne werden aangemeld door klinisch chemici, hematologen, pathologen en andere artsen. M-proteïnemie werd vastgesteld met agarose- of met cellulose elektroforese afhankelijk van de per ziekenhuis gebruikte methode. Om geïnccludeerd te worden moest elk M-proteïne bevestigd worden door immunotypering (immunofixatie). Patiënt kenmerken (leeftijd, geslacht, conditie), laboratorium uitslagen (o.a. hemoglobine gehalte, leukocy-

ten en trombocyten aantallen, nierfunctie, calcium, albumine en totaal eiwit spiegels, M-proteïne isotype en gehalte in serum en urine) evenals de uitslagen van beenmergonderzoek en skeletfoto's werden (anoniem) vastgelegd in een grote database. De M-proteïne gerelateerde diagnose, comorbiditeit en therapie werden eveneens geregistreerd. Bij inclusie werd een serummonster ingevroren (-80°C). Jaarlijks werden de patiënten gevolgd. Toestemming voor deze registratie werd verkregen bij alle medisch ethische commissies van alle ziekenhuizen. In totaal werden 1464 patiënten geïncludeerd.

De patiënten werden initieel ingedeeld in vijf groepen op basis van M-proteïne gerelateerde diagnose: 1. Multipel myeloom ($n=261$, 18%), 2. Andere hematologische maligniteit ($n=160$, 11%), 3. MGUS ($n=208$, 14%), 4. Interne ziekte ($n=209$, 14%) en M-proteïne eci (e causa ignota) ($n=626$, 43%).

Serum merkers

Interleukine 6 en syndecan-1

Beenmergonderzoek is momenteel het meest informatieve maar eveneens het meest patiënt onvriendelijke discriminerende onderzoek in M-proteïnemie. Voor de praktijk zou het veel handiger zijn als er een serum merker bestond waarmee er een onderscheid gemaakt zou kunnen worden tussen een kwaadaardige of goedaardige oorzaak van de gevonden M-proteïnemie.

In **hoofdstuk 2** is onderzocht of serum interleukine-6 (IL-6) als een discriminerende factor van waarde is bij patiënten met een nieuw gediagnosticeerde M-proteïnemie. IL-6 is niet alleen betrokken bij de vorming van acute fase eiwitten maar functioneert ook als een autocriene en paracriene groeifactor voor (maligne) plasma cellen. Serum IL-6 spiegels werden bepaald in 212 duidelijk omschreven patiënten met M-proteïnemie: multipel myeloom (60), andere hematologische ziekten (46), solide tumoren (35), auto-immuunziekten (17) en MGUS (54). IL-6 waarden in alle diagnostische groepen overlapt en serum IL-6 fungeert daarmee onvoldoende als een discriminerende marker in M-proteïnemie; zelfs wanneer patiënten met koorts of een infectie ($n=42$) van deze analyse uitgesloten werden. In patiënten met multipel myeloom waren hoge IL-6 spiegels (≥ 50 pg/ml) niet geassocieerd met een slechte prognose maar waren verhoogde waarden van bekende prognostische parameters als $\beta 2\text{M}$ and NCAM dat wel. Dit houdt in dat wij een representatieve patiënten populatie met multipel myeloom geselecteerd hadden.

Voorts bleek na een kritische revisie van alle gepubliceerde studies over serum IL-6 in M-proteïnemie en/of multipel myeloom dat IL-6 data gerelateerd moeten worden aan de gebruikte bepalingsmethode (biologische- of immunologische bepaling) en aan de status van multipel myeloom (nieuw gediagnosticeerd, gedurende therapie,

progressieve ziekte). Concluderend kan gesteld worden dat serum IL-6 noch fungeert als een discriminerende merker bij nieuw gediagnosticeerde M-proteïnemie, noch als een prognostische parameter in multipel myeloom.

In **hoofdstuk 3** wordt een volgende serum parameter, syndecan-1, geëvalueerd als discriminerende serum marker in M-proteïnemie. Syndecan-1 is een in de celmembraan ingebed eiwit en is betrokken bij celdeling, cel-cel en cel-omgeving interacties. Serum syndecan-1 (CD138) is een onafhankelijke prognostische marker in MM, komt tot expressie op pre-B-cellen, verdwijnt bij differentiatie en wordt vervolgens weer tot expressie gebracht op normale en maligne plasmacellen. Syndecan-1 werd in het serum van 226 patiënten van het IKW-cohort met nieuw gediagnosticeerde M-proteïnemie bepaald. M-proteïnemie gerelateerde diagnoses waren: multipel myeloom, (n=66), hematologische maligniteiten (n=37), MGUS (n=54), provisionele MGUS (geen beenmergonderzoek uitgevoerd; n=69) and 36 controle personen. Mediane serum spiegels van syndecan-1 waren hoger in multipel myeloom (226 ng/ml) vergeleken met alle andere diagnostische categorieën (hematologische maligniteiten 79 ng/ml, MGUS 128 ng/ml, provisionele MGUS 128 ng/ml, controle 5 ng/ml; $p < 0.0001$). Echter, tussen alle categorieën was er een sterke overlap van syndecan-1 waarden. Hoge waarden van serum syndecan-1 (>166 ng/ml) voorspelden geen progressie van MGUS naar multipel myeloom, maar waren wel geassocieerd met een slechtere overleving binnen de groep van multipel myeloom. De prognostische waarde in multipel myeloom werd dus bevestigd, maar met een sensitiviteit van 68% en een specificiteit van 78% is serum syndecan-1 van beperkte discriminerende waarde bij patiënten met nieuw gediagnosticeerde M-proteïnemie.

Solide tumoren en M-proteïnemie

In het verleden werd bij patiënten met M-proteïnemie een toegenomen prevalentie van solide (niet hematologische) tumoren tot 43% gerapporteerd hetgeen een paraneoplastisch verschijnsel suggereerde. We onderzochten in **hoofdstuk 4** deze mogelijke relatie door in het IKW M-proteïnemie cohort het totaal aan niet-hematologische maligniteiten te bepalen bij registratie en gedurende follow-up. De sterkte van database onderzoek is dat deze aan andere databases te koppelen zijn mits een gemeenschappelijke parameter gevonden kan worden, in dit geval dezelfde patiënt. Derhalve werd de IKW M-proteïnemie database gekoppeld aan de regionale database van de Kankerregistratie. Hierdoor kwam tevens additionele informatie vrij over de tumor (histologie, therapie) en werden tumoren toegevoegd die niet bekend waren in de M-proteïne database waarmee deze database compleet gemaakt werd. Van de 1464 patiënten in de IKW-database werden er bij 167 (11%) patiënten 173 solide tumoren gevonden zonder dat er een multipel myeloom of een andere maligne hematologische tumor aanwezig was. De typen tumor die gevonden werden staan in figuur 1;

hoofdstuk 4. Bijna alle tumoren (n=168; 96%) waren (adeno)carcinomen, de andere zes tumoren bestonden uit melanomen (4), één sarcoom en één leiomyosarcoom. We konden geen relatie aantonen tussen histologie en M-proteïne isotype. Ter vergelijking met ons cohort selecteerden we uit de literatuur alleen studies met ten minste 100 patiënten waarbij nauwkeurige gegevens over tumor histopathologie en informatie over het M-proteïne gegeven werden. Er bleven 8 studies over waarin overeenkomstige gegevens over tumortype, histologie en M-proteïne isotype gevonden werden. Tevens identificeerden we 21 patiënten met een solide tumor bij wie zich gedurende follow-up geen hematologische maligniteit ontwikkelde en het M-proteïne minstens nog één keer bepaald was. Deze patiënt gegevens werden gebruikt om een relatie tussen beide fenomenen aan te kunnen tonen dan wel uit te sluiten. In het geval van een relatie was de verwachting dat het M-proteïne gehalte zou dalen na therapie en stijgen bij progressie van de tumor. Dit bleek echter niet het geval te zijn, er was geen enkele relatie tussen tumorstatus en M-proteïne gehalte. Als laatste werd het relatieve risico op een solide tumor bepaald bij die patiënten die een M-proteïnemie zonder geassocieerde maligniteit hadden. Dit relatief risico was verhoogd in het eerste jaar voor alle tumortypes maar daalde daarna naar een normaal risico in de daarop volgende jaren. Aangezien de meeste tumoren binnen het jaar na detectie van het M-proteïne gevonden werden suggereert dit meer een diagnostische selectie van patiënten dan een causale rol. Het is makkelijk voor te stellen dat bij de verdenking op de aanwezigheid van een maligniteit, zeker bij lytische bothaarden, er laagdrempelig een eiwit elektroforese ingezet wordt. Er is dus geen causale relatie tussen M-proteïnemie en een solide tumor, evenmin is het risico op een maligniteit in de jaren daarna vergroot.

Follow-up M-proteïnemie cohort

Patiënten met MGUS lopen een jaarlijks risico van 1-2% om een maligne transformatie te krijgen naar een multiple myeloom of B-cel non-Hodgkin lymfoom. Deze gegevens komen uit grote retrospectieve studies. Er is slechts één prospectieve studie naar het risico op maligne transformatie in MGUS maar deze bevat slechts 64 patiënten met een te korte follow-up om duidelijke uitspraken te kunnen doen. In **hoofdstuk 5** worden de follow-up gegevens van het gehele IKW-cohort besproken. In totaal werden 1464 patiënten geregistreerd; 760 (52%) mannen, 704 (48%) vrouwen, met een mediane leeftijd van 73 (17-103) jaar. Multipel myeloom werd gediagnosticeerd in 294 patiënten (20%). Andere hematologische ziekten werden in 163 (11%) patiënten gediagnosticeerd en bestonden uit: myelodysplastisch syndroom (17), lymfoplasmacytoid lymfoom/Waldenström's macroglobulinemie (55), andere B-cel non-Hodgkin lymfomen (54), chronische lymfatische leukemie (21), cryoglobulinemie (2), Hodgkin lymfoom (4), chronische myeloïde leukemie (2), acute myeloïde leuke-

mie (4), plasma cel leukemie (1), hemolytische anemie (4). MGUS werd vastgesteld in 1007 (69%) patiënten; *definitieve* MGUS ondersteund door beenmergonderzoek in 213 (21%) patiënten en *provisieele* MGUS waarbij een bevestigend beenmergonderzoek ontbrak in 794 patiënten. Tevens werd van de laatste groep ook de M-proteïne gerelateerde diagnose geregistreerd: solide tumor, infectie en auto-immuun ziekte. Er waren verder geen klinisch relevante verschillen tussen beide MGUS-groepen. Totale follow-up voor alle 1464 patiënten was 6543 persoonsjaren (mediaan 3.9, range 0-12.4 jaren) gedurende welke 1007 (69%) overleden; van 19 patiënten waren geen follow-up gegevens bekend. Mediane follow-up was 8.9 (0.4-12.0) jaar voor diegenen die in leven waren en 2.0 (0-11.4) jaren voor diegenen die overleden waren. Totale follow-up voor alle MGUS patiënten was 4782 persoons-jaren met een mediaan van 4.5 (range 0-12.0) jaren. Significant voor overleving waren zowel in de univariate als in de multivariate analyse de M-proteïne-gerelateerde diagnose, definitieve MGUS versus provisieele MGUS, geslacht, leeftijd en serum albumine. Aangezien albumine ook voorspellend is voor overleving bij nieuw gediagnosticeerde myeloom patiënten in de recent ontwikkelde ISS score (International Staging System) dient deze associatie zeker verder onderzocht te worden. Alle MGUS-patiënten overleefden korter dan een op leeftijd en geslacht gematcht cohort van de Nederlandse bevolking. Factoren geassocieerd met een verhoogde kans op maligne transformatie naar een multipel myeloom of B-cel NHL waren alleen serum M-proteïne gehalte en isotype. De kans op maligne transformatie was trouwens lager dan eerder gerapporteerd in retrospectieve studies, ca 0,4% per jaar en totaal 3,9% op 10 jaar. Hierbij werd gebruik gemaakt van een zgn 'competing risk' analyse waarbij patiënten die overleden niet meer meetelden in de risicobepaling voor maligne transformatie. Concluderend kan gesteld worden dat de aanwezigheid van een MGUS wel implicaties heeft, zeker bij een verlaagd serum albumine is de overleving significant verminderd.

HOVON-16

Snelheid van monoclonaal proteïne afname tijdens therapie en kwaliteit van leven tijdens interferon-alfa

Seriële bepaling van het serum M-proteïne tijdens chemotherapeutische behandeling voor multipel myeloom een belangrijke rol bij de evaluatie hiervan. Het M-proteïne isotype en serumgehalte vormen deel van de diagnostische criteria van multipel myeloom en het gehalte aan serum M-proteïne is een directe afspiegeling van de plasmacel tumormassa. Het effect van anti-myeloom therapie wordt daarom gemeten aan de hand van de verandering van het serum M-proteïne concentratie. Een stijging van het M-proteïne gehalte staat gelijk aan ziekte progressie, een daling aan afname van ziekte activiteit en geen verandering van de M-proteïne concentratie aan

stabiele ziekte activiteit. Zo is één van de criteria voor complete respons het niet meer aantoonbaar zijn van het M-proteïne en spreekt men bij het stabiel blijven van het M-proteïne na therapie over de plateaufase.

Studies over de *snelheid* van respons op therapie en overleving in MM hebben in het verleden tegenstrijdige resultaten opgeleverd. De meeste studies lieten een overlevingsvoordeel voor langzame responders zien, sommige voor snelle responders op therapie. In **hoofdstuk 6** hebben we onderzocht of de snelheid waarmee het M-proteïne tijdens therapie daalde (= snelheid van respons op therapie) van prognostisch belang was. Data van de HOVON-16 fase III trial werden gebruikt waarin 262 patiënten met multipel myeloom het klassieke orale melfalan-prednison schema (melfalan 0.25 mg/kg & prednison 1.0 mg/kg oraal 5 dagen per 4 weken) kregen. Serum M-proteïne spiegels werden bij aanvang van therapie en telkens voor de start van elke nieuwe melfalan-prednison cyclus per 4 weken bepaald. Nadat plateaufase bereikt was werden de patiënten gerandomiseerd tussen wel of geen onderhoudstherapie met interferon-alfa. Uitgesloten werden patiënten met lichte keten myeloom (n=18), IgM-myeloom (n=1) en ontbrekende immunotypering. Van de 242 patiënten had 75% een IgG M-proteïne en 25% IgA; myeloom stadia: I: 1%, II: 35% and III: 64%.

De mediane M-proteïne daling na de eerste cyclus van melfalan-prednison was 21% voor IgG en 27% voor IgA, en nam af naar < 5% na 4 melfalan-prednison cycli. Een duidelijk overlevingsvoordeel werd gezien voor patiënten die een M-proteïne daling van minstens 30% meteen na de eerste melfalan-prednison kuur bereikt hadden; dit verschil werd significant wanneer ten minste een daling van 40% werd bereikt.

Aangezien bekende prognostische variabelen (Salmon & Durie stadium, serum creatinine, en hemoglobine) hun prognostische waarde behielden concluderen we dat snelle respons op melfalan-prednison therapie van prognostisch belang is in multipel myeloom. Eerder is gesuggereerd dat melfalan-prednison therapie drie maanden nodig heeft om effect te geven, onze resultaten laten zien dat respons op melfalan-prednison therapie al gezien wordt na één kuur. De mediane daling van het M-proteïne na één kuur melfalan-prednison was hoger in patiënten met een IgA isotype vergeleken met het IgG isotype (27% respectievelijk 21%). Dit kan komen door een kortere halfwaardetijd van IgA versus IgG (5.8 versus 23 dagen) maar dit vertaalde zich overigens niet in een overlevingsvoordeel voor patiënten met IgA-myeloom.

In **hoofdstuk 7** is wederom gebruik gemaakt van hetzelfde patiëntenbestand van de HOVON-16 studie. Na inductie behandeling met melfalan-prednisonkuren werd er bij die patiënten die een plateaufase behaalden gerandomiseerd tussen wel of geen onderhoudsbehandeling met interferon-alfa. De primaire eindpunten van de HOVON-16 studie waren progressievrije overleving en algehele overleving, het secundaire eindpunt was kwaliteit van leven. Slechts één derde van alle (typisch de oudere) patiënten in deze studie kwam in aanmerking voor onderhoudstherapie met interferon-alfa. Van deze patiënten moest vervolgens weer meer dan éénderde deze onderhoudstherapie stoppen vanwege toxiciteit. Dit betekent dat maar een minder-

heid van alle multipel myeloom patiënten deze onderhoudstherapie gedurende langere tijd aankon. Daarmee is het wel opmerkelijk dat hoewel de bestudeerde groepen klein waren, een significant langere progressievrije overleving werd gezien in die patiënten die interferon-alfa kregen. Dit vertaalde zich niet in een langere algehele overleving, zeer waarschijnlijk omdat de groepen te klein waren om significante verschillen te detecteren. De kwaliteit van leven werd niet beïnvloed door interferon-alfa. Concluderend moet gesteld worden dat interferon-alfa nog steeds een rol speelt in de behandeling van multipel myeloom en verder onderzocht moet worden samen met de nieuwste ontwikkeling in de therapie van multipel myeloom.

De kracht van de IKW database

Alle artikelen die uit het Paraproteïne Cohort zijn ontstaan zijn een teken van de kracht en het succes van een grote populatie database¹⁻¹⁴. Een populatie-database is niet eenvoudig op te zetten, maar onmisbaar als men hypothesen op bevolkingsniveau wil onderzoeken. Tevens hebben wij laten zien dat ook koppelen met een andere grote database, namelijk die van de regionale Kankerregistratie niet alleen mogelijk was maar ook meerwaarde bood. De benadering van de patiënt met nieuw gediagnosticeerde M-proteïnemie verschilde per arts en per ziekenhuis. Uiteindelijk bleek uit onze follow-up data dat het verrichten van een beenmergonderzoek om een MGUS goed te inventariseren niet relevant te zijn voor de inschatting op de kans op maligne ontaarding. Dit in tegenstelling tot het serum gehalte van het M-proteïne en isotype. Deze waren wel van belang, de kans op maligne ontaarding steeg met het stijgen van het serum M-proteïne gehalte, en tevens was de kans op maligne ontaarding het hoogst bij het IgA en het laagst bij het IgG isotype. Deze resultaten ondersteunen het in Nederland gevoerde beleid zoals verwoord in de CBO richtlijn 'Monoklonale Gammopathie' uit 2001 waarin een beenmerg onderzoek in het algemeen niet geadviseerd wordt bij een serum M-proteïne gehalte van minder dan 10 g/l of minder.

Overleving in patiënten met MGUS bleek afhankelijk van geslacht, leeftijd, MGUS-status maar ook opvallend van serum albumine. Serum albumine is een bekende marker van ziekte progressie in multipel myeloom, maar geeft ook de voedingsstatus weer en is onevenredig gecorreleerd met serum IL-6 spiegels. Aangezien serum albumine ook prognostisch van belang is in het nieuwe 'International Staging System' voor multipel myeloom valt er veel voor te zeggen deze relatie uit te diepen.

In conclusie, deze database heeft een gedetailleerd beeld van diagnostische strategieën in nieuw gediagnosticeerde M-proteïnemie in het IKW gebied gegeven maar is tevens ook de grootste prospectieve studie in M-proteïnemie tot nu toe.

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Appendices



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List of abbreviations

β2M	Beta-2-microglobulin
CCCW	Comprehensive Cancer Centre West (Integraal Kanker Centrum West; IKW)
CRP	C-reactive protein
HOVON	Hematologie Oncologie Volwassenen Nederland
IFN-alpha	Interferon-alpha
IL-6	Interleukin-6
IMWG	International Myeloma Working Group
MM	Multiple Myeloma, also known as 'Kahler's disease' or 'Rustizky's disease'
M-protein(aemia)	Monoclonal Protein(aemia)
MGUS	Monoclonal Gammopathy of Unknown Significance
MP	Melphalan-Prednisone chemotherapy
NCAM	Neural Cell Adhesion Molecule
NHL	Non Hodgkin's lymphoma
OS	Overall survival
Paraprotein(aemia)	Former name for M-protein(aemia)
PFS	Progression-free survival
QoL	Quality of life
SMR	Standardized Mortality Ratio
WHO	World Health Organisation

