Prognosis in monoclonal proteinaemia

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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 well[3].

M-proteinaemia is frequently encountered in persons older than 50 years[4-10]. The incidence increases as age advances rising from 1% in the fifth to 3% and 5.7% in the seventh[4] and eighth[5] decade, respectively, and has even been reported to rise to 19% in the ninth decade[11]. 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 Salmon[12], the British Columbia Cancer Agency[13] 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 ºC. 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 thesis[17]. 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-proteinaemia[14]. 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 exists[16]. Several other tumour markers have been reported in the literature, however with ambiguous results such as β2-microglobulin[18], serum NCAM[19] and C-reactive protein[20].

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 proteins[21] 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 prognosis[23-29]. 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 β2M 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/nl) 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 phenomenon[32-74]. 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 demonstrated[63]. 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 as 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-up[1]. 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 years[75]. 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 thereafter[76], 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 there after 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 increase[5]. 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 responders[88-91], 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 effect[90]. 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 chemotherapy[93], 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 hospitals[17]. 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 2001[105]). 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 levels[107]. 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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