Serum Neural Cell Adhesion Molecule Differentiates Multiple Myeloma From Paraproteinemias Due to Other Causes


Serum neural cell adhesion molecule (NCAM) has been described as a prognostic marker in multiple myeloma (MM). Both C-reactive protein (CRP) and β2-microglobulin (β2M) are established prognostic markers in MM. We tested the diagnostic value of these markers in 212 serum samples of patients with paraproteinaemia registered prospectively in a population-based registry. Sixty patients had MM and 152 had other monoclonal gammopathies (hematologic diseases [48], paraneoplastic disease [35], autoimmune disease [15], and monoclonal gammopathy of undetermined significance [56]). CRP and β2M had wide and overlapping ranges in all diagnostic categories. However, serum neural cell adhesion molecule (NCAM) was low (<20 U/mL) in all but 4 of 152 nonmyeloma cases and high (≥20 U/mL) in 31 (52%) of the 60 MM cases. Two patients with non-Hodgkin's lymphoma, leukemia cells, trophoblast cells, and small cell lung carcinoma. It was recently discovered that serum NCAM could be used as a prognostic marker in MM. Higher serum NCAM values were observed in patients with more advanced disease and shorter survival times. However, hardly any information on NCAM levels in paraproteinemias due to other causes exists.

The Comprehensive Cancer Center West (CCCW) initiated a population-based registry of all patients with a paraproteinaemia newly diagnosed between 1991 and 1994 in the midwestern part of The Netherlands. Because this project also comprised an archive of serum samples at first diagnosis of patients included in the registry, we decided to test the diagnostic value of serum NCAM on a number of serum samples of patients with MM and well-defined monoclonal gammopathies of other causes. In addition, we analyzed two established prognostic markers, β2-microglobulin (β2M) and C-reactive protein (CRP), which together form a prognostic staging system, and extracted data on other laboratory tests from the registry, to determine whether a combination of blood tests, without the help of bone marrow or x-ray examinations, serum NCAM, in combination with paraprotein type and concentration, can differentiate between MM and nonmyeloma patients.

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PATIENTS, MATERIALS, AND METHODS

Patients. Serum samples at first diagnosis were obtained in the course of a population-based registry performed in the CCCW region (1.7 million inhabitants), which included all patients newly diagnosed in 1991, 1992, and 1993 with a paraproteinaemia and/or MM. The study involved a registration of patient characteristics (age, sex, and performance status), results of x-ray and bone-marrow examinations, laboratory data (hemoglobin, leukocyte and thrombocyte counts, serum creatinine, lactate dehydrogenase [LDH], alkaline phosphatase, calcium, albumin, total protein, and type and concentration of the paraprotein in serum and urine), final classification in relation to the paraprotein, the presence of other diseases, therapy, a yearly follow-up, and the storage of 1 mL of serum at first diagnosis. The serum samples were stored at −80°C in one of the participating hospitals.

Paraproteins were detected by either agarose or celluloseacetate electrophoresis, depending on the hospital in which the patient was first seen (14 hospitals in the CCCW region, of which sera from 7 hospitals [9 locations] were used in this study). To be included in
from the database, each paraprotein had to be confirmed by immunotyping (usually immunofixation).

From 867 patients with serum available, 212 were evaluable for the present report. The following diagnoses had been made: MM, 60; hematologic diseases, 48; paraneoplastic, 35; autoimmune disease, 15; and MGUS, 56. Of 60 MM patients, 9 had indolent MM, 13 had stage I MM, 4 had stage II MM, and 34 had stage III MM. The hematologic diseases were subdivided into lymphoproliferative (n = 37; 15 immunocytoma, 18 other B-cell non-Hodgkin's lymphoma [NHL], and 4 B-cell chronic lymphatic leukemia [CLL]), myeloproliferative (n = 7; 6 myelodysplastic syndrome and 1 pro-myelocytic leukemia), and other (n = 4; 2 Hodgkin's disease, 1 cryoglobulinemia, and 1 hematocytic anemia without autoantibodies). The other 655 serum samples were not eligible for the following reasons: no definite diagnosis was made by the local physician or the diagnosis could not be confirmed; no clinical data were available as yet; or the serum had not been taken at first diagnosis or for analysis purposes. All samples were collected between 1983 and 1990.

The diagnosis MM was made when at least two of the following criteria were present: (1) paraprotein in serum or urine, (2) lytic bone lesion(s), or (3) more than 10% plasma cells in bone marrow cytology. A paraprotein in serum (but not in urine), with more than 10% plasma cells, in the absence of symptoms, anemia, leukocytopenia, thrombocytopenia, hypercalcemia, renal failure due to myeloma, or lytic bone lesions was termed indolent MM. Any paraprotein without indications of MM or other hematologic or malignant diseases was considered normal serum values (0–20 U/mL). The diagnosis of normal serum NCAM (0–20 U/mL) was based on (1) absence of symptoms, (2) no definite diagnosis was made by the local physician, or (3) the sample was not taken at first diagnosis or for analysis purposes. Normal serum NCAM values (<20 U/mL) were present in 31 of 60 myeloma cases (Table 1).

<table>
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<tr>
<th>Table 1. Number of Patients With Normal and High Serum NCAM Levels Related to Diagnosis</th>
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<td>Nonmyeloma</td>
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<tr>
<td>Serum NCAM &lt;20 U/mL</td>
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<td>Serum NCAM ≥20 U/mL</td>
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For serum NCAM determinations, the eNCAM RIA-gnost immunoassay (Behringwerke AG, Marburg, Germany) was used. The assay was performed using a solid-phase two-site chemiluminescent enzyme immunoassay (Immulite 82M; Diagnostics Products Corp, Los Angeles, CA). A human CRP mammalian antibody was used for determination of CRP by rate nephelometry (Array Protein System, Beckman Instruments Inc, Galway, Ireland). The assay coefficients of variation for serum NCAM were 2% to 12% (range, 8 to 113 U/mL); for CRP, 7% to 10% (range, 1.1 to 8.1 mg/L); and for P2M, <8% (range, 10 to 90 mg/L). Normal values were less than 20 U/mL for serum NCAM, 1 to 3 mg/L for CRP, and <10 mg/L for P2M. The serum NCAM, CRP, and P2M determinations were performed on blinded samples. Other laboratory results were extracted from the registry.

**Laboratory methods.** Serum samples which had been stored at −80°C were used for serum NCAM, CRP, and P2M determinations. Thawed samples were shipped on ice to Marburg University (Marburg, Germany), where serum NCAM determinations were performed. CRP and P2M determinations were performed immediately after thawing in the Westeinde Hospital (The Hague, The Netherlands).

For serum NCAM determinations, the eNCAM RIA-gnost immunoassay (Behringwerke AG, Marburg, Germany) was used. β2M was determined using a solid-phase two-site chemiluminescent enzyme immunoassay (Immulite 82M; Diagnostics Products Corp, Los Angeles, CA), and a human CRP mammalian antibody was used for determination of CRP by rate nephelometry (Array Protein System, Beckman Instruments Inc, Galway, Ireland). The assay coefficients of variation for serum NCAM were 2% to 8% (range, 8 to 113 U/mL); for CRP, 7% to 10% (range, 1.1 to 8.1 mg/L); and for CRP, <8% (range, 10 to 90 mg/L). Normal values were less than 20 U/mL for serum NCAM, 1 to 3 mg/L for β2M, and less than 10 mg/L for CRP. The serum NCAM, CRP, and β2M determinations were performed on blinded samples. Other laboratory results were extracted from the registry.

**Statistics.** Statistical methods to compare MM versus nonmyeloma included Mann-Whitney's test, Pearson's χ² test, and Mantel-Haenszel test for linear association. Subgroups of MM and nonmyeloma were compared using analysis of variance. Discriminant analysis was used to investigate stepwise whether a combination of laboratory tests was superior to differentiate between MM and nonmyeloma as compared with one single test. Analyses were performed using SPSS/PC+; data were entered in a database using SPSS Data Entry II (both SPSS Inc, Chicago, IL).

**RESULTS**

Normal serum NCAM values (<20 U/mL) were present in 148 of 152 nonmyeloma cases; high values (≥20 U/mL) occurred in 31 of 60 myeloma cases (Table 1). These figures yielded a sensitivity of 52% and specificity of 97%, with a positive predictive value (PV) of 31 of 35 (89%) and a negative PV of 148 of 177 (84%). The four nonmyeloma patients with high NCAM values had all MM (n = 30 U/mL); 2 had NHL, 1 had CLL, and 1 had autoimmune disease. Serum NCAM levels gradually increased, going from MGUS to stage III MM (analysis of variance, P < .001; Fig 1). The results of CRP, β2M, and serum NCAM determinations for all different diagnostic categories are listed in Table 2 and Fig 1. CRP and β2M levels varied widely in all diagnostic categories.

For all patients, the risk score by Bataille using CRP and β2M was calculated (low risk, both <6 mg/L; intermediate risk, either CRP or β2M ≥6 mg/L; high risk, both ≥6 mg/L). In the MM group, only 16 of 60 patients (27%) fell into the high-risk group, with 20 falling into the intermediate-risk group and 24 into the low-risk group. Most of the nonmyeloma patients fell into the intermediate-risk group (88 of 152 patients [58%]), with 41 falling into the low-risk group and 23 into the high-risk group. An increase in risk score did not correlate with a linear increase in probability of having MM (Mantel-Haenszel test for linear association, P = not significant [NS]). Serum NCAM levels were significantly higher for high risk patients as compared with low and intermediate risks (analysis of variance, P < .001).

The results of several laboratory tests for myeloma and nonmyeloma patients are given in Table 3. LDH was abnormal in 37 of 151 nonmyeloma patients and 7 of 60 MM patients (P = .038; not performed in 1 patient), whereas alkaline phosphatase was abnormal in 38 of 128 nonmyeloma and 11 of 55 MM patients (P = NS; not performed in 29 patients). Of nonmyeloma cases, 41 were IgM, 104 were IgG, and 7 were IgA, whereas of MM patients, 42 had IgG, 13 had IgA, 1 had IgD, and 4 had light chain disease.

A discriminant analysis was performed to determine the combination of laboratory tests contributing mostly to the diagnosis MM or nonmyeloma. The following variables were entered: serum NCAM, CRP, β2M, paraprotein type and concentration, hemoglobin, leukocyte and thrombocyte counts, and creatinine. Paraprotein type was used to test two dummy variables that discriminated IgM, IgG/IgA, and IgD/light chain only. Ten cases with missing values could not be included in the analysis, leaving 202 patients. The concentration of paraprotein was the most important factor to predict diagnosis, followed by paraprotein type and serum NCAM. Extending the analysis to include corrected serum calcium, LDH, and alkaline phosphatase (the latter 2 being entered as dichotomous variables coding normal and abnormal values) did not alter the results. Because of missing
Fig 1. Values of serum NCAM, CRP, and β2M for the different diagnoses made in 212 patients with monoclonal gammopathies. The dotted lines depict cut-off values for the normal range; outliers are represented by an arrow and accompanied by the actual value. ly-pro, lymphoproliferative; my-pro, myeloproliferative; other, other hematologic diseases; p-neopl., paraneoplastic phenomenon; auto-imm., autoimmune disorders; IMM, indolent MM.

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cancer. Values were high in this disease and low in other lung malignancies, indicating that serum NCAM may be used specifically for diagnostic purposes. In our material, the only patient with small cell lung carcinoma had a low serum NCAM concentration of 4.2 U/mL.

The prognostic value of serum NCAM reported by Kaiser et al. was tested using two prognostic staging systems: the system developed by Durie and Salmon and the one by Bataille using CRP and β2M. Serum NCAM levels increased significantly with increasing MM stage in both systems, thus confirming its prognostic value in MM. In our material, the risk score by Bataille could not be used to separate MM from nonmyeloma cases.

In a discriminant analysis, paraprotein type and concentration, together with serum NCAM, proved to be the most important combination of factors predicting the outcome MM or nonmyeloma. This was true even when IgM paraproteins (indicating a nonmyeloma condition) were excluded from the analysis. The low correlation coefficient between serum NCAM and paraprotein concentration indicates that serum NCAM has diagnostic value in myeloma additional to paraprotein concentration.

It has been observed that several initially promising immunological markers in monoclonal gammopathies (interleukin-6 [IL-6], CRP, and β2M) subsequently were negatively influenced by the presence of other malignancies, decreasing the discriminative difference found between MGUS and MM. A recent study showed that serum IL-6 was not diagnostic. For this analysis, we carefully selected a very large series of patients with nonmyeloma-associated paraproteinemias and found low serum NCAM levels in all nonmyeloma categories, including hematologic and other malignancies.

Therefore, we conclude that serum NCAM is of great value as a diagnostic marker in MM. A high percentage of patients will be correctly classified with the help of only three laboratory tests for which a single serum sample is sufficient. Even without the results of supplementary tests such as bone marrow and x-ray examinations, the majority of cases with a paraproteinemia were correctly classified as having MM or a nonmyeloma-associated monoclonal gammopathy.

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ADDENDUM

The following members participate in the Paraprotein Task Force of the CCCW: Dr J.C. Kluin-Nelemans (chairman), Dr W.B.J. Gerrits, Dr J. Hermans, Dr H. Kruijff, Dr W. de Kieviet, Dr Ph.M. Klun, Dr F. Ong, Prof Dr E.M. Noordijk, Dr P.J. Seelen, and Dr P.W. Wijermans. The sera used for this study were contributed by the following hospitals in The Netherlands: Diaconessenhuis Leiden (Leiden), Diaconessenhuis Voorburg (Voorburg), Groene Hart (Gouda), Leyenburg (The Hague), Reinier de Graaf Gasthuis (Delft), Rijnland (Leiderdorp and Alphen a/d Rijn), and Westeinde (The Hague).

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