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 paraproteinemia registered prospectively in a population-based registry. Sixty patients had MM and 152 had other monoclonal gammopathies (hematologic diseases [46], 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 μg/mL) in all but 4 of 152 nonmyeloma cases and high (≥20 μg/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 paraproteinemias 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 gammapathies 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, could be sufficient to separate MM from other monoclonal gammapathies.

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 paraproteinemias 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
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.\(^{1,12}\)

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 prol- myelocytic leukemia), and other (n = 4; 2 Hodgkin's disease, 1 cryoglobulinemia, and 1 hemolytic 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 not enough serum was left for combined NCAM, \(\beta_2M\), and CRP determinations.

The diagnosis MM was made when at least two of the following criteria were present:\(^{1,11}\) paraprotein in serum or urine, lytic bone lesion(s), or 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, leucocytopenia, thrombocytopenia, hypercalcemia, renal failure due to myeloma, or lytic bone lesions was termed indolent MM.\(^{1,11}\) Any paraprotein without indications of MM or other hematologic or other malignan-
cies or autoimmune disease with less than 10% plasma cells in bone
 marrow aspirate was termed MGUS.

**Laboratory methods.** Serum samples which had been stored at 
\(-80^\circ\text{C}\) were used for serum NCAM, CRP, and \(\beta_2M\) determinations. Thawed samples were shipped on ice to Marburg University (Marburg, Germany), where serum NCAM determinations were performed. CRP and \(\beta_2M\) determinations were performed immediately after thawing in the Westeinde Hospital (The Hague, The Netherlands).

For serum NCAM determinations, the eNCAM RIA-gnot antibody (Behringwerke AG, Marburg, Germany) was used. \(\beta_2M\) was determined with a solid-phase two-site chemiluminescent en-
zyme immunoassay (Immulite \(\beta_2M\); Diagnostics Products Corp, Los Angeles, CA), and a human CRP mammalian antibody was used for determination of CRP by the rate nephelometry (Array
Protein System, Beckman Instruments Inc, Galway, Ireland). In-
terassay coefficients of variation for serum NCAM were 2% to 8% (range, 8 to 113 U/mL); for \(\beta_2M\), 7% to 10% (range, 1.1 to 8.1 mg/
L); and for CRP, \(\pm 8\%\) (range, 10 to 90 mg/L). Normal values were less than 20 U/mL for serum NCAM, 1 to 3 mg/L for \(\beta_2M\), and less than 10 mg/L for CRP. The serum NCAM, CRP, and \(\beta_2M\) determinations were performed on blinded samples. Other laboratory results were extracted from the registry.

**Statistics.** Statistical methods to compare MM versus nonmy-
eloma included Mann-Whitney's test, Pearson's \(x^2\) test, and Mantel-
Haenszel test for linear association. Subgroups of MM and nonmy-
eloma 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 (\(\geq 20\) 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 all had levels \(\leq 30\) 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, \(\beta_2M\), and serum NCAM determinations for all different diagnostic categories are listed in Table 2 and Fig 1. CRP and \(\beta_2M\) levels varied widely in all diag-
nostic categories.

For all patients, the risk score by Bataille using CRP and \(\beta_2M\) was calculated (low risk, both <6 mg/L; intermediate
risk, either CRP or \(\beta_2M\) \(\geq 6\) mg/L; high risk, both \(\geq 6\) mg/
L).\(^{10}\) 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 nonmyel-
eloma 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 inter-
mediate risks (analysis of variance, \(P < .001\)).

The results of several laboratory tests for myeloma and nonmyeloma patients are given in Table 3. LDH was abnor-
mal 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, \(\beta_2M\), paraprotein type and concentration, hemoglobin, leucocyte and thrombocyte counts, and creatinine. Paraprotein type was entered using 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 abnor-
mal values) did not alter the results. Because of missing

<table>
<thead>
<tr>
<th>Table 1. Number of Patients With Normal and High Serum NCAM Levels Related to Diagnosis</th>
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<tr>
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<tr>
<td>Serum NCAM &lt; 20 U/mL</td>
</tr>
<tr>
<td>Serum NCAM (\geq 20) U/mL</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>
values, only 161 of 212 cases could be used in that analysis. Entering the variables CRP and β2M separately or combined in the risk score described by Bataille et al did not contribute significantly to the model. When we ran the same analysis using only the three selected factors (paraprotein concentration, paraprotein type, and serum NCAM), 209 cases were evaluable. All three factors made highly significant contributions (P < .001) to the final model to discriminate between MM and nonmyeloma, with serum NCAM being chosen immediately after paraprotein concentration and before paraprotein type. Of 209 cases, 89% could be correctly classified as belonging to the MM or nonmyeloma group. Even when we excluded IgM paraproteins from the analysis (using 171 of 212 cases) the result stayed the same, with 88% of cases correctly classified when serum NCAM, paraprotein type (IgG/IgA vs IgD/light chains), and paraprotein concentration were combined. The correlation between serum NCAM and paraprotein concentration was low (r = .36).

Of 105 patients with a paraprotein concentration of less than 10 g/L and hemoglobin levels >6.2 mmol/L (10 g/dL), 3 had serum NCAM levels ≥20 U/mL. Two patients diagnosed with CLL and autoimmune thrombocytopenia had only mildly elevated serum NCAM values (≥30 U/mL). The other patient had stage III MM and had a serum NCAM concentration of 70 U/mL.

**DISCUSSION**

In recent years, many potential prognostic markers for myeloma survival have been reported. Some of these are standard laboratory tests, such as thrombocyte count, LDH, and creatinine, and others are recently discovered prognostic markers, such as β2M, CRP, and serum NCAM. New markers to diagnose MM and differentiate this disease from other monoclonal gammopathies are seldom reported. Most diagnostic classification systems use paraprotein levels, the percentage of plasma cells in bone marrow aspirates, and the presence of lytic bone lesions to make a definite diagnosis of MM, often supplemented by symptoms and signs and standard laboratory tests. Only Kyle and Greipp incorporate specialized diagnostic tests into their system, such as β2M and the plasma cell and peripheral blood B-cell labeling index. Unfortunately, most of these are not readily available to most physicians. We evaluated whether a combination of blood tests only, without the help of additional tests, could sufficiently differentiate between MM and nonmyeloma patients with monoclonal gammopathies.

In patients with a paraproteinemia, serum NCAM alone turned out to be an extremely useful diagnostic test if elevated. A disadvantage was that, even though all nonmyeloma cases had low (<20 U/mL) or only slightly elevated (≥30 U/mL) NCAM values, only half of MM patients had high values. This accounts for the high specificity and positive PV and low sensitivity and negative PV. Of all laboratory tests evaluated in this study, serum NCAM was the second best marker to differentiate between MM and nonmyeloma-associated monoclonal gammopathies, with paraprotein concentration being the best marker.

In patients with low paraprotein concentration and normal hemoglobin values, 3 turned out to have elevated serum NCAM levels, of whom 2 with nonmyeloma conditions had levels less than 30 U/mL. In 1 patient with stage III MM, serum NCAM was high (70 U/mL), indicating its diagnostic value even when there was no suspicion of MM.

Serum NCAM has also been studied in small cell lung
It has been observed that several initially promising immunochemo 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.31 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.

ACKNOWLEDGMENT

We thank J.M. Hulshorst-Jansen, J. Borsje-van der Heijden, M. Grevelink, P.S. Kuiper, P. Houtsma, and K.P.H. van de Meys van

**Table 2. Median Values and Ranges of Age, Serum NCAM, CRP, and β2M in 212 Patients With a Monoclonal Gammopathy**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Subdiagnosis</th>
<th>n</th>
<th>Age (yr) (normal, 25-92)</th>
<th>NCAM (U/mL) (normal, &lt;20 U/mL)</th>
<th>CRP (mg/L) (normal, &lt;20 mg/L)</th>
<th>β2M (mg/L) (normal, 1-3 mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonmyeloma</td>
<td></td>
<td>212</td>
<td>73 (25-92)</td>
<td>6.9 (1-30)</td>
<td>20 (2-336)</td>
<td>3.3 (1.6-22.2)</td>
</tr>
<tr>
<td>Internal diseases</td>
<td></td>
<td>50</td>
<td>73 (50-92)</td>
<td>5.2 (1-24)</td>
<td>40 (2-336)</td>
<td>3.0 (1.6-16.1)</td>
</tr>
<tr>
<td>Paraneoplastic</td>
<td></td>
<td>35</td>
<td>74 (50-92)</td>
<td>5.3 (1-17)</td>
<td>50 (2-158)</td>
<td>3.2 (2.0-16.0)</td>
</tr>
<tr>
<td>Autoimmune</td>
<td></td>
<td>15</td>
<td>72 (56-82)</td>
<td>5.1 (2-24)</td>
<td>6 (2-236)</td>
<td>2.4 (1.6-16.1)</td>
</tr>
<tr>
<td>Hematologic</td>
<td></td>
<td>48</td>
<td>73 (25-92)</td>
<td>8.6 (2-30)</td>
<td>6 (2-236)</td>
<td>4.3 (1.5-22.2)</td>
</tr>
<tr>
<td>Lymphoproliferative</td>
<td></td>
<td>37</td>
<td>70 (28-85)</td>
<td>9.2 (2-30)</td>
<td>6 (2-236)</td>
<td>3.4 (1.5-14.7)</td>
</tr>
<tr>
<td>Myeloproliferative</td>
<td></td>
<td>7</td>
<td>81 (52-92)</td>
<td>4.1 (3-13)</td>
<td>33 (4-236)</td>
<td>7.4 (2.4-22.2)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>4</td>
<td>74 (25-85)</td>
<td>8.7 (3-10)</td>
<td>42 (6-48)</td>
<td>6.2 (2.7-8.1)</td>
</tr>
<tr>
<td>MGUS</td>
<td></td>
<td>54</td>
<td>74 (47-92)</td>
<td>7.1 (2-19)</td>
<td>25 (2-165)</td>
<td>3.2 (1.1-9.3)</td>
</tr>
</tbody>
</table>

**Table 3. Median Values and Ranges of Different Laboratory Tests in Myeloma and Nonmyeloma Patients**

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal Values</th>
<th>Nonmyeloma (n = 152)</th>
<th>Myeloma (n = 60)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum NCAM (U/mL)</td>
<td>&lt;20 U/mL</td>
<td>6.9 (1-30.2)</td>
<td>20.0 (2.4-219.7)</td>
<td>.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>&lt;10 mg/L</td>
<td>20 (2-336)</td>
<td>5 (2-334)</td>
<td>.004</td>
</tr>
<tr>
<td>β2M (mg/L)</td>
<td>1-3 mg/L</td>
<td>3.3 (1.6-22.2)</td>
<td>4.4 (1.3-48.6)</td>
<td>.003</td>
</tr>
<tr>
<td>Paraprotein concentration (g/L)*</td>
<td>Absent</td>
<td>2.7 (1.9-110.0)</td>
<td>28.9 (2.5-88.6)</td>
<td>.0001</td>
</tr>
<tr>
<td>Hemoglobin (mmol/L)</td>
<td>7.5-10.5 mmol/L</td>
<td>7.6 (2.6-10.6)</td>
<td>6.8 (2.5-10.1)</td>
<td>.003</td>
</tr>
<tr>
<td>Leukocyte count (10⁹/L)*</td>
<td>4.0-10.0×10⁹/L</td>
<td>8.1 (0.9-272)</td>
<td>6.4 (2.8-12.7)</td>
<td>.0001</td>
</tr>
<tr>
<td>Thrombocyte count (10⁹/L)*</td>
<td>150-450×10⁹/L</td>
<td>265 (9-1422)</td>
<td>241 (73-425)</td>
<td>.017</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>80-100 µmol/L</td>
<td>100 (51-1757)</td>
<td>100 (51-474)</td>
<td>.006</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>35-50 g/L</td>
<td>35 (19-57)</td>
<td>37 (16-52)</td>
<td>NS</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>60-80 g/L</td>
<td>70 (42-128)</td>
<td>85 (57-161)</td>
<td>.0001</td>
</tr>
</tbody>
</table>

* Incidental missing values (≤8).
† Missing data in 44 cases.
Winden, technicians of the Department of Clinical Chemistry, Westeinde Hospital (The Hague, The Netherlands), for their help in determining CRP and β2M.

**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. Kluin, 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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F Ong, U Kaiser, PJ Seelen, J Hermans, PW Wijermans, W de Kieviet, G Jaques and JC Kluin-Nelemans