Monoclonal Immunoradiometric Assay and Polyclonal Radioimmunoassay Compared for Measuring Neuron-Specific Enolase in Patients with Lung Cancer

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Neuron-specific enolase (NSE) is the most sensitive and specific tumor marker for small-cell lung cancer (SCLC). We evaluated a new monoclonal IRMA (Sangtec) for NSE and compared it with a polyclonal RIA (Pharmacia) in patients with SCLC or other lung cancers (NSCLC). We measured NSE concentrations in 100 healthy subjects (NI group), 100 patients with benign pulmonary diseases (BPD group), and 194 patients with advanced lung cancer (97 SCLC and 97 NSCLC). Intra- and inter assay CVs were <7% for both assays, and dose–dilution curves paralleled their respective standard curves. Values measured by both assays were highly correlated in all groups. NSE concentrations were significantly (P < 0.001) lower by IRMA than by RIA in NI and BPD groups. The upper 95th percentile values for NSE in the NI group were 11.7 μg/L in the RIA and 9.2 μg/L in the IRMA. In NSCLC, the values were significantly (P < 0.05) lower by IRMA but the percentage of subjects with increased values was higher (vs the NI group, 31% for RIA and 44% for IRMA, P < 0.005). Diagnostic sensitivity for SCLC was improved with IRMA: 83% of values with RIA and 93% with IRMA were increased above the NI group values (P < 0.005); the corresponding values for SCLC vs BPD were 81% and 89% (P < 0.05). NSE values measured in 39 patients with SCLC after chemotherapy were more often increased and were significantly higher with the IRMA than with the RIA (P < 0.005).

Additional Keyphrases: immunoassay comparison • tumor markers

Neuron-specific enolase (NSE) is currently viewed as the most sensitive and specific tumor marker for small-cell lung cancer (SCLC) (1–4). Sequential determinations of NSE concentrations in serum are efficient, noninvasive, and cost-effective for monitoring the response of SCLC to chemotherapy (4–7). However, production of NSE is not specific for SCLC, because expression and/or secretion of NSE can be observed in some patients with other histological types of lung cancer (non-small-cell lung cancers, NSCLC). However, NSE production might represent neuroendocrine differentiation of these tumors and possibly indicate a chemosensitivity and a prognosis comparable with those for SCLC (8, 9).

Most clinical studies and routine laboratory determinations use the Pharmacia radioimmunoassay (RIA), which gives reproducible values between laboratories (1, 4, 6, 7, 10). Here we compare the clinical performance of a recently marketed Immunoradiometric assay (IRMA) of NSE, adapted from the method of Paus and Nustad (11), with the traditional RIA in patients with advanced lung cancer.

Materials and Methods

We studied three groups of subjects: the NI group—100 healthy subjects, 70 men and 30 women, with a median age of 42 (range 18–66) years; the BPD group—100 patients with various benign acute or chronic pulmonary diseases, 61 men and 39 women, with a median age of 59 (range 16–87) years; and the SCLC and NSCLC group—194 patients with advanced lung cancer, 97 SCLC and 97 NSCLC (54 squamous cell lung cancers, 29 adenocarcinomas, 10 large-cell lung neoplasms, and 4 other forms), 171 men and 23 women, with a median age of 60 (range 33–81) years. Most patients (179 of 194) had not been treated before the evaluation; of the 15 pretreated patients, 8 had received only radiotherapy, 4 only chemotherapy, 1 had undergone surgery, and 2 had been treated by combined methods. All patients had progressive disease at the time of evaluation, which was done before starting chemotherapy in the framework of therapeutic trials of the European Lung Cancer Working Party (12, 13). Fifty patients (40 SCLC, 10 NSCLC) were reevaluated after three cycles of chemotherapy, and the pattern of change in NSE concentrations was then compared with the objective tumor response to therapy.

NSE concentrations were measured in each serum sample, by the same technique, both by the polyclonal RIA (Pharmacia, Uppsala, Sweden) and by a new monoclonal IRMA (Prolifina NSE IRMA; Sangtec Medical, Bromma, Sweden). Hemolyzed samples were excluded from the evaluation.

We performed classical statistical tests with SPSS software (14).
Results

Technical comparisons. Both assays were technically easy to perform and their turnaround times were similar. Dose-dilution curves of samples with increased values were roughly parallel to the standard curves for each assay; two examples are shown in Figure 1. Intra-assay coefficients of variation (CVs; n = 10–13 determinations) were 6.1% with the RIA and 3.5% with the IRMA for determinations of a serum with normal NSE concentrations (8 μg/L); corresponding figures for a serum with large NSE concentrations (115 μg/L) were 3.9% and 2.0%. Interassay CVs (n = 15–17 assays) were 5.0% with the RIA and 6.7% with the IRMA for determinations of serum with low NSE concentrations (12 μg/L) and were 4.1% and 5.8%, respectively, for serum with increased NSE concentrations (35 μg/L). We detected no loss of immunoreactivity with either assay when serum was left for 2 h at 20 or 37 °C, or for serum frozen and thawed as many as three times.

Comparison of reference values and assay specificity. Individual serum NSE concentrations in the NI and BPD groups are depicted in Figure 2. We defined the upper limits of normality as the 95th percentiles determined in the NI group, i.e., 11.7 μg/L for the RIA and 9.2 μg/L for the IRMA. Values measured by both assays were significantly correlated (r = 0.63; P < 0.001). Median values of the range of values in the NI population were 7.5 (2.5–18.4) μg/L for the RIA and 6.8 (3.2–13.2) μg/L for the IRMA. Comparison of the distributions by a paired Wilcoxon test was statistically significant, the values measured by the IRMA being lower than those measured by the RIA (P < 0.001).

Comparisons of the concentrations measured by both assays in the BPD group also gave significantly lower values with the IRMA (P < 0.001). Median (range) values in the BPD group were thus 7.5 (2.0–36.3) μg/L for the RIA and 6.4 (3.1–30.3) μg/L for the IRMA, the 95th percentiles being 11.9 and 10.3 μg/L, respectively. Compared with the 95th percentiles of the NI group, 6% of the values were "above normal" when measured by the RIA, compared with 11% by the IRMA (P = 0.06 by Cochran's test). Values measured by both assays were highly correlated (r = 0.90, P < 0.001), and the increased values were not specific for a particular lung disease.

Diagnostic sensitivity. In SCLC serum samples, the median (range) NSE values were 30 (5–924) μg/L when measured by the RIA and 34 (6–548) μg/L by the IRMA. The third quartiles (75th percentiles) were 56 and 60 μg/L, respectively. Individual values are depicted in Figure 3; a comparison of the distributions indicated that the values were significantly higher when measured by the IRMA (paired Wilcoxon test, P < 0.005). Values measured by both assays were again significantly correlated (r = 0.95; P < 0.001). Compared with the NI group, 92.5% of the SCLC patients had increased NSE values with the RIA vs 92.8% with the IRMA (P < 0.005). Compared with the 95th percentile of the BPD group, the corresponding figures were 81.4% and 88.7% (P < 0.05).

In NSCLC serum samples, the median (range) NSE values were 9.6 (3.7–184) μg/L when measured by the RIA and 9.0 (5.3–107) μg/L by the IRMA. The third quartiles were 13.5 and 13.1 μg/L, respectively. Individual values are shown in Figure 3; a comparison of the distributions showed that the values were significantly lower when measured by the IRMA (P < 0.05). There were no significant differences between the two assays for the various histological subgroups of NSCLC.
relation between the values measured by both assays was highly significant ($r = 0.95; P < 0.001$). Compared with the NI group, 30.9% of the NSCLC patients had increased values when determined by the RIA vs 44.3% by the IRMA ($P < 0.005$); the corresponding figures were 27.8% and 38.9% for squamous cell tumors ($P < 0.05$), and 31.0% and 44.8% for adenocarcinomas ($P = 0.05$). Compared with the 95th percentile of the BPD group, 27.8% of the values were increased for the RIA vs 34.0% for the IRMA ($P = 0.06$).

**Pattern of change in tumor marker concentrations compared with tumor response to chemotherapy.** Figure 4 indicates the individual data for the 29 SCLC patients studied after chemotherapy who presented an objective tumor response. The values were again significantly higher with the IRMA than with the RIA ($P < 0.005$). NSE concentrations significantly decreased in those who had an objective response but also in the patients classified as showing no change (data not shown). Of the 29 responders, NSE concentrations increased in none, returned to normal in 20 as measured with both assays, and remained increased in 3 when measured by the RIA and in 8 by the IRMA. In the 10 nonresponders, NSE concentrations were normal in 5 when measured by the RIA and in 2 by the IRMA. Normal NSE concentrations were thus observed in 27 patients with initially increased RIA values: 5 were complete responders, 15 were partial responders, and 7 were nonresponders; for the 24 similar patients with initially increased IRMA values, the corresponding numbers were 4, 16, and 4.

Of the 10 patients with NSCLC evaluated after three cycles of therapy, 7 were nonresponders; of the 5 patients with increased values, 4 still had high NSE concentrations by both assays but 1 patient returned to normal values after therapy.

**Discussion**

This study indicates that the recently introduced monoclonal IRMA for NSE measurement is somewhat superior to the traditional polyclonal RIA. Technical comparisons of the two assays gave similar results. The correlation between the values measured with both assays was generally excellent ($r > 0.90$ in diseased subjects). The main advantage of the IRMA over the RIA was improved sensitivity. The upper limit of normal values, defined as the 99.7th percentile, was lower for the IRMA (9.2 $\mu$g/L) than for the RIA (11.7 $\mu$g/L). This latter value is very close to the values found by other investigators using the same RIA (1, 3, 6, 10). Values were significantly lower when measured by the IRMA in the NI group and in the BPD group. If assay specificity was not improved, however, diagnostic sensitivity was higher, because NSE concentrations determined in patients with SCLC were significantly greater when measured by the IRMA than were the values measured by the RIA. Compared with the NI group, the sensitivity for SCLC patients increased from 83% to 93%; compared with the BPD group, the sensitivity for SCLC increased from 81% to 89%. The upper limit of normal values was higher in our study than in the report of Paus and Nustad (11), who used the original IRMA, but the sensitivities for SCLC patients were quite similar (93% vs 90%). Several new tumor markers have been proposed as markers for lung cancer (15–18), but none reaches the sensitivity of NSE, particularly NSE measured with the IRMA evaluated here.

Concentrations of NSE also were more often increased in NSCLC patients when measured by the IRMA than by the RIA. The clinical relevance of NSE expression in NSCLC patients remains to be clarified (9), and the increased sensitivity of the IRMA methodology should be helpful for this. However, it remains to be determined whether such increased sensitivity will also be found for
other malignancies characterized by increased NSE concentrations (19–21).

Our limited data on monitoring tumor mass by measuring NSE essentially confirm the data of other investigators (4–7). Changes in NSE concentrations generally agreed with the definition of an objective response to chemotherapy. A normal NSE concentration was always observed in patients who had a complete response to chemotherapy, in most patients having a partial response, and in some patients with apparently stable disease. Follow-up NSE data determined by both assays were very well correlated, but our preliminary findings suggest that values might remain increased after therapy more often when measured by the IRMA than when measured by the RIA. This could reflect the increased sensitivity of the IRMA and could be clinically important, but these data must be confirmed in a larger number of patients before conclusions are drawn.

In summary, our data from a large series of normal subjects and patients with lung cancer indicate that the recently introduced monoclonal IRMA for measuring NSE appears to be more sensitive than the polyclonal RIA used by most investigators and laboratories. These data further support the use of NSE as the marker for monitoring SCLC.

References
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