
6-D-erythro-Neopterin is synthesized in significantly increased amounts by human macrophages upon activation by γ-interferon. Neopterin has been used as a marker for cellular immune activation in various diseases (1). Measurement of neopterin is possible by high-performance liquid chromatography (HPLC) or by radioimmunoassay (RIA) (2–4). More recently, enzyme-linked immunosorbent assays (ELISAs) of neopterin have been described (5–8).

Neopterin measurement can be used to screen blood donors for presence of infections in a global sense, because increased neopterin concentrations in blood or urine are an early and sensitive indicator for the presence of a broad panel of infectious diseases. In the Austrian Tyrol, neopterin has been screened routinely in each blood sample donated by voluntary blood donors since 1986, with the rationale of providing improved safety for the recipient of blood donations (9). Recently, all Austrian blood-banking institutions have been ordered by the federal government to include in the panel of laboratory tests for blood donations an assay to detect cellular immune activation; at present, neopterin measurement is the only available candidate for such a test.

We have tested a commercially available ELISA kit (ELItest; Henning-Berlin, Berlin, Germany) in the particular context of screening of blood donations. For comparison, we have also determined neopterin concentrations in the same blood samples by RIA (Immutest; Henning-Berlin) and, in some of the samples, by HPLC too. Samples were drawn from 1040 consecutive apparently healthy blood donors. When the initial result by ELISA or RIA exceeded 8.0 nmol/L, we repeated the analysis. In a few of the blood donations that were reassayed, enough serum was available for neopterin determination by HPLC (4).

The mean (and SD) value obtained by ELISA (5.9 nmol/L, 2.5) agreed better than did the RIA data (7.3, 2.0) with mean values obtained previously by RIA (9) for >76 000 blood donors (5.4, 2.3). Linear correlation between the methods was r = 0.779. Frequencies of neopterin concentrations above the upper cutoff limit of 10.0 nmol/L were 42 of 1040 (4.0%) for ELISA, and 63 of 1040 (6.1%) for RIA. The methods agreed in 999 (96.0%) cases (967 <10.0 nmol/L; 32 >10.0 nmol/L); 31 were greater only by RIA, and 10 only by ELISA.

In 29 of the 42 (69.0%) donors with increased neopterin in the initial ELISA, reassy confirmed the increase. Similarly, in 33 of the 63 (52.4%) donors with increased neopterin in the initial RIA, the result was confirmed. Linear correlation between initial and repeat results was r = 0.93 for ELISA, but only 0.86 for RIA.

Samples from 119 donors were reassayed by ELISA and RIA; 70 of the second assay results were below the cutoff limit in both assays, 24 were above the cutoff limit in both assays, 21 were increased only by RIA, and 4 only by ELISA.

Among those donors for whom samples were reassayed by ELISA and (or) RIA, HPLC results also were available for 142. Of these, 116 had neopterin <10 nmol/L by HPLC, and 26 were above this limit. Of the 116 cases with low HPLC results, 105 (90.5%) were also low by ELISA, but only 99 (86.5%) were low by RIA. Of the 26 donors with high neopterin by HPLC, 23 (88.5%) were also high by ELISA, 22 (84.6%) by RIA.

As shown above, of the 73 donors with increased neopterin in the initial assay (either ELISA or RIA), 41 showed a discrepancy between both methods. HPLC analysis of these 73 samples suggested superior reliability of the ELISA results: In 51 samples ELISA and HPLC yielded concordant results, but RIA and HPLC results agreed in only 28 samples.

Receiver-operator characteristic (ROC) curves were constructed for the 109 donors for whom results were obtained by all three methods. Results were classified according to HPLC neopterin ≤10 nmol/L vs >10 nmol/L. Fig. 1 shows that, in agreement with all other analyses, ELISA performed slightly better than RIA, not only near the cutoff value of 10 nmol/L but over the whole range of data.

We conclude that this ELISA of neopterin evaluated is
suitable for use in the context of transfusion medicine. The analytical properties of the kit are at least as good as those of the commonly used RIA, which showed significantly more false-positive results (vs HPLC results), including three gross outliers (probably from pipetting errors). Additionally, the reproducibility of the ELISA was better than that of the RIA, and the absence of radioactive materials is an additional desirable feature.

References

Neuron-Specific Enolase Concentrations in Serum in Nonneoplastic Patients with Pneumonia, Julio Collazos,1,2 Cristobal Esteban,2 and Arantza Fernandez2 (Section of 1 Intern. Med., and 2 Respiratory Diseases, Hospital de Galdakao, 48960 Vizcaya, Spain; 3 address correspondence to this author; fax 34-4-4567043)

Enolase (EC 4.2.1.11) is a dimeric enzyme composed of various permutations of three immunologically distinct subunits, α, β, and γ (1). Although five isoenzymes have been identified, neuron-specific enolase (NSE) represents the concentration of the γ subunits contained in the γγ and in the heterodimeric αγ form (2). Immunohistochemical studies with antibodies to the γ subunit have localized NSE specifically within neuronal and neuroendocrine tissues. This limited distribution allows NSE to be used as a biochemical marker for neuroendocrine tumors (3). NSE is widely used as a tumor marker in the small-cell lung cancer; its measurement in serum has prognostic value (4, 5). Increased concentrations of NSE in serum have been also found in nonmalignant lung diseases, usually with moderate values (4–5). However, the existing literature on this topic reports only the above-normal rate, and no study has taken into consideration the clinical characteristics of the patients.

We have measured serum NSE concentrations in a group of patients with pneumonia, whom we examined by clinical and laboratory evaluations to investigate the behavior of NSE in these patients and to search for factors associated with the increase in this marker.

A total of 60 patients with bacterial, nontuberculous, radiologically proven pneumonia without malignancy (41 men and 19 women, mean age 55.2 years, range 19–88) underwent a clinical and laboratory evaluation. Fourteen patients had underlying chronic obstructive pulmonary disease (COPD); the remaining 46 had had no previous lung diseases.

All patients were included in a study protocol within 24 h of admission. The study procedures were in accordance with the guidelines of the Ethical Committee of our hospital. Blood was collected simultaneously for all laboratory determinations. Spirometric measurements were carried out in patients with COPD after resolution of the pneumonia. NSE was measured by radioimmunoassay (Pharmacia Diagnostics AB, Upssala, Sweden). The upper limit of normal was established at 10 μg/L, the mean ± 2SD for a control group of 100 healthy individuals of ages 19–63 years (mean 37.3).

Correlations were tested with the Spearman’s rank coefficient, and the Mann–Whitney U-test was used to compare NSE values in two groups. P < 0.05 for two-sided tests was considered statistically significant.

NSE serum concentrations were increased in eight patients (13.3%), six without underlying lung disease and two with COPD; the median value for this group was 6.0 μg/L (range 3.5–29). Table 1 shows the NSE values in these patients according to diagnosis. There were no significant differences in NSE concentrations with respect to patients’ sex or age.

A significant negative correlation was found between NSE serum concentrations and arterial PO2 in the patients as a whole (r = −0.27, P = 0.04) but not in the patients with normal NSE (r = −0.2, P = 0.16). On the contrary, in all patients no significant association was found with duration of symptoms, smoking habits, fever, hematocrit, leukocytes, erythrocyte sedimentation rate, urea, creatinine, arterial pH, or PO2. Also, we found no significant correlation between NSE and the spirometric measures (vital capacity, forced expiratory volume in 1 s, forced expiratory flow from 25% to 75% of the vital capacity, and the ratio between the latter two variables) in patients with COPD.

Patients reporting dyspnea (n = 30) had higher NSE concentrations than the other 30 patients who did not (P = 0.0004), and the 15 patients with pleural effusion had lower concentrations than the remaining 45 patients without effusion (P = 0.007). Conversely, no significant difference in NSE concentrations was observed in the patients as a

| Table 1. Neuron-specific enolase concentrations in serum in patients with pneumonia. |
|----------------|----------------|----------------|
|                |                |                |
| Pneumonia      | 46             | 14             |
| NSE, μg/L      | 5.6            | 7.0            |
| Pneumonia + COPD | 6 (13.0)       | 2 (14.3)       |
| All            | 6.0            | 6.0            |
| * >10 μg/L     |                |                |

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