Eur J Cancer Clin Oncol, Vol. 24, No. 5, pp. 825–828, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00

Perspectives and Commentaries

Neuron-Specific Enolase: How Useful as a Cancer Marker?

D.N. CARNEY and M. TEELING
Mater Hospital, Eccles Street, Dublin, Ireland

(A COMMENT ON: Splinter TA, Cooper EH, Kho GS, Oosterom R, Peake MD. Neuron-specific enolase as a guide to the treatment of small cell lung cancer. *Eur J Cancer Clin Oncol* 1987, **23**, 171–176).

THE GLYCOLYTIC enzyme enolase which is necessary for the anaerobic conversion of glucose to metabolites suitable for oxidation has three distinct subunits, alpha, beta and gamma. Within the brain three forms of enolase exist which represent the possible dimeric combinations of the two subunits alpha and gamma. The least acidic isozyme is non-neuronal enolase (NNE) which is composed of two alpha subunits. The most acidic isozyme is composed of two gamma subunits and is neuron-specific enolase (NSE). The intermediate form is a hybrid molecule containing an alpha and gamma subunit. Both the hybrid and NSE isozyme are only observed in tissue extracts derived from the brain or various neuroendocrine tissues [1].

It is now accepted that NSE is a highly specific marker for neurons in both the central and peripheral nervous system. Using immunostaining techniques, NSE is seen in all types of neurons including granule cells, Purkinje cells, projection neurons, and both sensory and autonomic neurons. NSE has also been demonstrated in a variety of normal cells including pinealocytes, pituitary glandular and peptide-secreting cells, thyroid parafollicular cells, adrenal medullary chromaffin cells, cells of the islets of Langerhans, Merkel's cells of the skin and neuroendocrine cells of the lung.

Because of the findings of NSE in specific tissues under normal conditions, it is possible that increased expression of NSE and increased serum levels of NSE may occur with malignant proliferation of these tissues and thus may be of value in diagnosis, staging and treatment of such cancers. The application of NSE determination in medical oncology can be assessed under various headings: (1) determination of NSE content in tissue biopsies; (2) serum measurements of NSE as a marker of tumour diagnosis, disease extent and response to therapy, and (3) determination of cerebrospinal fluid NSE as an indicator of cranial and CNS metastases.

Because NSE has been demonstrated in normal cells of the neuroendocrine system, studies of malignant tumours of these cells for the expression of NSE as tissue extracts may be of diagnostic value.

In a study of 90 neuroendocrine tumours by Tapia *et al.* [2], which included both primary and metastatic lesions, all tumours secreting one or more hormones stained strongly by immunohistochemical techniques for NSE. An excellent correlation was observed however between the intensity of NSE staining and the amount of extractable NSE determined by radioimmunoassay for 13 tumours thus assayed. In this study no correlation was noted between the degrees of immunostaining for NSE and the type of peptide hormone secreted. The tumours examined in this study included islet cell tumours, phaeochromocytomas, medullary thyroid carcinoma, apudomas of the GI tract, pancreas and lung, and small-cell lung cancer.

Further detailed immunohistochemical studies of a variety of neuroendocrine and non-neuroendocrine tumours have suggested that this technique may not be specific enough for evaluation of NSE contents in human tumours. In a comparative

Accepted 11 September 1987.

study of neuroendocrine and non-neuroendocrine tumours a number of the non-APUD tumours were noted to have stained focally positive for NSE. These included several types of CNS tumours including glioblastomas, astrocystomas, oligodendrocytomas, ependymomas, medulloblastomas, pineocytomas, meningiomas and choroid plexus papillomas [3]. However, with the exception of glioblastomas (12/14 cases positive) and astrocytomas (16/23 cases positive), only a small fraction of the other CNS tumours examined stained positive for NSE. As might be expected peripheral nervous system tumours (neuroblastomas, paraganglionomas) also stained positive. In these studies, however, it also appeared that staining for NSE added little further information to that already obtained by histological examination and was not of value in assessing the degree of differentiation of the CNS tumours. In addition and in contrast to this study a report by Royds *et al.*, also evaluating CNS tumours by immunostaining technique, demonstrated that NSE was not detected in a variety of gliomas and other intracranial tumours [4].

Since NSE was first identified in cells lining the respiratory tree, several investigators have reported on its value as a marker for neuroendocrine tumours of the bronchus. In detailed studies of extracts of cell lines of both small-cell lung cancer and non-small-cell lung cancer, NSE levels detected by RIA were significantly elevated in all lines of SCLC origin [5]. In contrast, NSE was rarely detectable in non-small-cell lung cancer lines. These data would suggest that differential expression of NSE by small-cell and non-small-cell lung cancer might be of diagnostic value. However, immunohistochemical staining of primary lung biopsies for NSE has not been shown to be of value in differentiating small-cell from non-small-cell tumours. While some small-cell tumours are positive, results are not uniform, and many non-small-cell lung cancer tumours also stain positive [6]. It is the opinion of these authors that immunohistochemical staining of lung tumours for NSE adds little information to that obtained from histological examination alone and that determination of extractable NSE within the tumours may be of better value, in particular when one is dealing with anaplastic tumours. This may also apply to immunostaining of all human tumours for NSE for with this technique many other non-APUD tumours including Schwannomas, fibroadenomas and carcinomas of the breast, renal cell tumours and chordomas also stain positive [3, 6]. Whether this non-specific staining of non-APUD tumours for NSE is a reflection of technical problems or non-specificity of the antibodies used remains to be determined. Further studies are required to elucidate this.

*Table 1. Serum NSE in newly diagnosed patients with lung cancer**

	NSE elevated	(No./%)
SCLC:		
Limited stage	65/124	52%
Extensive stage	144/168	85%
All patients	386/543	72%†
Non-SCLC	28/193	14%

*Data compiled from eight studies.
†Includes additional patients in whom stage was not listed.

Many investigators have studied the value of serum NSE determination in patients with cancer, in particular small-cell lung carcinoma and children with neuroblastomas. Among 122 children with widespread metastatic neuroblastomas, an elevated serum enolase was noted in 96%. The high frequency of raised serum NSE was similar to the frequency of elevated urinary catecholamines at diagnosis in the same group of patients. Although all patients had advanced stage IV disease, the initial pretherapy level of serum NSE was of prognostic value especially for infants less than 1 year of age [7].

For those children whose serum NSE was less than 100 ng/ml, survival was significantly longer than for similar patients with NSE levels greater than 100 ng/ml.

In several other studies of children with small round-cell malignancies including neuroblastomas, Ewing's sarcomas, rhabdomyosarcomas and lymphomas an elevated NSE was only detected in patients with neuroblastomas. Although the numbers of patients in these studies are small, the data do suggest that serum NSE determinations may be of value in differentiating tumours in this category where histology results are equivocal. Moreover, for patients with neuroblastoma pretherapy NSE level may be of prognostic value as previously noted [8].

Detailed studies of serum NSE determinations have been carried out by several investigators in patients with lung cancer, in particular small-cell lung cancer [9–11]. As indicated in Table 1, serum NSE levels show an excellent correlation with extent of disease, i.e., tumour burden, and response to therapy. In no studies, however, did initial NSE determinations provide further information to that which had been obtained by standard staging techniques and procedures. Moreover, an elevated NSE did not correlate with disease in specific organ sites, e.g., bone marrow or brain. In addition, while serum NSE levels were significantly more elevated among patients with extensive disease when compared with patients with limited

stage disease, considerable overlap did exist in the NSE levels between patients within these two groups. Thus serum NSE determinations at diagnosis in patients with small-cell lung carcinoma was of little value in predicting the stage or extent of disease or the sites of metastatic disease. Finally, as approximately 10% of patients with non-small-cell lung carcinoma had elevated NSE at diagnosis, serum NSE determinations in patients with lung cancer did not appear to be of value as an aid to histological diagnosis.

Serial measurements of serum NSE may be of value in monitoring responses to cytotoxic therapy in patients with small-cell lung carcinoma. In most studies serum levels elevated at diagnosis fall with therapeutic response and rise with recurrence of tumour. However, with few exceptions NSE levels provided little new information above that obtained by physical examination and standard staging procedures. In the study of Johnson *et al.* [10] serum NSE levels determined in patients on chemotherapy demonstrated a persistent rise in serum NSE up to 12 weeks before the clinical recognition of relapse in 15 of 23 (65%) of patients. While these data suggest that NSE levels may be of value in the early detection of relapse from cytotoxic therapy, the lack of highly effective second-line chemotherapy for small-cell lung carcinoma patients who relapse from primary therapy questions the value of routine serum NSE determination in the follow-up of these patients. While initial pretreatment levels of NSE appear to be of little value as independent prognostic factors or in aiding staging procedures, the study by Splinter *et al.* reported in this journal [12] suggests that serial NSE determination at 3–6-weekly intervals from the commencement of therapy may be of value in predicting the degree of response, i.e., complete and partial remission and subsequent survival. From their data the authors conclude that the initial slope of the fall in NSE levels plotted on semilogarithmic paper is of predictive value. These data provide new information that the initial slope or the fall of tumour markers may be of significant importance in determining outcome to cytotoxic therapy. Such information has been clearly demonstrated for patients with testicular carcinoma who have elevated beta HCG at the time of diagnosis. Further studies are required to verify this information. It should be noted that up to 17% of patients with non-small-cell lung carcinoma have been demonstrated to have an elevated serum neuron-specific enolase at diagnosis.

While little information is available on the NSE content of the tumours of patients with an elevated serum NSE, it is of interest that many of the patients with non-small-cell lung carcinoma and an elevated NSE have demonstrated a significant response to cytotoxic therapy [13]. These data suggest that the endocrine properties of lung carcinoma tumours may be of importance in predicting responses to cytotoxic therapy. Intracranial metastasis from small-cell carcinoma of the lung and other histological types of lung carcinoma is a frequent occurrence in patients with these tumour types. These metastases including both parenchymal metastases and carcinomatous meningitis remain major problems with high morbidity and mortality. Recent studies have suggested that determination of CSF NSE may be of value in the very early detection of tumour recurrence in these sites [14]. The finding of an elevated CSF NSE in patients with small-cell carcinoma of the lung is highly suggestive of metastasis, either intracranial or meningeal. Studies are required however to determine if routine CSF measurements may detect early CNS recurrence prior to it becoming clinically apparent.

Summary. Although determinations of NSE within tumours, either by RIA or immunohistochemistry techniques, may be of value in differentiating neuroendocrine tumours from non-neuroendocrine tumours, for several reasons these tests appear to be of limited value. For tumours such as neuroblastomas and other typical endocrine tumours, NSE determinations within the tumour add little value to the pathological tests. Immunohistochemical testing appears also to be of little value since not all well-documented endocrine tumours contain NSE, and since many non-endocrine tumours may stain positive for NSE. Whether these conflicting data are a reflection of the quality of the antibodies used or techniques remains to be determined.

As a serum marker in small-cell lung cancer patients NSE is neither specific enough nor sensitive enough to suggest its routine use in screening, diagnosis or monitoring responses to therapy as it provides little information above that of standard clinical procedures. However, the data provided by Splinter *et al.* [12] suggests that sequential measurements in the early phases of therapy may be of prognostic value and warrants further evaluation. For patients with non-small-cell lung carcinoma and an elevated serum NSE further studies are required to confirm if the NSE can be used as a marker for predicting chemosensitivity in this group. In paediatric tumours where pathological uncertainty exists serum NSE determination may be of value in differentiating small-cell round tumours where therapy and prognosis are very different within each tumour type. Finally, and perhaps most promising, it does appear that CSF determinations of NSE coupled with CSF levels of bombesin and calcitonin may not only be an excel-

lent preclinical marker of CNS relapse but also may be of value in distinguishing parenchymal metastases for meningeal carcinomatosis in small-cell carcinoma.

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