S-100 and NSE as Serum Markers in Melanoma

Anna Tofani, Raffaele P. Cioffi, Rosa Sciuto, Sandra Rea, Anna Festa, Franco Di Filippo, Renato Cavaliere and Carlo L. Maini

From the Department of Nuclear Medicine (A. Tofani, R.P. Cioffi, R. Sciuto, S. Rea, A. Festa, C.L. Maini), and 1st Division of Oncological Surgery (F. Di Filippo, R. Cavaliere), Regina Elena Cancer Institute, Rome

Correspondence to: Professor Carlo Ludovico Maini, La Pietra Pizzuta, 03010 Patrica (FR), Italy. Tel: + 39 6 4985 2236. Fax: + 39 6 4985 2346

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S-100 protein and neuron-specific enolase (NSE) have recently been proposed as serum markers for melanoma. In this study NSE and S-100 serum levels were assayed by commercial IRMA methods in 53 patients with melanoma. The overall prevalence of abnormal marker levels was similar for NSE (26%) and S-100 (30%). The 24 patients in stages I and II had uniformly normal S-100 levels, but abnormal NSE levels were observed in 3 out of the 12 patients in stage II (33%) and in 1 out of 12 in stage I. NSE appears thus to be the marker of choice in the early stages, where its increase points to disease progression. In patients in stages III and IV the prevalence of abnormal values was 34% for NSE and 55% for S-100 ($p = < 0.05$). In the latter group diagnostic sensitivity increased to 62% if isolated elevation of each marker was considered. In patients with advanced stage disease, both NSE and S-100 should be assayed.

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A common problem in dealing with patients with cancer is determining their individual prognosis. Serum markers of disease activity are currently used to evaluate the response to therapy and to monitor disease progression. Melanoma presents special challenges for early diagnosis of disease activity and outcome assessment. At present, the literature on tumor markers in malignant melanoma is scarce. Recently, neuron-specific enolase (NSE) and S-100 protein have been proposed as serum markers for melanoma (1–3).

NSE is an isoenzyme of glycolitic enzyme enolase restricted to neurons and peripheral neuroendocrine tissue. NSE is expressed in tumors of neuroectodermal origin including tumors arising from the APUD (amine precursor uptake and degradation) system and melanoma (4). Serum NSE is a well-known marker of neuroendocrine tumors (5). Serum elevation of NSE has also been described in stroke patients (6) and in patients suffering from severe and minor head injuries (7).

S-100 protein, a 21 kD calcium-binding protein, is a dimer composed of two subunits, 'a' and 'b'. Different arrangements of subunits give origin to three isoforms of the protein (8). The a-a (S-100a0) is located in heart and skeletal muscle, while a-b (S-100 a) and b-b dimers (S-100 b) are predominately located in nervous tissue (9). S-100 is found diffusely in the cytoplasm, but a fraction is bound to membranes (10). Although its biological role has not been fully elucidated, S-100 has been reported to regulate protein phosphorylation and cytoskeleton system activity (11, 12). S-100 is also thought to be secreted (13); in vitro, extracellular S-100 can stimulate neuronal differentiation and glial proliferation (14, 15). S-100 is a histochemical marker for melanocytes (16) and it has been recognized by immunohistochemical methods in different tumors of neuroectodermal origin such as melanoma (17), neurinoma (18), skin tumors (19), glioma and ependymoma (20). Serum S-100 is also a marker of damage of the CNS (21, 22) and may increase in patients with cerebral complications following cardiac surgery (23), head traumas (24) or ischemic stroke (21).

Clinical experience of NSE and S-100 as serum markers in melanoma is still limited. Increased NSE serum levels in patients with metastatic melanoma have been reported to be associated with a large tumor burden, with a gradual rise indicating disease progression and a poor prognosis (25). An increased S-100 serum level has been described in advanced melanoma (3). Recent studies suggest that NSE and S-100 have a good prognostic value in malignant melanoma (25, 26). As the two markers, even though both are mostly cytoplasmic proteins (10), have different biological roles, an increased diagnostic yield can be expected.

In the present study both NSE and S-100 serum levels were determined in 53 patients in different stages of melanoma to assess their reliability as indexes of disease activity.

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MATERIAL AND METHODS

Patients

Fifty-three consecutive patients with melanoma (26 males, 27 females; age range 16–81 years, mean and median age 52 and 50 years, respectively) admitted to 'Regina Elena' Cancer Institute between January 1995 and February 1996 entered the study. In accordance with AJCC criteria (27), 24 patients presented local disease (stage I or II), 29 had metastases (stage III or IV). Twenty healthy volunteers were used as a control group. All the patients gave their informed consent to participate in the study.

S-100 and NSE determination

Blood samples were drawn at hospital admittance or during outpatient follow-up examinations. After centrifugation (1 000 rpm for 10 min), sera were stored at $-20°C$ until assayed. S-100 and NSE serum concentrations were determined blind of clinical information with a two-site (sandwich) immunoradiometric assay using commercially available kits (Sangtec S-100 IRMA, Prolifigen NSE IRMA; Byk Gulden, Milan, Italy). Samples were assayed in triplicate.

In the first step of the S-100 assay, samples were incubated with a plastic bead coated with a monoclonal antibody to S-100. After washing, a second ^{125}I-labelled monoclonal antibody to S-100 was added and incubated. After further washing, the radioactivity bound to the bead was measured in a gamma-counter. S-100 concentrations were calculated using standards with known concentration of the protein and expressed in mcg/l. The detection limit of the S-100 IRMA was 0.1 mcg/l. Intra-assay coefficients of variation (CV) were 7.4% for low values (n = 10, mean 3.2 mcg/l) and 4.8% for high values (n = 10, mean 40.8 mcg/l). The corresponding interassay CVs were 9.8% (n = 10, mean 3.4 mcg/l) and 5.0% (n = 10, mean 39.2 mcg/l), respectively.

In the NSE assay, sera were incubated with a plastic bead coated with a monoclonal antibody to NSE and a second ^{125}I-labelled monoclonal antibody directed to a different epitope of the protein. After washing, radioactivity bound to the bead was measured and NSE concentrations determined using standard procedures. The detection limit was 2 mcg/l. Intra-assay CVs were 6.8% for low values (n = 10, mean 7.6 mcg/l) and 3.4% for high values (n = 10, mean 38.3 mcg/l). The corresponding interassay CVs were 7.4% (n = 10, mean 8.0 mcg/l) and 9.1% (n = 10, mean 36 mcg/l), respectively.

Statistical analysis

A χ^2-test and linear regression analysis were used as appropriate.

RESULTS

Normal values

Serum NSE values in the control group ranged from 3.0 to 10.2 mcg/l, median 6.45, while serum S-100 levels ranged from 0.11 to 0.43 mcg/l, median 0.31. Both S100 and NSE values were always under the 95th percentile values of the reference population tested by the manufacturer (0.5 and 12.5 mcg/l for S-100 and NSE, respectively). We therefore chose the 95th percentile values to define normal thresholds.

Serum S-100

In the whole group of patients with melanoma, serum S-100 was elevated in 16 out of 53 patients (30%). However, in the subgroup of 24 patients with melanoma in stages I and II, no elevated value was found. On the contrary, in the subgroup with metastatic disease (stage III or IV), 16 out of the 29 patients (55%) had increased S-100 levels. The values ranged from 0.5 to 290 mcg/l, with a mean value of 27.21 mcg/l and a median value of 0.86 mcg/l. The highest S-100 value was measured in a patient with lymph node, lung and bone metastases, who died three days after blood sampling. A value of 93 mcg/l was measured in another patient with lung and liver metastases. However, several patients with multiple metastases had normal S-100 values (Figure).

Serum NSE

Serum NSE levels were elevated in 14 out of 53 patients with melanoma (26%). Only 4 patients out of 24 (16%) with melanoma in stages I and II had increased NSE values ranging from 12.2 to 48 mcg/l (mean and median values of 24.15 mcg/l and 16.65 mcg/l respectively). Ten out of 29 patients (34%) with metastatic disease (stages III and IV) presented elevated levels of serum NSE ranging from 12.5 to 178 mcg/l (mean and median values of 30.24 mcg/l and 25 mcg/l respectively). The highest values of serum NSE were observed in the same two patients who presented the highest S-100 values and in a third patient with diffuse metastases (178, 51 and 54 mcg/l, respectively) (Figure).

Comparative results

For the whole group of patients with melanoma, no statistically significant differences were found when comparing S-100 and NSE sensitivity (30 and 26%, respectively). In patients with primary melanoma or with local invasion (stages I and II), NSE showed a poor sensitivity while S-100 values were normal in all patients.

In patients with advanced disease, i.e. with lymphonode and distant metastases (stages III and IV), sensitivity rose for both S-100 (55%) and NSE (34%). A slight, not statistically significant, difference was observed for S-100 ($p < 0.5$). Differences between sensitivity for S-100 and

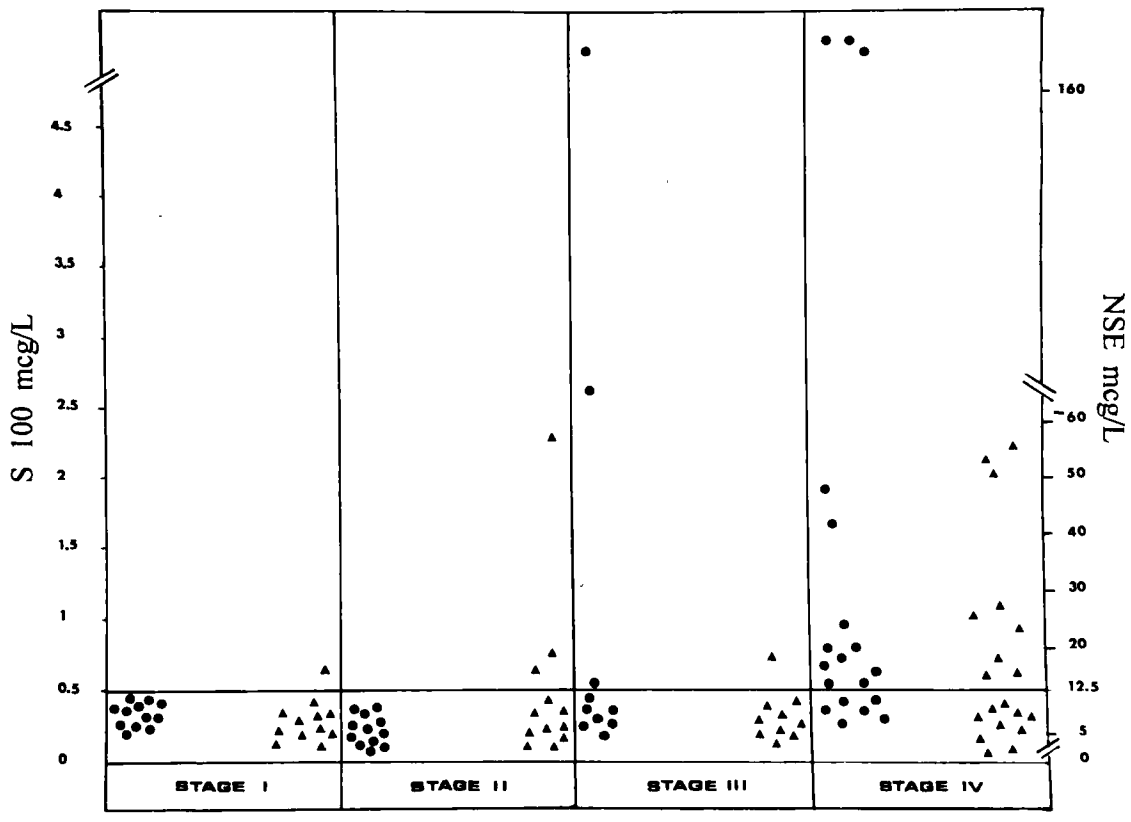

Figure. Serum S-100 (●) and NSE (▲) levels in 53 patients with melanoma (12 patients in stage I, 12 in stage II, 10 in stage III and 19 in stage IV). Cut-off values are < 12.5 mcg/l for NSE and < 0.5 mcg/l for S-100.

NSE in patients in stages III and IV compared with patients in stages I and II were significant only of S-100 ($p < 0.002$; $p < 0.5$ respectively).

On analyzing patients in stage IV separately, it was found that sensitivity was even higher, with 68% (13/19) for S-100 and 48% (9/19) for NSE. S-100 and NSE values correlated well in this subgroup of patients ($r = 0.657$; $p = 0.002$).

Eight patients out of 53 (15%), all in stage IV of the disease, had elevated values for both S-100 and NSE. S-100 alone was increased in 8 patients (5 in stage IV and 3 in stage III), while NSE alone was elevated in 6 patients (1 in stage IV, 1 in III, 3 in II and 1 in stage I).

DISCUSSION

NSE and S-100 values have been reported as possible prognostic factors in melanoma, as high levels of these proteins can be detected in sera of melanoma patients with advanced disease (25, 26). The results of the present study are in line with previous reports. The pattern of prevalence of pathological values in different stages of the disease was not the same for NSE and S-100.

A low overall prevalence (16%) of abnormal NSE values in the 24 patients with local disease was observed in this series, but the four abnormal values were from the 12 patients in stage II (33%). In patients in stage I–II, serum S-100 levels were uniformly within the normal range and thus its assay does not seem warranted. These findings confirm a reported prevalence of abnormal values of 1.3% for S-100 and 12.5% for NSE in patients in stages I and II (26).

Prevalences of abnormal NSE and S-100 values in patients with advanced disease (stages III and IV) are higher, being 34% and 55% respectively (Figure). These findings are in line with those reported by others. Wibe et al. (2) found pathological serum NSE levels in 48% of 63 patients with advanced melanoma (stages III–IV). Gou et al. (26) reported a sensitivity of 41.3% and 26.1% for S-100 and NSE in 46 patients in stages III–IV. In our series, the highest values for NSE and especially for S-100 were observed in patients with extensive metastases just before death, suggesting a possible prognostic role of these markers.

It is of practical interest that S-100 or NSE were elevated separately in several patients with advanced disease (10/29, 34%) In this subgroup diagnostic sensitivity increased to 62% if elevation of each marker was considered, suggesting that both markers should be assayed in these patients. The different behavior of NSE and S-100 cannot readily be explained: differences in their biological role,

differences in biokinetics and heterogeneity of melanomas may play an important role. Clinical correlates of selective increase of one marker only are not yet known.

CONCLUSIONS

The following conclusions can be drawn:

(a) In stages I and II, NSE is clearly superior to S-100 as a marker and should be included in the routine follow-up, since elevated serum NSE levels are probably associated with progression of the disease; (b) in patients in stages III and IV both markers may be elevated, but not necessarily both in the same patient; thus both NSE and S-100 should be assayed; (c) the true prevalence of false negatives is unknown and difficult to evaluate, as a normal serum level of either marker in a melanoma patient could be related to the known biological heterogeneity of melanoma, to small neoplastic mass, to inactive disease or a combination of these and other unknown factors; (d) longitudinal studies are warranted in order to assess clinical correlates of serum NSE and S-100 determinations in melanoma patients.

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