# Neuroendocrine Differentiation in Renal Cell Carcinoma

## *Evaluation of Chromogranin A and Neuron-Specific Enolase*

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Acta Oncologica Vol. 38, No. 5, pp. 623–628, 1999

Chromogranin A and neuron-specific enolase (NSE) as neuroendocrine markers were evaluated in 200 patients with renal cell carcinoma, and 15 patients with benign renal cysts. Immunoassays of serum levels and immunohistochemical staining of tumour tissue were performed. Serum chromogranin A was elevated in 28 (14%) patients with renal cell carcinoma, but the levels did not differ from those for patients with benign cysts. Serum NSE was elevated in 54 (27%) patients, significantly higher compared with controls ($p = 0.0002$). Serum chromogranin A level was positively correlated to serum creatinine and age, but not to tumour stage or grade. Serum NSE level was positively correlated to tumour stage and grade, but not to serum creatinine or age. Immunohistochemical staining for chromogranin A was positive in 1 of 24 (4%), and for NSE in all 18 (100%) tumours analysed. In a multivariate analysis, tumour stage, grade, and serum NSE, but not chromogranin A, were significant predictors of prognosis.

*Received 7 October 1998*
*Accepted 9 December 1998*

Chromogranin was first found in the chromaffin granules of the adrenal medulla (1, 2) and is co-released from the adrenals with the catecholamines. Chromogranin A stabilizes the granule cores and is cleaved into several active peptides (3), some of which may suppress neuroendocrine secretion. By means of different immunoreactive analyses, chromogranin A has been demonstrated in normal and neoplastic neuroendocrine tissues (4, 5). As a marker of neuroendocrine differentiation, chromogranin A is expressed in pheochromocytomas, carcinoid tumours, and in small cell lung cancer (4, 6, 7). Expression has also been demonstrated in tissue from non-endocrine tumours such as prostatic, breast, non-small cell lung, and colorectal carcinoma (8–11). Chromogranin A has also been demonstrated in serum, and increased serum levels have been observed in patients with neuroendocrine tumours (12–14), but also in prostatic (15), and colorectal carcinoma (16).

Neuron-specific enolase (NSE) is a glycolytic enzyme associated with neuroendocrine tumours. In renal cell carcinoma, NSE was demonstrated using immunohistochemistry (17), and later elevated serum levels of NSE were also found (18). We have in an earlier report shown the prognostic value of NSE in renal cell carcinoma (19).

The aim of this investigation was to quantify serum chromogranin A and NSE in patients with renal cell carcinoma, to evaluate the possible neuroendocrine differentiation of this tumour. In a subset of patients, chromogranin A and NSE expression was analysed in tumour tissue using immunohistochemistry.

## MATERIAL AND METHODS

Two hundred consecutive patients (119 males, 81 females, median age 66 years, range, 25–86) with histologically verified renal cell carcinoma were included in our investigation. The patients were admitted to the Urology Department, University Hospital, Umeå from 1982 to 1994. The patients were given a physical examination, chest radiography, ultrasound of the abdomen and computed tomography, and in the event of symptoms, bone scintigraphy and skeletal radiography. Staging was carried out according to Robson et al. (20) and the tumours were classified into nuclear grades according to Skinner et al. (21). One hundred and eighty-one patients were operated with radical, and 2 with partial nephrectomy, and 17 patients had palliative therapy because of advanced metastatic disease. Fifteen patients with benign renal cysts were used as clinical controls.

 ISSN 0284-186X

Serum samples were obtained with patients' informed consent before initiation of therapy, and stored at −80°C until analysis. Chromogranin A was analysed in duplicate using the DAKO Chromogranin A ELISA kit (Glostrup, Denmark). The reference interval according to the manufacturer's manual was 17–46 U/L. Neuron-specific enolase was analysed using the Prolifigen NSE IRMA (Sangtec Medical, Bromma, Sweden). Haemolytic sera were excluded, and the upper reference interval value was set at 12.5 μg/L. Serum creatinine was analysed using a method based on the reaction of Jaffé. For age and kidney filtration rate dependency analysis of chromogranin A and NSE, the median age (66 years) and 125 μmol/L creatinine were used.

Immunohistochemical staining of chromogranin A and NSE was performed on formalin-fixed, paraffin-embedded tumour specimens using standard techniques including microwave treatment of the sections in citrate buffer. The anti-chromogranin A antibody (Boehringer-Mannheim Gmbh, Germany) was used at a 1 : 5000 dilution and the anti-NSE antibody (DAKO, Denmark) at 1 : 400 dilution.

The median follow-up time from admission was 81 months (range, 3–152 months). During this period 109 patients died of renal cell carcinoma, and 26 of unrelated causes. Sixty-seven patients were alive at the time of follow-up.

For statistical analysis the Mann–Whitney and Jonckheere–Terpstra tests were used. Multivariate analysis was performed with the Cox method. Survival curves were according to the Kaplan–Meier method, and for survival analysis the logrank test was used.

## RESULTS

Serum levels of chromogranin A and NSE in patients with renal cell carcinoma and benign renal cysts are presented in Table 1. For NSE, the levels were significantly higher in

**Table 1**

*Serum chromogranin A and NSE in patients with renal cell carcinoma and benign renal cysts in relation to age and renal function assessed by serum creatinine*

| | Chromogranin A (U/L) | | | NSE (μg/L) Mean ± SD | |
|---|---|---|---|---|---|
| | No. | Mean ± SD | | | |
| Renal cell carcinoma | 200 | 28.6 ± 38.3 | N.S. | 12.4 ± 9.0 | p = 0.0002 |
| Benign cysts | 15 | 24.9 ± 13.9 | | 7.0 ± 0.6 | |
| Age (years) | | | | | |
| <66 | 99 | 21.8 ± 22.7 | p = 0.0013 | 13.6 ± 10.7 | N.S. |
| ≥66 | 101 | 35.3 ± 48.2 | | 11.3 ± 7.0 | |
| Renal function | | | | | |
| Serum creatinine μmol/l | | | | | |
| <125 | 182 | 26.4 ± 37.0 | p = 0.0047 | 12.5 ± 9.4 | N.S. |
| ≥125 | 18 | 51.3 ± 44.6 | | 11.4 ± 4.1 | |

Mann–Whitney test. SD = standard deviation, N.S. = not significant (p > 0.05).

**Table 2**

*Serum chromogranin A and NSE in patients with renal cell carcinoma in relation to disease stage and nuclear grade. The number of patients with serum levels above the normal limit is indicated*

| Stage | No. | Chromogranin A (U/L) | | | | NSE (μg/L) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean ± SD | | >46 | | Mean ± SD | | >12.5 | |
| | | | | No. | (%) | | | No. | (%) |
| I | 75 | 25.5 ± 28.5 | N.S. | 9 | 12 | 9.7 ± 6.1 | p = 0.0001 | 11 | 15 |
| II | 7 | 23.1 ± 12.3 | | 1 | 14 | 8.6 ± 1.6 | | – | – |
| III | 44 | 39.7 ± 60.8 | | 9 | 20 | 12.6 ± 8.5 | | 11 | 25 |
| IV | 74 | 25.8 ± 29.8 | | 9 | 12 | 15.4 ± 11.2 | | 32 | 43 |
| Grade | | | | | | | | | |
| 1 | 3 | 17.3 ± 10.3 | N.S | – | – | 7.8 ± 0.5 | p < 0.0001 | – | – |
| 2 | 41 | 30.9 ± 42.4 | | 6 | 17 | 9.3 ± 7.0 | | 3 | 7 |
| 3 | 99 | 27.0 ± 29.9 | | 13 | 13 | 11.3 ± 7.4 | | 22 | 22 |
| 4 | 47 | 32.8 ± 53.0 | | 8 | 17 | 17.1 ± 11.8 | | 23 | 49 |

Jonckheere–Terpstra test. SD = standard deviation, N.S. = not significant (p > 0.05).

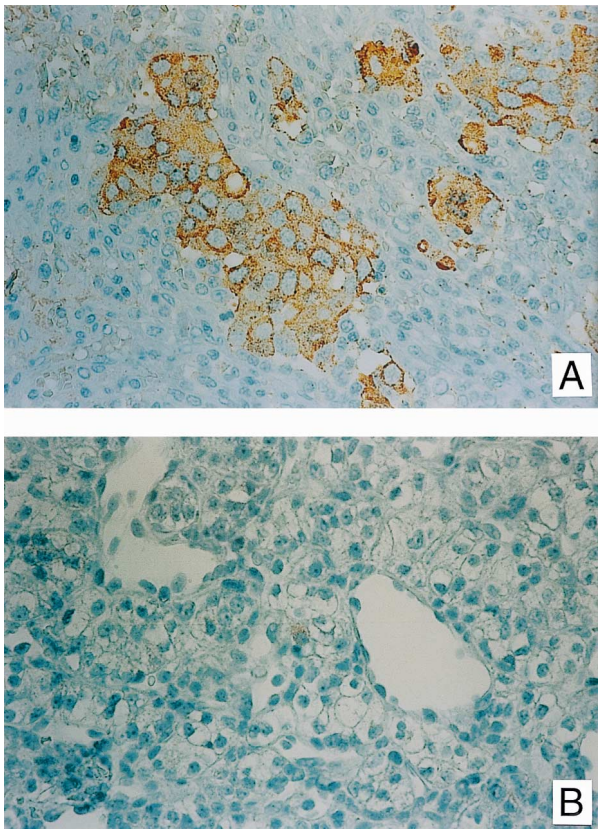


*Fig. 1.* Immunohistochemical staining for chromogranin A in renal cell carcinoma (A) positive, and (B) negative tumour.

patients with renal cell carcinoma compared with those with benign renal cysts. For chromogranin A no such difference was observed. Chromogranin A levels increased with age, and there was also a positive correlation with serum creatinine. Neuron-specific enolase showed no such correlation. Nor was there any difference in serum chromogranin A or NSE by gender (data not shown). The relation between serum chromogranin A and NSE was analysed using a linear correlation test, and a weak but significant correlation was observed ($r = 0.20$; $p = 0.0041$).

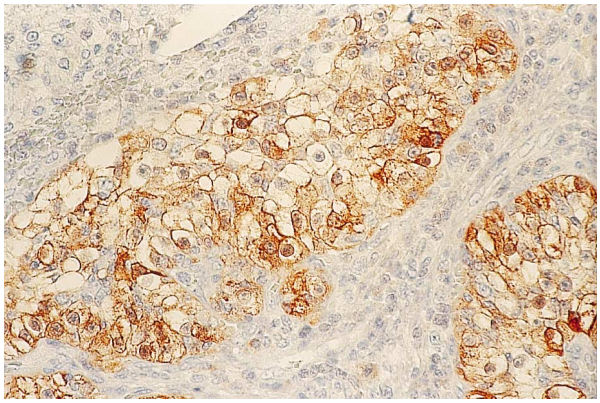

*Fig. 2.* Immunohistochemical staining for NSE in renal cell carcinoma.

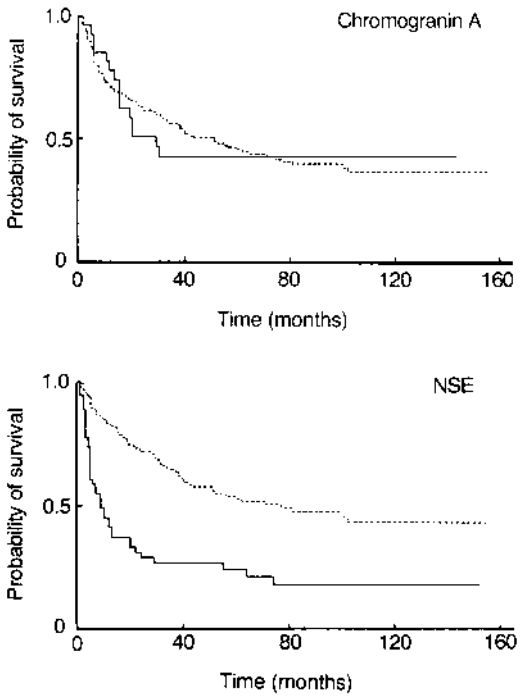


*Fig. 3.* Survival curves according to the Kaplan–Meier method in patients with elevated (—) and normal (- - -) serum level of chromogranin A (upper panel), and NSE (lower panel).

Sixty-six patients had either elevated chromogranin A or elevated NSE, but this was not significantly different from random frequency.

In Table 2 the correlations between serum chromogranin A and NSE and disease stage and tumour grade are presented. Twenty-eight (14%) patients had elevated serum chromogranin A and 54 (27%) had elevated NSE. No significant difference in serum chromogranin A level according to stage or nuclear grade was observed. For serum NSE, on the other hand, a positive correlation to both stage and grade was observed ($p = 0.0001$).

Twenty-four tumours were analysed immunohistochemically for chromogranin A. These were tumours from all seven patients with serum chromogranin A levels $\geqslant 100$ U/L, and 17 randomly sampled tumours from among patients with lower serum chromogranin A. Only one (4%) tumour had a positive staining for chromogranin A, and the serum level of chromogranin A in this patients was elevated (Fig. 1A and B). Histochemical staining for NSE was assessed in the 17 randomly sampled tumours, plus the one that stained for chromogranin A. All 18 tumours stained for NSE (Fig. 2), but the staining varied considerably from rather homogeneous to very heterogeneous, with scattered islands of positive cells. In some tumours only a few, small areas were NSE positive.

Survival curves for patients with elevated and normal serum levels of chromogranin A and NSE, respectively, are presented in Fig. 3. Neuron-specific enolase was identified

as a significant predictor of poor prognosis using the logrank test ($p = 0.001$).

In a multivariate analysis of prognostic factors, age, gender, disease stage, tumour grade, serum chromogranin A and NSE levels were evaluated (Table 3). Only stage, grade and serum NSE were identified as significant prognostic factors for survival.

## DISCUSSION

In renal cell carcinoma there are several indications of neuroendocrine differentiation. Expression of NSE, in both tumour tissue and serum, has been demonstrated (17–19), and Gazdar et al. (22) found L-Dopa decarboxylase expression. However, in an analysis of chromogranin A in tissue extracts from normal kidney and renal cell carcinoma, Wilson & Lloyd (4), found no expression. O'Connor et al. (13), and Eriksson et al. (14), assessed chromogranin A in serum from a limited number of patients and found normal concentrations. These results are consistent with the data in the present study, where no significant difference was found between serum levels in patients with renal cell carcinoma compared with patients with renal cysts. Furthermore, a lack of correlation between tumour stage and grade to serum chromogranin A was observed. However, on analysing chromogranin A, Edgren et al. (23), found elevated plasma levels in 19 out of 22 patients with renal cell carcinoma. In that study all patients had advanced disease and chromogranin A was analysed during therapy either with tamoxifen, interleukin-2 or interferon $\alpha$. Immunohistochemical analysis of chromogranin A was, however, negative (23). The reason for the contradictory results in these two studies is not clear.

In the present study only 14% of the patients had elevated serum chromogranin A. Our data also showed that serum chromogranin A was increased in patients with impaired renal function (Table 1). This observation accords with earlier results (24), and explains one-third of the elevated values of chromogranin A in the present study. However, 56% of the patients with impaired renal function had normal serum chromogranin A. Another reason for increased serum chromogranin A is treatment with emetogenic chemotherapy agents (25), probably due to release from neuroendocrine cells in the gastointestinal tract. None of the patients in this study received any chemotherapeutic agents at the time of sampling.

In the present study an increase in chromogranin A levels with age was found (Table 1). Conversely, O'Connor & Deftos (12) reported on the lack of influence of age on serum chromogranin A in healthy individuals. This discrepancy may be explained by the fact that our patients were older than those studied by O'Connor and Deftos. The correlation between age and serum chromogranin A was valid also when patients with serum creatinine $> 125$ μmol/L were excluded.

Immunohistochemical analysis of tumour tissue demonstrated chromogranin A expression in 1 out of 24 tumours. This rare expression is in accordance with earlier results (4, 23). The meagre expression of chromogranin A in tumour cells indicates that the serum levels mainly reflect other sources for this protein than release from renal cell carcinoma.

**Table 3**

*Multivariate analysis of prognostic factors according the Cox method*

| Prognostic factor | Relative risk | p-value | 95% Conf. Interval Lower | – | Upper |
|---|---|---|---|---|---|
| Age (years) | | | | | |
| <65 | 1.0 | | | | |
| ≥65 | 1.2 | 0.32 | 0.80 | – | 1.90 |
| Gender | | | | | |
| Male | 1.0 | | | | |
| Female | 1.2 | 0.29 | 0.82 | – | 1.89 |
| Stage | | | | | |
| I–II | 1.0 | | | | |
| III–IV | 10.8 | <0.0001 | 5.58 | – | 20.92 |
| Grade | | | | | |
| 1–2 | 1.0 | | | | |
| 3–4 | 3.3 | 0.01 | 1.31 | – | 8.62 |
| NSE (μg/L) | | | | | |
| <12.5 | 1.0 | | | | |
| ≥12.5 | 2.4 | 0.0001 | 1.58 | – | 3.89 |
| Chromogranin A (U/l) | | | | | |
| <46 | 1.0 | | | | |
| ≥46 | 0.63 | 0.15 | 0.34 | – | 1.19 |

Serum NSE was significantly higher in patients with renal cell carcinoma compared to controls, and a neat, positive correlation with disease stage and tumour grade was demonstrated. Our results are consistent with earlier reports (17, 18), and the present study confirms a less favourable prognosis for patients with elevated serum NSE (19). Furthermore, our findings on the expression of NSE in tumour tissue correspond to earlier results (17), demonstrating immunoreactivity for NSE in all renal cell carcinomas analysed. Owing to the heterogeneous NSE staining in tumour tissue, quantification of the expression is difficult to achieve, but it is reasonable to assume that the serum levels reflect the production of NSE by the tumour. The frequent immunohistochemical expression of NSE in tumour tissue may indicate that NSE is produced by the tumour cells.

In neuroendocrine tumours, such as carcinoids, chromogranin A and NSE are often co-expressed (6, 8, 10, 16). In the present study only a weak positive correlation between serum chromogranin A and NSE was demonstrated. However, the expression of chromogranin A and NSE in tumour tissue is rather dissociated, an observation that has been described earlier using immunoreactivity in tumour tissue (6), as well as in sera from patients with small cell lung carcinomas (26).

In conclusion, a lack of association between serum chromogranin A and tumour characteristics was demonstrated. In contrast, a strong correlation with tumour stage and grade was observed for NSE, an increased level of which also was identified as a significant predictor of poor prognosis for patients with renal cell carcinoma.

## ACKNOWLEDGEMENTS

This study was supported by grants from the Lions Research Foundation, Department of Oncology, the Medical Faculty, Umeå University, Sweden, and the Swedish Cancer Society.

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