EUROPEAN JOURNAL OF CANCER 48 (2012) 662–671

Available at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.ejconline.com

Choice of tumour markers in patients with neuroendocrine tumours is dependent on the histological grade. A marker study of Chromogranin A, Neuron specific enolase, Progastrin-releasing peptide and cytokeratin fragments

Catharina M. Korse [a,*], *Babs G. Taal* [b], *Andrew Vincent* [d], *Marie-Louise F. van Velthuysen* [c], *Paul Baas* [b], *Johanna C.G.M. Buning-Kager* [a], *Theodora C. Linders* [a], *Johannes M.G. Bonfrer* [a,d]

[a] *Department of Clinical Chemistry, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, P.O. Box 90203, 1006 BE Amsterdam, The Netherlands*

[b] *Department of Medical Oncology, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands*

[c] *Department of Pathology, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands*

[d] *Department of Biometrics, The Netherlands Cancer Institute – Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands*

ARTICLE INFO

Article history:
Available online 22 September 2011

Keywords:
Neuroendocrine tumours
Chromogranin A
NSE
ProGRP
Cytokeratins
MonoTotal

ABSTRACT

Background: Chromogranin A (CgA) is the most important tumour marker for well-differentiated neuroendocrine tumours (NET) and neuron specific enolase (NSE) for poorly differentiated neuroendocrine carcinoma (NEC). This study investigated whether the markers progastrin-releasing peptide (proGRP) and cytokeratin fragments (CKfr) CK8, CK18 and CK19 (MonoTotal®) can be of additional value to the histological classification and help predict survival in these patients.

Methods: CgA, NSE, proGRP and CKfr were measured in 242 patients with grade 1 NET (G1NET), 38 with grade 2 NET (G2NET), 42 with large cell NEC (LCNEC), 251 with small cell NEC (SCNEC) and in 282 healthy persons. Results were compared with tumour characteristics and survival by means of Receiver Operating Characteristics (ROC) curves and Cox regression analyses.

Results: The largest area under the ROC curve was for CgA (0.86, 0.91 and 0.90, respectively) when comparing patients with G1NET, G2NET and LCNEC with healthy persons. ProGRP showed the highest sensitivity (73%) at 95% specificity in patients with SCNEC. In a multivariate survival analysis, only CKfr was associated with survival ($P < 0.0001$) for patients with well-differentiated NET (G1NET and G2NET). For patients with poorly differentiated NEC, both CKfr and NSE were associated with survival ($P < 0.0001$ and $P = 0.003$, respectively).

Conclusion: Within all histological groups a combination of tumour markers proved to be more informative as diagnostic and prognostic marker than each marker alone. In patients with well-differentiated NET and LCNEC we recommend the use of CgA and CKfr, whilst in patients with SCNEC, proGRP and CKfr are preferred.

* *Corresponding author:* Tel.: +31 20 5122794; fax: +31 20 5122799.
E-mail address: t.korse@nki.nl (C.M. Korse).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2011.08.012

1. Introduction

Classification of neuroendocrine tumours (NET) is an area of ongoing debate.[32,33,39] In 2010, the WHO released a new classification scheme for the digestive system based on histological grade.[4] This classification divides NETs into well-differentiated NET, including grade 1 and 2 (G1NET and G2NET) and poorly differentiated (grade 3) neuroendocrine carcinoma (NEC), including large and small cell neuroendocrine carcinoma (LCNEC and SCNEC). Meanwhilst, the classification for lung tumours has not changed since 1994 and was already based on grade of differentiation.[39] These tumours are divided into typical (comparable to grade 1) and atypical (comparable to grade 2) carcinoid, based on the number of mitoses per high-power field in combination with the presence of necrosis.

Currently, chromogranin A (CgA) is the most frequently used marker, especially in the management of patients with well-differentiated NET, but has some limitations, as various assays are available and an international standardisation is lacking. In addition, elevated CgA levels may be caused by renal or liver failure, and the use of proton pump inhibitors.[13,22] For poorly differentiated NEC, neuron-specific enolase (NSE) is the marker of choice.[8,9,29] NSE is present in neurons and neuroendocrine cells and can therefore serve as biomarker. Progastrin-releasing peptide (proGRP) is a promising tumour marker for small cell lung cancer (SCLC).[23,25] ProGRP is the precursor of the neuropeptide gastrin-releasing peptide (GRP) and its production is increased in SCLCs.[23,25] Molina et al. described elevated levels in patients with NET, but the histological characteristics of these tumours were not mentioned. Cytokeratin fragments (CKfr) are sensitive indicators of tumour cell turnover and thus especially useful in the management of patients with malignancies of epithelial origin.[3] MonoTotal® is an assay used to determine cytokeratin 8, 18 and 19 (CK8, CK18 and CK19) fragments in serum. Moreover, CKfr is associated with angiogenesis factors which may play a role in NET; however, CKfr has not yet been investigated in NET.[5,10]

Therefore, this study evaluates the role of CgA, NSE, proGRP and CKfr in the diagnosis and prognosis of NET.

2. Patients and methods

2.1. Patients

Serum samples of all consecutive patients diagnosed with NETs from 1994 until 2009 were used for the present study with their consent. From each patient one blood sample was taken at the time of initial presentation to our institute. After centrifuging, serum was stored at −30 °C until measurement.

Patients were divided into two main groups according to the WHO classification[4,16,38]: well-differentiated NET (G1NET and G2NET); and poorly differentiated NEC (grade 3) with LCNEC and SCNEC. In addition, age, sex, survival data, stage of disease (limited/extensive in case of SCNEC, or locoregional/metastasised in other histological groups), localisation of the primary tumour and pretreatment (yes/no) were registered.

2.2. Healthy volunteers

Relatives of randomly chosen patients visiting our hospital were asked to donate blood for research, if they had no cancer in the past. Written informed consent was received.

2.3. Serum assays

CgA levels were measured by a solid-phase, two-site immunoradiometric assay, the CGA-RIA kit (CIS Bio-international, Gif-sur-Yvette, France) as described before.[12,18,20] NSE levels were measured with the Modular Analytics E170 (Elecsys module) analyser (Roche Diagnostics, Mannheim, Germany) using the electrochemiluminescence immunoassay (ECLIA) technique.[27] ProGRP levels were measured with the ARCHITECT immunoassay analyzer (Abbott, Wiesbaden, Germany). CKfr were determined by means of the MonoTotal™IRMA assay (IDL Biotech AB, Bromma, Sweden). CKfr measures defined epitopes of CK8, CK18 and CK19, using the monoclonal antibodies 6D7, 3F3 and IDLC4.[5]

2.4. Statistical analyses

Due to the asymmetric distribution of the biomarker values natural log-transformations were applied. Upper limits of normal (ULN) for healthy persons were defined as the 95th percentile according to the guidelines of the Clinical and Laboratory Standards Institute.[15] The sensitivity of the tumour markers for the different histological groups was calculated as the percentage of elevated levels according to the ULN. Differences in sensitivities between the primary sites or between the stages of diseases were calculated with Fisher's Exact Tests. Receiver Operating Characteristic (ROC) curves were constructed to compare the predictive ability of the markers to discriminate the patients with NET of different histological groups from healthy persons.[14]

Martingale residual plots were used to assess the appropriate functional form of the tumour markers in relation to overall survival.[37] Univariate and multivariate Cox proportional hazard models were constructed to determine the association between patient characteristics and tumour markers, with overall survival. The multivariate analysis included the covariates sex, age, metastatic disease, pretreatment, site and grade of differentiation. Additional stepwise analyses were performed to determine why different factors became non-significant in the presence of others. The Kaplan–Meier technique was employed to depict the association between the tumour markers (divided into quartiles) and survival.

3. Results

For this study a total of 855 serum samples were collected: 282 healthy persons, 280 well-differentiated NET and 293 poorly differentiated NEC (Table 1). Almost half of the patients (264/573 = 46%), were pretreated before referral to our

Table 1 – Patient and tumour characteristics according to histological groups.

	Healthy persons	Well-differentiated		Poorly differentiated	
		G1NET	G2NET	LCNEC	SCNEC
Number	282	242	38	42	251
Sex					
Male	142 (50%)	116 (48%)	20 (53%)	18 (43%)	149 (59%)
Female	140 (50%)	126 (52%)	18 (47%)	24 (57%)	102 (41%)
Age, years					
Median	51	61	54	60	60
Range	(18–76)	(21–85)	(26–80)	(23–82)	(27–86)
Pretreatment					
No		120 (50%)	22 (58%)	29 (69%)	138 (55%)
Yes		122 (50%)	16 (42%)	13 (31%)	113 (45%)
Site					
Small bowel		71 (29%)	0 (0%)	0 (0%)	0 (0%)
Appendix		8 (3%)	0 (0%)	0 (0%)	0 (0%)
Colon/rectum		33 (14%)	5 (13%)	3 (7%)	2 (<1%)
Pancreas		17 (7%)	3 (8%)	9 (21%)	0 (0%)
Lung/thorax		23 (10%)	19 (50%)	9 (21%)	200 (80%)
Bladder		0 (0%)	0 (0%)	2 (5%)	20 (8%)
Other Sites		9 (4%)	3 (8%)	6 (14%)	18 (7%)
Unknown		81 (33%)	8 (21%)	13 (31%)	11 (4%)
Stage of disease					
Locoregional/Limited		53 (22%)	6 (16%)	13 (31%)	110 (44%)
Metastatic/Extensive		189 (78%)	32 (84%)	29 (69%)	138 (55%)
Patient Status					
Alive		140 (58%)	15 (39%)	5 (12%)	30 (12%)
Dead		102 (42%)	23 (61%)	37 (88%)	221 (88%)
OS Duration					
Median		3.2	1.7	0.67	0.61
Range		(0.0014–12)	(0.03–9.8)	(0.038–10)	(0.0055–15)
OS					
Median survival		5.8	2.7	0.74	0.64
95% CI		(4.6–8.1)	(1.4–4.9)	(0.55–1.4)	(0.55–0.71)
Events/*N*		102/242	23/38	37/42	221/251

Abbreviations: G1NET, grade 1 neuroendocrine tumours; G2NET, grade 2 neuroendocrine tumours; LCNEC, large cell neuroendocrine carcinoma; SCNEC, small cell neuroendocrine carcinoma; OS, overall survival; CI, confidence interval.

institute. In patients with G1NET, most tumours originated in the gastro-intestinal tract ($n = 129$, 53%). In 81 patients with metastatic disease (33%), the primary tumour site was unknown. The lung was the predominant site in G2NET and SCNEC: 50% and 80%, respectively. In the LCNEC group, most tumours were located in the lung and the pancreas (21% for both sites). Median survival was associated with histological differentiation: 42% of the G1NET patients had died with a median overall survival of 5.8 years, whilst in 88% of the SCNEC patients had died with a median survival of 0.6 years.

In the healthy volunteers, no association was found between the four markers and age. However, there was an association between CKfr and sex (Mann–Whitney: $P < 0.001$). For CKfr the ULN for women was 83 U/l and for men it was 120 U/l. The ULN for CgA, NSE and proGRP were 87 μg/l, 13.0 μg/l and 53 ng/l, respectively.

In well-differentiated NET, the median CgA level was higher compared to poorly differentiated NEC (204 and 73 μg/l, respectively) as shown in Fig. 1. However, in LCNEC the median value of CgA (71 μg/l) did not differ from that in well-differentiated NET ($P = 0.49$). In contrast, the median levels of the other tumour markers (NSE, proGRP and CKfr) were higher in patients with poorly differentiated NEC compared to patients with well-differentiated NET. Although the median proGRP level in the small group of LCNEC ($n = 42$) was similar to those in well-differentiated NET ($P = 0.24$). Extremely high proGRP levels were also found in patients with well-differentiated NET.

CKfr levels were strongly associated with grade of differentiation: median values were 37, 68, 141 and 230 U/l for healthy persons, G1NET, G2NET and LCNEC, respectively. The median level for CKfr in SCNEC was lower than in LCNEC (109 versus 230 U/L, $P = 0.012$).

Fig. 2 shows that the diagnostic performance of the tumour markers in patients with NET compared to healthy controls differed between the histological groups. In patients with well-differentiated NET the highest area under the curve (AUC) was found for CgA: 0.86 (95% CI, 0.82–0.89) and 0.91 (95% CI, 0.84–0.98) for G1NET and G2NET, respectively. Comparable AUCs were found for CgA, NSE and CKfr in the LCNEC group: 0.90 (95% CI, 0.83–0.96), 0.83 (95% CI, 0.75–0.92) and 0.88 (95% CI, 0.82–0.95), respectively, whilst the AUC for proGRP was much lower: 0.66 (95% CI, 0.56–0.76). For the SCNEC the AUCs for proGRP and CKfr did not differ: 0.86 (95% CI, 0.82–0.90) and 0.87 (95% CI, 0.84–0.90), respectively). However, at 95% specificity, the sensitivity of proGRP was much higher than that of CKfr (73% and 51%, respectively).

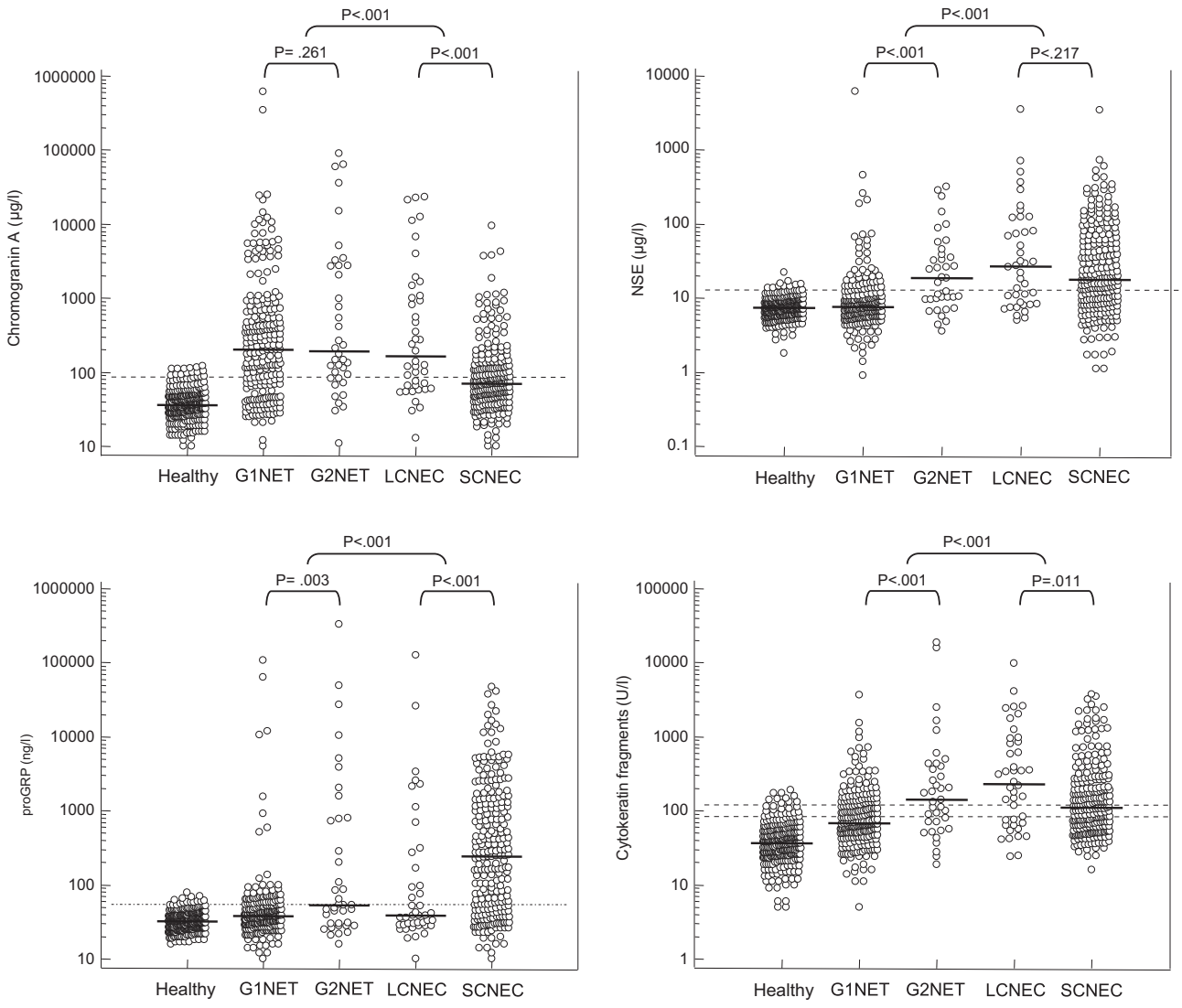

Fig. 1 – Scatterplots of chromogranin A, neuron specific enolase (NSE), progastrin releasing peptide (proGRP) and cytokeratin fragments CK8, CK18 and CK19 in healthy persons, in patients with grade 1 and grade 2 neuroendocrine tumours (G1NET and G2NET, respectively), and in patients with large cell and small cell neuroendocrine tumours (LCNEC and SCNEC, respectively). Comparisons are made with Mann–Whitney tests. Dotted lines present the upper limit of normal.

In general, there was no association between CgA and NSE and the primary site, except for CgA in the SCNEC: sensitivity (i.e. percentage of elevated values) in lung tumours was lower compared to non-lung tumours (33% and 54%, respectively) (Table 2). In the poorly differentiated group, an association was found between primary site and proGRP: in the LCNEC the sensitivity in the tumours of the gastrointestinal tract was 17% compared with 53% in the tumours of the non-gastrointestinal tract. In the SCNEC the sensitivity in the lung was 76% compared with 54% in the non-lung tumours. The sensitivity of the CKfr in the tumours of the gastrointestinal tract appeared to be higher than in the other tumours, but was not statistically proven.

Within all histological groups the percentage elevated values of all four markers were higher in patients with distant metastases than in patients with locoregional disease.

3.1. *Survival analyses*

The Martingale residual plots indicate that in the well-differentiated group NSE and proGRP appeared to have approximately the same thresholds as defined in the healthy group as ULN (13.0 µg/l and 53 ng/l, respectively). Both CgA and CKfr appeared to be log-linearly related to overall survival. In the univariate Cox regression analyses, sex, age, metastatic disease, primary site, grade of differentiation, and all four tumour markers were associated with overall survival (Table 3). In the multivariate model only age, metastatic disease and CKfr were independently associated with overall survival ($P = 0.0004$, $P = 0.01$ and $P < 0.0001$, respectively). Fig. 3A and B show the survival curves for CgA and CKfr divided by quartiles for patients with well-differentiated NET.

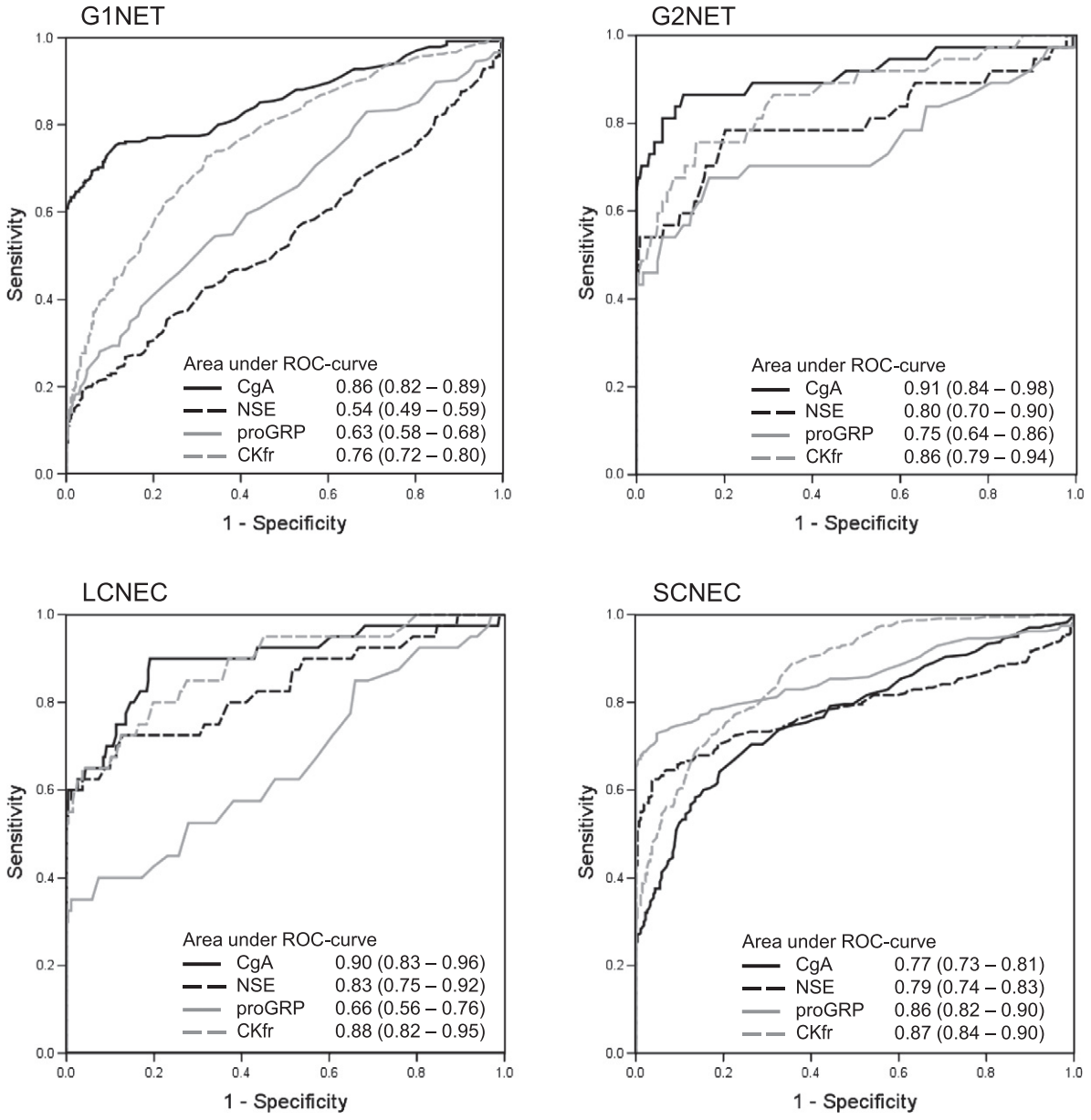

Fig. 2 – ROC curves illustrating the diagnostic performance of chromogranin A (CgA), neuron specific enolase (NSE), progastrin releasing peptide (proGRP) and cytokeratin fragments CK8, CK18 and CK19 (CKfr) when comparing patients with grade 1 and grade 2 neuroendocrine tumours (G1NET and G2NET, respectively), large cell neuroendocrine carcinoma (LCNEC) and small cell neuroendocrine carcinoma (SCNEC) with healthy persons. Area under the ROC curves are presented with 95% confidence interval.

In the patients with poorly differentiated NEC, the Martingale residuals indicate that CgA and proGRP had a dichotomised association with survival, whilst NSE and CKfr were log-linearly related with survival. In the univariate Cox regression analyses, stage of disease, pretreatment and all four markers were associated with survival. In the multivariate model, stage of disease, pretreatment, site of primary tumour, NSE and CKfr were significantly associated with overall survival. Patients with lung tumours tended to have a poorer survival than patients with other primary tumour sites (HR = 1.8, $P = 0.0004$) (Table 3). Fig. 3C and D show the survival curves for CgA and CKfr divided by quartiles.

4. Discussion

This is the first study in which the tumour markers progastrin-releasing peptide (proGRP) and cytokeratin fragments CK8, CK18 and CK19 (CKfr) in addition to the established markers CgA and NSE were investigated in patients with NET. Recently published studies demonstrated the relevance of classifying the NET according to improved histopathological grading.[21,30,34] Therefore, this grading system (from G1 to G3) was also applied in our study group.

In recent years CgA showed the highest sensitivities in patients with well-differentiated NET for G1NET and G2NET

Table 2 – Sensitivities (percentage elevated values according to ULN) of Chromogranin A, NSE, proGRP and Cytokeratin fragments (CK8, CK18 and CK19), in patients with neuroendocrine tumours according to histological grade, primary site and stage of disease.

		CgA (μg/l) (%)	P-value	NSE (μg/l) (%)	P-value	proGRP (ng/l) (%)	P-value	CKfr (U/l) (%)	P-value
G1NET1 (*N* = 242)		68		19		24			
Site	GI tract (*N* = 129)	60	0.33	17	1.00	15	0.19	29	1.00
	Non-GI tract (*N* = 32)	50		16		25		28	
Stage	Locoregional (*N* = 53)	28	<0.001	8	0.02	19	0.37	15	<0.001
	Distant (*N* = 189)	79		23		25		41	
G2NET (**N** = 38)		74		54		47		54	
Site	GI tract (*N* = 8)	63	0.67	63	0.68	38	0.42	75	0.10
	Non-GI tract (*N* = 22)	73		46		59		36	
Stage	Locoregional (*N* = 6)	50	0.31	33	0.38	50	1.00	17	0.08
	Distant (*N* = 32)	78		58		47		61	
LCNEC (**N** = 42)		67		63		36		64	
Site	GI tract (*N* = 12)	67	0.72	36	0.25	17	0.06	75	0.27
	Non-GI tract (*N* = 17)	59		65		53		53	
Stage	Locoregional (*N* = 13)	46	0.08	50	0.31	46	0.49	38	0.04
	Distant (*N* = 29)	76		68		31		76	
SCNEC (**N** = 251)		37		62		73		50	
Site	Non-Lung (*N* = 40)	54	0.02	70	0.22	54	0.006	54	0.50
	Lung (*N* = 200)	33		59		76		48	
Stage	Locoregional (*N* = 110)	26	0.001	45	<0.001	63	0.002	31	<0.001
	Distant (*N* = 138)	46		76		81		64	

Abbreviations: ULN, upper limit of normal; G1NET, grade 1 neuroendocrine tumours; G2NET, grade 2 neuroendocrine tumours; LCNEC, large cell neuroendocrine carcinoma; SCNEC, small cell neuroendocrine carcinoma; CgA, chromogranin A; NSE, Neuron-specific enolase; proGRP, progastrin-releasing peptide; CKfr, cytokeratin fragments CK8, CK18 and CK19.

(68% and 74%, respectively); these values are similar to those reported earlier by our group (74%)[20] and by others (68–80%).[2,11,24,29] We found a much lower sensitivity (37%) in the 251 patients with SCNEC. The same low sensitivity was reported by Cimitan et al. (37%) who compared CgA levels with somatostatin receptor scintigraphy findings in 20 patients with poorly differentiated NEC of whom 14 had SCNEC.[7] This low sensitivity of CgA in SCNEC might be explained by the rarity of large dense-core granules, which release CgA in the blood circulation.[22] The sensitivity of 67% in 42 patients included in this study, shows that CgA is a suitable marker for LCNEC.

NSE proved to be associated with the poorly differentiated NEC (sensitivity was 63% in LCNEC and 62% and SCNEC). A large difference was observed within patients with well-differentiated NET: 19% in G1NET and 54% in G2NET. These values are comparable to those in other studies (range 18%-43%), although no information was given about the grading of these tumours.[1,11,29] Due to a sensitivity of 54% in G2NET, NSE might be of additional value to CgA.

ProGRP is established as a promising marker for small cell lung cancer.[23,28,36] In the present study, proGRP was the most sensitive marker in patients with SCNEC (73%). However, in patients with non-lung SCNEC the sensitivity was only 54%. In spite of a reasonable sensitivity for non-lung tumours (54%), proGRP is a more suitable maker for patients with lung tumours (76%). This confirmed our results for proGRP in an earlier study, in which a high proGRP indicated a primary tumour in the lung.[19]

Cytokeratins in tissue as additional prognostic markers are reported[35], but thus far not in NET. The cytokeratin marker MonoTotal® measures the cytokeratin fragments CK8, CK18 and CK19 in serum and was initially developed for the management of patients with non-small cell lung cancer (NSCLC).[10] Another cytokeratin fragments assay, Tissue Polypeptide Antigen (TPA), measures the same fragments. TPA has been used as a serological marker in various epithelial cell-associated carcinoma. A direct comparison of MonoTotal® and TPA, only presented in an abstract, shows that MonoTotal® has a better sensitivity than TPA: 71% versus 53% at 95% specificity in patients with NSCLC (dr. L. Holubec, Charles University, Czech Republic). Cytokeratins are intermediate filament proteins found in the cytoskeleton and specific for epithelial cells. The expression profile of cytokeratins usually remains stable during transformation into malignant cells and might be of additional value as biomarker in pathology.[3,6] Cytokeratins are also useful as serum tumour marker for monitoring treatment as they reflect tumour cell turnover.[3,10] In the present study, a strong association between CKfr and histological grade was found, which underlines that CKfr might indeed be related to tumour cell turnover.

The association of CKfr with tumour activity was also found when we investigated the association of tumour markers with overall survival. CKfr was the only independent prognostic tumour marker for survival ($P < 0.0001$) in well-differentiated NET. As CKfr reflects tumour activity and CgA tumour burden, they provide essential information about tumour growth.[17]

Table 3 – Cox regression analyses of prognostic factors in patients with neuroendocrine tumours.

	Events/*N*	Well-differentiated							Events/*N*	Poorly differentiated					
		Univariate			Multivariate					Univariate			Multivariate		
		HR	95% CI	*P*-value	HR	95% CI	*P*-value			HR	95% CI	*P*-value	HR	95% CI	*P*-value
Sex															
Male	69/136	1		0.04	1		0.07	Male	143/167	1		0.93	1		0.46
Female	56/144	0.7	0.5–1.0		0.7	0.5–1.0		Female	115/126	1	0.8–1.3		1.1	0.8–1.4	
Age															
Years per decade	125/280	1.5	1.3–1.8	<0.0001	1.4	1.2–1.7	0.0004		258/293	1.1	1.0–1.2	0.17	1.01	1.0–1.0	0.18
Metastases															
No	11/59	1		<0.0.001	1		0.01	Limited	96/123	1		<0.0001	1		0.0004
Yes	114/221	4.2	2.2–7.8		2.3	1.1–4.5		Extensive	162/170	2.5	1.9–3.2		1.8	1.3–2.4	
Pretreatment															
No	66/142	1		0.47	1		0.28	No	143/167	1		<0.0001	1		<0.0001
Yes	59/138	0.9	0.6–1.2		1.2	0.8–1.8		Yes	115/126	1.9	1.5–2.5		2.5	1.9–3.3	
Primary site															
Small bowel	23/71	1		0.01	1		0.28								
Other sites	30/78	1.3	0.8–2.3		1.5	0.8–2.6		Other sites	48/60	1		0.14	1		0.001
Lung	18/42	1.7	0.9–3.1		2	1.0–3.9		Lung	188/209	1.4	1.0–1.9		1.8	1.3–2.6	
Unknown	54/89	2.2	1.4–3.7		1.2	0.7–2.1		Unknown	22/24	1.2	0.7–2.0		0.9	0.5–1.5	
Histological grade															
Grade 1	102/242	1		0.001	1		0.42	LCNEC	37/42	1		0.49	1		0.89
Grade 2	23/38	2.4	1.5–3.8		1.3	0.7–2.3		SCNEC	221/251	1.1	0.8–1.6		1	0.7–1.6	
CgA															
								Normal	147/172	1			1		0.57
log	125/280	1.4	1.3–1.5	<0.0001	1.1	1.0–1.2	0.08	Elevated	111/121	1.5	1.2–1.9	0.001	1.1	0.8–1.4	
NSE															
Normal	79/211	1			1		0.33					<0.0001			0.0003
Elevated	46/69	3.5	2.4–5.1	<0.0001	1.3	0.8–2.1		log	258/293	1.4	1.2–1.5		1.2	1.1–1.4	
proGRP															
Normal	84/204	1		0.001	1		0.08	normal	79/96	1		0.04	1		0.69
Elevated	41/76	2.0	1.4–2.9		1.5	1.0–2.2		elevated	179/197	1.3	1.0–1.7		1.1	0.8–1.5	
CKfr															
log	125/280	2.2	1.9–2.6	<0.0001	1.8	1.4–2.3	<0.0001	log	258/293	1.7	1.5–1.9	<0.0001	1.5	1.3–1.8	<0.0001

*Abbreviations:*HR, hazard ratio; CI, Confidence interval; CgA, chromogranin A; NSE, neuron specific enolase; proGRP, progastrin-releasing peptide; CKFR, Cytokeratin fragments CK8, CK18 and CK19; LCNEC, large cell neuroendocrine carcinoma; SCNEC, small cell neuroendocrine carcinoma

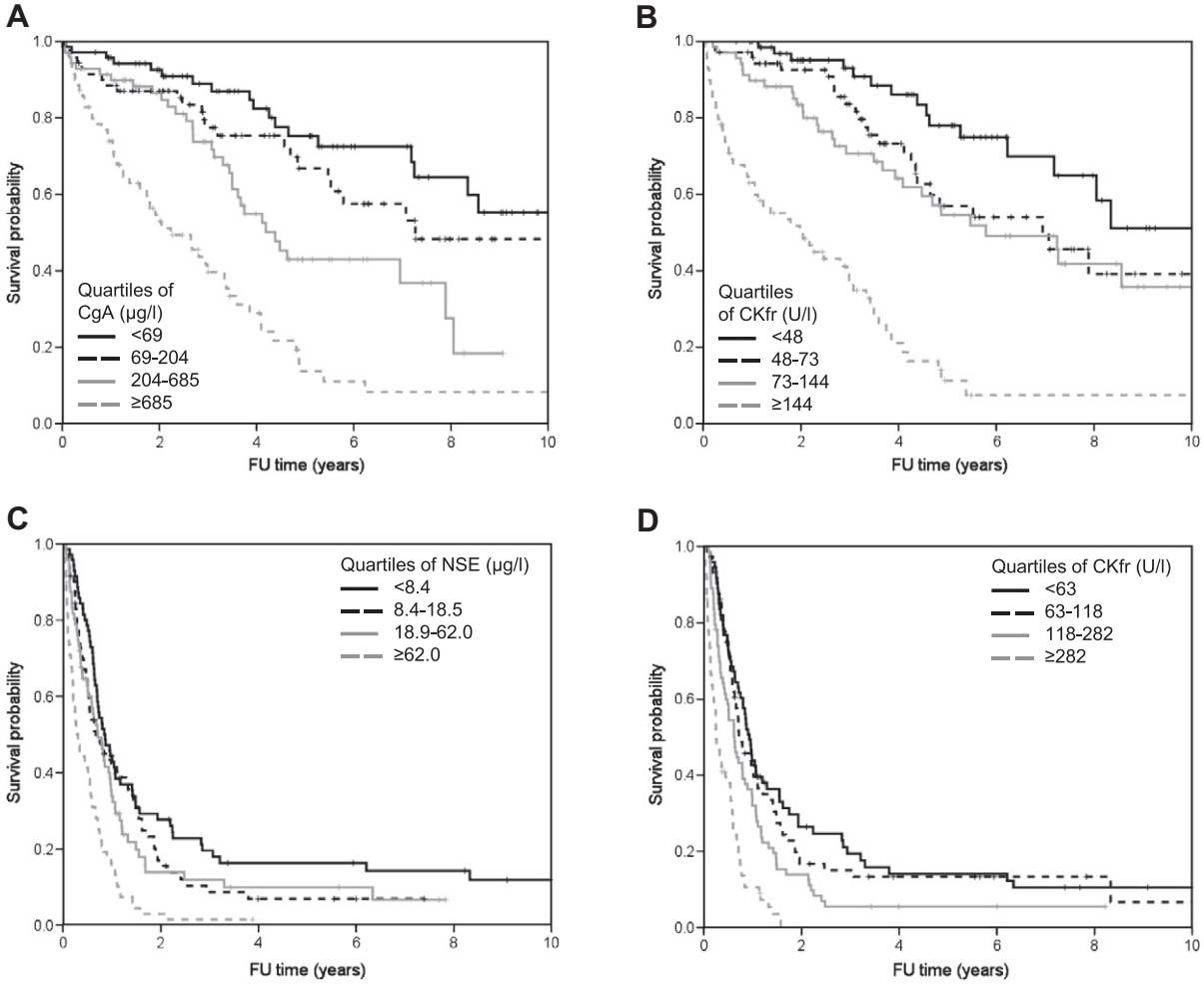

Fig. 3 – Overall survival curves according to the Kaplan–Meier method stratified by the quartiles of the tumour markers. Shown are chromogranin A (A), cytokeratin fragments (CKfr) (B) in patients with well-differentiated neuroendocrine tumours (grade 1 and 2) and NSE (C) and cytokeratin fragments (D) in patients with poorly-differentiated neuroendocrine carcinoma (large cell and small cell carcinoma).

CgA and CKfr were also the most informative markers in LCNEC. Although NSE was also an independent prognostic variable for overall survival, the clinician might consider CKfr or NSE in addition to CgA. This is in line with Molina et al. who reported that NSE had a strong prognostic value, independent of clinical parameters (such as age, Karnofsky Index, symptoms and smoking status) in NSCLC.[26] Since NSE in erythrocytes and platelets leads to elevated results in haemolysed[31], CKfr might be a better alternative as additional tumour marker to CgA.

In the present study proGRP was the most accurate marker to discriminate SCNEC from healthy persons, especially in lung tumours; this finding is in agreement with others.[25,36] Thus, the combination of proGRP, the most sensitive marker, and CKfr indicative of tumour is informative for the course of disease. NSE might also be added, as it is an independent prognostic variable for overall survival.

Since tumour markers are useful in evaluation of patient management, the markers should be further examined in a longitudinal study.

In conclusion, this cross-sectional study provides promising information about the four markers with respect to their diagnostic and prognostic value for the different histopathological groups: CgA is the most sensitive marker for diagnosing well-differentiated NET and LCNEC, and proGRP for SCNEC. CKfr is an important prognostic marker for survival in all patients with NET. Within all the histological groups a combination of tumour markers is more informative than each marker alone: in patients with well-differentiated NET and LCNEC, we recommend CgA and CKfr, whilst in patients with SCNEC, proGRP and CKfr are preferred.

Role of the funding source

Novartis Pharma B.V. and Abbott Diagnostics had no role in the study design or in the collection, analysis and interpretation of data.

Conflict of interest statement

None declared.

Acknowledgements

The authors would like to thank Abbott Diagnostics and Novartis Pharma B.V. for their funding.

REFERENCES

1. Bajetta E, Catena L, Procopio G, et al. Is the new WHO classification of neuroendocrine tumours useful for selecting an appropriate treatment? *Ann Oncol* 2005;**16**:1374–80.
2. Bajetta E, Ferrari L, Martinetti A, et al. Chromogranin A, neuron specific enolase, carcinoembryonic antigen, and hydroxyindole acetic acid evaluation in patients with neuroendocrine tumors. *Cancer* 1999;**86**:858–65.
3. Barak V, Goike H, Panaretakis KW, Einarsson R. Clinical utility of cytokeratins as tumor markers. *Clin Biochem* 2004;**37**:529–40.
4. Bosman FT, Carneiro F, Hruban RH, Theise ND. *WHO Classification of the tumours of the digestive system*. 4th ed. Lyon, France: IARC Press; 2010.
5. Brattstrom D, Wagenius G, Sandstrom P, et al. Newly developed assay measuring cytokeratins 8, 18 and 19 in serum is correlated to survival and tumor volume in patients with esophageal carcinoma. *Dis Esophagus* 2005;**18**:298–303.
6. Chu PG, Weiss LM. Keratin expression in human tissues and neoplasms. *Histopathology* 2002;**40**:403–39.
7. Cimitan M, Buonadonna A, Cannizzaro R, et al. Somatostatin receptor scintigraphy versus chromogranin A assay in the management of patients with neuroendocrine tumors of different types: clinical role. *Ann Oncol* 2003;**14**:1135–41.
8. De Herder WW. Biochemistry of neuroendocrine tumours. *Best Pract Res Clin Endocrinol Metab* 2007;**21**:33–41.
9. Eriksson B, Oberg K, Stridsberg M. Tumor markers in neuroendocrine tumors. *Digestion* 2000;**62**(Suppl 1):33–8.
10. Eriksson P, Brattstrom D, Hesselius P, et al. Role of circulating cytokeratin fragments and angiogenic factors in NSCLC patients stage IIIa–IIIb receiving curatively intended treatment. *Neoplasma* 2006;**53**:285–90.
11. Giovanella L, Ceriani L, Bandera M, Garancini S. Immunoradiometric assay of chromogranin A in the diagnosis of small cell lung cancer: comparative evaluation with neuron-specific enolase. *Int J Biol Markers* 2001;**16**:50–5.
12. Giovanella L, La Rosa S, Ceriani L, et al. Chromogranin-A as a serum marker for neuroendocrine tumors: comparison with neuron-specific enolase and correlation with immunohistochemical findings. *Int J Biol Markers* 1999;**14**:160–6.
13. Giusti M, Sidoti M, Augeri C, Rabitti C, Minuto F. Effect of short-term treatment with low dosages of the proton-pump inhibitor omeprazole on serum chromogranin A levels in man. *Eur J Endocrinol* 2004;**150**:299–303.
14. Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. *Radiology* 1982;**143**:29–36.
15. Horowitz GL, Altail S, Boyd JC, Zakowski J. *Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory. 3rd ed.*. Pennsylvania: Clinical and Laboratory Standards Institute; 2008.
16. Klimstra DS, Modlin IR, Coppola D, Lloyd RV, Suster S. The pathologic classification of neuroendocrine tumors: a review of nomenclature, grading, and staging systems. *Pancreas* 2010;**39**:707–12.
17. Kolby L, Bernhardt P, Sward C, et al. Chromogranin A as a determinant of midgut carcinoid tumour volume. *Regul Pept* 2004;**120**:269–73.
18. Korse CM, Bonfrer JM, Aaronson NK, Hart AA, Taal BG. Chromogranin A as an alternative to 5-hydroxyindoleacetic acid in the evaluation of symptoms during treatment of patients with neuroendocrine tumors. *Neuroendocrinology* 2009;**89**:296–301.
19. Korse CM, Taal BG, Bonfrer JM, et al. An elevated progastrinreleasing peptide level in patients with well-differentiated neuroendocrine tumours indicates a primary tumour in the lung and predicts a shorter survival. *Ann Oncol* 2011.
20. Korse CM, Taal BG, de Groot CA, Bakker RH, Bonfrer JM. Chromogranin A and N-terminal pro-brain natriuretic peptide: an excellent pair of biomarkers for diagnostics in patients with neuroendocrine tumor. *J Clin Oncol* 2009;**27**:4293–9.
21. La Rosa S, Inzani F, Vanoli A, et al. Histologic characterization and improved prognostic evaluation of 209 gastric neuroendocrine neoplasms. *Human Pathol* 2011.
22. Modlin IM, Gustafsson BI, Moss SF, et al. Chromogranin A – biological function and clinical utility in neuro endocrine tumor disease. *Ann Surg Oncol* 2010;**17**:2427–43.
23. Molina R. ProGRP: a new biomarker for small cell lung cancer. *Eur J Clin Med Oncol* 2010;**2**:1–8.
24. Molina R, Alvarez E, Aniel-Quiroga A, et al. Evaluation of chromogranin A determined by three different procedures in patients with benign diseases, neuroendocrine tumors and other malignancies. *Tumour Biol* 2011;**32**:13–22.
25. Molina R, Auge JM, Filella X, et al. Pro-gastrin-releasing peptide (proGRP) in patients with benign and malignant diseases: comparison with CEA, SCC, CYFRA 21–1 and NSE in patients with lung cancer. *Anticancer Res* 2005;**25**:1773–8.
26. Molina R, Filella X, Auge JM, et al. Tumor markers (CEA, CA 125, CYFRA 21–1, SCC and NSE) in patients with non-small cell lung cancer as an aid in histological diagnosis and prognosis. Comparison with the main clinical and pathological prognostic factors. *Tumour Biol* 2003;**24**:209–18.
27. Muley T, Ebert W, Stieber P, et al. Technical performance and diagnostic utility of the new Elecsys neuron-specific enolase enzyme immunoassay. *Clin Chem Lab Med* 2003;**41**:95–103.
28. Niho S, Nishiwaki Y, Goto K, et al. Significance of serum pro-gastrin-releasing peptide as a predictor of relapse of small cell lung cancer: comparative evaluation with neuron-specific enolase and carcinoembryonic antigen. *Lung cancer* 2000;**27**:159–67.
29. Nobels FR, Kwekkeboom DJ, Coopmans W, et al. Chromogranin A as serum marker for neuroendocrine neoplasia: comparison with neuron-specific enolase and the alpha-subunit of glycoprotein hormones. *J Clin Endocrinol Metab* 1997;**82**:2622–8.
30. Pape UF, Jann H, Muller-Nordhorn J, et al. Prognostic relevance of a novel TNM classification system for upper gastroenteropancreatic neuroendocrine tumors. *Cancer* 2008;**113**:256–65.
31. Ramont L, Thoannes H, Volondat A, et al. Effects of hemolysis and storage condition on neuron-specific enolase (NSE) in cerebrospinal fluid and serum: implications in clinical practice. *Clin Chem Lab Med* 2005;**43**:1215–7.
32. Rindi G, Kloppel G, Alhman H, et al. TNM staging of foregut (neuro)endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 2006;**449**:395–401.
33. Rindi G, Kloppel G, Couvelard A, et al. TNM staging of midgut and hindgut (neuro) endocrine tumors: a consensus proposal including a grading system. *Virchows Arch* 2007;**451**: 757–62.
34. Scarpa A, Mantovani W, Capelli P, et al. Pancreatic endocrine tumors: improved TNM staging and histopathological grading permit a clinically efficient prognostic stratification of patients. *Mod Pathol* 2010;**23**:824–33.

35. Schmitt AM, Anlauf M, Rousson V, et al. WHO 2004 criteria and CK19 are reliable prognostic markers in pancreatic endocrine tumors. *Am J Surg Pathol* 2007;**31**:1677–82.
36. Sunaga N, Tsuchiya S, Minato K, et al. Serum pro-gastrin-releasing peptide is a useful marker for treatment monitoring and survival in small-cell lung cancer. *Oncology* 1999;**57**:143–8.
37. Therneau T, Grambsch P. *Modeling survival data: extending the Cox model*. 1st ed. New York: Springer-Verlag; 2000.
38. Travis WD, Rush W, Flieder DB, et al. Survival analysis of 200 pulmonary neuroendocrine tumors with clarification of criteria for atypical carcinoid and its separation from typical carcinoid. *Am J Surg Pathol* 1998;**22**:934–44.
39. Travis W, Colby T, Corrin B, et al. *Histological Typing of Lung and Pleural Tumours. WHO International Histological Classification of Tumours*. 3rd ed. Berlin: Springer; 1999.