The Impact of Neural Invasion Severity in Gastrointestinal Malignancies

A Clinicopathological Study

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Objectives: Because neural invasion (NI) is still inconsistently reported and not well characterized within gastrointestinal malignancies (GIMs), our aim was to determine the exact prevalence and severity of NI and to elucidate the true impact of NI on patient's prognosis.

Background: The union internationale contre le cancer (UICC) recently added NI as a novel parameter in the current TNM classification. However, there are only a few existing studies with specific focus on NI, so that the distinct role of NI in GIMs is still uncertain.

Materials and Methods: NI was characterized in approximately 16,000 hematoxylin and eosin tissue sections from 2050 patients with adenocarcinoma of the esophagogastric junction (AEG)-I-III, squamous cell carcinoma (SCC) of the esophagus, gastric cancer (GC), colon cancer (CC), rectal cancer (RC), cholangiocellular cancer (CCC), hepatocellular cancer (HCC), and pancreatic cancer (PC). NI prevalence and severity was determined and related to patient's prognosis and survival.

Results: NI prevalence largely varied between HCC/6%, CC/28%, RC/34%, AEG-I/36% and AEG-II/36%, SCC/37%, GC/38%, CCC/58%, and AEG-III/65% to PC/100%. NI severity score was uppermost in PC (24.9 ± 1.9) and lowest in AEG-I (0.8 ± 0.3). Multivariable analyses including age, sex, TNM stage, and grading revealed that the prevalence of NI was significantly associated with diminished survival in AEG-II/III, GC, and RC. However, increasing NI severity impaired survival in AEG-II/III and PC only.

Conclusions: NI prevalence and NI severity strongly vary within GIMs. Determination of NI severity in GIMs is a more precise tool than solely recording the presence of NI and revealed dismal prognostic impact on patients with AEG-II/III and PC. Evidently, NI is not a concomitant side feature in GIMs and, therefore, deserves special attention for improved patient stratification and individualized therapy after surgery.

Keywords: gastrointestinal malignancies, neural invasion severity, perineural invasion, prognosis, survival

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Gastrointestinal malignancies GIMs are the second leading cause of cancer-related deaths, with an utmost bad prognosis, especially in pancreatic cancer (PC).[1] The most effective and promising chance for curative treatment in nearly all GIMs is neoadjuvant treatment, followed by resection or resection by adjuvant treatment. Currently, decision whether or not to give neoadjuvant/adjuvant therapy is mostly dependent on the TNM classification. Therefore, clinical and histopathological analysis is inevitable to avoid undertreatment and improve survival. Here, easy and accessible novel prognostic tools beyond the TNM classification may be helpful to attain better patient's stratification and thus more individualized and efficient cancer therapy. Neural invasion (NI) might represent such a novel tool.

Presently, in head/neck and prostate tumors, NI is recognized as an important route of cancer spread, and its presence has high impact on treatment.[2,3] Since the potential impact of NI has been recognized, the UICC has recently added NI as a novel parameter in the current TNM classification.[4] The incorporation of NI into the TNM classification was an important step, but until today, we do not exactly know what kind of additional information we gain and furthermore, how or whether we should react and change our therapy regimes according to the stage of NI. However, current literature on the existence of NI in GIM is confusing. There exists a great variability of NI prevalence rates not only between different GIMs but also even within 1 tumor entity. For example, in colorectal cancer, NI is present with a prevalence rate of 6% to 26%, in gastric cancer (GC) widely ranging between 2% and 76%, and in PC between 45% and 98%.[5–10] These great discrepancies are due to unfocused studies, divergent NI definitions, and especially due to lack of a common definition for NI in GIMs. Thus, the importance of a uniform characterization of NI within GIMs is urging, and the characterization of its true impact on patient prognosis within GIM is mandatory.

Therefore, in the present study, we aimed to establish a comprehensive and reliable characterization of NI and thereby assessing its true prevalence in GIMs. Furthermore, a robust NI severity score system was used to define the impact of NI on survival and prognosis within GIMs.

MATERIALS AND METHODS

Patient and Specimen Selection

In the present study, adenocarcinomas of the esophageal junction (AEG)-I-III, squamous cell carcinomas (SCCs) of the esophagus, GC, colon cancer (CC), rectal cancers (RCs), cholangiocellular

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cancers (CCCs), hepatocellular cancer (HCC) and PCs were included. In order not to distort the original state of NI with any kind of neoadjuvant therapy, patients with neoadjuvant therapy, additional malignancies, and emergency operations were excluded from the study. Thus, a total of 2050 patients with GIMs were consecutively selected between January 1987 and June 2009 at our institution (Table 1). The median age was 65 ± 11.6 years. Informed consent for tissue collection was obtained before surgery. Adjuvant therapy was initiated in 5% (3/58) in AEG-II, 3% (2/65) in AEG-III, 3% (6/184) in GC, 19% (19/114) in CCC, 6% (3/47) in HCC, 100% (132/132) in PC, 36% (385/1075) in CC, and 41% (77/187) in RC. Patients with AEG-I and SCC did not receive any kind of adjuvant therapy. To mimic daily routine pathological work, all hematoxylin and eosin–stained sections from regular pathologic examination were used to perform the comprehensive histopathological reevaluation. According to previous publications, at least 3 primary tumor slides and all slides showing lymph nodes and their surrounding tissue were used to classify and characterize NI.[11,12] On average, 8 tissue sections were analyzed for NI prevalence and severity in each patient with a total of approximately 16,000 sections. Histopathological analysis was performed by 3 independent observers (F.L., K.M., and J.G.D.) blinded to patient survival data, followed by resolution of any differences by joint review and consultation with third observers (K.B., R.L., and F.B.), as reported before.[11–13]

Definition and Establishment of a Novel NI Severity Score in GIMs

NI severity score was determined for all GIMs, as recently shown.[11–13] Three NI stages were determined: (i) *epineural tumor associations* (ENA), lesions in which cancer cells directly touch the epineural sheet without penetrating the perineurium (Fig. 1A); (ii) *perineural invasion* (PNI), defined as cancer cells within the perineurium (Fig. 1B); and (iii) *endoneural invasion* (ENI), as infiltration of cancer cells into the endoneurium (Fig. 1C). The presence of cancer cells inside the ganglionic capsules of the Auerbach plexus or the direct contact with the myenteric plexus cells was separately noted as *invasion to Auerbach plexus* (Fig. 1D).

All nerves in the entire tissue specimens were categorized and scored as *non–cancer-invaded* (0), *ENA* (1), *PNI* (2), or *ENI* (3). Individual NI severity score was generated by adding the number of invaded nerves (n) with the respective NI category, as shown in the following formula: Individual *NI severity score* = n(ENA) × 1 + n(PNI) × 2 + n(ENI) × 3, as recently shown.[11,12] The final NI severity score of each individual patient was calculated by the mean of the 3 different scores of the 3 independent observers.

Statistical Analysis

The χ^2 test was used for comparisons of frequency data between independent patient groups. The Mann-Whitney *U* test was conducted to compare level of semiquantitative data between 2 unrelated samples. Survival distribution was estimated and illustrated according to Kaplan-Meier method. The log-rank test was applied to assess survival differences between independent groups. Cox regression analysis was used for multivariable analysis, and resulting estimates of hazard ratios (HRs) were provided with 95% confidence intervals (CIs). Based on the fitted Cox regression models, predicted survival probability curves for 6, 12, 24, and 48 months were calculated and displayed in dependence on the observed NI severity score levels. Following general recommendations on regression modeling strategies, we considered well-established prognostic factors as adjustment variables in the multivariable analysis.[14] These variables included age, pathologic tumor stage, tumor grading, metastasis status, nodal status, and sex. For 2 reasons, we did not consider *P* value–based selection of potential confounder variables: first, due to the large sample size of the study data set, even clinically irrelevant factor-outcome associations would reveal small *P* values. Second, it has been shown that associational criteria are neither necessary nor sufficient to demonstrate presence or absence of confounding.[15] Interrater reliability regarding the NI severity score assessment was evaluated by the intraclass correlation coefficient (ICC). All tests were 2-sided, and a *P* value of less than 0.05 was considered to indicate statistical significance. No correction of *P* values was conducted to correct for multiple test issues.[16]

RESULTS

Prevalence of NI Varies Within GIMs

In the upper gastrointestinal tract, NI was present in 36% of patients with AEG-I, in 36% of patients with AEG-II, in 65% of patients with AEG-III, in 37% of patients with SCC, and in 38% of patients with GC. In the lower gastrointestinal tract, 28% of patients with CC and 34% of patients with RC exhibited NI. Patients with hepatobiliary cancer revealed NI in 6% of patients with HCC and in 58% of patients with CCC. Impressively, in PC, all patients presented NI (100%; Table 1).

The prevalence rates of Auerbach plexus were 29% in AEG-I, 28% in AEG-II, 29% in AEG-III, 14% in SCC and in GC and CC, and finally 12% in RC.

PC Has the Highest NI Severity Among All GIMs

Interrater reliability regarding the NI severity score assessment was evaluated by the intraclass correlation coefficient and revealed

TABLE 1. Patient Characteristics

Entity	Patients n =	NI Positive %	Sex (%) M:F	Age Media ± SD	pT (%)				pN (%)				cM (%)		G (%)			
					1	2	3	4	0	1	2	3	0	1	1	2	3	4
AEG I	96	36	94:6	65 ± 11.6	44	32	24	0	55	39	6	0	96	4	4	41	54	1
AEG II	58	36	76:24	66 ± 11.9	17	55	23	5	31	43	10	16	85	15	3	29	64	4
AEG III	65	65	66:34	72 ± 13.2	8	48	35	9	28	23	23	26	74	26	2	19	74	5
SCC esophagus	92	37	78:22	55 ± 9.1	36	26	34	4	46	53	1	0	86	14	1	34	61	4
Gastric cancer	184	38	55:45	67 ± 12.7	32	35	39	4	48	23	16	13	80	20	2	24	70	4
Colon cancer	1075	28	57:43	66 ± 11.6	10	13	53	24	56	21	23	—	75	25	3	59	37	1
Rectal cancer	187	34	63:37	62 ± 10.9	14	20	47	19	56	23	21	—	91	9	1	70	29	0
CCC	114	58	49:51	64 ± 10.3	10	37	44	9	48	42	10	0	97	3	3	44	50	3
HCC	47	6	68:32	61 ± 9.3	23	30	45	2	30*	0	—	—	100	0	7	59	32	2
Pancreatic cancer	132	100	59:41	64 ± 9.6	2	4	90	4	16	84	—	—	89	11	2	57	41	0†

*Only 14 patients had resected lymph nodes, all without infiltration of tumor cells.
†Missing data for grading of 23 patients.

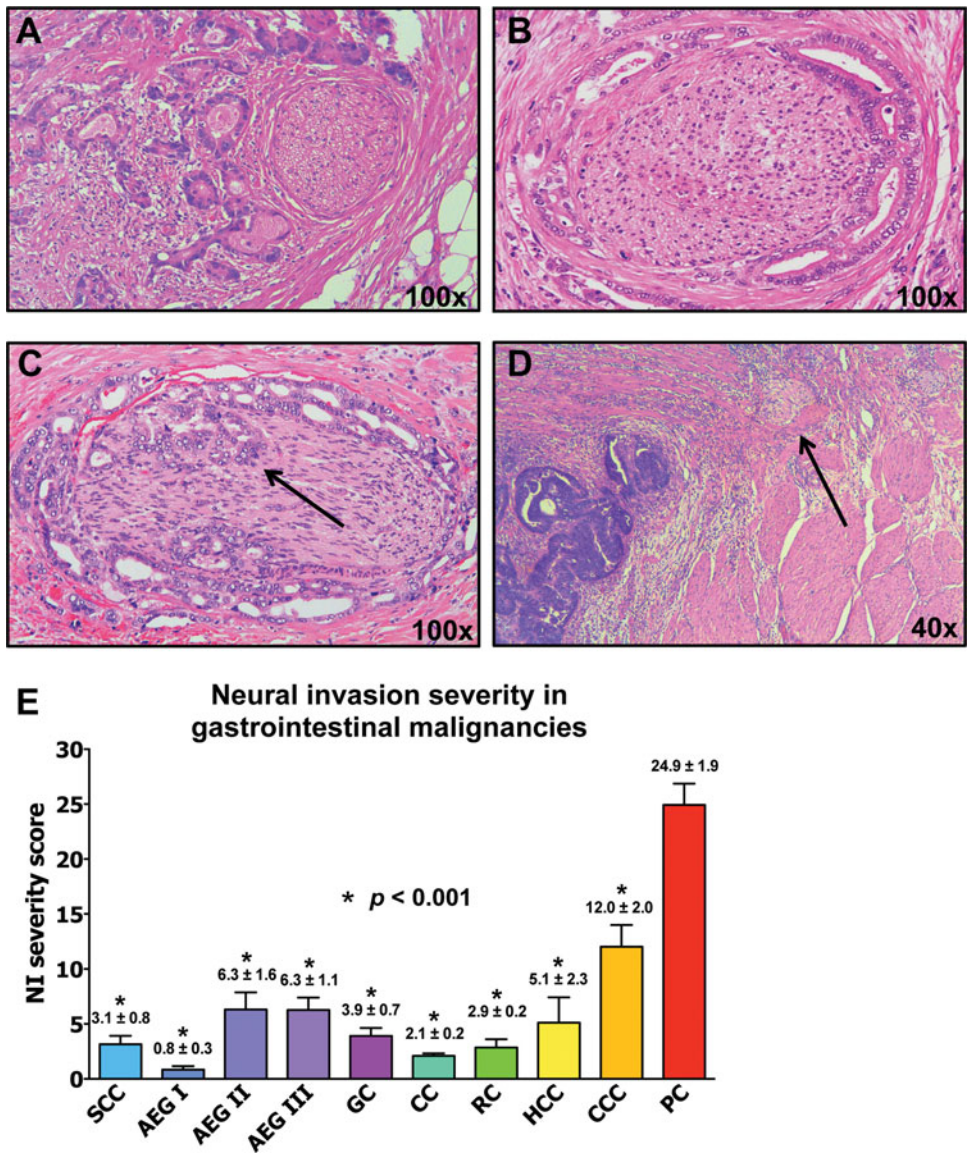

FIGURE 1. Definition of NI on representative hematoxylin and eosin–stained tissue sections: A, Epineural association: Cancer cells directly touch the epineurium but not penetrate it. B, Perineural invasion: Cancer cells are within the perineural sheet. The tumor cells directly touch and displace the nerve but are not destroying its bundle integrity. C, Endoneural invasion: Arrow shows cancer cells within the endoneural structures. They have invaded through the perineurium into the endoneural sheet. D, Representative hematoxylin and eosin–stained picture of cancer cell invasion into Auerbach plexus. The arrow shows normal myenteric plexus ganglia between both muscular layers. Magnifications are shown in the right lower corner of the images. E, Neural invasion severity score values for each tumor entity presented in mean ± standard error of the mean. Note significant smaller values for malignancies of the upper and lower gastrointestinal tract in comparison with pancreatic cancer. *indicates a significant difference $P < 0.05$ compared to pancreatic cancer/PC.

excellent agreement between the 3 raters (intraclass correlation coefficient = 0.991; 95% CI, 0.990–0.992). Significant differences in NI severity were present between GIMs ($P < 0.001$; Fig. 1E). The lowest NI severity score was detected in AEG-I: 0.8 ± 0.3 (mean ± SEM), followed by CC: 2.1 ± 0.2, RC: 2.9 ± 0.2, SCC: 3.1 ± 0.8, GC: 3.9 ± 0.7, and HCC: 5.1 ± 2.3. Medium level NI severity scores were detected in AEG-II: 6.3 ± 1.6 and AEG-III: 6.3 ± 1.1, followed by patients with CCC: 12.0 ± 2.0. The highest NI severity score of all GIMs was recorded in PC (24.9 ± 1.9).

Prevalence of NI and Impact on Survival

The median follow-up period of all 2050 patients was 37 months (interquartile range: 12.8–78.0). To attain a valid survival analysis, AEG-II and AEG-III were united to 1 group (AEG-II/III) because of their similar cancer biology and close location. The overall 5-year survival (5y-OS) for AEG-I was 40 ± 6.7%. In patients with AEG-I with NI, 5y-OS was 28 ± 9% compared with 49 ± 9% without NI (P value log-rank test = 0.038; Fig. 2A). In patients with AEG-II/III, 5y-OS was 36 ± 5%, which was significantly impaired

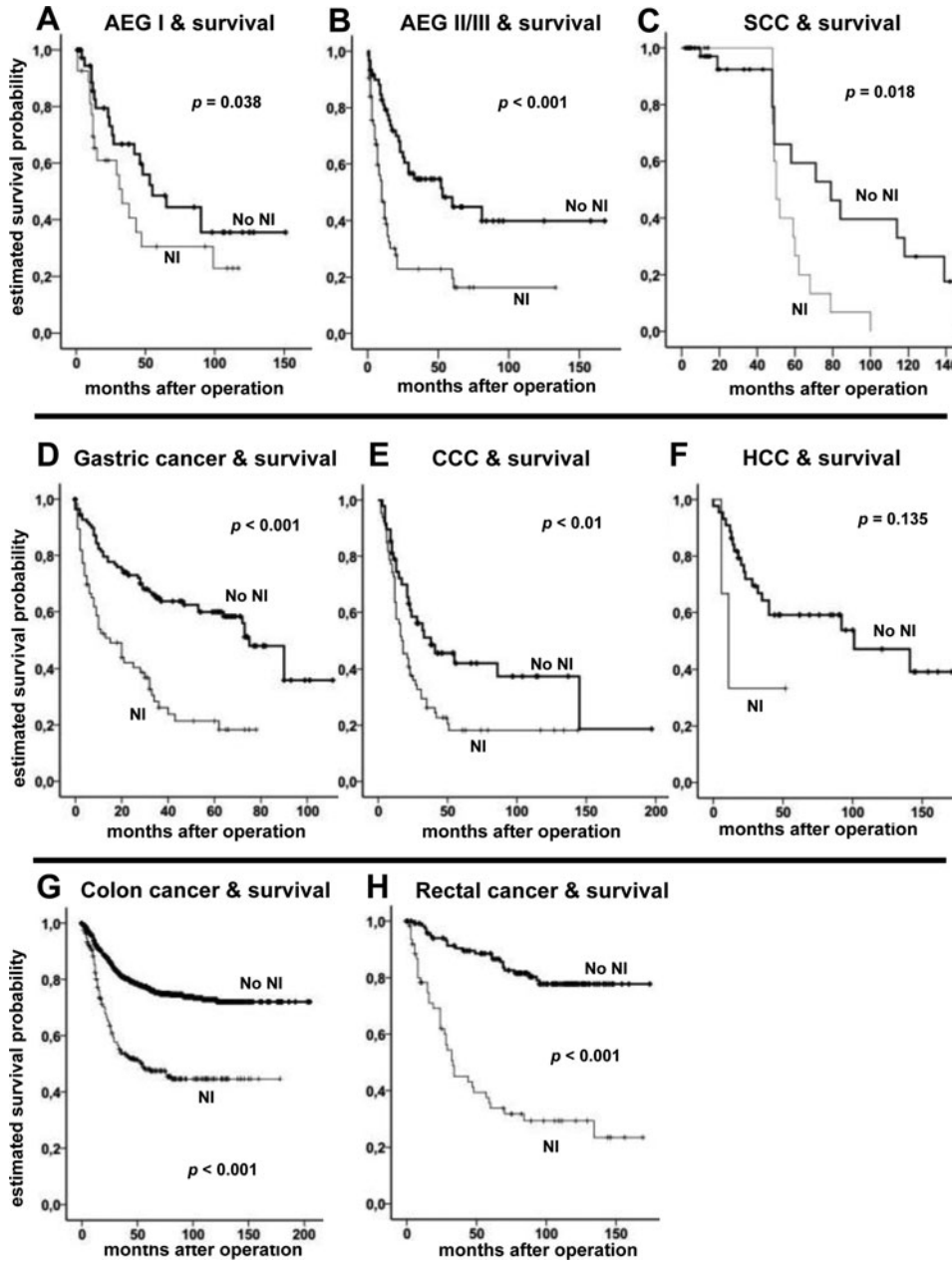

FIGURE 2. Graphs depict Kaplan-Meier analysis of survival depending on occurrence of NI for each tumor entity Upper gastrointestinal tumor including A, AEG I; B, AEGII/III; C, SCC; D, Gastric cancer/GC; E, Cholangiocellular cancer/CCC; F, hepatocellular cancer/HCC; G, colon cancer; H, rectal cancer. With the exception of HCC, patients' survival was significantly impaired when NI was detected in every tumor entity.

in patients with NI (20 ± 6%) compared with patients without NI (45 ± 8%; $P < 0.001$; Fig. 2A). Patients with SCC had a 5y-OS of 43 ± 9%. A significant difference was found in patients with NI positive versus NI negative SCC (27 ± 11% vs 59 ± 12%; $P = 0.024$, Fig. 2A). Patients with GC demonstrated a 5y-OS of 46 ± 4%, with a significant longer 5y-OS in patients without NI than in patients with NI (64 ± 5% and 21 ± 6%; $P < 0.001$; Fig. 2A).

In patients with HCC, the 5y-OS was 59 ± 8%, with no difference in 5y-OS between patients with or without NI (33 ± 27% vs 61 ± 8%; $P = 0.121$, Fig. 2B). 5y-OS in patients with CCC was 28 ± 5%, with a significant difference between patients without NI (43% ± 8%) compared with patients with NI (18 ± 5%, $P = 0.009$; Fig. 2C). 5y-OS of patients with PC was the lowest with 19 ± 3%. Because all patients with PC demonstrated NI, no subanalysis (NI positive vs NI negative) was performed.

In the lower gastrointestinal tract, 5y-OS for CC and RC was 69 ± 2% and 70 ± 4%, respectively. Patients with CC and RC without NI (77 ± 2% and 88 ± 3%) demonstrated a significantly better 5y-OS than patients with NI (48 ± 3% and 34 ± 6%, $P < 0.001$; Figs. 2D, E).

A noteworthy number of patients receiving adjuvant therapy were registered only in CC (36%), RC (41%), and CCC (19%), besides PC (100%). To test whether adjuvant therapy had an impact on overall survival within patients with CC, RC, and CCC, a multivariable analysis including NI (yes/no), sex, age, TNM, grading, and adjuvant therapy was performed. It was evident that only in CC (HR, 0.60; 95% CI, 0.45–0.79; $P < 0.001$; see Supplemental Digital Content Table 1, available at http://links.lww.com/SLA/A641) adjuvant therapy was identified as an independent prognostic factor, whereas in RC and CCC, this was not the case. To test the potential impact of adjuvant therapy in patients with NI positive CC, we performed an additional multivariable analysis including sex, age, TNM, grading, and adjuvant therapy. It was obvious that in this special subset of patients with NI, adjuvant therapy lost its function as an independent prognostic factor, demonstrating that adjuvant treatment of CC (HR, 0.69; 95% CI, 0.45–1.04; $P = 0.075$; see Supplemental Digital Content Table 1, available at http://links.lww.com/SLA/A641) had no distinct impact on survival in patients with NI.

Prognostic Potential of NI Prevalence and Severity in GIMs

To determine the prognostic quality of NI, 2 separate multivariate analyses were performed: (1) the simple presence of NI as covariate or (2) more accurate, the NI severity score, as it allows a more individualized analysis of NI. Because all patients with PC feature NI, prognostic analysis was performed only with the NI severity score. Cox regression analysis included also common prognostic factors such as sex, age, pTNM status, and tumor grading.

Within the multivariate analyses, the sole presence of NI did not show any prognostic power in AEG-I-III, SCC, CCC, HCC, and CC (Table 2). Only in GC and RC, the sole presence of NI significantly diminished patients' survival. In GC, the likelihood of dying among NI present patients was almost doubled (HR, 1.73; 95% CI, 1.10–2.73; $P < 0.02$), and in RC, the probability of dying was even 3 times higher in patients with NI (HR, 3.01; 95% CI, 1.59–5.67; $P = 0.001$; Table 3). Cox regression analysis, using NI severity as a covariate, demonstrated no independent prognostic power, neither in AEG-I and SCC nor in GC (Table 3). However, NI in AEG-II/III was an independent prognostic factor (HR, 1.16; 95% CI, 1.02–1.30; $P < 0.02$; Table 3). In patients with CCC, HCC, CC, and RC, NI severity showed no independent prognostic potential. However, in PC, NI severity was identified as an additional independent prognostic factor (HR, 1.09; 95% CI, 1.04–1.15, $P < 0.001$; Table 3).

Escalating NI Severity Scores Bear Diminished Survival

A regression model estimated the survival probabilities dependent on NI severity scores. This model revealed a firm illustration of the estimated association of higher NI severity levels and poorer survival in patients with AEG-II/III, HCC, colorectal cancer, and PC (Figs. 3A–E). For example, patients with AEG-II/III with a severity score of 20 points had a 40% chance to survive 12 months postoperatively. In contrast, if the NI severity score was 40, 12-month survival probability was only 10% (Fig. 3A). Although NI prevalence in HCC was the lowest among all GIMs, patients with HCC exhibited particularly impaired survival with increasing NI severity. When the NI severity score rose from 2 to 4, estimated survival probability was remarkably reduced by almost 50% (70% vs 34%; Fig. 3B). Although NI is not a frequently observed phenomenon in patients with HCC, it was evident that once NI was detected, these affected patients had an utmost bad prognosis with a more than 2-fold higher risk of dying and even a more than 7-fold higher risk of dying with increasing NI severity scores (Fig. 3B and Tables 2 and 3). Similarly, patients with PC with an NI score of 20 points had a 60% likelihood of surviving 12 months postoperatively. However, if the NI severity score was 40, estimated survival was decreased by more than 50% (Fig. 3D).

DISCUSSION

The present study provides the largest and most comprehensive currently available analysis on NI in more than 2000 patients with 10 different cancers of the gastrointestinal tract. It demonstrates in a standardized manner that the prevalence of NI and tumor-specific

TABLE 2. Multivariable Analysis NI

	AEG I			AEG II + III			SCC Esophagus			Gastric Cancer		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
NI presence (yes vs no)	1.01	0.38–2.68	0.977	1.63	0.97–2.75	0.064	1.51	0.21–11.0	0.687	1.73	1.10–2.73	0.018
Female vs male	1.27	0.27–6.04	0.762	1.03	0.61–1.76	0.897	0.63	0.18–2.14	0.455	0.93	0.61–1.42	0.745
Age	0.98	0.94–1.01	0.207	1.03	1.01–1.05	0.012	0.98	0.92–1.03	0.399	1.04	1.02–1.06	<0.001
pT 3/4 vs 1/2	1.58	0.63–3.97	0.331	2.04	1.14–3.62	0.016	1.63	0.28–9.57	0.589	1.50	0.89–2.52	0.131
pN												
1 vs 0	2.89	1.29–6.46	0.010	3.64	1.48–8.93	0.005	1.51	0.21–11.0	0.687	2.47	1.33–4.56	0.004
2 vs 0	3.68	1.00–13.5	0.50	5.00	1.84–13.6	0.002	1.24	0.35–2.45	0.489	4.16	2.13–8.12	<0.001
3 vs 0	—	—	—	7.60	2.83–20.4	<0.001	—	—	—	3.54	1.61–7.77	0.002
pM1 vs pM0	1.35	0.23–3.44	0.974	1.79	0.94–3.41	0.78	2.13	0.63–7.29	0.226	3.29	1.75–6.17	<0.001
G 3/4 vs 1/2	1.47	0.66–3.28	0.351	1.19	0.60–2.34	0.619	0.66	0.28–1.58	0.352	0.74	0.42–1.30	0.297
	HCC			**CCC**			**Colon Cancer**			**Rectal Cancer**		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
NI presence (yes vs no)	2.10	0.43–10.1	0.357	1.64	0.95–1.82	0.077	1.06	0.83–1.34	0.664	3.01	1.59–5.67	0.001
Female vs male	0.59	0.18–1.95	0.390	0.88	0.55–1.43	0.613	1.25	1.00–1.56	0.055	0.70	0.41–1.20	0.194
Age	1.00	0.96–1.06	0.871	1.03	1.01–1.06	0.012	1.01	1.00–1.02	0.065	1.01	0.99–1.04	0.278
pT 3/4 vs 1/2	1.77	0.63–4.93	0.276	2.54	1.50–4.30	0.001	2.85	1.60–5.09	<0.001	2.26	0.92–5.55	0.074
pN												
1 vs 0	—	—	—	2.96	1.75–5.00	<0.001	2.73	1.93–3.86	<0.001	1.23	0.59–2.56	0.575
2 vs 0	—	—	—	2.07	0.88–4.90	0.098	4.90	3.44–6.97	<0.001	3.30	1.65–6.63	0.001
pM1 vs pM0	—	—	—	2.57	0.81–8.17	0.110	5.47	4.19–7.15	<0.001	4.71	2.26–9.82	<0.001
G 3/4 vs 1/2	2.60	1.03–6.52	0.042	1.72	1.04–2.86	0.035	1.27	1.01–1.61	0.042	2.01	1.17–3.47	0.012

TABLE 3. Multivariable Analysis NI Severity Score

	AEG I			AEG II + III			SCC Esophagus			Gastric Cancer			Pancreatic Cancer		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
NI severity score*	0.99	0.67–1.44	0.940	1.16	1.02–1.30	0.019	0.97	0.78–1.19	0.733	1.04	0.94–1.15	0.485	1.09	1.04–1.15	<0.001
Female vs male	1.51	0.32–7.23	0.606	1.13	0.66–1.95	0.657	0.68	0.21–2.22	0.521	1.01	0.67–1.54	0.962	0.92	0.62–1.34	0.656
Age	0.98	0.94–1.01	0.211	1.03	1.01–1.05	0.009	0.98	0.92–1.03	0.387	1.04	1.02–1.06	<0.001	1.03	1.01–1.04	0.008
pT 3/4 vs 1/2	1.40	0.66–3.03	0.389	2.08	1.16–3.74	0.014	2.43	0.81–7.28	0.112	1.62	0.97–2.71	0.065	0.87	0.38–2.02	0.753
pN															
1 vs 0	2.48	1.13–5.43	0.023	3.54	1.43–8.76	0.006	2.10	0.82–5.40	0.123	2.68	1.45–4.96	0.002	1.47	0.87–2.49	0.151
2 vs 0	3.33	1.04–10.7	0.043	5.31	1.91–14.7	0.001	1.20	0.76–2.33	0.274	4.37	2.23–8.56	<0.001	—	—	—
3 vs 0	—	—	—	9.44	3.55–25.1	<0.001	—	—	—	4.06	1.85–8.92	<0.001	—	—	—
pM1 vs pM0	0.81	0.72–2.34	0.648	1.58	0.84–2.95	0.155	3.00	0.83–10.9	0.095	3.53	1.87–6.66	<0.001	1.32	0.75–2.34	0.343
G 3/4 vs 1/2	1.84	0.83–4.07	0.940	1.31	0.66–2.60	0.442	0.66	0.27–1.61	0.361	0.77	0.43–1.35	0.356	1.73	1.13–2.65	0.011

	CCC			HCC			Colon Cancer			Rectal Cancer		
Variable	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
NI severity score*	1.04	0.98–1.10	0.187	7.52	0.90–62.8	0.063	1.00	0.94–1.07	0.916	1.03	0.95–1.11	0.500
Female vs male	0.91	0.56–1.48	0.708	0.60	0.18–1.99	0.407	1.25	1.00–1.57	0.048	0.67	0.39–1.17	0.159
Age	1.03	1.01–1.05	0.017	1.00	0.95–1.05	0.969	1.01	1.00–1.02	0.060	1.02	1.00–1.04	0.114
pT 3/4 vs 1/2	2.67	1.60–4.45	<0.001	1.68	0.60–4.68	0.325	2.87	1.61–5.12	<0.001	3.88	1.70–8.86	0.001
pN												
1 vs 0	3.00	1.78–5.06	<0.001	—	—	—	2.74	1.93–3.87	<0.001	1.47	0.73–2.96	0.279
2 vs 0	1.97	0.82–4.75	0.132	—	—	—	5.00	3.52–7.11	<0.001	5.02	2.57–9.81	<0.001
pM1 vs pM0	2.26	0.73–6.95	0.156	—	—	—	5.58	4.27–7.29	<0.001	3.72	1.81–7.64	<0.001
G 3/4 vs 1/2	1.65	0.98–1.10	0.047	2.47	0.98–6.23	0.056	1.28	1.01–1.62	0.042	1.90	1.11–3.25	0.020

*The corresponding hazard ratio values display the estimated risk increment associated with a NI severity score gain of 5 points.

NI severity and their impact on prognosis differ extensively between GIMs and confirm PC as the most neuroaffine gastrointestinal cancer entity.

The biological impact of NI was first recognized in prostate and head/neck cancers and was followed in the past years by intensifying research in PC.[3,13,17–19] Research of NI in GIMs is limited, and there is a noticeable discrepancy in the definition and analysis of NI.[10,17,20–25] This inconsistency resulted in highly variable NI prevalence rates even in studies on the same tumor entity. Moreover, most NI data were retrospectively extracted from routine pathology reports rather than targeted reexamination of tissue specimens, which is known to yield more precise prevalence rates.[25]

To determine the true prevalence and impact of NI in GIMs, all patients with neoadjuvant therapy regimens were excluded because of the well-known influence of neoadjuvant therapy on prevalence and severity of NI in patients with AEG and RC.[11,26,27] By using a standardized definition, a very wide range of prevalence rates for NI in GIMs from 6% in HCC up to 100% in PC could be detected. There may be several reasons for this remarkable difference: First, the natural anatomic density of peripheral nerves in visceral organs is different. This may explain the very low prevalence of NI in HCC, because the liver is distant to major nerve plexus (ie, celiac and hypogastric plexus) as opposed to the pancreas, which is—in addition to possessing a large amount of autonomic nerves—also located in the immediate neighborhood of the celiac plexus. This anatomical difference may also account for the nearly 10-fold higher NI prevalence of 58% in extrahepatic CCC and of 65% in AEG-III with greater proximity to the celiac plexus. However, nerve density may not be the only reason for this discrepancy, because in RC with its dense rectal neural plexus, NI prevalence reaches a mere 34% when compared with 100% for PC.[28,29] Nevertheless, PC is the most aggressive neuroinvasive tumor among all GIMs, because regardless of tumor stage, there was not a single patient in whom NI was absent.

In recent studies, we demonstrated that cancer cells of different origin exhibit varying extents of neuroaffinity, as best seen in the 3-dimensional neural-migration assay.[12,18,30] In such an in vitro setting, PC cells demonstrate an evidently faster and more targeted migration toward nerves as opposed to CC and RC cells.[12] Furthermore, it is known that neuroplastic changes actively support the generation of NI in PC, but not all GIM subtypes are able to induce these neuroplastic alterations.[12,13,31] In PC, neuroplastic alterations like increased neural density and hypertrophy are induced by potent neurotropic factors such as nerve growth factor and Artemin.[11,13,19] In CC, the amount of nerve growth factor and Artemin in colonic nerves are much less than in pancreatic nerves in PC. Furthermore, CC cells as opposed to PC cells are not capable of inducing neuroplastic alterations in cultured neurons. Therefore, it seems that the observed noticeable differences in NI prevalence between the GIMs may be due to different neurotropic attributes of each cancer cell type with distinct neurocancer affinities. In this context, it is of importance that not only the frequency of NI was different within the GIMs but also their aggressiveness to invade nerves, as seen in the noticeable different NI severity scores. It was evident that the more destructive the NI behavior was, the more impaired prognosis was detected in the majority of all GIMs. Here again, PC showed the most aggressive NI phenotype with the highest NI severity scores (24.9), whereas patients with CC revealed only very low scores (2.9).

Apart from PC, AEG-II/III turned out to be the only other GIM where NI severity had a noticeable impact on survival. Only little information is available on the presence of NI and its impact on survival in AEG, because NI was not well defined and not the main focus of the analysis.[20,26,27] However, these limited data corroborate our data by indicating that patients with AEG-II and -III reveal higher prevalences of NI than patients with AEG-I, that NI was identified as an independent prognostic factor in patients with AEG and GC, and that neoadjuvantly treated patients with AEG seem to exhibit significantly reduced NI.[20,26,27] Whether AEG tumors also feature neurotropic attributes as known in PC must be investigated in future studies.

Now the important question remains whether this novel knowledge has any clinical consequence. Because all patients with PC

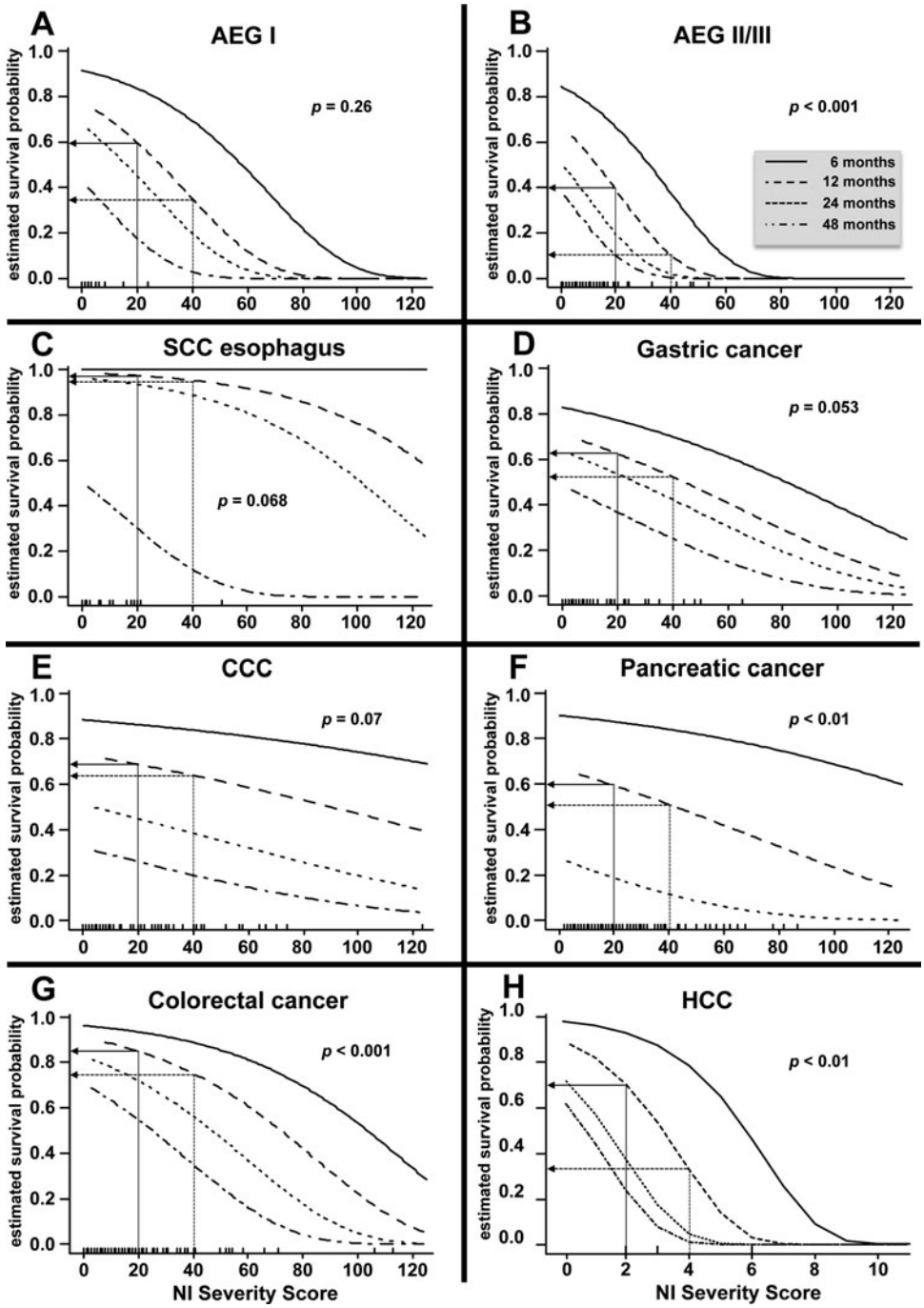

FIGURE 3. Estimated survival probability (*y* axis) for 6, 12, 24, and 60 months according to the NI severity score (*x* axis) Upper gastrointestinal tumor including A, AEG I; B, AEGII/III; C, SCC; D, Gastric cancer/GC; E, Cholangiocellular cancer/CCC; F, pancreatic cancer; G, colorectal cancer; H, hepatocellular cancer. Note the strikingly diminished predicted survival with increasing NI severity scores. Arrow lines and dotted arrow lines exemplify increasing NI severity score and its corresponding estimated survival probability. Dash bars at the *x* axis depict actually observed severity score levels of all patients in each cancer entity.

receive adjuvant chemotherapy, one can discuss whether patient with a primarily resectable PC with a distinct NI severity score can be upgraded and categorized as a patient with a locally advanced cancer. To improve the efficacy of such an adjuvant regime in this special patient population with a defined NI severity cutoff value, physicians may consider more aggressive adjuvant therapy options including chemotherapy (eg, FOLFIRINOX) and/or radiochemotherapy.[32] Similarly, patients with CC with T3–T4 N0 (stage II) tumors do normally not receive any adjuvant chemotherapy.[33] However, one may consider adjuvant therapy for these patients if they demonstrate

extraordinarily high NI severity scores. These novel data raise a lot of important questions that have to be taken seriously. The first step must be the identification of the different NI severity cutoff scores via prospective controlled studies.

Advances in systematic histopathological assessment and molecular cancer phenotyping allowed identification of leading prognostic factors in GIMs. Between the UICC stages I to III, lymphovascular invasion significantly shortens the 5y-OS of patients with esophageal cancer, GC, or CC.[34] Similarly, microsatellite instability is encountered in 15% of sporadic CC and GC and is increasingly recognized to identify a subpopulation of patients with a favorable prognosis.[35,36] Patients with metastatic CC and mutated *BRAF* gene have a mere 10.4-month overall survival time when compared with 34.7 among *BRAF* wild-type patients.[37] Therefore, a major future objective shall be to identify the prognostic significance of NI in relation to established prognostic factors in GIMs.

CONCLUSIONS

Our present data clearly demonstrate that NI is not a concomitant side feature in GIMs and deserves special attention due to its prognostic and potential therapeutic impact. Therefore, implementation of a standardized routine-based analysis of NI prevalence in combination with the individual NI severity score may enable us now to react and improve patient stratification and individualized therapeutic decision making after surgery.

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Drs. Florian Liebl, MD, and Ihsan Ekin Demir, MD, have contributed equally to this manuscript.

REFERENCES

1. Jemal A, Siegel R, Xu J, et al. Cancer statistics, 2010. *CA Cancer J Clin.* 2010;60:277–300.
2. Ayala GE, Wheeler TM, Shine HD, et al. In vitro dorsal root ganglia and human prostate cell line interaction: redefining perineural invasion in prostate cancer. *Prostate.* 2001;49:213–223.
3. Batsakis JG. Nerves and neurotropic carcinomas. *Ann Otol Rhinol Laryngol.* 1985;94:426–427.
4. Sobin LH. *TNM Classification of Malignant Tumours*. 7th ed. Oxford: Wiley-Blackwell; 2009.
5. Weiser MR, Landmann RG, Kattan MW, et al. Individualized prediction of colon cancer recurrence using a nomogram. *J Clin Oncol.* 2008;26:380–385.
6. Scartozzi M, Galizia E, Verdecchia L, et al. Lymphatic, blood vessel and perineural invasion identifies early-stage high-risk radically resected gastric cancer patients. *Br J Cancer.* 2006;95:445–449.
7. Pour PM, Bell RH, Batra SK. Neural invasion in the staging of pancreatic cancer. *Pancreas.* 2003;26:322–325.
8. Zhu Z, Friess H, diMola FF, et al. Nerve growth factor expression correlates with perineural invasion and pain in human pancreatic cancer. *J Clin Oncol.* 1999;17:2419–2428.
9. Bilici A, Seker M, Ustaalioglu BB, et al. Prognostic significance of perineural invasion in patients with gastric cancer who underwent curative resection. *Ann Surg Oncol.* 2010;17:2037–2044.
10. Fujita S, Nakanisi Y, Taniguchi H, et al. Cancer invasion to Auerbach's plexus is an important prognostic factor in patients with pT3-pT4 colorectal cancer. *Dis Colon Rectum.* 2007;50:1860–1866.
11. Ceyhan GO, Liebl F, Maak M, et al. The severity of neural invasion is a crucial prognostic factor in rectal cancer independent of neoadjuvant radiochemotherapy. *Ann Surg.* 2010;252:797–804.
12. Liebl F, Demir IE, Rosenberg R, et al. The severity of neural invasion is associated with shortened survival in colon cancer. *Clin Cancer Res.* 2013;19:50–61.
13. Ceyhan GO, Bergmann F, Kadihasanoglu M, et al. Pancreatic neuropathy and neuropathic pain—a comprehensive pathomorphological study of 546 cases. *Gastroenterology.* 2009;136:177–186. e1.
14. Harrell FE Jr. Variable selection. In: Harrell FE, Jr, ed. *Regression Modeling Strategies With Applications to Linear Models, Logistic Regression, and Survival Analysis*. New York, NY: Springer;2001:56–60.
15. Pearl J. Failing sufficiency via marginality. In: Pearl J, ed. *Causality Models, Reasoning and Inference*. Cambridge: Cambridge University Press; 2000:185–186.
16. Saville DJ. Multiple comparison procedures: the practical solution. *Am Stat.* 1990;44:174–180.
17. Liebig C, Ayala G, Wilks JA, et al. Perineural invasion in cancer: a review of the literature. *Cancer.* 2009;115:3379–3391.
18. Ceyhan GO, Demir IE, Altintas B, et al. Neural invasion in pancreatic cancer: a mutual tropism between neurons and cancer cells. *Biochem Biophys Res Commun.* 2008;374:442–447.
19. Ceyhan GO, Giese NA, Erkan M, et al. The neurotropic factor artemin promotes pancreatic cancer invasion. *Ann Surg.* 2006;244:274–281.
20. Reynolds JV, Ravi N, Muldoon C, et al. Differential pathologic variables and outcomes across the spectrum of adenocarcinoma of the esophagogastric junction. *World J Surg.* 2010;34:2821–2829.
21. Torres CM, Wang HH, Turner JR, et al. Pathologic prognostic factors in esophageal squamous cell carcinoma: a follow-up study of 74 patients with or without preoperative chemoradiation therapy. *Mod Pathol.* 1999;12:961–968.
22. Hatzaras I, George N, Muscarella P, et al. Predictors of survival in periampullary cancers following pancreaticoduodenectomy. *Ann Surg Oncol.* 2010;17:991–997.
23. Shen FZ, Zhang BY, Feng YJ, et al. Current research in perineural invasion of cholangiocarcinoma. *J Exp Clin Cancer Res.* 2010;29:24.
24. Shirai K, Ebata T, Oda K, et al. Perineural invasion is a prognostic factor in intrahepatic cholangiocarcinoma. *World J Surg.* 2008;32:2395–2402.
25. Liebig C, Ayala G, Wilks J, et al. Perineural invasion is an independent predictor of outcome in colorectal cancer. *J Clin Oncol.* 2009;27:5131–5137.
26. Fareed KR, Ilyas M, Kaye PV, et al. Tumour regression grade (TRG) analyses in patients with resectable gastro-oesophageal adenocarcinomas treated with platinum-based neoadjuvant chemotherapy. *Histopathology.* 2009;55:399–406.
27. Mansour JC, Tang L, Shah M, et al. Does graded histologic response after neoadjuvant chemotherapy predict survival for completely resected gastric cancer? *Ann Surg Oncol.* 2007;14:3412–3418.
28. Knudsen JB, Nilsson T, Sprechler M, et al. Venous and nerve invasion as prognostic factors in postoperative survival of patients with resectable cancer of the rectum. *Dis Colon Rectum.* 1983;26:613–617.
29. Olsson C, Chen BN, Jones S, et al. Comparison of extrinsic efferent innervation of guinea pig distal colon and rectum. *J Comp Neurol.* 2006;496:787–801.
30. Wang K, Demir IE, D'Haese JG, et al. The neurotropic factor neurturin contributes toward an aggressive cancer cell phenotype, neuropathic pain and neuronal plasticity in pancreatic cancer. *Carcinogenesis.* 2014;35:103–113.
31. Demir IE, Schafer KH, Tieftrunk E, et al. Neural plasticity in the gastrointestinal tract: chronic inflammation, neurotropic signals, and hypersensitivity. *Acta Neuropathol.* 2013;125:491–509.
32. Michl P, Gress TM. Current concepts and novel targets in advanced pancreatic cancer. *Gut.* 2013;62:317–326.
33. Figueredo A, Coombes ME, Mukherjee S. Adjuvant therapy for completely resected stage II colon cancer. *Cochrane Database Syst Rev.* 2008;3:CD005390.
34. Langheinrich MC, Schellerer V, Perrakis A, et al. Molecular mechanisms of lymphatic metastasis in solid tumors of the gastrointestinal tract. *Int J Clin Exp Pathol.* 2012;5:614–623.
35. Yamamoto H, Adachi Y, Taniguchi H, et al. Interrelationship between microsatellite instability and microRNA in gastrointestinal cancer. *World J Gastroenterol*. 2012;18:2745–2755.
36. Merok MA, Ahlquist T, Royrvik EC, et al. Microsatellite instability has a positive prognostic impact on stage II colorectal cancer after complete resection: results from a large, consecutive Norwegian series. *Ann Oncol.* 2013;24:1274–1282.
37. Tran B, Kopetz S, Tie J, et al. Impact of BRAF mutation and microsatellite instability on the pattern of metastatic spread and prognosis in metastatic colorectal cancer. *Cancer.* 2011;117:4623–4632.

DISCUSSANTS

M. Krawczyk (Warsaw, Poland):

First of all, I would like to thank the authors for the permission to read the article and would also like to thank the European Surgical Association for the privilege of being the first discussant of this

interesting study. In the article, the authors present a large cohort of 2050 patients with adenocarcinoma of the esophago-gastric-junction, squamous cell carcinoma of the esophagus, gastric cancer, colon and rectal cancer, cholangiocellular, hepatocellular cancer (HCC), and pancreatic cancer. The aim of the study was to determine whether the neural invasion differentiated in various GIM tumors, and the clinical impact, if any, of this.

I have 1 remark regarding the material. The authors excluded patients who had undergone neoadjuvant therapy from the analysis. However, they did not exclude patients who had received adjuvant therapy. Such a difference may have had an influence on the results and overall survival.

Moreover, I have 2 other remarks.

It is true that the authors have found the answer to the aim of their study, as in pancreatic cancer and in adenocarcinomas of the esophageal-junction/AEG II/III NI-severity was significant. These results are comparable with those supplied by the authors of the TNM classification. Nevertheless, paying attention to the neural invasion in pancreatic cancer and adenocarcinoma of the esophago-gastric-junction is of academic significance because it is known that the prognosis for both cancers is very poor. Thus, the outcome of pancreatic cancer and adenocarcinoma of the esophago-gastric-junction highlights only the problem.

However, it is much more difficult to analyze the clinical impact and, particularly, determine whether the outcome of NI invasion can help us to individualize treatments after surgery. To accomplish this task, it is necessary that we analyze a comparable number of patients in each group. For example, in the HCC group, you studied only 47 cases, whereas in the colon cancer group, you analyzed 1075 cases. We require a prospective study.

Response From G.O. Ceyhan, H. Friess (Munich, Germany):

Thank you very much for your comments and excellent questions. First of all, we excluded all patients treated with neoadjuvant therapy from the analysis because we did not want to distort the natural biology of neural invasion in our patients. We wanted to be sure that this is the clear-cut picture of neural invasion within our patient population. Concerning our data obtained from patients with rectal cancer and other patients, especially patients with AEG, we know that neoadjuvant treatment reduces the prevalence of neural invasion. To answer the question of whether adjuvant treatment had an effect on our results, we analyzed this important aspect in our entire study population. Most of the patients did not receive adjuvant treatment. For instance, none of the patients with AEG I and a squamous cell carcinoma of the esophagus received adjuvant therapy. Only 3% of the patients with AEG II/III and gastric cancer and up to 6% of those with HCC received adjuvant therapy.

Furthermore, 36% of patients with colon cancer, 41% of patients with rectal cancer, and 19% of patients with cholangiocellular carcinoma received adjuvant treatment. We performed a multivariable analysis, including neural invasion, and identified only adjuvant therapy as an independent prognostic factor in patients with colon cancer. Moreover, we performed another subanalysis of the patients with colon cancer with the presence of neural invasion and investigated whether adjuvant therapy had an impact, finding that this was not the case. Therefore, adjuvant therapy seems to not have had an impact on our results. All patients with pancreatic cancer received adjuvant therapy. To exclude pancreatic cancer from the study, due to its 100% penetrance of adjuvant regimens, would be excluding the golden control group, in which we know that neural invasion has a major impact on survival.

Regarding your second remark, you're completely right. Patients with AEG and pancreatic cancer have a poor prognosis. We face the following problem in our daily practice in patients with pancreatic cancer, where we operate these patients who have advanced tumor disease; they are resected and intraoperative frozen sections are used to identify neural invasion. What is the consequence of this important information? In short, nothing; we completed the operation and the patient received adjuvant therapy. Seven months later, the patient came back with back pain; a computed tomographic scan is obtained and the local recurrence is identified, typically at the superior mesenteric artery and/or at the coeliac plexus, indicating local recurrence due to the neural invasion of cancer cells. To avoid this in the future, we have to identify patients who have a high risk of developing early local recurrence. One possible way to do this is to generate a clear defined neural invasion severity cutoff and with this information, identify patients with an advanced case of the disease and offer them a more aggressive adjuvant therapy regimen.

With regard to your third question, I completely agree with you. At the moment, there's a prospective study running at our department, in which we want to identify the cutoff value for neural invasion severity in pancreatic cancer.

DISCUSSANTS

N. Senninger (Münster, Germany):

I just have a brief comment. I think that it would be worthwhile to pursue the whole thing with native specimens because then you can better examine the inflammatory potential surrounding this invasive process. My question is the following: did you measure inflammatory cells in these tumors, such as tumor-invading lymphocytes, to get a feeling of what the mechanism is that propels the cells into the nerves? Perhaps it is not the metastatic potential but rather, inflammatory side effects.

Response From G.O. Ceyhan, H. Friess (Munich, Germany):

This is also a very interesting question and we have also worked on this subject. We have recently published our findings in *Plos One*. We reported that the more perineural inflammatory cell infiltrations were present around the intrapancreatic nerves, the more aggressive the neural invasion of cancer cells observed was. This means that the inflammatory cell infiltration runs in parallel with neural cancer cell invasion and is somehow augmenting it. A more detailed analysis of this issue is still undergoing.

DISCUSSANTS

J. Reynolds (Dublin, Ireland):

I enjoyed your article. I just have two questions. First, lymphovascular invasion is often paralleled with perineural invasion. Was this evaluated in your multivariate analysis? Second, some people think Siewert type 2 tumors have a gastric origin, whereas others argue that they are esophageal. Are we to read anything in the close parallel between type 2 and type 3 tumors from your study data?

Response From G.O. Ceyhan, H. Friess (Munich, Germany):

With regard to the first question, because we did not have data on lymphovascular invasion in all patients, we had to exclude this object from our study.

In answer to your second question, due to the limitations of our study, we found that AEG II and III behave similarly regarding neural invasion and were clearly different from patients with AEG I and other esophageal cancer. Therefore, this observation supports the theory that Siewert type 2 tumors behave more like gastric cancer. However, this hypothesis needs more investigation.