Neural invasion in gastric carcinoma

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Abstract

***Aims*—To determine whether neural invasion in advanced gastric cancer is of clinicopathological significance.**

***Methods*—The study population comprised 121 cases of primary advanced gastric carcinoma. Two paraffin wax embedded blocks taken from the central tissue slice in each primary tumour were used. For definitive recognition of neural invasion, immunostaining for S-100 protein was applied to one slide; the other slide was stained with haematoxylin and eosin.**

***Results*—Neural invasion was recognised in 34 of 121 (28%) primary gastric carcinomas. There were significant differences in tumour size, depth of tumour invasion, stage, and curability between patients with and without neural invasion. The five year survival rates of patients with and without neural invasion were 10 and 50%, respectively. Multivariate analysis, however, demonstrated that neural invasion was not an independent prognostic factor.**

***Conclusions*—Neural invasion could be an additional useful factor for providing information about the malignant potential of gastric carcinoma. This may be analogous to vessel permeation which is thought to be important, but is not an independent prognostic factor.**

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Keywords: Neural invasion, gastric carcinoma, prognostic factor.

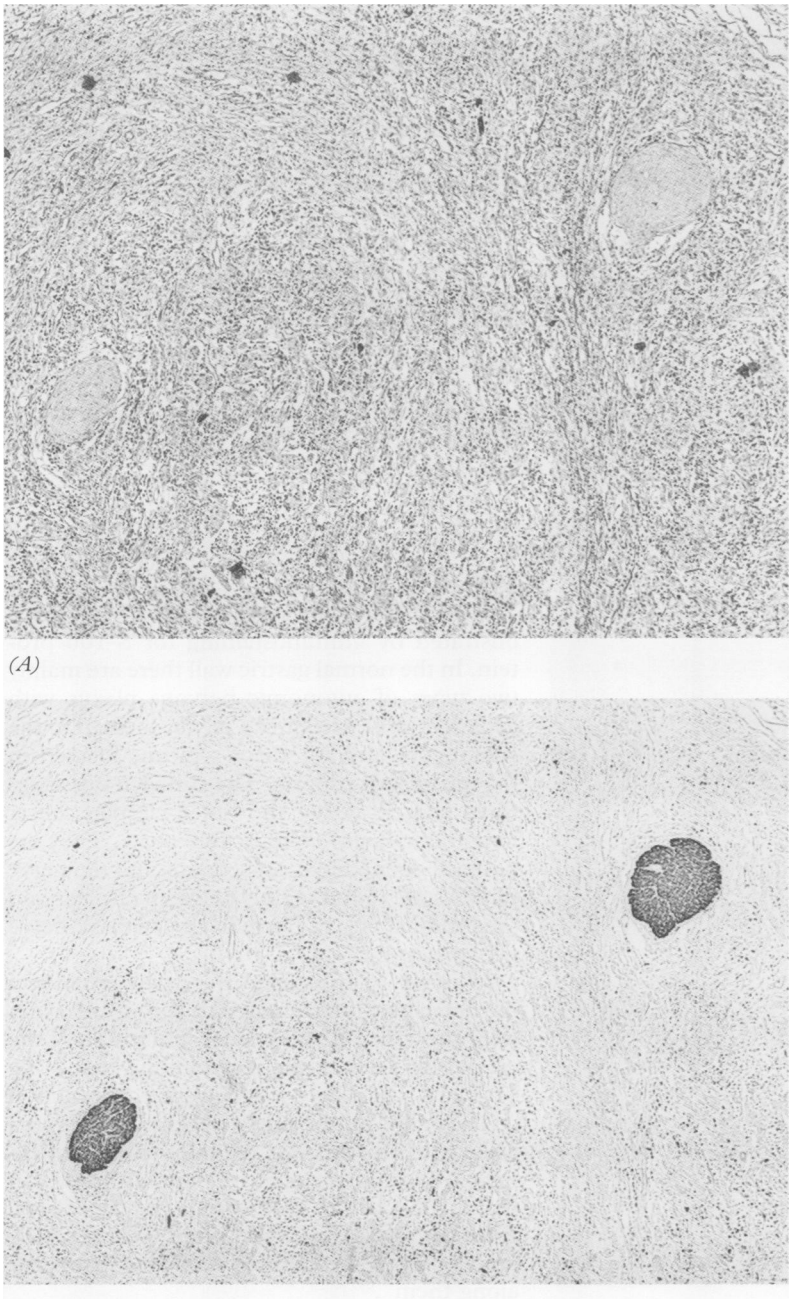


Figure 1 Two nerve bundles in the muscular layer proper are well preserved despite the dense infiltration of carcinoma cells in the gastric wall. Inflammatory cell infiltrate is also present. (A) Haematoxylin and eosin (× 55). (B) Immunohistochemical stain for S-100 protein (× 55). Note the clear demonstration of two nerve bundles by the immunohistochemical stain.

Gastric carcinomas arise from the mucosal epithelium and directly invade the surrounding tissues with expansive or infiltrative growth patterns. They also metastasise to distant organs, such as liver, lung, lymph nodes, peritoneum, and ovary, through vascular or lymph vessel invasion or direct (peritoneal) dissemination.[1] Vascular or lymphatic invasion is one of the most important pathological factors affecting the prognosis of patients with gastric carcinoma.[2–4]

Neural tissue in the stomach can be invaded by carcinoma cells, but so far this has received little attention. However, the concept of neural invasion has received attention in pancreatic carcinoma.[5 6] Neural invasion of the extrapancreatic nerve plexus is reported to be the result of continuous spread of the carcinoma cells within the perineural space, and en bloc resection of the tissue involving the nerve plexus is emphasised in the surgical treatment.[6] The importance of neural invasion has also been reported in other carcinomas.[7–12]

Methods

The study population comprised 121 patients with primary gastric carcinoma who had undergone gastrectomy in the Department of Surgery II, Kyushu University Hospital, Fukuoka, Japan, between 1983 and 1988. Of these, 76 had undergone curative resection. All

specimens were examined both macroscopically and histologically according to the criteria proposed by the Japanese Research Society for Gastric Cancer.[13]

The resected stomachs were opened along the greater or lesser curvature, pinned onto a wooden board, and fixed in 10% formalin. The central tissue slice taken from each tumour contained the largest longitudinal dimension. Accordingly, two to eight blocks were obtained in each case. Histological diagnosis was made using sections stained with haematoxylin and eosin. The lymph nodes located alongside the left gastric, the common hepatic, and the coeliac arteries were dissected en bloc. These tissues contained not only lymph nodes but also nerve fibres in and/or around the coeliac plexus; these were also examined to determine whether neural invasion was present or not.

In all cases two paraffin wax blocks were recut and stained immunohistochemically for S-100 protein; one paraffin wax block contained both cancerous and adjacent non-cancerous tissue; the other had cancerous tissue which had invaded the stomach wall. The two sections were dewaxed in xylene, rehydrated through alcohol, and then immersed in 3% hydrogen peroxide in methanol for 10 minutes to block endogeneous peroxidase activity. Sections were subsequently washed in phosphate buffered saline. Normal goat serum was then applied for 20 minutes to reduce non-specific binding. The sections were incubated for one hour with primary antibody (Dako, Carpinteria, California, USA) (diluted 1 in 100) at room temperature, then with biotinylated goat antirabbit IgG diluted 1 in 200 for one hour (Vector Laboratories, Burlingame, California, USA), and finally with avidin biotin peroxidase complex for 30 minutes (Vector Laboratories). Peroxidase labelling was developed with 3,3′-diaminobenzidine and hydrogen peroxide, and the sections were counterstained with methyl green.

The χ^2 test and the Mann–Whitney U test were used to compare data on patients with and without neural invasion. Survival analysis was performed using the Kaplan–Meyer and Mantel–Cox methods. Stepwise Cox regression analysis[14] was performed to determine which of the many covariates had the most prognostic significance.

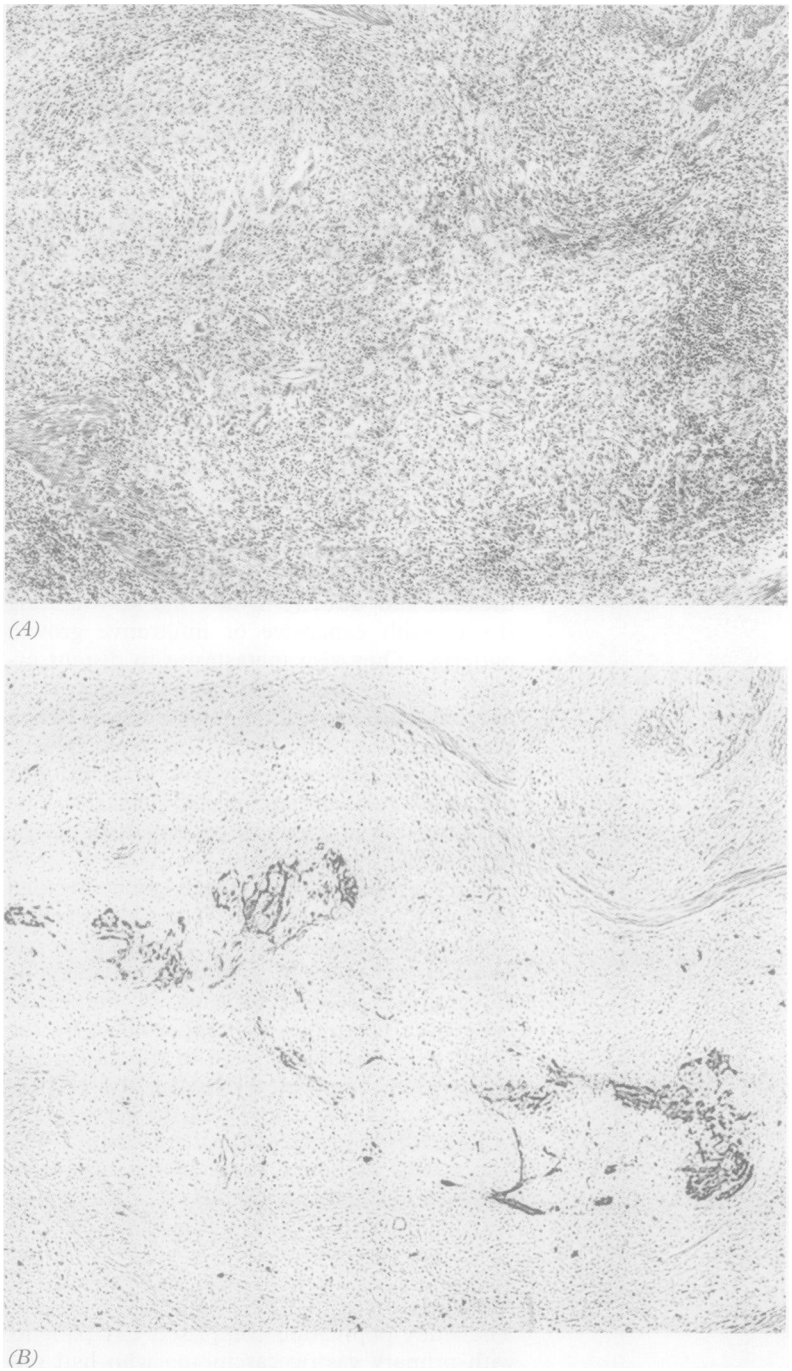


Figure 2 The nerve bundle in the muscular layer proper is destroyed by direct infiltration of carcinoma cells. The muscle layer is severely infiltrated by carcinoma cells (A) (haematoxylin and eosin, × 55). (B) Immunohistochemical stain for S-100 protein revealed the destruction of the nerve bundles in the muscle layer (× 55). This is not regarded as neural invasion.

Results

The nerve fibres and bundles were clearly demonstrated by immunostaining for S-100 protein. In the normal gastric wall there are mainly two types of autonomic nervous plexus with ganglion cells: Meissner's plexus located in the submucosa and Auerbach's plexus located in the muscularis propria. The stomach possesses three muscle coats: the inner circular, outer longitudinal, and oblique fibre coats. They are not well defined in most sections and the nerve bundles are found mainly between circular and longitudinal coats. There is an extensive nerve network in the wall.[15]

In many of the cases of gastric carcinoma the nerve bundles encircled by the perineurium were preserved despite dense infiltration of the carcinoma cells in the surrounding tissue (fig 1). In 12 cases there was complete destruction or disruption of the nerve fibres by carcinoma cells, but this was not regarded as neural invasion because it might be part of the overall penetration of the gastric wall (fig 2). Neural invasion was defined as "carcinoma cells observed inside the perineurium and extending along them".[6 8]

Neural invasion of the submucosal layer, the proper muscular layer, or the subserosal layer was observed in 34 of 121 (28%) cases. In 22 cases perineural invasion was prominent and the nerve fibres encircled by the endoneurium

were preserved (fig 3). Invasion of the endoneurium and perineural space was prominent in 12 cases (fig 4). In several cases there were oedematous or atrophic changes in the nerve bundles; these might be secondary changes due to neural invasion because severe neural invasion was seen just adjacent to this (fig 5).

Neural invasion around the coeliac plexus was recognised in eight cases. These cases showed severe neural invasion in the primary carcinoma of the stomach. Interestingly, four of the eight showed both lymph node metastasis and neural invasion, and the remaining four showed only neural invasion in the tissues located alongside the left gastric, the common hepatic, and the coeliac arteries.

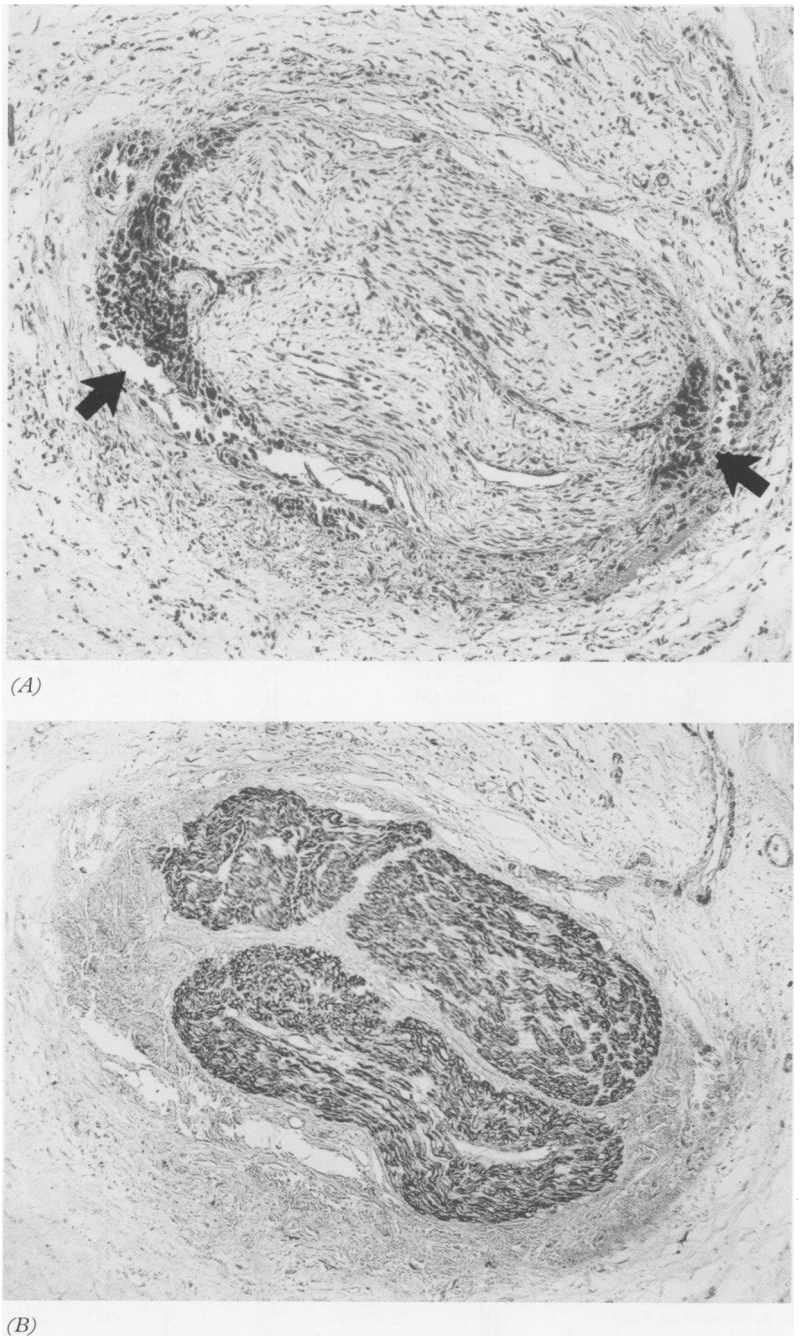


Figure 3 Perineural invasion of gastric carcinoma in the subserosal layer. Cancer cells (arrows) are present in the perineural space. (A) Haematoxylin and eosin (× 100). (B) Immunohistochemical stain for S-100 protein (× 100).

The clinicopathological characteristics of patients with and without neural invasion are summarised in table 1. There was a significant difference in tumour size, depth of tumour invasion, stage, and curability between those with and without neural invasion. No differences were seen for age, sex, tumour location, histological type, lymphatic permeation, vascular permeation, lymph node metastasis, hepatic metastasis, or peritoneal dissemination.

The survival curves of both groups are presented in fig 6. Five year survival rate was 10·2% in patients with and 50·3% in those without neural invasion (fig 6A). This represents a significant difference between these groups ($p<0·01$). With respect to the patients who had undergone curative resection, the five year survival rate was 24·1 and 67·8% in the neural invasion positive and the negative groups, respectively, ($p<0·01$) (fig 6B).

Stepwise Cox regression analysis was performed in 76 patients who had undergone curative resection to determine which of the many covariates had the most prognostic significance with regard to survival. The covariates studied were age, sex, tumour size, tumour location, gross type, histological type, depth of wall invasion, lymphatic permeation, vascular permeation, lymph node metastasis, and neural invasion. The analysis revealed that the depth of wall invasion and the tumour size were independent prognostic factors after curative resection in patients with gastric carcinoma (table 2). Logistic regression analysis disclosed that the only factor which correlated with neural invasion was the size of the tumour ($p=0·004$).

Discussion

It has long been recognised that carcinoma cells spread via the blood and lymph vessels. Spread within peripheral nerves has received little attention, although del Regato and Spjut[16] suggested that invasion of perineural spaces by carcinoma cells is more common than is generally suspected. The perineural space has often been referred to as a lymph vessel, and the relation between the perineural space and lymph vessels has been studied. The perineural space is currently recognised as an independent space, distinct from a lymph vessel, following electron microscopic,[10] peroxidase injection,[17] and histopathological studies.[5 6 18–20]

The perineurium acts as a barrier to neural invasion.[6 7] We consider this to be true in certain cases of gastric cancer because the peripheral nerves were well preserved even though the surrounding tissues were replaced by carcinoma cells, as shown in fig 1. Carcinoma cells could invade the perineural space from a fragile part of the perineurium—that is, a site invaded by the blood vessels. Subsequently, carcinoma cells spread longitudinally within nerve bundles to the proximal nerve plexus.[6 7 9–12 20]

In pancreatic cancer no consistent tendencies were recognised with respect to the relations

between the histological type, infiltration pattern, or the quantity of interstitial connective tissue and the degree of neural invasion.[5 6] The incidence of invasion of the extrapancreatic nerve plexus was high among cases with severe lymph vessel invasion.[6] The prognosis was poor when neural invasion was severe.[21] However, a significant correlation was observed between macroscopic type, microscopic type, depth of invasion, and perineural invasion in bile duct carcinomas.[8] The five year survival rate was also significantly lower when neural invasion was present.[8] In colorectal carcinoma the survival rates were lower and the incidence of metastases much higher when neural invasion was present.[11 22] Neural invasion had the strongest association with local recurrence.[11]

The positive rate of neural invasion is high (80% or more) in pancreatic and bile duct carcinomas[5 6 8]; however, it is relatively low in colorectal carcinomas (14–32%).[11 22] The positive rate in gastric carcinoma in this study was 26% and is similar to that in colorectal carcinoma. The prognosis of patients with gastric carcinoma was much worse when neural invasion was present. Multivariate analysis, however, disclosed that neural invasion was not an independent prognostic factor. The contribution of vascular and lymphatic permeation to the malignant potential of the tumour is thought to be important, but neither of these is an independent prognostic factor. We suggest that the same applies to neural invasion.

Many authors have emphasised the importance of resection of the nerve and lymph nodes together with a primary tumour as a more

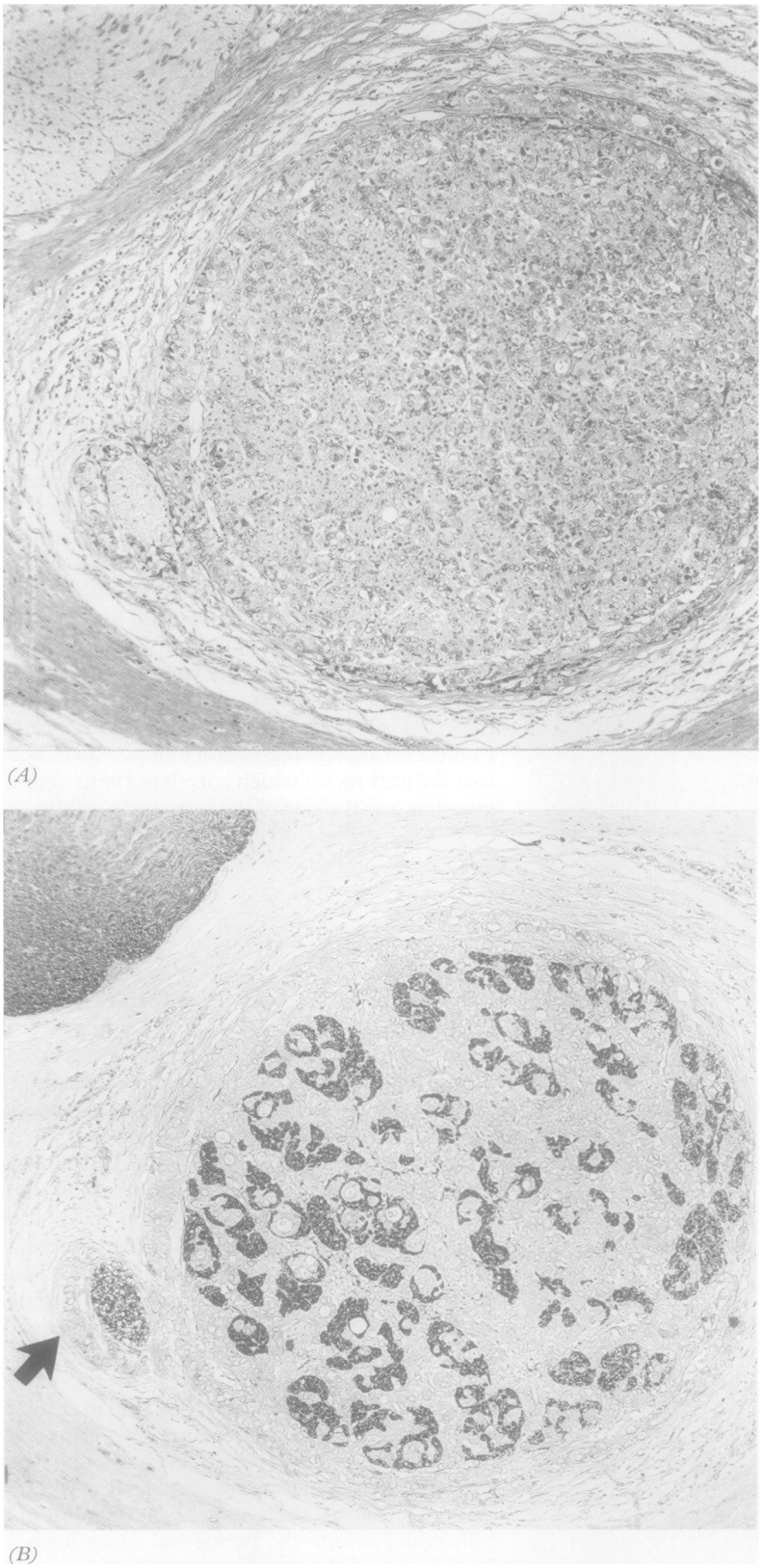


Figure 4 Perineural and endoneural invasion of gastric carcinoma in the subserosal layer. (A) The cut surface of the swollen nerve bundle (haematoxylin and eosin, × 100). It is difficult to recognise the nerve fibres. (B) Immunohistochemical staining for S-100 protein, demonstrating that this is a nerve bundle (× 100). The perineural and the endoneural spaces are invaded by carcinoma cells and the nerve fibres are dispersed. Only the perineural space is invaded by carcinoma cells in the small branch (arrow). Note that there is no cancer cell invasion in the surrounding tissue. One of the nerve bundles is free of invasion by carcinoma cells (upper left corner in both figures).

Table 1 Clinicopathological findings and neural invasion

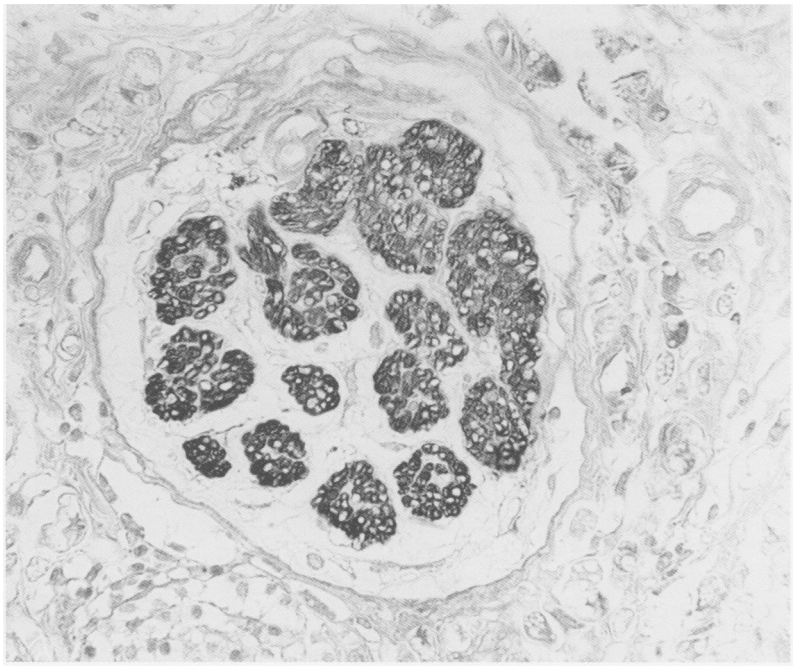
Variable	*Neural invasion*		*p value*
	Positive (n=34)	*Negative (n=87)*	
Age (mean, years)	53·8	60·2	NS
Sex			
male	16	51	NS
female	18	36	
Size (mean, cm)	9·9±4·6	7·6±3·3	p<0·01
Tumour location			
upper (C)	12	28	NS
middle (M)	10	33	
lower (A)	12	26	
Gross type			
expansive	4	28	NS
infiltrative	27	48	
intermediate	3	11	
Histology			
intestinal	12	40	NS
diffuse	20	44	
mixed	2	3	
Depth of invasion			
within muscular layer	1	10	p<0·01
invading the subserosa	0	19	
invading the serosa	15	39	
invading other organs	18	19	
Lymphatic permeation			
absent	13	28	NS
present	21	59	
Vascular permeation			
absent	27	74	NS
present	7	13	
Lymph node metastasis			
absent	8	29	NS
present	26	58	
Hepatic metastasis			
absent	31	83	NS
present	3	4	
Peritoneal dissemination			
absent	27	76	NS
present	7	11	
Stage			
I	1	12	p<0·01
II	1	11	
III	12	32	
IV	20	32	
Curability of operation			
radical	16	60	p<0·02
non-radical	18	27	

Table 2 Independent prognostic variables in patients who underwent curative resection for gastric carcinoma

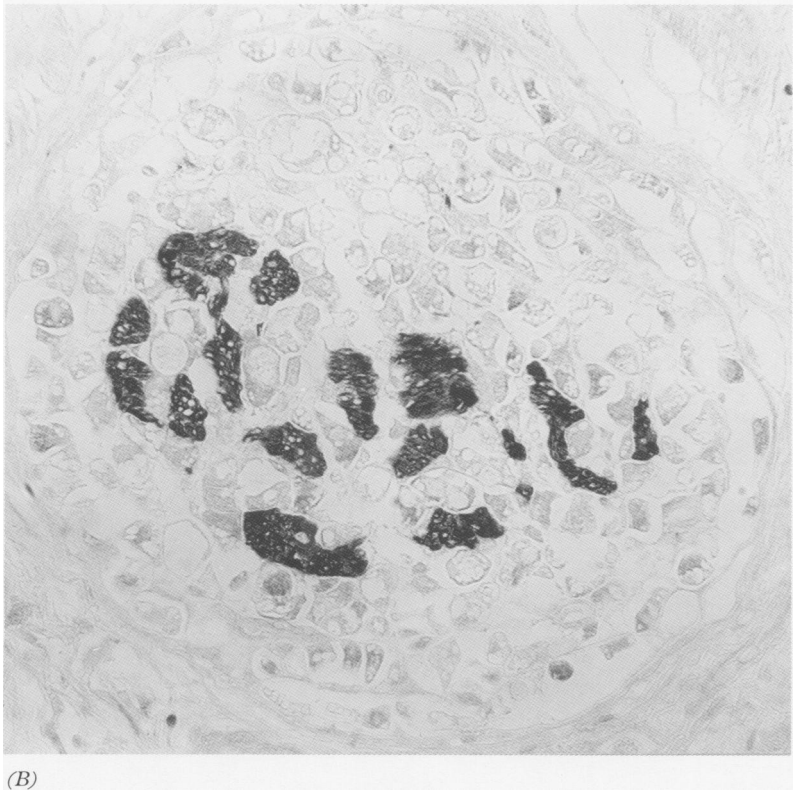
Variable	*Regression coefficient (R)*	*Standard error (S)*	*R/S*	*Relative risk*	*p value*
Depth of tumour invasion	0·460	0·233	1·977	1·585	p<0·001
Tumour size	0·144	0·066	2·193	1·155	p<0·001

curative approach to prevent recurrence of the tumour.[6 7 9 11 21] In this study eight of 34 cases with positive neural invasion in the primary gastric carcinoma had neural invasion in the coeliac nerve plexus. Four of the eight showed no lymph node metastasis in the roots of the left gastric, common hepatic or coeliac arteries. In our institution we perform an R2 operation as a standard procedure for gastric carcinoma,[23] and the N2 lymph nodes (alongside the left gastric, common hepatic, and coeliac arteries) are dissected together with the surrounding soft tissue which includes the coeliac nerve plexus. Therefore, this procedure is important not only from the view point of lymph node metastasis but also from that of neural invasion.

In conclusion, although neural invasion was not an independent prognostic factor, the presence or absence of neural invasion may provide an additional basis for diagnosing patients with gastric carcinoma. En bloc resection of the autonomic nerve fibres and plexus around the left gastric, common hepatic, and coeliac arteries together with the lymph nodes may be necessary to completely remove the cancer tissue.



(A)



(B)

Figure 5 Oedematous changes in the nerve. The perineural and endoneural spaces are enlarged. (A) (Immunohistochemical stain for S-100 protein, × 400). (B) Neural invasion. Cancer cells are present within the perineural space, and bundles of nerve fibres are detached (immunohistochemical stain for S-100 protein, × 400).

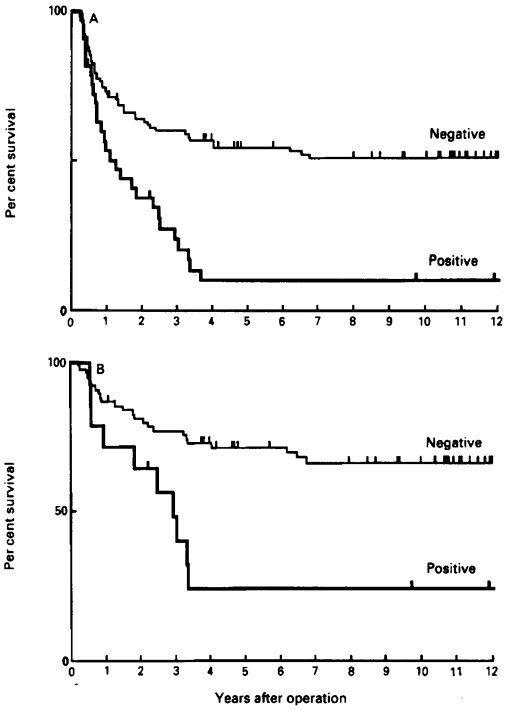


Figure 6 Survival curves of patients with or without neural invasion in primary gastric carcinoma. A, all patients; B, patients who underwent curative surgery. There is a significant difference between patients with and without neural invasion.

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