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Clinicopathological and prognostic significance of EGFR, VEGF, and HER2 expression in cholangiocarcinoma

D Yoshikawa[1,2], H Ojima[3], M Iwasaki[4], N Hiraoka[3], T Kosuge[5], S Kasai[2], S Hirohashi[3] and T Shibata[*,1,3]

[1]Cancer Genomics Project, National Cancer Center Research Institute, Tokyo, Japan; [2]Division of Gastroenterological and General Surgery, Department of Surgery, Asahikawa Medical College, Asahikawa, Japan; [3]Pathology Division, National Cancer Center Research Institute, Tokyo, Japan; [4]Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo, Japan; [5]Hepato-Biliary and Pancreatic Surgery Division, National Cancer Center Hospital, Tokyo, Japan

Epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and human epidermal growth factor receptor 2 (HER2) have been considered as potential therapeutic targets in cholangiocarcinoma, but no studies have yet clarified the clinicopathological or prognostic significance of these molecules. Immunohistochemical expression of these molecules was assessed retrospectively in 236 cases of cholangiocarcinoma, as well as associations between the expression of these molecules and clinicopathological factors or clinical outcome. The proportions of positive cases for EGFR, VEGF, and HER2 overexpression were 27.4, 53.8, and 0.9% in intrahepatic cholangiocarcinoma (IHCC), and 19.2, 59.2, and 8.5% in extrahepatic cholangiocarcinoma (EHCC), respectively. Clinicopathologically, EGFR overexpression was associated with macroscopic type ($P = 0.0120$), lymph node metastasis ($P = 0.0006$), tumour stage ($P = 0.0424$), lymphatic vessel invasion ($P = 0.0371$), and perineural invasion ($P = 0.0459$) in EHCC, and VEGF overexpression with intrahepatic metastasis ($P = 0.0224$) in IHCC. Multivariate analysis showed that EGFR expression was a significant prognostic factor (hazard ratio (HR), 2.67; 95% confidence interval (CI), 1.52–4.69; $P = 0.0006$) and also a risk factor for tumour recurrence (HR, 1.89; 95% CI, 1.05–3.39, $P = 0.0335$) in IHCC. These results suggest that EGFR expression is associated with tumour progression and VEGF expression may be involved in haematogenic metastasis in cholangiocarcinoma.
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Cholangiocarcinoma arises from the ductal epithelium of the bile duct tree and is classified anatomically into intrahepatic cholangiocarcinoma (IHCC) and extrahepatic cholangiocarcinoma (EHCC). The incidence and mortality rates of cholangiocarcinoma, especially those of IHCC, are increasing worldwide (Khan *et al*, 2005). Complete resection is the only way to cure the disease at present. Moreover, because cholangiocarcinoma is difficult to diagnose at an early stage and extends diffusely, most patients have unresectable disease at clinical presentation, and prognosis is very poor (5-year survival is 0–40% even in resected cases) (Khan *et al*, 2005; Sirica, 2005). Therefore, novel effective therapeutic strategies are urgently required to improve the prognosis. Among potential therapeutic targets, several studies have revealed overexpression of epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor 2 (HER2) protein, amplification, and mutation of these genes (Ito *et al*, 2001; Aishima *et al*, 2002; Ukita *et al*, 2002; Altimari *et al*, 2003; Gwak *et al*, 2005; Nakazawa *et al*, 2005; Leone *et al*, 2006) as well as overexpression of vascular endothelial growth factor (VEGF) protein (Hida *et al*, 1999; Tang *et al*, 2006) in cholangiocarcinoma.

*Correspondence: Dr T Shibata; E-mail: tashibat@ncc.go.jp
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Epidermal growth factor receptor and HER2 are members of the ErbB receptor tyrosine kinase family. Binding of ligands, such as epidermal growth factor and transforming growth factor alpha (TGFα), to their extracellular ligand-binding domain initiates intracellular signalling cascades, leading to progression, proliferation, migration, and survival of cancer cells (Olayioye *et al*, 2000; Yarden and Sliwkowski, 2001). Vascular endothelial growth factor plays a key role in tumour-associated neo-angiogenesis, which contributes to providing a tumour with oxygen, nutrition, and a route for metastasis. It binds to VEGFR (vascular endothelial growth factor receptor), and leads to survival, proliferation, and migration of endothelial cell (Tabernero, 2007). Expression of these molecules has been reported to have prognostic significance in several cancers (Gusterson *et al*, 1992; Han *et al*, 2001; Nicholson *et al*, 2001; Des Guetz *et al*, 2006; Mohammed *et al*, 2007). Recently, agents targeted at these molecules have been used clinically, such as trastuzumab in breast cancer (Gonzalez Angulo *et al*, 2006), gefitinib, and erlotinib in non-small cell lung cancer, and bevacizumab in colorectal cancer (Tabernero, 2007). In cholangiocarcinoma, a phase II study of erlotinib (Philip *et al*, 2006) and some case reports of combined chemotherapy including cetuximab (Sprinzl *et al*, 2006; Huang *et al*, 2007) have been reported.

However, no previous studies have clarified associations between the expression of these molecules and clinicopathological

factors or prognosis in patients with cholangiocarcinoma. To elucidate the biological significance and potential of these molecules as therapeutic targets, we investigated EGFR/VEGF/HER2 expression and attempted to elucidate their associations with various clinical features as well as patient survival in 236 cases of cholangiocarcinomas.

MATERIALS AND METHODS

Patients

A total of 236 patients with cholangiocarcinoma (male 160; female 76) who had undergone tumour resection and been diagnosed histologically as having adenocarcinoma of the bile duct at the National Cancer Center Hospital, Tokyo, between January 1991 and August 2004, were enrolled in the present study. Median patient age and follow-up period were 65 years and 875 days, and median tumour sizes of IHCC and EHCC were 4.8 and 3.0 cm, respectively. Detailed characteristics of patient with IHCC and EHCC are presented in Tables 1 and 2. All patients were followed for more than 100 days. Follow-up examination was performed using computed tomography, abdominal ultrasonography, and measurement of the serum carcinoembryonic antigen and carbohydrate antigen 19-9 (CA19-9) levels every 3-6 months. Recurrence was diagnosed by clinical, radiological, or pathological methods, but mainly by radiological evaluation including computed tomography and ultrasonography. Clinical and pathological profiles were obtained from the database of hepatobiliary tumours based on the medical records of the patients. This study was approved by the Ethics Committee of the National Cancer Center, Tokyo, Japan, and written informed consent was obtained from all patients.

All cases were anatomically classified into two groups: IHCC and EHCC. Tumours arising from the bilateral hepatic duct or distal common bile duct were classified as EHCC. The numbers of IHCC and EHCC cases were 106 and 130, respectively.

Histological assessment

Tumour staging and histological classification were assessed according to *TNM Classification of Malignant Tumours* (Sobin and Wittekind, 2002) defined by the International Union Against Cancer (UICC) and the *World Health Organization Histological Classification of Tumours* (Hamilton and Altonen, 2000). Macroscopic types of IHCC were defined with reference to *General Rules for the Clinical and Pathological Study of Primary Liver Cancer* (Liver Cancer Study Group of Japan, 2003): (1) the mass-forming type (MF), which develops an apparent tumour in the liver; (2) the periductal infiltrating type (PI), which spreads along the bile duct; (3) the intraductal growth type (IG), which is confined within the bile duct, and divided into two groups: the mass-forming group (MF and MF mixed with PI or IG) and the non-mass forming group (PI and/or IG). Macroscopic types of EHCC were divided into polypoid type and non-polypoid type (including nodular, scirrhous constricting, and infiltrating types). Other clinicopathological factors were categorised into groups that are presented in Table 1 (IHCC) and Table 2 (EHCC). Because the classifications and clinicopathological factors used in IHCC and EHCC are different, statistical analyses were performed separately.

Immunohistochemistry

Immunohistochemistry (IHC) for EGFR, VEGF, and HER2 was performed using a polymer-based method (Envision™ + Dual Link System-HRP (Dako, DK-2600 Glostrup, Denmark)). Sources and dilutions of primary antibodies were as follows: anti-EGFR (mouse monoclonal, clone 31G7; Zymed, South San Francisco, CA, USA; 1:100), anti-VEGF (rabbit polyclonal; Zymed; 1:50), and anti-HER2 (rabbit polyclonal; Dako; 1:300).

Formalin-fixed, paraffin-embedded serial tissue sections (4 μm) were placed on silane-coated slides for IHC. Sections cut through the maximum tumour diameter were selected for IHC evaluation. The sections were deparaffinised and rehydrated in xylene and grade-diluted ethanol (50-100%), and submerged for 20 min in 0.3% hydrogen peroxide with absolute methanol to block endogenous peroxidase activity. Antigen retrieval for EGFR, VEGF, and HER2 was carried out by adding Digest-all™3 pepsin solution (Zymed) at 37°C for 10 min for EGFR, near boiling in 0.01 M citrate buffer (pH 6.0) for 15 min for VEGF, and heating in 0.01 M citrate buffer at 121°C for 10 min by pressure cooker for HER2. After protein blocking, the sections were incubated with each primary antibody at room temperature for 1 h, followed by incubation with

Table 1 Characteristics of the IHCC patients

Factors	Categories	Population
Age	<65 years old	54 (50.9%)
	⩾65 years old	52 (49.1%)
Gender	Male	64 (60.4%)
	Female	42 (39.6%)
Tumour size	⩽5.0 cm	55 (55.6%)
	>5.0 cm	44 (44.4%)
Macroscopic type	Non-mass forming	17 (16.0%)
	Mass forming	89 (84.0%)
Invasion of portal vein	Negative	23 (21.9%)
	Positive	82 (78.1%)
Invasion of hepatic vein	Negative	56 (54.9%)
	Positive	46 (45.1%)
Intrahepatic metastasis	Negative	75 (70.8%)
	Positive	31 (29.2%)
Lymph node metastasis	Negative	62 (58.5%)
	Positive	44 (41.5%)
UICC pT	1+2	71 (68.3%)
	3+4	33 (31.7%)
UICC stage	1+2	45 (42.5%)
	3A+3B+3C	61 (57.5%)
Histological classification	Well	22 (20.8%)
	Mod	79 (74.5%)
	Por	5 (4.7%)
Lymphatic vessel invasion	Negative	20 (18.9%)
	Positive	86 (81.1%)
Venous invasion	Negative	19 (17.9%)
	Positive	87 (82.1%)
Perineural invasion	Negative	29 (27.4%)
	Positive	77 (72.6%)
Hepatic surgical margin	Negative	89 (84.0%)
	Positive	17 (16.0%)
Bile duct margin	Negative	91 (85.8%)
	Positive	15 (14.2%)

Well = well differentiated adenocarcinoma; Mod = moderately differentiated adenocarcinoma; Por = poorly differentiated adenocarcinoma. In some factors, data were not available for all cases.

Table 2 Characteristics of the EHCC patients

Factors	Categories	Population
Age	<65 years old	60 (46.2%)
	≥65 years old	70 (53.8%)
Gender	Male	96 (73.8%)
	Female	34 (26.2%)
Tumour size	≤3.0 cm	72 (56.3%)
	>3.0 cm	56 (43.7%)
Macroscopic type	Polypoid	21 (16.8%)
	Non-polypoid	104 (83.2%)
Depth of tumour invasion	Within FM	13 (10.0%)
	Beyond FM	117 (90.0%)
Invasion of portal vein	Negative	97 (74.6%)
	Positive	33 (25.4%)
Invasion of hepatic artery	Negative	127 (97.7%)
	Positive	3 (2.3%)
Lymph node metastasis	Negative	71 (54.6%)
	Positive	59 (45.4%)
UICC pT	1+2	49 (37.7%)
	3+4	81 (62.3%)
UICC stage	1A+1B	37 (28.5%)
	2A+2B+C	93 (71.5%)
Histological classification	Pap	20 (15.4%)
	Well	31 (23.8%)
	Mod	62 (47.7%)
	Por	17 (13.1%)
Lymphatic vessel invasion	Negative	16 (12.3%)
	Positive	114 (87.7%)
Venous invasion	Negative	19 (14.6%)
	Positive	111 (85.4%)
Perineural invasion	Negative	23 (17.7%)
	Positive	107 (82.3%)
Dissected periductal structures margin	Negative	109 (83.8%)
	Positive	21 (16.2%)
Bile duct margin	Negative	92 (70.8%)
	Positive	38 (29.2%)
Invasion to other organ	Negative	53 (40.8%)
	Positive	77 (59.2%)

FM = fibromuscular layer; Pap = papillary adenocarcinoma; Well = well differentiated adenocarcinoma; Mod = moderately differentiated adenocarcinoma; Por = poorly differentiated adenocarcinoma. In some factors, data were not available for all cases.

Envision+ Dual Link reagent at room temperature for 30 min, and visualised using 3,3′-diaminobenzidine tetrahydrochloride as a chromogen. Finally, the sections were counterstained with haematoxylin. Sections were gently rinsed in phosphate-buffered saline between the incubation steps.

Evaluation of immunohistochemistry

All sections were evaluated by DY, HO, and TS without the knowledge of any clinical or pathological information, and cases for which consensus could not be reached were discussed to decide the evaluation. Based on the Herceptest™ (Dako) criteria, intensities of both EGFR and HER2 were defined as follows: 0, no membrane staining or membrane staining in ≤10% cancer cells; 1+, faint and partial membrane staining in >10% cancer cells; 2+, moderate and complete membrane staining in >10% cancer cells; 3+, strong and complete membrane staining in >10% cancer cells. Intensities of VEGF were defined as follows: 0, no cytoplasmic staining or cytoplasmic staining in ≤30% cancer cells; 1+, faint cytoplasmic staining, equivalent to the intensity of normal bile duct epithelium within the same section, in >30% cancer cells; 2+, moderate cytoplasmic staining in >30% cancer cells; 3+, strong cytoplasmic staining in >30% cancer cells. For cases showing mixed intensity, the predominant intensity was selected as the final IHC score. A final IHC score of 2+ or 3+ was defined as positive for expression of each protein.

Statistical analysis

Associations between results of IHC and clinicopathological factors were assessed by χ^2 test. Cumulative survival rates and survival curves were calculated by the Kaplan–Meier method, and log-rank test was performed for the comparison of survival curves. Cox's proportional hazard model was performed to estimate hazard ratio (HR) and 95% confidence interval (CI) of each outcome (death and recurrence). Multivariate analyses were performed using the factors identified to be risk factors for each outcome by univariate analyses, without UICC pT and UICC Stage, which are composed of other factors. All *P*-values reported are two-sided, and significance level was set at $P<0.05$. All statistical analyses were performed with the Statview 5.0 statistical software package (Abacus Concepts, Berkeley, CA, USA).

RESULTS

Expression of EGFR, VEGF, and HER2 protein in cholangiocarcinoma

Representative cases of positive staining for each protein are shown in Figure 1 (A, EGFR; B, HER2; C, VEGF). Epidermal growth factor receptor, VEGF, and HER2 were expressed in 29 (27.4), 57 (53.8), and 1 (0.9%) of the 106 IHCCs, respectively, and in 25 (19.2), 77 (59.2), and 11 (8.5%) of the 130 EHCCs, respectively. Microscopically, EGFR was mostly overexpressed in the moderately and/or poorly differentiated component, which is characterised by infiltration (52 of 54 EGFR-positive cases, Figure 1D), whereas only two cases showed EGFR overexpression in the well-differentiated component. In contrast, HER2 was preferentially expressed in the well-differentiated component. In 6 of 12 HER2-positive cases, HER2 was expressed only in well-differentiated component (Figure 1E), and 5 progressive cases showed positive HER2 staining in both the well and moderately and/or poorly differentiated components and 1 case only in moderately differentiated component. There was no association between VEGF expression and histological features.

Associations between EGFR, VEGF, and HER2 expression and clinocopathological factors

Statistical analyses of HER2 were performed only in EHCC cases because of the small number of HER2-positive cases in IHCC. In IHCC, VEGF expression was significantly associated with intrahepatic metastasis ($P=0.0224$). There was no significant association between EGFR expression and any clinicopathological factors.

In EHCC, EGFR expression was significantly associated with macroscopic type (0% in the polypoid type, 24.0% in the non-polypoid type; $P=0.0120$), lymph node metastasis ($P=0.0006$), UICC Stage ($P=0.0424$), lymphatic vessels invasion ($P=0.0371$), and perineural invasion ($P=0.0459$). Human epidermal growth factor receptor 2 expression was significantly associated with

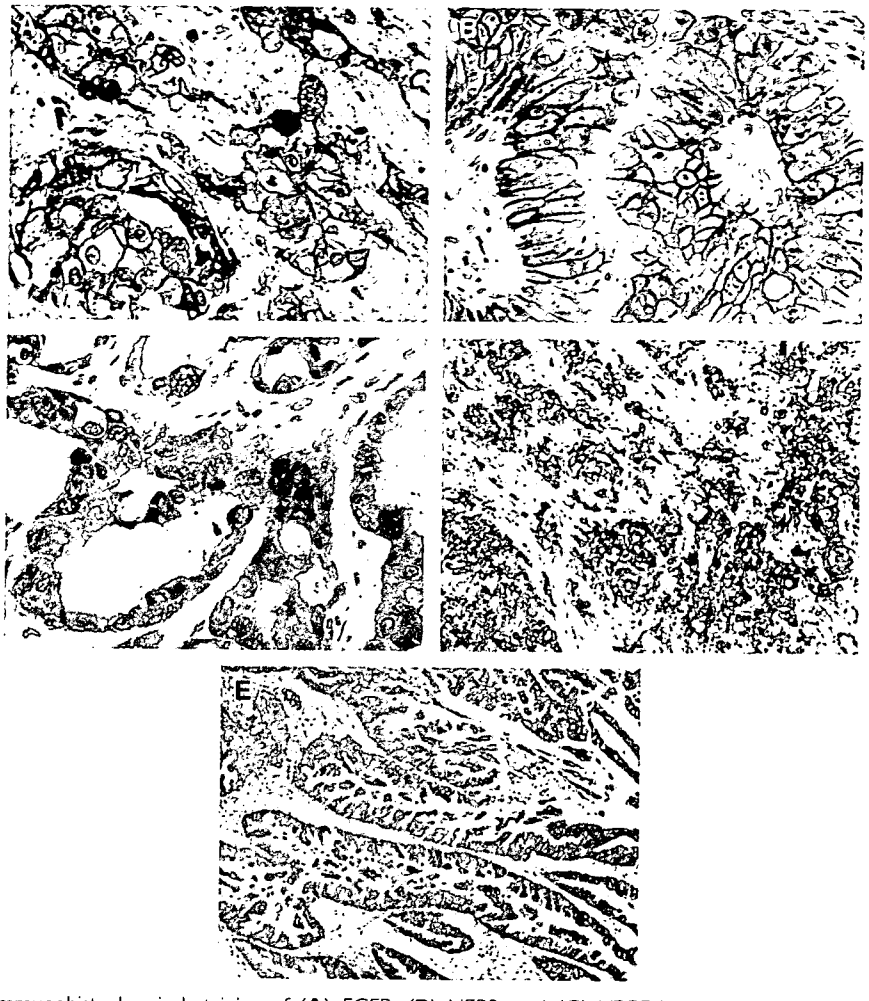

Figure 1 Representative immunohistochemical staining of (**A**) EGFR, (**B**) HER2, and (**C**) VEGF in cholangiocarcinoma (× 400 magnification). (**D**) Epidermal growth factor receptor tends to be expressed in the poorly differentiated component (× 100 magnification). (**E**) Human epidermal growth factor receptor 2 is preferentially expressed in more differentiated areas such as the glandular or papillary component (× 100 magnification).

macroscopic type (23.8% in the polypoid type, 5.8% in the non-polypoid type; $P=0.0078$), histological classification (25% in papillary adenocarcinoma, 9.7% in well differentiated adenocarcinoma, 3.2% in moderately differentiated adenocarcinoma, 5.9% in poorly differentiated adenocarcinoma; $P=0.0237$), and invasion to other organs (3.9% in invasive cases, 15.1% in non-invasive cases; $P=0.0242$). VEGF expression was not significantly associated with any factors in EHCC.

Detailed results of associations between EGFR/VEGF/HER2 expression and clinicopathological factors are shown in Supplementary information 1 (IHCC) and Supplementary information 2 (EHCC).

Univariate and multivariate analyses regarding overall survival and tumour recurrence in cholangiocarcinoma

The number of dead and the median survival time were 70 cases and 724 days in IHCCs, and 76 cases and 1197 days in EHCCs, respectively. The number of recurrence and the median recurrence time were 64 cases and 522 days in IHCCs, and 78 cases and 960 days in EHCCs, respectively.

Overall 5-year cumulative survival for patients with IHCC and EHCC was 33.0 and 41.6%, respectively, and no significant difference was identified between the groups ($P=0.0599$). The survival curves stratified by EGFR expression status are shown as Figure 2. Five-year survival for patients with EGFR-positive and EGFR-negative tumours was 17.7 and 47.1% for IHCC, and 26.4 and 45.6% for EHCC, respectively. There was a significant difference between EGFR-positive and -negative cases for both IHCC ($P=0.0008$) and EHCC ($P=0.0204$).

The results of multivariate analyses following univariate analyses regarding overall survival and tumour recurrence are shown in Table 3 (IHCC) and Table 4 (EHCC).

In IHCC, 13 factors including EGFR expression were identified as significantly prognostic by univariate analysis. Multivariate analysis revealed that EGFR expression was an independent prognostic factor (HR, 2.67; 95% CI, 1.52–4.69; $P=0.0006$), along with mass-forming macroscopic group (HR, 2.96; 95% CI, 1.06–8.31; $P=0.0390$), intrahepatic metastasis (HR, 2.91; 95% CI, 1.60–5.29; $P=0.0005$), and lymph node metastasis (HR, 1.96; 95% CI, 1.04–3.69; $P=0.0375$). In EHCC, 14 factors including EGFR expression were identified as significantly prognostic by univariate analysis. Multivariate analysis revealed that lymph node metastasis (HR, 2.03; 95% CI, 1.16–3.55; $P=0.0133$) and a histological classification of moderately differentiated adenocarcinoma (HR for papillary adenocarcinoma, 4.23; 95% CI, 1.08–16.50; $P=0.0380$) and poorly differentiated adenocarcinoma (HR for papillary adenocarcinoma, 13.22; 95% CI, 3.10–56.45; $P=0.0005$) were significant prognostic factors.

Multivariate analysis following univariate analysis for risk factors of tumour recurrence revealed that EGFR expression in IHCC was a significant risk factor of tumour recurrence (HR, 1.89;

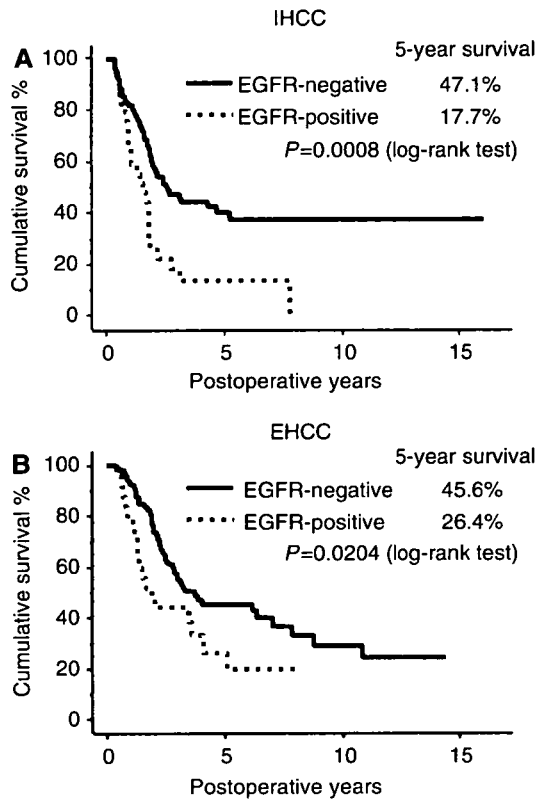

Figure 2 Survival curves stratified by EGFR expression in (**A**) IHCC and (**B**) EHCC (Kaplan–Meier method). The outcome of EGFR-positive cases was significantly worse than that of EGFR-negative cases in both IHCC ($P=0.0008$) and EHCC ($P=0.0204$) (by log-rank test).

95% CI, 1.05–3.39; $P=0.0335$), along with intrahepatic metastasis (HR, 2.36; 95% CI, 1.31–4.25; $P=0.0044$), lymph node metastasis (HR, 2.24; 95% CI, 1.19–4.22; $P=0.0126$), and venous invasion (HR, 6.74; 95% CI, 1.31–34.73; $P=0.0225$), whereas, in EHCC, lymph node metastasis (HR, 1.75; 95% CI, 1.03–2.98; $P=0.0394$) and dissected periductal structures margin (HR, 1.81; 95% CI, 1.03–3.16; $P=0.0383$) were independent risk factors of tumour recurrence, but EGFR expression was not associated with tumour recurrence even in univariate analysis.

DISCUSSION

This study, analysing EGFR/VEGF/HER2 expression in the largest cohort of cholangiocarcinoma reported so far, showed for the first time that EGFR expression in IHCC is significantly associated with poor prognosis. In addition, our study confirmed previously reported prognostic factors in cholangiocarcinoma, such as macroscopic type, intrahepatic metastasis, lymph node metastasis, and histological classification (Yamamoto *et al*, 1998; Ohtsuka *et al*, 2002; Morimoto *et al*, 2003; DeOliveira *et al*, 2007). Expression of EGFR or HER2 is known to be a prognostic factor in some cancers (Gusterson *et al*, 1992; Nicholson *et al*, 2001), but no previous study has clarified the influence of these molecules on prognosis in cholangiocarcinoma (Ito *et al*, 2001; Altimari *et al*, 2003; Nakazawa *et al*, 2005), probably because cholangiocarcinoma is a relatively rare cancer and collection of a large cohort is difficult. Indeed, most previous studies were performed on the basis of only 50 cases at most. Although it is unclear why EGFR expression in IHCC is an independent prognostic factor, it may be associated with frequent relapse of cancer because EGFR expression is also a risk factor for tumour recurrence.

Table 3 Multivariate analyses regarding overall survival and tumour recurrence in IHCC (Cox's proportional hazard model)

	Overall survival			Tumour recurrence		
	HR	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Macroscopic type						
Non-mass forming	1.00			1.00		
Mass forming	2.96	1.06–8.31	0.0390	3.06	1.00–9.40	0.0505
Invasion of portal vein						
Negative	1.00			1.00		
Positive	0.67	0.30–1.47	0.31	1.01	0.43–2.41	0.98
Invasion of hepatic vein						
Negative	1.00			1.00		
Positive	1.19	0.66–2.12	0.57	1.17	0.65–2.14	0.60
Intrahepatic metastasis						
Negative	1.00			1.00		
Positive	2.91	1.60–5.29	0.0005	2.36	1.31–4.25	0.0044
Lymph node metastasis						
Negative	1.00			1.00		
Positive	1.96	1.04–3.69	0.0375	2.24	1.19–4.22	0.0126
Histological classification						
Well differentiated	1.00			1.00		
Moderately differentiated	1.24	0.56–2.75	0.60	0.65	0.28–1.53	0.32
Poorly differentiated	2.09	0.58–7.49	0.26	1.35	0.32–5.72	0.69
Lymphatic vessel invasion						
Negative	1.00			1.00		
Positive	3.31	0.80–13.65	0.0982	1.37	0.41–4.56	0.61
Venous invasion						
Negative	1.00			1.00		
Positive	4.07	0.97–17.09	0.0551	6.74	1.31–34.73	0.0225
Perineural invasion						
Negative	1.00			—		
Positive	0.60	0.26–1.36	0.22	—	—	—
Bile duct margin						
Negative	1.00			—		
Positive	1.84	0.91–3.73	0.0923	—	—	—
EGFR expression						
Negative	1.00			1.00		
Positive	2.67	1.52–4.69	0.0006	1.89	1.05–3.39	0.0335

Abbreviations: CI = confidence interval; HR = hazard ratio.

In contrast to IHCC, EGFR expression was not an independent prognostic factor in EHCC, but was associated with clinical features that may represent tumour progression and invasion, such as lymph node metastasis and apparent stromal invasion in EHCC. Because cancer tissue tends to be heterogeneous, histological diagnosis is generally decided on the basis of the degree of differentiation that predominates. In order to elucidate the biological significance of each protein, we microscopically examined positive cases in detail and compared their expression with histological components, and found that EGFR tended to be expressed in the poorly differentiated component, which is characterised by infiltration in both IHCC and EHCC. Similar results have been reported in bladder cancer (Neal *et al*, 1985), oesophageal adenocarcinoma (Wilkinson *et al*, 2004), and IHCC (Ito *et al*, 2001), although the studies were based on small cohorts. These findings indicate that EGFR expression may be a relatively late event in the development of cholangiocarcinoma and

Table 4 Multivariate analyses regarding overall survival and tumour recurrence in EHCC (Cox's proportional hazard model)

	Overall survival			Tumour recurrence		
	HR	**95% CI**	***P*-value**	**HR**	**95% CI**	***P*-value**
Tumour size						
⩽3.0 cm	1.00			—		
>3.0 cm	1.29	0.71–2.35	0.41	—	—	—
Macroscopic type						
Polypoid	1.00			—		
Non-polypoid	0.44	0.16–1.26	0.13	—	—	—
Depth of tumour invasion						
Within FM	1.00			1.00		
Beyond FM	1.26	0.19–8.60	0.81	1.16	0.24–5.57	0.85
Invasion of portal vein						
Negative	1.00			1.00		
Positive	1.48	0.81–2.69	0.20	1.59	0.92–2.75	0.94
Lymph node metastasis						
Negative	1.00			1.00		
Positive	2.03	1.16–3.55	0.0133	1.75	1.03–2.98	0.0394
Histological classification						
Papillary	1.00			1.00		
Well differentiated	3.40	0.85–13.66	0.0849	0.91	0.33–2.51	0.85
Moderately differentiated	4.23	1.08–16.50	0.0380	1.19	0.47–3.02	0.72
Poorly differentiated	13.22	3.10–56.45	0.0005	2.80	0.99–7.87	0.0516
Lymphatic vessel invasion						
Negative	1.00			1.00		
Positive	1.78	0.29–11.10	0.54	2.36	0.45–12.37	0.31
Venous invasion						
Negative	1.00			1.00		
Positive	3.93	0.81–19.12	0.0898	1.89	0.52–6.92	0.34
Perineural invasion						
Negative	1.00			1.00		
Positive	1.94	0.58–6.53	0.29	0.98	0.38–2.51	0.97
Dissected periductal structures margin						
Negative	1.00			1.00		
Positive	1.20	0.67–2.17	0.54	1.81	1.03–3.16	0.0383
Invasion to other organ						
Negative	1.00			1.00		
Positive	1.02	0.53–1.94	0.96	0.94	0.53–1.69	0.84
EGFR expression						
Negative	1.00			—		
Positive	1.04	0.55–1.96	0.90	—	—	—

HR = hazard ratio; CI = confidence interval; FM = fibromuscular layer.

associated with invasion and progression. Because it has been previously reported that poor differentiation is associated with unfavourable outcome in other cancers (Sohn *et al*, 2000; Hassan *et al*, 2005), the association between EGFR expression and poor differentiation may also be a reason that EGFR expression is a prognostic factor.

Though the prognostic factors were different between IHCC and EHCC, it may be due to the difference of anatomical character, which extrahepatic bile duct is near from other organs and is not surrounded by liver parenchyma in contrast to intrahepatic bile duct. The intrahepatic epithelium is distinct from the extrahepatic epithelium in terms of development and differentiation (Shiojiri, 1997), and the risk factors, pathogenesis, and clinical features of IHCC and EHCC are different (Strom *et al*, 1985; Nakeeb *et al*, 1996; Shaib *et al*, 2007). Although no previous studies have elucidated EGFR function in normal bile duct epithelium, EGFR overexpression might play distinct roles in IHCC and EHCC.

Vascular endothelial growth factor expression was detected frequently, being evident in about 60% of our study cases, which is consistent with previous studies (31.4–75.6%) (Hida *et al*, 1999; Tang *et al*, 2006). Our study revealed that VEGF expression was significantly associated with intrahepatic metastasis in IHCC. Vascular endothelial growth factor is a key molecule in angiogenic pathway. Angiogenesis is an essential component in the process of metastasis, and this has been partly confirmed by studies showing that microvessel density (MVD) is associated with metastasis and a poorer outcome in a range of cancers (Weidner *et al*, 1991; Zetter, 1998). It has also been reported that high MVD is an independent prognostic factor in node-negative IHCC (Shirabe *et al*, 2004) and is associated with VEGF expression in IHCC (Tang *et al*, 2006), although no study has clarified the involvement of angiogenesis in the process of metastasis in cholangiocarcinoma. Our result suggests that VEGF plays an important role in the process of cholangiocarcinoma metastasis by promoting angiogenesis.

Human epidermal growth factor receptor 2 was expressed in only 11 of 130 EHCC cases (8.5%) and in one of 106 IHCC cases (0.9%). The proportion of HER2-positive cases reported previously has varied from 4.2 to 81.8% (Ito *et al*, 2001; Aishima *et al*, 2002; Ukita *et al*, 2002; Altimari *et al*, 2003; Nakazawa *et al*, 2005), and the discrepancy may be due to differences in staining procedure or tumour location. In contrast to EGFR expression, HER2 expression was associated with more favourable clinical features, such as a polypoid macroscopic type and absence of other organ involvement. The proportion of HER2-positive cases in papillary adenocarcinoma was higher than in other histological types, consistent with some previous reports claiming that HER2 expression in cholangiocarcinoma is associated with an early disease stage (Endo *et al*, 2002; Nakazawa *et al*, 2005). Microscopically, HER2 is preferentially expressed in well differentiated component, and it is also expressed in dedifferentiated components (moderately and/or poorly differentiated components) in progressive cases. This indicates that HER2 overexpression is maintained from an early stage of carcinogenesis in cases that are HER2-positive.

Recently, the efficacy of molecular targeting therapy for various molecules including EGFR/VEGF/HER2 has been proved clinically in a wide range of cancers. Epidermal growth factor receptor inhibitor has been reported to be effective in a cholangiocarcinoma cell line (Yoon *et al*, 2004), and a phase II study of erlotinib, an EGFR inhibitor, in patients with advanced biliary cancer has been reported. In this study, the progression-free rate at 6 months as a primary end point was 17% (7/42) despite the fact that disease condition was severe, and the disease control rate was 50% (20/42) (Philip *et al*, 2006). This study suggested the clinical applicability of the EGFR inhibitor to cholangiocarcinoma. Several clinical trials demonstrating the efficacy of VEGF inhibition for other cancers have been reported (Hurwitz *et al*, 2004; Sandler *et al*, 2006), and VEGF upregulation in tumour cells is considered to be a mechanism of resistance to EGFR inhibitors (Viloria Petit *et al*, 2001). Therefore, dual inhibition of both EGFR and VEGF may exert a synergistic effect.

In summary, we have shown that EGFR and VEGF expression is relatively common in cholangiocarcinoma. Moreover, in IHCC, EGFR expression is an independent prognostic factor and VEGF expression is associated with intrahepatic metastasis. In EHCC, EGFR expression is associated with clinical factors involved in tumour progression and invasion. Our results suggest the validity and significance of molecular targeting agents for EGFR and/or VEGF pathway, and that further preclinical and clinical studies are warranted for improving the clinical outcome of cholangiocarcinoma.

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Characterisation and protein expression profiling of annexins in colorectal cancer

R Duncan[1], B Carpenter[2], LC Main[1], C Telfer[2] and GI Murray*[,1]

[1]Department of Pathology, University of Aberdeen, Aberdeen, UK; [2]Auvation Ltd, Crombie Lodge, Aberdeen Science Park, Balgownie Drive, Aberdeen, UK

The annexins are family of calcium-regulated phospholipid-binding proteins with diverse roles in cell biology. Individual annexins have been implicated in tumour development and progression, and in this investigation a range of annexins have been studied in colorectal cancer. Annexins A1, A2, A4 and A11 were identified by comparative proteomic analysis to be overexpressed in colorectal cancer. Annexins A1, A2, A4 and A11 were further studied by immunohistochemistry with a colorectal cancer tissue microarray containing primary and metastatic colorectal cancer and also normal colon. There was significant increase in expression in annexins A1 ($P=0.01$), A2 ($P<0.001$), A4 ($P<0.001$) and A11 ($P<0.001$) in primary tumours compared with normal colon. There was increasing expression of annexins A2 ($P=0.001$), A4 ($P=0.03$) and A11 ($P=0.006$) with increasing tumour stage. An annexin expression profile was identified by *k*-means cluster analysis, and the annexin profile was associated with tumour stage ($P=0.01$) and also patient survival. Patients in annexin cluster group 1 (low annexin expression) had a better survival (log rank = 5.33, $P=0.02$) than patients in cluster group 2 (high annexins A4 and A11 expression). In conclusion, this study has shown that individual annexins are present in colorectal cancer, specific annexins are overexpressed in colorectal cancer and the annexin expression profile is associated with survival.

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The annexins are a multigene family of calcium-regulated phospholipid-binding proteins (Gerke and Moss, 2003; Gerke *et al*, 2005). The annexins are classified into five groups (A–E), and within each of these groups, individual annexins are identified numerically. Annexins in group A are human annexins, with group B referring to animal annexins without human orthologs, group C to fungi and moulds, group D to plants and group E to protists (Liemann and Huber, 1997; Rand, 2000; Hayes and Moss, 2004; Rescher and Gerke, 2004; Lim and Pervaiz, 2007). The characteristic annexin structural motif is a 70-amino-acid repeat, called the annexin repeat. Four annexin repeats packed into an α-helical disk are contained within the C-terminal polypeptide core (Gerke and Moss, 2003). While all annexins share this core region, the N-terminal varies widely between annexins, and it is this diversity of N-terminal amino-acid sequence that gives the individual annexins their functional differences and biological activities (Gerke and Moss, 2003; Gerke *et al*, 2005). There are 12 human annexin subfamilies (A1–A11 and A13) that have been found to have various intra- and extracellular roles in a range of cellular processes such as cell signalling, ion transport, cell division and apoptosis (Gerke and Moss, 2003; Gerke *et al*, 2005).

*Correspondence: Professor GI Murray, Department of Pathology, University Medical Buildings, Foresterhill, Aberdeen AB25 2ZD, UK;
E-mail: g.i.murray@abdn.ac.uk
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All annexins share an ability to bind to negatively charged phospholipid membranes in a calcium-dependent manner. This property is found within the annexin core motif where the calcium- and membrane-binding sites are located. Annexins bind to the cytosolic surface of the plasma membrane and to organelle membranes such as the Golgi apparatus. This binding can be reversed by the removal of calcium, freeing the annexin from the phospholipid membrane. However, the functional significance of their reversible membrane-binding ability remains unknown in many annexins, although in some it is thought to be important for vesicle aggregation and membrane organisation (Liemann and Huber, 1997; Rand, 2000; Rescher and Gerke, 2004; Lim and Pervaiz, 2007). Although all annexins share this binding property, there is variation in calcium sensitivity and phospholipid specificity between individual annexins. For example, within one cell there can be differences in the distribution of annexins, with annexin A1 having an endosomal localisation, A2 to be found in cytosol and A4 being associated with the plasma membrane (Liemann and Huber, 1997).

Some annexins are capable of calcium-independent binding and several have roles in vesicle aggregation. Annexins A1, A2 and A11 function in cooperation with other calcium-binding proteins to form complexes while annexins A1, A2 and A5 interact with cytoskeletal proteins. Many annexins are involved in exocytic and endocytic pathways and some have roles in ion channel regulation (Gerke and Moss, 2003). Extracellularly, annexin A1 has a role in controlling the inflammatory response while annexin A2 is present on the external surface of endothelial cells, where it may act as a receptor for ligands, including plasminogen and tissue plasminogen

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Current Status of Surgery for Pancreatic Cancer

Koichi Hirata[a] Shinichi Egawa[b] Yasutoshi Kimura[a] Takayuki Nobuoka[a]
Hidenori Oshima[a] Tadashi Katsuramaki[a] Toru Mizuguchi[a] Tomohisa Furuhata[a]

[a]Department of Gastrointestinal Surgery and Oncological Surgery, Sapporo Medical University School of Medicine, Sapporo, and [b]Department of Gastroenterological Surgery, Tohoku University Graduate School of Medicine, Tohoku, Japan

Key Words
Pancreatic cancer, incidence · Surgery, pancreatic cancer

Abstract
Background: In Japan the annual incidence of pancreatic cancer has increased over the last decade, but no advancement has been made in the long-term prognosis after resection. The significant differences in the surgical procedures between Western countries and Japan have been discussed. Therefore, an adequate comparison and analysis of the data from Japan, Europe and the USA is required. This review evaluates many important published reports from Japan which influence surgical procedure. ***Methods:*** Several important highlights and controversies regarding the concept of surgical treatment and surgical procedure are discussed comparing the results in Japan with those in Western countries. ***Results:*** No significant difference in diagnostic strategy using various imaging methods was observed between Japan and Europe. The stage classification for pancreatic cancer by the Japanese Pancreatic Society (JPS) seems to be superior to others, because the results on long-term prognosis after pancreatectomy of cases with pancreatic head cancer, diagnosed as tubular adenocarcinoma, has been arranged logically. Pancreatectomy with extended radical dissection is recommended in Japan, but several clinical studies from Europe and the USA suggest that this is ineffective. The basic concepts of this controversy have recently come closer altogether. Scientific clinical trials for instance on the necessity of adjuvant treatment, etc., are now on-going. ***Conclusion:*** The characteristics on diagnosis and treatment of pancreatic cancer in Japan are described. The JPS registration system for pancreatic cancer can provide much more information, i.e. dependency on diagnostic methods, highly frequent sites of lymph node and of distant metastases, the prognosis of small pancreatic cancers, etc. The indication for any surgical treatments should be limited to cases with the possibility of cancer free margins.

Introduction

Pancreatic cancer is the fifth leading cause of cancer death in Japan. The lethality of this malignant neoplasm is demonstrated by the annual incidence which is roughly more than 17,000 patients/year. Unfortunately, the incidence of pancreatic cancer is increasing in Japan and, aside from tobacco, its exact risk factors remain poorly understood. Pancreatic cancer registration has been carried out by the Japanese Pancreas Society (JPS) since 1981, and the 5-year survival on this registry after pancreatectomy is 13.1% [1]. According to the JPS classification, the 5-year survival in the cases with stage I, defined as no metastasis to regional lymph nodes and no neural invasion, is 61.0%, and those for stages II, III, IVa and IVb are arranged in a parallel manner. This might suggest the

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Koichi Hirata
S-1, W-16, Chuo-ku
Sapporo 060-8543 (Japan)
Tel. +81 11 611 2111, ext. 3280, Fax +81 11 613 1678
E-Mail a.narita@sapmed.ac.jp

accuracy of the JPS stage classification and the effectiveness of the surgical procedure for carcinoma of the pancreas. However, the number of the patients with stage I is extremely low (about 1.4%/year) in spite of the development of imaging diagnoses, and most of the cases are still in stage IVa and IVb.

Surgical techniques in Japan have been standardized on a basic treatment concept for invasive pancreatic cancer, which aims for a safe pancreatectomy procedure and aggressive dissection with a negative surgical margin. The results from this concept were expected to show qualitatively better treatment results for advanced pancreatic cancer in Japan during the 1990s. However, a longer survival rate has not yet come about because it is still difficult to diagnose patients with earlier stages of pancreatic cancer even though the screening system should have enabled this. It is well recognized that, upon surgical treatment, pancreatic cancer very often occurs in the retropancreatic extension, has lymph node metastases and neural invasion upon surgical treatment. The Japanese concept in this field recommends extended radical pancreatectomy, but this does not ascertain a better result as opposed to our expectations. A few institutes have had higher 5-year survival results of more than 30% [2–4]. However, these results were not based on a prospective randomized study and include various stages. The similar result on long-term prognosis have been observed in Europe and the USA [5–7], although extreme lymph node dissection and neural plexus dissection have not been performed. The effectiveness of postoperative adjuvant chemotherapy has recently been reported in randomized control trials (RCTs) [8–10]. Also in Japan, several trials on surgical procedures and adjuvant treatment have been reported [11]. In this review, the current status of surgery for pancreatic cancer in Japan is discussed in accordance with the introduction of international and Japanese trends mainly concentrating on the highlights and the controversies.

Diagnosis and Assessment of Resectability

In a patient with confirmed or suspected pancreatic cancer, the first clinical step in management is to determine the resectability and to evaluate of the tumor staging. Most of the clinical features, such as marked and rapid weight loss, persistent back pain, ascites, supraclavicular lymphadenopathy and ascites, are known as risk factors which reflect one or some of distant metastases such as hepatic metastases, systemic lymph node metastases, major stenosis in large vein (portal or superior mesenteric vein), neural invasion and peritoneal dissemination. In most cases these pathophysiologies are generally detected by ultrasound sonography, contrast computed tomography (CT), multi-detected CT and MRI. The existence of these features often make a patient select a palliative method such as bypass operation. However, resection of the tumor should be performed whenever no contraindicating risk is found. Pancreatic tumors are considered resectable when CT shows an isolated pancreatic mass without contiguous organ invasion, vascular involvement, nodal metastases, liver metastases or ascites. However, poor preoperative assessment of resectability by CT scan is known in detecting lymph node metastases, scattered local extension and small hepatic metastases. Is helical CT or dynamic MRI better for diagnosis [12]? Following diagnosis of a resectable pancreatic carcinoma, reliable detection of lymph node status is most important with regard to a curable resection. However, it has been reported that the diagnostic accuracy of CT imaging of nodal metastases varies from 42 to 58%, sensitivity 19–37%, specificity 60–92%, positive predictive value 47–83%, and negative predictive value 34–67% [13–16]. According to recent studies on ultrasonographic diagnosis, endoluminal ultrasonography is highly sensitive to detect invasion of major vascular strictures [17, 18]. The effectiveness of endoluminal ultrasonography in the diagnosis of pancreatic cancer gave a sensitivity of 95%, and a specificity of 80%, and negative predictive value of 80% [17]. Kaneko et al. [18] reported similar results with a slightly higher sensitivity of 96.9%, specificity of 91.2% and overall accuracy of 93.9% in the diagnosis of portal invasion. On the other hand, CT analysis resulted in a sensitivity of 83.9%, specificity of 74.3% and overall accuracy of 78.9%. Involvement of the venous system exceeding half the circumference of the vessel on CT is very suggestive of invasion, but this is not so when less than half of the vessel is involved, and it is not adaptable in artery systems. Accordingly, an indicative factor in the diagnosis of local cancer extension has not been established. Direct macroscopic observation and laparoscopic diagnosis are indicated in patients with pancreatic tumors not isolated from the surrounding tissue and vessels.

Importance of Staging

In Japan more than 70% of the pancreatic tumors requiring surgical treatment are located in the head of the pancreas (table 1) [1]. Others are located in the body and/or tail of the pancreas. The possibility of better prognosis

Table 1. Tumor location in the pancreas [1]

	Location				Total
	head	body-tail	head and body/body and tail	whole	
Tumor size					
TS1 (<2 cm)	638	159	25	2	824
TS2 (2–4 cm)	2,929	598	240	20	3,787
TS3 (4–6 cm)	1,519	476	479	31	2,505
TS4 (>6 cm)	652	366	773	214	2,005
Unknown	346	122	134	54	656
Total	6,084	1,721	1,651	321	9,777
Operation					
Resected	4,913	1,254	921	135	7,223
Palliative	965	230	517	117	1,829
Others	206	237	213	69	725
Total	6,084	1,721	1,651	321	9,777

by operation is limited to resected cases with Ro operation and no lymph node metastasis. There are several classifications for pancreatic cancer and we would like to compare some representative classifications, i.e. the Japanese Pancreas Society (JPS) classification (table 2a) [1], the 2002 Union International contre la Cancer (UICC) tumor node metastasis (TNM) classification (table 2b) [19], and the American Joint Committee on Cancer (AJCC) (in cooperation with the TNM committee of the International Union Against Cancer) staging system. In their initial versions, there were wide differences in determining the rules on stage-for-stage comparison, but have become much closer together with the latest revisions (table 3). These systems may be contributive as predictive prognostic factors for overall survival, but they are sometimes not useful for planning treatment because patients with advanced stages of disease may not be candidates for surgical resection. This fact has remained the difficulty of staging based on the skill and efforts of surgeons of pancreatic cancer. Otherwise, highly qualified surgeons have given much effort to curability under the rules of the staging systems, but it is very difficult to definitely identify regional metastases and invasion at the macroscopic level in the perioperative period. Most of these cases unfortunately resulted in non-curable operations according to the pathological diagnosis, which contributes to the scientific support of clinical knowledge. Recent improvements in diagnostic systems before/after surgery may contribute somewhat to the prognosis and new treatment, i.e. molecular target therapy, etc., in near future. Pancreatic cancer is very malign with a high ability to metastasize to the lymph nodes and to invade vessels (lymph canals, arteries and veins) and the perineural region. Therefore, pathological descriptions for these areas should be made.

Much molecular research for the diagnosis of micrometastases via the lymph system and via the blood stream are of clinical significance; some have proven the significant influence of micrometastases in the resected lymph nodes and/or cancer-positive conditions in the blood stream on survival (table 4) [21–30]. However, diagnostic methods using immunohistochemical or molecular analysis are not supported by medical insurance in Japan. Some molecular research concluded that the relationship between morphological and molecular diagnoses is very useful for prognosis, but each diagnostic value is proven as an independent factor on statistical analysis. In future the development of molecular diagnosis could contribute not only to the strategy for treatment but also to the decision of targeting treatment. At present, no meaningful treatment method, except surgery, has been invented, and a breakthrough, such as the appearance of molecular target drugs, is awaited.

Comparison between JPS and UICC Staging Classifications

Advancements in the treatment of pancreatic cancer in Japan have been supported by the National Pancreatic Cancer Registry of the JPS. The success of this registry has resulted in the provision of macroscopic and microscopic standard criteria, standard guidelines for the diagnosis, treatment, and introduction of risk factors on prognosis. Finally, the JPS classification was established on the basis of these data and it has been recognized that the stage classifications for pancreatic cancer reveal the more stratified and informative criteria. Many Japanese surgeons depend on these staging criteria to determine treatment strategy and obtain informed consent. By analyzing the JPS data on 3,979 patients who underwent resection for tubular adenocarcinoma of the pancreatic head, Isaji et al. [31] recently reported that the JPS classification is more reliable for predicting outcomes as compared with the UICC classification. In the past there have been wide discrepancies in the prognostic results for pancreatic cancer at each stage between Japan and the United States. This might be due to differences in clinical staging between the JPS and the UICC. In 1998, Kawarada et al. [32] compared the JPS 4th edition (1993) and the

Table 2. Stage groupings according to the JPS [1] (**a**) and UICC [19] (**b**) classifications

a JPS

	M0			M1
	~~N0~~	~~N1~~	~~N2~~	~~N3~~
Tis	0			
T1	I	II	III	IVb
T2	II	III	III	
T3	II	III	IVa	
T4	IV			

b UCICC

	M0		M1
	~~N0~~	N0	
Tis	0		IV
T1	IA		
T2	IB	IIIB	
T3	IIA	IIIB	
T4	III	III	

Wide differences with regard to the grouping are observed for tumor status and lymph node metastasis.

Table 3. Comparison of the definitions for T number in the latest publications between the JPS 5th edition (2002) [1] and the UICC 6th edition (2002) [19]

Classification	JPS 5th ed. (2002)	UICC 6th ed. (2002)
T1	Tumor limited to the pancreas, ≤2 cm in greatest dimension	Tumor limited to the pancreas, ≤2 cm in greatest dimension
T2	Tumor limited to the pancreas, >2 cm in greatest dimension	Tumor limited to the pancreas, >2 cm in greatest dimension
T3	Tumor that has extended into any of the following: bile duct (CH), duodenum (DU), peripancreatic tissue (S, RP)	Tumor extends beyond the pancreas but without involvement of celiac axis or superior mesenteric artery
T4	Tumor that has extended into any of the following: adjacent large vessels (PV, A), extrapancreatic nerve plexus (PL), other organs (OO)	Tumor involves celiac axis or superior mesenteric artery

Table 4. Detection of disseminated pancreatic cancer cells in the peripheral blood samples from Japanese reports

Markers	Samples	Positive/total patients (detection rates, %)	References
K-ras mutation (codon 12)	Peripheral blood	2/6 (33.3%)	Tada et al. [21], 1993
K-ras mutation (codon 12)	Liver	13/17 (76.5%)	Inoue et al. [22], 1995
K-ras mutation (codon 12)	Peripheral blood	10/10 (100%)	Nomoto et al. [23], 1996
K-ras mutation (codon 12)	Lymph nodes	4/6 (66.6%)	Tamagawa et al. [24], 1997
K-ras mutation (codon 12)	Lymph nodes	8/13 (61.5%)	Ando et al. [25], 1997
CEA mRNA	Peripheral blood	3/9 (33.3%)	Funaki [26], 1998
Keratin 19 mRNA (stage IV)	Peripheral blood	2/19 (10.5%)	Aihara et al. [27], 1997
	Portal blood	1/18 (5.6%)	
CEA mRNA	Peripheral blood	13/21 (61.9%)	Miyazono et al. [28], 1999
Chymotrypsinogen mRNA	Peripheral blood	7/10 (70%)	Kuroki et al. [29], 1999
α4GnT mRNA (stage IV)	Peripheral blood	33/43 (76.7%)	Ishizone et al. [30], 2006

UICC 5th edition (1997), and the results showed that the JPS system was more reliable for long-term prognosis. However, the opinion leaders in the Western countries suggested that the rule of classification of the JPS 4th edition was very complicated and not useful clinically. Japanese researchers surely also have a similar impression. Since then, further efforts by the JPS Review Committee of the General Rules on the Study of Pancreatic Cancer have been asked to establish more a simple and reliable staging classification. Finally, the JPS published the 6th

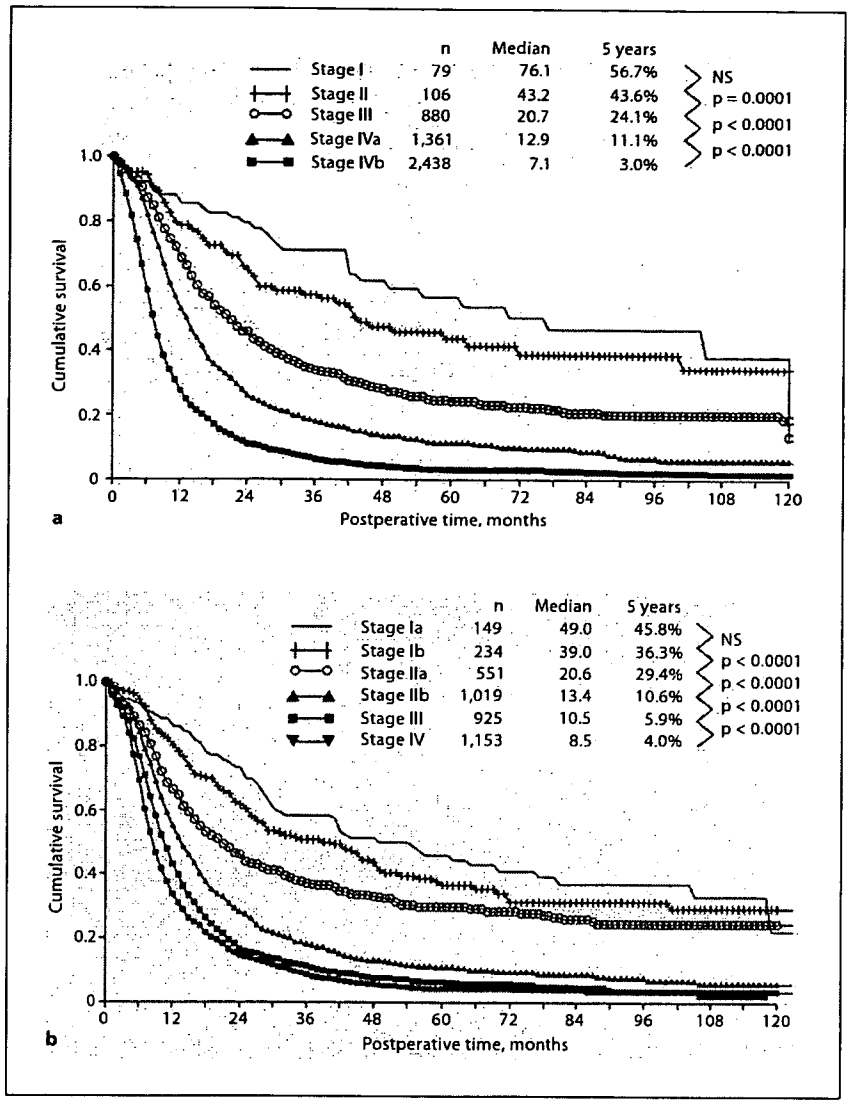

Fig. 1. a Survival curves according to the JPS staging of the patients who underwent resection for tubular adenocarcinoma of the pancreas head. Altogether the survival rates of the five stages differed significantly. **b** Survival curves according to the UICC stages. No difference could be found between stages Ib and IIa, and stages IIb, III and IV [19].

Japanese edition in 2002 and the 2nd English edition in 2003. On the other hand, the 6th edition of UICC was published in 2002, which showed even wider differences in staging from that of the JPS (table 3). Therefore, the first purpose of Isaji et al. [31] was to analyze the results of operative treatment over the last 15 years (18,629 cases) to determine whether the prognosis of pancreatic cancer had improved, and secondly to compare the usefulness of the two classifications on outcome. Generally, it is understood that it is difficult to decide the best research method for such a comparison. Therefore, they focused on the reliability of predicting outcome for 3,979 resected cases with tubular adenocarcinoma localized in the head of pancreas. The results were as follows: (1) the survival rate was correlated with the Japanese stage classification (fig. 1a); (2) the extent of the primary tumor (T category) indicates the significant difference in the survival rates among the 4 groups in both classifications; (3) the extent of lymph node involvement and of extrahepatic tissue invasion better reflects prognosis by the JPS rules than the UICC rules, and (4) the UICC staging system does not reflect differences in prognosis among the stages, especially between stages Ib and IIa, and stages IIb, III and IV (fig. 1b).

These results indicate that the JPS classification may offer a better prediction of prognosis.

Surgical Treatment

Surgical treatment of pancreatic cancer unfortunately has only a low success rate with regard to its long-term prognosis, and there is only a likelihood of cure following operation [33]. Recent studies in Japan and also in Western countries show that pancreaticoduodenectomy is associated with a 5-year survival of 10–20% [1], which has remained unchanged over the last 10 years. The surgical mortality rate of less than a few percent has improved. The most important prognostic factor for long-term survival after radical resections has been shown to be nodal status. In general, the 5-year survival after pancreaticoduodenectomy is roughly 10% for node-positive disease, while it can be 25–30% for node-negative disease. However, it is impossible to definitely detect the positive lymph nodes before and/or during surgery. Therefore, patients without the contraindications for curative resection, i.e. the presence of distant metastases, peritoneal seeding, tumor infiltration to the celiac artery or superior mesenteric artery extension of tumor tissue into the mesentery, etc., should receive the appropriate radical operation to improve their outcome.

Most hospitals in Japan have experience with extensive radical resection including excision of the portal vein, total or extensive regional pancreatectomy and extensive retroperitoneal lymphadenectomy. Some have suggested the effectiveness of extensive radical resection [2, 3]. However, no evidence from RCTs has been reported.

Current Concept in Japan

In 2004, Matsuno et al. [34] reported the results of 20 years experience with the pancreatic cancer registry in Japan. The total number of cases was 23,302, of which the number of epithelial and non-epithelial tumors were 11,819 and 0, respectively, and the number of the cases without histological diagnosis was 11,483. Data analysis was performed using SPSS software.

The male to female ratio was 1.58:1.00. The overall resectability rate was approximately 40% for the patients who underwent pancreatectomy for invasive cancer in the head of the pancreas. The 5-year survival in the invasive carcinoma group was 9.7%, and wide differences were observed between the various histologies of the resected cases ranging from 10.7 to 44.8% as follows: tubular adenocarcinoma 10.7% (well differentiated type 13.1%, moderately differentiated type 9.3%, poorly differentiated type 9.3%); papillary adenocarcinoma 26.1%; adenosquamous carcinoma 15.8%, etc. Comparing the 5-year survival limited to cases with tubular adenocarcinoma,

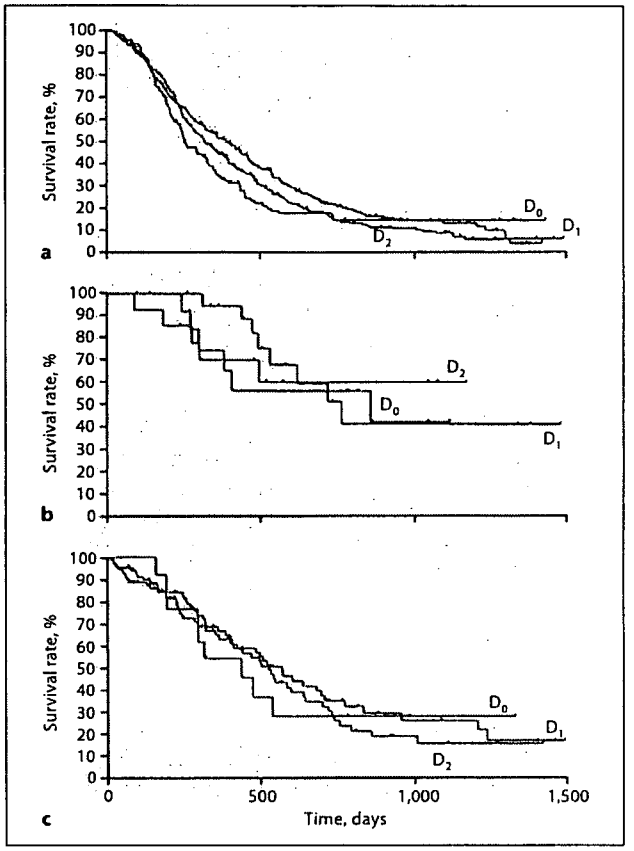

Fig. 2. Cumulative survival rates in relation to lymph node dissection in all patients (**a**; log rank test, $p = 0.10$ for D_0, D_1, and D_2), patients with stage I and stage II cancer (**b**; $p = 0.95$), and patients with stage III cancer (**c**; $p = 0.81$) [36]. There were no significant differences. D_0 = No lymph node dissection; D_1 = N_1 lymph node dissection; D_2 = N_2 lymph node dissection.

no differences have been observed over the past 20 years. Namely, no improvement in outcome has been observed after surgical treatment. On the other hand, in extensive radical pancreatectomies performed from 1991 to 2000 as the standard operation for pancreas cancer, the higher respectability was found to be more than 40%, in relation to the result of about 25% seen around 1980. But no significant improvement in survival rate has been seen.

Nakasako et al. [35] reported their experience with the extensive radical operation at one institute (186 cases) and no difference in 5-year survival was found: 7% during 1968–1979, and 8% during 1978–1995. Hirata et al. [36] and Mukaiya et al. [37] tried to analyze cases collected from multi-institutional experience. The effectiveness of extensive radical pancreatectomies was poor (fig. 2). On

Table 5. Number of cases with invasive ductal pancreatic carcinoma [1]

Stage	Location				Total
	head	body-tail	head and body/body and tail	whole	
I	87	46	3	0	136
II	126	41	26	7	200
III	938	137	28	9	1,112
IVa	1,507	370	140	19	2,036
IVb	2,407	781	1,112	210	4,570
Unknown	1,019	346	342	76	1,753
Total	6,084	1,721	1,651	321	9,777

the other hand, Ishikawa et al. [2] and Nagakawa et al. [3] reported better results with the extended radical operation, and also Hiraoka et al. [38] showed the effectiveness of combination therapy with the intraoperative radiation added to the extended radical operation.

The 5-year survival of the stage I cases with pancreas head carcinoma was 56.7%, and that for cancer of the pancreas body and tail was 58.5%.

The high-quality prognosis in stage I suggested that the diagnosis of such small cancers should be required to in order to obtain better results in pancreatic cancer. However, the proportion of tumor size 1 cases and stage I cases among all cases were very low: 8.4% (table 1) and 1.4%, respectively (table 5).

The absolute number and proportion of small pancreatic cancers have gradually been increasing year by year, but advances in treatment methods have not kept pace. Therefore, most Japanese surgeons still often face extremely advanced cases of pancreatic cancer.

Based on expert opinion, the concept of the surgery was synchronous resection of the artery or portal vein, wide dissection of the plexus nerve and extended dissection of lymph nodes. This concept has recently been changed due to the data from the JPS registration system.

Extensive Radical Pancreatectomy versus Standard Pancreatectomy

Due to the extremely high incidence of histological non-curative results with standard dissection, extended radical dissection is used in pancreatectomy to prevent the frequent local recurrence which tends to occur in spite of a clinically curative operation. Extended radical dissection, which has major complications such as severe diarrhea, uncomfortable intestinal condition due to dissection of the plexus nerve, malnutrition and lower quality of life, continued to be of interest compared with the standard operation during the 1990s in Japan. Several Japanese reports on extensive dissection suggested the benefit of clearance of lymph nodes and retroperitoneal connective tissue [2, 3], which might have somewhat influenced this field in other countries. However, no significant difference between extensive radical and standard pancreatectomy was suggested by multicenter prospective randomized trials [39, 40] in Western countries, and the reason for this difference was not scientifically clear among Japanese surgeons. A difference in the background of the patients undergoing surgical treatment has been suggested. Western institutes with a record of relatively good prognosis have introduced low resectability, but most Japanese institutes with high resectability have not experienced an advance in post-surgical prognosis. For example, the highly advanced cases might be included more often in Japan than in Western countries. One retrospective study tried to address this issue [37], and indicated no survival advantage of extended dissection, except for a limited group of patients with a small number of microscopic lymph node metastases. No significant difference between extended and standard operations was found in an RCT study by Yeo et al. [39] in the USA and Pedrazzoli et al. [40] in Italy. The patients who received either the extended or standard operation had high rates of local recurrence and hepatic metastases, and they died. This poor prognosis may be due to the poor condition of the patients at operation who already had systemic disease. Nagino and Nimura [41] recently reported no statistical difference between the extended radical and standard operations for patients with stage II, III and IVa pancreatic cancer among Japanese patients by RCT. The result showed that the 1- and 3-year survival rates were 76.5 and 29.3% for the standard procedure, and 53.8 and 15.1% for the extended procedure. A slightly worse prognosis was suggested for the extended operation.

Accordingly, there is doubt about the significance of extensive dissection not only for advanced stages but also for earlier stages of pancreatic cancer.

In the near future, patients with or without indications for surgical treatment may be selected preoperatively according to the biological behavior of the cancer cells.

Indication of Vascular Resection

Nakao et al. [42] recommended extended radical resection for elective patients and concluded that the most important indication of this procedure is to obtain surgical cancer-free margins. There is no indication that the

Table 6. Portal and/or superior mesenteric vein resection in pancreatic cancer in Japan

References	Patients	nPVR	Type of procedure	Year
Mimura et al. [44]	71	55	PD:28, TP:27	1994
Takahashi et al. [45]	137	79	PD:42, TP:32	1994, 1995
Nakao et al. [46]	200	146	TP:69, PD:57, DP:9, PPPD:11	1995, 2001
Imaizumi et al. [47]	480	172	Extend PD:150, Extend TP:22	1998
Ishikawa et al. [48]	43	27	PD:27	1998
Naganuma et al. [49]	83	30	PD, TP, DP, PPPD	1998
Shibata et al. [50]	74	28	PD:23, TP:3, DP:2	2001
Kawada et al. [51]	66	28	PD:20, TP:5, PPPD:3	2002
Aramaki et al. [52]	69	22	PD:14, TP:7, DP:1	2003
Nakagohri et al. [53]	81	33	PD, DP	2003

nPVR = Number of patients who underwent synchronous portal vein resection; PD = pancreatoduodenectomy; TP = total pancreatectomy; DP = distal pancreatectomy; PPPD = pylorus-preserving pancreaticoduodenectomy.

surgical margins will become cancer positive if extended resection is used in these patients.

Because of the absence of any RCT, Siriwardana and Siriwardana [43] made a detailed systematic review of outcome in patients following superior mesenteric vein (SMV) and/or portal vein (PV) resection during pancreatectomy. Japanese studies are shown in tables 6 and 7 [44–53]. Although regional pancreatectomy was recommended by Fortner [54] in 1974, this procedure is unfortunately associated with extremely high morbidity and no improvement in prognosis. Therefore, tumor extension to SMV/PV, superior mesenteric or celiac artery was recognized as a contraindication to surgical resection.

In 1996, Fuhrman et al. [55] reported no difference in hospital stay, morbidity, mortality, tumor size, margin positivity, modal positivity or tumor DNA content between two groups without or with SMV/PV resection. This study suggested that the development of SMV/PV resection was not significant and also that there is an inherent biological difference. However, when the purpose is to obtain cancer-free margins by PV/SMV resection, most Japanese surgeons would be eager to resect them simply for the low possibility of a good prognosis. Among those patients with systemic disease, only a few could be supported by adjuvant chemotherapy.

Effectiveness of Surgical Treatment for Advanced Pancreatic Cancer of the Pancreas

It has been discussed whether highly advanced but locally resectable pancreatic cancer can be adapted to surgical treatment or not. Imamura and Doi [56] faced this problem in a multicenter RCT comparing surgical resection and radiochemotherapy for locally advanced pancreatic cancer (limited strictly only to cases with JPS stage IVa). This study was performed using strict selection criteria, the final decision being made by direct observation and judgment during laparotomy after the preoperative diagnosis of stage IVa. It was concluded that such cancers, without involvement of the common hepatic artery or superior mesenteric artery, can be successfully treated by experienced surgeons at specialized centers, so-called

Table 7. Morbidity, mortality and pathohistological results after portal and/or superior mesenteric vein resection in pancreatic cancer in Japan

References	nPVR	Morbidity, %	Mortality, %	PV(+) %	RM(+) %
Mimura et al. [44]	55		11		43.6
Takahashi et al. [44]	79		9.5	61	38
Nakao et al. [46]	146		5.5	71	58.2
Imaizumi et al. [47]	172	23	5	60.4	
Ishikawa et al. [48]	27			85.1	
Naganuma et al. [49]	30	16	1.2		36.6
Shibata et al. [50]	28	32	4	58.3	29
Kawada et al. [51]	28	46	4	75	64
Aramaki et al. [52]	22	9	4.5	63.4	
Nakagohri et al. [53]	33		6.1	51.5	24.2

PV(+) = Percentage of patients with portal vein involvement in surgical specimen; RM(+) = percentage of patients with resection margin-positive.

high-volume centers. Therefore, a substantial number of patients with stage IVa cancer still have curatively resectable disease and could have a more favorable outcome with surgery. Most skillful surgeons continue to resect stage IV tumors today.

No-Touch Isolation Technique

In order to prevent blood stream metastasis, the concept of isolated pancreatectomy [57] was created. With this aggressive procedure the patient undergoes bypass catheterization of the portal vein to decompress the congestion and prevent the shedding of cancer cells induced by the surgical manipulations of the pancreas head. Japanese reports on the incidence of pancreatic cancer cells in peripheral blood, bone marrow and liver tissue (table 4) have shown that this is the cause of distant metastases, which is supported by immunohistochemistry and molecular biological studies. Research has suggested the meaningful relationship between positive cancer cells in peripheral blood and distant metastases in cancer.

Kobayashi et al. [58] and Nakao and Takagi [57] suggested that the non-touch isolation technique (NTIT) could prevent liver metastases. During NTIT, isolation of the portal vein precedes ligature of the surrounding veins after dividing the duodenum and pancreas. Hirota et al. [59] proposed a different method of NTIT: ligation of Henle's gastrocolic trunk vein at the communicating point to the superior mesenteric vein, then division of the stomach or the upper duodenum, pancreas, choledochus, and jejunum. The pancreatic duct and choledochal duct should be ligated to prevent dissemination. Thereafter, the ligation of the portal vein branches follows. It is characteristic that no kocherization is performed until all vascular branches are completed and no catheterization to the portal vein is needed. A comparative study of the NTIT and the conventional procedure with extensive intraoperative peritoneal lavage revealed: (1) the rate of molecular detection determines the rate of cancer cells in the portal venous blood and in the lymphatic fluid, and (2) the different frequency of hepatic metastasis, local recurrence and peritoneal dissemination. Further comparative study is necessary to confirm the significance of the NTIT procedure in pancreatic cancer surgery.

Mortality after Pancreatic Resection

Pancreatic resection is a high-risk surgical procedure with considerable postoperative morbidity and mortality. The hospital mortality rate after pancreatic resection has decreased during last 15 years, but there is a very wide variation in rates between institutes and countries. Reports on the relationship between hospital volume and mortality after pancreatic resection provide a convincing evidence of an need for centralization, as several studies have assessed the impact of referral to high-volume centers on morbidity and mortality after pancreaticoduodenectomy [60–62]. Mortality rates at the high-volume centers are less than 5% and most reported less than 2%. Otherwise, centers with less experience continue to report mortality rates ranging from 7 to 15%. Birkmeyer et al. [61] reported the adjusted in-hospital mortality (1994–1999) among Medicare patients undergoing pancreatic resections: 16.3% (1 case/year), 14.6% (1–2 cases/year), 11.0% (3–5 cases/year), 7.2% (6–16 cases/year) and 3.8% (>16 cases/year). Therefore, Birkmeyer et al. [61] analyzed the summarized surgeon-specific and institute-volume outcome. Surgeon volume was divided into 3 groups: low (<2 cases/year); middle (2–4 cases/year), and high (>4 cases/year). Institute volume was divided into 3 groups: low (<3 cases/year); middle (3–13 cases/year), and high (>13 cases/year). Low-volume surgeons could have better results at higher-volume institutes. Further study is expected to clarify the influence of pancreatic condition on morbidity, i.e. parenchymal fibrosis and main pancreatic duct size and coexistent disease.

In some European countries such as the United Kingdom and Germany, centralization of institutes with a system of high-risk surgical procedures has been recommended, but its effects have not yet been analyzed and no precise report has been made [63]. It seems that the overall results are not changed. The data on hospital volume and mortality after pancreatic resection are too heterogeneous to perform a meta-analysis, but a systematic review shows convincing evidence of an inverse relation between hospital volume and mortality, and enforces the plea for centralization [64]. In Japan, there is no national registry concerning the outcomes of surgical treatment but the Japanese health insurance system is undergoing objective change which may lead to centralized systems. Cases will be optimized and medical costs minimized when patients with pancreatic cancer are referred to high-volume institutes.

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