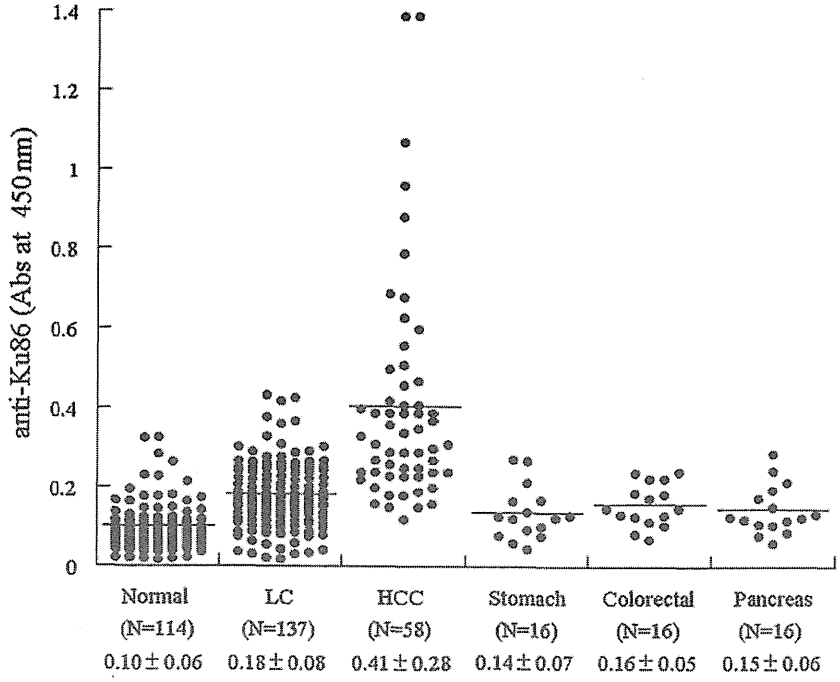

Fig. 2. Serum anti-Ku86 levels in patients with HCV-related chronic liver diseases, HCC, other gastrointestinal cancers and in healthy volunteers. Serum anti-Ku86 levels in HCC patients were significantly higher than those in patients with liver cirrhosis (LC) and other gastrointestinal cancers. Significance of the differences was assessed by Mann–Whitney *U*-test.

2.4. Data collection and statistical analysis

Serum levels of AFP and PIVKA-II were measured using commercial enzyme immunoassay kits (Fujirebio Inc., Tokyo, Japan), with cut-off values set at 40 ng/ml and 40 mAU/ml, respectively, to give 90% specificity in patients with liver cirrhosis. Numerical data are presented as the mean ± SD. The significance of differences in above analyses was examined using IBM SPSS Statistics 19 (SPSS Inc., Chicago, IL, USA). The overall diagnostic accuracies of each tumor marker were evaluated by receiver-operating characteristic (ROC) analysis using R statistical software, version 2.12.1 (http://www.r-project.org/) with the pROC add-on package. $P < 0.05$ was considered significant in all analyses.

3. Results

3.1. Immunohistochemistry of Ku86 in HCC tissues

Although staining of Ku86 in nontumor tissues was minimal, strong staining was noted in tumor tissues mainly in the nucleus. In some tumor cells, weak staining was also seen in the cytoplasm (Fig. 1A). Similar results were obtained in four other comparisons. It was noteworthy that HCC was distinguished from adjacent nontumor tissue by stronger staining of Ku86 (Fig. 1B).

3.2. Serum anti-Ku86 levels in patient groups

Serum anti-Ku86 levels in patient groups and healthy subjects are presented in Fig. 2. Serum anti-Ku86 levels were significantly higher in patients with HCC (0.42 ± 0.25) compared to those with liver cirrhosis (0.18 ± 0.08) (all $P < 0.001$). The levels in gastric, colorectal and pancreatic cancers were increased but were significantly lower than those of HCC ($P < 0.001$). In 12 cases, serum anti-Ku86 levels were determined just before and 2 months after surgical resection of the tumors. As shown in Fig. 3A, the levels significantly decreased after surgery (0.49 ± 0.33 vs. 0.19 ± 0.16, $P < 0.001$). Preoperative serum anti-Ku86 levels as related to expression levels of Ku86 assessed by immunohistochemistry are presented in Fig. 3B. Anti-Ku86 level tend to be higher in patients with greater Ku86 expression in HCC tissues.

3.3. Comparison with AFP and PIVKA-II (DCP)

There was no significant correlation of anti-Ku86 with the two conventional HCC tumor markers, AFP and PIVKA-II (Supplementary Fig 1).

Serum levels of AFP, PIVKA-II and anti-Ku86 in HCC patients are summarized in Table 2A. Also, serum levels of the three markers in all the 28 Stage I cases are shown in Table 2B.

The cut-off values of the three markers were all set at levels that gave 90% specificity compared with patients with liver cirrhosis: 0.28 Abs, 40 ng/ml, 40 mAU/ml for anti-Ku86, AFP, and PIVKA-II, respectively. In 28 HCC patients with solitary small (<2 cm) tumor (Stage I), serum anti-Ku86 levels were above the cut-off value in 17 cases (60.7% sensitivity). In these 28 patients, the sensitivities of AFP and PIVKA-II at cut-off levels that gave 90% specificity were 17.8% and 21.4%, respectively (Fig. 4A). Anti-Ku86 levels were above the cut-off level in 11 (61.1%) of 18 Stage I cases in which the serum levels of AFP and PIVKA-II were both below their respective cut-off values. Thus, combination assays of AFP, PIVKA-II and anti-Ku86 could detect 21 out of 28 Stage I HCC cases (Table 2B).

ROC curves for anti-Ku86, PIVKA-II, AFP and a combination of AFP and PIVKA-II in Stage I and Stage I–II HCC cases compared with LC patients are presented in Fig. 4B . The area under the curve (AUC) for anti-Ku86 was significantly greater than those for PIVKA-II, AFP and a combination of AFP and PIVKA-II ($P < 0.001$).

4. Discussion

The data presented in this study provides the first evidence that anti-Ku86 could be an early indicator of HCV-related HCC. The study is also a good example of potential HCC tumor marker

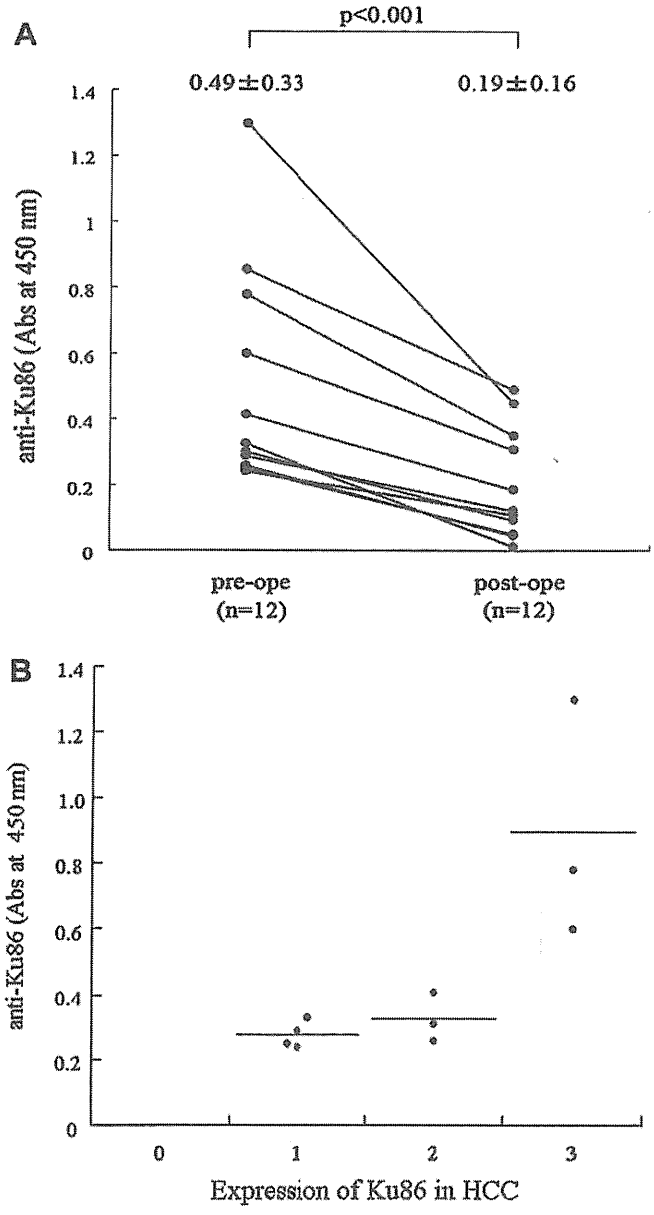

Fig. 3. Serum anti-Ku86 before and after surgical resection of tumors. (A) Serum anti-Ku86 levels before and 2 months after surgical resection of HCC in 12 cases. The levels significantly decreased after surgery ($P < 0.001$ assessed by the Wilcoxon signed rank sum test). (B) Preoperative serum anti-Ku86 levels as related to expression levels of Ku86 assessed by immunohistochemistry. Anti-Ku86 level tend to be higher in patients with greater Ku86 expression in HCC tissues.

Table 2A
Serum levels of AFP, PIVKA-II and anti-Ku86 in patients with hepatocellular carcinoma. (A) The mean values (mean + SD) of each marker in patients with liver cirrhosis and HCC.

	Liver cirrhosis ($N = 137$)	HCC	
		Stage I ($N = 28$)	Stage II ($N = 30$)
AFP (ng/mL)	14.2 ± 14.5	24.5 ± 47.5	473.9 ± 2010.6
PIVKA-II (mAU/mL)	21.6 ± 13.3	87.8 ± 218.6	136.7 ± 224.4
Anti-Ku86 (Abs)	0.18 ± 0.08	0.35 ± 0.17	0.46 ± 0.34

discovery originating from comprehensive proteome analysis of HCC tissues.

Table 2B
Serum AFP, PIVKA-II and anti-Ku86 levels in 28 patients with solitary and small (<2 cm) HCC (Stage I). Positive data are underlined.

No.	Age	Sex	Noncancerous tissue	Child-Pugh	Tumor size (mm)	AFP (ng/mL)	PIVKA-II (mAU/mL)	Anti-Ku86 (Abs)
1	73	Female	LC	B	14	5.6	14	0.50
2	69	Female	LC	A	20	4.8	12	0.18
3	80	Female	LC	A	19	14.3	30	0.19
4	74	Female	LC	A	20	28.7	20	0.34
5	61	Male	LC	C	20	43.8	12	0.96
6	81	Female	LC	C	20	9.0	586	0.41
7	73	Male	LC	A	17	8.6	1041	0.23
8	56	Female	LC	B	8	14.0	13	0.22
9	56	Male	LC	A	12	4.6	68	0.24
10	60	Male	LC	C	16	14.5	241	0.15
11	71	Female	LC	A	17	5.5	19	0.46
12	70	Male	LC	B	10	2.3	12	0.25
13	67	male	LC	A	7	6.6	16	0.56
14	76	Female	LC	A	10	8.7	15	0.40
15	80	Female	LC	B	14	227.7	50	0.60
16	79	Male	LC	A	15	137.9	18	0.24
17	61	Male	LC	B	8	40.5	20	0.37
18	81	Male	LC	A	12	6.4	26	0.39
19	76	Male	LC	B	10	41.7	13	0.29
20	51	Male	LC	A	12	7.1	12	0.24
21	81	Female	LC	B	10	12.9	10	0.30
22	75	Female	LC	A	17	6.1	15	0.35
23	63	Male	LC	A	12	7.8	33	0.36
24	76	Female	LC	A	18	6.5	20	0.18
25	71	Female	LC	B	17	5.5	19	0.33
26	75	Male	CH		14	3.6	37	0.47
27	69	Male	LC	A	17	2.4	17	0.23
28	49	Female	LC	A	11	8.9	78	0.29

LC, liver cirrhosis; CH, chronic hepatitis, Child-Pugh, Child-Pugh classification to indicate the severity of liver cirrhosis.

Although direct analyses of serum or plasma by mass spectrometry may provide biomarker candidates for a variety of diseases, the spectrum of observed proteins and peptides suggests that they are not easily applicable to early detection of solid tumors [25]. Glycomic and glycoproteomic approaches might be more promising [26,27].

We previously conducted proteome analyses to compare protein expression levels between surgically resected HCC tissues and adjacent non-tumor tissues using agarose 2D-DIGE [23]. Expression levels of 83 proteins differed between the tumor and non-tumor tissues, and immunoblotting showed significantly increased expression of clathrin heavy chain (CHC) and Ku86 in the tumor tissue [23]. Since autologous proteins overexpressed in tumor cells can be altered in a way that renders them immunogenic, we compared the serum anti-CHC and anti-Ku86 levels in HCV-related HCC patients with those in patients with liver cirrhosis without HCC. The results of preliminary experiments showed that the increase of serum anti-Ku86 in HCC sera was much greater than that of anti-CHC (data not shown). Therefore, in the current study, we focused on anti-Ku86.

The Ku complex is composed of two subunits of 70 and 86 kDa, which are designated as Ku70 and Ku86 (also referred to as Ku80), respectively [28]. Ku70 and Ku86 are the regulatory region of a DNA-dependent protein kinase that is involved in multiple biological processes, including DNA double-strand break repair, V(D)J recombination, telomere length maintenance, cell cycle progression, and transcriptional regulation [29]. We detected Ku86 overexpression in HCC by direct 2-DE proteome analysis of HCC tissues. Overexpression of Ku86 in HCC was also shown by Luk

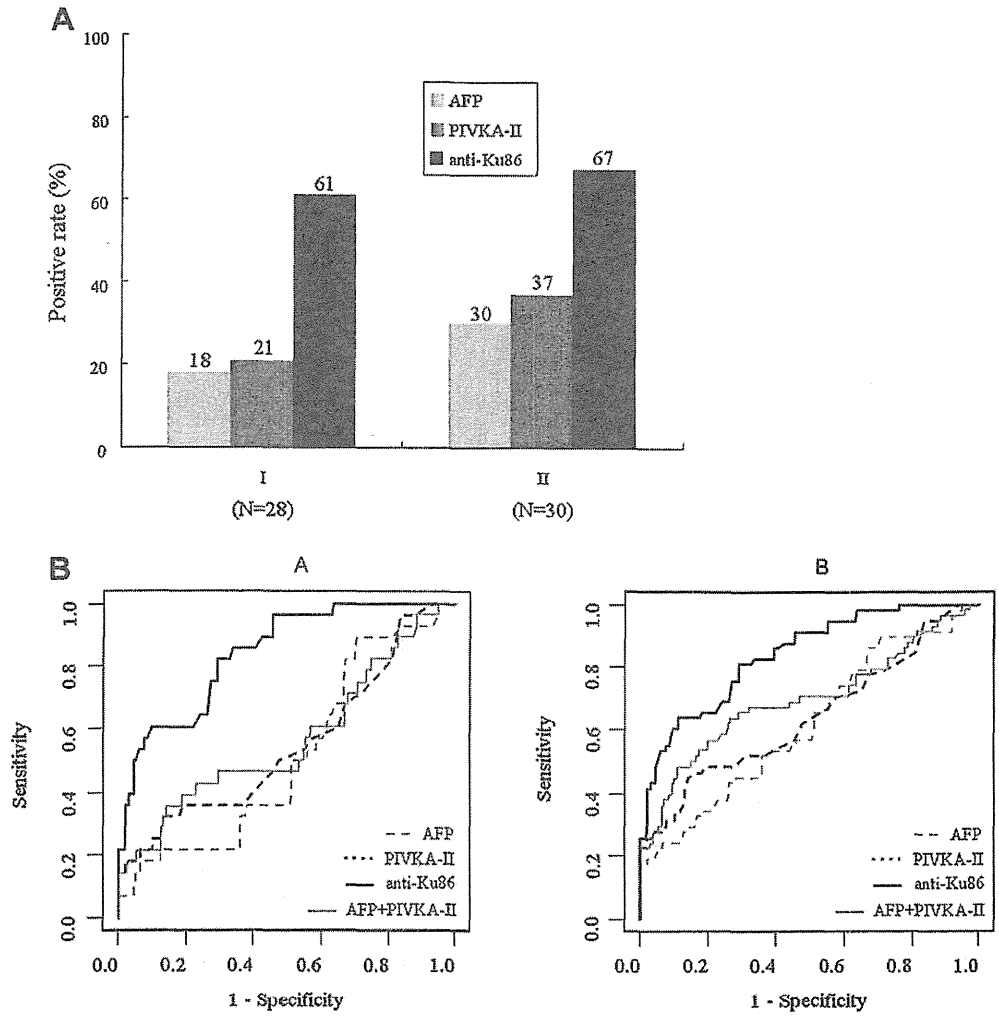

Fig. 4. Comparison of anti-Ku86 with the conventional tumor markers of HCC. (A) Sensitivity of AFP, PIVKA-II and anti-Ku86 in early (Stage I) and relatively early (Stage II) HCC cases. Sensitivities were obtained at cut-off levels that gave 90% specificity in cirrhotic patients without HCC: 40 ng/ml for AFP, 40 mAU/ml for PIVKA-II, and 0.28 Ab for anti-Ku86. The sensitivity of anti-Ku86 was significantly higher than those of AFP and PIVKA-II in Stage I and Stage II cases. The differences between anti-Ku86 and AFP or PIVKA-II in Stage I and Stage II cases were statistically significant ($P < 0.05$) as assessed by Fisher's exact test. (B) ROC curves for anti-Ku86, PIVKA-II, AFP in Stage I (early) and Stage I–II HCC cases, compared with LC patients. The area under the curve (AUC) for anti-Ku86 was significantly greater than those for PIVKA-II, AFP and a combination of AFP and PIVKA-II ($P < 0.001$).

et al. by a different approach [30]; using a murine monoclonal antibody generated against HCC samples, overexpression of the heterodimer Ku70/Ku80 (=Ku86) in the nucleus and/or cytoplasm was shown in HCC cell lines and in liver cancer tissues [30]. Ku70 is also present in the plasma membrane [31], which makes this antigen more accessible to the immune system.

There are many ways in which autologous proteins become immunogenic in tumor cells, including overexpression, mutation, misfolding, and aberrant degradation. In addition, proteins that are mislocalized during malignant transformation can also provoke a humoral response. Overexpressed proteins appear to increase the antigenic load in HCC, as in the case of cyclin B1 [24]. Indeed, in the present study, immunohistochemical staining of anti-Ku86 tended to be stronger in HCC cases in which preoperative serum anti-Ku86 levels were highly elevated. The possibility of a missense mutation of the *XRCC5* gene that codes for Ku86 should also be considered. The exact reasons of the increased antigenicity of Ku86 in HCC tissues remain to be clarified.

Autoantibodies have various characteristics and advantages as cancer biomarkers [21,22]. First, the immune response to tumor associated antigens (TAAs) can occur at a relatively early stage of carcinogenesis. Second, autoantibodies are stable and remain elevated for a relatively long period, in contrast to other biomarkers including TAAs themselves, which are less stable and rapidly degraded and cleared. Serum levels of the autoantibodies are also much higher than their respective TAAs, as a result of amplification by the immune system in response to a single autoantigen.

Based on a proteome analysis of HCC tissues, we have provided the first evidence that anti-Ku86 is a promising tumor marker for early detection of HCV-related HCC. Ku86 appears to develop antigenicity at a relatively early stage of tumorigenesis. Since mechanisms of hepatocarcinogenesis are variable depending on the etiology, it is possible that the antigenic potential of Ku86 differs in HCC of other etiologies. Therefore, a larger multicenter prospective study including HCC of various etiologies including HBV and non-alcoholic steatohepatitis (NASH) is required for further evaluation of the diagnostic and pathophysiological roles of elevation of serum anti-Ku86 in early HCC.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.04.099.

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胆道癌の治療方針と外科治療

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はじめに●

胆道癌(胆管癌，胆嚢癌，乳頭部癌)において，外科切除後の術後5年生存率は，胆管癌：33.1%，胆嚢癌：41.6%，乳頭部癌：52.8%と報告され[1]，他の消化器癌と比較し，依然として予後不良の疾患であり，その罹患数も増加傾向にある．

胆道癌の治療は，現時点では，治癒切除が唯一の根治性が得られる治療法であり[2]，できる限りその可能性を検討すべきであるが，その術式に関しては胆道癌の発生部位はもちろん，その進展範囲によりさまざまであり，厳密な進展度診断は欠かすことができない．一方，腹膜播種，肺などの遠隔臓器への転移，多発肝転移，遠隔リンパ節転移があった場合は，通常，切除の対象外とされ，外科切除以外の緩和治療を含む治療法が選択される．本稿では，胆道癌の治療方針と外科治療につき，胆管癌，胆嚢癌，乳頭部癌に分け概説する．

胆管癌●

胆管癌の臨床症状として多くは閉塞性黄疸を認めるため，内視鏡的経鼻胆道ドレナージendoscopic nasobiliary drainage(ENBD)などによる胆道ドレナージが必要となるが，正確な部位診断，進展度診断を行うために，ドレナージ処置前(チューブ挿入前)に，腹部超音波検査，multidetector-row CT(MDCT)，磁気共鳴胆管膵管造影検査magnetic resonance cholangiopancreatography(MRCP)を行うべきである．これはその後の術式選択にきわめて重要(図1)なため，安易なドレナージは避け，ドレナージ前に高次施設へ紹介することも念頭におくべきである．その際に遠隔臓器への転移，リンパ節転移の有無なども診断し，治療法として外科切除が適当かどうかを検討する．ついで，切除範囲を決定するために，胆管癌の局所進展様式を考慮し，胆管壁に沿った長軸方向への水平浸潤と胆管壁外に向かう垂直浸潤を正確に診断する必要がある．それにはMDCTが有用ではあるが，それに加えて内視鏡的逆行性胆管造影endoscopic retrograde cholangiography(ERC)などの直接胆道造影は表層上皮内進展以外の水平浸潤範囲の診断に有用(図2)である場合があり，経口胆道鏡検査peroral cholangioscopy(POCS)は直接胆管内腔を観察でき，生検も可能なため，表層上皮内進展の進展範囲診断

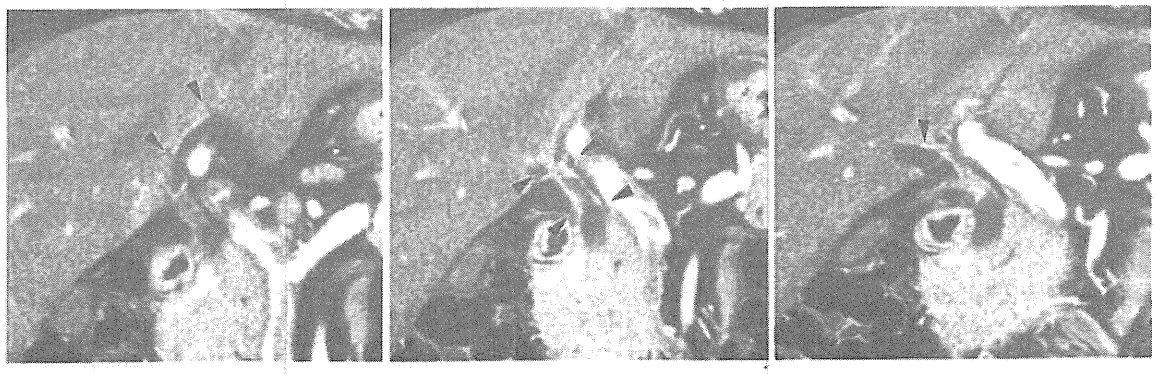

図1　肝門部胆管癌MPR像
左右肝管合流部を中心に左はB4合流部を越えて，膵臓側は膵上縁まで造影効果のある壁肥厚を認めた(矢頭)．
⇒肝左葉切除＋尾状葉切除＋肝外胆管切除を施行．

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◎胆道癌の治療は，現時点では，治癒切除が唯一の根治性が得られる治療法である．
◎拡大肝葉切除後の術後肝不全を避けるために術前門脈塞栓術は有効である．

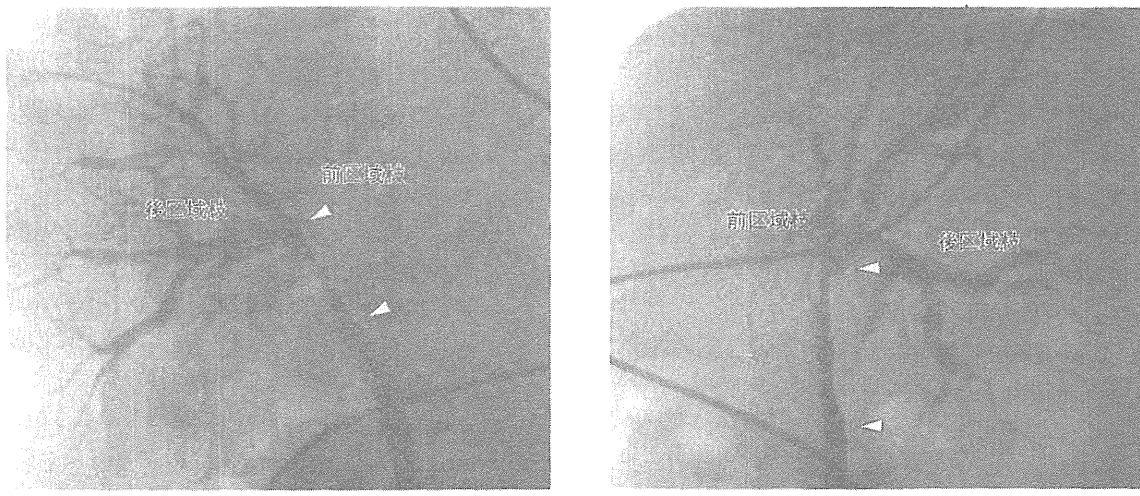

図2　左3区域切除症例，進展範囲(矢頭)
左肝管は描出されず，右肝管は，前後分岐部近傍にかけて不整像を認めた．

に有用とされる．胆管腔内超音波 intraductal ultrasound (IDUS)は深達度診断，血管浸潤などの胆管壁垂直浸潤の診断にすぐれている．以上のような検査により，切除の可否，術式が決定されるが，基本術式は胆管癌の主座により異なる．

1．肝門部胆管癌

基本的な手術術式は肝切除＋肝外胆管切除＋リンパ節郭清であるが，肝切除術式としては，右葉切除，右三区域切除，左葉切除，左三区域切除が，前述の進展度診断に基づき選択される場合が多い．それに加え，尾状葉切除を要することがほとんどであるが，これは尾状葉枝が左右肝管合流部背側に合流する解剖学的特徴のため，尾状葉胆管起始部に癌浸潤を認めることが多く，それを切除するためには尾状葉切除が必要である，という理由による．このような術式選択の場合，胆道ドレナージは基本的に予定残肝側の片葉ドレナージで十分とされる．

肝葉切除以上の肝切除を施行するにあたり，切除後の残肝容積が不足するために起こる肝不全が重大な合併症となるため，CT volumetry による術前肝容積測定にて残肝容積率が40%以下の場合は，術前に門脈塞栓術を施行し，非塞栓肝葉の代償性肥大をはかり肝不全のリスクを軽減することを考慮する必要がある．門脈塞栓術の導入により術後肝不全の発症は減少しており，肝門部胆管癌の切除術式として以前行われていたような肝実質温存手術は肝機能不良例や高齢者，肝膵同時切除例などのハイリスク例を除いてはあまり行われなくなってきている．

血管浸潤例においては，現在では，門脈浸潤症例に対しても治癒切除を目的とした積極的な外科切除，つまり門脈合併切除・再建を行うべきとする報告が多くみられ，肝門部胆管癌の手術手技としては必須のものとなっている．われわれの施設での治癒切除例のうち，血管合併切除例は，5年生存率は，22%と非血管合併切除例の41%と比較して，不良であるものの，非切除例に比べると明らかにその予後は改善されており，進行肝門部胆管癌において門脈合併切除・再建を併施した術

●進行肝門部胆管癌において門脈合併切除・再建を併施した術式は根治術として意義あるものとされる.
●胆嚢癌において根治切除を目指すために,EUSを用いて正確な深達度診断を行うことはきわめて重要である.

式は根治術として意義あるものと考えられる[3].

2. 中・下部胆管癌

中・下部胆管癌に対する基本術式は幽門輪温存や亜全胃温存を含めた膵頭十二指腸切除術 pancreatico-duodenectomy (PD) + リンパ節郭清である.そのなかで,中部胆管癌は,癌の進展範囲がより肝側に及ぶ頻度が高く,肝側胆管断端の癌浸潤の有無が問題となる場合が少なくない.そのため,肝門部胆管切除や肝膵同時切除 hepatectomy with pancreatoduodenectomy (HPD) が必要となることもある.また,一方では癌が限局していた場合,胆管切除のみで治癒切除が得られる症例もある.したがって,MDCTによる進展度診断に加え,術前に胆道造影およびPOCS下生検にて水平浸潤診断を正確に行うことと,術中においては,迅速病理診断にて,胆管断端を正確に評価できる病理医の存在が重要となる.いずれにせよ治癒切除を得ることが術後の予後に最も重要であるとされる.また,肝門部胆管癌手術と同様,血管浸潤がある場合でも積極的に血管合併切除再建を施行すべきとする報告が多い.

胆嚢癌●

胆嚢の解剖学的特徴として,胆嚢壁は,粘膜筋板を欠いた上に固有筋層が非常に薄く,漿膜下層がリンパ網に富んでいることと,胆嚢漿膜は肝臓の漿膜に引き続いており,胆嚢漿膜下層が直接,肝臓の実質に接しているということから,胆嚢癌が漿膜下に一旦浸潤すると,容易に転移・周囲への浸潤をきたし,特に肝床側に位置する癌では,肝臓への浸潤および肝内転移の頻度が高くなるという特徴を有する.一方,いわゆる早期胆嚢癌(mおよびmpまでの壁深達度のもの)では,切除後の予後は5年生存率で85.9%と良好である[1].したがって,根治切除を目指すために,正確な深達度診断,特にmpまでかss以深なのか,を行うことはきわめて重要である.

術前壁深達度診断においては超音波内視鏡検査 endoscopic ultrasonography (EUS) が現在最も有用であるとされる.また,進行胆嚢癌においては,進展様式は多様であり[4],リンパ節転移や遠隔転移,血管浸潤の評価においてはMDCTが,胆嚢管および肝十二指腸間膜浸潤の評価には,胆管癌に準じ,MDCTとともにMRCPまたはERCが有用である.

その一方,慢性胆嚢炎(黄色肉芽腫性胆嚢炎を含む)や胆嚢腺筋症と胆嚢癌の鑑別が,さまざまな診断 modality を駆使しても,ときに困難なことがある.このような場合は,安易に胆嚢摘出術を施行するのではなく,胆嚢癌の存在を念頭において手術に臨むべきである.われわれの施設では開腹下に胆嚢全層切除を行い,術中迅速組織診に提出し,癌の有無,癌であればその深達度を術中に診断している.このような症例の場合の腹腔鏡下胆嚢摘出術は,癌が存在していた場合,特に胆嚢床部で癌に切り込む可能性があること(深達度ssやm-RAS ss癌の場合),胆嚢損傷に伴う胆汁漏出とその結果による port site recurrence や腹膜再発の可能性が増加することから現状では避けるべきである.

胆嚢癌の術式は,深達度に応じて選択される.

1. mおよびmpまでの深達度

術前,画像診断にてm,mpまでの壁深達度と判断される症例では,理論的には胆嚢摘出術で対処しうる.しかしながら,深達度mpまでの術前診断の正診率はあらゆる modality を駆使しても約80%と報告されている[2].したがって,われわれの施設ではm,mpの癌と術前診断される場合では,胆嚢全層切除術+術中迅速組織診を適応としている.術中迅速にてmp癌の場合では,そ

- 乳頭部癌に対する標準術式は，膵頭十二指腸切除術＋リンパ節郭清術である．
- 胆嚢癌を疑う症例の術式は，原則的に開腹胆嚢摘出術を行うことが望ましい．
- 胆嚢癌で肝十二指腸間膜浸潤陽性の場合には，拡大肝右葉切除または肝右三区域切除が選択される．

のリンパ節転移の有無については一部の報告においてリンパ節転移を認める報告もあるため，われわれは肝十二指腸間膜内のリンパ節サンプリングを追加している．また，術中迅速組織診断で，胆嚢管断端，壁深達度を診断し，胆嚢管断端陽性またはss以深であった場合は，追加切除を施行すべきと考える．

2. ss以深の深達度

ss胆嚢癌に対する切除術式はいまだ一定の基準はないが，施設によって，肝については胆嚢床切除，あるいは肝中央下区域切除(S4a + S5)が選択されている．われわれは，術中迅速組織診にてss以深，あるいは，術前画像診断にてss以深，肝十二指腸間膜浸潤陰性と診断した際には，原則として，肝中央下区域切除＋肝外胆管切除＋D2郭清およびNo.16a2，b1リンパ節サンプリングを基本術式としている．肝中央下区域切除は，ss以深胆嚢癌の場合，肝実質直接浸潤部の切除，初期肝転移巣の切除を目的とし[5]，肝外胆管切除は，肝十二指腸間膜内の転移リンパ節やリンパ管浸潤，神経周囲浸潤巣などを確実に郭清する目的から行っている．一方，肝直接浸潤が右グリソン鞘に及ぶ場合は中央下区域切除ではなく，肝拡大右葉切除が選択されるが，肝実質浸潤範囲によっては肝中央二区域切除が行われることもある．

肝十二指腸間膜浸潤陽性の場合には，通常肝門部から右グリソン鞘に沿って癌浸潤を認めることが多いため[5]，拡大肝右葉切除や肝右三区域切除が選択されることが多い．たとえ，門脈などの血管浸潤がみられていても，それを合併切除再建することにより治癒切除が得られることが少なくなく，積極的に外科切除を施行すべき，とする報告がわが国では多くみられる．拡大肝右葉切除以上の肝切除においては，切除後の残肝容積が不足する場合は，胆管癌同様に術前に門脈塞栓術を考慮する必要がある．また，肝十二指腸間膜浸潤にて閉塞性黄疸をきたした場合は，術前にENBDを行い減黄処置を行っている．膵浸潤や膵頭部リンパ節からの膵直接浸潤，肝十二指腸間膜浸潤が広範囲に及び胆管十二指腸側断端が癌陽性となる症例で治癒切除が可能な場合は，肝切除＋膵頭十二指腸切除術が選択される．また，大腸や十二指腸に浸潤がみられる場合はそれらを合併切除する．

乳頭部癌

乳頭部癌に対する標準術式は，中下部胆管癌手術と同様，幽門輪温存や亜全胃温存を含めた膵頭十二指腸切除術＋リンパ節郭清であり，大半は根治切除可能である．

癌進展度からみると，深達度でOddi筋を越えない乳頭部癌ではリンパ節転移率はきわめて低いことから，縮小手術も検討されるが，Oddi筋浸潤の有無を含めた正確な術前診断はEUSやIDUSを含めたさまざまなmodalityを駆使してもまだ困難であり，現時点では乳頭部癌に対する縮小手術のコンセンサスは得られていない[2]．

おわりに

胆道癌は，治癒切除が唯一の根治性が得られる治療法であり，根治切除が可能であれば積極的に治療を行うべきである．また，門脈などの血管浸潤がみられていても，それを合併切除再建することにより治癒切除が得られるならば，積極的に外科切除を施行すべきである．拡大肝葉切除においては，肝不全などの術後合併症を予防するために切除後の残肝容積が不足する場合は，術前の門脈塞栓術を施行し，リスクを軽減する必要がある．現在のところ，術前，術後の補助療法は確立されていないが，今後，治癒切除の向上，生存率の改善に向けた化学，放射線療法などのプロトコール

のデータの蓄積が期待される．

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ORIGINAL ARTICLE

The alterations in hepatic microcirculation and Kupffer cell activity after biliary drainage in jaundiced mice

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Abstract

Background/purpose The aim of this study is to examine the effects of biliary drainage on hepatic microcirculation and Kupffer cell activity in the liver with obstructive jaundice.

Methods Common bile duct ligation and division was performed on C57BL/6 mice to induce obstructive jaundice. Seven or 14 days after surgery, some mice underwent biliary drainage. Three days after biliary drainage, sinusoidal perfusion, leukocyte rolling and sticking in the postsinusoidal venules, and the diameters of sinusoids containing blood flow were evaluated using intravital microscopy. Kupffer cell phagocytic activity was estimated as the ratio of Kupffer cells that phagocytosed fluorescent-labeled particles to sinusoids containing blood flow.

Results Sinusoidal perfusion after biliary drainage was significantly increased compared with that in livers with obstructive jaundice, but remained decreased compared with controls. Although the number of rolling leukocytes and sticking leukocytes was significantly decreased, the diameters of sinusoids remained reduced, associated with an increase in Kupffer cell phagocytic activity compared with controls even after biliary drainage.

Conclusions Leukocyte–endothelial cell interaction is ameliorated but sinusoids remain narrowed due to swelling of activated Kupffer cells; this might cause deterioration of hepatic microcirculation during the early phase of biliary drainage.

Keywords Mice · Biliary drainage · Intravital microscopy · Hepatic microcirculation · Kupffer cell

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Introduction

It has been noted that obstructive jaundice (OJ) is one of the major causes of postoperative complications after surgery [1]. In particular, morbidity and mortality rates have been reported to be high after major hepatectomy in patients with OJ [2, 3], and preoperative biliary drainage (BD) is recommended in patients with OJ undergoing major hepatectomy [4]. However, the mechanisms underlying the high incidence of postoperative complications and the effects of BD in patients with OJ are still not clearly understood.

Intravital microscopy (IVM) is one of the best methods to examine microenvironmental events in the normal physiological state. Using IVM, we have previously shown that OJ reduces hepatic microcirculation and activates individual Kupffer cells in mice [5, 6]. The aim of this

study was to clarify the mechanisms underlying the effects of BD on hepatic function by examining hepatic microcirculation and Kupffer cell activity in mice with OJ using IVM.

Methods

Animals

Eight-week-old male C57BL/6 mice (Shizuoka Laboratory Animal Center, Shizuoka, Japan), individually housed in cages and fed standard laboratory food and water ad libitum, were used for these experiments. Approval for this study was obtained from Chiba University Animal Care and Use Committee and was in compliance with the guidelines established by the National Institutes of Health.

Obstructive jaundice

Mice were anesthetized with pentobarbital sodium (75 mg/kg ip). The mice underwent a midline laparotomy and common bile duct ligation and division (CBL) to induce OJ in the OJ group. Briefly, the common bile duct was mobilized, doubly ligated and divided using 5-0 silk. A silicone catheter with an inner diameter of 0.5 mm was inserted into the gallbladder. The other end of the catheter was ligated and was placed in the nape of the neck through the subcutaneous tunnel. In the sham group, the common bile duct was mobilized but not ligated. A silicone catheter was inserted into the gallbladder in the same manner as the OJ group.

Biliary drainage

Mice were anesthetized by ether inhalation. In the OJ/BD group, the ligated end of the catheter was exposed by incising the skin of the neck, and opened for external BD 7 or 14 days after CBL. In the Sham/BD group, the ligated end of the catheter was also opened for external BD 7 or 14 days after the sham operation. On the third day after BD, the following experiments were performed.

Assay

At the time of sacrifice, blood was withdrawn from the inferior vena cava to obtain serum samples for later analysis. Serum total bilirubin levels and alanine aminotransferase (ALT) levels were determined by commercial kits (Wako Pure Chemical Industries, Co., Osaka, Japan).

Intravital microscopy

The mice were anesthetized with pentobarbital sodium (75 mg/kg ip) and air patency was maintained by a tracheotomy and a tracheal stent. The carotid artery was cannulated and blood pressure was monitored continuously using a blood pressure analyzer. The respiratory rate was counted every 10 min and body temperature was monitored continuously using a thermometer. The mice were studied using established high-resolution intravital microscopic methods as previously described [7]. The liver was gently exteriorized through a midline and left subcostal abdominal incision and positioned over a window of mica cover glass in a specially designed microscope stage with drainage for irrigating fluid. The stage was positioned under a fluorescent microscope (BX50WI; Olympus, Tokyo, Japan) so that the liver was observed by transmitted light or fluorescent microscopy. A closed circuit television system, a CCD video camera (DXC-108; SONY, Tokyo, Japan) and a videotape recorder were used to monitor and record each experiment for subsequent analysis.

The relative adequacy of sinusoidal perfusion was evaluated by counting the number of sinusoids containing blood flow in five to ten periportal and five to ten centrilobular microscopic fields (Fig. 1a, b).

Two postsinusoidal venules (20–40 μm in diameter, 150–200 μm in length) for each mouse were identified for counting leukocytes that had adhered to the endothelial surface. Rolling leukocytes were counted as those that rolled along the endothelial surface. They were categorized by sinusoidal circumference ($\pi \times$ diameter of vessel observed). Sticking leukocytes were counted as those that did not move for 20 s during the observation period. They were categorized by endothelial surface area ($\pi \times$ diameter $\times$ length of vessel segment observed) (Fig. 2a, b).

The diameters of more than fifty randomly selected sinusoids containing blood flow were measured in each mouse.

Kupffer cell function was assessed by observing the phagocytosis of 1-μm fluorescent latex particles

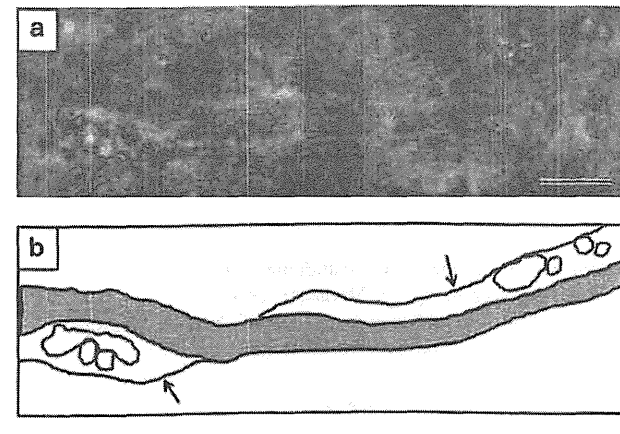

Fig. 1 Intravital light microscopic image (**a**) and schema (**b**) of sinusoids containing blood flow (*shaded area*) and Kupffer cells (*arrow*). *Bar* = 10 μm

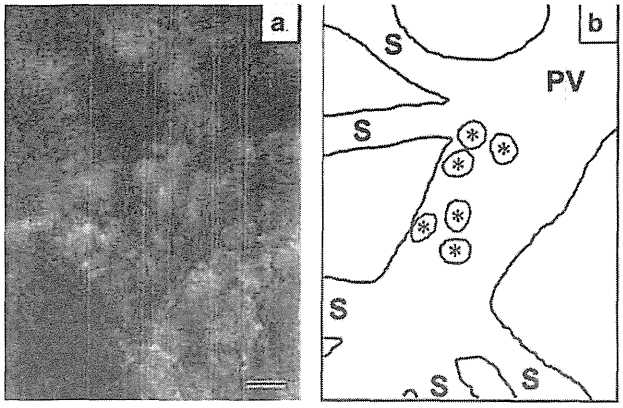

Fig. 2 Intravital light microscopic image (**a**) and schema (**b**) of the postsinusoidal venule (*PV*). *S* sinusoids, *bar* = 10 μm

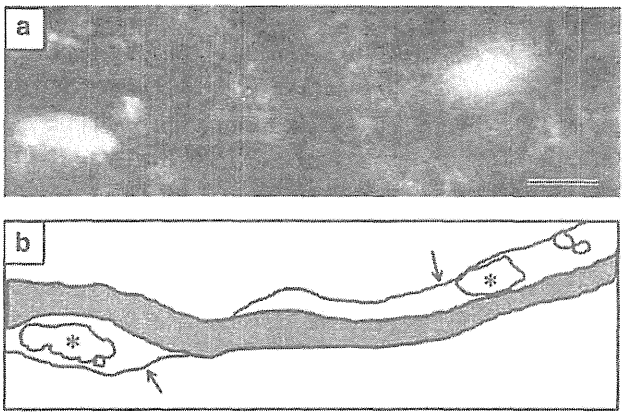

Fig. 3 Intravital fluorescent microscopic image (**a**) and schema (**b**) of sinusoids containing blood flow (*shaded area*) and Kupffer cells (*arrow*) for the same field as Fig. 1. *Asterisk* phagocytosed beads, *bar* = 10 μm

(Polyscience, Inc., Warrington, PA, USA) by individual cells. The latex was diluted with sterile saline (1:15) and administered intravascularly into the tail of each mouse at a dose of 0.1 ml (3×10^8 particles). The number of cells that phagocytosed latex was counted in the same periportal and centrilobular microscopic fields 15 min after each mouse had received the latex solution. Since reduced perfusion of individual sinusoids can reduce the delivery of the latex to Kupffer cells in these vessels, the ratio of Kupffer cells that phagocytosed latex to sinusoids containing blood flow was used as a measure of Kupffer cell phagocytic activity (Fig. 3a, b)

Statistical analysis

All data were expressed as means ± SD. Data were analyzed with an analysis of variance with subsequent Fisher's PLSD test. Differences were considered significant when $p < 0.05$.

Results

Serum parameters

CBL significantly increased the serum ALT levels. BD significantly decreased the serum ALT levels, and no significant differences were detected in the serum ALT levels between the sham/BD group and the OJ/BD group (Fig. 4). CBL significantly increased the serum total bilirubin levels. BD significantly decreased the serum total bilirubin levels, but levels in the OJ/BD group were slightly, but significantly, higher than those in the sham/BD group (Fig. 5).

Intravital microscopy

The mean arterial blood pressure, respiratory rate and rectal temperature showed no significant differences among the 4 groups during the observation period (data not shown).

CBL significantly decreased the number of sinusoids containing blood flow on days 7 and 14 after surgery. BD significantly increased the number of sinusoids containing blood flow, but that in the OJ/BD group was significantly lower than that in the sham/BD group (Fig. 6). No significant differences were observed between the periportal area and the centrilobular area (data not shown).

CBL significantly increased the number of rolling leukocytes on days 7 and 14 after surgery. BD significantly decreased the number of rolling leukocytes, and no significant differences were detected in the number of rolling leukocytes between the sham/BD group and the OJ/BD group (Fig. 7). CBL significantly increased the number of sticking leukocytes after 7 days of OJ. BD significantly decreased the number of sticking leukocytes, and no significant differences were detected in the number of sticking leukocytes between the sham/BD group and the OJ/BD group. Similar findings, but not statistically significant, were observed in mice undergoing BD after 14 days of OJ (Fig. 8).

CBL significantly reduced the diameters of sinusoids containing blood flow on days 7 and 14 after surgery. However, BD had no effect on the diameters of sinusoids containing blood flow at all, and the diameters of sinusoids containing blood flow in the OJ/BD group was significantly reduced compared with those in the sham/BD group (Fig. 9). The narrowest points of sinusoids were always found in the area where phagocytosing Kupffer cells were present in both the OJ and the OJ/BD group (Figs. 1, 3). No significant zonal differences were observed (data not shown).

CBL significantly induced Kupffer cell phagocytic activity on days 7 and 14 after surgery. However, BD had no effect on Kupffer cell phagocytic activity on days 7 and 14 after CBL. Kupffer cell phagocytic activity in

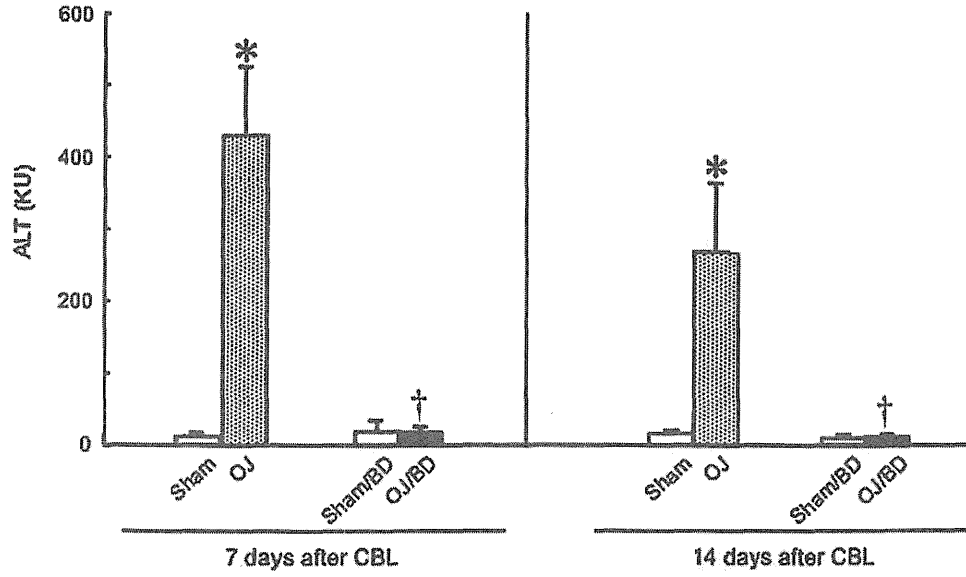

Fig. 4 Changes in the serum alanine aminotransferase (*ALT*) levels. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\dagger}p < 0.05$ compared with OJ

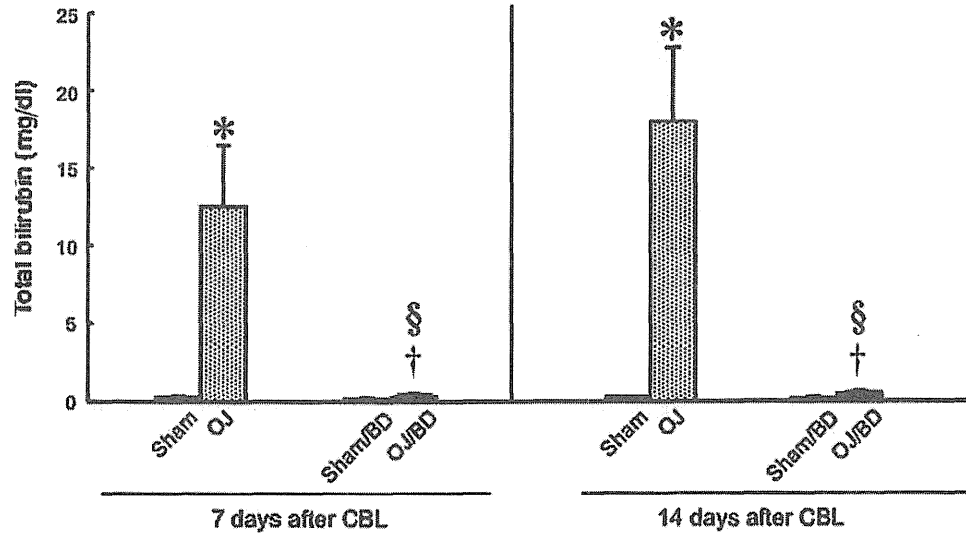

Fig. 5 Changes in the serum total bilirubin levels. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\dagger}p < 0.05$ compared with OJ; $^{\S}p < 0.05$ compared with sham/BD

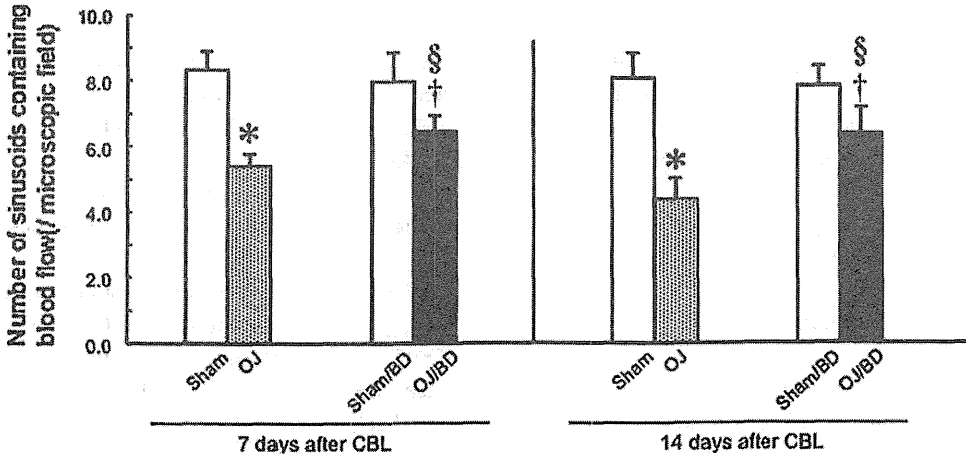

Fig. 6 Changes in the number of sinusoids containing blood flow. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\dagger}p < 0.05$ compared with OJ; $^{\S}p < 0.05$ compared with sham/BD

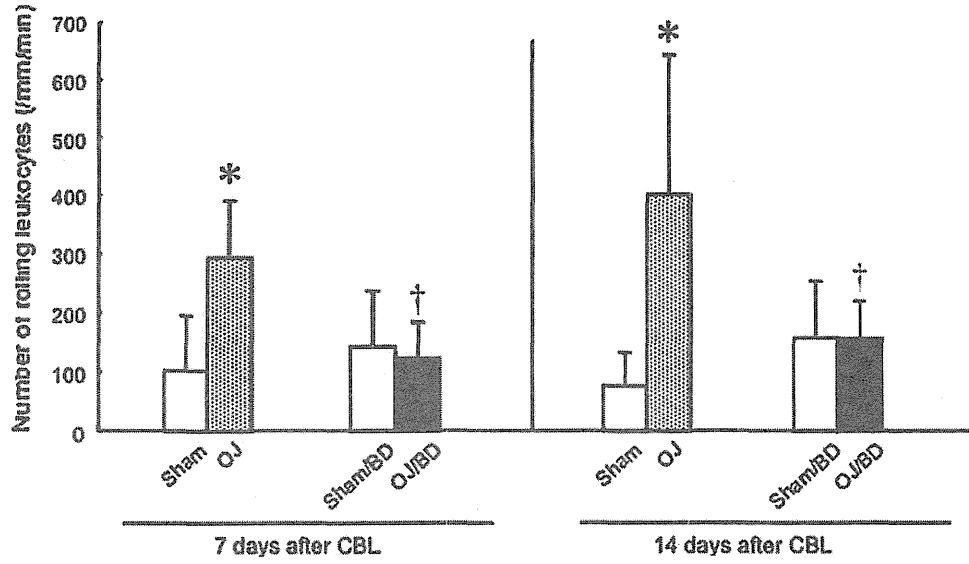

Fig. 7 Changes in the number of rolling leukocytes. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\dagger}p < 0.05$ compared with OJ

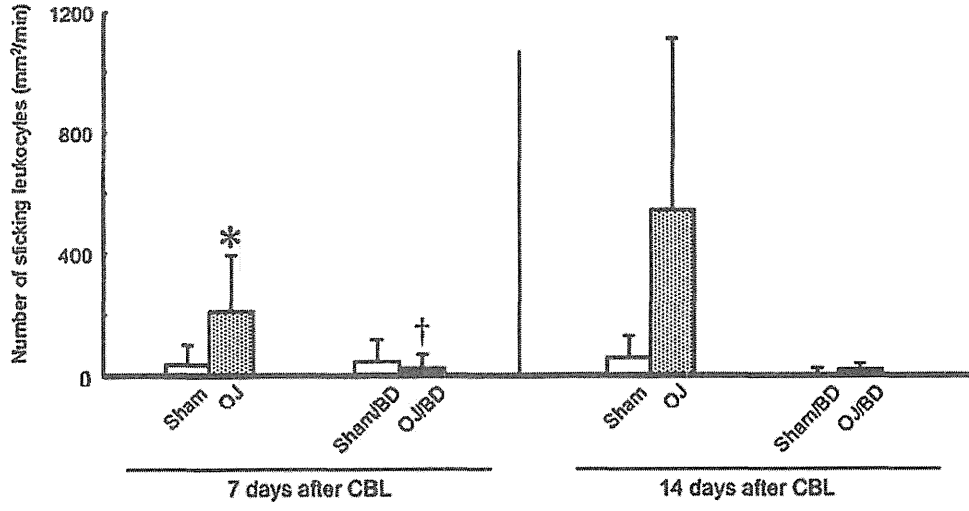

Fig. 8 Changes in the number of sticking leukocytes. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\dagger}p < 0.05$ compared with OJ

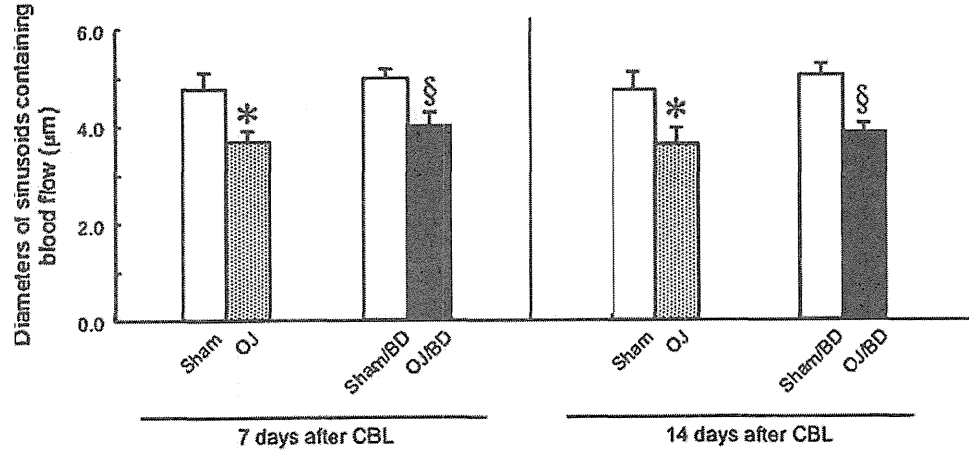

Fig. 9 Changes in the diameters of sinusoids containing blood flow. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\S}p < 0.05$ compared with sham/BD

the OJ/BD group was significantly increased compared with that in the sham/BD group after 14 days of OJ and BD. Similar findings, but not statistically significant, were observed in mice undergoing BD after 7 days of OJ (Fig. 10). No significant zonal differences were observed (data not shown).

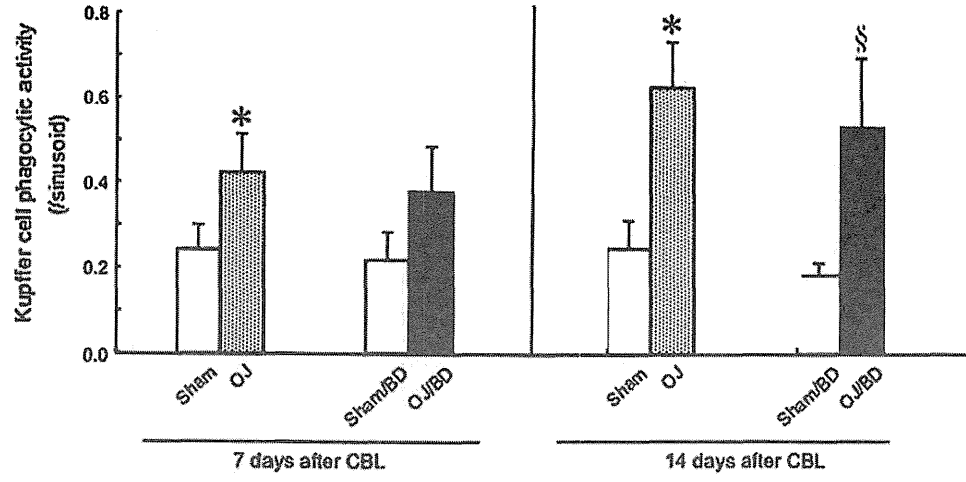

Fig. 10 Changes in Kupffer cell phagocytic activity. *CBL* common bile duct ligation and division, *OJ* obstructive jaundice, *Sham/BD* sham operation followed by biliary drainage, *OJ/BD* obstructive jaundice followed by biliary drainage. Values represent means ± SD with $n = 10$ mice per group. $^{*}p < 0.05$ compared with sham; $^{\S}p < 0.05$ compared with sham/BD

Discussion

We employed mice to examine the effects of BD in this study although rats have been employed in many studies with small animals. This is because, although the amount of sample from mice is smaller than that from rats, mice are superior to rats in terms of cost, space to cage and the convenience of obtaining many kinds of reagents for experiments such as commercially available antibodies. Moreover, mice have a significant advantage in that hepatic microcirculation and leukocytes (rolling and sticking) can be observed using not fluorescent but light IVM because the livers of mice are thin (Figs. 1, 2). Mice underwent CBL to induce OJ and insertion of a catheter for biliary drainage into the gallbladder which exists in mice, but not in rats, during the same laparotomy. This procedure avoided relaparotomy. Kishimoto et al. [8] reported an animal model of BD after OJ using mice, in which the common bile duct was clamped with a surgical clip to induce OJ, and the clip was removed for BD with relaparotomy. Each model has its respective benefits because our model is for external BD and the other is for internal BD. However, our model has the advantages that there is no risk that clipping might injure the mucosa of the common bile duct, and that it is unnecessary to perform relaparotomy for BD, compared with the other model.

The effect of preoperative BD in patients with OJ remains controversial. Some reports have shown that preoperative BD is unnecessary before pancreaticoduodenectomy [9, 10]. However, it has been reported that there is a high mortality rate in patients with OJ after extended hepatectomy, caused mainly by hepatic failure [11]. Some reports have shown that BD contributes to improvement in the mortality rate after major hepatectomy [12, 13]. These data suggest that BD is important for patients with OJ undergoing major hepatectomy. However, the mechanisms underlying the effect of BD on hepatic function are not clear. Serum ALT levels were decreased to normal levels by BD for 3 days after both 7 and 14 days of OJ (Fig 4). However, the serum total bilirubin levels were decreased, although they remained slightly but significantly higher compared with controls with BD for 3 days (Fig. 5). To investigate these inconsistent data, we examined changes in hepatic microcirculation 3 days after BD using IVM. Sinusoidal perfusion was significantly ameliorated but remained significantly deteriorated compared with controls with BD for 3 days (Fig. 6). The rolling and sticking leukocytes were reduced to the levels of controls after BD. Leukocyte rolling and sticking is known to be mediated by leukocyte–endothelial cell interaction, which is regulated by various adhesion molecules. It has been reported that OJ has induced expression of intercellular adhesion molecule-1 in the livers of rats by Western blot analysis [14] and by immunohisotochemistry [15]. Moreover, it has been reported that expression of vascular cell adhesive molecule-1 has been induced by OJ and reduced by internal BD in mice [8]. These data suggest that leukocyte–endothelial cell interaction is induced by OJ and reduced by BD. BD might ameliorate hepatic microcirculation in part by improved leukocyte–endothelial cell interaction which caused deterioration of hepatic microcirculation during OJ.

In this study, we evaluated the diameters of sinusoids containing blood flow which are thought to be involved in alteration of hepatic microcirculation. Diameters of sinusoids containing blood flow were not ameliorated and remained reduced compared with controls provided with BD for 3 days (Fig. 9). Using a laser flow meter, Matsumoto et al. [16] showed that BD ameliorates hepatic perfusion in rats with OJ and that changes in the ultrastructure and the number of fenestrae in the sinusoids were associated with alterations in hepatic perfusion observed by scanning electron microscopy. On the other hand, it is known that Kupffer cells are activated causing swelling by some stimuli such as endotoxin [7]. We previously showed

that swollen Kupffer cells cause a reduction in the diameters of sinusoids leading to hepatic microcirculatory disturbance in mice with OJ [5, 6]. In this study, Kupffer cell phagocytic activity remained induced after BD for 3 days in mice with OJ. These data suggest that narrowing of sinusoids due to swollen Kupffer cells might cause deterioration of hepatic microcirculation during the early phase of BD.

Whereas many reports have shown that phagocytic activity of the whole liver is decreased by OJ and ameliorated by BD [17–20], few studies have demonstrated phagocytic activity of individual Kupffer cells. Movement of endotoxin from the gut into the portal circulation is eliminated mainly by Kupffer cells [21]. It has been reported that OJ causes endotoxin translocation from the gut [22] which is thought to activate individual Kupffer cells [23]. In this study, Kupffer cell phagocytic activity in sinusoids containing blood flow was increased in livers with OJ and remained activated after BD for 3 days (Fig. 10). The period of adequate BD required for recovery of Kupffer cell activity was reported to range from days to months [20]. This might be due to differences in the procedures and models. Further analysis is needed to ascertain the adequate period of BD.

Phagocytic activity of the whole liver depends on both the activity of individual Kupffer cells and the sinusoidal perfusion delivering substances to individual Kupffer cells. We showed in this study that Kupffer cell phagocytic activity remained induced and that sinusoidal perfusion was ameliorated compared with that in livers with OJ, but remained deteriorated compared with controls when the serum total bilirubin levels were decreased after BD. These data suggest that sinusoidal perfusion deterioration compared with controls during the early phase of BD might reduce clearance of endotoxin, leading to postoperative complications associated with endotoxemia in patients with OJ. Alternatively, it might be possible that activated Kupffer cells concomitant with ameliorated sinusoidal perfusion compared with that in livers with OJ contribute to elimination of endotoxin and avoid postoperative complications during the early phase of BD.

We did not examine whether internal BD or external BD is superior. Many studies have shown that internal BD is superior to external BD. Gouma et al. [24] showed that restoration of gastrointestinal bile by internal BD prevented portal and systemic endotoxemia in rats. Kamiya et al. [25] showed that bile replacement during external BD restored the intestinal barrier function in patients with OJ. On the other hand, it has been reported that relief of biliary obstruction is more important than restoration of gastrointestinal bile in prevention of endotoxemia [26]. This study also showed that external BD resulted in ameliorated sinusoidal perfusion compared with that in livers with OJ. There is another argument regarding the procedures for BD: percutaneous transhepatic biliary drainage (PTBD) and endoscopic nasobiliary drainage (ENBD) provide external BD, and endoscopic biliary stenting (EBS) provides internal BD. A recent report has recommended ENBD because of fewer complications related to the procedure [27]. Whereas PTBD was shown to be associated with a risk of cancer dissemination via the BD tract [27], cholangiography via PTBD or ENBD catheter, which are both external drainage, provides a precise indication of longitudinal cancer spread along the bile duct for patients with hilar cholangiocarcinoma. PTBD is also useful when several tubes for BD are needed or when endoscopic BD was unsuccessful. Further investigation is needed to discover the appropriate procedure for preoperative BD for patients with OJ.

In summary, leukocyte–endothelial cell interaction is ameliorated, but sinusoids remain narrowed due to swelling of activated Kupffer cells, which might reduce hepatic microcirculation during the early phase of BD. These data suggest that hepatic microcirculatory disturbance could cause postoperative complications associated with endotoxemia even after BD in patients with OJ. Whereas the optimal duration of BD is not clear, it has been reported that at least 4–6 weeks of drainage is needed for full recovery of hepatic function [9]. This study has shown that liver damage due to decreased sinusoidal perfusion does not correlate with the serum total bilirubin levels during the early phase of BD. The serum hyaluronic acid level is known to be an indicator of sinusoidal injury [28, 29], and might be a good marker of changes in hepatic microcirculation after BD. A recent report demonstrated the usefulness of contrast-enhanced ultrasonography using Sonazoid to examine hepatic microcirculatory disturbance in patients with acute liver injury [30], suggesting that the procedure might be useful to determine the alteration of hepatic microcirculation during BD. It is important to establish the timing of hepatectomy in patients with OJ by examining the optimal period of BD required for recovery of hepatic microcirculation and Kupffer cell activity.

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（分担研究報告書）

がん登録からみたがん診療ガイドラインの普及効果に関する研究
－診療動向と治療成績の変化－

（研究分担者　山口幸二　産業医科大学　第一外科教授）

研究要旨

膵癌診療ガイドラインの普及について調査を行うとともに、実際の膵癌臨床における効果、影響についての研究を行う。現在、2009年度改訂版（第2版）およびWeb化が完成し、出版数およびホームページへのアクセス数などについても調査中である。また、2013年の改訂版発行に向けて改訂委員会および公聴会を重ねており、同時にガイドライン普及効果についての議論も行っているところである。

A．研究目的
ガイドライン作成をより多くのがん種において完結させ、さらに改訂の継続性を維持していくための適切な環境の在り方を検討することにある。

B．研究方法
膵癌診療ガイドライン（2009年度改訂版）およびこのWeb公開について、その普及率について出版数、ウェブアクセス数から調査する。同時に、アンケート調査にてガイドライン有効性の評価を行う。（倫理面への配慮）ガイドラインの普及に伴い、患者個人の意見が無視されないように配慮する。

C．研究結果
現在までのところ、膵癌診療ガイドライン（2009年度改訂版）の出版数およびウェブアクセス数について調査を進めているところである。

D．考察
ガイドラインの評価スケールとしてAGREE, AGREE II, Shanyfelt法、COGSなど様々な方法があるが、どれを用いるべきかについての議論が必要であろう。また、実際の患者ALLや予後の向上の評価については、NCD（National Clinical Database）など、どのデータベースを用いて調べるかにつての検討が必要であろう。

E．結論
膵癌診療ガイドラインの普及率および実臨床における影響を調査中である。

F．健康危険情報
なし

G．研究発表
1.　論文発表

Yamaguchi K, Tanaka M; Committee for Revision of Clinical Guidelines for Pancreatic Cancer of Japan Pancreas Society.

EBM-based Clinical Guidelines for Pancreatic Cancer 2009 from the Japan Pancreas Society: a synopsis.

Jpn J Clin Oncol. 2011 Jul;41(7):836-40.

H．知的財産権の出願・登録状況
なし

JJCO Japanese Journal of Clinical Oncology

Jpn J Clin Oncol 2011;41(7)836–840
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Review Article

EBM-based Clinical Guidelines for Pancreatic Cancer 2009 From the Japan Pancreas Society: A Synopsis

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Clinical Practice Guidelines for Pancreatic Cancer Based on Evidence-based Medicine, 2006, were published by the Japan Pancreas Society (Committee for Revision of Clinical Guidelines for Pancreatic Cancer) in March 2009 in Japanese[1] and were revised to Clinical Practice Guidelines for Pancreatic Cancer Based on Evidence-based Medicine 2009 in July 2009 in Japanese.[2] These guidelines were established according to Evidence-Based Medicine. A total of 443 papers were collected from 2544 reports concerning pancreatic cancer that were listed on PubMed and Igakuchuo Zasshi from July 2004 to April 2007. This new guidelines were written by members of the Committee for Revision of Clinical Practice Guidelines for Pancreatic Cancer in the Japan Pancreas Society. The guidelines show algorithm for the diagnosis (Fig. 1) and treatment (Fig. 2) of pancreatic cancer, address five subjects: diagnosis, chemotherapy, radiation therapy, surgical therapy and adjuvant therapy, and include 25 clinical questions (CQs) and 39 recommendations. The corresponding CQ numbers are inserted in the algorithms. There are five degrees of recommendation:

A Strongly recommended because there is strong scientific evidence.
B Recommended because there is scientific evidence.
C1 Recommended although there is no scientific evidence.
C2 Not recommended because there is no scientific evidence.
D Not recommended because there is evidence showing that it is ineffective or harmful.

This article presents a synopsis of the guidelines in English.

Diagnosis

CQ1-1 What are risk factors for pancreatic cancer?

The below-mentioned risk factors have been reported to have evidences supporting the relationship between the factors and pancreatic cancer:

(i) Family history: pancreatic cancer and hereditary pancreatic cancer syndrome.
(ii) Accompanying diseases: diabetes mellitus, obesity, chronic pancreatitis, hereditary pancreatitis, intraductal papillary mucinous neoplasm (IPMN).
(iii) Habits: tobacco.

RECOMMENDATION 1-1

(i) Patients with more than one risk factor are recommended to undergo further examination to detect pancreatic cancer (Grade B).
(ii) IPMN progresses to invasive cancer and accompanies pancreatic cancer. IPMN should be adequately assessed and carefully followed up (Grade B).

CQ1-2 What are the clinical symptoms of pancreatic cancer?
The below-mentioned clinical symptoms have been reported as those of pancreatic cancer:

(i) Abdominal pain is the most frequent symptom, followed by jaundice, back pain and body weight loss.
(ii) Clinically silent pancreatic cancer.
(iii) Fifty percent of pancreatic cancer patients show early-onset diabetes mellitus (glycogen metabolism disturbance) within 3 years.

RECOMMENDATION 1-2

(i) Patients with unexplainable abdominal pain, back pain, jaundice and/or body weight loss should undergo further examination for pancreatic cancer. However, the clinical outcome of symptomatic pancreatic cancer is poor (Grade B).