ISSN 2218-4333 (online)

World Journal of *Clinical Oncology*

World J Clin Oncol 2011 May 10; 2(5): 195-236

Baishideng Publishing Group
www.wjgnet.com

WJCO World Journal of Clinical Oncology

A peer-reviewed, online, open-access journal of oncology

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WJCO World Journal of Clinical Oncology

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Monthly Volume 2 Number 5 May 10, 2011

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World Journal of Clinical Oncology
Volume 2 Number 5 May 10, 2011

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NAME OF JOURNAL
World Journal of Clinical Oncology

LAUNCH DATE
November 10, 2010

SPONSOR
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
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http://www.wjgnet.com

PUBLISHING
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No.90 Jaffe Road, Wanchai, Hong Kong, China
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http://www.wjgnet.com

PUBLICATION DATE
May 10, 2011

ISSN
ISSN 2218-4333 (online)

EDITORIAL OFFICE
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World Journal of Clinical Oncology
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ONLINE SUBMISSION
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World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i5.195

World J Clin Oncol 2011 May 10; 2(5): 195-202
ISSN 2218-4333 (online)

TOPIC HIGHLIGHT

Michiko Yamagata, MD, PhD, *Series Editor*

Genetically-engineered mouse models for pancreatic cancer: Advances and current limitations

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Received: November 30, 2010 Revised: April 17, 2011
Accepted: April 24, 2011
Published online: May 10, 2011

Abstract

Recently, there has been significant progress in the development of genetically-engineered mouse (GEM) models. By introducing genetic alterations and/or signaling alterations of human pancreatic cancer into the mouse pancreas, animal models can recapitulate human disease. Pancreas epithelium-specific endogenous Kras activation develops murine pancreatic intraepithelial neoplasia (mPanIN). Additional inactivation of p16, p53, or transforming growth factor-β signaling, in the context of Kras activation, dramatically accelerates mPanIN progression to invasive pancreatic ductal adenocarcinoma (PDAC) with abundant stromal expansion and marked fibrosis (desmoplasia). The autochthonous cancer models retain tumor progression processes from pre-cancer to cancer as well as the intact tumor microenvironment, which is superior to xenograft models, although there are some limitations and differences from human PDAC. By fully studying GEM models, we can understand the mechanisms of PDAC formation and progression more precisely, which will lead us to a breakthrough in novel diagnostic and therapeutic methods as well as identification of the origin of PDAC.

Key words: Pancreatic ductal adenocarcinoma; Genetically-engineered mouse; Pancreas epithelium-specific; Kras; Tumor-stromal interaction; Tumor microenvironment; Origin of pancreatic ductal adenocarcinoma; Murine pancreatic intraepithelial neoplasia; Acinar-ductal metaplasia; Inducible genetically-engineered mouse

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Ijichi H. Genetically-engineered mouse models for pancreatic cancer: Advances and current limitations. *World J Clin Oncol* 2011; 2(5): 195-202 Available from: URL: http://www.wjgnet.com/2218-4333/full/v2/i5/195.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i5.195

INTRODUCTION

Pancreatic cancer and biliary cancer are the most lethal cancers and the incidence rate is increasing. Currently, pancreatic cancer is the fifth leading cause of cancer death in Japan and the fourth in the USA[1,2]. Biliary cancer is found most frequently in Japan where it is the sixth leading cause of cancer death[3]. The annual incidence and number of deaths is very close, which indicates the high lethality of both cancers. To overcome these lethal cancers, disease models that can recapitulate human conditions would be of great help in understanding the details of the disease and developing novel therapeutic approaches.

Previously, xenograft models (i.e. subcutaneous tumor and orthotopic tumor) have been used as *in vivo* tumor models by inoculating human cancer cell lines or tis-

sues into immunocompromised mice. Scientists creating genetically-engineered mouse (GEM) models have strived to mimic human pancreatic cancer for years, and recently models close to the human disease have been established. As described below, there are clear differences between xenograft tumors and GEM model tumors and the latter model is considered a closer approximation of human disease conditions; therefore, using GEM models in preclinical studies will provide various benefits.

Recent progress in GEM models of pancreatic cancer can be called a breakthrough in pancreatic cancer research. In this review, I discuss the advances and current limitations of GEM models of pancreatic cancer. On the other hand, in the biliary cancer field, there is no GEM model yet and we are waiting for the establishment one of such a model. I apologize in advance to colleagues whose work could not, unfortunately, be sited in this review.

MULTI-STEP CARCINOGENESIS HYPOTHESIS OF PANCREATIC DUCTAL ADENOCARCINOMA ALONG WITH GENETIC ALTERATIONS

Since most pancreatic cancers found in the clinic are "conventional" pancreatic ductal adenocarcinoma (PDAC), we should target and model PDAC. Hereafter, I focus on PDAC in this review.

Like the famous adenoma-carcinoma sequence in colorectal cancer, a multi-step carcinogenesis hypothesis of PDAC, which is linked to an accumulation of genetic alterations, has currently been consensually accepted clinically: As genetic alterations accumulate in normal pancreatic epithelial cells, precancer lesions, pancreatic intraepithelial neoplasia (PanIN) emerge, they progress in stage and eventually progress into invasive cancer, when tumor cells invade beyond the basal membrane[4] (Figure 1). A constitutive active point mutation of the *Kras* gene codon 12 has already been found at the stage of early, low grade PanIN and found nearly 100% at the invasive cancer stage. There is no such highly frequent spot mutation as this in sporadic solid cancers, which suggests that the Kras activation might truly initiate the PDAC carcinogenesis process. Inactivation of tumor suppressor genes (TSGs); i.e. $p16^{INK4a}$, *p53*, *Smad4*, are found along with the PanIN stage progression, which suggests that these are also involved in the process of PDAC formation.

However, PanIN cannot be detected by current diagnostic imaging modalities, and, therefore, we usually have no chance to observe the transition from PanIN to invasive PDAC.

Recently, by introducing PDAC-related genetic alterations into mouse pancreas, several GEM models recapitulating human PDAC have been established.

To achieve pancreas-specific genetic engineering, a Cre-loxP system driven by the *PDX1* or *Ptf1a* (*p48*) gene promoter is mainly employed. *PDX1* and *Ptf1a* are expressed from embryonic days 8.5 and 9.5, respectively. Both are required for pancreas development and differentiation. The pancreatic epithelium at the adult stage contains three lineage cells: acinar cells, duct cells (both are exocrine cells) and islet cells (endocrine cells) (Figure 2). Since *PDX1* and *Ptf1a* are expressed before divergence into these three lineages, Cre-loxP recombination is executed in all the three lineages. If PDAC really originates from normal pancreatic duct cells, pancreatic duct cell-specific genetic alteration might be the best approximation. However, to date, there is no available pancreatic duct-specific promoter. Therefore, the *PDX1* or *Ptf1a*-driven models cannot provide definite answers as to whether the duct cells are the real origin of PDAC. However, these models show murine PanINs (mPanINs) and develop PDAC. Considering that previous GEM models resulted in only acinar cell carcinoma or islet cell tumors, current models are very close to human PDAC.

ENDOGENOUS $KRAS^{G12D}$ EXPRESSION MODEL

Current GEM models of PDAC have been improved greatly by the establishment of the "endogenous" $Kras^{G12D}$ expression model[5]. When a constitutively active mutant $Kras^{G12D}$ protein is expressed in a pancreas epithelium-specific manner, the mice demonstrate a gradual mPanIN progression, which is very close to human PanIN. This history-making model elucidates that the *Kras* mutation, almost always found in human PDAC, is necessary and sufficient for an initiation of PDAC carcinogenesis. Subsequently, this endogenous $Kras^{G12D}$ expression model became a platform for the following GEM models of PDAC.

In this endogenous $Kras^{G12D}$ expression model, one *Kras* gene locus is substituted by a sequence of *LSL-*$Kras^{G12D}$ (Figure 3). The *LSL-*$Kras^{G12D}$ contains a loxP-stop-loxP (LSL) sequence inserted in the promoter region upstream of the $Kras^{G12D}$ protein coding sequence. Therefore, only in the pancreas epithelium, where Cre recombinase is expressed, the stop sequence is cut out and downstream $Kras^{G12D}$ protein expression is switched on. Here it is referred to as "endogenous", because the $Kras^{G12D}$ protein is expressed at a physiological level under the control of the native *Kras* promoter. The "expression at a physiological level" seems very important in this context. Previous *Kras* transgenic models might have had an excess level of transcript, which then failed to recapitulate PDAC formation in human disease.

In this pancreas epithelium-specific endogenous $Kras^{G12D}$ expression model, mPanIN emerges at a couple of weeks of age and progresses in stages over time. mPanIN shows close similarity with human PanIN including strong COX-2 (cyclooxygenase-2) and Hes1 expression. However, they do not progress into invasive PDAC within a year. This suggests that *Kras* activation might be sufficient to initiate PDAC carcinogenesis, but a second event might be required to accelerate the process into invasive PDAC formation.

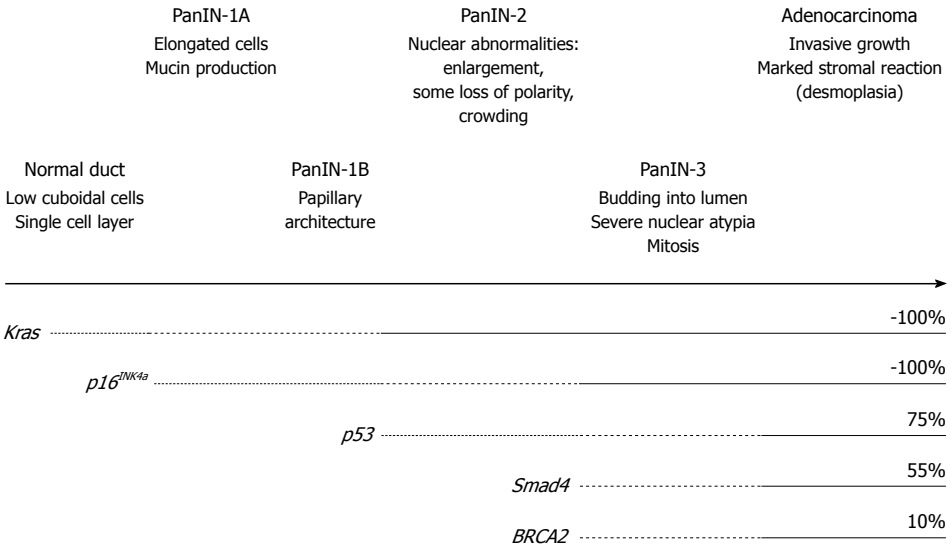

Figure 1 Multi-step carcinogenesis hypothesis of pancreatic cancer. As genetic alterations of *Kras*, *p16*^{INK4a}, *p53*, *Smad4* and *BRCA2* accumulate, the pre-cancer lesion pancreatic intraepithelial neoplasia (PanIN) occurs and progresses from low-grade (1A, 1B) to high-grade (2, 3) and to invasive cancer. The frequency of each genetic alteration at the invasive cancer stage is also shown.

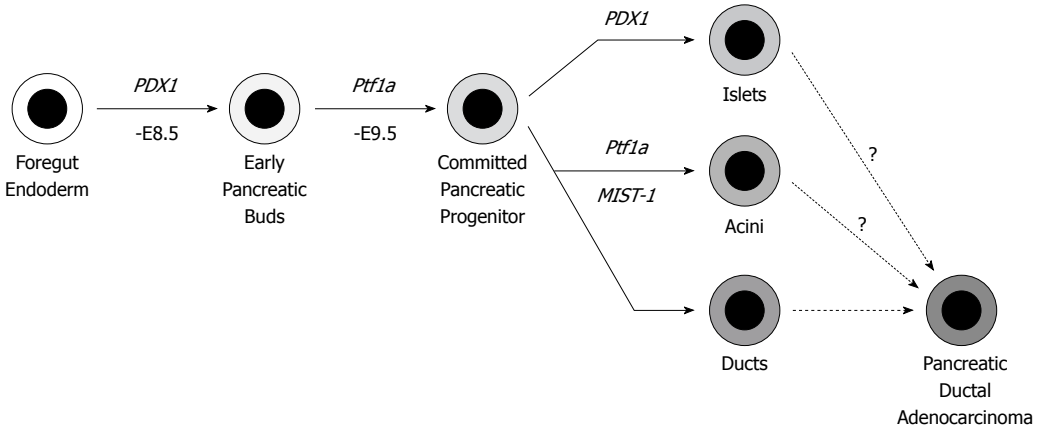

Figure 2 Cell differentiation program in the pancreas. *PDX1* and *Ptf1a* genes are expressed on E8.5-9.5 d, which determines the cell fate in the pancreas epithelium. By using the *PDX1* or *Ptf1a* gene promoter-induced Cre-loxP system, all three lineages (islet, acini and duct) have the designed genetic alterations. The cell of origin of pancreatic ductal adenocarcinoma (PDAC) is still under discussion. Experimentally, PDAC can be derived from all three lineages.

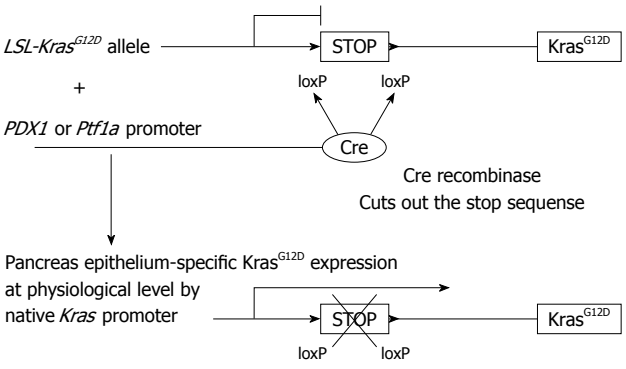

Figure 3 Pancreas epithelium-specific *Kras*^{G12D} expression. Cre recombinase is expressed under the promoter of *PDX1* or *Ptf1a* gene, which is pancreas epithelium-specific. Without cre recombinase expression, a stop sequence between the loxP sites prevents mutant *Kras*^{G12D} expression. In cells where only cre recombinase is expressed, the stop sequence is cut out, which turns on the pancreas epithelium-specific *Kras*^{G12D} expression at a physiological level under the native *Kras* promoter.

MODELS OF ENDOGENOUS *KRAS*^{G12D} EXPRESSION PLUS INACTIVATION OF TUMOR SUPPRESSOR

In combination of endogenous *Kras*^{G12D} expression and inactivation of TSGs, as shown Figure 1, invasive PDAC models have been established. They have been established with endogenous *Kras*^{G12D} expression plus *p16*^{INK4a}/*Arf* knockout[6], or mutant p53 expression[7], or *p53* knockout[8], or transforming growth factor-β receptor 2 (*Tgfbr2*) knockout[9]. In December 2004, an epoch-making meeting "Pancreatic cancer in mice and man: the Penn Workshop 2004" was held at the University of Pennsylvania. A number of well-known pathologists of human pancreatic cancer joined and reviewed existing GEM models of pancreatic cancer, and completed a consensus report on GEM models of PDAC[10]. In the report, models includ-

ing the endogenous *Kras*^{G12D} expression were recognized as the closest approximation of human PDAC carcinogenesis through PanIN, although acinar-ductal metaplasia was commonly observed and might have progressed into PDAC in the GEM models.

Among these endogenous *Kras*^{G12D} plus TSG inactivation models, *Kras*^{G12D} plus *p16*^{INK4a}/*Arf* knockout was the first published model of invasive PDAC[6]. In this model, PDAC grows very rapidly and aggressively invades other organs directly. The median survival time is nearly 8 wk and all animals die by 11 wk of age. It seems that they die too quickly to form distant metastasis. The second one was the *Kras*^{G12D} plus mutant *p53* expression model[7]. In this model, mutant p53 protein is expressed at a physiological level by the endogenous *p53* promoter. This model develops PDAC and frequent metastasis to the liver and/or lung, the most frequent sites of metastasis in human PDAC. The median survival is nearly 5 mo. Inactivation of p53 also causes chromosomal instability. These endogenous *Kras*^{G12D} plus TSG inactivation models, including *Kras*^{G12D} plus *p16* knockout and *Kras*^{G12D} plus *p53* knockout[8], basically demonstrate differentiated PDAC with expanded stromal components, which is very close to human PDAC compared to previous models. However, these models also frequently contained undifferentiated tumors, sarcomatoid tumor or anaplastic carcinoma, which are infrequent in human PDAC.

ENDOGENOUS *KRAS*^{G12D} EXPRESSION PLUS *TGFBR2* KNOCKOUT MODEL

We established the endogenous *Kras*^{G12D} expression plus *Tgfbr2* knockout model[9]. The mice demonstrate only differentiated ductal adenocarcinoma without any undifferentiated or sarcomatoid tumor histology, which suggests that this model might have the closest histology with human PDAC.

TGF-β, a well-known cytokine with multiple functions in various conditions, has a growth inhibitory effect on epithelial cells and is recognized as a tumor suppressor in the early stages of carcinogenesis[11]. The TGF-β ligand binds to two membranous receptors of serine/threonine kinase and the downstream signals are mainly transduced through the Smad2/3/4 pathway. In human PDAC, *Smad4* gene deletion or mutation is found in more than 50% of patients, which is characteristically frequent compared with other cancers[12]. *Tgfbr2* gene mutations are less than 5%[13], however, it is also reported that down regulation of *Tgfbr2* gene expression is observed in nearly 50% of PDAC[14].

We mimicked a blockade of TGF-β signaling by *Tgfbr2* knockout, a little upstream of Smad4. The endogenous *Kras*^{G12D} expression plus *Tgfbr2* knockout model shows mPanIN-like lesions at 3 wk of age and a rapid progression to PDAC in a few weeks. Almost all normal pancreas structure is lost by 6-7 wk of age, followed by death, with a median survival of 59 d (8 wk). In this clinical course, the mice demonstrate abdominal distension (92%), body weight loss (80%), ascites (60%) and jaundice (12%), which were frequently found in human PDAC patients. The histology is differentiated ductal adenocarcinoma with abundant stromal components and marked fibrosis (desmoplasia), which is very close to human PDAC (Figure 4). Furthermore, this model does not contain sarcomatoid tumor histology as described above, which is considered an advantage of this model. Most of the mice die too quickly to form distant metastasis, however, some mice infrequently lived over 20 wk and all of them showed metastasis to lung and liver as well as peritoneal dissemination, which suggests a highly invasive potential.

The endogenous *Kras*^{G12D} expression plus *Smad4* knockout model was also published by three groups. Surprisingly, all of them showed cystic tumors in the pancreas, which is considered as an approximation of intraductal papillary mucinous neoplasm (IPMN) or mucinous cystic neoplasm (MCN), different precancer lesions of PDAC[15-17]. Therefore, although the *Smad4* gene is frequently altered in human PDAC, *Tgfbr2* knockout in the context of the *Kras*^{G12D} expression can model human PDAC better than *Smad4* knockout in mice. The *Kras*^{G12D} plus *Smad4* knockout model is rather useful for understanding IPMN. IPMN is considered a pre-cancer lesion with a long latent period and much better prognosis than conventional PanIN to PDAC. Recently, however, cases of concomitant PDAC distant from benign IPMN lesions have gained attention. The IPMN models might help in dissecting the relation of PDAC and IPMN.

FUTURE DIRECTIONS OF TRANSLATIONAL RESEARCH USING GEM MODELS OF PDAC

Advantages of GEM models and evaluation of novel therapeutic methods

To date, xenografts, subcutaneous or orthotopic tumors injected with human PDAC cell lines into immunocompromised mice, have been mainly used as *in vivo* models of PDAC. Evaluation of new therapeutic drugs has also been performed by using the xenografts. In the future, GEM models are to be mainly used in various investigations instead of xenograft models.

The GEM models have the following two major advantages compared to the xenografts: intact tumor progression processes after the engineered genetic alterations and intact tumor microenvironment including tumor-stromal interactions. In xenografts, invasive tumor is suddenly implanted without any pre-cancer processes. In addition, the significance of the tumor microenvironment has been recently drawing attention. PDAC tissues characteristically contain a relatively small number of cancer cells and abundant stromal components, which is difficult to mimic by xenograft models. Cancer-associated fibroblasts, tumor-associated macrophages and neutrophils might play important tumor-promoting roles, which

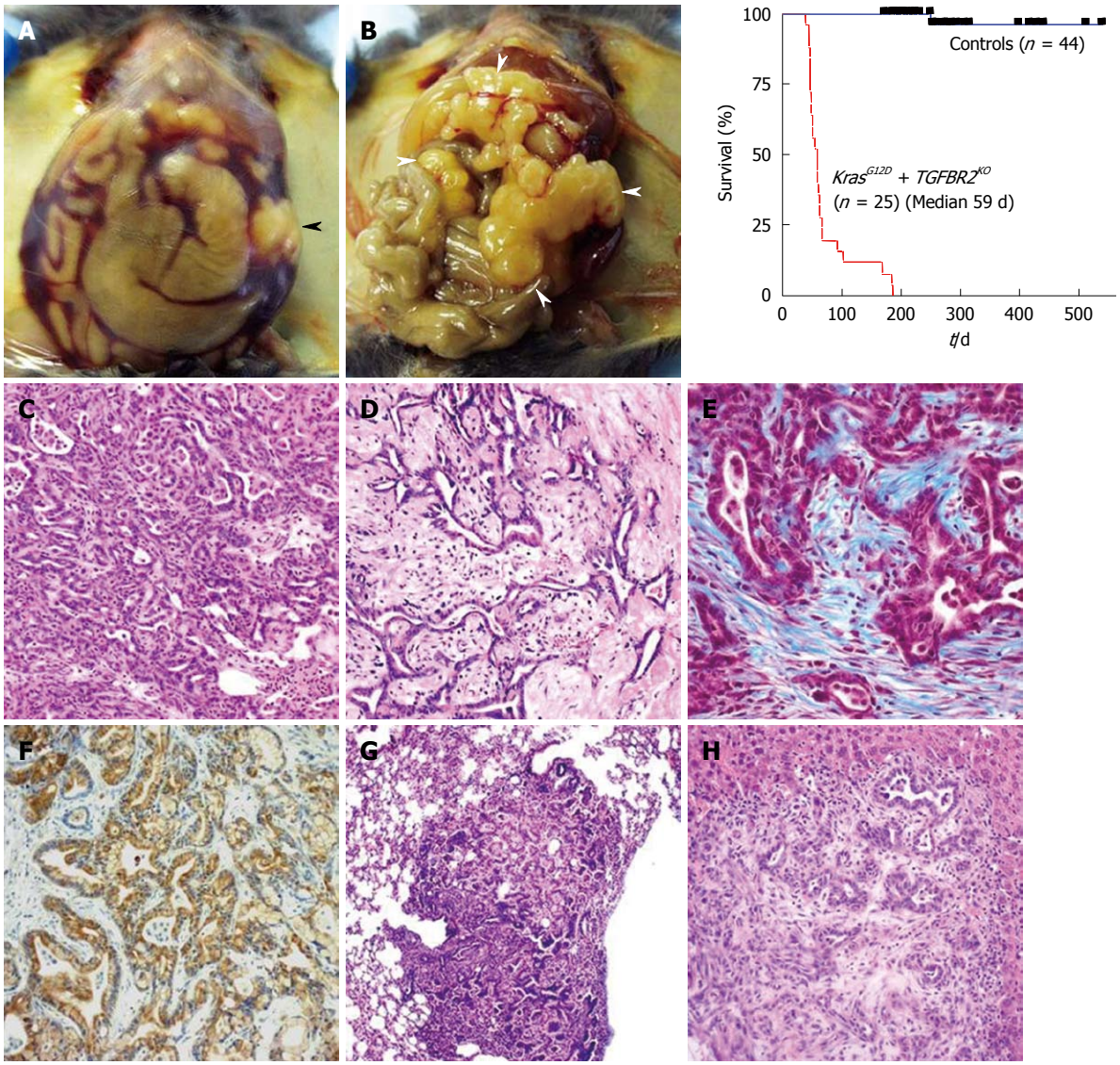

Figure 4 Endogenous *Kras*^{G12D} plus transforming growth factor-β receptor 2 knockout pancreatic ductal adenocarcinoma. A, B: Macroscopic appearances and a survival curve of the endogenous *Kras*^{G12D} plus transforming growth factor-β receptor 2 (*Tgfbr2*) knockout pancreatic ductal adenocarcinoma (PDAC) mice; A: Abdominal distension and bloody ascites are observed. Black arrowhead indicates the tumor; B: The whole pancreas is occupied by tumor and enlarged (white arrowheads). Jaundice is also observed here; C-H: Microscopic appearance of the endogenous *Kras*^{G12D} plus *Tgfbr2* knockout PDAC; C, D: Differentiated ductal adenocarcinoma with abundant stroma is observed in HE sections; E: Marked fibrosis and desmoplasia is observed. The blue color indicates fibrosis in trichrome blue staining; F: Positive immunostaining of cytokeratin 19 indicates a ductal phenotype tumor; G, H: The tumor has a metastatic potential to the lung (G) and liver (H).

might be also incomplete in immunocompromised mice.

Olive *et al*[18] recently directly compared tumors of xenografts and GEM models of PDAC (endogenous *Kras*^{G12D} expression plus mutant p53 expression) and reported that in xenograft models, blood vessels are very close to tumor cells and chemo reagents are effectively delivered into the tumors, whereas, in GEM tumors there is dense stroma between the tumor cells and blood vessels, which results in impaired delivery and anti-tumor effects. To date, a number of clinical trials for PDAC have been executed. Although every therapeutic regimen has had a significant anti-tumor effect in preclinical studies, almost all have failed to show superiority to gemcitabine, a current standard chemo reagent. This might also be explained by the difference in the tumor microenvironment between the xenografts and real human tumors, which might be a parallel between xenografts and GEM tumors as described above. Therefore, GEM models might be better for evaluating novel drugs in preclinical studies. Singh *et al*[19] also compared responses of chemotherapeutic regimens between a GEM model (endogenous *Kras*^{G12D} expression plus *p16*^{INK4a}/*Arf* knockout) and human PDAC patients and stated that the GEM model faithfully reproduces similar survival results of previous human clinical trials. Most xenograft studies have evaluated anti-tumor effects by tumor volume or size and number of metastases, but not by survival. In the preclinical studies using GEM models, novel therapeutics can be evaluated by overall survival rate as a primary endpoint (and also by progression-free survival as a secondary endpoint by using imaging modalities), which is also advantageous and closer to the human situation.

Discovery of early diagnostic methods for PDAC

In trying to establish an early diagnostic method, proteomic analysis of peripheral blood samples from the endogenous *Kras*^{G12D} or endogenous *Kras*^{G12D} plus *p16*^{INK4a}/*Arf* knockout models has been performed[5,20]. These studies revealed several molecules whose plasma levels change between PDAC- and mPanIN-bearing mice, or mPanIN and normal mice. The molecules are considered as potential candidates for novel tumor markers that dramatically renovate an early diagnostic strategy of PDAC. Imaging modalities are also important, especially for evaluating tumors in live animals. Progress in ultrasound, CT and MRI for small animals as well as contrast or sensitizing agents will also open the pathways for the development of novel diagnostic strategies in PDAC.

Elucidating underlying mechanisms of PDAC carcinogenesis

Since the constitutively active *Kras* mutation is observed in almost all PDAC patients, the downstream MAPK and PI3K signals are also activated in these patients. On the other hand, amplification of the epidermal growth factor receptor gene, upstream of Kras, is also frequently found in PDAC[4]. Hedgehog, Notch signal activation and COX-2 overexpression are also clinically observed. This activated signaling is reproduced in the GEM models described above, therefore, inhibition of this signaling may lead to potential therapeutic targets. Inhibition of Hedgehog or Notch signaling has already been reported with significant anti-tumor effects using some GEM models[18,21,22]. The impact of anti-tumor effects (the extent of survival elongation) might be associated with the fundamental mechanisms of PDAC carcinogenesis and progression. Understanding the entire image of intracellular signaling in the GEM PDAC cells and dissecting underlying mechanisms of PDAC formation and progression will allow us to select the most effective combination of targeted molecules or signaling to treat or prevent PDAC carcinogenesis and progression.

Understanding a tumor microenvironment and its contribution to PDAC progression

Stromal expansion and marked fibrosis is the primary feature of PDAC tissue, which suggests that tumor-stromal interactions might be associated with the extent of biological malignancy of PDAC. Thus, we screened for secreted factors from PDAC cells into the tumor microenvironment using the endogenous *Kras*^{G12D} expression plus *Tgfbr2* knockout model and found that several CXC chemokines are much more highly produced and secreted by the PDAC cells compared with the mPanIN cells. The CXC chemokines mainly affect the receptor CXCR2 in the stromal fibroblasts, rather than the PDAC cells autonomously, to induce connective tissue growth factor (CTGF) expression. CTGF strongly promotes fibrosis and tumor angiogenesis, resulting in tumor progression. Moreover, treating the mice with a CXCR2 inhibitor demonstrates anti-tumor effects and prolongs survival significantly (manuscript in submission). Inhibition of Hedgehog signaling described above also reduces the stromal volume significantly and modulates tumor vasculature[18]. Previously, chemotherapies have been developed to target only cancer cells, however, blocking tumor-stromal interactions and modulating the tumor microenvironment, including angiogenic components and/or inflammatory/immune cell regulation, can have a synergistic therapeutic effect in combination with conventional chemotherapies (Figure 5).

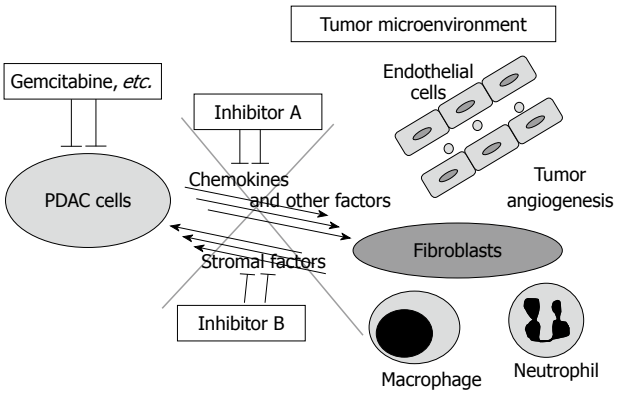

Figure 5 Tumor-stromal interaction as a therapeutic target of pancreatic ductal adenocarcinoma. In pancreatic ductal adenocarcinoma (PDAC) tissue, PDAC cells and stromal cells are interacting with each other by certain factors including chemokines, which might have tumor-promoting effects. The tumor microenvironment contains, for example, fibroblasts, macrophages, neutrophils and vascular endothelial cells. The combination of conventional chemo reagents (e.g. gemcitabine) and the inhibition of the tumor-stromal interaction might be a more effective therapeutic strategy for PDAC.

Approaching the cell of origin for PDAC

In the multi-step carcinogenesis hypothesis, as shown Figure 1, the cell of origin for PDAC has been considered as a normal pancreatic duct cell. This seems to be a clinical consensus, since mutations of *Kras*, *p16INK4a* and *p53*, for example, have not been detected in the acinar cells closely located to cancer cells in the clinical samples.

The GEM models described above generally use the *PDX1* or *Ptf1a* promoter, which results in genetic alterations occurring in all pancreatic epithelial cells. Therefore, these models cannot answer whether the duct cells are really the only cells of origin for PDAC or not (Figure 2). More recently, GEM models that have genetic alterations in more localized cell lineages and/or inducible alterations at the adult stages have been reported, which allowed us to approach the cell of origin for PDAC. "Inducible" GEM models contain the tamoxifen-inducible CreER system or tetracycline-inducible Tet-ON/OFF system.

Recent reports revealed that endogenous *Kras*^{G12D} expression in acinar cell lineages at the adult stages, using the acinar cell marker *Elastase I* or *Mist1* gene promoter, demonstrate mPanIN formation, which indicates that PDAC could be derived from acinar cells in mouse models[23,24]. Another report describes Kras activation in acinar cells or insulin-producing endocrine cells at the adult

stage, followed by pancreatitis using the chemical reagent caerulein, frequently demonstrated mPanIN, which indicates that inflammation could promote transdifferentiation from acinar or islet cells to duct-like cells and also promote carcinogenesis in mouse models[25,26]. These results suggest that PDAC could be derived from acinar and islet cells in mouse models. However, mature acinar and islet cells seemed refractory to mPanIN formation and required inflammation[25,26]. To date, pancreatic duct cell-specific GEM models have not been established. The duct-specific "inducible" GEM model is the closest approximation of the human PDAC carcinogenesis hypothesis and will give us a chance to understand PDAC completely. On the other hand, the *Nestin-cre*; *LSL-Kras*^{G12D} model also shows mPanIN formation, which indicates that nestin-positive cells could be the cells of origin for PDAC[27]. Nestin is an intermediate filament protein predominantly expressed in stem cells of the central nervous system and is also known to be expressed in progenitor cells of the exocrine pancreas epithelium. Taken together, PDAC might be derived from certain immature cell populations that can differentiate into the three mature lineages.

GEM model-specific differences compared to human PDAC

As described above, use of GEM models has made significant advances, yet there still is room for refinement and discrepancies with human conditions need to be elucidated.

There are GEM model-specific differences compared to human PDAC, which were also documented in the consensus report of GEM models of PDAC. The most prominent difference might be multi-focal tumorigenesis in GEM models. In humans, tumors usually emerge as a single neoplastic focus, whereas GEM models show multi-focal tumor progression, which results in lobular tumor formation occupying the entire pancreas. Therefore, tumor margins are difficult to delineate and tumor volume might be analyzed as the size of entire (tumor-occupied) pancreas.

In GEM models, acinar-ductal metaplasia and the ductular-insular complex (duct formation inside or in the periphery of the islet) are frequently observed, especially in the models using the *PDX1* or *Ptf1a* promoter[10]. In humans, these are occasionally observed and are frequently non-neoplastic; however, in GEM models, most of them should be considered as neoplastic lesions on the way to cancer progression. In the GEM models using the *PDX1* or *Ptf1a* promoter-cre, any epithelial cells can have Kras activation, every acinar cell demonstrates acinar-ductal metaplasia and every islet shows the ductular-insular complex, all of which might progress into PanIN-like ductal neoplasia and eventually into invasive PDAC. Since acinar cells occupy nearly 80% of the normal pancreas, acinar-ductal metaplasia is observed abundantly in GEM models (especially in the endogenous *Kras*^{G12D} plus TSG inactivation models), which might also be one of the greated differences in GEM models compared to human PDAC. The consensus report noted that the acinar-ductal metaplasia should be distinguished from duct-derived mPanIN lesions, however, in a few weeks, acinar-ductal metaplasia rapidly progresses into PanIN-like lesions, which are already difficult to distinguish from duct-derived mPanIN lesions. The final appearance of invasive PDAC recapitulates human disease, suggesting that acinar-ductal metaplasia, which definitely progresses into PDAC in the GEM models, might also contribute to PDAC formation in humans.

CONCLUSION

Recent progress in the use of GEM models can be called a breakthrough, although there are still limitations and differences compared to human PDAC. Analyzing the GEM models, with knowledge of the advances and limitations, will allow us to understand the entire image of PDAC and to develop effective therapies, diagnosis and prevention based on the underlying mechanisms of PDAC carcinogenesis and progression. Using GEM models and combining bench and bedside closely together might provide a breakthrough in the PDAC field and ultimately overcome the most lethal cancer.

ACKNOWLEDGMENTS

I thank Anna Chytil, Mary E Aakre and Harold L Moses for lots of comments and suggestions.

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S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Zheng XM

World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i5.203

World J Clin Oncol 2011 May 10; 2(5): 203-216
ISSN 2218-4333 (online)

TOPIC HIGHLIGHT

Michiko Yamagata, MD, PhD, *Series Editor*

Endoscopic diagnosis of extrahepatic bile duct carcinoma: Advances and current limitations

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Received: July 28, 2010 Revised: September 16, 2010
Accepted: September 23, 2010
Published online: May 10, 2011

Abstract

The accurate diagnosis of extrahepatic bile duct carcinoma is difficult, even now. When ultrasonography (US) shows dilatation of the bile duct, magnetic resonance cholangiopancreatography followed by endoscopic US (EUS) is the next step. When US or EUS shows localized bile duct wall thickening, endoscopic retrograde cholangiopancreatography should be conducted with intraductal US (IDUS) and forceps biopsy. Fluorescence *in situ* hybridization increases the sensitivity of brush cytology with similar specificity. In patients with papillary type bile duct carcinoma, three biopsies are sufficient. In patients with nodular or infiltrating-type bile duct carcinoma, multiple biopsies are warranted, and IDUS can compensate for the limitations of biopsies. In preoperative staging, the combination of dynamic multidetector low computed tomography (MDCT) and IDUS is useful for evaluating vascular invasion and cancer depth infiltration. However, assessment of lymph nodes metastases is difficult. In resectable cases, assessment of longitudinal cancer spread is important. The combination of IDUS and MDCT is useful for revealing submucosal cancer extension, which is common in hilar cholangiocarcinoma. To estimate the mucosal extension, which is common in extrahepatic bile duct carcinoma, the combination of IDUS and cholangioscopy is required. The utility of current peroral cholangioscopy is limited by the maneuverability of the "baby scope". A new baby scope (10 Fr), called "SpyGlass" has potential, if the image quality can be improved. Since extrahepatic bile duct carcinoma is common in the Far East, many researchers in Japan and Korea contributed these studies, especially, in the evaluation of longitudinal cancer extension.

Key words: Bile duct cancer; Bile duct carcinoma; Cholangiocarcinoma; Endoscopic retrograde cholangiopancreatography; Intraductal ultrasonography

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Tamada K, Ushio J, Sugano K. Endoscopic diagnosis of extrahepatic bile duct carcinoma: Advances and current limitations. *World J Clin Oncol* 2011; 2(5): 203-216 Available from: URL: http://www.wjgnet.com/2218-4333/full/v2/i5/203.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i5.203

INTRODUCTION

Most patients with bile duct cancer are diagnosed in an advanced stage[1-3]. From 1998 to 2004, the 5-year survival rate after surgical resection was 33.1% for bile duct cancer in Japan[3]. To improve the therapeutic effect of bile duct carcinoma, efforts have been focused on diverse areas: early detection of the lesions, accurate differentiation of benign and malignant biliary stenosis, assessment of locoregional tumor extension, development of surgical methods, biliary stenting, and chemoradiotherapy for unresectable bile duct cancer.

In the diagnosis of intrahepatic cholangiocarcinoma, non-invasive, cross-sectional imaging tests including computed tomography (CT) and magnetic resonance imaging (MRI) are useful. MRI in the form of magnetic resonance cholangiopancreatography (MRCP) and multi-detector low CT (MDCT) are the most commonly performed imaging tests in these patients. In contrast, for the diagnosis of extrahepatic bile duct cancer, an endoscopic approach is essential. Endoscopic techniques are more invasive and include the use of endoscopic retrograde cholangiopancreatography (ERCP) and endoscopic ultrasonography (EUS). In this review, we describe the advances and current limitations of our professional area: endoscopic procedures in the diagnosis of extrahepatic bile duct carcinoma.

EARLY DETECTION OF EXTRAHEPATIC BILE DUCT LESIONS

Tompkins *et al*[1] reported that 91% of patients with bile duct cancer who underwent surgery had serum bilirubin levels greater than 2.0 mg/dL. Tio *et al*[2] reported that almost all patients were diagnosed after developing obstructive jaundice, and only two of 76 patients showed stage T1 disease. In contrast, Sugiyama *et al*[4] reported that 18 of 103 patients showed no jaundice, and eight of 103 patients had stage T1 disease. When a patient complained of abdominal discomfort or showed an elevation of serum biliary enzymes, they routinely performed ultrasonography (US) to screen for pancreatobiliary ductal diseases, which resulted in the early detection of lesions with good prognosis.

US

In the middle and distal bile duct, US cannot assess tumors sufficiently due to disturbance by gastrointestinal gas[5]. Even now, the sensitivities of US in demonstrating hilar tumor, middle bile duct tumor, and distal bile duct tumor are 85.6%, 59.1%, and 33.3%, respectively[5]. Therefore, bile duct dilatation on US findings is an important sign for the early diagnosis of bile duct cancer[5]. Our group has reported an asymptomatic unicteric patient with bile duct carcinoma, in whom US at the time of health examination showed dilatation of the bile duct[6]. In most countries, routine US examination for these patients might be difficult due to cost-effectiveness. In Japan, US equipment has become popular even in small clinics in the past two decades, and screening of the biliary tract using US is increasingly performed.

MRCP

Until recently, when US showed dilatation of the bile duct, ERCP was the next step to obtain cholangiography. Recently, MRCP has become an alternative to ERCP as it is a non-invasive modality[7,8]. When US shows intraductal tumor, initial ERCP rather than MRCP should be carried out for cost-effectiveness, even if the patient shows no jaundice. However, when US shows only dilatation of the extrahepatic bile duct, MRCP is a safe modality to obtain a clear cholangiogram.

A recent excellent prospective study by Sai *et al*[7] demonstrated that MRCP showed a sensitivity of 90% in evaluating extrahepatic bile duct carcinoma in the non-icteric stage. They investigated non-icteric patients who had abnormal concentrations of serum biliary enzymes and whose common hepatic duct was more than 8 mm in diameter on abdominal US due to unknown reasons. In this study, 10 patients were diagnosed with extrahepatic carcinoma including 5 patients in T1 stage during a 7-year period.

EUS

By using intraduodenal scanning, in the extrahepatic bile duct, EUS can provide high resolution power without echo attenuation and without the influence of gastrointestinal gas. When evaluating extrahepatic bile duct carcinoma in the non-icteric stage, the sensitivity and specificity of MRCP followed by EUS were 90% and 98%, respectively[7]. In another study, in 32 patients with normal serum liver enzymes and whose common bile duct was dilated on US findings, EUS did not show biliary malignancy[9]. Therefore, the patients who had abnormal concentrations of serum biliary enzymes and whose common hepatic duct was dilated on abdominal US will be good targets for EUS. EUS was also useful in diagnosing distal biliary strictures without a mass on CT[10].

Fernández-Esparrach *et al*[8] also performed a prospective study of MRCP and EUS in the evaluation of 63 patients with unexplained common bile duct dilation on standard US, although most of these patients had jaundice. The sensitivity and specificity of MRCP in diagnosing malignancy in these patients were 95% and 98%, respectively. The sensitivity and specificity of EUS were 100% and 100%, respectively[8].

ACCURATE DIFFERENTIATION OF BENIGN AND MALIGNANT BILIARY STRICTURES

ERCP

An ERCP image of a patient with extrahepatic bile duct carcinoma in the non-icteric stage is shown in Figure 1.

On ERCP or MRCP findings, benign diseases including post-operative stenosis, chronic pancreatitis, primary sclerosing cholangitis, or autoimmune pancreatitis show bile duct stenosis as well as malignant disease[11-13]. Cholangiography shows filling defects at the common bile duct in patients with adenomyoma[11] or inflammatory strictures[12]. Therefore, accurate distinction between benign and malignant biliary structures is essential to avoid unnecessary surgery.

The accuracy of MRCP is comparable with that of ERCP[13]. Malignancy is suggested when cholangiography shows long (greater than 10 mm), asymmetric, and irreg-

Table 1 Sensitivity rates for detection of malignancy by endoscopic brush cytology of a biliary stricture

Authors and year	Country	Panc cancer	Bile duct cancer	Specificity
Venu *et al*[21], 1990	USA	60% (3/5)	80% (20/25)	100% (88/88)
Rupp *et al*[22], 1990	USA	91% (21/23)	100% (6/6)	88% (7/8)
Foutch *et al*[23], 1991	USA	0% (0/6)	100% (5/5)	100% (3/3)
Ryan *et al*[24], 1991	USA	30% (6/20)	44% (4/9)	100% (17/17)
Howell *et al*[25], 1992	USA	0% (0/18)	50% (2/4)	100% (5/5)
Kurzawinski *et al*[26], 1993	Great Britain	65% (15/23)	60% (6/10)	100% (7/7)
Ferrari Júnior *et al*[27], 1994	USA	66% (16/29)	20% (2/10)	100% (22/22)
Ponchon *et al*[28], 1995	France	15% (3/20)	44% (12/25)	97% (64/66)
Sugiyama *et al*[29], 1996	Japan	36% (5/14)	59% (10/17)	100% (12/12)
Mansfield *et al*[30], 1997	Great Britain	38% (10/28)	63% (10/16)	100% (2/2)
Vandervoort *et al*[31], 1999	USA	11% (5/46)	30% (3/10)	100% (37/37)
Glasbrenner *et al*[32], 1999	Germany	35% (11/31)	80% (16/20)	90% (19/21)
Jailwala *et al*[33], 2000	USA	24% (11/46)	23% (7/30)	100% (29/29)
Farrell *et al*[34], 2001	USA	78% (14/18)	60% (6/10)	83% (10/12)
Fogel *et al*[35], 2006	USA	36% (32/88)	26% (10/38)	100% (8/8)
Kitajima *et al*[36], 2007	Japan	60% (9/15)	71% (15/21)	100% (7/7)

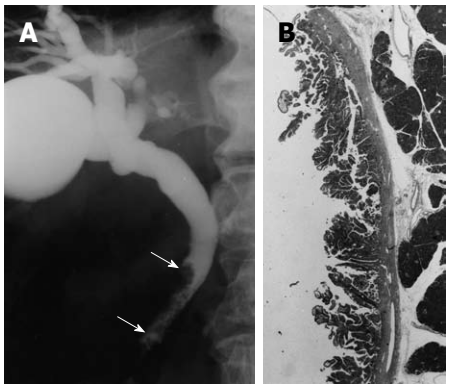

Figure 1 Cholangiographic finding of extrahepatic bile duct carcinoma in the non-icteric stage. A: Cholangiography shows a papillary tumor at the distal common bile duct (arrows); B: The histologic findings of the resected specimen showed papillary adenocarcinoma confined to the mucosal layer (hematoxylin and eosin, × 1).

ular strictures. Benign disease is suggested when cholangiography shows short, regular, and symmetric strictures. Using these criteria, the diagnostic sensitivity and specificity for ERCP were 74% and 70%, respectively. The diagnostic sensitivity and specificity for MRCP were 70% and 72%, respectively[13].

Although ERCP is conducted for the purpose of biliary drainage to release obstructive jaundice, the utility of preoperative biliary drainage is controversial. Some reports indicate that preoperative biliary drainage increased infectious complications after hepatectomy for proximal bile duct tumor[14]. In preoperative biliary drainage for cholangiocarcinoma, endoscopic nasobiliary drainage (NBD) is preferable to endoscopic biliary stenting, because secondary cholangitis due to the retrograde flow of duodenal fluid into the biliary tree does not occur[15-17]. NBD is also useful to obtain a clear cholangiogram to evaluate longitudinal cancer extension along the bile duct, which is common in cholangiocarcinoma[15-17]. In contrast, a clear cholangiogram is unnecessary in patients with pancreatic cancer, since longitudinal cancer extension is rare in these patients. One randomized controlled trial of preoperative biliary drainage for cancer of the head of the pancreas has been reported[18]. This report concluded that routine preoperative biliary drainage in patients undergoing surgery for cancer of the pancreatic head, with obstructive jaundice and a bilirubin level less than 14.6 mg/dL, increases the rate of complications. Therefore, we agree that routine ERCP is not required in patients with pancreatic cancer.

Bile cytology during ERCP

In some prospective studies, bile exfoliative cytology aspirated after insertion of an external biliary catheter showed disappointing results (sensitivity 6%-24%)[19,20]. Brush cytology has a specificity of nearly 100%[21-38]. When its specificity is 100%, its sensitivity for cholangiocarcinoma ranges from 23% to 80%[21-36] as shown in Table 1. Its sensitivity for pancreatic cancer is low, ranging from 0% to 66%[21-36], even now. The low sensitivity is related to low cellularity of these tumors and the demoplastic reaction. A new long brush did not improve results[35]. Repeated brushing improved the sensitivity from 35% to 44% ($P = 0.01$), although dilation of the stenosis did not improve its sensitivity[38] (Table 1).

Percutaneous transhepatic cholangioscopy

Percutaneous transhepatic cholangioscopy (PTCS) is predominantly performed in Asian countries such as Japan, Korea, and Taiwan, where there is a high prevalence of intrahepatic stones and cholangiocarcinoma[39-53]. Even in patients with non-dilated intrahepatic bile duct, percutaneous transhepatic biliary drainage (PTBD) can be performed with the assistance of cholangiography *via* NBD[52,53]. Once the PTBD tract is established, insertion of the cholangioscope from the percutaneous tract is relatively easy. On cholangioscopic findings, irregularly dilated and tortuous vessels, so called "tumor vessels", are good targets for biopsy[39-50]. Although PTCS is an excellent procedure to obtain the target biopsy with a

Table 2 Sensitivity rates for detection of malignancy by endoscopic forceps biopsy of a biliary stricture

Authors and year	Country	Panc cancer	Bile duct cancer	Specificity
Kubota *et al*[54], 1993	Japan	50% (2/4)	89% (16/18)	100% (5/5)
Ponchon *et al*[28], 1995	France	46% (6/13)	44% (7/16)	97% (35/36)
Sugiyama *et al*[29], 1996	Japan	71% (10/14)	88% (15/17)	100% (12/12)
Jailwala *et al*[33], 2000	USA	33% (15/46)	30% (9/30)	100% (10/10)
Tamada *et al*[59], 2002	Japan	50% (6/12)	84% (21/25)	100% (18/18)
Kitajima *et al*[36], 2007	Japan	60% (9/15)	57% (12/21)	100% (7/7)

sensitivity of 93%-96%[39-41], it requires an invasive technique compared to the transpapillary approach. The sensitivities of the target biopsy for bile duct carcinoma, pancreatic carcinoma, and gallbladder carcinoma are 95.7% (135/141), 67.2% (45/67), and 76.2% (48/63), respectively[39,41].

The numbers and locations of the biopsies required to make a diagnosis of carcinoma depend on the origin and cholangioscopic appearance of the tumor[45]. A diagnosis of carcinoma was made in all patients (n = 4) with a tumor of the papilla of Vater and in all patients (n = 15) with a polypoid bile duct tumor, with two biopsies from the tip of the polypoid mass. In patients with bile duct cancer of the stenotic type (n = 19), cancer was diagnosed in 95% of cases when three biopsies were taken from the margin of the stenotic area rather than within the area of the stenosis. When cholangioscopy showed a tortuous, dilated vessel (n = 10), the diagnosis of cancer was made with two biopsies taken from the margin of the stenosis rather than inside the stenosis. In the patients with metastatic bile duct cancer (n = 14), the diagnosis was made in only 43% of cases when three biopsies were taken from the margin of the area of stenosis. When combined with results from the three biopsies taken from within the area of stenosis, the sensitivity for diagnosing pancreatic cancer improved from 20% to 60%[45].

Transpapillary biopsy

The sensitivity of transpapillary bile duct biopsy is reported to be 52%-81%[28,29,34,54-62] as shown in Table 2. In these series, various biliary diseases, including pancreatic cancer and gallbladder cancer are included. When the tumor is located outside the bile duct (pancreatic or gallbladder cancer), the sensitivity of the biopsy is low (50%-71%). However, in patients with bile duct carcinoma, the sensitivity of transpapillary bile duct biopsy is 84%-89%[29,54,56,57,59]. Sugiyama *et al*[29] designed new biopsy forceps which could be introduced into the bile duct without sphincterotomy. Once the guidewire is introduced into the bile duct, the forceps can be inserted into the bile duct along the placed guidewire[56-59]. In the patient with cholangiocarcinoma, the diagnostic results of this clamshell type forceps with a soft outer Teflon sheath (Olympus Optimal. Co. Ltd.)[29,54,59] is superior to that of triple tissue sampling using the Howell system[33,55]. Ropeway-type biopsy forceps are also commercially available now[57]. Selective biopsy of both hepatic ducts is also possible[58]. Dumonceau *et al*[62] used a giant basket to grasp the tissue, and reported a sensitivity of 80%.

The required number and the location of biopsy should be selected according to the type of tumor. In patients with papillary (polypoid) type bile duct carcinoma, three biopsies from the tip of the polypoid lesion were sufficient for the diagnosis with a sensitivity of 100%[59,63]. In patients with nodular or infiltrating type cholangiocarcinoma, multiple biopsies from the margin of the stenosis and within the stenosis improve sensitivity[59,63]. Since endoscopic skill is an art, endoscopists must sufficiently manipulate the tip of the forceps by free hand according to the gross finding of the tumor to improve the sensitivity of the method. The passion to improve the results is important as well as objective comparison with published data. In patients with pancreatic cancer, this modality has limitations, and other methods should be selected if ERCP tissue sampling shows negative results[29,54,59].

Percutaneous transhepatic intraluminal biopsy

Via the PTBD route, multiple intraluminal biliary ductal biopsies using a sheath with a side port show good results[63]. In patients with polypoid-type cholangiocarcinoma, the sensitivities of a single biopsy and 2 biopsies were 67% (4/6) and 100% (6/6), respectively. In patients with nodular-type cholangiocarcinoma, the sensitivities of a single biopsy, 3 biopsies, 6 biopsies, and 9 biopsies were 40% (4/6), 80% (16/20), 90% (18/20), and 95% (19/20), respectively. These results suggest that repeated biopsies may improve the sensitivity of transpapillary biopsy in patients with nodular-type cholangiocarcinoma.

Advanced techniques in cytology

Advanced cytologic techniques, including digitized image analysis (DIA) and fluorescence *in situ* hybridization (FISH), have been used to increase the sensitivity of bile cytology[64-67]. The DIA technique quantitates nuclear DNA *via* special stains to assess the presence of aneuploidy, whereas FISH analysis detects chromosomal polysomy by using fluorescent probes.

In a prospective study, when routine cytology was negative, FISH had an increased sensitivity (35%-60%) compared to routine cytology, however, the sensitivity and specificity of DIA was intermediate as compared to routine cytology[64]. In another prospective study, DIA increased the sensitivity from 15% to 43%, but decreased the specificity from 100% to 92%[65]. FISH increased the

sensitivity from 15% to 44%, with similar specificities (98% for FISH and 100% for routine cytology)[65]. In patients with negative brush cytology and forceps biopsy, DIA, FISH, and composite DIA/FISH were able to predict malignant diagnoses in 14%, 62%, and 67%, respectively[66]. In another comparative study, FISH increased the sensitivity from 20.1% to 42.9% compared to routine cytology, with similar specificities (99.8% for FISH and 100% for routine cytology). DIA was not a significant independent predictor of malignancy[67]. These results show that FISH is a useful technique to improve the diagnostic ability of cytology.

"Mother-baby" system - peroral cholangioscopy

A small caliber (3.2-4.1 mm) "baby" cholangioscope is inserted into the common bile duct through the channel of a large caliber "mother" duodenoscope[68-75]. To date, there have been only a few large studies on diagnostic peroral cholangioscopy (POCS) in biliary-tract diseases, despite many reports on therapeutic POCS and diagnostic PTCS. The utility of POCS is further limited by the fragility of the cholangioscopes and insufficient optical resolution. Biopsies can be performed through the cholangioscope, but adequate sampling remains challenging due to the small size of the working channel (1.2 mm) and the limited maneuverability of the long baby scope.

Fukuda *et al*[68] reported that the diagnostic criteria of PTCS[39-41] was useful in POCS, and that the addition of POCS improved diagnostic ability compared with endoscopic retrograde cholangiography/tissue sampling (accuracy 78%-93.4%, sensitivity 57.9%-100%). In recent years, the development of video cholangioscopy has largely overcome the issue of poor image quality[71-75]. This improvement due to video cholangioscopy can provide better quality images, resulting in the ability to observe each lesion clearly and to perform the correct target biopsy. Furthermore, video cholangioscopy makes it possible to use the narrow-banding imaging (NBI) system[71,73]. Itoi *et al*[71] reported that POCS combined with NBI could clarify the fine surface structure of lesions and mucosal vessels compared with conventional white-light observation in all cases. These results suggest that POCS combined with NBI may lead to higher detectability of biliary-tract lesions, even minute lesions. One problem of NBI cholangioscopy is that bile is recognized as an incoming reddish fluid similar to blood[71]. This is a significant issue that requires improvement, because it leads to poor images, and it is time consuming to clean the bile duct.

Carbon dioxide insufflation is a tactic for obtaining clear images of the bile duct during POCS[75].

Direct cholangioscopy

In one of the first reports of POCS in 1977, a straight-view fiberscope of 8.8 mm diameter could be directly inserted through the mouth, into the biliary system after an endoscopic sphincterotomy, without the need for a mother scope[76]. Our research group also reported the utility of direct POCS in 1982, using a previously placed balloon catheter in the intrahepatic bile duct as the anchor system[77]. Since this system has the limitation of poor insertion rates of cholangioscopy, the modality was replaced by the "mother baby" system. However, recently a thin-caliber gastroscope made it possible to perform this method again[78-80]. Only one endoscopist is necessary, and the larger working channel (2.0 mm) of the endoscope allows for large biopsies[78-80]. This modality may be useful for the evaluation of intraductal papillary mucinous neoplasms of the bile duct[81].

SpyGlass

Recently, the SpyGlass peroral cholangio-pancreatoscopy system (Boston Scientific Co., Natick, MA, USA) has been introduced[82-84]. This system uses a reusable optical probe, a disposable access and delivery catheter (SpyScope), and disposable biopsy forceps. The outer diameter of the SpyScope is 10 French. This system offers several advantages over previous cholangioscopes. It allows for single-operator control of both the duodenoscope and the SpyScope because the SpyScope catheter is mounted on the duodenoscope by a silastic belt. The endoscopist can sequentially manipulate both the duodenoscope and the SpyScope with one hand; thus, two endoscopists are not needed. This system also uses 4-way tip deflection, which allows for improved access of tertiary ducts. Furthermore, the irrigation channel (0.6 mm) is separate from the working channel (1.2 mm), which allows for sustained continuous irrigation even if the working channel is in use. Therefore, the SpyGlass system can be used for cholangioscopic-guided target biopsy[84]. The sensitivity and specificity of SpyGlass-directed biopsy was 71% and 100%, respectively, in an evaluation of intraductal lesions in 20 patients[84].

Intraductal US

When the findings of ERCP or MRCP are equivocal, we have been performing transpapillary intra-bile ductal US to detect small tumors or localized wall thickening[6,59]. Ultrasound imaging of the intra-bile duct using a thin (2.0 to 2.4 mm in diameter), high-frequency (15 to 20 MHz) ultrasonic probe, called "intraductal ultrasonography", is capable of producing high quality cross-sectional images of the bile duct, and is used for the differential diagnosis of biliary strictures[6,44,59,60,66,85-89].

Many investigators have reported that intraductal US (IDUS) could compensate for the false negative results of ERCP tissue diagnosis[59,60,66,85-89]. Multiple regression analysis showed that the presence of a sessile tumor (intraductal or outside the bile duct: $P < 0.05$), tumor size greater than 10.0 mm ($P < 0.001$), and interrupted wall structure ($P < 0.05$) were independent variables that predicted malignancy[59]. We must bear in mind, however, that asymmetric localized bile duct wall thickening with normal bile duct structure on IDUS images occurs in primary sclerosing cholangitis and other inflammatory changes as well as in bile duct carcinoma[44,59].

Optimal coherence tomography

Optimal coherence tomography (OCT) is a new technique that produces cross-sectional images using infrared light. OCT has an axial resolution which is 10-fold better than that of high-frequency ultrasound. However, its depth penetration is limited to approximately 1 mm *vs* 10 mm for a 20 MHz ultrasound probe.

Preliminary studies have demonstrated the ability of OCT to generate high resolution images of the biliary tree that correlate with histological findings[90,91]. OCT has the potential to identify small bile duct lesions, however, it is not widely available except in a few centers. Therefore the role of OCT in the diagnostic workup of bile duct carcinoma is not yet established.

EUS-fine-needle aspiration

In patients with extrahepatic bile duct carcinoma, percutaneous US-guided fine-needle aspiration (FNA) is difficult, since these tumors are too small. On the other hand, EUS has high-resolution power imaging and can puncture lesions of 3 mm or greater[92-98].

In one study, sensitivity was better for ERCP-based techniques (brush cytology and forceps biopsy) in biliary tumors (ERCP 75% *vs* EUS 25%), whereas EUS-guided biopsy was superior for pancreatic masses (EUS 60% *vs* ERCP 38%)[93]. Therefore, pancreatic cancer is a good target for EUS-FNA. In recent studies of EUS-FNA in patients with hilar strictures with negative brush cytology, the diagnostic sensitivity, specificity, and accuracy were 77%-89%, 100%, and 79%-91%, respectively[94,96]. A potential risk of this modality is intraperitoneal seeding. In patients with unresectable bile duct cancer, EUS-FNA may be conducted after negative ERCP results[97,98].

ASSESSMENT OF CANCER DEPTH INFILTRATION

Accurate diagnosis of the extent of the cancer is essential to enable selection of the appropriate medical and surgical therapy. On dynamic CT findings, extrahepatic cholangiocarcinoma may be seen as a focal thickening of the ductal wall with various enhancement patterns. Until now, in many cases of extrahepatic cholangiocarcinoma, visualization of the tumor was not definitive because they were too small to be detected, as previously reported[3-10,59,60,66,86-89]. More recent studies, however, have shown the utility of modern contrast-enhanced MDCT in the preoperative staging of hilar cholangiocarcinoma[99-105]. The diagnostic ability of 16-channel MDCT is excellent[99,104]. The diagnostic ability of dynamic MRI combined with MRCP using a 1.5 T MR system is comparable with MDCT[105-107]. The reports which clarified the utility of MDCT for the staging of extrahepatic cholangioma are limited compared to hilar cholangiocarcinoma[101,108].

IDUS is utilized to compensate for dynamic MDCT to demonstrate the tumor extension in the hepatoduodenal ligament. IDUS has become a promising modality in assessing the depth of cancer infiltration in the bile duct[109-118]. In recent years, three-dimensional IDUS has become an excellent modality for assessing tumor staging[114,116]. However, IDUS could not assess tumor invasion outside the hepatoduodenal ligament, for example, in the superior mesenteric vein, proper hepatic artery or left hepatic artery due to echo attenuation. In addition, IDUS can not assess distant metastases. Therefore, the combination of dynamic CT and IDUS is essential for accurate preoperative staging.

Vascular invasion

MDCT correctly revealed hepatic artery invasion with 75%-100% sensitivity and 90%-100% specificity and portal vein invasion with 92.3%-100% sensitivity and 80%-100% specificity[99-105,108].

On IDUS, when the high-echoic layer between the tumor and a vessel disappeared, it was diagnosed as positive for vascular invasion. Using this criterion, the accuracies of IDUS in assessing tumor invasion to the right hepatic artery and portal vein were 86%-100% and 92%-100%, respectively[87,109-118]. However, visualization of the left hepatic artery and proper hepatic artery was poor (14%/18%) due to anatomical reasons which caused ultrasound attenuation[112,113]. Some investigators reported that 3D-IDUS may improve diagnostic accuracy for the detection of tumor invasion into the portal vein and the hepatic artery[114,116].

Lymph node metastases

Dynamic CT detected lymph node swelling, however, it was not effective in differentiating whether the swelling indicated an inflammatory or a malignant change[108,119-121]. The accuracy of MDCT in evaluating lymph node metastases was 57%-69%[108,119-121]. The accuracy of CT for the characterization of paraaortic nodes was not different from that of MRI[119]. A short axis-diameter over 5.3 mm, irregular margin, and the presence of central necrosis were suggestive morphologic features of malignant nodes[119]. Another report also showed that a round node with a short-axis diameter exceeding 18 mm showed high positive predictive values of malignancy (67%). However, CT was not useful since nodes of this size and character were rare[120].

On IDUS, although high-resolution US might improve detection of small epicholedochal lymph nodes, due to the limited depth of ultrasonic penetration, IDUS was inferior to conventional EUS with respect to detection of lymph node metastases[110,117,118]. A hypo-echoic, clear margin, and round shaped lymph node was judged as malignant swelling. An irregular or angle shaped lymph node was judged as inflammatory swelling. Using these criteria, the accuracy of IDUS in assessing lymph node metastases was 75%-78%[110,117,118,122]. As these results show, the assessment of lymph node metastases using CT and IDUS is difficult.

Distinction of T1 and T2 biliary tumors

The inside hypo-echoic layer on IDUS images corresponded not only to the fibromuscular layer but also to a part of the peri-muscular connective tissue. Therefore, even if the tumor was limited to the inside low-echoic layer, it suggests a T2 tumor (the tumor invaded the peri-muscular connective tissue) as well as a T1 tumor (the tumor which is confined to the fibromuscular layer). Therefore, accurate distinction of T1 and T2 tumors by IDUS as well as CT is difficult[110,123].

Invasion of the serosa

When the outside hyper-echoic layer was interrupted, IDUS assessed it as positive serosal invasion. Using these criteria, the accuracy of IDUS in assessing tumor invasion to the serosa was 86%-93%[109,110].

Invasion of the pancreatic parenchyma

Sonographic detection of a bile duct tumor protruding into the pancreatic parenchyma or disruption of the outer bile duct layer were diagnosed as positive for invasion of the pancreatic parenchyma. Using these criteria, the accuracy of IDUS in assessing tumor invasion to the pancreas was 93%-100%[109,110,124].

ASSESSMENT OF LONGITUDINAL CANCER EXTENSION

Cholangiography

Extrahepatic bile duct carcinoma shows longitudinal spread along the bile duct, often resulting in residual tumor at the surgical margin. Conventional cholangiography can inadequately assess this as previously reported[115-144].

Longitudinal extension of cholangiocarcinoma consists of mucosal (superficial) or submucosal (invasive) infiltration depending on the tumor growth pattern. Mucosal extension is predominantly seen with papillary (intraductal) and nodular (mass-forming) tumors, while submucosal extension is mainly seen with infiltrating (sclerosing) and nodular-infiltrating tumors[125-128]. The length of longitudinal extension is determined by the type of invasion, with a mean length of 6-10 mm for the submucosal spread and 10-20 mm for the mucosal spread[125-128]. Therefore, a gross surgical margin of more than 1 cm in the infiltrating type and more than 2 cm in the papillary and nodular types is recommended to achieve negative microscopic resection margins[127,128].

Dynamic MRI

The addition of contrast-enhanced dynamic images to unenhanced and MRCP images did not significantly improve the diagnostic accuracy for assessment of the longitudinal extent of bile duct cancer[133].

CT

MDCT correctly revealed longitudinal extension of hilar cholangiocarcinoma in 77.8%-87% of patients[99,100,103,134], and extrahepatic cholangiocarcinoma in 62.5%-78.6% of patients[134,135]. MDCT revealed wall thickening of the bile duct accompanied by submucosal cancer extension, which is common in hilar cholangiocarcinoma[99,100,103,134]. However, CT has a strong tendency to underestimate longitudinal mucosal spread, which is common in extrahepatic cholangiocarcinoma[125-132,135]. In these patients, at the hepatic margin of the mucosal spread, the width of the mucosa is too thin to be demonstrated by CT or MRI[133-135].

PTCS

Preoperative assessment of longitudinal spread of bile duct cancer has been conducted by mapping biopsy using PTCS[39-42,44,45,47,110,136-138]. With the combination of PTCS and cholangiography, its accuracy improved to 80%-92%[42,110,138]. Observation of the fine mucosal structure is essential to compensate for the false-negative study of mapping biopsy. Nodular, finely reticulo-granular and highly papillary forms of papillo-granular mucosa were characteristic of superficial spreading carcinoma[39-42,44,45,47,136-138]. Regular papillo-granular mucosa was seen even in the non-cancerous area, and methylene blue satin was useful, since the mucosa that did not stain was characteristic of mucosal spread[42,136]. The presence of irregularly dilated and tortuous vessels, so-called tumor vessels, and the patterns of luminal narrowing, suggested intramural cancer extension[39-42,44-46,136-139]. Regular non-dilated vessels were seen even in the non-cancerous area[39-42,44,45,136-138]. Lee *et al*[137] reported that PTCS was essential to evaluate longitudinal cancer extension in patients with polypoid-type cholangiocarcinoma, however, MRCP was sufficient for stenotic-type cholangiocarcinoma. Kim *et al*[138] reported the utility of the combination of PTCS and IDUS in evaluating longitudinal cancer extension of extrahepatic bile duct carcinoma. Since PTCS requires an invasive procedure and may lead to seeding along the PTCS tract, this information should be utilized by POCS from now on.

IDUS

The assessment of longitudinal cancer extension along the bile duct is a promising aspect of IDUS[138-145]. However, to establish the diagnostic system of longitudinal spread by IDUS, some problems have been solved. Bile duct wall thickening occurs by inflammatory change due to mechanical stimulation of the drainage catheter as well as intra-wall extension of the cancer which shows asymmetric thickening, as previously reported[140-144].

A possible solution to this problem might be accurate assessment of the appearance and the internal echo of the wall thickening. When IDUS shows a papillary pattern of the bile duct mucosal surface, heterogeneous bile duct wall thickening (width $\geqslant$ 1.8 mm) with irregular outer marginal, or asymmetric bile duct wall thickening (width $\geqslant$ 1.8 mm) with rigid inner edge, it may be judged as a sign of longitudinal spread of the cancer. However, asymmetric bile duct wall thickening without a rigid inner

Table 3 Intraductal ulytrasonography for the evaluation of longitudinal cancer extension of extrahepatic bile duct carcinoma

Authors and year	Country	Route	Accuracy of IDUS
Tamada *et al*[110], 1995	Japan	PTBD/ERCP	68% (13/19)
Inui *et al*[115], 1998	Japan	PTBD/ERCP	85% (11/13)
Fujita *et al*[116], 1998	Japan	ERCP	80% (12/15)
Menzel *et al*[118], 2000	Germany	ERCP	80% (24/30)
Tamada *et al*[143], 2001	Japan	PTBD/ERCP	71% (25/35)
Tamada *et al*[145], 2001	Japan	ERCP	84% (16/19)
Kim *et al*[138], 2010	Korea	PTBD	92% (18/19)

PTBD: Percutaneous transhepatic biliary drainage; ERCP: Endoscopic retrograde cholangiopancreatography.

edge without an irregular outer marginal border should be judged as a sign of inflammation[143].

On IDUS findings, Inoue evaluated the asymmetry of the thickened bile duct wall by measureing the maximum thickening of the medial hypoechoic layer and the minimum thickening of this layer. The maximum/minimum thickening rate of the cancer spread site and the non-spread site were 2.7 (1.1-4.5) and 1.9 (1.3-3.3), respectively[144].

Our research group has already reported that in patients who had not undergone biliary drainage, 95% did not show bile duct wall thickening over 1.8 mm at the common hepatic duct on IDUS images *via* the transpapillary route, when they did not have primary sclerosing cholangitis or longitudinal cancer extension along the bile duct[139]. Once a biliary catheter was inserted, accuracy of IDUS in assessing longitudinal cancer extension was 71%-72%[110,142,143].

Transpapillary IDUS prior to biliary drainage is useful to reduce artifacts associated with bile duct drainage tubes. When employing this technique any asymmetrically bile duct wall detected with IDUS was judged to be a phenomenon of longitudinal tumor spread allowing for an accuracy of 85%[145]. In the remaining 15% of patients, at the border of the longitudinal cancer extension, the thickening of the mucosal spread was too thin to be visualized on IDUS. The results of IDUS in this area are listed in Table 3.

POCS

Itoi *et al*[71] suggested that NBI cholangioscopy is expected to make it possible to detect not only polypoid lesions but also flat superficial cancerous lesions. They also suggested some limitations of this method. First, observation of the proximal portion of the biliary tumor was possible only in limited cases, since the cholangioscope could not easily be passed through. Secondly, submucosal cancerous progression with non-neoplastic bile-duct epithelium could not be identified even by NBI. These data suggest that POCS by using NBI is limited in cases that show surface structure changes at this stage. IDUS should be conducted to compensate for this limitation[137,139]. Until now, only one report has described the utility of POCS in evaluating the longitudinal cancer extension of extrahepatic bile duct carcinoma in contrast to PTCS (Table 4).

The development of a baby scope the size of "SpyGlass" and with excellent image quality is warranted.

OCT

Since OCT has an axial resolution 10-fold better than that of high-frequency ultrasound, and its depth penetration is limited to approximately 1 mm *vs* 10 mm for a 20 MHz ultrasound probe[90,91], this modality is expected to be utilized for the diagnosis of longitudinal cancer extension. Unfortunately, there is no previous report of OCT in this area.

CONCLUSION

When US shows dilatation of the bile duct, MRCP followed by EUS is the next step to diagnose bile duct carcinoma. When US or EUS shows localized bile duct wall thickening, ERCP should be conducted with IDUS and forceps biopsy (Figure 2). FISH increases the sensitivity of brush cytology with similar specificity. In patients with papillary (polypoid) type bile duct carcinoma, three biopsies are sufficient for the diagnosis. In patients with nodular-type bile duct carcinoma, multiple biopsies are warranted, and IDUS can compensate for the limitations of biopsies. In patients with pancreatic cancer, the sensitivities of forceps biopsy and brush cytology are low. In patients with hilar cholangiocarcinoma, dynamic MDCT provides excellent information for the detection of vascular invasion. In patients with extrahepatic bile duct carcinoma, the combination of MDCT and IDUS is useful to evaluate vascular invasion and cancer depth infiltration (Figure 3). However, assessment of lymph node metastases is difficult. In cholangiocarcinoma, assessment of longitudinal cancer spread is important. Its extension consists of mucosal (superficial) or submucosal (invasive) infiltration depending on the tumor growth pattern. Mucosal extension is predominantly seen with papillary and nodular tumors, while submucosal extension is mainly seen with infiltrating and nodular-infiltrating tumors. The length of longitudinal extension is determined by the type of invasion, with a mean length of 6-10 mm for submucosal spread and 10-20 mm for mucosal spread. The combination of IDUS and MDCT is useful for revealing submucosal cancer extension, which is common in hilar cholangiocarcinoma. To estimate mucosal extension, which is common in extrahepatic bile duct carcinoma, the combination of IDUS and cholangioscopy is required. The utility of current POCS is limited by the maneuverability of the "baby scope". The new thin baby scope (10 Fr), called "SpyGlass", has potential, if the image quality can be improved. In patients with unresectable cholangiocarcinoma, EUS-FNA is useful to compensate for the negative results of ERCP tissue sampling.

Since extrahepatic bile duct carcinoma is common in the Far East, many researchers (histopathologists, sur-

Table 4 Cholangioscopy for the evaluation of longitudinal cancer extension of extrahepatic bile duct carcinoma

Authors and year	Country	Modality	Accuracy
Tamada *et al*[110], 1995	Japan	PTCS + mapping biopsy; PTCS + mapping biopsy + IDUS	80% (12/15); 93% (14/15)
Sato *et al*[42], 1998	Japan	PTCS	81% (13/16)
Kawakami *et al*[72], 2009	Japan	POCS; POCS + mapping biopsy	77% (10/13); 100% (13/13)
Kim *et al*[138], 2010	Korea	PTCS + mapping biopsy; PTCS + mapping biopsy + IDUS	90% (18/20); 95% (18/19)

IDUS: Intraductal ultrasonography; PTCS: Percutaneous transhepatic cholangioscopy; POCS: Peroral cholangioscopy.

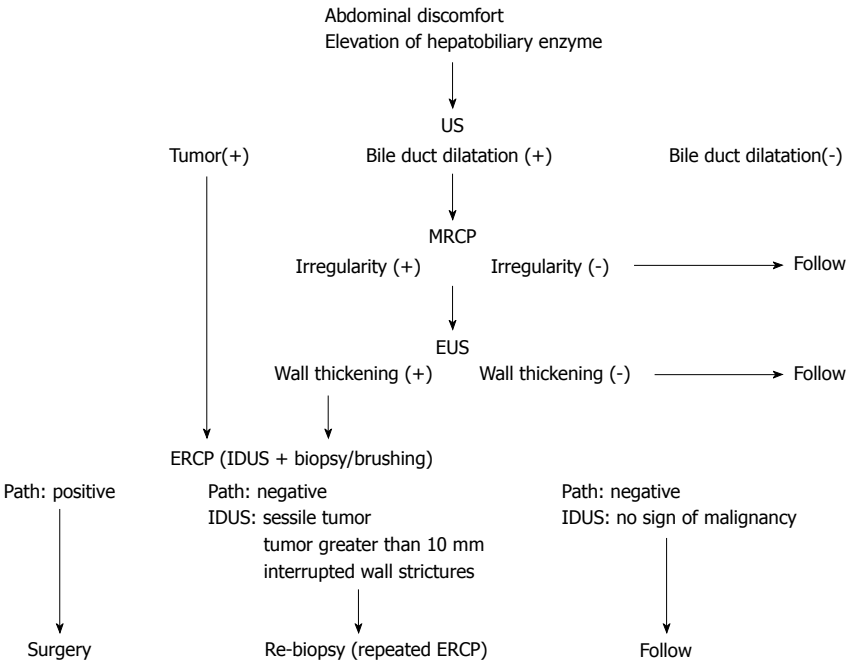

Figure 2 Diagnostic methods for extrahepatic bile duct carcinoma without jaundice. US: Ultrasonography; MRCP: Magnetic resonance cholangiopancreatography; EUS: Endoscopic ultrasonography; ERCP: Endoscopic retrograde cholangiopancreatography; IDUS: Intraductal ultrasonography.

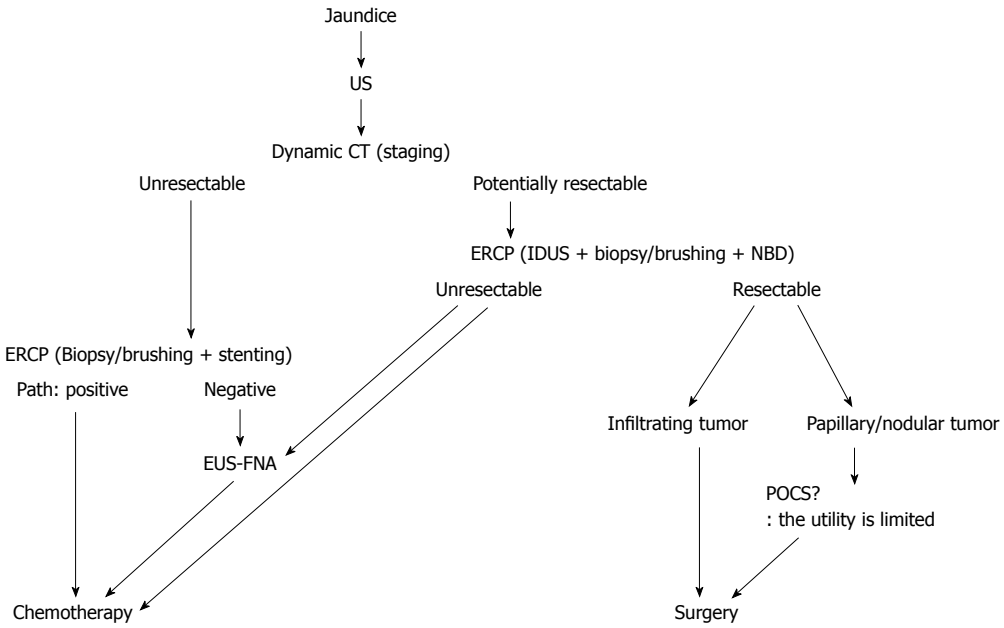

Figure 3 Diagnostic methods for extrahepatic bile duct carcinoma with jaundice. US: Ultrasonography; CT: Computed tomography; NBD: Naso-biliary drainage; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration; ERCP: Endoscopic retrograde cholangiopancreatography; IDUS: Intraductal ultrasonography; POCS: Peroral cholangioscopy.

geons, radiologists, and endoscopists) in Japan and Korea contributed these studies, especially, the evaluation of longitudinal cancer extension, as shown in Table 3 and references.

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S- Editor Cheng JX **L- Editor** Webster JR **E- Editor** Zheng XM

World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i5.217

World J Clin Oncol 2011 May 10; 2(5): 217-224
ISSN 2218-4333 (online)

TOPIC HIGHLIGHT

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Clinical oncology for pancreatic and biliary cancers: Advances and current limitations

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Received: October 30, 2010 Revised: December 15, 2010
Accepted: December 22, 2010
Published online: May 10, 2011

Abstract

In the early 2000s, the main stream of endoscopic ultrasonography (EUS) changed from a mechanical scanning method to electronic radial or linear scanning methods. Subsequently, useful applications in trans-abdominal ultrasonography came within reach of EUS. In particular, contrast-enhanced EUS (CE-EUS) and EUS-elastography became cutting-edge diagnostic modalities for pancreatic disorders. Each type of pancreatic disorder has characteristic hemodynamics. CE-EUS uses color Doppler flow imaging and harmonic imaging to classify pancreatic lesions. EUS-elastography can assess tissue hardness by measuring its elasticity. This parameter appears to correlate with the malignant potential of the lesions. Tissue elasticity studies can provide information on both its pattern and distribution. The former is the conventional method of morphological diagnosis, but it is restricted to observations made in a region of interest (ROI). The latter is an unbiased analysis that can be performed by image analysis software and is theoretically constant, regardless of the ROI. Though EUS-fine needle aspiration (FNA) is also a very useful diagnostic tool, there are several limitations. Diagnostic EUS-FNA of pancreatic cystic lesions has marginal utility mainly due to low sensitivity. Therefore, in particular, endoscopists should keep this limitation in mind.

Key words: Contrast-enhanced endoscopic ultrasonography; Endoscopic ultrasonography-elastography; Endoscopic ultrasonography-fine needle aspiration; Pancreatic cystic lesions; Dissemination; Track seeding; Marginal utility for pancreatic cystic lesions of endoscopic ultrasonography-fine needle aspiration

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INTRODUCTION

Endoscopic ultrasonography (EUS) is thought to be one

of the most reliable and efficient diagnostic modalities for the pancreato-biliary diseases, especially when the diagnostic targets are limited to the locoregional area. Recently, electronic scanning EUS (both electronic radial and curved linear types) has been introduced in clinical settings and many applications in the field of transabdominal ultrasonography (US) are being used. Here, we review the current situation and limitations of EUS in the diagnosis of pancreatic diseases, mainly based on our experiences.

RECENT PROGRESS IN EUS

Recent progress in EUS is summarized in Figure 1. Mainstream EUS was a mechanical radial scanning method (MR-EUS) until the early 2000s. In the early 2000s, we first developed the endosonoscope with an electronic radial scanning method[1,2]. From that time, both electronic radial and linear (curved linear) type EUS was employed using the same utilities as those used with a high-end transabdominal ultrasound apparatus.

Despite many new emerging applications, the essence of ultrasonographic imaging still remains associated with B-mode image quality. In fact, conventional B-mode EUS images have been considered the most sensitive for diagnosing pancreatic tumors, permitting the detection of tumors smaller than 1 cm with the limitation of operator dependency[3-5]. Therefore, an important step in the development of new electronic radial scanning EUS (ER-EUS) was to keep the B-mode image quality at the same level as provided by EUS with a mechanical radial scanning method. A case of branch-duct type intraductal papillary mucinous neoplasm (IPMN; adenoma in this case) is shown in Figure 2. The leftmost image obtained by MR-EUS could not depict the mural nodule due to a near field artifact. A mural nodule is recognized as the most reliable predictor of diagnosing malignancy or benignancy[6]. From this standpoint, MR-EUS may not be useful for the diagnosis of IPMN. On the other hand, the right two images obtained by ER-EUS revealed the mural nodule, which was about 10 mm in diameter. The rightmost image was made more sophisticated by tissue harmonic imaging (THI) technology, which results in clearer ultrasonographic images by omitting acoustic artifacts. B-mode image quality, including that of THI modified images from ER-EUS, proved to be even better than the image quality from MR-EUS, which we considered as encouraging results.

On the basis of the above, a review of the efficacy and limitations of EUS with a variety of applications, listed in Figure 1, is offered here.

CONTRAST-ENHANCED EUS

Diagnostic modalities, such as computed tomography (CT) and magnetic resonance imaging (MRI), need the combination of non-contrast and contrast-enhanced images to provide an accurate diagnosis. EUS makes use of color/power Doppler flow imaging and harmonic imaging and, therefore, diagnosis with vascular information is possible.

EUS is performed in the left lateral position under diazepam-induced sedation with heart rate monitoring. The electronic radial scanning mode is employed in all cases. An electronic radial-type endoscope and the ultrasound observation system EG-3670URK (Pentax Co., Ltd., Tokyo, Japan) with Hi Vision 900 (Hitachi Co., Ltd., Tokyo, Japan) are used in EUS (Figure 3), or a GF-UE260-AL5 (Olympus Co., Ltd., Tokyo, Japan) with Prosound α-10 (Aloka Co., Ltd., Tokyo, Japan, Figure 4). The former employs a wide-band pulse inversion method (WPI) with the mechanical index (MI) automatically set at 0.16-0.23 in accordance with the focal point. The latter employs the extended pure harmonic detection method (ExPHD) with the MI set at 0.25. A single focus is set on the distal side of the target lesion. WPI and ExPHD are essentially identical to each other in that both methods transmit the two reversal phased waves at the same time to cancel the fundamental wave, resulting in enhancement of the second harmonics. To perform contrast-enhanced EUS (CE-EUS) and study tissue hemodynamics, we first image the pancreas and targeted lesions with B-mode EUS and then administer Levovist® (Nihon Schering Co., Ltd., Tokyo, Japan) or Sonazoid® (Daiichi Sankyo Co., Ltd., Tokyo, Japan) through a peripheral vein[7]. The use of Sonazoid® for pancreatic diseases was approved by the Institutional Review Board of our institute, and was used after obtaining written informed consent from the patients.

In general, there are two main categories of CE-EUS, the first is contrast-enhanced color/power Doppler imaging and the second is contrast-enhanced harmonic imaging[7-9]. CE-EUS using a Doppler method provides the image that divides the target into vascular-rich areas and hypovascular areas clearly. CE-EUS using harmonic imaging methods presents a more detailed view of the vasculature of the target lesions. In addition, it gives quantitative information, such as a time-intensity curve showing the change of an echo-intensity over time. Those two methods should be selected in accordance with the intended use.

Figure 5 shows images of a pancreatic endocrine tu-

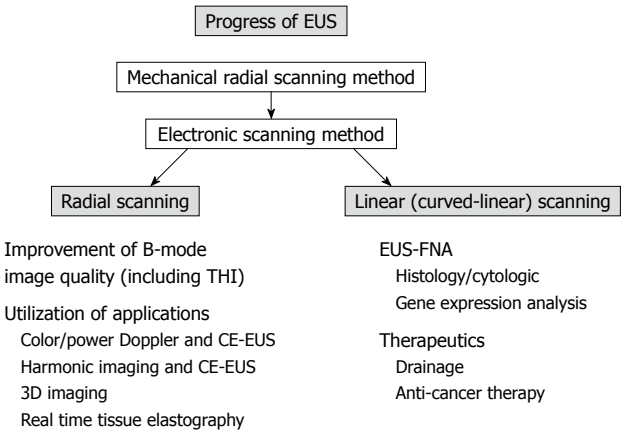


Figure 1 Progress of endoscopic ultrasonography. In the early 2000s, mainstream use of endoscopic ultrasonography (EUS) changed from a mechanical radial scanning method to an electronic radial or electronic linear scanning method, which enabled the endosonography to make use of many applications. FNA: Fine needle aspiration; THI: Tissue harmonic imaging; CE-EUS: Contrast-enhanced EUS; 3D: Three-dimensional.

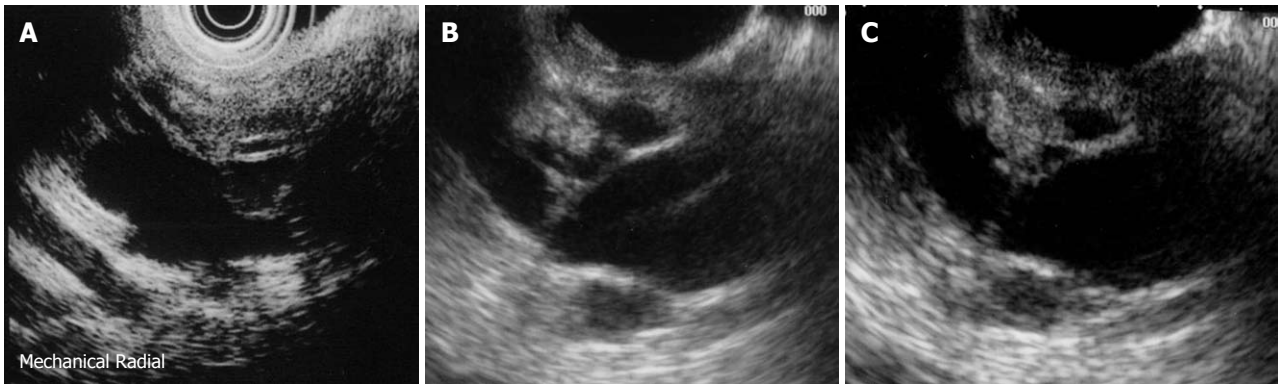


Figure 2 Branch-duct type intraductal papillary mucinous neoplasm. A: An mechanical radial scanning endoscopic ultrasonography (EUS) image could not depict the mural nodule due to a near field artifact; B, C: Electronic radial scanning EUS images revealed a mural nodule about 10 mm in diameter; C: The image was made more sophisticated by tissue harmonic imaging (THI) technology, which brought a clearer ultrasonographic image by omitting acoustic artifacts.

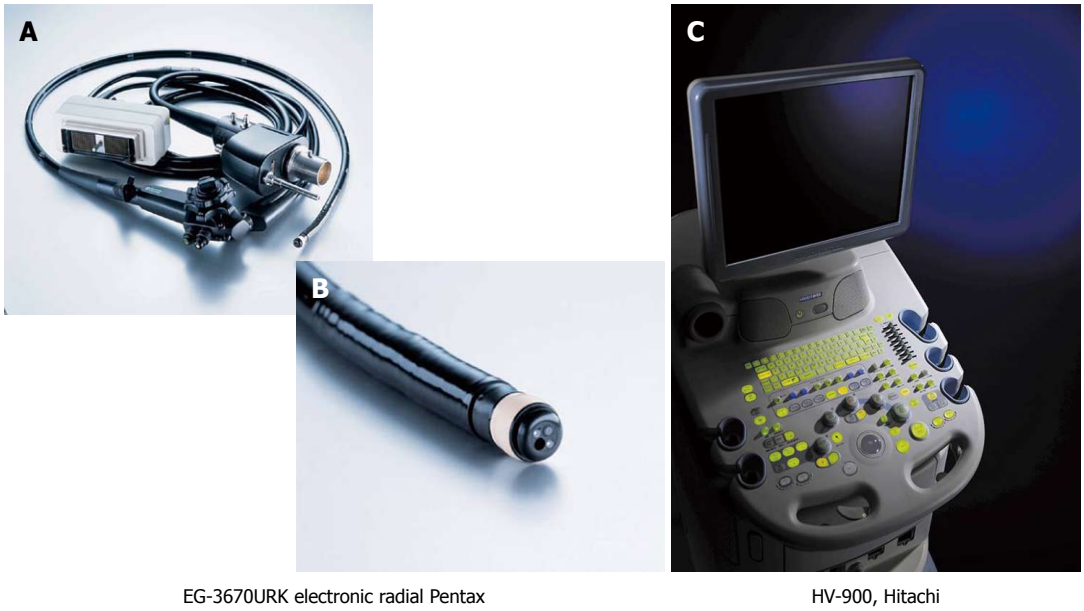


Figure 3 Endoscope and ultrasound machine (Setting-1). A, B: The images indicate the whole view of the Pentax endosonoscope (EG-3670URK) and the tip of the scope; C: The image shows the ultrasound machine (HV-900, Hitachi, Japan).

mor. Plain color Doppler EUS (non-enhanced color Doppler EUS) only showed few color signals inside the clearly delineated iso-echoic tumor (left image). In contrast, there were abundant color signals inside the tumor, indicating it was hypervascular. B-mode imaging combined with CE-EUS imaging provides an important clue to the diagnosis of pancreatic endocrine tumors. What is important to remember is that plain color Doppler EUS images give only limited information on color signals.

Figure 6 presents CE-EUS images using harmonic methods. There was a hypoechoic mass at the pancreatic body with an irregular margin (pre-enhanced). The leftmost image of the bottom indicated an increase in the echo-intensity close to that of the surrounding normal parenchyma. The center and rightmost images were those obtained 3 min and 5 min after injection of contrast-enhancing agents. The tumor became hypoechoic (i.e. hypovascular) compared with surrounding tissues. Moreover, the time-intensity curve showed quantitative information on the changing echo-intensity in each region of interest (ROI, Figure 7). This case was, of course, one example of pancreatic ductal adenocarcinoma. Other types of enhanced pattern can be studied as well[10].

In summary, these two methods are both useful and efficient methods of diagnostic imaging. We must be careful to employ the appropriate method for each case.

THREE-DIMENSIONAL IMAGING

Electronic scanning brought three-dimensional (3D)-EUS imaging into reality. Figure 8 shows an IPMN with high-grade dysplasia case. There are numerous papillary growths in the IPMN. The volume-rendering images (Figure 8B) reflect the surface architecture with reality. Furthermore, the combination of volume-rendering imaging and color Doppler imaging may be more useful in the diagnosis of the malignancy potential of IPMN (Figure 8C). At this point,

Figure 4 Endoscope and ultrasound machine (Setting-2). A-C: The left three images indicate the whole view of the Olympus endosonoscope (GF-UE260-AL5) and the tip of the scope (both bare tip and tip with inflated balloon images are presented.); D: The image shows the ultrasound machine (ProSound α-10, Aloka, Japan).

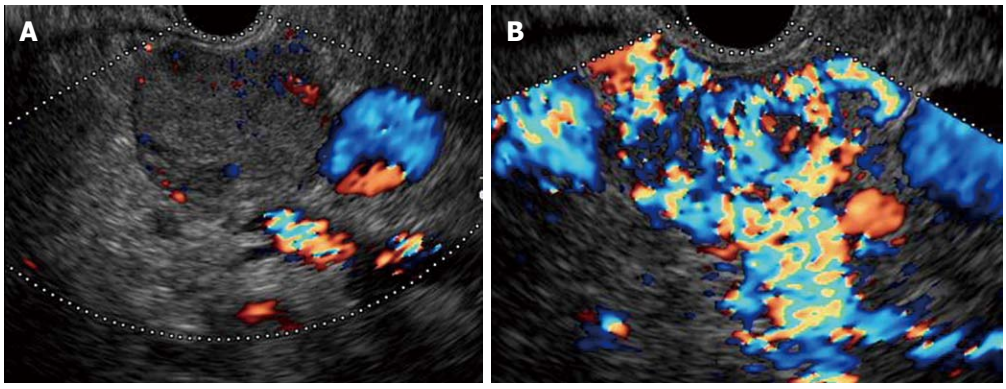


Figure 5 Endocrine tumor of the pancreas. A: Plain color (non-enhanced color) Doppler endoscopic ultrasonography (EUS) only showed few color signals inside the clearly delineated iso-echoic tumor; B: In contrast, there were abundant color signals inside the tumor, indicating it was hypervascular. B-mode image combined with contrast-enhanced EUS images provided an important clue for the diagnosis of pancreatic endocrine tumors. Levovist® was used in this case.

there is no positional information in the images obtained with our system. 3D-EUS may be more useful and informative with the addition of precise positional information.

EUS-ELASTOGRAPHY

In addition to B-mode observation, the diagnosis of pancreatic disorders can also be aided by evaluating tissue hemodynamics. Elastography has the potential to provide new information that differs from B-mode imaging or hemodynamic information, but without the need for contrast medium[11].

Generally, tissue hardness is thought to correlate with malignant potential; malignant tumors are harder than those that are benign. Changes in tissue elasticity are generally correlated with pathological phenomena. Many cancers, such as scirrhous carcinoma of the breast, appear as extremely hard nodules that are a result of increased stromal density. Other diseases involve fatty and/or collagenous deposits that increase or decrease tissue elasticity[12]. On the basis of this concept, several techniques to evaluate tissue hardness, also called tissue elastic imaging, have been developed[13,14]. Tissue elastic imaging with MRI or CT has been introduced for clinical use but, in this review, we will focus on tissue elastic imaging with an ultrasonographic approach that is named real-time tissue elastography (Hitachi Co., Ltd.) and is combined with EUS. The principles of elastography can be explained by using a spring model[11,12]. Thus, when a one-dimensionally connected hard spring and soft spring are compressed, the hard spring is negligibly deformed, but the soft spring is compressed. This difference in deformation results in differences in displace-

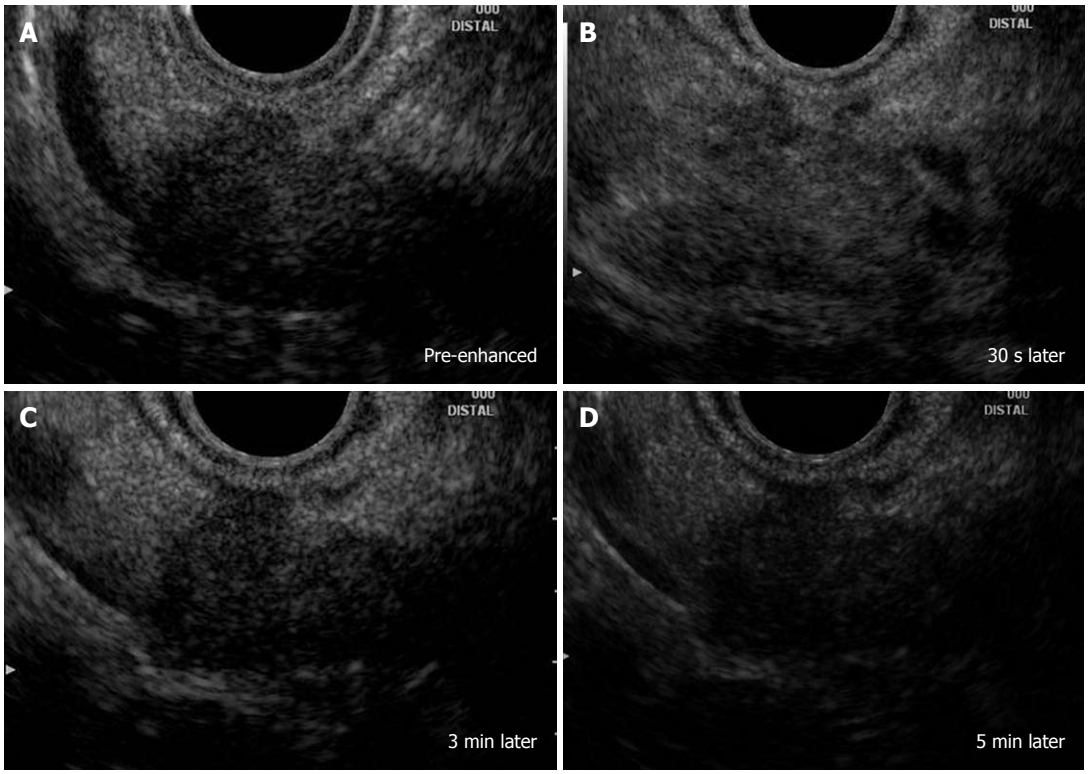
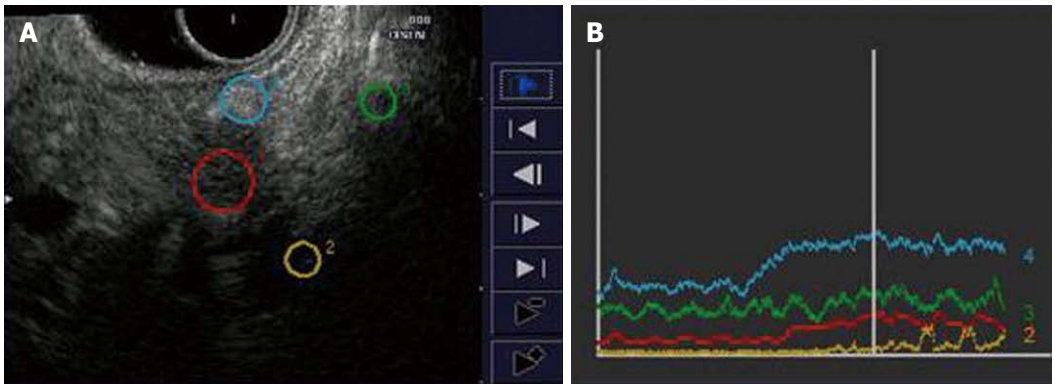


Figure 6 Pancreatic ductal adenocarcinoma. A: There was a hypoechoic mass at the pancreatic body with an irregular margin (pre-enhanced); B: The image indicated an increase in echo-intensity close to that of the surrounding normal parenchyma (30 s later after injection of Sonazoid); C, D: The center and the rightmost images were obtained 3 min and 5 min after injection. The tumor became hypoechoic (i.e. hypovascular) compared with surrounding tissues.



Zero to 1 min later

Figure 7 Time intensity curve for pancreatic ductal adenocarcinoma. A, B: Both settings (settings 1 and 2) can provide a quantitative analysis graphic curve of the changing echo-intensity named the "time intensity curve".

ment among various areas, and the amount of distortion obtained by spatial differentiation of this displacement distribution provides elasticity information.

One should appreciate that real-time tissue elastography provides information about the distributed pattern of tissue hardness as well as hardness at a specific point. The information regarding hardness at a specific point is classified further into 2 categories: (1) pattern recognition; and (2) quantitative assessment (strain ratio) (Table 1).

Figure 9 shows a case of pancreatic ductal adenocarcinoma. The EUS-elastographic image (left side) shows a markedly hard area at the site of the low-echo tumor area (right side) and distribution of slightly soft spots in the interior. Histopathologic examination confirmed that the hard area contained a large amount of fibrous tissue, and the internal soft spots were aggregations of atypical ducts (of various sizes).

Table 1 Interpretation of real time tissue elastography[8]

Hardness at the specific point
Pattern recognition
Quantitative assessment: strain ratio
Distributed pattern of tissue hardness

EUS-elastography provides additional important infor-

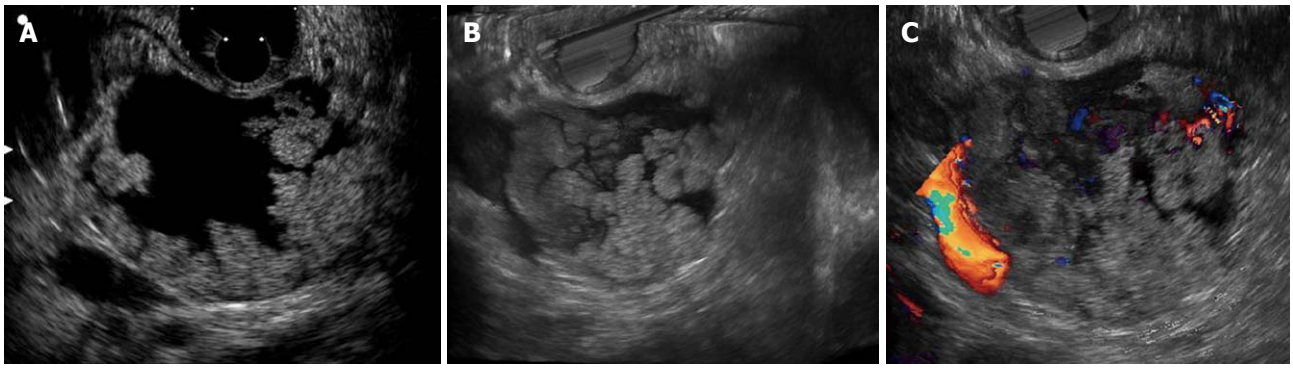


Figure 8 Image of a intraductal papillary mucinous neoplasm with high-grade dysplasia. A: There are numerous papillary growths in the intraductal papillary mucinous neoplasm (IPMN) maximum intensity projection image; B: The volume-rendering images reflect the surface architecture with reality; C: The combination of volume-rendering imaging and color Doppler imaging may be more useful in the diagnosis of the malignant potential of the IPMN.

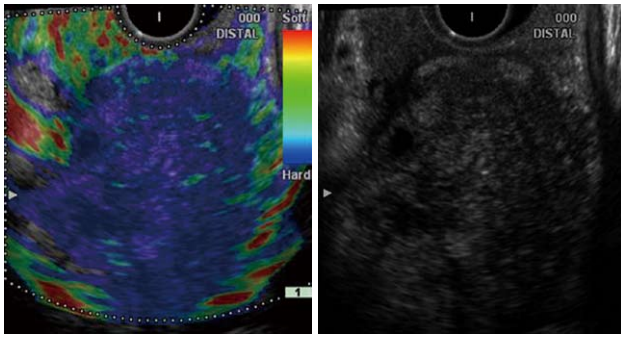


Figure 9 Pancreatic ductal adenocarcinoma. Endoscopic ultrasonography-elastographic image (left side) shows a markedly hard area at the site of the low-echo tumor area (right side) and distribution of slightly soft spots in its interior. Histopathologic examination confirmed that the hard area contained a large amount of fibrous tissue, and the internal soft spots were aggregations of ducts atypical (of various sizes).

mation relating to hardness; that is, the distributed pattern (Table 1). Regardless of ROIs, the distributed pattern is theoretically a constant. The distributed pattern of hardness in a case of chronic pancreatitis is analyzed in Figure 10. The prototype image analysis software used here extracted various features from real-time tissue elastography images. It converted the red-green-blue value inside the ROI of the elastography image into relative strain value and calculated other features of the elastography image, such as the mean of relative strain value, the standard deviation of the relative strain value, and the proportion of blue (low strain) region in the analysis region, and determined the complexity of the blue (low strain) region in the analysis region [(Perimeter of blue region)2/(Area of blue region)]. With this software (produced in cooperation with Hitachi Co., Ltd.), we can demonstrate the uniformity, or lack thereof, of a target lesion and quantify a number of objective parameters of the distribution of hardness described above.

The fourth tissue characterization, following B-mode imaging, color/power Doppler imaging and CE-EUS, must be EUS-elastography. Nevertheless, what we must keep in mind is that represented colors in this system are relative in each ROI. We cannot compare the images among individuals precisely. An absolute value or image with elastic information is eagerly awaited.

EUS-FINE NEEDLE ASPIRATION RELATED PROCEDURES (SPECIAL FOCUS ON THE DIAGNOSIS OF CYSTIC NEOPLASMS)

The usefulness of EUS-fine needle aspiration (FNA) has been well recognized in the diagnosis of intramural lesions (e.g. gastrointestinal stromal tumor: GIST, leiomyoma) and extramural lesions, such as pancreatic tumors, lymph nodes and mediastinal masses. In 1995, Hammel *et al*[15] reported its usefulness for the differential diagnosis of cystic lesions of the pancreas by analyzing cyst fluid collection obtained by transabdominal US guided FNA. In the early 2000s, enthusiasm for preoperative fluid collection analysis reported positive results. Recently, however, there have been reports[16,17] that preoperative analysis of the pancreatic cyst fluid obtained by EUS-FNA has marginal utility. Moreover, dissemination due to EUS-FNA was reported[18].

According to ASGE guidelines (on the role of endoscopy in the diagnosis and the management of cystic lesions and inflammatory fluid collections of the pancreas)[19], it was recommended that aspirated cyst contents may be submitted for cytologic, chemical and/or tumor marker analysis.

As to cytology, ASGE guidelines indicated that FNA can provide material for a cytologic diagnosis in up to 80% of cases of pancreatic cystic lesions, and the accuracy for diagnosing various cystic lesions by EUS-FNA was 54% to 97%. In addition, ASGE guidelines stated that malignancy within a cystic neoplasm can be identified by cytology with 83% to nearly 100% specificity, despite marginal sensitivity varying from 25% to 88%. Moreover, ASGE guidelines pointed out that low sensitivities, combined with the reported results of chemistry analysis and tumor markers, had broad ranges, which made interpretation difficult. There have been several reports[16,17] related to this issue with negative tones. Furthermore, concern for potential dissemination caused by EUS-FNA of pancreatic cystic neoplasms[18] still remains unresolved. Enthusiastic exploration may be important, but the attempts in this field cannot be fully encouraged at this time.

DISCUSSION

In this review, we have described the potential of CE-

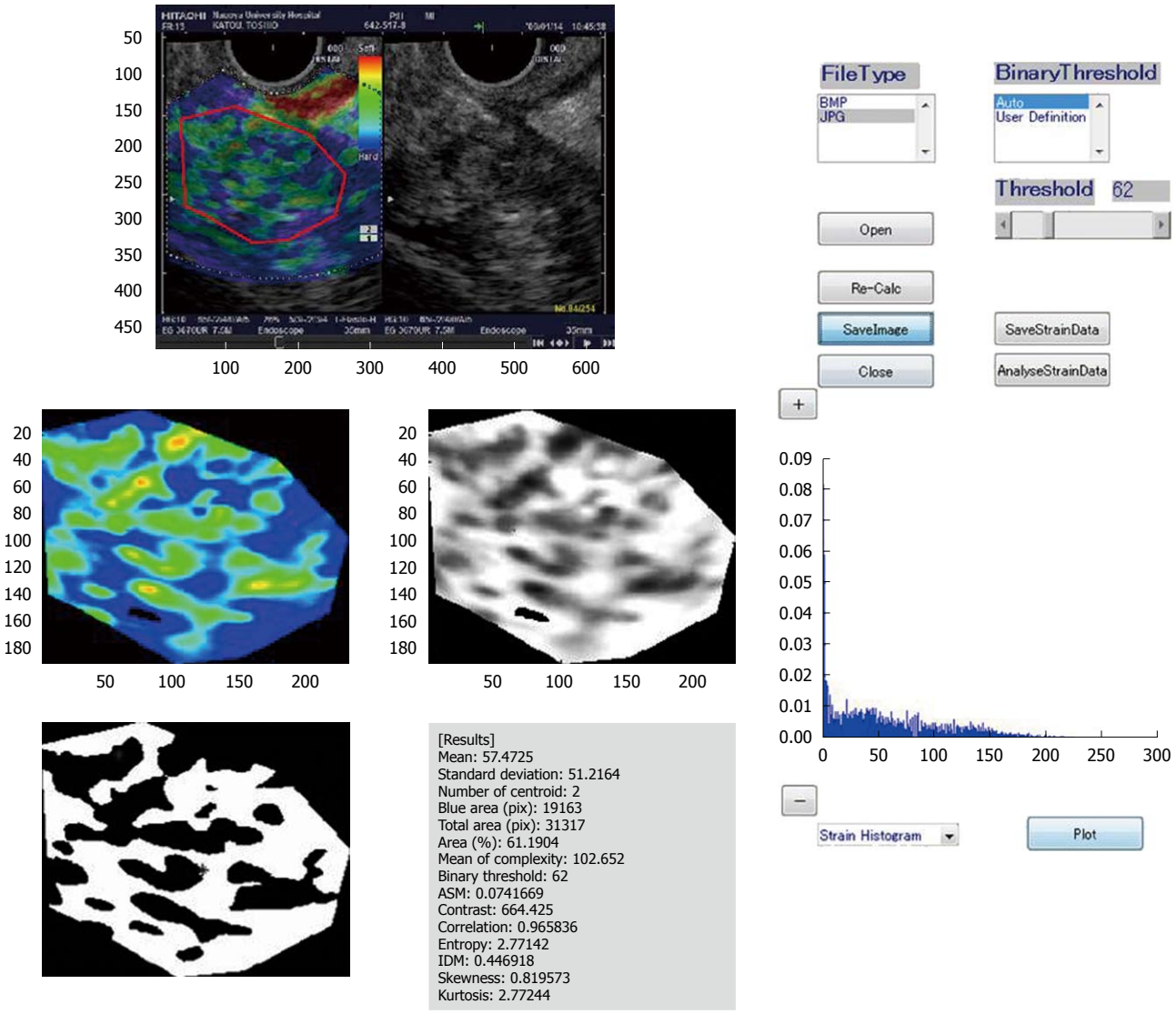


Figure 10 Prototype image analysis software (chronic pancreatitis in this case). The prototype image analysis software used here extracts various features from real-time tissue elastography images. It converts the red-green-blue value inside the region of interest of the elastography image into a relative strain value and calculates other features of the elastography image, such as the mean of the relative strain value, the standard deviation of the relative strain value, and the proportion of the blue (low strain) region in the analysis region, and determines the complexity of the blue (low strain) region in the analysis region [(Perimeter of blue region)2/(Area of blue region)].

EUS (both Doppler and harmonic methods), 3D-EUS, EUS-elastography and EUS-FNA to be used as cutting-edge diagnostic modalities. In addition, we summarized our experience with these technologies. The performance of EUS depends on both the efficiency of an endoscope and ultrasonographic technologies.

In 2003, we first developed the endosonoscope with electronic radial scanning in cooperation with PENTAX (PENTAX Co., Ltd., Tokyo, Japan) to combine ultrasound techniques that were being used for transabdominal US. An electronic scanning method made it possible for us to perform CE-EUS, 3D-EUS and EUS-elastography. Tissue characterization by EUS was only made by B-mode imaging before the advent of CE-EUS, 3D-EUS and EUS-elastography. CE-EUS can now provide hemodynamic analysis of pancreatic disorders at the same level as CT or MRI.

EUS-elastography has introduced a new form of pathologic analysis; that is, tissue elasticity. Tissue elasticity as detected by this system can be divided into 2 major categories. One is pattern recognition, which has been the conventional method of morphologic diagnosis. Importantly, the image of EUS elastography indicates the relative value in a ROI, so the same lesion might display different colors in a different ROI. This is a limitation of EUS-elastography. The other is the distribution of tissue elasticity. With the prototype image analysis software, we can now capture and analyze features of real-time tissue elastography by using computer software. Theoretically, this will limit interpretation bias and provide a measure of pattern distribution that is constant and independent, regardless of ROIs.

CE-EUS and EUS-elastography, as well as other methods, have the potential to provide clinical utility for the diagnosis of pancreatic disorders, however, additional studies and greater experience are needed before their place in our diagnostic armamentarium can be fully understood.

TECHNICAL TERMS FOR BETTER UNDERSTANDING

Near field artifact (Otherwise known as reverberation artifact): Reverberation is the persistence of sound in a particular space after the original sound is removed. Reverberation artifact is created when a sound is produced in an enclosed space causing a large number of echoes to build up and then slowly decay as the sound is absorbed by the walls and air.

THI: Imaging method produced by tissue harmonic component which is generated during the propagation of ultrasound in the media such as a body tissue.

WPI® (wideband pulse inversion: Hitachi Medico, Tokyo, Japan) and ExPHD® (extended pure harmonic detection: Aloka, Tokyo, Japan): Most of the same technique of ultrasound imaging. They work by sending two trains of pulses out of phase to each other, and summing the returning echoes. The signal from tissue cancels, whereas the signal from the collapsing or vibrating microbubbles is recorded.

MI: MI is used as an estimate for the degree of bioeffects which a given set of ultrasound parameters will induce. A higher mechanical index means a larger bioeffect. Currently the FDA stipulates that diagnostic ultrasound scanners cannot exceed a mechanical index of 1.9.

Volume-rendering image: Volume rendering is a technique used to display a 2D projection of a 3D discretely sampled data set.

Relative strain value: Real-time tissue elastography® represents 256-stepwise colors corresponding to the relative strain values in the ROI.

MIP: A MIP is a computer visualization method for 3D data that projects in the visualization plane the voxels with maximum intensity that fall in the way of parallel rays traced from the viewpoint to the plane of projection.

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S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Zheng XM

World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i5.225

World J Clin Oncol 2011 May 10; 2(5): 225-228
ISSN 2218-4333 (online)

TOPIC HIGHLIGHT

Michiko Yamagata, MD, PhD, *Series Editor*

Recent advances and limitations of surgical treatment for pancreatic cancer

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Received: December 24, 2010 Revised: March 8, 2011
Accepted: March 15,2011
Published online: May 10, 2011

Abstract

Recent advances in surgical treatment for pancreatic cancer have been remarkable. Pancreatoduodenectomy is a standard surgical procedure for cancer of the pancreatic head, and is now indicated even for elderly patients over 80 years of age. Pancreatoduodenectomy with combined resection of the peripancreatic vessels has improved survival, but extended resection including lymph nodes is considered to have no extra survival benefit. Furthermore, laparoscopic resection procedures including pancreatoduodenectomy, distal pancreatectomy, enucleation and central pancreatectomy can now be performed safely. Neoadjuvant or adjuvant chemotherapy using gemcitabine may further improve the surgical outcome. An understanding of the oncological aspects of pancreatic cancer and the development of surgical techniques and chemotherapy may further contribute to improving the outcome of surgery for pancreatic cancer.

Key words: Chemotherapy; Laparoscopic surgery; Pancreatic cancer; Pancreatoduodenectomy; Pancreatic fistula

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Kubota K. Recent advances and limitations of surgical treatment for pancreatic cancer. *World J Clin Oncol* 2011; 2(5): 225-228 Available from: URL: http://www.wjgnet.com/2218-4333/full/v2/i5/225.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i5.225

INTRODUCTION

Although curative surgical resection is the best treatment for pancreatic cancer, morbidity and mortality after surgery are still high and prognosis is still unsatisfactory. Pancreatic cancer requires pancreatoduodenectomy (PD) or distal pancreatectomy. Advanced cancers may invade the portal vein and nerve plexus, and subsequently these structures may also need to be resected[1,2]. Recent advances in preoperative management and surgical techniques have facilitated safe and successful resection of such cancers. Herein, the recent advances and limitations of surgical treatment for pancreatic cancer are reviewed.

PREOPERATIVE BILIARY DRAINAGE

It has not been clarified whether PD or major hepatectomy should be performed without preoperative biliary drainage (PBD) in patients with jaundice. Although PBD is considered to improve liver function, the benefits of the procedure are controversial. Van der Gaag *et al*[3] performed a multicenter, randomized trial involving 202 patients with jaundice due to cancer of the pancreatic head (96 undergoing early surgery with no drainage and 106 undergoing PBD). The rates of serious complications were 39% in the early-surgery group without drainage and 74% in the PBD group. Surgery-related complications occurred in 35 patients in the early-surgery group and in 48 patients in the PBD group. Mortality and the length of hospital stay did not differ significantly between the groups. They concluded that routine PBD in patients

undergoing surgery for cancer of the pancreatic head increases the rate of complications. Furthermore, Mezhir *et al*[4] analyzed the complications related to PBD in patients with cancer of the pancreatic head and concluded that PBD increased the incidence of infectious complications including wound infection and intra-abdominal abscess, although the incidence of anastomotic leakage was unaffected. On this basis they considered that routine use of PBD remains unjustified.

SURGICAL TREATMENT

Although surgical treatment for pancreatic cancer is usually indicated for patients younger than 80 years, the postoperative results for patients older than 80 years still remain unclear. Tani *et al*[5] found that the incidence of delayed gastric emptying in such elderly patients was higher, although not to a significant degree. The other outcomes in the elderly group were similar to those of patients younger than 80 years of age. They concluded that PD was a feasible surgical procedure for elderly patients who had a good performance status.

Pancreatic ductal adenocarcinoma is an aggressive disease. Surgical resection with negative margins (R0) offers the only opportunity for cure. Patients who have advanced disease that limits the chance for R0 surgical resection may undergo margin positive PD. Lavu *et al*[6] reported that median survival was 27.2 mo for R0, 15.6 mo for margin-positive PD, 6.5 mo for palliative bypass and 5.4 mo for celiac plexus neurolysis alone. They concluded that margin-positive PD in highly selected patients can be performed safely, with low perioperative morbidity and mortality, and is superior to palliative bypass for locally advanced pancreatic ductal adenocarcinoma. Furthermore, Hüser *et al*[7] recommended that prophylactic gastroenterostomy should be performed during surgical exploration of patients with unresectable pancreatic head tumors because it reduces the incidence of long-term gastroduodenal obstruction without impairing short-term outcome.

The characteristics of pancreas cancer include invasion to the superior mesenteric vein or nerve plexus, and lymph node metastasis. Boggi *et al*[2] evaluated the operative risk and prognostic implications of pancreatectomy plus resection and reconstruction of peripancreatic vessels in patients with pancreatic adenocarcinoma. They found that pancreatectomy plus resection and reconstruction of peripancreatic vessels could be performed as safely as palliation or conventional pancreatectomy, and was associated with better survival when compared to palliation. Makino *et al*[1] investigated the patterns of nerve plexus invasion. Extrapancreatic nerve plexus invasion by carcinoma of the pancreatic head could be divided into two patterns based on the embryological structure of the pancreas and the location of the tumor. Patients with carcinoma in the ventral pancreas frequently had pancreatic head plexus 1, pancreatic head plexus 2, and superior mesenteric arterial plexus invasion. Patients with carcinoma in the dorsal pancreas had invasion into the common hepatic artery plexus and the plexus within the hepatoduodenal ligament. They considered that this information could be useful for determining the surgical strategy for carcinoma of the pancreas head. Hernandez *et al*[8] evaluated the survival benefit of extending resections to obtain microscopically negative margins after intraoperative frozen sections had revealed cancer positivity, but found that this approach did not improve survival.

Recently, visceral ischemic complications have attracted the attention of surgeons[9]. Ischemic complications are an underestimated cause of death after PD, and are due to pre-existing stenosis of the celiac axis and superior mesenteric artery, or intraoperative hepatic artery injury. Pre-existing arterial stenosis can be detected by routine multi-detector CT. Preoperative endovascular stenting for intrinsic stenosis, division of the median arcuate ligament for extrinsic compression, and meticulous dissection of the hepatic artery can help to minimize ischemic complications.

Pancreatic fistula is one of the most common complications after PD. Berger *et al*[10] investigated the utility of duct-to-mucosa pancreatojejunostomy in a randomized, prospective, dual-institutional trial and found considerably fewer fistulas with invagination compared with duct-to-mucosa anastomosis after PD. Higher incidence rates of pancreatic fistula were confirmed in patients with soft pancreas than in those with hard pancreas.

Laparoscopic resection

The role of laparoscopy in pancreatic surgery was originally relegated to staging and palliation for pancreatic surgery. Laparoscopic staging of pancreas tumors was shown to be superior to dynamic CT for visualizing small, occult liver and peritoneal metastases and was useful for avoidance of unnecessary laparotomy in patients with unresectable disease[11,12]. The addition of laparoscopic ultrasound during laparoscopic staging enhanced the ability of laparoscopy to determine tumor resectability with an accuracy approaching that of open exploration without any significant increase in morbidity or mortality. Gagner and Pomp were the first to report a successful laparoscopic pancreatic resection in 1994[13]. Two years later, Sussman and colleagues published the first report of a laparoscopic distal pancreatectomy for insulinoma[14]. Currently, five operative procedures are commonly performed on the pancreas for neoplastic disease: diagnostic laparoscopy with or without biopsy, PD, tumor enucleation, central pancreatectomy and distal pancreatectomy with or without splenectomy[15]. These laparoscopic procedures are particularly recommended for benign tumors, but it is very difficult to make clear recommendations with regard to laparoscopic resection of malignant pancreatic tumors due to a lack of conclusive data.

Radiofrequency ablation

Girelli *et al*[16] employed radiofrequency ablation for locally advanced pancreatic cancer. The 30-d mortality rate was 2%, and abdominal complications occurred in 24% of patients, being associated with the procedure in half of such cases. They concluded that radiofrequency abla-

tion of locally advanced pancreatic cancer is feasible and relatively well tolerated.

Chemotherapy

Heinrich *et al*[17] investigated the clinical utility of neoadjuvant chemotherapy using gemcitabine and cisplatin. The surgical morbidity was low without perioperative death, and one pancreatic fistula occurred. A histologic response was documented in 54% of patients, and cytopathic effects in 83%. Neoadjuvant chemotherapy elicited a significant metabolic and histologic response, which was best predicted by PET. Most importantly, surgery after neoadjuvant chemotherapy was shown to be safe. Furthermore, Ohigashi *et al*[18] evaluated the feasibility and efficacy of preoperative full-dose gemcitabine, concurrent 3D-conformal radiation, surgery and postoperative liver perfusion chemotherapy for T3 pancreatic cancer. They were able to effectively reduce the incidence of both local and liver recurrence, possibly contributing to improving the long-term outcome. Pancreatic resection combined with neoadjuvant or adjuvant chemotherapy undoubtedly contributes to improvement in outcome.

PROGNOSIS

The prognosis of pancreatic ductal adenocarcinoma is dismal, and influenced by tumor stage. Massucco *et al*[19] analyzed the prognostic significance of variables related to nodal involvement (node status, number of disease-positive nodes, node ratio and site of nodal metastases) in patients with resected pancreatic head cancer. They found that the level of nodal metastatic spread was a statistically significant prognostic factor, and that both the number of disease-positive nodes and the node ratio were an accurate proxy for node level (1: peri-pancreatic nodes, 2: nodes along the main arteries and hepatic hilum, 3: pre-aortic nodes), perhaps contributing to patient risk stratification.

Obesity is reported to influence cancer-related outcome. Fleming *et al*[20] considered that obese patients with a body mass index (BMI) of more than 35 were more likely to have node-positive pancreatic cancer and to show shorter survival after surgical resection. Data suggest that the negative influence of a BMI exceeding 35 on the incidence of lymph node metastasis and disease-free and overall survival is unrelated to the potential complexity of performing major oncologic surgery in obese patients. In fact, it has been shown that increased pancreatic fat promotes dissemination and lethality of pancreatic cancer. Pancreatic steatosis is considered to alter the tumor microenvironment, enhance tumor spread and contribute to the early demise of patients with pancreatic adenocarcinoma[21,22].

Readmission after PD is not uncommon. One report has indicated that 59% of patients were readmitted within 1 year following PD and that 47% were readmitted to a secondary hospital. Readmission was associated with poorer median survival in comparison to patients who were not readmitted (10.5 *vs* 22 mo)[23].

Long-term survival after pancreatectomy for pancreatic duct adenocarcinoma has been rarely reported. Adam *et al*[24] reported a French multicenter series of long-term survivors (>5 years) comprising 20 men and 10 women. Three patients underwent portal vein resection, 1 underwent hepatic artery resection-reconstruction, and 1 underwent segmentectomy for liver metastasis. However, all the resections were complete, both macroscopically and microscopically (R0). They concluded that pancreatic duct adenocarcinoma can be cured, and that long-term survival after R0 curative surgery has become a reality. These long-term survivors did not fulfill the ideal prognostic criteria, and some even presented with advanced disease.

CONCLUSION

Although pancreatic cancer is still associated with poor prognosis, an understanding of the oncological aspects of pancreatic cancer and the development of surgical techniques, including laparoscopic surgery, and chemotherapy may further contribute to improving the outcome of surgery for pancreatic cancer.

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S- Editor Tian L **L- Editor** Webster JR **E- Editor** Tian L

World Journal of Clinical Oncology

Online Submissions: http://www.wjgnet.com/2218-4333office
wjco@wjgnet.com
doi:10.5306/wjco.v2.i5.229

World J Clin Oncol 2011 May 10; 2(5): 229-236
ISSN 2218-4333 (online)

TOPIC HIGHLIGHT

Michiko Yamagata, MD, PhD, *Series Editor*

FDG-PET for hepatobiliary and pancreatic cancer: Advances and current limitations

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Received: January 24, 2011 Revised: March 8, 2011
Accepted: March 15, 2011
Published online: May 10, 2011

Abstract

In Japan, the use of ^{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) for some malignant tumors came to be covered by the National Health Insurance in 2002. In 2010, the health insurance coverage was expanded to all types of malignant tumors. However, since PET examination requires a large amount of capital investment, facilities at which PET is available are still limited. On the other hand, PET equipment has rapidly been introduced in large hospitals and in the diagnostic imaging centers of major cities during the past few years. Although numerous middle-sized and small hospitals cannot afford to perform PET, physicians can refer their patients to facilities where PET is available. Therefore, it is essential for general physicians to gain accurate knowledge on PET, including the appropriate indications for PET, in order to select patients for referral to PET facilities. PET is not always a useful tool, especially for lesions of the pancreas and hepatobiliary system, which is the main topic of this review. The indications of PET for lesions in these organs vary depending on the purpose of the examination. In this article, we review the indications for PET (or PET/computed tomography [CT]) using FDG of the liver, biliary tract, and pancreas.

Key words: Positron emission tomography; ^{18}F-fluorodeoxyglucose; Pancreatic cancer

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Murakami K. FDG-PET for hepatobiliary and pancreatic cancer: Advances and current limitations. *World J Clin Oncol* 2011; 2(5): 229-236 Available from: URL: http://www.wjgnet.com/2218-4333/full/v2/i5/229.htm DOI: http://dx.doi.org/10.5306/wjco.v2.i5.229

FDG-PET EXAMINATION FOR LIVER CANCER

Liver cancer can be classified as hepatocellular carcinoma (HCC), cholangiocellular carcinoma (CCC) or metastatic hepatic carcinoma, and the degree of 18F-fluorodeoxy glucose(FDG) uptake and clinical usefulness of FDG-positron emission tomography (PET) differ according to the histological type.

PET FOR HEPATOCELLULAR CARCINOMA

HCC is known to show a faint FDG uptake. This can be explained based on the mechanism of FDG uptake in tumors. FDG is an analogue of glucose, and when injected into the body, it is taken up by the cells and phosphorylated in the same pathway as glucose. The metabolic process of FDG is the same as that of glucose up to this point, but the reactions of FDG do not proceed further (Figure 1). In other words, the FDG remains in the cells. On the other hand, because dephosphorylating enzyme activity is higher

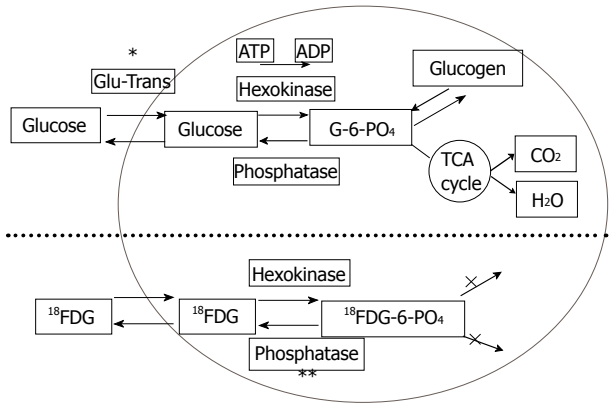

Figure 1 Schema of the metabolic pathway of glucose and 18-F-fluorodeoxyglucose in cells. fluorodeoxyglucose has the same pathway as glucose up to the phosphorylation process, and does not progress further. Most malignant cells are known to have overexpression of the glucose transporter (*) and low activity of phosphatase(**).

in normal liver cells than in other tissues, it is likely that the glucose accumulated by normal cells is dephosphorylated again and excreted out of the cells. Since such enzyme activity is retained in well-differentiated HCC, equilibrium is reached when the FDG in the cells is also excreted out of the cells. Moreover, the activity of the glucose transporter is known to be weak as compared with that in other types of malignant tumors. Therefore, HCC shows relatively weak FDG uptake (Figure 2). On the other hand, since the enzyme activity is correlated with the degree of differentiation of HCC, poorly-differentiated HCC shows weak enzyme activity and strong FDG uptake[1,2]. Since the FDG uptake appears to vary with the degree of differentiation of HCC, we may be able to predict, to some extent at least, the degree of differentiation of HCC by the degree of FDG uptake, even though FDG-PET is still not very useful for the diagnosis of HCC: the lower the degree of histological differentiation of HCC, the higher the FDG uptake level. Furthermore, since poorly differentiated HCC is frequently associated with metastasis and recurrence, FDG/PET is useful for detecting such metastasis/recurrence, as it has the merit of imaging the whole body (Figures 3 and 4)[3]. Moreover, the degree of histological differentiation is thought to be correlated with prognosis, and the poorer the degree of differentiation of the HCC, the poorer the prognosis. Thus, FDG-PET may be a promising and useful tool in the future for predicting the prognosis of HCC[4,5]. On the other hand, some reports have mentioned the usefulness of non-FDG radiopharmaceuticals such as choline[6] and acetate[7]. Although the efficacy and the role of these drugs in HCC are not yet established, it is possible that non-FDG PET may also be a promising tool in the future.

PET EXAMINATION FOR CHOLANGIOCELLULAR CARCINOMA

CCC is histologically classified as adenocarcinoma, and usually shows increased FDG uptake (Figure 5)[8,9].

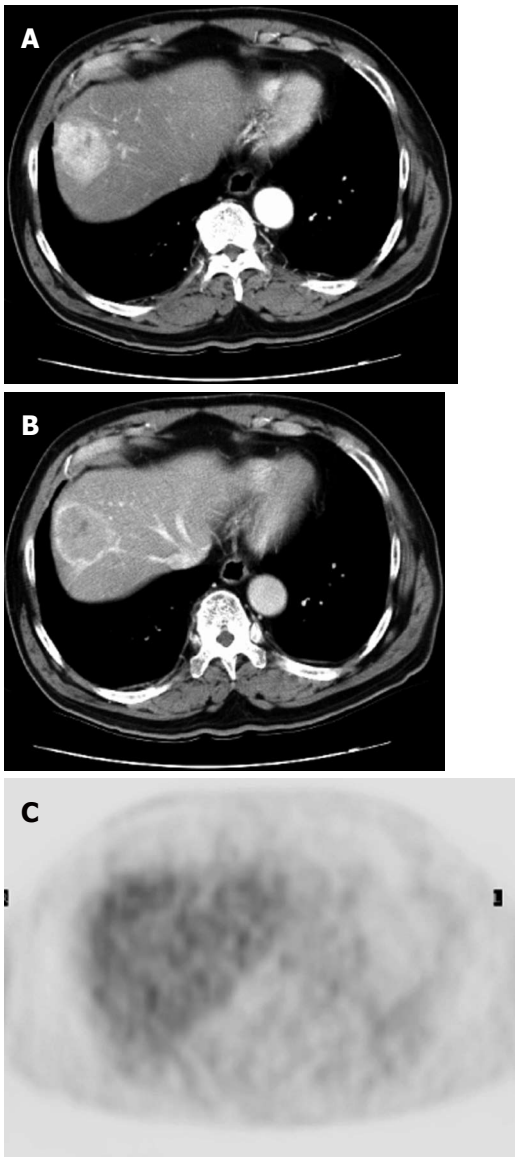

Figure 2 A case of well-differentiated hepatocellular carcinoma. A, B: Arterial and portal phase of dynamic computed tomography. Typical enhancement pattern of hepatocellular carcinoma is shown; C: FDG-PET. There is no fluorodeoxyglucose uptake.

However, since both poorly differentiated HCC and metastatic hepatic carcinoma show marked FDG uptake, as stated above, it is difficult to differentiate between the two types of cancer based on the uptake of FDG alone. Thus, other morphological diagnostic imaging techniques such as CT or magnetic resonance imaging (MRI) are indispensable for reference.

Moreover, diagnostic "high-resolution" imaging tools, such as direct contrast radiography, endoscopic ultrasound (EUS), intraductal ultrasound (IDUS), contrast-enhanced CT and MRI, are sufficient for diagnosing the stage of primary lesions, thus, the clinical significance of PET is of little value for diagnosis of the T factor in CCC. On the other hand, PET may be used as a complementary diagnostic tool for the diagnosis of lymph node metastases when lesions are around 10 mm, when they are difficult to assess by CT alone. However, it is difficult to detect

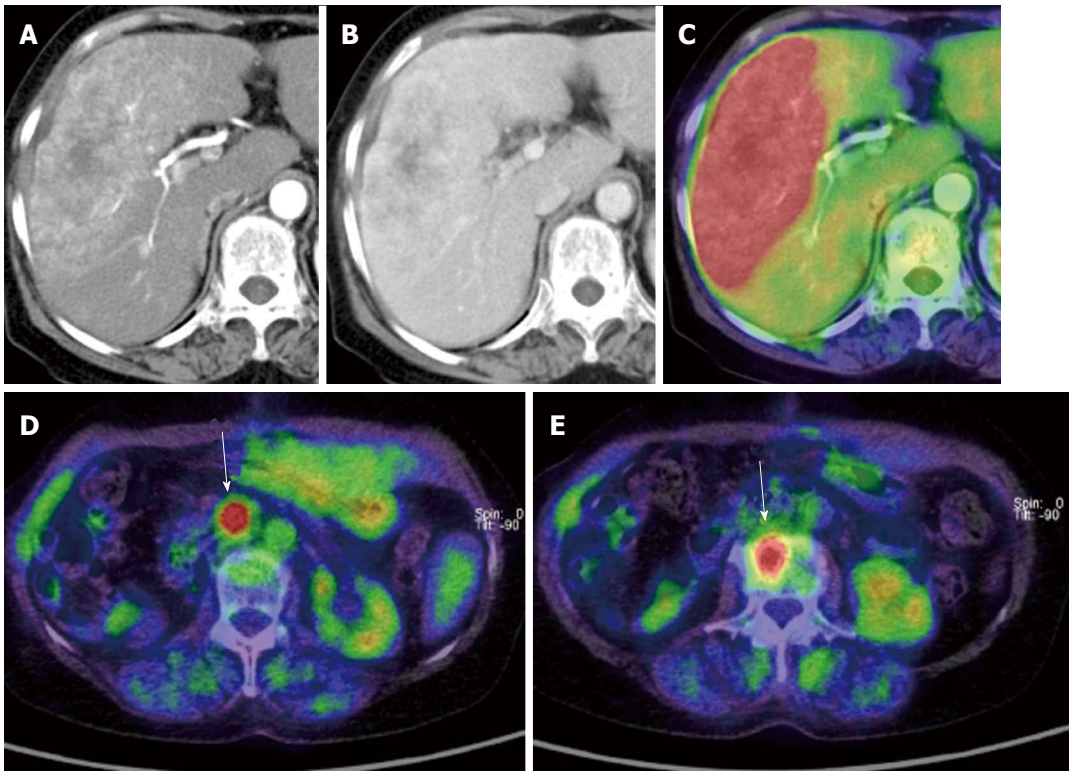

Figure 3 A case of mixed-type hepatocellular carcinoma with extrahepatic metastases. A: Arterial phase of dynamic computed tomography (CT); B: Portal phase of dynamic CT. The tumor shows early enhancement which is a feature of hepatocellular carcinoma (HCC), although it also has a lobular border and delayed enhancement which are features of cholangiocellular carcinoma. Pathological diagnosis was mixed-type HCC; C: This type of HCC shows strong accumulation; D, E: This case also has lymph node and bone metastases (arrows).

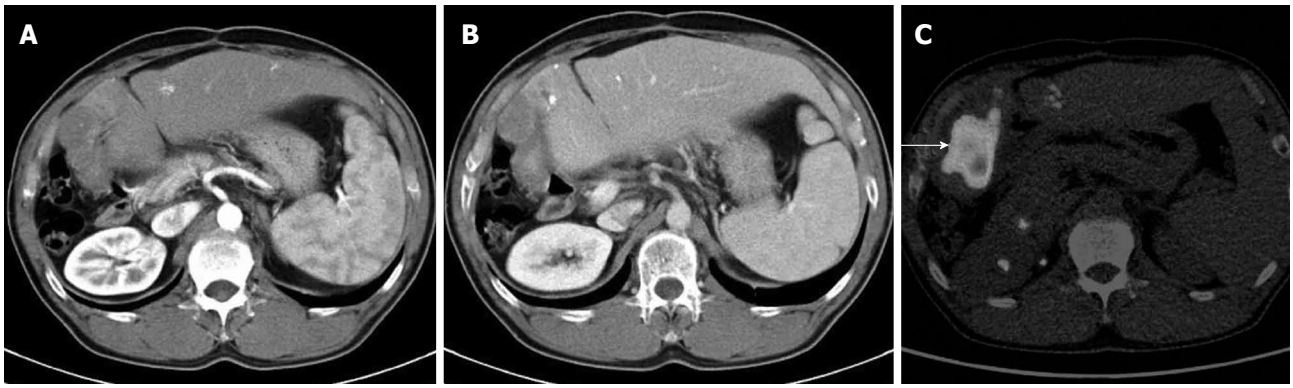

Figure 4 A case of recurrent hepatocellular carcinoma after right lobectomy. A, B: Arterial and portal phase of dynamic computed tomography (CT). Although tumor shadow was noted at the cut surface of the liver, it is difficult to determine recurrence due to poor enhancement; C: The tumor was diagnosed as recurrence of hepatocellular carcinoma because of strong fluorodeoxyglucose deposit (arrows).

microscopic metastases by PET; thus, PET remains a diagnostic imaging procedure with "high specificity but low sensitivity". As in the case of other cancers, PET is expected to be useful for detecting the presence/absence of distant metastases and diagnosing recurrent disease in CCC. In particular, PET is useful for the diagnosis of distant metastasis, as demonstrated by a study which showed that the treatment policy was determined by PET in 17% of cases[10], and another study which showed that PET was helpful in changing the treatment policy in 30% of cases[11]. PET is excellent for diagnosing recurrent disease, which is difficult to detect after hepatic resection or bile duct resection, due to its excellent contrast resolution. However, FDG uptake is reduced even in cases of CCC when recurrent cancer cells grow only gradually; thus, one of the pitfalls of FDG-PET is its low detection rate of recurrence.

PET EXAMINATION FOR METASTATIC LIVER CANCER

The visualization of liver metastases may depend on the histological features of the primary lesion. In general,

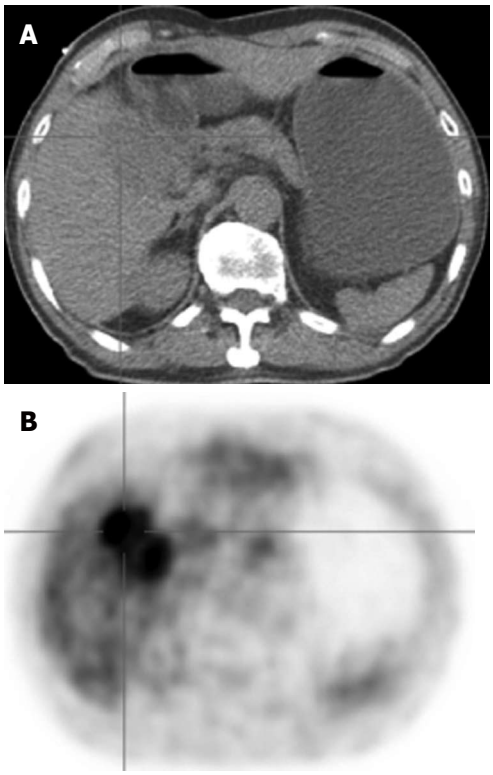

Figure 5 A case of cholangiocellular carcinoma. A: Non-contrast computed tomography (CT) obtained by PET/CT (low-dose CT); B: FDG-PET. Cholangiocellular carcinoma is usually depicted as an FDG-avid tumor unlike hepatocellular carcinoma.

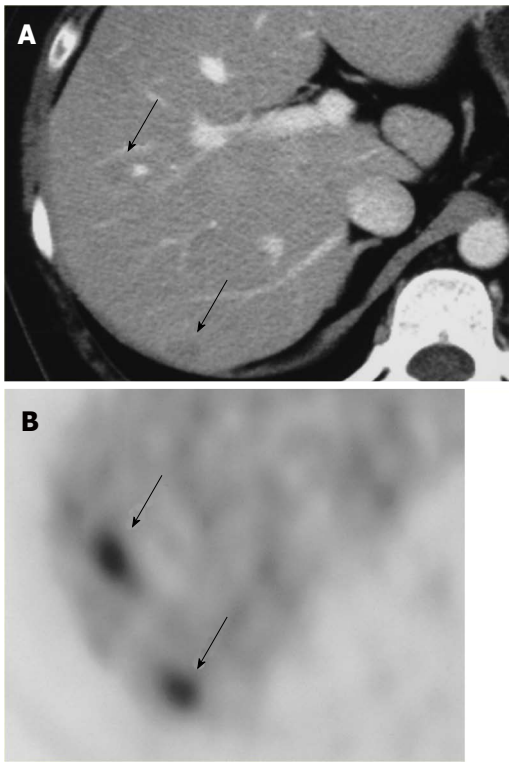

Figure 6 Liver metastases due to cervical cancer; A: CECT shows unclear low density areas in the liver (arrows). B: FDG-PET clearly depicts two liver metastases (arrows).

when the primary lesion shows marked FDG uptake, the metastases also show increased FDG uptake. However, the visualization of metastases on PET is also influenced by other factors, e.g., tumor-related factors, such as tumor size, cell density, presence of bleeding and necrosis, and external factors, such as blood glucose level and respiratory movements during data acquisition. Thus, the FDG uptake may differ between the primary and metastatic lesions depending on the aforementioned factors.

As compared with other imaging modalities, PET is not the most suitable for detecting small lesions because of its poor spatial resolution. Even if liver tumors show FDG avidity, tumor uptake of FDG must be stronger than the physiological liver uptake to be clearly recognized. It is evident that the contrast resolution of PET is superior to that offered by plain or contrast-enhanced CT (Figure 6). However, contrast-enhanced dynamic CT performed at an appropriate contrast timing using multi-detector row CT may allow the detection of small lesions that measure φ5 mm or less. MRI, which offers a good balance of both contrast resolution and spatial resolution, can also be an excellent diagnostic tool for visualizing liver metastases. Ruers *et al*[12] focused on the usefulness of PET for the detection of metastatic lesions in addition to primary hepatic tumors. Another report also emphasized the merit of FDG-PET to identify restaging disease, and FDG-PET has additional clinical value in the management of solitary liver metastases[13].

CT alone is sometimes inadequate for differentiating liver tumors, such as small cysts from hemangiomas or hepatic metastases. However, FDG-PET is useful for differentiating malignant from benign tumors because of its high specificity. Thus, a combination of modalities, i.e., CT with high sensitivity and PET with high specificity, may be the most effective combination for the diagnosis of liver metastases. As plain CT alone is inadequate for detecting liver metastases, we sometimes perform PET/contrast-enhanced CT at our facility to avoid performing contrast-enhanced CT and PET separately.

PET is expected to play an important role in the future for the assessment of therapeutic response to molecular-targeted drugs. Molecular-targeted drugs have been reported to be less effective in decreasing tumor size compared to conventional anticancer drugs. Consequently, the findings of PET have attracted attention as surrogate markers for the effects of molecular-targeted drugs. At present, molecular-targeted drugs are widely used in the treatment of lung cancer, breast cancer and gastrointestinal stromal tumors, which frequently occur with liver metastases. Since PET allows detection of not only liver metastases but also metastases elsewhere in the body, it is expected to play a more important role in the future for surrogate markers (Figure 7)[14].

PET EXAMINATION FOR BILIARY TRACT CANCER

PET examination for extrahepatic bile duct cancer: Ac-

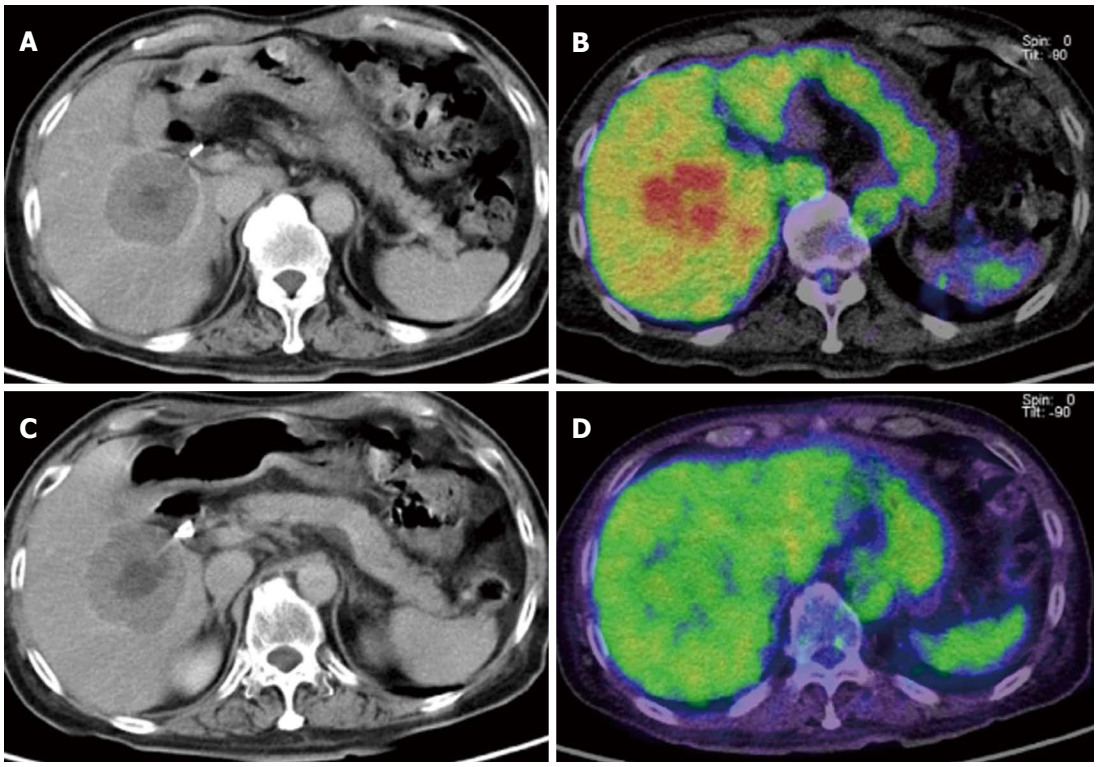

Figure 7 Monitoring therapeutic effect in liver metastases due to GIST gastrointestinal stromal tumor using Gleevec (imatinib mesylate). A, B: Before chemotherapy. A: CECT demonstrated bull's eye like low density in the liver, which was consistent with metastases; B: The metastatic tumor shown as an FDG-avid mass; C, D: After chemotherapy. (C) CECT shows similar mass before chemotherapy although (D) fluorodeoxyglucose (FDG) uptake significantly decreased. FDG-PET may more correctly reflect the therapeutic effect than CECT.

cording to the report of Petrowsky *et al*[10] the diagnostic accuracy of FDG-PET was 53% for extrahepatic bile duct cancer, indicative of a poor diagnostic performance. The flat "infiltrating" type, which is the most common type of extrahepatic bile duct cancer, is characterized by tubular adenocarcinoma with abundant fibrosis and endoluminal extension. Such histological and morphological features are major reasons for the apparently reduced uptake of FDG in these tumors.

On the other hand, the papillary type (one of the minor subtypes of bile duct cancer) which is characterized by a massive form and protruding growth into the lumen sometimes shows increased uptake of FDG. PET has been shown to have high sensitivity for the detection of this histological type of bile duct cancer[11,15].

It is desirable that PET examination for bile duct cancer be performed prior to the insertion of a PTCD tube, because stimulation due to the tip of the inserted tube causes cholangitis. It may cause a pseudo-positive result.

Although FDG also accumulates due to lymph node metastases of extrahepatic bile duct cancer, it is incapable of revealing microscopic metastases. In other words, FDG-PET is not useful for the detection of lymph node metastases from extrahepatic bile duct cancer because of its low sensitivity[15]. Thus, FDG-PET appears to have limited usefulness in the diagnosis of bile duct cancer.

PET EXAMINATION FOR GALLBLADDER CANCER

FDG-PET has a sensitivity of 75-100% and specificity of 80-89% for the detection of primary gallbladder cancer as mentioned in the literature (Figure 8). However, ultrasound, MRI, and contrast-enhanced CT are better for the detection of this cancer because of their high spatial resolution. FDG-PET is reported to be useful for differentiating benign from malignant gallbladder tumors[16], although acute cholecystitis and mass-forming xanthogranulomatous cholecystitis may also show marked FDG uptake (Figure 9). Thus, the ability of this modality to differentiate these tumors remains controversial. Moreover, FDG-PET appears to be a poor tool for detecting early gallbladder cancer because of its poor spatial resolution. For gallbladder cancer, the primary aim of performing FDG-PET is to identify distant metastases and recurrence.

PET EXAMINATION FOR PANCREATIC CANCER

PET examination of the pancreas is covered by the National Health Insurance for "differentiating pancreatitis from pancreatic cancer." In 2006, the health insurance coverage was expanded to the diagnosis of metastasis

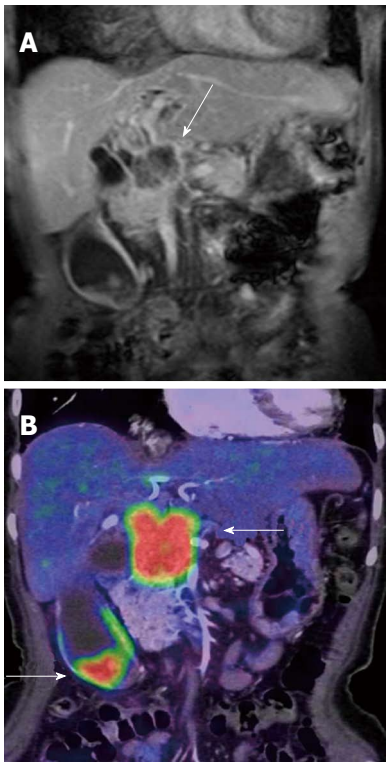

Figure 8 Gall bladder cancer with lymph node metastases near the pancreatic head. A: CE-MRI (coronal section) shows poorly enhanced tumor near the pancreatic head (arrow). The tumor was thought to be a primary lesion at first; B: PET/CT (with CE) demonstrated two FDG-avid lesions (arrow). Gall bladder cancer and its metastases usually show strong FDG deposits.

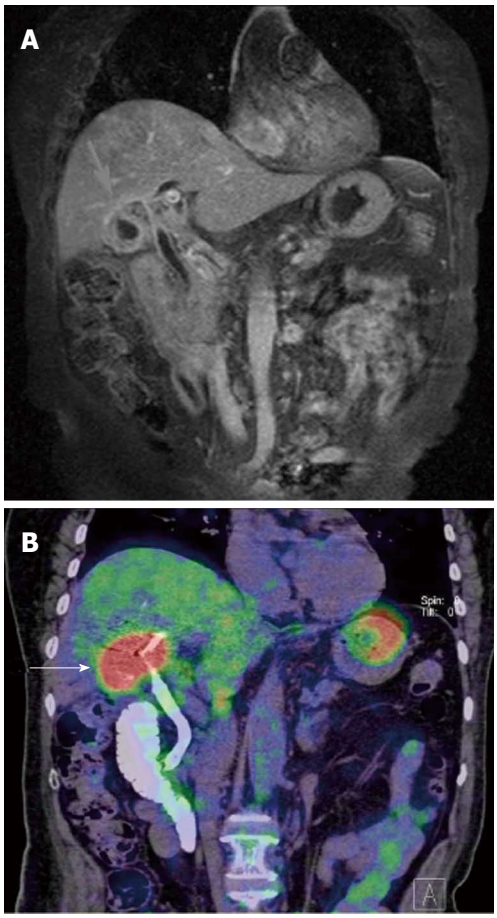

Figure 9 A case of acute cholecystitis. A: CE-MRI (coronal section) shows irregular wall thickening of gall bladder (arrow) with hilar bile duct stenosis; B: PET/CT performed after PTC. Gall bladder shows strong FDG accumulation (arrow) although pathological diagnosis was acute cholecystitis. Discrimination between active inflammation and tumor is difficult using accumulation of fluorodeoxyglucose.

and recurrence.

Conventionally, it has been thought that FDG-PET would be useful for differentiating pancreatic cancer from tumor-forming pancreatitis, as cancers show more marked FDG uptake as compared to pancreatitis. Chronic pancreatitis can be differentiated from cancer due to its lower FDG uptake compared to that of cancer. However, inflammatory cells also show increased FDG uptake because of accelerated glucose metabolism, therefore, the differentiation between acute pancreatitis and cancer is difficult. Accordingly, positive findings obtained in patients who have clinical symptoms of pancreatitis or biochemical evidence of inflammation should be interpreted with caution. Imdahl A *et al*[17] reported that delayed PET imaging is useful for the differentiation of cancer from acute pancreatitis as cancer shows increased deposits in the delayed phase. However, a controversial study has reported that FDG uptake is enhanced in the delayed phase even in cases of inflammation. Thus, FDG-PET cannot be regarded as a reliable imaging tool for differentiating between acute pancreatitis and cancer even when delayed images are obtained.

A possible diagnosis of pancreatitis can be made when a tumor shows gradually decreasing FDG uptake within a short interval.

FDG-PET has been reported to play a significant role in the differentiation of IgG4-related pancreatitis among cases of pancreatitis. This disease entity has been widely recognized in recent years, and an increasing number of patients are diagnosed with IgG4-related pancreatitis. This disease has been defined as a systemic disease complicated by inflammation in various organs other than the pancreas. FDG-PET is reported to be an effective tool for evaluating these lesions[18] because various organs, such as the salivary glands, hilar lymph nodes, lungs (interstitial pneumonia), kidney (nephritis) and retroperitoneum are sometimes involved simultaneously. In other words, abnormal FDG uptake other than in the pancreas may raise suspicion of IgG4-related pancreatitis rather than pancreatic cancer (Figure 10).

In cases of pancreatic cancer, PET is most useful for identifying distant metastasis and recurrence. Local recurrence is sometimes difficult to evaluate by conventional morphological imaging alone because it is associated with treatment-related morphological changes, such as fibrosis, hemorrhage, etc. Moreover, as pancreatic cancer has poor vascularity, it is difficult to evaluate the tumor based on the dynamic contrast study. Under these circumstances, PET may be of significant value for visualizing the lesion

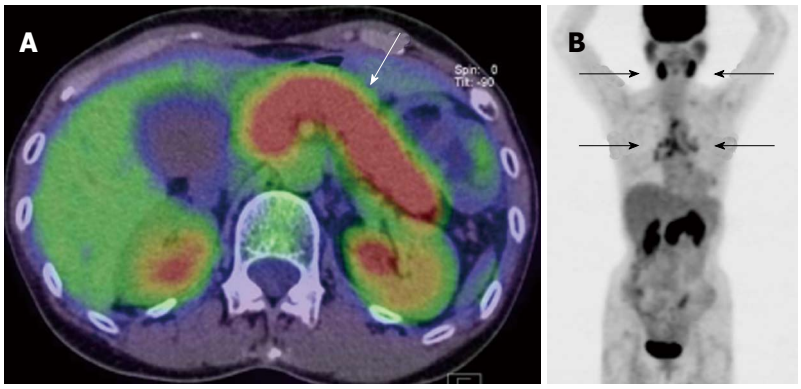

Figure 10 IgG4 related pancreatitis. A: PET/CT shows strong fluorodeoxyglucose (FDG) accumulation in the whole pancreas with swelling (arrow); B: MIP image of PET. Besides diffuse uptake in the pancreas, symmetrical FDG deposits were noted in the bilateral salivary glands and hilar, mediastinal lymph nodes (arrows). Distribution in the involved organs is characteristic of this disease.

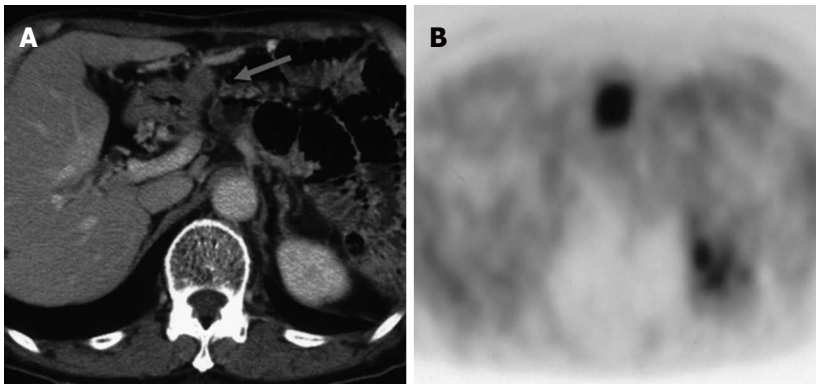

Figure 11 A case of elevated tumor marker after resection of pancreatic cancer. A: Small nodule (arrow) was missed by initial CECT; B: PET detected the nodule much more clearly.

due to its high contrast resolution.

Another reason for the difficulty in detecting distant metastasis based on conventional imaging is that it is hard to predict the site of metastasis. PET whole body imaging is of great value particularly when recurrence is suspected by clinical symptoms such as the development of pain or increased serum levels of tumor markers, *etc.* (Figure 11). Ruf *et al*[19] performed PET, CT and MRI in 23 patients with clinically suspected recurrence of pancreatic cancer based on the development of postoperative pain, decreased body weight and increased serum levels of tumor markers, and confirmed recurrence by PET in 22 patients (96%) on PET, but in only 9 patients (39%) by CT/MRI.

Even PET alone has been shown to be superior to CT in previous publications. However, it is difficult to differentiate between physiological and pathological accumulation in the ureter, bladder and intestinal tract by PET alone because of a lack of anatomical information. To resolve this issue, a PET/CT system was developed. PET/CT can offer combined images of PET with CT to add anatomical information to FDG uptake. PET/CT may replace dedicated PET scanners in the near future.

CONCLUSION

In this review, we have outlined the usefulness and limitations of PET for the evaluation of lesions in the liver, gallbladder, and pancreas. Ultrasound and dynamic CT are the simplest and most economical imaging modalities for the diagnosis of lesions in these organs. In addition, many other imaging tools, such as MRI, EUS and IDUS, are also available for detailed evaluation of these organs. All of these methods are used as "high-resolution" diagnostic imaging tools for visualizing "locoregional areas," and PET is unlikely to play an important role in the local diagnosis of these lesions. In contrast, PET (PET/CT) involves whole-body imaging and is useful for visualizing distant metastases and unexpected recurrences. Therefore, PET/CT appears to be of significance in evaluation of the whole body in cases with advanced or atypical tumors. Since PET/CT began to be covered by the National Health Insurance in 2002, we perform PET/contrast-enhanced CT in cases of advanced cancer for evaluation of the presence of distant metastases, for evaluation of therapeutic outcomes, and for the early diagnosis of recurrence. I have also recommended performing "PET/contrast-enhanced CT scans first" for examination of the whole body (except for the head). Simultaneous PET and contrast-enhanced CT scanning appears to be an efficient method with improved diagnostic accuracy, and it is unnecessary to perform PET and contrast-enhanced CT separately.

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World J Clin Oncol 2011 May 10; 2(5): I
ISSN 2218-4333 (online)

Acknowledgments to reviewers of *World Journal of Clinical Oncology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Clinical Oncology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

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World J Clin Oncol 2011 May 10; 2(5): I
ISSN 2218-4333 (online)

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January 15-16, 2011
Melanoma 2011: 21st Annual Cutaneous Malignancy Update, San Diego, CA, United States

January 15, 2011
Current Trends in Breast Cancer: Updates From the 2010 San Antonio Breast Cancer Symposium, Dallas, TX, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA, United States

January 21-23, 2011
8th Meeting of the EAU Section of Oncological Urology, London, England, United Kingdom

January 27-28, 2011
2nd National Conference: Recent Advances in Renal and Bladder Cancer, London, United Kingdom

January 27-28, 2011
8th Annual Cancer Drugs Research & Development, San Diego, CA, United States

February 10-12, 2011
17th Annual NOCR Meeting, Las Vegas, NV, United States

February 19-22, 2011
Scripps Cancer Center's 31st Annual Conference: Clinical Hematology and Oncology, San Diego, CA, United States

February 24-26, 2011
European Multidisciplinary Conference in Thoracic Oncology (Lung 2011-EMCTO), Lugano, Switzerland

February 25-27, 2011
7th European Congress on Hematologic Malignancies: From Clinical Science to Clinical Practice, Budapest, Hungary

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64th Society of Surgical Oncology Annual Cancer Symposium 2011, San Antonio, TX, United States

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8th Annual Oncology Nursing Advanced Practice: Innovation through Practice, San Diego, CA, United States

March 07-09, 2011
9th International Symposium on Targeted Anticancer Therapies, Paris, France

March 09-13, 2011
16th National Comprehensive Cancer Network Annual Conference (NCCN 2011), Hollywood, FL, United States

March 11-12, 2011
12th European Congress: Perspectives in Lung Cancer, Torino, Italy

March 14-18, 2011
Oncology Imaging Update in Costa Rica, Guanacaste, Costa Rica

March 17-19, 2011
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March 18-22, 2011
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April 29-30, 2011
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June 03-07, 2011
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June 20-23, 2011
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June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 23-25, 2011
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July 03-07, 2011
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World Journal of Clinical Oncology

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World J Clin Oncol 2011 May 10; 2(5): I-V
ISSN 2218-4333 (online)

INSTRUCTIONS TO AUTHORS

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ISSN 2218-4333 (online)

Indexed and Abstracted in

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Acknowledgments

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

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Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

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