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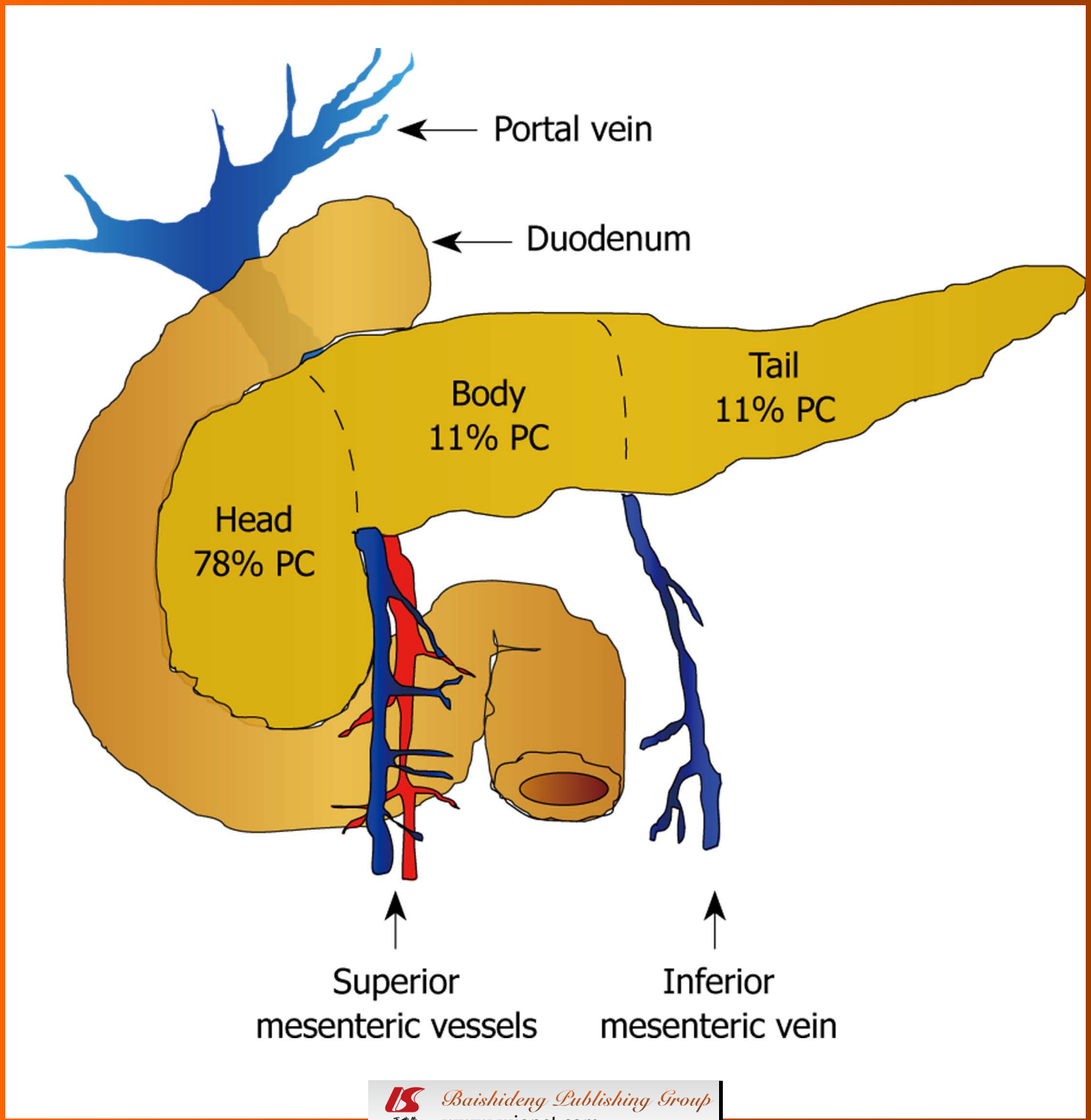

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EDITORIAL

MicroRNAs in pancreatic ductal adenocarcinoma

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Abstract

Ductal adenocarcinoma of the pancreas is a lethal cancer for which the only chance of long-term survival belongs to the patient with localized disease in whom a potentially curative resection can be done. Therefore, biomarkers for early detection and new therapeutic strategies are urgently needed. miRNAs are a recently discovered class of small endogenous non-coding RNAs of about 22 nucleotides that have gained attention for their role in downregulation of mRNA expression at the post-transcriptional level. miRNAs regulate proteins involved in critical cellular processes such as differentiation, proliferation, and apoptosis. Evidence suggests that deregulated miRNA expression is involved in carcinogenesis at many sites, including the pancreas. Aberrant expression of miRNAs may upregulate the expression of oncogenes or downregulate the expression of tumor suppressor genes, as well as play a role in other mechanisms of carcinogenesis. The purpose of this review is to summarize our knowledge of deregulated miRNA expression in pancreatic cancer and discuss the implication for potential translation of this knowledge into clinical practice.

Key words: MicroRNAs; Pancreatic cancer

Peer reviewer: Yoshiharu Motoo, MD, PhD, FACP, FACG, Professor and Chairman, Department of Medical Oncology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan

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INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related mortality in the United States, with 36 800 estimated deaths in 2010, with the great majority being due to ductal adenocarcinomas[1]. Due to the asymptomatic onset of pancreatic cancer, most patients are in advanced or metastatic condition at the time of diagnosis, resulting in poor prognosis. Most patients found to have pancreatic cancer die within 12 mo, and few survive 5 years after diagnosis. The poor prognosis of these patients is due to its late clinical presentation with symptoms, early and aggressive local invasion, and high metastatic potential[2]. Advances in chemo-radiation therapy have been slow over the last few decades, and the overall prognosis in pancreatic cancer has remained essentially unchanged. The only chance of long-term survival with pancreatic adenocarcinoma belongs to

patients with localized disease in whom a potentially curative resection can be performed. Earlier diagnosis and better treatments are urgently needed to improve the survival rate of pancreatic cancer.

Histologically, the pancreas is divided largely into the exocrine and endocrine pancreas, the former consists of ducts and acini, and the latter constitutes the islets that have a hormone secretory function. The most common type of pancreatic cancer, representing about 85% of all pancreatic cancer types[3], arises from the epithelial lining of the exocrine pancreatic duct. Therefore, in this review, we mainly focus on miRNA expression in pancreatic ductal adenocarcinoma (PDAC).

Pancreatic cancer originates from the sequential accumulation of multiple genetic alterations[4]. In the past several decades, significant progress in the identification and characterization of cancer-related gene abnormalities has been made. However, this progress has not yet been effectively translated into new reliable biomarkers that lead to the earlier diagnosis or more effective treatment of this deadly disease. Specific miRNAs affecting tumor suppressor genes or oncogenes may be critical biomarkers that lead to early detection, or potential drug targets for pancreatic cancer.

Although regulation of oncogenes and tumor suppressor genes, by genetic and epigenetic changes has been regarded as being important in the development of pancreatic cancer[5-8], the exact molecular mechanisms of carcinogenesis and of pancreatic cancer progression remain unknown. Gene silencing is frequently caused by epigenetic changes, such as DNA methylation or altered miRNA expression rather than by genetic events such as mutation or deletion. miRNA binding at the 3' untranslated region (UTR) in tumor suppressor genes is an epigenetic change that may contribute to carcinogenesis and cancer progression. Although relatively few genetic mutations have been identified in PDAC, aberrant miRNA expression has been found in both pancreatic tumor tissues and cell lines.

BIOGENESIS, FUNCTION AND TARGETS OF miRNAs

miRNAs are about 22-nucleotide non-protein-coding RNA molecules that regulate gene function in various gene silencing pathways. These molecules are phylogenetically conserved and play important roles in cell survival, proliferation, differentiation, apoptosis and angiogenesis[9,10]. miRNA expression patterns differ, depending upon cell, tissue, and disease types, and changes in these expression patterns have been implicated as an important player in carcinogenesis.

The miRNA, lin-4, was first discovered in 1993 as a small non-coding RNA that regulates *Caenorhabditis elegans* development by negative regulation of lin-14 protein expression[11]. In 2000, the second miRNA, let-7, was identified from *C. elegans* and confirmed as a 21-nucleotide small RNA[12]. Since the discovery of lin-4 and let-7, many more miRNAs have been identified using various experimental and computational methods[13]. In the most recent database (miRBase 15 release), over 15000 mature miRNAs are identified in 133 species[14]. Although they do not encode proteins, miRNAs are transcribed by RNA polymerase Ⅱ as independent units in the nucleus (Figure 1). The primary transcript (pri-miRNA) is processed by the nuclear RNase Ⅲ Drosha and its cofactor DGCR8/Pasha to generate precursor miRNA (pre-miRNA), a 60-70-nucleotide RNA that has a stem loop structure[15-17]. Pre-miRNA is rapidly exported to the cytoplasm by exportin 5 in a Ran-GTP-dependent manner, where it is further processed by a second RNase Ⅲ, dicer, which cuts off the terminal loop and generates a mature about 22-nucleotide miRNA. Mature miRNA is initially part of an imperfect double-stranded RNA duplex called miRNA/miRNA*. This double-stranded RNA duplex binds to a protein (Argonaute 2) as a part of the RNA induced silencing complex (RISC), while the strand of the duplex that is complementary miRNA* is released. The RISC, containing its miRNA, binds to the target mRNA and triggers either mRNA degradation or inhibition of translation, depending on the degree of complementarity between miRNA and its target[18-21].

Each miRNA regulates multiple target genes. In fact, bioinformatics predict that miRNAs may regulate about 50% of all human genes[22]. Therefore, precise identification of miRNA targets is critical to advance our understanding of the role of miRNA regulation in carcinogenesis. Accurate identification of physiologically active miRNA targets is now a considerable impediment to the functional characterization of individual miRNAs.

miRNAs negatively regulate their target mRNAs primarily through base-pairing interactions, which leads to either mRNA degradation or translational inhibition depending upon the degree of match between the "seed sequence" (positions 2-7 at the 5' side) of miRNA and 3'UTR of mRNA (Figure 1). When the seed sequence perfectly or partially matches with target 3' UTR of mRNA, then it may lead to degradation of the mRNA or inhibit translation[18-21]. Based upon publicly available algorithms, each miRNA has several hundred potential target mRNAs. Recent reports have further indicated that secondary structures of mRNA contribute to target recognition sites, due to the fact that there is energetic cost to free base-pairing interactions for accessible targets[23-25]. Kertesz *et al*[26] have shown that target site accessibility is as important as sequence match in the seed sequence region, and that effective miRNA binding requires unpairing of local regions that flank the target, as well as that the target region is unpaired in thermodynamic equilibrium. Thus, simultaneous profiling of miRNA and mRNA, as well as protein expression, has recently been shown to be a timely strategy to achieve the required precision in the identification of functional miRNA targets[27-30].

In summary, miRNAs regulate their targets by direct mRNA cleavage or translational inhibition. miRNAs are coded by genes and are transcribed by RNA polymerase Ⅱ. They have their own regulatory elements and appear as transcriptional units containing either unique or multiple

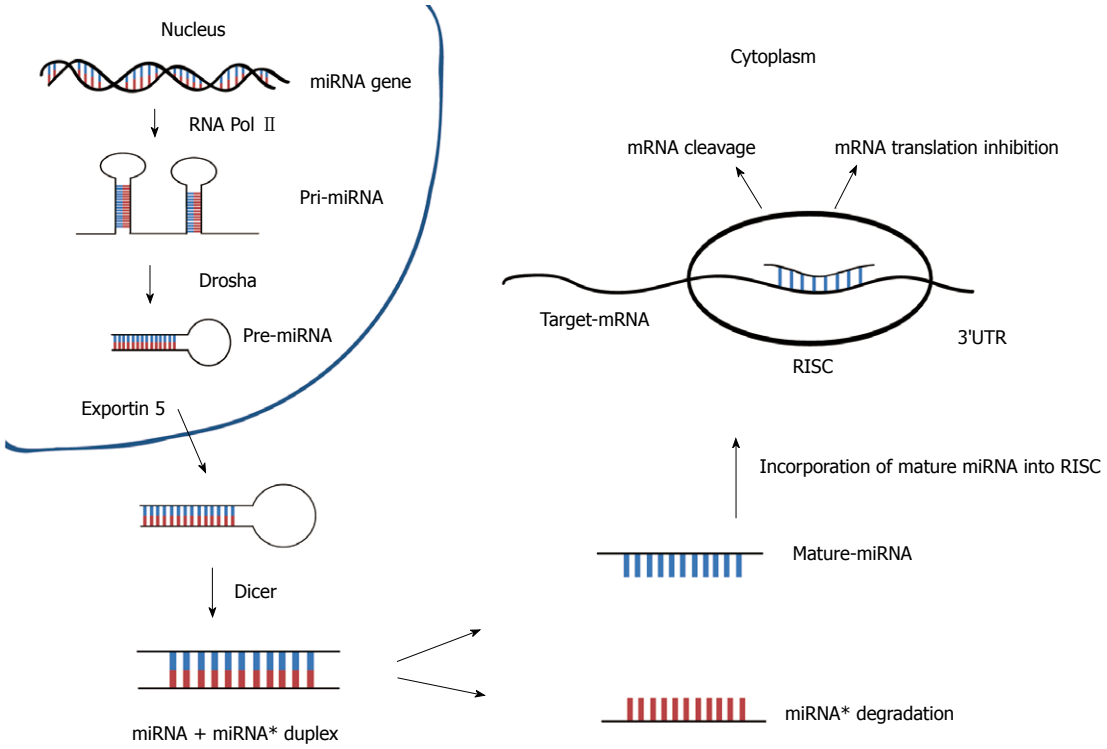

Figure 1 Schematic representation of miRNA biogenesis. miRNA genes are transcribed by RNA polymerase Ⅱ (RNA pol Ⅱ) into long transcripts called primary miRNAs (pri-miRNA) that contain multiple stem-loop/hairpin structures as independent units in the nucleus. pri-miRNA is processed by the nuclear RNase Ⅲ Drosha and its cofactor DGCR8/Pasha to generate precursor miRNA (pre-miRNA). The pre-miRNA is rapidly exported to the cytoplasm by exportin 5, where it is further processed by a second RNase Ⅲ, Dicer, that cuts off the terminal loop and generates a mature about 22-nucleotide miRNA. The mature miRNA is an imperfect double-stranded RNA duplex called miRNA/miRNA*. The double-stranded RNA duplex binds to a protein (Argonaute 2) as a part of the RNA induced silencing complex (RISC), while one of the strands of the duplex, which is complementary miRNA*, is released. The RISC, which contains its miRNA, binds to the target mRNA and triggers either mRNA degradation or inhibition of translation, depending on the degree of complementary between miRNA and its target.

miRNAs (polycistronic). Circumstantial evidence linking miRNAs and carcinogenesis has been observed in over 50% of miRNA genes, which are located within regions of loss of heterozygosity, amplification, fragile sites, viral integration sites, and other cancer-associated genomic regions. Recent high-throughput methodologies have shown deregulated miRNA expression in an increasing number of human cancers, including pancreatic cancer. Differences in miRNA expression patterns have been found to distinguish tumors of different developmental origin, even better than traditional mRNA expression profiling[31].

miRNA AND HUMAN CANCER

The first evidence of miRNA involvement in human cancer came from a study that characterized chromosome 13q14 in chronic lymphocytic leukemia (CLL)[32]. Calin *et al*[32] have shown that miR-15 and miR-16 are deleted or downregulated in about 70% of CLL cases. The tumor suppressive role of miR-15a and miR-16-1 has been supported further by the discovery that expression of both miRNAs inversely correlates with expression of the anti-apoptotic BCL2 protein[33]. BCL2 expression is inhibited by miR-15a and miR-16-1 and these repressions induce apoptosis in leukemic cells. These data suggest a model whereby somatic deletions of miR-15a and miR-16-1 aid leukemogenesis by allowing tumors to escape apoptosis.

Since this first report of aberrant miRNA expression in CLL, deregulation of a number of miRNAs has been found in other human cancers. While some miRNAs, including miR-125b and miR-145 in breast cancer, and let-7 in lung cancer, are reduced, others such as miR-21 and miR-155 in breast cancer, miR-155 in lung cancer, the precursor of miR-155 in Burkitt lymphoma, miR-17-92 cluster and miR-155 in B-cell lymphoma, are overexpressed[31,34-39]. These studies also have shown that miRNA expression signatures correlate well with specific clinical cancer characteristics, and could be used to differentiate normal and cancerous tissues, as well as subtypes of malignancy[40-43].

Deregulation of miRNA in cancer could be caused by: (1) chromosomal regional gain, loss or translocation; (2) aberrant expression and activation of transcriptional factors; (3) epigenetic alterations; and (4) changes in miRNA processing[44]. As described above, the association between chromosomal abnormality and miRNA expression in CLL is due to downregulation of the miR16-1/15a cluster in chromosome 13q14.3[32]. In contrast, upregulation of miR-155 in tumor appears to be due to transcriptional regulation and aberrant miRNA processing[36,45]. miR-155 is encoded in non-coding DNA known as BIC (B-cell integration cluster), located at chromosome 21q21.3, where neither amplification nor loss of heterozygosity is observed. Several studies have shown that miR-155 is in-

Table 1 miRNA deregulation in human pancreatic cancer

miRNA	Lee *et al*[49]	Szafranska *et al*[50]	Bloomston *et al*[51]	Zhang *et al*[52]	Other	Outcome
let-7					↓[53]	
let-7d	↑[1]					
let-7f-1	↑					
miR-10a			↑		↑[54]	
miR-10b			↑			
miR-15b	↑			↑		
miR-16-1	↑					
miR-18a		↑				
miR-21	↑		↑		↑[55, 56]	Poor[55]
miR-23a			↑			
miR-23b			↑			
miR-24-1,2	↑					
miR-29c		↓				
miR-31		↑				
miR-92-1	↑					
miR-93		↑				
miR-95				↑		
miR-96		↓				
miR-99			↑			
miR-100	↑		↑			
miR-100-1/2			↑			
miR-103-2			↑			
miR-107	↑		↑			
miR-125a			↑			
miR-125b-1	↑		↑			
miR-130b		↓				
miR-139	↓					
miR-141		↓				
miR-142-P	↓					
miR-143		↑	↑			
miR-145		↑				
miR-146			↑			
miR-146a		↑				
miR-148a		↓	↓			
miR-148b		↓	↓			
miR-150		↑				
miR-155	↑	↑	↑			Poor[57]
miR-181a	↑		↑			
miR-181b			↑			
miR-181b-1			↑			
miR-181b-2			↑			
miR-181c	↑		↑			
miR-181d			↑			
miR-186				↑		
miR-190				↑		
miR-196a		↑		↑		miR-196a-2; Poor[51]
miR-196b		↑				
miR-199a-1			↑			
miR-199a-2			↑			
miR-200b				↑		
miR-203		↑				Poor[57]
miR-205		↑	↑			
miR-210		↑	↑			Poor[57]
miR-212	↑					
miR-213			↑			
miR-216		↓				
miR-217		↓				
miR-220			↑			
miR-221	↑	↑	↑	↑		
miR-222		↑	↑	↑		Poor[57]
miR-223		↑	↑			
miR-224		↑				
miR-301	↑					
miR-345	↓					
miR-375			↓	↓		
miR-376a	↑					
miR-424	↑					

[1]Arrows indicate increased (↑) or decreased (↓) expression of the specified miRNA.

duced at the transcriptional level by transforming growth factor β/Smad, nuclear factor-κB and activator protein-1 family transcription factors through direct interaction with the miR-155/BIC promoter[46-48]. Further studies have shown that miR-155 processing also regulates mature miR-155 expression levels[36,45], suggesting that overexpression of miR-155 in cancer is due to transcriptional activation and miRNA processing.

miRNA EXPRESSION PROFILE IN NORMAL PANCREATIC TISSUE AND PANCREATIC TUMOR

miRNA expression profiles in pancreatic tumor tissues are different from those identified in normal pancreas or in chronic pancreatitis. Most miRNA expression profile analyses show that miRNAs are deregulated in tumor tissues as compared to normal pancreas, and that the expression pattern is tissue specific. Several studies focusing on miRNA expression profiles in pancreatic tissues have identified a number of differentially expressed miRNAs. Table 1 summarizes the aberrantly expressed miRNAs in human pancreatic cancer and their association with patient survival.

Szafranska *et al*[50] have performed the first comprehensive miRNA expression profile study in tissues from normal pancreas (n = 7), chronic pancreatitis (n = 7), PDAC (n = 10) and 33 human tissues of different non-pancreatic origin, to identify miRNA candidates with a potential for future clinical application from a pool of 377 known and novel miRNAs. The authors have found that two miRNAs, miR-216 and miR-217, are pancreas-specific. These results were in agreement with those of two previous studies[58,59]. Furthermore, both miR-216 and miR-217 are absent or only minimally expressed in pancreatic carcinoma tissues and cell lines. Therefore, miR-216 and miR-217 are potential biomarkers. Based upon clustering analysis, the three pancreatic tissues types can be classified according to their respective miRNA expression profiles. Among 26 miRNAs that have been identified as most prominently deregulated in PDAC, only miR-217 and miR-196a have been found to discriminate between normal pancreas, chronic pancreatitis and tumor tissues. These miRNAs are also potential biomarkers.

Recently, expression of 201 miRNA precursors (representing 222 miRNAs) was profiled in pancreatic adenocarcinoma, paired with benign tissue, normal pancreas, chronic pancreatitis and pancreatic cancer cell lines with the real-time PCR miRNA array[49]. These three cell types could be classified by the clustering algorithm. One hundred miRNA precursors have been identified as aberrantly

expressed miRNAs including known ones in other cancers and novel ones in pancreatic tumor. A list of the top 20 aberrantly expressed miRNA precursors has been proposed as a signature for pancreatic adenocarcinoma.

Bloomston *et al*[51] have identified a large global expression pattern of miRNAs that can differentiate PDAC from chronic pancreatitis with 93% accuracy. Among several deregulated miRNAs in the pancreatic cancers, most notably, miR-21 and miR-155 are uniquely overexpressed in pancreatic tumor, as compared to tissues from normal pancreas and chronic pancreatitis. Both miR-21 and miR-155 have been suggested to play an important role in functioning as a proto-oncogene and have been shown to be overexpressed in several cancers. These authors have performed an miRNA microarray profiling with about 1100 miRNA probes, which included 326 human miRNAs, using microdissected pancreatic tumor tissues.

Zhang *et al*[52] have evaluated 95 miRNAs, selected from pancreatic cancer profiling, and correlated them to their potential biological functions related to cancer biology, cell development, and apoptosis. Among them, eight miRNAs (miR-196a, miR-190, miR-186, miR-221, miR-222, miR-200b, miR-15b, and miR-95) are differentially expressed in most pancreatic cancer tissues and cell lines. All of these eight genes are significantly unregulated, from 3- to 2018-fold, in pancreatic tumors as compared with normal control samples.

In summary, these profiling data may provide novel insights into the miRNA-driven mechanisms involved in pancreatic carcinogenesis, and offer new potential targets for early detection and therapeutic strategies in pancreatic cancer.

miRNAS AS BIOMARKERS FOR PANCREATIC CANCER DIAGNOSIS

Development of biomarkers for pancreatic cancer is especially critical because most patients with this disease remain asymptomatic until the disease progresses to become locally advanced or develops distant metastases. Therefore, most of these patients are surgically inoperable at the time of diagnosis. Sensitive and specific biomarkers for pancreatic cancer are urgently needed to offer better therapeutic options and survival outcome.

Over the years, a number of protein- and DNA-based biomarkers have been proposed as markers of early detection for pancreatic cancer. However, most of these markers fail to have clinical potential, and they have not influenced patients' survival. Since the first discovery of miRNAs by Lee *et al*[11] in 1993, many researchers have investigated expression profiles, biological functions and targets of miRNAs in carcinogenesis and tumor progression, with the purpose of translating the results to clinical settings.

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) of the pancreas is not likely to be used routinely for screening for PDAC because of its invasive nature. However, this procedure has recently emerged as a specific and minimally invasive modality for preoperative diagnosis and staging of pancreatic cancer. Furthermore, EUS-FNA may also be useful for screening high-risk individuals, as well as for the prognosis and predicting the response to treatment in cases in which the tumor is inoperable[60-62]. Szafranska *et al*[63] have identified potential miRNA markers in EUS-FNA biopsies of pancreatic tissue. The combination of expression pattern of miR-196a and miR-217 can differentiate PDAC cases from healthy controls and chronic pancreatitis in the FNA samples. Furthermore, miR-196a expression is likely specific to PDAC cells and is positively associated with the progression of PDAC.

Carcinogenesis in PDAC develops with a multistep progression from morphologically distinct non-invasive precursor lesions within exocrine pancreatic ducts[64]. These precursors include the intraductal papillary mucinous neoplasms (IPMNs), the mucinous cystic neoplasms, and pancreatic intraepithelial neoplasia (PanIN). Two studies have been carried out to detect expression patterns of miRNA in IPMNs and PanIN. IPMNs are grossly visible, non-invasive, mucin-producing precursors of pancreatic cancer within the main pancreatic duct or one of its branches[65,66]. In contrast, PanINs are non-invasive, microscopic epithelial neoplasms, arising within smaller pancreatic ducts, < 5 mm in diameter, and characterized by cytological and architectural atypia[65,67]. Habbe *et al*[68] have reported significant overexpression of 10 miRNAs in IPMNs (n = 15). miR-155 and miR-21 show the highest relative fold-changes in the precursor lesions. These results have been validated by *in situ* hybridization analysis. miR-155 and miR-21 are upregulated in most IPMNs [83% (53/64) and 81% (52/64)] as compared to normal ducts [7% (4/54) and 2% (1/54)]. With these promising data, the potential use of these miRNAs as biomarkers has been evaluated in pancreatic juices. A total of 15 pancreatic juice samples from 10 patients with IPMNs, and five with other pancreatobiliary disorders obtained at the time of surgical resection were measured for relative levels of miR-155 and miR-21 by quantitative real-time RT-PCR. Upregulation of both miR-155 and miR-21 in the subset of IPMN-associated pancreatic juices was observed, as compared with control samples. These results indicate that aberrant miRNA expression occurs early in the precursor lesion during the multiple stages of pancreatic cancer development, and miRNA profiles may be assessed with more accessible clinical samples, such as pancreatic juice, and could be used as a diagnostic tool.

du Rieu *et al*[69] have investigated miRNAs in PanIN tissues from a conditional Kras (G12D) mouse model (n = 29) and from human origin (n = 38). Expression of miR-21, miR-205 and miR-200 has been found to be positively associated with PanIN progression in the Kras (G12D) mouse model. In the human tissues, expression of miR-21, miR-221, miR-222 and let-7a increases with PanIN grade. The authors, using *in situ* hybridization analysis, have observed that miR-21 expression is concen-

Table 2 miRNAs and their targets involved in human pancreatic cancer

miRNA	Function	Targets	Related cellular events	Ref.
let-7	Suppress	RAS[71]	Inhibit cell proliferation, KRAS expression, and mitogen-activated protein kinase activation	[53]
let-7, miR-200	Suppress		Reverse EMT	[72]
Let-7a	Suppress	RAS	Attenuate KRAS expression and radiosensitize tumor cell	[73]
miR-10a	Oncogenic	HOXB1, 3	Promote metastatic behavior	[54]
miR-21	Oncogenic		Induce cell proliferation, invasion, chemoresistance	[56]
miR-21	Oncogenic		Potentially associated with cell proliferation	[74]
miR-200c	Suppress		Potentially associated with G0/G1 arrest and increased apoptotic rate	
miR-21, miR-221	Oncogenic	PTEN, RECK, CDKN1B	Arrest cell cycle, induce apoptosis, and sensitize the effects of gemcitabine with inhibition of miR-21 or -221	[75]
miR-22	Suppress	SP1, ESR1	Potentially inhibit tumorigenesis	[76]
miR-34	Suppress	BCL2, NOTCH1/2	Inhibit clonogenic cell growth and invasion, induce apoptosis and G1 and G2/M arrest in cell cycle, sensitize to chemotherapy and radiation, and potentially inhibit pancreatic cancer stem cells	[77]
miR-107	Suppress	CDK6	Induce in vitro cell growth downregulation	[78]
miR-155	Oncogenic	TP53INP1	Inhibit apoptosis	[79]
miR-194, miR-200b, miR-200c, miR-429	Oncogenic	EP300	Potentially promote metastatic behavior	[80]
miR-224, miR-486	Oncogenic	CD40	Potentially associated with invasion and metastasis	[81]

BCL2: B-cell CLL/lymphoma 2; CD40: CD40 molecule; CDK6: Cyclin-dependent kinase 6; CDKN1B: Cyclin-dependent kinase inhibitor 1B; EP300: E1A binding protein p300; ESR1: Estrogen receptor 1; HOXB1, 3: Homeobox B1, 3; KRAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; NOTCH1/2: Notch 1/2; PTEN: Phosphatase and tensin homolog; RECK: Reversion-inducing-cysteine-rich protein with kazal motifs; SP1: Sp1 transcription factor; TP53INP1: Tumor protein p53 inducible nuclear protein 1; EMT: Epithelial-to-mesenchymal transition.

trated in the dysplastic ductal epithelial cells. Using PDAC-derived cell lines, they also have noted that miR-21 expression is regulated by Kras (G12D) and epidermal growth factor receptor (EGFR).

Wang *et al*[70] have studied plasma samples from patients with PDAC and have found that four miRNAs (miR-21, miR-210, miR-155 and miR-196a) are able to differentiate pancreatic cancer patients from healthy controls, with moderate accuracy (sensitivity: 64%, and specificity: 89%). In summary, these studies suggest a potential value of miRNAs in the clinical setting as a potential diagnostic tool for PDAC.

miRNAS AS ONCOGENES AND TUMOR SUPPRESSORS

miRNAs are functionally classified into oncogenes or tumor suppressors based upon their targets, thus binding to oncogenes or tumor suppressor genes. Therefore, oncogenic miRNAs are upregulated in tumors, whereas tumor suppressor miRNAs are downregulated. The functions and targets of a handful of miRNAs have been investigated in pancreatic cancer (Table 2).

Torrisani *et al*[53] have reported that tumor suppressor let-7 miRNA is expressed in normal acinar pancreatic cells, but is extensively downregulated in PDAC samples, as compared with adjacent non-involved tissues. Transfection of pancreatic cancer cell lines with let-7 miRNA inhibits cell proliferation, Kras expression, and mitogen-activated protein kinase activation. This study has demonstrated that intracellular restoration of let-7 miRNA reverts neoplastic characteristics of PDAC, suggesting that let-7 miRNA functions as a tumor suppressor in pancreatic cancer. In addition, the results of this study suggest let-7 miRNA as a replacement therapy for pancreatic cancer.

miRNAS AS THERAPEUTIC TARGETS IN PANCREATIC CANCER

Most epithelial tumors, including pancreatic cancer, are believed to progress toward loss of epithelial differentiation and acquisition of a mesenchymal phenotype that leads to enhanced cancer cell invasion and migration[82,83]. The aggressiveness of pancreatic cancer is, in part, due to its drug resistance characteristics, which are also associated with the epithelial-to-mesenchymal transition (EMT). Several studies have shown that the events leading to EMT are regulated by miRNAs[84-89]. Li *et al*[72] have investigated the effects of let-7 and miR-200 on the morphological changes of EMT in gemcitabine-resistant pancreatic cancer cells (GRPCCs). They have found that: (1) the expression of miR-200 and let-7 is significantly downregulated in GRPCCs, which have EMT characteristics; and (2) transfection of GRPCCs with miR-200 rescues the epithelial phenotype by upregulating the epithelial marker E-cadherin and downregulating the mesenchymal markers ZEB1 and vimentin. These authors also have demonstrated that tumor cell sensitivity to gemcitabine is increased after re-expression of miR-200b. These results suggest that EMT could be regulated by miRNAs, and provide a potential strategy for treatment.

RAS mutations are frequent in human tumors and are known to be one of the responsible factors for radiation-induced cell death[90,91]. Using transfection of Lin28 siRNA into pancreatic cancer cells harboring Kras mutation,

Oh *et al*[73] have shown that upregulation with let-7a results in attenuated expression of Kras and increased radiosensitization of pancreatic cancer cells. This suggests that miRNA could be used as a valuable therapeutic option in radioresistant tumors that have Kras mutations.

The main reason for poor survival in pancreatic cancer is the presence of metastasis at the time of diagnosis. Weiss *et al*[54] have shown that miR-10a expression promoted metastasis, and repression of miR-10a inhibited invasion and metastasis in xenotransplantation experiments using zebrafish embryos. They have further identified tumor suppressors HOXB1 and HOXB3 as targets of miR-10a, and have reported that retinoic acid receptor antagonists inhibit miR-10a expression and suppress metastasis. These data suggest new therapeutic applications for miRNA in patients with metastatic pancreatic cancer.

Several studies have reported significant overexpression of miR-21 in pancreatic tumors[49,51], suggesting the potential role of miR-21 in pancreatic cancer. Moriyama *et al*[56] have confirmed that miR-21 is overexpressed in pancreatic cancer cells. They also have observed that miR-21 contributes to cell proliferation, invasion, and chemoresistance. They also have found that mRNA expression of invasion-related genes, matrix metalloproteinase (MMP)-2 and MMP-9, and vascular endothelial growth factor is positively correlated with miR-21 expression. The above studies show that miR-21 functions as an oncogene, and that it is involved in pancreatic cancer chemoresistance. Therefore, miR-21 could be a target for a therapeutic strategy for patients with chemoresistant pancreatic cancer.

Zhang *et al*[74] have found that pancreatic cancer cells treated with trichostatin A (TSA), one of the common histone deacetylase inhibitors[92,93], are arrested in G0/G1 phase, and exhibit an increased in apoptotic rate. The treatment also induces downregulation of miR-21 and upregulation of miR-200c. The data support the oncogenic function of miR-21, and the tumor suppressor function of miR-200, suggesting that epigenetic regulation of miRNAs with histone deacetylase inhibitor could be used as a therapeutic option in pancreatic cancer.

It has been shown that antisense oligonucleotides (ASOs) can inhibit upregulated miRNAs in tumors[94]. Park *et al*[75] have investigated miR-21 and miR-221 biological function using ASOs in pancreatic cancer. ASOs for miR-21 and miR-221 both reduce proliferation of pancreatic cancer cell lines, increase apoptosis by 3-6-fold, and induced G1 arrest. ASOs also increase the levels of the miR-21 targets PTEN and RECK, and the miR-221 target, CDKN1B, at the protein level. The authors have found that ASO targeting of miR-21 and miR-221 sensitizes tumor cells to the effects of gemcitabine, and that ASO-gemcitabine combination treatments generate synergistic antiproliferative effects in pancreatic cancer cells. These results imply that targeting miRNAs with ASOs could be a potential new therapeutic strategy for pancreatic cancer.

In vitro and *in vivo* studies have reported the anticancer activity, with low toxicity, of curcumin (diferuloylmethane)[95,96], a naturally occurring flavonoid from the rhizome of *Curcuma longa*[97,98]. Sun *et al*[76] have investigated whether curcumin affects the expression profiles of miRNAs in pancreatic cancer, and have reported overexpression of miR-22 and downregulation of miR-199a* in pancreatic cancer cells treated with curcumin. The predicted target genes of miRNA-22 are Sp1 transcription factor (SP1) and estrogen receptor 1 (ESR1). The expression of these genes (SP1 and ESR1), which are involved in cell growth, metastasis and apoptosis, is suppressed by upregulation of miR-22. Thus, Sun *et al* have suggested that one of the important anticancer mechanisms of curcumin is modulation of miRNA expression, such as miR-22.

Some cancer stem cells are involved in tumor initiation, self-renewal and survival[99], and miRNAs have been shown to have critical roles in cancer stem cell differentiation. Ji *et al*[77], using cell sorting of $CD44^+/CD133^+$, have examined the roles of miR-34 in p53-mutant human pancreatic cancer cell lines, to find a potential link between stem cells and pancreatic cancer. These authors have observed that miR-34 upregulation results in significant inhibition of clonogenic growth and cell invasion, induction of apoptosis, G1 and G2/M cell cycle arrest, and sensitization of the cells to chemotherapy and radiation. They also have detected an 87% reduction in tumor initiating cells (or cancer stem cells), which was mediated by downregulation of its downstream targets BCL2 and NOTCH. This study has shown that restoration of miR-34 could have significant promise as a novel molecular therapy for human pancreatic cancer *via* inhibiting pancreatic cancer stem cell differentiation.

Aberrations in epigenetic regulation are common in human cancers, and tumor suppressor genes are frequently silenced by this mechanism in nearly all malignancies[100,101]. Recent studies have shown that subsets of miRNAs are also silenced by the same mechanism[102,103]. For example, Lee *et al*[78] have shown that miR-107 is silenced by promoter DNA methylation in pancreatic tumors. These authors treated human pancreatic cancer cell lines with the demethylating agent, 5-aza-2'-deoxycytidine or the histone deacetylase inhibitor, TSA, or with a combination of the two, and identified the upregulation of 14 miRNAs, including miR-107. Retroviral expression of miR-107 in pancreatic cancer cells downregulates *in vitro* cell growth by repressing cyclin-dependent kinase 6, a putative miR-107 target. This study shows that epigenetic mechanisms of miRNA may be involved in pancreatic carcinogenesis.

Tumor protein p53 inducible nuclear protein 1 (TP53INP1) is a pro-apoptotic stress-induced gene. TP53 is able to activate TP53INP1 transcription as a target[104,105]. However, overexpression of TP53INP1 induces cell cycle arrest and apoptosis *in vitro*, independently from TP53. Gironella *et al*[79] have reported that TP53INP1 is expressed in normal tissues but is markedly downregulated or lost in early stages of pancreatic cancer development. TP53INP1 repression by transfection of miR-155 causes loss or significant decrease in expression of TP53INP1. These data suggest that TP53INP1 is an additional potential target of miR-155.

Several studies have suggested that EP300 may func-

tion as a tumor suppressor. This gene is located on chromosome 22q; a region known for its frequent loss of heterozygosity in different cancers, including pancreatic cancer[106-109]. Mees *et al*[80] have classified 16 human PDAC cell lines into three hierarchical groups according to their metastatic potential, and have profiled their mRNA and miRNA expression. The highly metastatic PDAC cell lines, when compared to the non-metastatic cell lines, have shown decreased mRNA and protein expression of EP300, which is related to significant upregulation of EP300-targeting miRNAs (miR-194, miR-200b, miR-200c and miR-429). Using the same 16 human PDAC cell lines, these authors have found markedly reduced expression of CD40 protein, which is involved in the host antitumor immune response[110,111]. CD40-targeting miR-224 and miR-486 are upregulated in the highly invasive and metastatic PDAC[81]. These results show that miRNAs are involved in regulating the metastatic behavior of PDAC, and in modulating metastasis-specific tumor suppressor genes. Targeting of these miRNAs may have potential therapeutic value in PDAC.

miRNAS AS CLINICAL ASPECTS IN PANCREATIC CANCER

Most tumors show deregulation of miRNAs for the initiation and progression of human cancer, therefore, many researchers have been trying to exploit these miRNAs for therapeutic applications, and to develop novel therapies for human cancer[112-115]. Thus, oncogenic miRNAs can be suppressed with ASOs to their precursor or mature forms[94,116], and tumor suppressor miRNAs can be upregulated[53,72].

Numerous miRNA studies have demonstrated that miRNA-directed targeting therapy has therapeutic potential in human cancer. Recent studies have further demonstrated synergistic effects when miRNA-directed therapy is used in combination with conventional chemotherapy or radiotherapy for pancreatic cancer[73,75]. However, currently, there is no miRNA that is used in the clinical setting for treatment of cancer patients. Significant work needs to be done before miRNA-directed therapeutic strategies can be applied. However, current data have shown encouraging preliminary results to support their clinical applications in human cancer.

Several investigators have attempted to utilize miRNA expression profiles as a diagnostic tool to differentiate tumors from normal tissues[43,117,118], and as predictors of clinical outcome. However, there have not been sufficient studies that have investigated the correlation between alterations in miRNA expression and patient outcome in PDAC.

A few miRNA expression patterns have been investigated to predict prognostic outcome from specimens of patients with pancreatic cancer[51,55,57]. Bloomston *et al*[51] have analyzed the association between survival of patients and miRNA expression patterns. In the subgroup analysis of patients with lymph-node positive disease, a panel of six miRNAs (miR-452, miR-105, miR-127, miR-518a-2, miR-187 and miR-30a-3p) was able to differentiate between long-term survivors and short-term survivors who died within 2 years. Furthermore, high expression of miR-196a-2 is associated with poor outcome; patients with high miR-196a-2 expression have a shorter median survival of 14.3 mo when compared with patients with low miR-196a-2 expression, who have a median survival of 26.5 mo.

Dillhoff *et al*[55] have performed *in situ* hybridization after microdissection and tissue microarray analysis of 80 resected pancreatic cancer specimens, and found 79% of the pancreatic cancer samples, 27% of the chronic pancreatitis samples, and 8% of the normal pancreatic samples had positive miR-21 expression. Among the subset of patients with node-negative disease, high miR-21 expression resulted in poorer survival than in patients with low miR-21 expression (median: 27.7 mo *vs* 15.2 mo, $P = 0.037$), although miR-21 expression did not correlate with tumor size, differentiation, nodal status, or T stage.

Greither *et al*[57] have measured the levels of miR-155, miR-203, miR-210, miR-216, miR-217 and miR-222, which are known to be differentially expressed in pancreatic tumors. From 56 microdissected PDACs, they found that elevated levels of miR-155, miR-203, miR-210 and miR-222 were associated with poorer overall survival rates. They further noted that higher expression of all four miRNAs had a 6.2-fold increased risk of tumor-related death as compared to cases in which the expression of these miRNAs was low.

CONCLUSION

Since the discovery of miRNAs, growing evidence has confirmed a link between miRNAs and malignant diseases, and has identified their functions and targets that affect the complex process of carcinogenesis. Like other malignant tumors, PDAC has its unique miRNA expression patterns, which are different from those of other human tumors, and are able to differentiate normal pancreas from benign inflammatory pancreatic tissues and pancreatic cancer. At present, several important oncogenic and tumor suppressor miRNAs, and their molecular targets, have been identified in PDAC. More importantly, this information will lead to new development of prognostic, diagnostic, and treatment strategies. However, additional studies are required to find ways to utilize miRNAs as a therapeutic target in the clinical setting.

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TOPIC HIGHLIGHT

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Current trends in staging rectal cancer

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Abstract

Management of rectal cancer has evolved over the years. In this condition preoperative investigations assist in deciding the optimal treatment. The relation of the tumor edge to the circumferential margin (CRM) is an important factor in deciding the need for neoadjuvant treatment and determines the prognosis. Those with threatened or involved margins are offered long course chemoradiation to enable R_0 surgical resection. Endoanal ultrasound (EUS) is useful for tumor (T) staging; hence EUS is a useful imaging modality for early rectal cancer. Magnetic resonance imaging (MRI) is useful for assessing the mesorectum and the mesorectal fascia which has useful prognostic significance and for early identification of local recurrence. Computerized tomography (CT) of the chest, abdomen and pelvis is used to rule out distant metastasis. Identification of the malignant nodes using EUS, CT and MRI is based on the size, morphology and internal characteristics but has drawbacks. Most of the common imaging techniques are suboptimal for imaging following chemoradiation as they struggle to differentiate fibrotic changes and tumor. In this situation, EUS and MRI may provide complementary information to decide further treatment. Functional imaging using positron emission tomography (PET) is useful, particularly PET/CT fusion scans to identify areas of the functionally hot spots. In the current state, imaging has enabled the multidisciplinary team of surgeons, oncologists, radiologists and pathologists to decide on the patient centered management of rectal cancer. In future, functional imaging may play an active role in identifying patients with lymph node metastasis and those with residual and recurrent disease following neoadjuvant chemoradiotherapy.

Key words: Rectal cancer; Staging; Investigations; Magnetic resonance imaging; Ultrasound; Endoanal ultrasound; Positron emission tomography; Computerized tomography

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INTRODUCTION

Nearly one million patients are diagnosed with colorectal cancers (CRC) annually in the world[1]. The incidence of CRC is highest in the western world where it is the second commonest cause of cancer death and fourth commonest cause of death from cancer worldwide[2]. In the western world there is a life time risk of CRC of 5%. Overall the 5 year survival has improved in the UK (55% in males and 51% in females) but to a lesser extent than in the USA and Europe[3].

Around 30%-40% of colorectal cancer is defined to arise from the rectum which is defined as the distal margin of tumor within 15 cm of the anal verge[4,5].

Colonoscopy and biopsy is considered as the gold

standard investigation to confirm the diagnosis of rectal cancer and to exclude synchronous lesions. Patients are then staged to assess the extent of local disease and to identify the distant spread.

Traditional rectal cancer surgery is associated with high rates of local recurrence of 5%-20%[6]. However, with the combination of high quality surgery using total mesorectal excision[7] along with use of neoadjuvant and adjuvant treatment there has been a significant reduction in local recurrence and improved survival[8]. The surgeon aims to achieve a microscopic tumor free (R_0) resection. Despite this, there is a risk of local failure. Careful preoperative assessment of the pelvis identifies high risk patients in whom the resection margins are either involved or within 1 mm of the mesorectal fascia. Involvement or threatened CRM (tumors within 1 mm of the mesorectal fascia) have a reduced chance of obtaining complete clearance. Thus, the status of the CRM has become more important than the TNM staging. In Europe and the UK, patients with involved CRM/threatened CRM are considered for long course chemoradiation prior to surgery.

IMPORTANCE OF PREOPERATIVE STAGING IN RECTAL CANCER

Accurate pre-operative staging of rectal cancer is crucial in planning the surgical treatment and is the strongest predictor for recurrence[9]. The staging helps us to formulate a structured multidisciplinary management care plan and assess the prognosis. It is also used to compare the results of hospitals offering rectal cancer treatment and to define the role of different treatment modalities.

Preoperative staging of rectal cancer can be divided into either local or distant staging. Local staging incorporates the assessment of mural wall invasion, circumferential resection margin involvement, and the nodal status for metastasis. Distant staging assesses for evidence of metastatic disease.

Rectal cancer is palpable in 40%-80% of cases[10]. Digital rectal examination helps in documentation of the size, location, distance from the anal verge, and fixity. Lesions felt by digital rectal examination can be visualized using a rigid proctoscope. The procedure allows an accurate localization and assessment of the tumor including fixity. Biopsies can be carried out where necessary. Rectal examination using proctoscopy may be considered as an important tool for newly diagnosed rectal cancers. Painful local perineal and anal conditions such as fissures or abscesses can restrict the use of this excellent tool. A trial comparing the use of CT virtual proctoscopy with rectal ultrasound examination in determining the stage of rectal cancer is being conducted in the USA and its results are awaited (http://clinical trials.gov/ct2/show/NCT00585728).

Currently, several modalities exist for the preoperative staging of rectal cancer. A combination of modalities involving use of computed tomography (CT), magnetic resonance imaging (MRI), and/or endorectal ultrasonography (EUS) is used to precisely assess the extent of spread of rectal cancer. The choice of investigations performed, however, is influenced by local expertise, guidelines and availability. Imaging in rectal cancer plays a crucial role in optimizing radiotherapy target definition to avoid adjacent vital structures[11]. EUS and MRI of the pelvis are used to assess the local spread while CT is the main modality to assess systemic spread. PET is indicated when there is clinical, biochemical or radiological suspicion of local recurrence or systemic disease.

Computerized tomography and computerized tomography colonography or virtual colonoscopy

CT scan of the entire chest, abdomen and pelvis is used for the detection of metastatic disease. CT is widely available and has faster acquisition times. However, it is not considered as the investigation of choice when it comes to assessing the layers of the rectal wall; hence it is not useful for local staging in rectal cancer and certainly is poor at evaluating superficial rectal cancers. The accuracy of CT to assess the tumor has been reported to be between 80%-95% in patients with advanced local disease[12]. The accuracy, however, decreased to around 63% when a broader spectrum of tumor sizes was analyzed. Sensitivity to pick up nodal disease has been found to be between 55%-70%[13]. In a meta-analysis involving 5000 patients, CT showed an accuracy for T staging of 73% and for nodal staging of 22%-73%[14].

The use of contrast enhanced multidetector CT colonography has improved the staging accuracy[15], by achieving superior spatial resolution and visualizing pictures in a variety of planes. However, its role in staging remains to be determined and currently it is used mainly to assess the distant metastatic disease (Figure 1A and B).

Virtual colonoscopy or CT colonogram (CTC) has been reported to be safer than colonoscopy[16] while being more sensitive than barium enema, and appears to be more acceptable to patients than either of the other tests[17]. The procedure can be performed by technicians thus saving clinicians time. In principle the data could be analysed by computer-assistance thus accelerating diagnosis time[18]. The results of the SIGGAR trial evaluating CTC versus colonoscopy or barium enema in symptomatic elderly patients are awaited[19]. CTC is the best radiological imaging for assessing the colon and rectum and at the same time identifies nodal disease and distant metastasis. The diagnosis of rectal cancer still needs to be confirmed by colonoscopy and biopsy.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is routinely used for preoperative staging of rectal cancer as it provides an accurate assessment of the tumor and the surrounding mesorectal fascia. It identifies patients at risk of local recurrence and those likely to benefit from neoadjuvant therapy. When compared with CT and ultrasound, MRI is more reliable for the evaluation of the extent of locoregional disease, planning radiation therapy, assessing postoperative changes and pelvic recurrence. The evaluation of nodal metastases remains a challenge with MRI (Figure 2).

Earlier MRI studies used body coils which lacked the

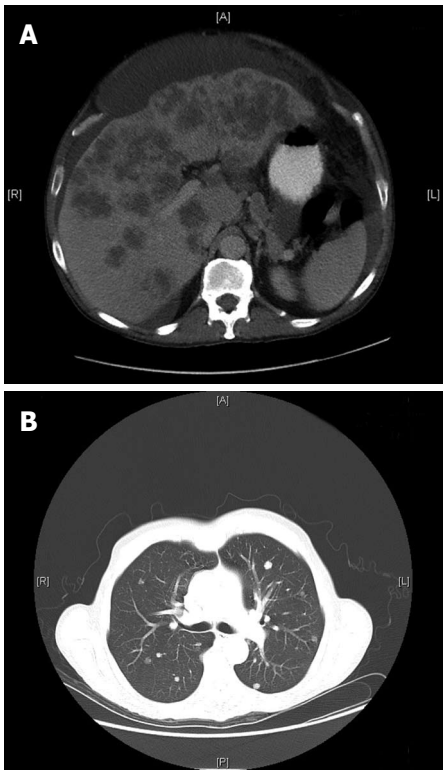

Figure 1 Computerized tomography. A: Computerized tomography (CT) abdomen showing a patient with rectal cancer having liver metastasis and ascites; B: CT Chest showing a patient with rectal cancer having lung metastasis.

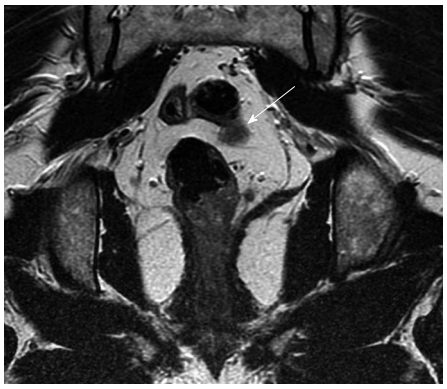

Figure 2 Coronal magnetic resonance imaging (arrow) showing possible lymph node or early vascular involvement.

resolution to differentiate the different layers of the rectal wall and added no advantage to conventional CT[20]. Subsequent use of phased-array coils permitted reliable identification of the mesorectal fascia which is crucial in the management of rectal cancer[21]. Initial studies suggested a histological clearance of at least 10 mm could be accurately predicted when the radiological clearance from the mesorectal fascia and critical structure was at least 5 mm[22]. Subsequent single centre study showed 92% accuracy in prediction of CRM involvement when the CRM cutoff of 1 mm was used and this is now confirmed from the multicentre European MERCURY study[21,23]. In Europe, MRI is now routinely used in the preoperative investigation for rectal cancer. Techniques for obtaining optimal

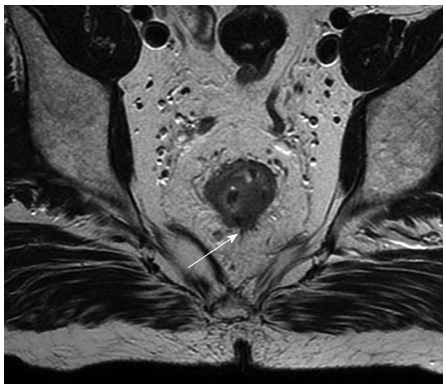

Figure 3 Magnetic resonance imaging (arrow) showing possible extension beyond the muscularis propria, radiologically staged as early T_3.

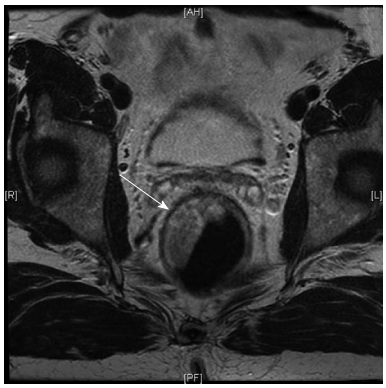

Figure 4 Coronal T2 W magnetic resonance imaging (arrow) showing the intact muscularis propria in a patient with rectal cancer. Radiologically staged as T_1 or T_2.

MRI images are described in the literature[24]. An axial picture enables identification of the distance of the CRM to the tumor. Coronal sections are useful in low rectal tumors to identify the relation to anal sphincter complex, pelvic floor, and pelvic side wall[25]. High signal intensity of the tumor on T2 w images suggest the presence of mucinous carcinoma which has poor prognosis compared to non-mucinous carcinoma[26]. The standard phased array MRI produces good quality images with good contrast resolution and a relatively large field of view. Routine use of intravenous contrast does not appear to improve the accuracy[27]. MRI cannot differentiate between T_2 and early T_3 lesions; a nodular or rounded advancing margin at the interface between muscularis propria and perirectal fat is suggestive of T_3 (Figure 3). Sometimes spiculations in the perirectal fat are considered as T_3 when in fact they are T_2 with desmoplastic reaction[22,28]. MRI certainly cannot differentiate between a T_1 and T_2 cancer (Figure 4). Another area of drawback is restaging following long course chemoradiotherapy. Studies by Chen and Hoffmann found T staging accuracy was 52% and 54% when compared to histology[29]. This is due to the inability to distinguish fibrosis from tumor with MRI similar to EUS. In low anterior tumors where the mesorectal fascia is close to the muscularis propria early T_3 can still infiltrate the mesorectal fascia[24]. Extramural vascular invasion is known to be an independent predictor of local recurrence[30,31]. The presence of a tubular structure in

proximity to a T_3 tumor or nodules with an irregular margin probably represents vascular invasion[21,32]. Recently there has been interest in the use of functional imaging such as diffusion weighted MRI imaging (DWI) and CT/PET to distinguish fibrosis from tumor[33].

MRI has been found to be useful in more advanced disease by providing clearer definition of the mesorectum and mesorectal fascia and seems to be a promising tool in assessing the locally advanced disease. With the advent of endorectal coils, the T staging accuracy has been reported to be between 70%-90%[34]. However, this technique has its limitations specially when evaluating the surrounding tissue, owing to signal attenuation at a short distance from the coil. Patient's compliance, limited availability and cost also contribute to its less wide application. Obstructing or nearly obstructing lesions can be difficult to negotiate as are high rectal cancers leading to failed/improper coil insertion in approximately 40% of patients[34].

Nodal accuracy has also been found to be variable although use of superparamagnetic iron oxide particles appears to be promising[35] as evidenced by studies in head, neck and urological cancers.

Ultrasound

Abdominal ultrasound (USS) is used to evaluate liver for metastasis, ascites, adenopathy, and for omental cake. The false negative rate is reported to be around 8%[36]. The technique, although inexpensive and widely available, is operator dependent. Intraoperative USS is rarely used apart from when synchronous rectal and liver resections are planned. Rapid advancement in imaging modalities has made USS a less favoured imaging modality in rectal cancer staging[37].

Endorectal ultrasound

Endorectal ultrasound (EUS) is sensitive for early rectal cancers (T_1 and T_2 lesions) with an accuracy of 69%-97%[38-43] and is useful in the surveillance following post transanal surgery. The standard technique involves a transanal probe enclosed in a water filled balloon introduced into the rectum to allow radial visualization of the rectum. High resolution allows the assessment of the rectal wall but the assessment of the mesorectal fascia is not possible and the assessment of the lymph nodes can be an issue and overstating has been a concern. Peritumor inflammation and artifacts due to faeces may lead to an ultrasound appearance which can be misinterpreted as tumor. These drawbacks can be exaggerated between the muscle layer and the surrounding fat which makes T_2 and T_3 lesions difficult to distinguish[44]. The accuracy of the T stage evaluation varies from 62%-92%[45]. In a meta-analysis of 11 studies it has been shown that sensitivities for superficial tumors are better than advanced lesions[46]. A 20 year (1984-2004) systematic review looking at studies with a minimum of 50 patients, evaluating the use of endorectal ultrasound and magnetic resonance imaging (MRI) in the local staging of rectal cancer, have found a complementary role for these imaging modalities in the assessment of tumor depth. Ultrasound was found to be highly accurate in early lesions ($T_{1,2}$ 40%-100%; $T_{3,4}$ 25%-100%, overall 82%). The review also found a similar accuracy in the assessment of nodal metastases[47]. Two meta-analyses in literature have shown that the sensitivity is affected by T stage[48]. A meta-analysis including 84 studies found EUS to be slightly superior in assessing the local involvement such as lymph nodes, however, no significant differences were noted when compared to other imaging modalities such as MRI. The results suggest that none of the current imaging modalities enable reliable detection of metastatic nodal disease[49].

EUS however, has its limitations as it cannot reliably distinguish an irregular outer rectal wall due to peritumoral inflammation or transmural tumor extension. Obstructing lesions may be difficult to scan especially with rigid probes leading to suboptimal staging. The scanning, although less expensive and portable, is operator dependent and has a steep learning curve. Bulky, high, stenotic, advanced (T_3) lesions or post-neoadjuvant therapy downstaged tumors can be a challenge[50-52].

EUS nodal staging accuracy is around 75%[53]. Morphologic characteristics suggestive of malignant involvement include hypoechoic appearance, round shape, peritumoral location, and size > 5 mm[45,46,51-53]. The loco-regional tumor assessment using three-dimensional EUS consists of transverse, coronal and sagital scan and has been found to be superior to CT and two-dimensional EUS. The 3D-reconstructed image shows tumor protrusion infiltrating into adjacent structures, thus, allowing for improved T and N staging[54]. Further, EUS-guided fine-needle aspiration can be carried out at the same time from the lesion or suspiciously looking lymph nodes.

Positron emission tomography

The principle of positron emission tomography (PET) is based on the differential metabolic profile of tumors compared to normal tissue. Fluoro-deoxy-glucose (FDG) is the most common PET tracer used. Due to increased metabolic activity, and change in the tumor biology, tumors preferentially show an increased uptake which results in radiolabelling[55]. Although selective, FDG accumulates in areas of infection, inflammation, in organs of increased metabolic activity such as brain, myocardium, liver or kidneys leading to false positive results[55]. FDG uptake is also influenced by the presence of mucin. PET is useful in identifying non-mucinous tumors compared to mucinous tumors. FDG/PET is mainly useful in the assessment of local recurrence and metastatic disease when conventional imaging is not helpful[56,57]. Currently it is not used as a primary staging modality in rectal cancers. Interpretation of PET without anatomic correlation poses difficulties hence PET-CT fusion scans where the pictures of both investigations are fused using software is used. This offers a detailed anatomical and functional imaging and is gaining rapid popularity and acceptance. The combination provides additional value to localize the hot spots. There are some technical limitations with this combination imaging and with the false positive rates due to other disease and physiological processes. The role of PET CT fusion scan has not changed compared to PET scans.

However, a recent study has found preoperative PET

changed the management in 17% of patients[58] with improved staging accuracy in combination with CT[56]. Another study carried by Gearhart in 37 patients reported an altered management plan for 27% of patients using FDG-PET/CT imaging modality for low rectal cancer[59].

Staging accuracy post-neoadjuvant therapy

With the increasing use of pre-operative neoadjuvant therapy, rectal tumor re-staging is increasingly performed prior to curative resection.

A reduction in staging accuracy has been noted which may be as a result of effects of neoadjuvant treatment due to post-radiation edema, inflammation, fibrosis, and necrosis[60].

A recent study of 29 patients undergoing neoadjuvant therapy and pretreatment and post-treatment staging with CT, MRI, and PET showed that PET was 100% sensitive in predicting response to therapy (compared with 54% for CT and 71% for MRI). Corresponding specificity for predicting tumor response to treatment was 60%, 80%, and 67% for PET, CT, and MRI, respectively[61], thus suggesting a further possible role of PET in predicting response to neoadjuvant therapy.

Tumor re-staging following post-neoadjuvant therapy remains problematic and it is hoped that a combination of imaging technique (CT, MRI, and EUS) and functional (PET) imaging may improve staging accuracy.

Suggested investigations for tumor staging of rectal cancer

On review of the literature, phased array MRI and EUS should be considered as the initial modalities to stage the local tumor. A fixed, locally advanced rectal cancer may be imaged better by MRI (Figure 5), whereas EUS is more appropriate for an early mobile rectal tumor (T_1-T_2 lesions). MRI has been shown to be highly accurate in predicting a clear circumferential resection margin in patients undergoing TME. Although both MRI and EUS provide a comparable overall T- and N-staging, use of these modalities is limited by issues such as availability, costs and technical expertise. CT scanning, although still the current standard for distant staging, may not be an effective tool to stage the local disease. A combination of CT and PET offering a detailed anatomical and functional imaging, however, seem to be promising and gaining popularity and acceptance for recurrent rectal cancers.

Suggested investigation for nodal staging of rectal cancer

The accuracy of MRI, CT and EUS for identifying malignant nodes is poor. Current criteria are based on size, shape and morphology. Any node of 1 cm and over is taken as significant[62]. The enlarged lymph node can be as a result of the inflammatory process but normal size nodes can have micrometastases. Brown *et al*[54] found 58% of positive malignant nodes were less than 5 mm. Morphological characteristics such as round shape, irregular borders and heterogenous signal intensity suggest nodal involvement[63].

Nodal accuracy has also been found to be variable, although use of superparamagnetic iron oxide particles (SPIO) seem to be promising as evidenced by studies in head, neck and urological cancers. The technique involves use of a contrast media containing SPIO which accumulates in normal lymph nodes, whereas due to defective phagocytosis, the uptake is poor or absent in malignant nodes. Hence by using T2 weighted imaging, these nodes can be identified. Initial studies are promising but further research is needed[35].

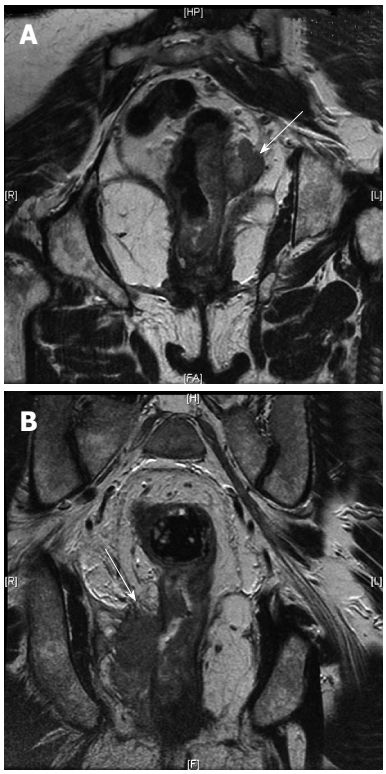

Figure 5 Magnetic resonance imaging. A: Magnetic resonance imaging (MRI) (arrow) showing the rectal cancer involving the circumferential resection margin; B: MRI (arrow) showing the rectal cancer invading the ischiorectal fat on the right (T_4).

CHOOSING THE CORRECT MANAGEMENT BASED ON STAGING IN THE ELDERLY

Over the age of 80, there is 10% mortality with rectal cancer surgery[64]. Studies from Brazil have shown a complete pathological response with chemoradiation[65] and it is well known that the elderly respond better to radiotherapy. Hence in a selected group of patients, imaging with EUS and MRI can identify patients who can be treated with neoadjuvant treatment and those with a complete radiological response can be followed by active surveillance with an intensive imaging protocol to identify those who recur to be considered for standard salvage surgical treatment or for local excision, thereby avoiding the risks associated with major rectal cancer surgery and possibly avoiding the need for permanent stoma and enabling organ preservation. This is possible only with high quality imaging techniques to assess the loco-regional disease.

CONCLUSION

Imaging in rectal cancer helps in deciding the treatment and determining the prognosis. The newer techniques help in superior image resolution, three-dimensional viewing, with decreased image acquisition times, minimal bowel preparation, and sometimes with functional qualities. This may be important following neo-adjuvant treatment. The most accurate method of rectal wall staging of rectal cancer is endorectal ultrasound and MRI but accurate staging of mesorectal fascia and lymph nodes is by phased array MRI. The management of rectal cancer is based on the proximity of the tumor to the mesorectal fascia. Hence the phased array MRI is the best overall technique for local staging of rectal cancer. Neoadjuvant treatment is not without risks; hence careful staging is important in obtaining good oncological and functional results and improving patient experience in the management of rectal cancer. In symptomatic patients local excision is beneficial in only 5% and this is the group which benefits most from EUS. With the introduction of colorectal screening it is felt nearly 50% of cancers may be of early stage disease which can be identified by EUS and managed by organ preserving intervention. Hence the role of EUS is likely to increase as part of the staging investigations in future and all these investigations are complementary in the management of rectal cancer.

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TOPIC HIGHLIGHT

Imran Hassan, MD, Assistant Professor, *Series Editor*

Management of stage Ⅳ rectal cancer: Palliative options

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Abstract

Approximately 30% of patients with rectal cancer present with metastatic disease. Many of these patients have symptoms of bleeding or obstruction. Several treatment options are available to deal with the various complications that may afflict these patients. Endorectal stenting, laser ablation, and operative resection are a few of the options available to the patient with a malignant large bowel obstruction. A thorough understanding of treatment options will ensure the patient is offered the most effective therapy with the least amount of associated morbidity. In this review, we describe various options for palliation of symptoms in patients with metastatic rectal cancer. Additionally, we briefly discuss treatment for asymptomatic patients with metastatic disease.

Key words: Palliative therapy; Rectal cancer; Malignant bleeding; Malignant obstruction; Endorectal stenting; Laser ablation

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INTRODUCTION

In 2009, there were approximately 41 000 new cases of rectal cancer in the United States[1]. In general, 70%-80% of those presenting patients have resectable disease and are treated curatively. Of these patients, nearly 40% develop recurrence, with the majority not being candidates for re-treatment with curative intent[2]. The goal of curative-intent operations is to remove all disease present. In contrast, the goal of palliative intent operations is to relieve symptoms, and by definition, leave local or metastatic residual disease. Approximately half of patients with rectal cancer may be candidates for palliative therapy at some point during their disease process, either because of locally advanced or metastatic disease at the time of presentation, or the late development of metastases[3].

Palliative treatment strategies for advanced stage rectal cancer should be individualized to patients according to their symptoms. Chemotherapy for metastatic disease is the current recommendation for asymptomatic patients[4]. Symptomatic patients can present particularly difficult challenges and can be treated with chemotherapy or combined chemoradiation therapy in conjunction with a procedure, if necessary, to relieve their symptoms. Local interventions can often effectively treat symptoms and increase quality of life. Options include extirpative resection, diversion procedures, endoscopic stenting, and laser or argon photocoagulation. The choice of treatment is partially dependent upon the patient's symptoms, age, comorbid conditions, and extent of disease.

Although the most appropriate treatment option is not always evident, a careful multidisciplinary approach with the surgeon playing the central role of determining when

aggressive operative intervention is warranted can ensure the most appropriate treatment strategy is devised. The goals in palliation should include the alleviation of symptoms, enhancing quality of life and improving comfort[5]. Herein, we review the current relevant literature on various treatment strategies as they are related to the palliative treatment of rectal cancer.

EVALUATION

Rectal cancer is defined as a malignant lesion within 15 cm of the anal verge as seen by rigid proctoscopy[6-8]. Subsequent to histological confirmation of diagnosis *via* tumor biopsy, initial work-up of the extent of disease guides subsequent treatment[4,9]. Proper staging is essential as decisions regarding neoadjuvant versus adjuvant therapy and operative versus palliative surgical intent will be based on clinical stage. The patient should undergo proctoscopy to determine distance from anal verge, as well as colonoscopy to interrogate the entire colon for synchronous lesions. Cross-sectional imaging of the chest, abdomen and pelvis in conjunction with endoscopic ultrasound (EUS) can assess depth of tumor penetration or invasion of local structures, lymph node status, and presence of metastatic disease[9,10]. Although EUS has appropriate sensitivity and specificity for differentiating muscularis propria invasion (94% and 86%), as well as perirectal tissue invasion (90% and 75%), magnetic resonance imaging (MRI) has proven to be an important adjunct for accurate staging of rectal cancer as well[9,11,12]. MRI has been found to have an 85% diagnostic accuracy for T-stage with 57%-85% accuracy for correctly identifying spread to lymph nodes; furthermore, the relationship to mesorectal fascia in conjunction with detection of adjacent organ invasion is superior utilizing MRI versus EUS[13-18]. In addition to imaging, a preoperative carcinoembryonic antigen level combined with basic laboratory values, comprehensive history and complete physical examination to assess performance status and comorbidity play important roles in the preoperative workup, because these factor significantly for choice of intervention[19].

When the pretreatment evaluation has determined a patient to no longer be appropriate for curative intent due to the presence of distant metastases or local invasion precluding a margin-negative resection, quality of life and symptom relief must become the main focus. In general, findings indicative of unresectability are utilized to predict the ability to achieve resection with negative margins. In those situations presented in Table 1, negative margins are obtained in 6%-36% of cases and surgical extirpation can result in significant postoperative disability[20]. However, resectability of the disease should be assessed by an experienced surgeon. In a study by Mathis *et al*[21], patients who were initially deemed locally unresectable, secondary to advanced primary colon and rectal cancer, were treated with aggressive multimodal therapy and found to have median survival of 3.7 years. Conversely, decision stratification must be influenced by expected survival in those patients evaluated properly and determined not to be candidates for aggressive resection. Consideration of operative interventions is more appropriately included in the conversation of palliative treatment for patients with expected outcomes exceeding 6 mo[19,22-25].

Table 1 Contraindications to resective operative intervention

Sciatic nerve pain
Bilateral ureteral obstruction
Extensive fixation to lateral pelvic side wall (CT/MRI or trial dissection)
Sacral involvement above S2 (resection produces spinal instability or post-operative complications)
Bilateral lymphedema or bilateral venous thrombosis (indicating encasement of major vascular structures)
Multiple peritoneal metastasis or metastasis fixed to or invading vital structures

CT: Computed tomography; MRI: Magnetic resonance imaging.

Approximately 50% of patients either present with distant metastases or develop distant metastases after primary treatment. Those that cannot be treated curatively should have care guided by patient wishes, functional status, expected life duration, and extent of disease and debilitating symptoms. In a study by Law *et al*[26], the most common presenting symptoms of patients undergoing palliative intervention for colorectal cancer were intestinal obstruction and rectal bleeding. In another study, 42% of patients presenting for palliative treatment were obstructed, 37% of patients experienced rectal bleeding, and 5% were asymptomatic, with the remainder (16%) experiencing pain or rectal discharge[27]. Taking into consideration the presenting symptoms and the underlying condition of the patient, palliative management can be divided into operative versus non-operative treatment.

CLINICAL SCENARIOS AND MANAGEMENT OPTIONS

Obstruction

Patients with rectal cancer can present with any number of symptoms that prompt evaluation (e.g. bleeding, perforation, abdominal pain, anemia, hematochezia, tenesmus, and malaise) and 10%-25% of patients present with obstructive symptoms[19,22,26,28]. Such a clinical scenario requires expedient yet thorough evaluation of the patient for resectability and potential for cure, because these patients often necessitate urgent, if not emergency, surgical intervention[28]. Rosen retrospectively analyzed 116 patients initially presenting with stage IV colorectal cancer and found that 26% presented with obstructive symptoms[22]. In another study, although the most common symptom precipitating medical evaluation in advanced colorectal cancer was bleeding (24%), Law *et al*[26] found that obstruction (23%) in conjunction with change in bowel habits (15%) comprised a significant proportion of patient presentations. Phang *et al*[29] found that nearly 10% of patients with rectal cancer presented with a bowel obstruction and required some emergency intervention. In that series, patients who underwent primary resection of the tumor at the time of emergency surgery had

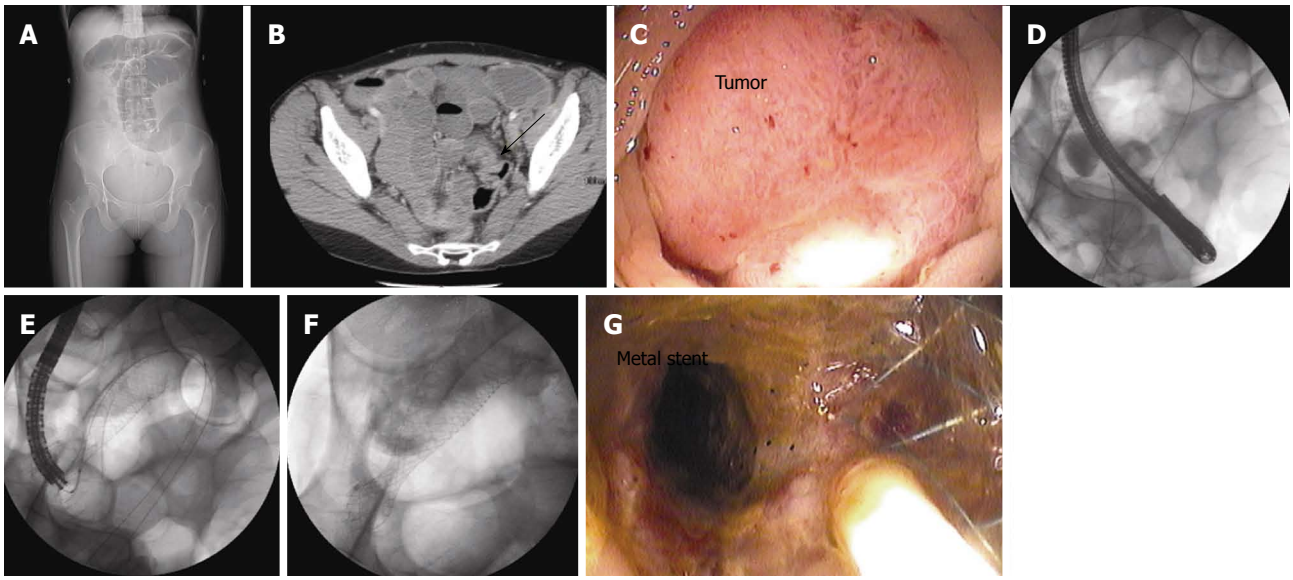

Figure 1 A young patient was diagnosed with an obstructing cancer in the upper rectum. Computed tomography demonstrated findings consistent with peritoneal metastases. She was referred for an endorectal stent to relieve the obstruction. A: Single-view plain radiography demonstrated colonic distension; B: A single-axial section with the arrow demonstrated the tumor; C: Luminal view of the tumor at time of sigmoidoscopy; D: Fluorography during stent placement demonstrated the wire across the tumor; E: Fluoroscopic view demonstrated the endoluminal stent being deployed; F, G: Fluoroscopic and endoscopic views of the stent in place.

worse overall survival and higher local recurrence rates than those patients who had elective surgery. Data such as these support the notion that interventions other than surgical resection should be entertained in those patients with rectal cancer who present in an emergency situation.

Non-operative approach: Self-expanding metallic stents have been widely utilized for maintaining patency in the biliary tree and esophagus. Transition to endorectal stenting was described in case reports in 1995, and since then, its use has increased with development of stents specifically designed for use in the large intestine[30]. Endorectal stents present one potential option to treat the obstructing rectal cancer. When utilized in this setting, they can be definitive treatment in the patient with widespread disease, or serve as a bridge to elective primary resection and anastomosis in the patient with acute obstruction.

Self-expanding metallic stents (SEMSs) are expandable metallic tubes placed in a collapsed state across the obstructing tumor under fluoroscopic guidance, endoscopic guidance or a combination procedure[31]. Various stents are utilized and when deployed expand to relieve the obstruction caused by tumor growth. Dedicated colonic stents are generally flared at the ends with a smaller mid-body diameter and differ with respect to length and diameter. Therefore, appropriate stents can be selected based on location and length of lesion as well as severity of obstruction. Examples of available stents include the colonic Z-stent (Wilson-Cook Medical, Winston-Salem, NC, USA) with 25-mm mid-body and 28-mm end diameters and the Ultraflex Precision Colonic Stent (Microvasive, Boston Scientific Corp., Natick, MA, USA) with 25-mm mid-body and 30-mm end diameters. The patient scenario presented in Figure 1 demonstrates a successful stent placement using a combination of fluoroscopic and endoscopic guidance.

Once deployed, the stent eventually becomes incorporated into the tumor and surrounding tissue *via* pressure necrosis, which allows anchoring and prevents migration[32]. Stent procedures are generally well tolerated with minimal sedation required for placement, which make them an enticing option for palliation of obstruction. In fact, a recent systematic review of 88 studies with 1785 patients who underwent SEMS placement for the relief of malignant colorectal obstruction reported a median success rate of 96.2%, with relief of obstructive symptoms 92% of the time[31]. When failure did occur, the most common cause was inability to pass a guidewire through the tortuous anatomy. On follow-up, 90.7% of patients in 11 of the studies reporting outcome had a patent stent upon death or at end-point for a mean duration of 106 d[31]. Studies such as these indicate that stents can be placed successfully in most situations, whether as a bridge to surgery or for definitive palliation.

Unfortunately, few randomized controlled trials have compared effectiveness of SEMSs and surgery for incurable, obstructing rectal cancer. In a non-randomized, prospective study, patients underwent SEMS placement or palliative surgery for obstructing, non-resectable rectal cancer. SEMS was successfully placed in 38/40 patients with mean duration of 269 d[33]. Although the stent group was statistically older with higher ASA classification, median survival was 296 d in the stent group *vs* 234 d for the surgery group. The length of hospital stay in the stent group was 2 d *vs* 9.5 d in the surgery group. Furthermore, complications requiring intervention occurred in 19% of the stent patients with no postoperative mortality *vs* 32% complication rate in the surgery group with 5% mortality. These results are consistent with the conclusion that surgical intervention confers no significant survival advantages and that SEMSs should be considered a reasonable alternative[33].

Another series from Germany has found that many patients are relieved of their obstruction and never require further surgery. Hünerbein *et al*[34] has found that 26 of 33 (79%) patients had long-term relief of bowel obstruction. Furthermore, 20 patients died with the stent in place at a mean of 5.3 mo and required no surgical interventions. The findings of this group corroborates those of others indicating the SEMSs are a safe option for the treatment of a malignant large bowel obstruction.

Overall, SEMSs are associated with less risk, shorter hospital stay and less morbidity and mortality than surgical resection or diversion. Although a certain percentage of patients with stent placement may require subsequent surgical intervention, SEMSs appears to have an appropriate role in the therapeutic options for palliation of obstruction. In fact, mortality after surgery for malignant large bowel obstruction in most series is 5%-10%, with one study reporting 18% mortality after surgery for obstructing colon cancer[22,35-40]. Postoperative complications have been found to range between 20% and 30% in most series,with one study reporting 54% postoperative complications[22,35-40].

Complications after stent placement such as bleeding, malposition and perforation can occur early after deployment. Late complications after stent placement include stent migration and occlusion. Given the limited life expectancy of the patient population in whom stents are typically placed, long-term complications or failures have been difficult to assess. Long-term complications such as obstruction have been documented to occur in approximately 15% of patients. These complications were successfully treated in all cases with another endoscopic procedure[34]. Bleeding was a rare complication (< 5% of patients) that was treated with endoscopic electrocoagulation. In this same series, short-term failure occurred in approximately 20% of patients and included stent migration, severe pelvic pain, incomplete stent expansion, and incontinence[34].

Perforation is an especially morbid complication in that violation of the colon or rectum carries significant consequences for these patients who are often quite debilitated from their primary disease process. This complication can occur as a result of over-expansion in the tumor bed or pressure necrosis in the normal colon. Rates of perforation are approximately 5% and surgical treatment requires a relatively high-risk operative intervention[31]. Song *et al*[41] have found the rate of perforation to be approximately 10%. Although one patient in their series ultimately died as a direct result of the perforation, there were no significant differences in median survival between patients with and without perforation.

Operative approach: Patients with obstruction who require an operative approach can be treated with either resection of the primary tumor or a diverting stoma. Because of the constraints associated with the pelvis and proximity of structures with tumor extension and fixation, complete resection often requires pelvic exenteration or removal of other organs along with the primary tumor[19]. These operations tend to be morbid and a less than ideal option in the patient with a limited life expectancy. Therefore, a colostomy is the preferred operation in the patient with an acute malignant obstruction of the large bowel. The sigmoid and transverse colon are the most commonly used conduits for creating a loop colostomy[42].

Other situations that necessitate operative intervention are those in which a SEMS is contraindicated. For example, the patient with cancer in close proximity to the anal canal (within 3 cm) can have intractable anal pain, tenesmus, and incontinence after placement of a SEMS[34]. Diverting colostomy can relieve the obstructing symptoms effectively and avoid these intractable symptoms. Additionally, an extended narrowing involving a long segment of the lumen with significant angulation can make SEMS placement impossible and the attempt can be high risk. Difficulty with passing the wire or pre-stent balloon dilation of the stricture may result in perforation, with these difficult obstructions necessitating emergency surgery[41]. Colostomy formation may be the better alternative in these cases[42].

A diverting colostomy can be placed using a laparoscopic or open approach. Laparoscopic fecal diversion is an attractive alternative in patients presenting with obstruction. Patients have smaller incisions with less associated pain, shorter hospital stay, quicker onset to return of bowel function, fewer postoperative complications, and the potential to initiate chemotherapy at a shorter interval when compared to open operations[19,43,44]. However, the laparoscopic approach can be difficult in this setting as the colon is often massively dilated and manipulation of the large organ can be impossible.

A particularly treacherous situation is presented in the setting of emergency decompressive surgery in which mortality approaches 20%, a complication occurs in nearly 50%, with half of patients incurring a permanent stoma[45]. Furthermore, complications resulting from the stoma are higher in patients undergoing emergency surgery[46]. In this setting, an expandable rectal stent can be placed as a bridge to surgery or as definitive palliation. In the recent comprehensive review of endorectal stents, patients were able to undergo elective surgery 2-16 d after stent placement. Rates of primary anastomosis for elective surgery after stent placement were twice that of emergency surgery for obstruction with shorter hospital stay, decreased morbidity, and decreased mortality in the elective surgery group[31].

Negative effects on quality of life and associated complications with a permanent colostomy are other reasons only to approach the obstruction operatively in those patients not amenable to other non-operative approaches[47]. Complications directly related to the colostomy can occur in up to one-third of patients, and include skin irritation, leakage, prolapse, pain, partial necrosis and retraction[19,48,49]. In conjunction with these complications, patients are more likely to feel socially restricted as a result of their colostomy when events such as leakage, prolapse

or retraction occur[50]. Furthermore, many patients are unhappy after the operation, contending that their education was not sufficient to prepare them to deal with the colostomy[51]. In a study evaluating patient satisfaction after colostomy placement in colorectal cancer, 31% of patients were dissatisfied with the information received regarding the colostomy procedure[52]. An additional study by Nugent *et al*[53] has revealed only 65% of patients felt sufficiently informed regarding what an ostomy entails. Moreover, 20%-35% of patients felt significant impact on quality of life including change in work, travel or social habits; consequently, patients expressed desire to supplement deficiencies with further counseling and follow-up. In fact, it has been shown that intensive preoperative education directed by a nurse with expertise in stoma care improves postoperative outcomes[54]. Despite these problems, fecal diversion remains an option for relief of symptoms in this patient population, and conversation with the patient to address any concerns may alleviate reservations and improve outcomes.

Primary tumor resection is occasionally indicated and can provide a reasonable quality of life postoperatively in selected patients. The most commonly performed procedures for palliative resection include abdominoperineal resection (APR), Hartmann procedure, low anterior resection (LAR), and exenteration. These operations are less commonly utilized for obstruction due to the expected short duration of survival of the patient. The decision between APR, LAR or Hartmann depends on tumor location and size, comorbidity, and ability to achieve clear margins. When addressing a rectal tumor in which resection does not preclude preservation of sphincter function, intervention would likely include low resection versus Hartmann procedure. An advantage of utilizing LAR is the maintenance bowel continuity and fecal continence. However, if there is poor predicted anal function, or concern for the anastomosis in an irradiated field, the formation of a proximal diverting ostomy negates the advantages of LAR over the Hartmann procedure[42,55]. With regard to low-lying rectal cancer, an advantage of the Hartmann operation over APR is the avoidance of a perineal wound and associated wound healing complications[56-58]. The Hartmann operation requires surgical dissection below the tumor for appropriate resection, therefore, studies have reported higher incidence of pelvic abscess than occurs with APR[57,59]. However, investigating patient outcomes following the Hartmann procedure versus APR for palliation in low-lying rectal cancer (approximately 5-5.5 cm from the anal verge), patients had similar rates of abdominal wound infection, pelvic/abdominal pain and stoma complications, whereas the APR group had a 46% occurrence of perineal wound sepsis and 38% incidence of perineal wound pain[57]. In contrast, if the rectal cancer involves the anal sphincter, APR is the preferred surgical option[42].

Pelvic exenteration is considered an extended radical resection in which surrounding organs are removed. This operation should be avoided when the goal of the operation is that of symptom palliation because the operation is generally fraught with complications and provides little if any improvement in quality of life[60]. Anterior exenteration includes resection of anterior pelvic organs; posterior exenteration involves a partial sacrectomy when excising the tumor; and complete exenteration is performed when significant invasion of most surrounding structures occurs[20]. Mortality rate from these procedures when performed for recurrent rectal cancer ranges from 0.6% to 5% at 30 d, with morbidity of 30%-60% and sphincter salvage of 5%-15%[20]. Therefore, patients who undergo an extended resection may experience prolonged hospital stay as well as higher rates of postoperative complications and re-admissions, while still requiring the formation of a stoma. There have been reports of symptom improvement and enhanced quality of life when performed in symptomatic individuals with unresectable disease[19]. However, pelvic exenteration is rarely performed for symptom palliation in symptomatic patients with unresectable rectal cancer.

Bleeding

Non-operative approach: Laser ablation is a well established treatment modality for palliation of rectal cancer, in which endoscopy is utilized to deliver focused energy to the rectal lesion[61]. The most frequently used laser is the neodymium yttrium argon garnet (Nd:YAG) laser, which has the ability to treat bleeding lesions and vaporize tumor tissue. Energy can be delivered to promote coagulative necrosis or vaporization depending on the goal of the treatment, with repeated treatments usually necessary[61]. Laser ablation has been utilized to palliate obstruction in inoperable rectal carcinoma, especially in cases in which tumor ingrowth causes obstruction, urgency or tenesmus after stent placement. However, laser ablation has been best utilized in cases in which bleeding is the prominent symptom. Coagulation is usually achieved after 2-5 sessions in 80%-90% of patients with complications ranging from 2% to 15%[61]. In a study by Rao *et al*[62], 8/11 patients were treated *via* endoscopic laser ablation for bleeding, with a median symptom-free interval of 10 mo. The average number of treatment episodes was six, with an immediate overall success rate of 91%. Another group that utilized endoscopic diode laser therapy for unresectable rectal cancer found lifelong symptom relief to be achieved in 51/57 patients. Obstruction was relieved in 22/24 patients and bleeding controlled in 29/30[27].

Complications associated with laser ablation occur in 2%-15% of patients[61,62]. The majority of complications reported tend to be minor, however, perforation requiring laparotomy occurred in 2/57 patients in a study of laser therapy[27]. Furthermore, successful palliation becomes less likely to be achieved with improvement in overall survival. Additionally, ablation is relatively ineffective with long-segment or circumferential tumors, or with angulated segments of the rectum. Despite these negative aspects, laser ablation is a relatively low cost, minimally invasive modality for palliation of bleeding that provides acceptable results in high-risk individuals.

Argon plasma coagulation (APC) utilizes electrocautery to ionize argon gas that acts to fulgurate the neoplasm and bleeding vessels. It has been utilized in open surgery to achieve hemostasis in superficial diffuse hemorrhage. This surface coagulation is fairly effective and thus APC has become more widely utilized than laser therapy in many centers for palliation of bleeding[61]. Because of the minimal depth of penetration (2-3 mm), with concomitant, efficient tissue coagulation, the risk of perforation is decreased compared to that with laser therapy. However, due to its limited penetration, it is not as effective for relieving obstruction. Compared to laser therapy, APC is easier to use, cheaper and more portable, which provides for an attractive option for palliating bleeding in an advanced-stage rectal cancer patient.

Chemotherapy has also been found to provide symptomatic improvement within 1-2 wk of initiating therapy, especially in cases of imminent obstruction or bleeding. In a study by Poultsides of 233 patients with synchronous metastatic disease and unresected primary tumor, 217 (93%) never required surgical palliation of their primary tumor, with only 16 patients (7%) requiring emergency surgery for primary tumor obstruction or perforation[63]. These data indicate that many patients can be treated with systemic therapy alone as preventive palliation, with the caveat that it requires a certain time period to produce desired effect.

In addition to bleeding, patients who present with locally advanced or recurrent disease often experience pelvic pain secondary to involvement of nerve structures within the pelvis, or from involvement of the sacrum. Radiotherapy can provide relief of pain and bleeding in 75% of patients for a median duration of 6-9 mo[64]. The range of doses studied varied from 20 to 60 Gy. However, radiotherapy has not been shown to confer a survival benefit and is best utilized for palliation of symptoms in patients with short life expectancy (6 mo)[65]. Outside of palliation for pain and bleeding, external beam radiation plays an integral role in the multimodal treatment of rectal cancer. In patients with locally advanced or recurrent disease, radiation should be utilized as multimodal therapy for potentially resectable disease[64].

Operative approach: Surgical options for the treatment of bleeding are similar to those for the treatment of obstruction. However, unlike the patient with a large obstructing lesion, the bleeding tumor may be smaller and more amenable to local or transanal excision (TAE) options. Although not a curative operation for locally advance rectal cancer, TAE for rectal cancer may provide symptomatic relief of bleeding. Transanal endoscopic microsurgery has been successfully used for this indication[66,67].

Asymptomatic patients

One of the principal concerns when evaluating an asymptomatic patient with metastatic rectal cancer is whether the primary lesion itself will become symptomatic, and necessitate intervention in order to avoid debilitating complications. This concern is what traditionally prompted surgical resection of primary disease, even in asymptomatic individuals. Proponents state that extirpation of the primary tumor can preclude development of obstruction, perforation or bleeding, thus avoiding a surgical emergency in already compromised patient receiving chemotherapy[68]. However, patients who are unfit candidates for complete resection do not achieve survival benefit with excision of the primary tumor[38,69,70]. Additionally, multiple studies have confirmed that asymptomatic or minimally symptomatic patients with incurable colon and rectal cancer have a low risk of developing debilitating symptoms prior to death from progressive disease[19,38,63,65,71-73]. Tebutt *et al*[71] have evaluated patients undergoing chemotherapy for metastatic disease, of whom, a subset had undergone resection of the primary, while another cohort initiated chemotherapy immediately after diagnosis. There was no difference in obstruction, peritonitis, gastrointestinal bleed or fistula formation between the two groups. Similarly, in a report by Scoggins *et al*[38], operative intervention was required in only 9% of patients managed initially without resection (chemotherapy subset), while morbidity and mortality were 30% and 5%, respectively, for asymptomatic patients undergoing initial operation. In a study by Poultsides *et al*[63] which has investigated outcomes in patients with synchronous colorectal metastases treated with chemotherapy, 217 out of 233 (93%) patients never required intervention for perforation, bleeding, obstruction or any other cancer-related complication. From these studies, it is apparent that, for asymptomatic individuals with unresectable metastatic disease, chemotherapy is the appropriate first-line therapy, and surgical resection without removal of all tumor burden will result in delay in starting therapy.

In contrast to systemic treatment alone, certain patients with advanced stage metastatic rectal cancer benefit from combined surgical resection and systemic therapy. The discussion regarding resection of metastatic foci for curative intent is extensive, therefore, it will be briefly reported here. When resection of a primary tumor combined with metastectomy was performed with curative intent, overall 5-year survival rates range from 35% to 58%, which significantly surpassed the 5-year survival attained by non-curative resection or systemic treatment alone[74-79]. With the development of newer biological agents, combined with more efficacious combination chemotherapy and improvement in surgical techniques that increase the efficacy and safety of resection, the number of potentially curable patients with disease amenable to resection has increased. Patients who present with widespread disease should be evaluated for surgical resectability at 2-mo intervals during cytotoxic chemotherapy[4]. The purpose of re-evaluation is to ascertain whether response to therapy has reduced the malignant neoplasm to a state in which R0 resection may be achieved[80]. Coincident with this, expanding criteria for patients amenable to safe resection of rectal cancer metastases has allowed allocation of patients into the "cure" category versus palliative measures. Therefore, understanding which patients should be considered for curative treatment provides an appropriate cohort that should be considered for palliation.

Traditionally, specific characteristics of metastases in colorectal cancer have governed suitability for liver resection. These include ≤ 3 metastases, no evidence of additional extra-hepatic disease, ability to achieve 1-cm resection margin, and small size of metastases (< 5 cm)[76,78,81-83]. Fortunately, with improvement in medical therapy and surgical proficiency (including imaging modalities, techniques such as portal vein embolization, and adjunct procedures such as radiofrequency ablation), these previous contraindications have become less absolute[74,76,84]. In a recent study, patients with > 3 hepatic metastases undergoing hepatic resection achieved similar survival as those with < 3 metastases given a microscopically negative resection margin (R0) and sufficient liver remnant[85]. As a result, the focus has shifted towards potential hepatic function after surgical extirpation instead of quantifying numerically disease preresection[68,76]. Additionally, while hepatic metastasis size > 5 cm has historically predicted poor outcome, tumor size is now only considered a contraindication if attaining a negative margin is impossible (i.e. insufficient remnant liver or proximity to critical structures, which precludes complete resection)[76,78]. Moreover, despite earlier reports of worse outcome when margins were < 1 cm, the extent of the negative margin has not been shown to confer increased survival (< 1 cm *vs* > 1 cm); rather, only microscopically negative margins are a requisite for survival benefit[75,82,83]. Furthermore, addressing extra-hepatic disease (specifically pulmonary metastases), plausibility of R0 resection should be the preferential concern dictating tumor resectability. Investigations have demonstrated survival benefit in those patients with both liver and pulmonary metastases that were amenable to margin-negative resection[86-90]. Similar to evaluation of liver metastases, isolated pulmonary metastases have been extensively investigated with the consensus that resection of pulmonary metastases with microscopically negative margins portends a favorable prognosis compared to chemotherapy alone[81,86,88,89,91,92]. From these types of data, it is obvious that evaluation of a patient with metastatic disease is complicated and the treatment plan should be jointly developed by a team of well-trained medical, surgical, and radiation oncologists.

Chemotherapy

The presence of synchronous metastases clearly decreases survival. However, those patients who are surgical candidates and can have all sites of disease removed have a better overall prognosis[93-95]. On the other hand, individuals who do not fall into the category of resectable advanced stage disease, and are also asymptomatic, should have systemic treatment initiated expeditiously after diagnosis.

Since the approval by the FDA in 1962 of 5-fluorouracil (5-FU) for systemic treatment of colorectal cancer, advances in our understanding of the molecular alterations that accrue in malignant colorectal disease have enabled significantly more efficacious chemotherapeutic regiments[96]. Moreover, specific characterization of the mechanism of action of various cytotoxic agents also has contributed to increasingly potent combination therapies. Utilized as monotherapy, 5-FU has generated response rates of 10%-15% in patients with advanced colorectal cancer[97]. Early modifications included addition of folinic acid (leucovorin) which increases the efficacy of 5-FU, as well as varying the method of administration (i.e. bolus *vs* continuous infusion), which demonstrates a higher response rate and increased overall survival (OS) in the continuous infusion group[96-98]. An oral formulation, capecitabine, also has become available and was approved by the FDA in 2001. A subsequent landmark in the development of a pharmaceutical regimen arose upon inclusion of agents such as irinotecan and oxaliplatin in the armamentarium against colorectal cancer.

Irinotecan, a topoisomerase I inhibitor, had initially demonstrated improved outcomes (overall survival, quality of life) *vs* supportive care alone in patients whose metastatic disease had progressed while on the standard chemotherapeutic regimen of 5-FU and leucovorin[99]. Additionally, in a similar study comparing irinotecan and 5-FU infusion in patients not responding to or progressing while on first-line 5-FU/leucovorin, patients within the irinotecan arm benefitted from increased progression-free survival (PFS) in conjunction with OS[100]. Consequently, irinotecan has been evaluated as first-line therapy in combination with bolus 5-FU and leucovorin (IFL), as well as infusional 5-FU and leucovorin (FOLFIRI). Because the addition of this new agent generated favorable results (increased PFS and OS), irinotecan has been incorporated into the armamentarium of primary chemotherapeutic treatments for metastatic disease[96,101,102].

Concordantly, oxaliplatin, a third-generation platinum complex, has demonstrated efficacy as an antitumor agent in advanced stage colorectal cancer patients with documented progression on standard fluorouracil-based chemotherapy[103,104]. This again prompted further evaluation of the efficacy of the platinum agent when administered in conjunction with 5-FU/leucovorin. In an equivalent manner to irinotecan, oxaliplatin demonstrated comparably favorable results (longer duration of PFS, higher response rate) when incorporated with leucovorin and 5-FU compared with the latter two agents alone[105]. Oxaliplatin potentiation of 5-FU cytotoxic activity has resulted in modification of first-line chemotherapy in which folinic acid, fluorouracil, and oxaliplatin (FOLFOX) has emerged as a therapeutic standard for metastatic disease.

In order to determine the best first-line agent for treatment of colorectal cancer, a randomized controlled trial was conducted evaluating FOLFOX and IFL[106]. This trial demonstrated an improved response to FOLFOX, thereby establishing this regimen as the new gold standard for the treatment of metastatic disease. This postulation was succeeded by the hypothesis that utilization of all three active drugs (5-FU, oxaliplatin, irinotecan), as well as infusional (and not bolus) 5-FU, were the underlying etiologies of increased survival[96]. A trial comparing first-line FOLFOX6 *vs* FOLFIRI (folinic acid, fluorouracil, irinotecan) followed by FOLFIRI and FOLFOX6 respectively was conducted to determine the appropriate sequence of

combination chemotherapy. OS was comparable in both groups (20.6 mo *vs* 21.5 mo), which was longer than OS in previous studies that have evaluated protocols with only two active drugs (oxaliplatin and 5-FU or irinotecan and 5-FU)[95]. These results were corroborated by a meta-analysis that has investigated the synergistic impact on survival when implementing therapy with 5-FU, leucovorin, irinotecan and oxaliplatin during the course of treatment[107].

The improved understanding of the biology of colorectal cancer has led to the development of several new agents that are active against members of the growth factor family. Although several novel agents have been evaluated in a number of diseases, three select therapies have been approved by the FDA for use in metastatic colorectal cancer: bevacizumab (2004), cetuximab (2004), and panitumumab (2006)[96,108]. FDA approval of cetuximab and panitumumab was contingent upon tumor expression of epidermal growth factor receptor (EGFR) (which occurs in 70%-80% of human colorectal carcinomas), as demonstrated by immunohistochemistry[96,109,110]. However, differential expression of EGFR *via* immunohistochemistry does not seem to correlate with response to or benefit from anti-EGFR therapy[110-112]. This finding engendered the question of whether different methods to ascertain EGFR levels in tumors (i.e. fluorescence *in-situ* hybridization, RT-PCR) are needed, or whether more specific markers exist that predict response to anti-EGFR treatment. As a result of numerous studies demonstrating association between KRAS mutation status and response to cetuximab/panitumumab therapy (discussed below), current recommendations are for use in colorectal cancer without specified KRAS mutations[4,113,114].

Cetuximab and panitumumab are high-affinity monoclonal antibodies (chimeric mouse/human IgG1 and human IgG2, respectively) directed against the extracellular ligand binding domain of EGFR. Their efficacy has been demonstrated in both irinotecan-based (FOLFIRI) and oxaliplatin-based (FOLFOX4) treatments[111,115-117]. When bound by ligand, EGFR activation triggers a cascade of events that propagate growth signals that ultimately promote cell proliferation and survival[118-120]. Within this signaling cascade lies KRAS, an intracellular G-protein that is mutated in 30%-50% of colorectal cancers; when this genetic aberration occurs in specific codons (12 and 13), the resultant constitutively active protein is no longer dependent upon upstream input from EGFR[96,109,112,121,122]. The relevance of KRAS mutations becomes apparent for patients treated with anti-EGFR therapy: abolishing the upstream signal does not likely provide any benefit. This principle has been validated by several studies that have evaluated cetuximab treatment in metastatic colorectal cancer, and KRAS mutational status, in which only patients with wild-type KRAS show improved response, PFS and OS[109,116,120-124]. Furthermore, this disparity in efficacy has also been observed in a study of KRAS mutational status and panitumumab therapy in refractory metastatic colorectal cancer[112,125]. Moreover, when evaluated as first-line treatment in conjunction with FOLFOX4, panitumumab increased PFS in patients with wild-type KRAS, while those with mutant KRAS suffered a decrease in PFS[115]. Heinemann has provided an excellent review of the clinical relevance of EGFR and KRAS status with respect to anti-EGFR therapy in patients with metastatic colorectal cancer[109].

Bevacizumab is a humanized monoclonal antibody directed against soluble vascular endothelial growth factor A (VEGF-A). The biological agent inhibits VEGF-A binding to vascular endothelial growth factor receptor, thus restricting angiogenesis, a process critical to tumor formation, invasion and metastasis[126-128]. In 2004, a cardinal study investigating the benefit of bevacizumab addition to IFL therapy compared to IFL alone in patients with previously untreated metastatic colorectal cancer demonstrated increased response rate, PFS and OS in the group receiving the anti-angiogenic biological agent[129]. Additionally, in patients with disease progression after first-line irinotecan-based therapy, bevacizumab supplementation of FOLFOX4 generated increased PFS and OS versus FOLFOX4 or bevacizumab alone[130]. A subsequent study evaluating first-line bevacizumab or placebo combined with FOLFOX4 as well as capecitabine/oxaliplatin (XELOX) revealed two important findings. Addition of bevacizumab to oxaliplatin-based therapy increased PFS when used as first-line therapy[131]. Combination of capecitabine and oxaliplatin was not inferior to FOLFOX4 therapy[132]. To explore further the clinical effects of targeted therapeutics, in a phase ⅢB trial in 2009, patients received oxaliplatin or irinotecan with bevacizumab, leucovorin and 5-FU as initial treatment for advanced systemic disease[133]. These patients were then randomly assigned to receive panitumumab or placebo. Remarkably, in the oxaliplatin-based group, those that received panitumumab showed decreased PFS and OS compared to the control group, while there was no difference seen with panitumumab addition in the irinotecan-based group[133]. In confirmation of this detrimental effect of combined anti-VEGF and anti-EGFR therapy, capecitabine, oxaliplatin and bevacizumab were administered as first-line therapy with or without cetuximab in patients with metastatic colorectal cancer, and addition of cetuximab resulted in decreased PFS[122].

Currently, according to the National Comprehensive Cancer Network guidelines, patients with unresectable, asymptomatic metastatic disease should undergo initial therapy consisting of one of the following: choice of FOLFOX, CapeOX or FOLFIRI, with or without bevacizumab; or FOLFOX or FOLFIRI with or without cetuximab/panitumumab (specifically for disease characterized by wild-type KRAS gene)[4]. Alternatively, FOLFOX or FOLFIRI alone can be utilized in an attempt to render patients possible candidates for resection[4]. Additionally, concomitant use of anti-EGFR and anti-VEGF therapy should be avoided[4]. Patients should be re-evaluated after 2 mo to determine if conversion to resectability has been achieved. Symptomatic improvement is often seen within weeks of initiating chemotherapy, thus negating the need for local intervention[63]. With these regimens, response

rates of approximately 50% have been achieved, with 50% reduction in bi-dimensional measurements occurring, and another 25% of patients demonstrating a minor response or stabilization[64]. In addition, chemotherapy is the only modality that has been demonstrated to increase survival in stage Ⅳ colorectal cancer, with median OS of 18-20 mo, which indicated that chemotherapy itself is effective for survival benefit and palliation of disease[4].

CONCLUSION

Approximately 20% of patients presenting with rectal cancer have stage Ⅳ disease[1,134]. Therefore, a thorough knowledge of palliative options is required to optimize quality of life and provide the best chance of long-term survival. Patients undergoing palliative treatment have a relatively short duration of survival (median: 6-9 mo), with dismal 5-year survival rates (0%-5%)[64]. This is especially true for patients who present symptomatically with obstruction, pain, bleeding and perforation. Patients undergoing chemotherapy for disseminated metastatic colorectal cancer have demonstrated median survival of 15-20 mo with various treatment options[4]. Therefore, when evaluating patients with metastatic rectal cancer, the patient's age, comorbidity, extent of disease, functional status, tumor characteristics, and symptoms must be taken into account to determine the best possible treatment approach. Given the fact that the majority of patients ultimately succumb to their disease, the constellation of factors must be utilized to provide the most effective relief with the minimum amount of morbidity and mortality. The patient with significant metastatic burden and a relatively unobtrusive primary tumor seems to benefit from the initiation of chemotherapy without further surgical therapy. Complications necessitating surgery are quite rare in this group of patients. However, symptomatic patients with significant burden of disease require a multidisciplinary team consisting of a surgeon, medical oncologist, gastroenterologist, and/or a radiation oncologist, to develop the most efficacious palliative intervention, to achieve the best goal-directed outcome for patients and family members.

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TOPIC HIGHLIGHT

Imran Hassan, MD, Assistant Professor, *Series Editor*

Neoadjuvant *vs* adjuvant pelvic radiotherapy for locally advanced rectal cancer: Which is superior?

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Abstract

The treatment of locally advanced rectal cancer including timing and dosage of radiotherapy, degree of sphincter preservation with neoadjuvant radiotherapy, and short and long term effects of radiotherapy are controversial topics. The MEDLINE, Cochrane Library databases, and meeting proceedings from the American Society of Clinical Oncology, were searched for reports of randomized controlled trials and meta-analyses comparing neoadjuvant and adjuvant radiotherapy with surgery to surgery alone for rectal cancer. Neoadjuvant radiotherapy shows superior results in terms of local control compared to adjuvant radiotherapy. Neither adjuvant or neoadjuvant radiotherapy impacts overall survival. Short course versus long course neoadjuvant radiotherapy remains controversial. There is insufficient data to conclude that neoadjuvant therapy improves rates of sphincter preserving surgery. Radiation significantly impacts anorectal and sexual function and includes both acute and long term toxicity. Data demonstrate that neoadjuvant radiation causes less toxicity compared to adjuvant radiotherapy, and specifically short course neoadjuvant radiation results in less toxicity than long course neoadjuvant radiation. Neoadjuvant radiotherapy is the preferred modality for administering radiation in locally advanced rectal cancer. There are significant side effects from radiation, including anorectal and sexual dysfunction, which may be less with short course neoadjuvant radiation.

Key words: Locally advanced rectal cancer; Neoadjuvant radiation; Adjuvant radiation; Rectal neoplasm; Chemoradiotherapy; Neoadjuvant chemoradiotherapy

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INTRODUCTION

Colorectal cancer is the third most frequent cancer in men and women. In 2009, in the United States 40 000 new cases of rectal cancer alone were diagnosed[1]. The past 2 decades have seen many advances in the treatment of patients with rectal cancer. Surgery remains the mainstay. The standard of surgical care now includes total mesorectal excision (TME), which was shown to significantly decrease local recurrence rates[2]. Evolution of Combined Modality Treatment (CMT) revolutionized care of locally advanced rectal cancer with the most considerable change the introduction of pelvic radiation. Improvements in preoperative staging with endorectal ultrasound and magnetic resonance imaging have allowed experimentation with different regimens of neoadjuvant (preoperative) and adjuvant (postoperative) radiotherapy (RT).

The goals of this review are to provide a critical over-

view of the most relevant clinical trials, and to evaluate the advantages and disadvantages of different RT regimens, in the adjuvant and neoadjuvant setting, for patients with locally advanced rectal cancer (stages ⅡB and C, ⅢA through C).

ADJUVANT RADIATION

RT for rectal cancer was first introduced in the 1980s, in an attempt to decrease rates of local recurrence in patients with locally advanced rectal cancer; at that time, the local recurrence rates after surgical resection were as high as 50%[3].

One of the first randomized controlled trials (RCTs) to demonstrate success in control of local recurrence with the use of adjuvant therapy was published in 1985 by the Gastrointestinal Tumor Study Group[4]. That study randomized 227 patients (data from 202 collected) to 4 arms: (1) no adjuvant therapy (the control arm) (n = 58); (2) adjuvant RT (n = 50); (3) adjuvant chemotherapy (n = 48); or (4) adjuvant CMT (n = 46). Patients in the CMT arm had significantly decreased local recurrence rates ($P < 0.009$), as compared with the control arm, but the overall survival rates did not significantly differ ($P = 0.07$). That 1985 publication ushered in the era of adjuvant therapy with RT for patients with locally advanced rectal cancer.

In the United States, the first official recommendation for the use of adjuvant chemoradiation in patients with rectal cancer came from the National Institutes of Health (NIH) consensus statement, published in 1990[5]. The NIH set the standard of care for patients with stage Ⅱ and Ⅲ rectal cancer to include adjuvant chemoradiation without specifying the optimal regimen. Subsequently, extensive research has been conducted on the most advantageous timing and dosage of pelvic RT in patients with locally advanced rectal cancer (Table 1). In 1997, the Norwegian Adjuvant Rectal Cancer Project Group published the results of one of the early trials evaluating the chemotherapy dose in adjuvant chemoradiation for patients with locally advanced rectal cancer[6]. Previous studies had shown improved locoregional control with adjuvant RT, but high toxicity and poor compliance with adjuvant CMT[4,7]. The Norwegian trial addressed the important issue of clinically significant complications in the setting of adjuvant CMT for rectal cancer. In that trial, 144 patients were randomized to surgery alone or to adjuvant CMT (chemoradiation with long-course RT and short-term 5-fluorouracil (5-FU)-based chemotherapy). The short-term chemotherapy was tolerated by patients without sacrificing the benefits of improved local control. The minimum follow-up time was 4 years. The 5-year recurrence free rates significantly differed (64% in the CMT arm *vs* 46% in the surgery alone arm, $P = 0.01$), as did the 5-year survival rates (64% in CMT arm *vs* 50% in surgery alone arm, $P = 0.05$). Further, a meta-analysis in 1988 reviewed all RCTs evaluating adjuvant therapy (8 RT *vs* surgery alone, 17 chemotherapy *vs* surgery alone) with the endpoint of overall survival and found only a small improvement in the adjuvant chemotherapy arm [odds ratio (OR), 0.83, 95% CI: 0.70-0.98]. No effect on survival was found in the RT arm[8].

NEOADJUVANT RADIATION

Efforts aimed at improving local control and long term survival stimulated experimentation with adjuvant RT in the 1990s and gave birth to the concept of neoadjuvant RT. Initial reports from small studies suggested that efficacy with neoadjuvant RT was comparable or improved compared to adjuvant RT, and toxicity was less severe. Delineating the veracity of these small studies intrigued investigators over the subsequent decade. Specifically two different regimens of neoadjuvant RT were being assessed: (1) long course RT, used mainly in the United States; and (2) short course RT, used mainly in Europe.

The European Organization for Research and Treatment of Cancer (EORTC) designed a study to evaluate the efficacy and toxicity profile of neoadjuvant RT (long-course). Four hundred and sixty-six patients were enrolled: 175 were ultimately randomized to surgery alone, and 166 randomized to neoadjuvant RT followed by surgery. Patients in the neoadjuvant arm tolerated the treatment adequately, had significantly decreased local recurrence rates (15% *vs* 30%, $P = 0.003$), but had no improvement in overall survival[9]. The Swedish Rectal Cancer Trial[10] was the first major trial to demonstrate significant improvement in local control with short-course RT (25 Gy in 5 consecutive daily fractions) followed by surgery, compared with surgery alone (11% local recurrence rate with short-course RT *vs* 27% without, $P < 0.001$). In addition, the Swedish trial was the only trial to demonstrate improved 5-year survival rates for patients in the neoadjuvant arm (58% with short-course RT *vs* 48% without, $P = 0.004$). The patient population included those with stage I rectal cancer as well as locally advanced disease. Note that the results of that trial, published in 1997, preceded surgical standardization to TME; hence, one of its drawbacks was the lack of standardization in surgical technique.

In response, the Dutch colorectal group performed a similar investigation, with the notable exception of standardizing surgery to TME[11]. Again, patients were randomized to either short course neoadjuvant RT followed by surgery within 1 wk (n = 695) or surgery alone (n = 719). A significant decrease in local recurrence rates was found at 2 years in the neoadjuvant RT arm (2.4% *vs* 8.2%, $P < 0.001$), but no difference in overall survival (82% *vs* 81.8%, $P = 0.84$). An additional variable examined in this study was the import of a positive circumferential margin (CRM). Positive CRM was significantly correlated with an increased risk of local recurrence; and patients with positive CRM received post operative long course RT. The Dutch colorectal group confirmed the findings of the Swedish rectal trial in terms of local control, contradicted findings of improved survival, and raised a new question regarding the role of selective adjuvant RT with positive CRM. That question was addressed with the Medical Research Council (MRC) CR07 trial, whose results were

Table 1 Randomized control trials evaluating timing and dose of radiation therapy

Trial (year results published)	Study design	Patients	Follow-up (mo)	Treatment	Outcome: overall survival	Outcome: local recurrence
Swedish Rectal Cancer Trial (1997)[10]	RCT	1168	60	Neoadjuvant short-course RT *vs* surgery alone	58% *vs* 48% (P = 0.004)	11% *vs* 27% (P < 0.001)
Dutch TME Trial (2001)[11]	RCT	1861	24	Neoadjuvant short-course RT (standard TME) *vs* surgery alone	82% *vs* 81.8% (P = 0.84)	2.4% *vs* 8.2% (P < 0.001)
German Rectal Cancer Study Group (2004)[14]	RCT	799	60	Neoadjuvant long-course RT + chemotherapy *vs* adjuvant long-course RT + chemotherapy	76% *vs* 74% (P = 0.80)	6% *vs* 13% (P = 0.006)
Polish Colorectal Group (2006)[16]	RCT	312	48	Neoadjuvant short-course RT *vs* neoadjuvant long-course RT	67.2% *vs* 66.2% (P = 0.96)	14.2% *vs* 9% (P = 0.17)
MRC-NCIC (2009)[17]	RCT	1350	60	Neoadjuvant short-course RT *vs* selective adjuvant long-course RT + chemotherapy	70% *vs* 67.9% (HR 0.91, 95% CI: 0.73 to -1.13, P = 0.40)	4% *vs* 11% (HR 0.39, 95% CI: 0.27 to 0.58, P < 0.0001)
NSABP R-03 (2009)[18]	RCT	267	60	Neoadjuvant long-course RT + chemotherapy *vs* postoperative long-course RT + chemotherapy	74.5% *vs* 65.6% (P = 0.065)	10.7% *vs* 10.7% (P = 0.69)
Stockholm Ⅲ (2010)[1]	RCT	303	Ongoing	Neoadjuvant short-course RT + surgery within 1 wk *vs* neoadjuvant short-course RT + surgery 4 to 8 wk later *vs* neoadjuvant long-course RT + surgery 4 to 8 wk later		Ongoing

[1]Interim results. RCT: Randomized control trial; RT: Radiotherapy; TME: Total mesorectal excision; MRC-NICI: Medical Rectal Council-National Cancer Institute of Canada; NSABP: National Surgical Adjuvant Breast and Bowel Project; HR: Hazard ratio.

published in 2009 (see below).

As more data became available, two meta-analyses were published in 2000 and 2001 asking two important questions. First, what is the efficacy of neoadjuvant RT in improving survival, and decreasing local recurrence rates[12] and second, what is superior in improving survival and decreasing local recurrence: adjuvant or neoadjuvant therapy[13]? Cammà *et al*[12] addressed the first question; their analysis included 14 RCTs and found that neoadjuvant RT significantly improved the 5-year survival rates (OR, 0.84, 95% CI: 0.72-0.98, P = 0.03), the cancer-related mortality rates (OR, 0.71, 95% CI: 0.61-0.82, P < 0.001), and the local recurrence rates (OR, 0.49, 95% CI: 0.38-0.62, P < 0.001). The Colorectal Cancer Collaborative Group evaluated 22 RCTs (involving a total of 8507 patients) to determine the answer to the second question. The RCTs compared neoadjuvant therapy, adjuvant therapy, or surgery alone and included both short-course and long-course RT. The group found a significant improvement in the yearly local recurrence rate in the neoadjuvant RT arm (a 46% decrease *vs* surgery alone, P = 0.00001) and in the adjuvant RT arm (a 37% decrease *vs* surgery alone, P = 0.002). But the 5-year survival rate (45% with RT *vs* 42.1% with surgery alone) and the overall survival rate (62% with RT *vs* 63% with surgery alone, P = 0.06) did not significantly differ. Of note, 30 Gy was identified as the biologically active dose of RT.

The issue of neoadjuvant *vs* adjuvant RT is further clouded by the inclusion of chemotherapy into treatment regimens. In 2004, the German Rectal Cancer Group compared neoadjuvant CMT with adjuvant CMT in patients with locally advanced rectal cancer[14]. Patients were randomly assigned to 2 arms: (1) neoadjuvant CMT (n = 421); and (2) adjuvant CMT (n = 402). All patients received long-course RT and 5-FU-based chemotherapy. The 5-year survival rates (76% with neoadjuvant CMT *vs* 74% with adjuvant CMT, P = 0.8) did not significantly differ. But the local recurrence rates significantly improved in the neoadjuvant arm (6% with neoadjuvant CMT *vs* 13% with adjuvant CMT, P = 0.006). The adjuvant arm had higher rates of acute and long-term toxicity (acute: 27% with neoadjuvant CMT *vs* 40% with adjuvant CMT, P = 0.001; long-term: 14% *vs* 24%, P = 0.01). Another important finding was that overstaging of patients resulted in unnecessary administration of neoadjuvant CMT.

In 2005, Law *et al*[15] contributed to the controversy surrounding overstaging and overtreatment by suggesting that low risk stage Ⅱ patients do not benefit from neoadjuvant therapy. They reported data on 224 patients with stage Ⅱ disease who underwent TME surgery without neoadjuvant or adjuvant CMT. They hypothesized that the benefit of treating stage Ⅱ disease with adjuvant therapy was less than the risk of complications or toxicity from CMT. Median follow up was 43 mo. Five years recurrence rate was reported as 6% which is comparable to previously reported values for patients undergoing neoadjuvant RT and surgery (2.4%-14.2%, Table 1)[10,11,14,16-18]. Overall survival was reported as 71% which is also similar to data from previous trials for patients undergoing neoadjuvant RT and surgery (58%-82%, Table 1)[10,11,14,16-18]. They conclude that there is no advantage to treating low risk stage Ⅱ rectal cancer patients with negative margins with neoadjuvant therapy. There was an emphatic response to this statement from many authors who felt that that not treating stage Ⅱ patients with neoadjuvant CMT was egregious[19].

Once short-course neoadjuvant RT was established to

be safe and effective, the next step was to compare its efficacy with that of long-course neoadjuvant RT. In 2006, the Polish Colorectal Study Group randomized 312 patients to either (1) neoadjuvant short-course RT, surgery within 1 wk, and optional adjuvant chemotherapy or (2) neoadjuvant long-course RT, neoadjuvant chemotherapy, and surgery 6 to 8 wk later. Early RT toxicity was higher in the long-course RT arm (18.2% with long-course RT *vs* 3.2% with short-course RT, $P < 0.001$), but the 5-year survival rates (66% *vs* 67%, $P = 0.96$) and the local recurrence rates (9% *vs* 14%, $P = 0.17$) did not significantly differ. The study concluded that short-course and long-course RT had comparable efficacy, but short-course RT remains the standard of care in Poland because of the lower toxicity distribution and higher compliance rates. In 2009, Guckenberger *et al*[20] introduced a new regimen for short-course RT, administering twice-daily doses of 2.9 Gy for 1 wk (total dose, 29 Gy) to 118 patients. That regimen lowered the single dose and allowed a 6-h tissue recovery period between treatments, but the daily dose was the same as with standard short-course RT (5 Gy daily × 5 d). The 188 patients had clinical stage Ⅱ (50%), Ⅲ (41.5%), and Ⅳ (8.5%) rectal cancer; they all received adjuvant 5-FU-based chemotherapy. The median follow-up time was 46 mo. Late toxicity (grade Ⅱ) occurred in 11% of the patients. The local control rate was 92%. The 5-year survival rate of 67% compared favorably with previously reported rates in randomized trials that also evaluated daily dosing of short-course RT (58%-82%, Table 1)[10,11,16].

In the United States, the National Surgical Adjuvant Breast and Bowel Project (NSABP) R-03 trial also compared neoadjuvant CMT and adjuvant CMT in patients with locally advanced rectal cancer[18]; the NSABP R-03 trial was similar to the German rectal cancer group trial published in 2004[14]. Both arms of the NSABP R-03 trial used long-course RT, and the chemotherapy regimen was 5-FU-based with leucovorin. The study was initially powered for a sample size of 900, but had to close early due to poor accrual. In all, 123 patients were randomized to neoadjuvant CMT and 131 to adjuvant CMT. The surgical technique was not standardized, but rather left to the discretion of the surgeon. Primary endpoints were the disease-free survival and overall survival rates. The overall survival rates (74.5% with neoadjuvant CMT *vs* 65.6% with adjuvant CMT, $P = 0.065$) and the locoregional recurrence rates [Hazard ratio (HR), 0.86, 95% CI: 0.41-1.81, $P = 0.693$] did not significantly differ - in contrast to the 5-year disease-free survival rates (64.7% *vs* 53.4%, $P = 0.011$). Of note, the rate of complete pathologic response was 15% in the neoadjuvant CMT group but the rates of sphincter preservation (48% with neoadjuvant CMT *vs* 39% with adjuvant CMT) did not significantly differ, per the opinion of the operating surgeon. It is difficult to draw conclusions from the NSABP R-03 trial, because it was underpowered and not standardized in operating technique.

In 2009, the MRC and National Cancer Institute of Canada (NCIC) combined CR07/CTG C016 trial[17] addressed the issue of selective adjuvant CMT based on operative margins. The trial randomized 1 350 patients to 2 arms: (1) neoadjuvant short-course RT; or (2) initial surgery with selective adjuvant long-course RT and 5-FU-based chemotherapy based on circumferential (CRM) involvement. The surgical technique was not standardized. Median follow-up time was 4 years; the primary outcome measure was local recurrence. In the selective adjuvant arm, 12% of the patients had a positive CRM, 78% of whom then underwent adjuvant RT. In the neoadjuvant arm, a 61% relative risk reduction (HR, 0.39, CI: 0.27-0.58, $P < 0.0001$) was found for local recurrence, and a 24% improvement (HR, 0.76, CI: 0.62-0.93, $P = 0.013$) was found for disease-free survival. But the 2 arms did not significantly differ in overall survival rates. The MRC CR07/NCIC-CTG C016 investigators concluded that neoadjuvant short-course RT was effective therapy in patients with operable rectal cancer.

BENEFITS OF NEOADJUVANT RT

With the advent of neoadjuvant therapy, reliable methods to evaluate its efficacy and to determine the significance of response to treatment have been necessary. Pathologic tumor response has risen to the forefront, although several tumor grading systems are currently in use. Two recent prospective studies evaluated the impact of tumor response on overall survival in patients with locally advanced rectal cancer[21,22]. Both studies concluded that tumor downstaging was the only variable that significantly and independently correlated with improved survival.

Most significantly the addition of neoadjuvant radiation has resulted in significant downsizing and downstaging of low locally advanced rectal cancers making sphincter preserving procedures feasible and with good oncologic outcomes. Weiser *et al*[23] performed a retrospective analysis of 148 patients with locally advanced rectal cancer (within 6 cm of the anal verge) who were treated with neoadjuvant CMT (long-course RT) and selective adjuvant chemotherapy. The decision to perform sphincter-preserving surgery was made intraoperatively. The likelihood of sphincter-preserving surgery was associated with significant tumor downstaging. They concluded that neoadjuvant CMT facilitated sphincter-preserving surgery in addition to intersphincteric resection.

However, short course neoadjuvant radiation does not seem to offer the same results. Sauer *et al*[14] did not find a significant difference in the rates of sphincter-preserving surgery between their neoadjuvant and adjuvant treatment arms. However, they did note that, within the subgroup of patients deemed to require abdominoperineal resection preoperatively ($n = 194$), the number of abdominoperineal resections actually performed was significantly lower in the neoadjuvant arm ($P = 0.004$). Bujko *et al*[24] specifically looked at whether neoadjuvant short-course RT offered a benefit for sphincter preservation over neoadjuvant CMT in 316 patients and found no significant difference:

61% of patients in the RT arm and 58% in the CMT arm underwent sphincter-preserving surgery (P = 0.57). In conclusion, although short-course RT improves local control, no strong evidence exists that it also improves rates of sphincter-preserving surgery indicating short-course neoadjuvant RT does not have a significant effect on preoperative tumor downsizing or downstaging.

A significant benefit of neoadjuvant RT is patient compliance with treatment. Adjuvant RT has been associated with higher rates of treatment interruption. Lebwohl *et al*[25] assessed for principle factors associated with treatment interruption in 113 RT patients. Patients in the adjuvant arm had a significantly increased chance of RT interruption, as compared with the neoadjuvant RT arm (OR, 14.08, CI: 1.55-127.87). Development of an adverse event was also significantly correlated with RT interruption (OR, 20.66, CI: 1.76-242).

ANORECTAL FUNCTION OUTCOMES

One of the most important variables evaluating quality of life in rectal cancer is anorectal function, specifically bowel function and sexual function[26]. This is affected by both chemoradiation and surgical technique. The Dutch colorectal group assessed anorectal functional outcomes after short-course preoperative RT and TME and found significant differences between patients who did *vs* did not undergo RT[27]. RT patients had higher rates of fecal incontinence (62% with RT *vs* 38% without, $P < 0.001$), pad wearing as a result of incontinence (56% *vs* 33%, $P < 0.001$), and anal blood loss (11% *vs* 3%, $P = 0.004$). RT patients also reported significantly lower satisfaction with bowel function.

A second prospective study randomized 316 patients to (1) short-course neoadjuvant RT or (2) long-course neoadjuvant chemoradiation[26]. The goal was to evaluate anorectal and sexual dysfunction and quality of life. Early complications were more common in the chemoradiation arm, but no significant differences were found in the degree of anorectal and sexual function or in quality of life.

In addition to bowel and sexual dysfunction, RT patients may experience acute and late RT toxicity, including nausea/vomiting, postoperative hernia, femoral neck fracture, skin problems (nonhealing perineal wounds), ileus, anastomotic stricture, and fistula. The Dutch colorectal group assessed RT toxicity, intraoperative and postoperative complications, and other variables in patients who underwent short-course neoadjuvant RT *vs* TME alone[27]. No differences were found in operative time, intraoperative complications, or hospital stay; however, the amount of intraoperative blood loss was higher in the RT arm ($P < 0.001$). Rates of perineal complications were also higher (29% with RT *vs* 18% with TME alone, $P = 0.008$). But no significant differences were found in the rate of abdominal wound complications (4.0% with RT *vs* 3.3% with TME alone) or in the overall postoperative mortality rate.

Frykholm *et al*[28] looked at long-term complications (minimum follow-up time, 5 years) after either neoadjuvant short-course RT (n = 255) or adjuvant long-course RT (n = 127), as compared with surgery alone (control group, n = 82). Long-term complications (defined as occurring at least 6 mo postoperatively) included recurrent abdominal pain, diarrhea, fecal incontinence, ileus, cystitis, paresthesias, delayed wound healing, and any neurologic dysfunction. The percentage of patients with small bowel obstruction did not significantly differ between the neoadjuvant RT group and control group. In the adjuvant RT group, the risk of developing a small bowel obstruction was significantly higher ($P < 0.01$). Overall, the frequency of complications possibly related to RT in the neoadjuvant group was 20%; in the adjuvant group, 41%. However, in the control group, the percentage of similar complications was 23%. In addition to finding a significant decrease in local recurrence after neoadjuvant short-course RT (13% in the neoadjuvant group *vs* 22% in the adjuvant group, $P = 0.02$), the cumulative risk of bowel obstruction was significantly higher in the adjuvant group.

Minsky *et al*[29] also demonstrated significantly lower rates of adverse events and improved compliance in patients treated with neoadjuvant CMT compared to patients treated with adjuvant CMT. Despite receiving higher doses of chemotherapy, the neoadjuvant arm experienced a 13% incidence of acute grade 3 or 4 toxicity compared to a 48% incidence in the adjuvant arm ($P = 0.045$). A meta-analysis by Birgisson *et al*[30] found that the most common late adverse effects of RT were bowel obstruction, bowel dysfunction (fecal incontinence), and sexual dysfunction. Several different RT regimens were included in the meta-analysis, offering some insight into how complications correlated with dosage. Overall, in the more recent studies which used lower doses and better techniques, the rates of adverse events were lower. Unfortunately, to date, no specific markers have been identified that might help predict which patients have a higher risk of acute RT toxicity. Further work is needed in this important area of ongoing research.

CONCLUSION

Patients with locally advanced rectal cancer clearly benefit, in terms of locoregional control, from both neoadjuvant and adjuvant RT; and patient compliance is better with neoadjuvant RT. No definitive evidence demonstrates the superiority of using short *vs* long-course RT.

The current standard treatment for patients with locally advanced rectal cancer in the United States consists of neoadjuvant radiation (45 to 55 Gy administered over 5 to 6 wk), followed by neoadjuvant chemotherapy (5-FU-based infusion + leucovorin), surgery 6 to 8 wk after completion of chemotherapy, and additional adjuvant chemotherapy after surgery[31]. In contrast, the standard regimen in most of Europe is now neoadjuvant short-course RT. The most recent European Rectal Cancer Consensus Conference concluded that neoadjuvant short-course RT (25 Gy administered over 1 wk), especially when combined with

5-FU-based chemotherapy, improved local control for patients with locally advanced rectal cancer[32].

Several important trials are currently in progress. The next interim analysis from Stockholm Ⅲ should provide some clues in the debate concerning short-course neoadjuvant RT and timing of surgery. Given the lack of data supporting improved overall survival rates with neoadjuvant or adjuvant RT, treatment failure in patients with stage Ⅱ and Ⅲ rectal cancer likely arises from distant metastases.

Current research trials focus on evaluating the impact of chemotherapy regimens on systemic disease in patients with locally advanced rectal cancer. The NSABP R-04 trial (radiation therapy and either capecitabine or fluorouracil with or without oxaliplatin before surgery in treating patients with resectable rectal cancer is designed to compare capecitabine (with or without oxaliplatin) *vs* 5-FU (with or without oxaliplatin) in patients with operable rectal cancer who undergo neoadjuvant RT. The EORTC is also currently enrolling patients in a similar trial comparing neoadjuvant CMT and adjuvant chemotherapy with (1) capecitabine and oxaliplatin *vs* (2) capecitabine alone in patients with locally advanced rectal cancer (PETACC-6).

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TOPIC HIGHLIGHT

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Sphincter preservation for distal rectal cancer - a goal worth achieving at all costs?

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Abstract

To assess the merits of currently available treatment options in the management of patients with low rectal cancer, a review of the medical literature pertaining to the operative and non-operative management of low rectal cancer was performed, with particular emphasis on sphincter preservation, oncological outcome, functional outcome, morbidity, quality of life, and patient preference. Low anterior resection (AR) is technically feasible in an increasing proportion of patients with low rectal cancer. The cost of sphincter preservation is the risk of morbidity and poor functional outcome in a significant proportion of patients. Transanal and endoscopic surgery are attractive options in selected patients that can provide satisfactory oncological outcomes while avoiding the morbidity and functional sequelae of open total mesorectal excision. In complete responders to neo-adjuvant chemoradiotherapy, a non-operative approach may prove to be an option. Abdominoperineal excision (APE) imposes a permanent stoma and is associated with significant incidence of perineal morbidity but avoids the risk of poor functional outcome following AR. Quality of life following AR and APE is comparable. Given the choice, most patients will choose AR over APE, however patients following APE positively appraise this option. In striving toward sphincter preservation the challenge is not only to achieve the best possible oncological outcome, but also to ensure that patients with low rectal cancer have realistic and accurate expectations of their treatment choice so that the best possible overall outcome can be obtained by each individual.

Key words: Rectal cancer; Survival; Local recurrence; Morbidity; Anorectal function; Quality of life; Patient preference

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INTRODUCTION

In the management of patients with rectal cancer, sphincter preservation is a priority and regarded a marker of surgical quality. Technical and technological advances have led to an increase in sphincter preserving surgery and a fall in the rate of abdominoperineal excision (APE)[1]. Furthermore, the recognition of the oncological importance of the circumferential, rather than distal resection margin, has allowed an increasingly aggressive surgical approach. The knowledge that a distal margin of 1 cm will safely allow complete tumor removal affords an ever greater proportion of patients the opportunity of sphincter preserving surgery for low rectal cancer[2]. In addition, our ever increasing understanding of tumor behaviour gives patients new options in the form of non-operative

treatment (following complete response to neo-adjuvant treatment), or transanal excision in selected circumstances. On the other hand, tumor down-staging following neo-adjuvant chemoradiotherapy has not led to the expected increase in sphincter preserving surgery.

Thus, for patients with low rectal tumors, and for whom APE would formerly have been the only option, a number of sphincter preserving options are now available. However, while it may be technically possible to reconstruct (or avoid radical surgery altogether) an increasing majority of patients with rectal cancer, we should pause to consider the overall merits of this approach and consider the patient's overall outcome (both oncological and functional), while remembering that there remain acceptable non-reconstructive alternatives (APE or low Hartmann's procedure). In doing so, a number of factors must be considered and the 'costs' of sphincter preservation evaluated.

ONCOLOGICAL OUTCOME IN THE TREATMENT OF RECTAL CANCER

The oncological outcome is of paramount importance whether anterior resection (AR), APE, transanal excision, or a non-operative approach is adopted in the treatment of low rectal cancer.

High rates of circumferential resection margin (CRM) positivity (up to 40%) following APE in some series and consequent high local recurrence rates have led to suggestions that the outcome following APE is inherently worse than that following AR. It does appear that rectal tumors in patients who undergo APE are often more locally advanced, more poorly differentiated, and show a lesser response to neo-adjuvant chemoradiotherapy[3]. However, with meticulous surgery and the avoidance of tumor perforation and margin positivity, results following APE can be similar to those after AR[4]. Indeed, local recurrence rates in the order of 5% can be achieved following the application of a standardised approach[5,6].

Undoubtedly the technique of APE has drifted from that originally described by Miles[7] in which a wide dissection of the rectum was performed to produce a cylindrical specimen. Application of TME principles and evolution in technique have resulted in an APE in which the specimen tapers (Morson's waist) at the level of the pelvic floor with a consequent narrow circumferential resection margin and risk of CRM positivity and tumor perforation. Recourse to originally described principles *via* an extra-levator approach avoids "waisting" of the specimen[8] and reduces the rate of CRM involvement[9]. Nonetheless, rates of CRM involvement may still lag behind those seen in AR[10] and there remains a need to further examine surgical technique in APE and develop a standardised approach with appropriate training if needed.

Inter-sphincteric resection represents the most extreme form of sphincter preserving surgery in which part, or all, of the internal sphincter is resected. This approach may be applied to tumors within 2 cm of the sphincter complex and is made feasible by the recognition that distal intramural tumor spread beyond 1 cm is uncommon. Thus, inter-sphincteric resection becomes an option for patients with tumors within 2 cm of the sphincter complex, in whom pre-operative continence is intact, and for whom the tumor, at least in its distal part, is confined to the rectal wall. Follow-up suggests that local (6.6%) and distant (8.8%) recurrence rates[11] are comparable to those in published series of APE. Patients with locally advanced (T3-T4) tumors may become candidates for inter-sphincteric resection if a favourable down-staging response to neo-adjuvant chemoradiotherapy is demonstrated[3]. Those who are not suitable for inter-sphincteric resection and require APE are likely to self-select as they have locally advanced tumors, that are poorly differentiated and show poor response to neo-adjuvant treatment[3].

Laparoscopy is increasingly employed as a less invasive approach in the management of rectal cancer. While the initial results from the UK MRC CLASSIC trial highlighted increased rates of margin positivity following laparoscopic rectal cancer surgery (when compared to conventional, open TME)[12], the long-term oncological outcomes do not appear to be compromised[13,14]. This study remains the only randomised controlled trial to assess the role of laparoscopy in rectal cancer, however results from prospective series of laparoscopic resection have also demonstrated similar oncological outcomes to those reported following open TME[15].

Transanal surgery for rectal cancer represents an attractive approach that may allow the morbidity and functional sequelae of total mesorectal excision (TME) to be avoided. Better surgical results with lower margin positivity are achieved following transanal endoscopic microsurgery (TEMS) than conventional transanal (TA) excision (2% *vs* 16%)[16], however outcomes are generally inferior to those following radical resection with a 3-5 fold increased local recurrence risk[17]. TEMS appears to be a reasonable option (LR < 5%) in selected patients with favourable pathological features (pT1 Sm1; well or moderately differentiated; < 3 cm diameter; no lymphovascular invasion)[18]. For tumors with less favourable features, the oncological result following TEMS is inferior to that seen after TME. Difficulty in reliably predicting the T-stage pre-operatively remains an obstacle to patient selection. Likewise, prediction of N-stage is problematic as up to 18% of T1 tumors will have associated nodal disease. However, in patients with adverse pathological features after TEMS, subsequent conversion to radical surgery does not appear to be associated with significantly increased LR rates[18]. In reality, the decision to adopt a transanal approach is frequently based upon the fitness of the patient.

One-fifth to one-quarter of patients following neo-adjuvant chemoradiotherapy will show a complete pathological response. Predicting those likely to respond and those who have had a complete pathological response remains difficult - up to 40% of patients who appear to have had a complete clinical response have residual disease following

resection[19]. Conversely, approximately 10% of patients who have an incomplete clinical response will show a complete pathological response[20]. Observation alone may be a viable alternative in selected patients who show a complete clinical response to neo-adjuvant therapy[20]. Local recurrence has been reported in 11% of those who had a sustained complete clinical response. These patients appear amenable to salvage therapy without adverse oncological outcome in the event of local recurrence[21].

There may also be a role for full thickness transanal excision of tumor in selected patients with T3 tumors who show an excellent response to neo-adjuvant chemoradiotherapy and who are deemed unfit for or refuse TME, or who had a perceived complete response to neo-adjuvant treatment. The limited available data point to local recurrence and survival figures that are comparable to those achieved with radical surgery[22]. This approach requires further validation.

Finally, endoscopic submucosal dissection is an evolving technique that may represent an alternative sphincter preserving approach in the management of rectal tumors. This technique has been reported with low complication rates and in patients in whom complete resection is achieved (approximately 70%) recurrence rates at short-term follow-up are low[23]. Further studies are required to establish the role of this technique.

FUNCTIONAL OUTCOME AND QUALITY OF LIFE FOLLOWING SURGERY FOR RECTAL CANCER

Functional outcome

Frequency, urgency, and soiling (anterior resection syndrome) are common problems after anterior resection that reflect loss of the capacitance and compliance of the rectal reservoir. Approximately 60% of patients experience some degree of incontinence, while one-third experience frequent symptoms of urgency and frequency. Post-operative studies suggest that anorectal dysfunction after low anterior resection is more a factor of reduced compliance and capacity, than diminished sphincter function[24,25]. Furthermore, reflexes of the anal sphincter that help to maintain continence are preserved after low anterior resection[26].

Patients undergoing inter-sphincteric resection have the additional insult of reduced internal sphincter function[24]. Inter-sphincteric resection is associated with a fall in resting anal canal pressures[27] and continence when compared to conventional anastomosis, but not with a worsening of stool frequency (typically averaging 2/24 h[28]) and urgency[29]. Long-term satisfactory continence rates are achievable in 75% of patients[11]. Outcomes, particularly in the first post-operative year, can be improved by performing only a partial or subtotal resection of the internal sphincter and through construction of a colonic J-pouch[27,30-32]. Pre-operative radiotherapy significantly worsens the functional outcome following inter-sphincteric resection[11].

Following straight anastomosis progressive dilatation of the neorectum can allow some improvement in compliance[33] and function over time. Colonic reservoirs (J-pouch or coloplasty) may allow early preservation of function by providing a neorectum functionally comparable to the resected rectum. It is technically possible to create a J-pouch in the majority of patients (95%)[34]. With optimum pouch size (5 cm)[35,36] and level of anastomosis (< 8 cm from the anal verge)[37], there appear to be functional advantages to the creation of a colonic J-pouch. Patients undergoing low anterior resection with J-pouch reconstruction have less stool frequency and urgency when compared to those with a straight anastomosis, however this benefit is not maintained beyond two years[34]. Surprisingly, this functional gain may not impact positively on quality of life after surgery[38]. Evidence would suggest that there is no significant advantage to coloplasty over straight anastomosis[38]. Side-to-end anastomosis using a short side limb may represent an alternative to colonic pouch with the limited available data suggesting comparable functional and surgical outcomes, however further studies are needed[39-41].

The benefits of the colonic pouch may not be attributable to an increased capacity when compared to straight anastomosis, but rather due to the interruption of normal propulsive motility[42,43].

Pre- or post-operative irradiation has a significant negative impact on function following anterior resection. In the Dutch TME study, pre-operative radiotherapy was associated with a significant increase in bowel frequency and incontinence (62% *vs* 38% for surgery alone) and this had a significant negative impact on patient satisfaction and daily activity[44]. Incontinence was worst in patients with lower tumors[44]. These findings have been replicated in other studies with long-term follow-up showing an approximate doubling of symptoms of faecal incontinence, soiling and bowel frequency when compared to patients treated with surgery alone[45]. Anorectal manometry has shown irradiated patients to have significantly lower resting and squeeze pressure, while endoanal ultrasound has shown increased scarring of the anal sphincter when compared to non-irradiated patients[24,45]. Short course pre-operative radiotherapy and pre-operative long-course chemoradiotherapy appear to impact similarly on anorectal function[46]. The functional outcome following post-operative radiotherapy is worse than following pre-operative treatment with patients experiencing increased frequency of defecation and clustering[47].

While reduced following pre-operative radiotherapy, the functional result in patients undergoing low anterior resection with colo-anal anastomosis appears to better with a colonic J-pouch rather than straight anastomosis or coloplasty at 24 mo follow-up[48].

Despite increased tumor down-staging, pre-operative conventionally fractionated radiotherapy does not appear to confer an advantage with respect to sphincter preservation over short-course radiotherapy[49].

Extended pelvic lymphadenectomy is frequently performed in Japan as an adjunct to TME, and often without neo-adjuvant treatment. This approach does not appear to confer an oncological advantage when compared to

TME alone (with neoadjuvant treatment) and is associated with an increased incidence of urinary and sexual dysfunction[50-52].

Quality of life

There is an absence of randomised studies comparing outcomes following APE and AR for low rectal tumors (due to presumption that AR is superior). As a result, inferences as to their comparative quality of life outcomes can only be drawn from individual studies. None-theless, the available data challenges the presumption that a permanent stoma automatically renders an inferior quality of life outcome when compared to that following restorative surgery. A meta-analysis of over 1400 patients from 11 studies showed no difference in general quality of life scores between patients who underwent APE and AR. While APE was associated with better emotional and cognitive function scores and superior future perspectives (patients' understanding of disease stage), vitality and sexual function scored better in patients undergoing AR[53]. These findings were consistent with those of an earlier meta-analysis[54], however, their interpretation must be tempered by the poor quality of a number of individual studies, and the limited follow-up duration which fails to allow for the progressive functional improvement patients often experience following AR.

MORBIDITY

The argument in favour of observation (and/or transanal excision) in complete responders to neo-adjuvant treatment is the avoidance of the morbidity and functional loss associated with TME, with or without a temporary or permanent stoma. Anorectal dysfunction, sexual dysfunction, difficulty voiding, and urinary incontinence are seen in up to one-third of patients following TME. Furthermore, these problems are exacerbated by pre-operative radiotherapy. Post-operative morbidity following laparoscopic and open rectal resection appears to be similar[12], while a benefit to the laparoscopic approach with respect to long-term complications such as adhesion small bowel obstruction and incisional hernia remains to be proven[55]. Laparoscopic resection appears to impact similarly on bladder function when compared to open TME, but may be associated with a worse outcome with regard to male sexual function[56].

For patients undergoing TME, larger studies have shown overall rates of early morbidity of approximately 40%. This figure increases to almost 50% following pre-operative radiotherapy. Of patients undergoing APE, approximately one-fifth develop perineal wound problems[57]. The incidence of perineal wound problems rises to 30% following radiotherapy[57] and doubles following extralevator APE (38%)[10]. Eleven percent of patients undergoing AR developed clinical anastomotic leaks in the Dutch TME trial. The leak rate was not affected by pre-operative radiotherapy, but was reduced with proximal defunctioning stoma (8% *vs* 16%)[57]. The mortality rate for non-irradiated patients was 3.3% in the same study.

Again, from the Dutch study we know that approximately 50% of patients undergoing AR will have a defunctioning stoma. It is worth noting that at long-term follow-up (median 48 mo) 21% of patients in one study who had undergone sphincter preserving surgery still had a stoma[58]. Loop ileostomy closure is associated with 17% morbidity, however the majority (80%) of patients can be managed non-operatively[59].

PATIENT PREFERENCE

The limited available evidence suggests that a majority (65%) of patients with rectal cancer are willing to defer decision making about their surgery to their surgeon[60].

What is not known, unlike for breast cancer, is the role that patients with rectal cancer would like to adopt in decision making, and how their given role influences their satisfaction with decision making and outcomes. We do know however that the relative importance that surgeons place on various outcomes such as permanent stoma and incontinence is often not matched by their patients[61]. Surgeons may in particular underestimate their patients' concerns. Furthermore, surgeon's choices may frequently be at odds with their patient's inherent and perhaps unrecognised true preference[62]. Patients, for example, express a stronger desire to avoid chemotherapy than to avoid permanent stoma, while doctors express the opposite view.

Multimedia decision aids (incorporating patient values into evidence based data) have been used to assess and quantify the relative importance patients with rectal cancer place on different quality of life outcomes. Patients who have had surgery place greater emphasis on the avoidance of incontinence post-operatively than the avoidance of a permanent stoma[61].

Trade-off techniques are another useful means of gauging patient's true preferences and will often highlight disparity between patients' preferences and those of their physicians[62]. Using this technique, the strength of a preference is measured by determining the degree of risk of a particular (poor) outcome that the patient would be willing to accept in order to have the treatment. When patient preferences are assessed using time-trade methods, patients strongly express a desire to avoid a stoma with 65% willing to trade a mean of 34% of their life expectancy to avoid this outcome[63]. Furthermore, patients expressed a stronger desire to avoid the option of APE and thus permanent stoma than their treating physicians. Again, in patients who have had surgery for rectal cancer, the majority of those without a stoma would be willing to trade frequent (monthly) episodes of incontinence in order to avoid a permanent stoma[64]. APE patients would however hypothetically trade fewer years of remaining life to be without a stoma, than AR patients would to be without incontinence[65].

While patients may often be happy to defer decisions as to the type of surgery to their surgeons, the majority of those patients who do choose, would favour AR over APE[60]. More patients who have had AR would choose

that option again, than patients who have had APE (69% *vs* 46%)[60]. Interestingly, at longer term follow-up 80% of patients who had APE indicate that they would choose the same option given the benefit of their experience[60].

CONCLUSION

Sphincter preservation in rectal cancer - a goal worth achieving at all costs? The answer must be no. While we should strive toward sphincter preserving options, we must recognize the limitations of currently available approaches and accept that sphincter preservation may not be the best overall option for each individual patient.

Oncological outcomes following AR and APE should be equivalent, however there remains room to uniformly improve and standardise approaches and outcomes in APE. If equivalence for oncological outcome is achieved, then functional outcome, quality of life, and ultimately patient preference become of paramount importance in decision making for the treatment of low rectal cancer. Anorectal dysfunction and poor functional outcome are common following AR. The alternative of APE or low Hartmann's procedure imposes a permanent stoma. Quality of life following APE appears to be similar to that following AR. Given the choice, most patients would choose AR over APE. It is doubtful however that patients appreciate fully the functional outcome following AR, and also likely that patients harbour excessively negative misconceptions about life with a permanent stoma. Patients must be informed that function may not be as good as they expect after AR, and also that patients who have undergone APE positively appraise this option at follow-up. The morbidity associated with stoma reversal (following AR), and the significant risk of perineal wound problems following APE must also be considered. Non-radical and even non-operative approaches are increasingly an option in the management of selected patients with low rectal cancer that obviate the morbidity and outcomes following TME. Ultimately we must ensure that patients with low rectal cancer have realistic expectations of their treatment options and that their decisions are truly informed.

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TOPIC HIGHLIGHT

Imran Hassan, MD, Assistant Professor, *Series Editor*

Minimally invasive surgery for rectal cancer: Are we there yet?

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Abstract

Laparoscopic colon surgery for select cancers is slowly evolving as the standard of care but minimally invasive approaches for rectal cancer have been viewed with significant skepticism. This procedure has been performed by select surgeons at specialized centers and concerns over local recurrence, sexual dysfunction and appropriate training measures have further hindered widespread acceptance. Data for laparoscopic rectal resection now supports its continued implementation and widespread usage by expeienced surgeons for select patients. The current controversies regarding technical approaches have created ambiguity amongst opinion leaders and are also addressed in this review.

Key words: Laparoscopic; Rectal cancer; Minimally invasive; Mesorectal excision

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INTRODUCTION

The benefits of laparoscopic colon surgery compared to the open approach are well established[1-4]. Furthermore, laparotomy has been associated with an increased morbidity when compared to minimally invasive techniques for colorectal disease[5]. More recently, the implementation of enhanced care programs coupled to laparoscopic resection has also resulted in a significant reduction in length of stay after both colon and rectal resection[6,7]. Laparoscopic colon surgery for select cancers is slowly evolving as the standard of care but minimally invasive approaches for rectal cancer have been viewed with significant skepticism.

Laparoscopic rectal resection for cancer is performed by select surgeons at specialized centers. The variability in anatomic definitions of the rectum, technique, selection criteria, and need for neoadjuvant therapy amongst this group of surgeons have made parallel comparisons difficult and ambiguous. Concern over local recurrence, sexual dysfunction and appropriate training measures have further hindered widespread acceptance of this approach. This opinion addresses short-term and oncological outcomes for laparoscopic resection of rectal cancer, the aforementioned obstacles, and current controversies regarding technical approaches.

ONCOLOGICAL OUTCOMES

There are many potential endpoints for determining success for laparoscopic rectal resection. Undoubtedly, the

most significant is ensuring oncologic equivalence when compared to the open technique. This variable can primarily be measured by the adequacy of circumferential radial margins, recurrence rates, and both disease free and overall survival. Furthermore, the incidence of sexual dysfunction and other complications after laparoscopic pelvic dissection should approximate that with the open approach.

Circumferential radial margin

A positive circumferential resection margin (CRM) is a known marker for increased risk of future recurrence[8]. Strict adherence to the principles of "total mesorectal excision" is essential to preserve the mesorectal envelope, obtain an adequate circumferential margin and therefore reduce local recurrence rates. The first randomized trial for laparoscopic rectal resection showed a trend towards increased CRM positivity (6% open *vs* 12% laparoscopic, $P = 0.19$) for anterior resection[3]. Although this was initially alarming, several surgeons involved were on their learning curve, and preoperative chemoradiotherapy (CRT) was not standardized. Fortunately, three year outcomes showed that the difference in CRM positivity between laparoscopic and open approaches for anterior resection did not influence local recurrence rates. More recently, five year outcomes revealed no difference between groups in survival, disease-free survival, and local and distant recurrence[9,10]. Wound/port-site recurrence rates in the laparoscopic arm were 2.4% and also unchanged[10]. Conversion was associated with significantly worse outcomes overall but not disease-free survival.

In the largest retrospective review to date, Ng *et al*[11] reported 579 laparoscopic rectal resections for cancer with a CRM positivity of 2.14%. These encouraging results were further substantiated by two recent randomized controlled trials that reported CRM positivity rates of 2.9% (open) *vs* 4% (laparoscopic)[12] and 1.4% (open) and 2.6% (laparoscopic)[13].

In 2006, the Spanish Association of Surgeons started an audited teaching program to both make known the results of rectal cancer treatment and improve the outcomes by the teaching process. The quality of the pathologic specimens for laparoscopic and open rectal resection patients was scored and the circumferential radial margin was positive if tumor was located 1 mm or less from the surface of the specimen. No differences between groups for the completeness of the mesorectum or distance of the tumor from the CRM were observed[14]. Although laparoscopic TME amongst this experienced group approximates that for their open resection for select tumors, the results may not be as favorable for low bulky lesions or those in an obese male or narrow pelvis.

Local recurrence

As highlighted above, the five year results of the MRC CLASSIC trial reported similar regional recurrence for laparoscopic *vs* open resection of rectal cancer. Several other studies have also shown acceptable regional recurrence rates. In their retrospective review, Ng and colleagues reported two port site recurrences and a pelvic recurrence rate of 7.4%[11]. Similarly, ten year outcomes from a prospective randomized trial for the laparoscopic resection of upper rectal cancers demonstrated a regional recurrence rate of 7.1% with no port-site recurrences[13]. Laurent and colleagues aimed to assess long-term oncologic outcomes after laparoscopic versus open surgery for rectal cancer from in a retrospective comparative study[15]. 471 patients had rectal excision for invasive rectal carcinoma during the trial period: 238 were treated by laparoscopy and 233 by open procedure. At 5 years, there was no difference of local recurrence (3.9% *vs* 5.5%, $P = 0.371$) between laparoscopic and open surgery[15].

The multi-institutional series from Japan reported 1057 selected patients with rectal cancer that underwent laparoscopic surgery[16]. All the data regarding the patient details and operative and postoperative outcome were collected retrospectively. At thirty months recurrence was found in 6.6% of the 1011 curatively treated patients. Specifically, local recurrence occurred in 11 patients (1.0%) and there was no port-site metastasis (Table 1)[15].

Table 1 Overall survival for laparoscopic rectal resection with minimal 5 yr follow-up

Authors	Survival (laparoscopic)	Survival (open)	Follow-up (yr)
MRC CLASSIC (Jayne *et al*)	57.9%	58.1%	5
Sartori *et al*	75.4%	NA	5
Ng *et al*	63.9%	55.0%	10
Lam *et al*	64.0%		5
Laurent *et al*	82.0%	79.0%	5
Ng *et al*	70.0%	NA	5
Siami *et al*	80.2%	NA	5
Bianchi *et al*	81.4%	NA	5
Tsang *et al*	81.3%	NA	5

NA: Not applicable.

FUNCTIONAL OUTCOMES

Laparoscopic rectal surgery proponents argue that the view in the pelvis is superior compared to the open approach. This magnification theoretically provides better visualization of the pelvic nerves. However, in the first randomized trial for laparoscopic rectal cancer male sexual function, erection and ejaculation were all significantly reduced with laparoscopic surgery. This should be interpreted with caution considering the aforementioned learning curve and that more patients in the laparoscopic group underwent a full TME, as compared to the open group. Bladder function remained similar between groups.

In a prospective evaluation of sexual function Stamopoulos and colleagues[17] used the international index of erectile function (IIEF) for 56 patients who underwent rectal cancer surgery (38 open *vs* 18 laparoscopic procedures, 38 low anterior *vs* 18 abdominoperineal resections). Rectal cancer resections were associated with a significant reduction in IIEF scores and high rates of sexual dysfunction at 3 and 6 mo. The IIEF and domain scores at different assessment points were comparable between the laparoscopic and open surgery groups[17].

Morino *et al*[18] also analyzed male sexual and urinary function after laparoscopic total mesorectal excision. They found that sexual desire was maintained by 55.6%, ability to engage in intercourse by 57.8%, and ability to achieve orgasm and ejaculation by 37.8% of the patients. The distance of the tumor from the anal verge and adjuvant or neoadjuvant treatments were the significant predictors of poor postoperative sexual function. Seven patients (14%) presented transitory postoperative urinary dysfunction, all of whom were medically treated. Tumor stage and distance from the anal verge were independently associated with the postoperative global international prostatic symptom score (IPSS). No differences were observed in urinary quality of life. The authors concluded that laparoscopic resection did not reproduce or improve on sexual and urinary dysfunction outcomes obtained in the best open TME series[18].

In another series with investigators well beyond their learning curve, urinary dysfunction was reported by 6 (6%) patients and 6 (6%) patients had sexual dysfunction, manifesting as retrograde ejaculation in four patients and erectile dysfunction in a further two patients. The low rates of sexual dysfunction in this unit may be attributable to pelvic dissection only being undertaken by experienced, dedicated laparoscopic colorectal surgeons. Previous studies reporting poorer functional outcomes have probably included a significant number of patients on the surgeons' learning curve.

CONVERSION

The conversion rate for laparoscopic rectal resection is variable between centers and levels of expertise. The MRC CLASSIC randomized trial had a conversion rate of 32% for rectal cancer[3], yet a previous experience of only 20 laparoscopic colon and rectal cases was sufficient to participate. A similar conversion rate (30%) was realized by Ng *et al*[11] in their ten year experience with laparoscopic rectal resection. After the inception of this trial significant improvements in energy devices, ports, cameras, and stapling devices have occurred that, combined with their experience, would likely decrease their current conversion rate.

Further analysis has shown that factors associated with conversion are BMI, male sex, and locally advanced tumors[19].

More recently, conversion rates reflect the beneficial impact of extensive experience. Three large retrospective series (2008-2010) have reported conversion rates as low as 5.4%[11], 15%[15], and 4.9%[20]. The multi-center retrospective series from Japan also demonstrated a reasonable conversion rate of 7.3%[16].

Conversion rates are as dependent on a reasonable inclusion or selection criteria as surgeon experience. Very low bulky tumors, anterior lesions in men with previous intervention for prostate cancer, T4 lesions, reoperative pelvic dissections and morbidly obese patients should be reserved for the open approach in most cases.

DEFINING THE RECTUM

There has been considerable debate as to the exact length of the rectum, the site of transition from sigmoid to rectum and most importantly the point of reference from where measurements are made. Within the surgical literature, numerous series have reported rectal cancer as being within 15, 16 and even 18 cm from the verge, although several other series use the dentate line as the reference point. Currently, the variability of these definitions not only impacts surgical decision making between centers but also the timing and need for neoadjuvant therapy, which in turn impacts oncologic outcomes and morbidity rates.

There are also significant differences in practice internationally with respect to the selection criteria used for CRT. In the United States, most practitioners adhere to the NCCN guidelines that recommend neoadjuvant CRT for patients with T3 or N1 disease with tumors within 10 cm of the dentate line[21]. The Mercury study group[22] has provided evidence that pre-operative MRI can accurately predict surgical resection margins. This report has led to a paradigm shift in the preoperative investigation and treatment of rectal cancer in the UK. With this approach, CRT is predominantly used when the tumor threatens or involves the mesorectal fascia and in all low rectal cancer where there is an inherent increased risk of involving the CRM.

Despite these apparent discrepancies most surgeons and oncologists generally agree that rectal cancer consists of extraperitoneal and intraperitoneal lesions. Tumors at or below the anterior reflection should be grouped together in investigations and are the real subject of this and other discussions surrounding laparoscopic rectal cancer.

TECHNICAL ISSUES

The most important variable being assessed with laparoscopic vs open rectal resection for cancer is the pelvic dissection. Surgeons must analyze their own ability to perform a laparoscopic total mesorectal excision with the same precision achieved by their open technique. Although this fact seems obvious it cannot be understated. Several studies continue to populate the literature describing a "hybrid" technique. With this approach the mobilization of the left colon is performed laparoscopically and the pelvic dissection and transection of the rectum are performed through a Pfannenstiel or lower midline incision. Outcomes with this technique have been favorable and it certainly has inherent advantages but unquestionably it is not laparoscopic rectal surgery. Therefore, although published results substantiate its role, ideally it should not be included in trials or case series for laparoscopic rectal resection and should not be billed or coded as such. If this procedure continues to demonstrate favorable outcomes and has a shorter learning curve it may require its own procedure code in the future.

Internationally, the straight laparoscopic approach with three or four abdominal trocar sites and a left lower quadrant or periumbilical extraction incision is preferred. Outcomes with this approach (outlined in previous section) were initially concerning but have now more consistently been favorable. As discussed above, the protracted opera-

tive times and concerns over both local recurrence and sexual function have been diminished with increased operative experience. This may be the most technically demanding method and surgeons preferring this technique recognize its limitations. Dividing the lower rectum, providing adequate traction low in the pelvis, and teaching trainees how to perform an appropriate total mesorectal excision are the current challenges. This procedure is less daunting for patients requiring an abdominal perineal resection. They are left without the morbidity of an abdominal wound as the specimen is routinely removed through the perineum.

Proponents of hand-assisted laparoscopy in the United States continuously have demonstrated equivalent outcomes for laparoscopic colon resection with reduced operative times. More recently results with hand-assisted methods for rectal cancer have also been reported with success[23,24]. When the hand-assisted device is left in place and the pelvic dissection is performed laparoscopically these cases should be included with other minimally invasive approaches to rectal cancer. This approach may be favorable in patients with a bulky mesorectum or when additional tension is required to facilitate accurate transection of the low rectum.

Dividing the rectum laparoscopically is not always technically feasible The limited angulation of the stapler and physical limitations of working in the bony confines of the pelvis are common deterrents[25]. In this situation, having an assistant apply perineal pressure may elevate the pelvic floor enough to allow the first cartridge of the stapler to reach the anorectal junction. Furthermore, utilizing a suprapubic port or medicalizing the right lower quadrant port may help. Lastly, if these techniques are unsuccessful a limited lower midline or Pfannenstiel incision can be made and a 30 mm open stapler can be introduced. If an appropriate distal margin is not obtainable with these methods a mucosectomy with partial inter-sphincteric resection and hand-sewn coloanal anastomosis is performed.

In addition to the difficulty with transection, very low anteriorly based and bulky lesions are often challenging. Entering the appropriate plane anterior to Denonvillier's fascia laparoscopically, respecting the need for an adequate radial margin, and maintaining meticulous hemostasis is essential. In this location, tissue planes can be more ambiguous and any bleeding further obscures the appropriate anatomy. If there is considerable doubt that the correct tissue plane is being violated, immediate conversion is warranted. Ideally these tumors are approached by surgeons who are well past their learning curve for laparoscopic pelvic dissection.

The recognition of these technical limitations and the ongoing development of advanced technology led to the introduction of robotic applications for low pelvic dissection. Data for robotic approaches to rectal cancer have recently been published and presented in national and international forums. The advantage of operating with more degrees of freedom for low rectal cancer is apparent and is of particular benefit in a narrow male pelvis. However, concerns over significantly increased cost, operative times, and training have limited its widespread adoption. Furthermore, proponents seem to be employing this approach *carte blanche* and looking for opportunities to expand its indications rather than using it as a tool. In the era of economic constraints and limited resident exposure to cases a costly technique with ill defined training methods should be used for select cases only.

CONCLUSION

Technical advances in the field of coloproctology have unquestionably improved patient outcomes. However, it is essential that we continue to strive to define the appropriate inclusion criteria for new approaches in regards to patient, disease, and surgeon experience. Historically, new technology, such as the PPH stapler, robotics, and laparoscopy, has become more than an optional approach or "tool". Surgeons inherently develop extraordinary comfort with the technology and tend to expand its indications, often illogically. Creativity and "pushing the envelope" should not be discouraged but when it becomes apparent that new approaches become simply a "means to an end" patients outcomes may be less than ideal.

The abundance of data for laparoscopic rectal resection for cancer supports its continued implantation and widespread usage by experienced surgeons for select patients. Until we become more adept at operating in the low narrow pelvis and transecting the rectum we must recognize that this approach is complementary to our open technique. To ensure the best outcomes we must continue to recognize the difference between the questions, "can you?" and "should you?" in regards to minimally invasive surgery.

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REVIEW

Advances in diagnosis, treatment and palliation of pancreatic carcinoma: 1990-2010

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Abstract

Several advances in genetics, diagnosis and palliation of pancreatic cancer (PC) have occurred in the last decades. A multidisciplinary approach to this disease is therefore recommended. PC is relatively common as it is the fourth leading cause of cancer related mortality. Most patients present with obstructive jaundice, epigastric or back pain, weight loss and anorexia. Despite improvements in diagnostic modalities, the majority of cases are still detected in advanced stages. The only curative treatment for PC remains surgical resection. No more than 20% of patients are candidates for surgery at the time of diagnosis and survival remains quite poor as adjuvant therapies are not very effective. A small percentage of patients with borderline non-resectable PC might benefit from neo-adjuvant chemoradiation therapy enabling them to undergo resection; however, randomized controlled studies are needed to prove the benefits of this strategy. Patients with unresectable PC benefit from palliative interventions such as biliary decompression and celiac plexus block. Further clinical trials to evaluate new chemo and radiation protocols as well as identification of genetic markers for PC are needed to improve the overall survival of patients affected by PC, as the current overall 5-year survival rate of patients affected by PC is still less than 5%. The aim of this article is to review the most recent high quality literature on this topic.

Key words: Diagnosis; Epidemiology; Palliation; Pancreatic cancer; Therapy

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INTRODUCTION

The vast majority (90%) of pancreatic cancers (PC) are malignant tumors originating from pancreatic ductal cells[1]. Anatomically, 78% of PCs are located in the head, and the remaining 22% are equally distributed in the body and in the tail[2]. The most common clinical presentations are progressive weight loss and anorexia, mid abdominal pain and jaundice[3-5]. Over the past two decades many advances in the diagnosis, therapy and palliation of PC have taken place although the overall survival of affected patients has not improved significantly. The aim of this article is to review the most recent high quality literature on this topic.

SEARCH STRATEGY AND SELECTION CRITERIA

The literature search was targeted at studies that reported at least one of the following aspects of PC: epidemiology, diagnosis, therapy (e.g. surgery, radiotherapy, chemotherapy) and palliation. Randomized controlled trials (RCT) and prospective observational studies were given preference. Each of the topics was searched in MEDLINE, Ovid MEDLINE In-process, Cochrane Database of Systematic Reviews, Database of Systematic Reviews, Database of Abstracts of Review of Effects, EMBASE, PubMed, National Library of Medicine Gateway by established systematic review methods (Jadad Scale for RCT, as well as Downs and Black checklist for observational studies)[6-8]. Articles from the authors' libraries and reference lists were further reviewed. We limited our search to English-language articles published from January 1990 to September 2010. We then developed a comprehensive and current database to catalog the medical literature on PC. To identify all potential papers, we searched the medical subject headings reported in Table 1. Three authors (Sharma C, Eltawil KM and Molinari M) independently performed the selection of the articles based on the content of titles and abstracts. When in doubt, each article was reviewed entirely. The decision to include articles in this review was reached by consensus. For conciseness, a full list of search strategies, search results, and quality assessment for each included study are available on request from the corresponding author.

EPIDEMIOLOGY

PC is the fourth leading cause of cancer related mortality in the United States with an estimated 42 500 new cases and 35 000 deaths from the disease each year[9]. In industrialized countries, the incidence of PC (11 per 100 000 individuals) ranks second after colorectal cancer among all gastrointestinal malignancies[10]. While the mortality rate for males has decreased by 0.4% from 1990 to 2005, the mortality rate for females has increased by 4.4%[9]. More than 80% of PCs are diagnosed in patients older than 60 and almost 50% have distant metastases at the time of presentation[10-12]. Men are more frequently affected than women [relative risk (RR) = 1.3] and individuals of African American descent in comparison to Caucasians (RR = 1.5)[10]. Analysis of overall survival shows that the prognosis of PC is still quite poor despite the fact that 1-year survival has increased from 15.2% (period between 1977-1981) to 21.6% (period between 1997-2001) and 5-year survival has increased from 3% (period between 1977-1986) to 5% (period between 1996-2004)[10].

RISK FACTORS

Smoking

The risk of PC in smokers ranks second to lung cancer[13] and it is proportionate to the frequency [≥ 30 cigarettes per day: odds ratio (OR) = 1.75], duration (≥ 50 years: OR = 2.13) and cumulative smoking dose (≥ 40 pack/years: OR = 1.78)[14]. A meta-analysis of 82 studies from 4 continents has shown that cigarette smokers were diagnosed at significantly younger age and had a 75% increased risk of developing PC in comparison to the regular population[15] and the risk persisted for 5 to 15 years after cessation[16]. In a case-control study of 808 PC patients matched against 808 healthy controls, female smokers were at increased risk in comparison to males as they suffered from a synergistic interaction between cigarette smoking, diabetes mellitus (OR = 9.3) and family history of PC (OR = 12.8)[17].

Diabetes

Nearly 80% of PC patients have either frank diabetes or impaired glucose tolerance[18]. Diabetes is usually diagnosed either concomitantly or during the two years preceding the diagnosis[19]. Several studies have assessed the role of diabetes in PC with conflicting results. A meta-analysis of 11 cohort studies found that the relative risk for diabetics was 2.1 [95% confidence interval (95% CI): 1.6-2.8][20]. These findings were supported by another cohort study of 100 000 Danish diabetic patients which found a standardized incidence ratio of 2.1 (95% CI: 1.9-2.4) in a 4-year follow-up[21]. A large prospective cohort study of 20 475 men and 15 183 women in the United States, has shown that the relative risk of PC mortality adjusted for age, race, cigarette smoking, and body mass index (BMI) was proportionate to the severity of abnormal glucose metabolism: RR was 1.65 for post load plasma glucose levels between 6.7 and 8.8 mmol/L; 1.60 for levels between 8.9 and 11.0 mmol/L, and 2.15 for levels equal or more than 11.1 mmol/L[22]. Diabetes can be an early manifestation of PC as about 1% of new onset of diabetes in patients older than 50 is linked to PC[23], but there is no evidence that screening for recent onset diabetes would reduce the mortality[12] or lead to early diagnosis[24].

The link between abnormal glucose and PC exists only for type Ⅱ diabetes. A meta analysis of 36 studies has shown that the OR of PC for patients with type Ⅱ diabetes for more than 5 years was 2.1[25], while there are no reports on the association between PC and type I diabetes[26].

Family history of diabetes does not appear to be a risk for PC. Compared to subjects with no family history, diabetics with a positive family history have an OR of 0.8 while non-diabetics with a positive family history have an OR of 1.0[27].

A recent prospective study found that women with gestational diabetes have a relative risk of PC of 7.1 (95% CI: 2.8-18.0)[28]. Gapstur and colleagues have proposed a mechanism to explain these findings[22] by the fact that at high levels, insulin binds to the insulin-like growth factor I (IGF1) receptor[24] and downregulates IGF binding protein 1[25] causing an increase in cell growth in PC cell lines[29,30].

Alcohol

The role of alcohol is controversial and several studies

Table 1 Summary of the terms used singly or in combination for evidence acquisition

Primary MeSH terms	Secondary MeSH terms (epidemiology, diagnosis)	Secondary MeSH terms (treatment, palliation)
Pancreatic neoplasm(s)	Epidemiology	Pancreaticoduodenectomy
Adenocarcinoma(s)	Classification	Resection
Carcinoma(s)	Diagnosis	Therapeutic(s)
Pancreatic diseases	Differential diagnosis	Treatment outcome(s)
Pancreas	Risk factor(s)	Surgery
Carcinoma, pancreatic ductal	Diagnostic imaging	Surgical procedures
Pancreatic duct(s)	Magnetic resonance imaging	Clinical trial(s)
Humans	Endosonography	Controlled clinical trial(s)
Adult	Ultrasonography	Randomized controlled trial(s)
	Emission computed tomography	Clinical trial (phase Ⅰ)
	Radionuclide imaging	Clinical trial (phase Ⅱ)
	Positron emission tomography	Clinical trial (phase Ⅲ)
	Tomography	Clinical trial (phase Ⅳ)
	X-ray computed	Drug therapy
	Biopsy (fine needle)	Chemotherapy
	Biopsy (needle)	Neoadjuvant therapy
	Cytology	Adjuvant
	Cytodiagnosis	Antineoplastic combined chemotherapy protocols
	Tumor markers (biological) antigen(s)	Antineoplastic agent(s)
	Carcinoembryonic antigen	Antimetabolites, antineoplastic
	Ca 19-9 antigen	Combined modality therapeutic antineoplastic
	Ca 125 antigen	Combined chemotherapy protocols neoadjuvant
	Antigens, tumor-associated, carbohydrate	Therapy
	Endoscopic retrograde cholangiopancreatography	Radiotherapy
	Computed assisted image processing	Drainage
	Sensitivity and specificity	Cholestasis
	Endoscopy	Obstructive jaundice
		Celiac plexus
		Autonomic nerve block
		Nerve block
		Ethanol
		Injections, intralesional
		Cisplatin
		Deoxycytidine
		Epidermal growth factor
		Fluorouracil
		Endostatin
		Biological products
		Neoplasm proteins
		Immunotherapy
		Antibodies, monoclonal

have shown inconsistent findings. This might be attributed to multiple associations with confounding variables mainly smoking, socio-economic status[31] and pancreatitis[30]. A recent pooled analysis of 14 cohort studies with a sample of 862 664 individuals has shown a slight positive association between PC and alcohol intake only for consumption above 30 g/d (RR = 1.22; 95% CI: 1.03-1.45)[32]. Contrasting findings were reported by a European epidemiological study with a smaller sample size (n = 555) that did not show any association between PC and alcohol consumption[33].

Compared with light drinkers, men consuming large amounts of hard liquor suffered from a 62% increased risk of PC (95% CI: 1.24-2.10)[16,34], but this was not observed for women or for beer and wine drinkers[34].

Although moderate alcohol consumption is not a risk factor, African Americans were found to have a significantly higher OR when adjusted for their drinking habits, suggesting that racial differences might play a role in the development of PC[35].

Pancreatitis

Several studies have shown a positive association between PC and history of pancreatitis, although the magnitude is still controversial[36,37]. An international epidemiological study reported that both genders with chronic pancreatitis had an increased risk independently of the cause of pancreatitis[37]. A large case-control study showed that chronic pancreatitis lasting more than 7 years was associated with a higher risk of PC (RR = 2.04; 95% CI: 1.53-2.72)[38]. A large Italian study from 1983 to 1992 found similar results, as the risk increased after 5 or more years of chronic pancreatitis (RR in the first 4 years = 2.1, RR after 5 years = 6.9)[34]. These findings have been challenged by an international study, as the risk was significantly increased only in the early years after diagnosis. This would suggest that pancreatitis might represent a manifestation of PC that becomes apparent only several years later, rather than a risk factor. The risk of PC in chronic pancreatitis has been shown to be especially true for patients affected by hereditary pancreatitis, who were found to have 53 times

the risk in comparison to normal individuals[39]. This was confirmed by another study that estimated a 40% cumulative risk of PC in patients with hereditary pancreatitis by the age of 70. For patients with paternal inheritance, the cumulative risk of PC was even higher with risk up to 75%[40]. Cytokines, reactive oxygen molecules and pro-inflammatory compounds seem to be responsible, as inflammation is a risk factor for many other solid tumors[38].

Genetic predisposition for PC

Genetic predisposing factors have been a topic of intense research in the last decades. Case reports of families with multiple affected members suggest that PC might have a hereditary background[41]. Yet, a large population study on twins identified hereditary factors for prostatic, breast and colorectal cancers, but not for PC[42]. A Canadian study on patients with suspected hereditary cancer syndromes found that the standardized incidence rate of PC was 4.5 (CI 0.54-16.) when cancer affected one 1st degree relative, and increased to 6.4 (CI 1.8-16.4) and 32 (CI 10.4-74.7) when two and three 1st degree relatives were affected, respectively[43]. This translates to an estimated incidence of PC of 41, 58 and 288 per 100 000 individuals, respectively, compared to 9 per 100 000 for the general population[44].

Brentnall *et al*[45] and Meckler *et al*[46] described examples of autosomal dominant PC in individuals presenting at early age (median age 43 years) and with high genetic penetrance (more than 80%). A mutation causing a proline (hydrophobic) to serine (hydrophilic) amino acid change (P239S) within a highly conserved region of the gene encoding paladin (PALLD) was found in all affected family members and was absent in non-affected individuals of the same family (family X). Another study has shown that the P239S mutation was only specific for family X and was not a common finding in other individuals with suspected familial PC[47]. Currently, genetic predisposition is thought to be responsible for 7% to 10% of all PC[48]. Genetic factors including germline mutations in p16/CDKN2A[49], BRCA2[50-52] and STK 11[53] genes increase the risk of PC. The combination of all these known genetic factors accounts for less than 20% of the familial aggregation of PC, suggesting the role of other additional genes.

A systematic review and meta analysis of studies that quantified familial risk of PC has shown that individuals with positive family history have an almost two-fold increased risk (RR = 1.80, CI 1.48-2.12)[54]. Therefore, families with two or more cases may benefit from a comprehensive risk assessment involving collection of detailed family history information and data regarding other risk factors[55]. A case-control study of PC in two Canadian provinces (Ontario and Quebec) assessed a total of 174 PC cases and 136 healthy controls that were compared for their family histories of cancer. Information regarding the ages and sites of cancer was obtained in 966 first degree relatives of the PC patients and for 903 first degree relatives of the control group. PC was the only malignancy in excess in relatives of patients with PC, compared to the control group (RR = 5, $P = 0.01$). The lifetime risk of PC was 4.7% for the first degree relatives and the risk was 7.2% for relatives of patients diagnosed before the age of 60[56].

Besides the isolated aggregation of PC in some families, several other hereditary disorders predispose to PC in known familial cancer conditions[57]. These include hereditary pancreatitis, Puetz-Jeghers syndrome, familial atypical multiple mole melanoma, familial breast and ovarian cancer, Li-Fraumeni syndrome, Fanconi anaemia, Ataxia-telangiectasia, familial adenomatous polyposis, cystic fibrosis and possible hereditary non-polyposis colon cancer or Lynch syndrome[11,55,58-60].

Familial PC registries

As the prognosis of PC is generally poor, there has been a strong interest in detecting genes or other markers that could help identify high risk patients at an early stage. Although a precise genetic marker for this scope is not currently available, geneticists and epidemiologists have been profiling traits of high risk families enrolled in registries established in North America and Europe[61]. Even if there is no standardized definition for familial PC, most authors apply the term to families with at least two first degree relatives affected by PC in the absence of other predisposing familial conditions[61]. The creation of familial PC registries has been used not only for identification of genetic mutations, but also for the screening of high risk individuals. In selected centers in North America and Europe, screening programs for high risk individuals have been implemented with the use of endoscopic ultrasound (EUS) and computed tomography (CT) scanning or magnetic resonance imaging (MRI). Such early diagnosis of PC within a comprehensive screening program is hoped to ultimately result in improved survival[62]. The discovery of the genetic bases of inherited PC continues to be an active area of research, and in 2001 a multi-center linkage was formed to conduct studies aimed at the localization and identification of PC susceptibility genes (PACGENE)[63]. The complex nature of pedigree data makes it difficult to accurately assess risk based upon the simple counting of the number of affected family members, as it does not adjust for family size, age of onset of PC, and the exact relationship between affected family members. Therefore, computer programs have been developed to integrate these complex risk factors and pedigree data. In April 2007, the 1st risk prediction tool for PC, PanaPro was released[64]. This model provides accurate risk assessment for kindreds with familial PC as the receiver operating characteristic (ROC) curve was 0.75 which is considered good for predictive models.

Nutritional status

A number of studies have explored the relationship between BMI, lifestyle, diet and the risk of PC, but uncertainty regarding the strength of this relationship still exists. A recent case-control study of 841 patients and 754 healthy controls showed that individuals with a BMI of 25-29.9 had an OR of 1.67 (95% CI: 1.20-2.34) in comparison to obese patients (BMI of $\geqslant 30$) who had an OR of 2.58 (95% CI: 1.70-3.90) independently of their diabetes status[65]. The duration of being overweight was

Table 2 Known risk factors for pancreatic cancer

Age (more than 60 yr)
Smoking
Diabetes
Type II
Gestational diabetes
Impaired glucose tolerance
Alcohol
Pancreatitis
Acute
Chronic
Genetic predisposition
Family history
Hereditary disorders
Hereditary pancreatitis
Puetz-Jeghers syndrome
FAMMM
Familial breast and ovarian cancer
Li-Fraumeni syndrome
Fanconi anaemia
Ataxia-telangiectasia
Familial adenomatous polyposis
Cystic fibrosis
HNPCC
Lynch syndrome
Obesity

FAMMM: Familial atypical multiple mole melanoma; HNPCC: Hereditary non polyposis colon cancer.

significantly longer among patients with PC than controls. Being obese or overweight, particularly in early adulthood, resulted in earlier onset of PC (age at presentation of PC was 61 years for overweight patients and 59 years for obese) when compared to the median age of diagnosis (64 years) in the general population[66]. A number of studies reported that central weight gain measured by waist circumference and/or waist-to-hip ratio had a statistically significant increased risk compared to those with peripheral weight gain (RR = 1.45, 95% CI: 1.02-2.07)[67,68]. The known risk factors for PC are summarized in Table 2.

CLASSIFICATION

Anatomical classification

According to the location, PC can be divided in three groups: tumors of the head, body and tail. PCs of the head are at the right side of the superior mesenteric vessels, and tumors of the neck and body are located between the superior mesenteric vessels and the inferior mesenteric vein. PCs of the tail are located to the left of the inferior mesenteric vein.

A large epidemiological study[2] of 100,313 patients in the United States has shown that 78% of PC presents in the head, 11% in the body and 11% in the tail (Figure 1).

Pathological classification

Recent advances in surgical pathology techniques integrated with molecular biology have allowed advances in the modern classification of PC. A summary of the clinicopathological features of the different categories of PC is shown in Table 3.

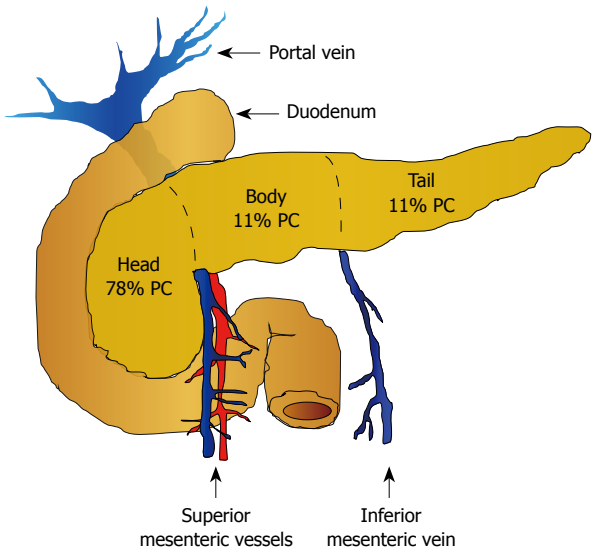

Figure 1 Graphical representation of the pancreas and frequency of pancreatic cancer in the three anatomical sections: head, body and tail. PC: Pancreatic cancer.

Ductal infiltrating adenocarcinoma

Ductal infiltrating adenocarcinoma (DIA) represents the most common type (85%-90%) of PC originating from ductal epithelial cells. Most DIAs appear as whitish masses, hard at palpation and poorly defined from surrounding tissues, predominantly solid although cystic degenerations can be seen in larger tumors[89]. The microscopic appearance of DIA ranges from well-differentiated neoplasms difficult to distinguish from reactive gland, to poorly differentiated. The majority of DIAs are moderately to poorly differentiated and develop a dense desmoplastic stroma[89]. Mutations in the KRAS2 or p16/CDKN2A genes are observed in 90% of patients, TP53 gene abnormalities in more than 75% and more than 55% of cases have changes in MADH4/DPC4 genes. Tumors showing loss of DPC4 expression have a worse outcome than those with intact DPC4[90], and immunolabeling for DPC2 protein can help to classify metastatic carcinomas of unknown primary etiology[91].

Solid pseudopapillary neoplasm

Solid pseudopapillary neoplasms (SPPN) represent a small proportion of PCs (3%) and present as solid, or solid and cystic masses. They are malignant epithelial neoplasms made of poorly cohesive cells that form pseudo-papillae around the blood vessels[91]. The majority of SPNN are grossly well demarcated, but typically do not have a well formed capsule. The majority are solid, yellowish and soft[92]. Larger tumors usually develop cystic degeneration filled with blood and necrotic debris. Cases that are almost completely cystic without a solid component have also been reported[91].

Molecular analyses have shown that SPNN are different from ductal adenocarcinomas as they do not harbor mutations in the Kras 2, p16/CDKN2A, TP53, MADH4/DPC4 genes[93]. In contrast, 90% of SPNN have a mutation on chromosome 3p (CTNNB1) respon-

Table 3 clinico-pathological features of the most frequent classes of pancreatic cancer

Classification	Frequency (%)	Author	yr	Survival (5-yr survival after surgical resection)
DIA (incidence per 100000 patients at risk = 8.37)[69]	85-90[1]	Conlon *et al*[70]	1996	10%
		Winter *et al*[71]	2006	18%
		Poultsides *et al*[72]	2010	19%
SPPN (incidence per 100000 patients at risk = NA)[69]	0.1-3[73]	Papavramidis *et al*[74]	2005	95%
IPMN (incidence per 100000 patients at risk = 0.03)[69]		Shin *et al*[76]	2010	Benign: 95% Malignant: 64%
IPMN with simultaneous DIA: (incidence per 100000 patients at risk = NA)[69]	5[75]	Poultsides *et al*[72]	2010	42%
		Fan *et al*[77]	2010	57%
		Sohn *et al*[78]	2004	43%
Pancreatoblastoma (incidence per 100000 patients at risk = NA)[69]	0.50[79]	Dhebri *et al*[80]	2004	50%
		Saif *et al*[79]	2007	80%
Undifferentiated (incidence per 100000 patients at risk = 0.03)[69]	2-7[81]	Paal *et al*[82]	2001	3% (3-yr survival)
		Connolly *et al*[83]	1987	5 mo (average survival)
Medullary carcinoma (incidence per 100000 patients at risk = NA)[69]	NA	Wilentz *et al*[84]	2000	11% 14 mo (average survival)
Mucinous cystadenocarcinoma (incidence per 100000 patients at risk = 0.43)[69]	1	Ridder *et al*[85]	1996	56%
Adenosquamous carcinoma (incidence per 100000 patients at risk = 0.05)[69]	4	Madura *et al*[86]	1999	5-7 mo (median survival)
		Mulkeen *et al*[87]	2006	
Acinar cell carcinoma (incidence per 100000 patients at risk = 0.02)[69]	2	Holen *et al*[88]	2002	38 mo after surgical resection (median survival) 14 mo for unresectable disease (median survival)

DIA: Ductal infiltrating adenocarcinoma; SPPN: Solid pseudo-papillary neoplasm; IPMN: Intraductal papillary nucinous neoplasm; NA: Not applicable.

sible for the metabolism of β-catenin protein causing its accumulation in the cytoplasm and nucleus of neoplastic cells[94]. As a result alteration in β-catenin protein expression disrupts E-cadherin which is a key regulator of cell junctions causing poor adhesion of neoplastic cells[95]. Although there is some histological overlap between SPNN and other tumors of the pancreas, immunolabeling for β-catenin protein may help establish the diagnosis.

Intraductal papillary mucinous neoplasm

Intraductal papillary mucinous neoplasms (IPMNs) represent 5% of all PCs and are papillary epithelial mucin-producing neoplasms arising in the main pancreatic duct or in one of its branches. IPMNs are relatively common with increasing age of the population[91] and the mean age at presentation is 65 years[96]. IPMN is a potential pre-malignant condition and the risks of developing invasive adenocarcinoma increase with tumor size and when originating in the main pancreatic duct.

Adenocarcinoma is present in up to one-third of patients with IPMN and current guidelines recommend surgical resection when IPMNs are greater than 3 cm, in the presence of main pancreatic duct dilatation and when mural nodules are detected[97].

Neoplastic cells of IPMN are columnar with gene profiles similar to infiltrating ductal carcinoma. About 25% of patients show loss of heterozygosity of the STK11/LKB1 gene[98,99]. Other frequent gene mutations are TP53, KRAS2, and P16/CDKN2A[100].

Pancreatic intraepithelial neoplasia

Pancreatic intraepithelial neoplasia (PanIN) represents a neoplastic proliferation of mucin producing epithelial cells confined to the smaller pancreatic ducts and is considered a precursor to invasive ductal carcinoma[101].

PanINs are usually characterized by lesions too small to be symptomatic or to be detected by current imaging technologies[89]. Microscopically, PanINs are classified into three grades (PanIN-1, PanIN-2 and PanIN-3) based on the progressive degree of architecture abnormality and cellular atypia[102]. PanIN-1 shows minimum cellular atypia, PanIN-2 moderate changes and PanIN-3 is equivalent to PC-*in-situ*. The discovery of specific molecular changes present in both PanIN and PC has helped to establish that these small lesions are the precursors to DIA[103]. Early abnormalities of IPMNs are telomerase shortening and activating point mutations in the KRAS2 gene while intermediate mutation is the activation of the p16/CDKN2A gene and late events are alterations in the TP53, MADH4/DPC4, and BRCA2 genes[102]. The understanding that many DIAs arise from PanIN lesions has prompted screening efforts on the detection of these small and potentially curable lesions[104].

Pancreatoblastoma

Pancreatoblastoma is a rare malignant tumor (0.5% of PC) usually presenting in the pediatric age group. Generally, it appears as a soft and well demarcated mass with epithelial or acinar differentiation, but often it has cells with endocrine and mesenchymal characteristics[79]. Most pancreato-blastomas affect children with a mean age of 5 years and are frequently associated with elevated levels of serum alpha fetoprotein. The median survival of patients with pancreato-blastomas is 48 mo and the 5-year

survival rate after successful resection is 50% (95% CI: 37%-62%)[80,105].

The majority of pancreato-blastomas have loss of heterozygosity of chromosome 11p from the maternal side[106]. These molecular findings unite pancreatoblastoma with other primitive neoplasms such as hepatoblastoma and nephroblastoma[107]. Genetic alterations in the adenomatous polyposis coli (APC)/β-catenin pathway have also been detected in most pancreato-blastomas including mutations in β-catenin (CTNNB1) and APC genes[107].

Undifferentiated carcinoma

Undifferentiated PC (UPC) lacks differentiation direction[91] and presents with symptoms similar to patients with DIA, but has a worse prognosis as it has a more aggressive behavior and tends to metastasize and infiltrate surrounding organs in early stages[82]. The average time from diagnosis to death is about 5 mo and only 3% of patients are alive at 5 years after undergoing surgical resection. UPCs can form large locally aggressive masses and may present with severe hemorrhage and necrosis. The majority of UPCs have KRAS2 gene mutation suggesting that they arise from pre-existing ductal adenocarcinomas that transform into poorly differentiated tumors during their progression[108].

Medullary carcinoma

Medullary carcinoma (MC) is a variant of PC characterized by poor differentiation and syncytial growth that has been described and recognized only in recent years[84]. Patients with MC have a better prognosis and are more likely to have a family history of any kind of cancer[109]. MC does not differ significantly from other classes of PC in its clinical presentation, age and gender. These tumors tend to form well demarcated soft masses and microscopically they are usually poorly differentiated with pushing rather than infiltrating features[110]. Focal necrosis and intratumoral lymphocytic infiltration can be prominent similar to MC of the colon and other tumors with microsatellite instability[89]. MCs have been shown to have loss of expression of one of the DNA mismatch repair proteins (M1h1 and Msh2) and mutation in the BRAF gene, which is a downstream effector of the k-ras pathway[111]. Patients with MC and their families may benefit from genetic counseling and more frequent screening for early detection of other common cancers. The prognosis of MC is better than adenocarcinoma, although it is not responsive to adjuvant chemotherapy based on fluorouracil (5-FU), similar to colon cancer with microsatellite instability[112].

Other rare classes of PCs

Mucinous cystadenocarcinoma: Malignant cystic neoplasms are rare entities that account for only 1% of all pancreatic tumors[113]. Both serous and mucinous cystic neoplasms are tumors of the exocrine pancreas with different biological behaviors. Serous cystadenomas are considered benign tumors with almost no malignant potential often managed expectantly unless symptomatic. However, the preoperative differentiation between a benign serous cystadenoma and malignant serous cystadenocarcinoma remains difficult[114]. Histologically, cystadenocarcinomas appear identical to serous cystadenomas and are distinguished only by the presence of lymphovascular invasion or metastases[115]. Mucinous cystadenocarcinomas resemble DIAs although some cell populations can present with undifferentiated features and other histological characteristics such as osteoclast-like giant cells, adenosquamous carcinoma, choriocarcinoma, or high-grade sarcoma[116-119]. Mucinous cystic neoplasms of the pancreas are slowly growing and only about 20% show invasive features[120,121].

The prognosis of cystadenocarcinoma is favorable compared to DIA with 5-year survival rates of 56% after radical resection[85]. There is limited evidence on the role of chemotherapy for cystadenocarcinomas of the pancreas as they appear to be unresponsive to current chemotherapy agents and radiation therapy[122,123].

Adenosquamous carcinoma: Adenosquamous carcinoma has previously been referred as adenoachantoma, mixed squamous and adenocarcinoma, and mucoepidermoid carcinoma. Histologically, they are characterized by mixed populations of adenomatous cells and cells with varying amount of keratinized squamous features. Usually this tumor affects patients in their seventh decade of life, with symptoms and pancreatic distribution similar to DIAs. Although it is reported that adenosquamous carcinomas represents 4% of all PCs (range 3%-11%), the literature on the natural history and survival is limited to case series only[86]. The prognosis seems to be worse than DIAs, with a mean survival of 5-7 mo even after surgical resection[86,87]. Lymphovascular and perineural invasion appear to be common and early features of adenosquamous carcinomas and the role of adjuvant chemo and radiation therapy is still not clear[124].

Acinar cell carcinoma: Acinar cell carcinomas (ACCs) represent less than 2% of all pancreatic malignancies[87,88]. ACCs are predominantly constituted by neoplastic cells with immunohistochemical staining characteristic for exocrine enzymes such as trypsin, chymotripsin or lipase, and they present in older patients than DIAs and the prognosis is slightly better, although the literature is somewhat limited[125,126]. Symptoms at presentation are aspecific and include abdominal pain and weight loss that are similar to all other PCs[125]. Very rarely, patients with ACC can develop subcutaneous fat necrosis secondary to exceedingly high concentrations of serum lipase and contrary to DIAs, bile duct obstruction causing jaundice is not as common[125]. Median survival for ACC confined to the pancreas treated by surgical resection is 38 mo, whereas it is 14 mo for individuals with unresectable disease[88]. For the majority of patients, surgical management is not curative as distant recurrent disease is more frequent than in DIA, suggesting the presence of early micrometastases even when the tumors are in the early stages[88]. Because ACCs are rare, there is a lack of studies on the role of chemotherapy, although radiation therapy seems to provide good responses in patients with regional unresectable disease[88].

DIAGNOSIS

Clinical presentation

Early symptoms of PC are notoriously difficult to measure as educational and economic factors influence their perception and reporting[127,128]. Cholestatic symptoms are more common in early PC of the head, while abdominal and back pain are more common in patients with distal PC and in patients with tumors infiltrating peripancreatic nerve tissue[129]. The appearance of these symptoms usually indicates advanced disease (Table 4)[129,130].

Early symptoms are usually vague such as anorexia, moderate weight loss, and early satiety[131]. Diabetes might be a sign of PC particularly when presenting during or beyond the sixth decade of life in the absence of risk factors and family history[20]. Diabetes is detected in 60%[132] to 81%[133] of PC patients within two years of their diagnosis. Early detection is possible if symptoms raise clinicians' suspicion, as 25% of patients report upper abdominal discomfort up to 6 mo prior to their diagnosis[134,135].

In two European studies[128,130], weight loss was present in 66%-84% of patients, jaundice (bilirubin level > 3 mg/dL) in 56%-61%, recent onset of diabetes in 97% and distended palpable gall bladder in 12%-94%, energy loss in 86%, abdominal pain in 78%, back pain in 48%, nausea in 50%, clay-coloured stools in 54%, dark urine in 58%, jaundice in 56% and pruritis in 32% of patients.

Serum tumor markers

Several serum tumor markers are associated with PC, however, to date, no single marker has been found to be optimal for screening.

Carbohydrate antigen 19-9: Carbohydrate antigens have been used as markers for several cancers[136,137]. The production of these antigens seems to be caused by the up-regulation of glycosyl transferase genes[138]. Among these carbohydrate antigen epitopes, Sialyl Lewis^a (sLe^a) detected by the 1116NS19-9 monoclonal antibody is commonly called carbohydrate antigen 19-9 (CA19-9)[139]. The serum levels of CA19-9 at the time of diagnosis and during follow-up of PC provide useful diagnostic and prognostic information[140,141]. Its sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) are 70%-90%, 43%-91%, 72% and 81%, respectively[142-145]. A worse survival was observed in patients with pre-operative CA19-9 levels above 370 U/mL (median survival 4.4 mo *vs* 9.5 mo if CA19-9 < 370 U/mL, *P* value < 0.01)[146]. In another study, serum levels of CA19-9 > 200 U/mL were associated with a survival rate of 8 mo compared to 22 mo for patients with lower tumor antigen levels ($P < 0.001$)[147]. In a prospective study of patients undergoing curative resection for PC, post-operative CA19-9 < 37 U/mL was associated with a longer median and disease-free survival compared to the control group[148-150]. One of the limitations of CA19-9 is that high serum bilirubin can falsely increase its level and therefore the risk of false positive results in patients with jaundice. This is not observed for other markers such as carcinoembryonic antigen (CEA) and carbohydrate antigen 242 (CA 242)[141].

Table 4 Presenting symptoms of advanced pancreatic cancer

Symptom	Percentage
Abdominal pain	78-82
Anorexia	64
Early satiety	62
Jaundice	56-80
Sleep disorders	54
Weight loss	66-84
Diabetes	97
Back pain	48
Nausea and weight loss	50-86

CEA: CEA is part of a subgroup of glycoproteins functioning as intracellular adhesion molecules. CEA was first detected in pancreatic secretions, and several studies have shown high levels of CEA in the pancreatic juice of patients with PC[151-153]. A Japanese study found significantly higher CEA levels in the pancreatic juice of PC patients compared to those with benign pancreatic diseases. When the CEA cut off level in pancreatic juice was 50 ng/mL, the PPV, NPV, and the accuracy for diagnosis of carcinoma were 77%, 95% and 85%, respectively. CEA levels in pancreatic juice were higher in smaller tumors in comparison to advanced PC due to the incomplete obstruction of the pancreatic duct[154]. A recent study examining single *vs* combined efficacy of tumor markers showed that CEA (> 5 ng/mL) alone had a sensitivity of 45% and a specificity of 75% in comparison to CA19-9 which had a sensitivity of 80% but lower specificity (43%) ($P = 0.005$)[141,155]. The combination of CEA (> 5 ng/mL) and CA 19-9 (> 37 U/mL) decreased the sensitivity to 37%, but increased the specificity to 84%. Similarly, the combination of CEA (> 5 ng/mL) and CA242 (> 20 U/mL) decreased the sensitivity to 34% and increased the specificity to 92%. Yet, CEA and CA242 are currently not used as single tumor markers for PC, and the simultaneous use of CEA and CA19-9 provides the same information as CA19-9 alone[156-158].

CA 242: CA 242, a sialylated carbohydrate was first defined by Lindholm *et al* in 1985 and has been used for diagnostic and prognostic purposes[159,160]. For PC, its diagnostic sensitivity and specificity are 60% ($P = 0.073$) and 76% ($P = 0.197$), respectively, comparable to CEA. It also seems to be valuable in differentiating PC from benign pancreatic tumors as well as other hepatobiliary cancers and to predict outcomes as survival rates in CA 242 positive patients are lower than those with negative serum levels ($P = 0.002$)[141].

In a study comparing CA 242 and CA19-9[161], CA 242 appeared to be an independent prognostic factor for patients with resectable disease as serum levels of CA 242 < 25 U/mL were associated with a significantly better survival ($P < 0.05$). For patients with unresectable disease, poorer outcomes were observed when CA 242 levels were > 100 U/mL.

Similar results have been confirmed by Ni *et al*, who found that CA 242 is an independent prognostic factor

Table 5 Summary of the performance characteristics of serum tumor markers for the diagnosis of pancreatic cancer

Serum tumor marker	Author	Yr	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
CA19-9	Boeck *et al*[141]	2006	70-90	43-91	72	81	67
	Ni *et al*[142]	2005					
	Steinberg *et al*[143]	1990					
	Safi *et al*[144]	1997					
	Mu *et al*[162]	2003					
CEA in pancreatic juice	Ozkan *et al*[155]	2003	NA	NA	77	95	85
	Futakawa *et al*[154]	2000					
	Ni *et al*[142]	2005					
CEA in serum	Boeck *et al*[141]	2006	45	75	NA	NA	NA
CA19-9 + CEA	Ni *et al*[142]	2005	37	84	91	90	89
	Ozkan *et al*[155]	2003					
	Ma *et al*[163]	2009					
CA 242	Nilsson *et al*[160]	1992	60	76	63	61	71
	Röthlin *et al*[164]	1993					
	Carpelan-Holmström *et al*[165]	2002					
	Pålsson *et al*[166]	1993					
CEA + CA 242	Ni *et al*[142]	2005	34	92	67	90	87
	Ozkan *et al*[155]	2003					
	Hall *et al*[167]	1994					
CA19-9 + CA 242	Ni *et al*[142]	2005	59	77	65.3	87.8	65.1
	Röthlin *et al*[164]	1993					
	Jiang *et al*[158]	2004					
CA19-9 + CA 242 + CEA	Ni *et al*[142]	2005	29	96	NA	NA	NA

PPV: Positive predictive value; NPV: Negative predictive value; CA19-9: Carbohydrate antigen 19-9; CEA: Carcinoembryonic antigen; CA 242: Carbohydrate antigen 242; NA: Not applicable.

in PC yielding more information than CA 19-9[142,161]. In this study the use of combined tumor markers resulted in lower sensitivity, but higher specificity (Table 5). Despite these findings, CA 242 is not used in clinical practice as commonly as Ca 19-9 due to the limited number of laboratories equipped to run this test.

Other tumor markers

Recent studies have identified other serum molecules such as CA494[168], CEACAM1[169], PTHrP[170], TuM2-PK[171], CAM 17.1[172] and serum beta HCG[173] as potential markers for PC. Although preliminary results appear promising with sensitivity and specificity comparable and sometimes superior to CA19-9 and CEA, their clinical use has to be confirmed in larger studies and their role is currently confined to a limited number of medical centers and for research purposes.

Imaging modalities

Although PC may be detected with one particular diagnostic test, proper staging often requires the use of several imaging modalities[174].

Abdominal ultrasound: Trans-abdominal ultrasound (US) is currently used as a screening test for patients with suspected PC[175]. Its sensitivity ranges between 48%[176] and 89%[177], specificity between 40%[178] and 91%[179] and accuracy between 46%[176] and 64%[180]. PCs measuring less than 1 cm are detected by US in only 50% of cases, while the sensitivity increases to 95.8% for tumors larger than 3 cm[177]. Other factors affecting the sensitivity of US are the operator's experience[181] and the technical characteristics of the machine. Newer US machines such as tissue harmonic imaging decrease artefacts and improve tissue contrast and therefore diagnostic accuracy[182]. US has a relatively low performance profile for the staging of PC as its sensitivity for lymph node involvement only ranges between 8%[159] and 57%[177].

Color Doppler US has been used to assess the possible involvement of the portal vein and superior mesenteric vessels with a sensitivity ranging between 50%[183] and 94%[184], specificity between 80% and 100%[183] and accuracy between 81% and 95%[175].

The recent introduction of intravenous contrast has been shown to improve evaluation of the vascularity of pancreatic lesions allowing differentiation between PC and other conditions with 90% sensitivity, 100% specificity and 93% accuracy[185]. Currently, US is considered a useful imaging modality for the initial screening of PC based on its ability to document unresectability (PPV = 94%)[176]. However, the PPV for resectabiltiy is only 55%[186], therefore, other imaging techniques are usually employed for better staging.

EUS: EUS provides high resolution images of the pancreas without interference by bowel gas[187]. Despite the advancement of CT scans, EUS appears to have a higher sensitivity in detecting small PCs (98%) in comparison to CT (86%)[188]. EUS has higher sensitivity compared to CT for local tumor staging (67% *vs* 41%), similar sensitivity for lymph node involvement (44% *vs* 47%) and potential tumor resectability (68% *vs* 64%)[185]. EUS has a NPV of 100% for PC of the head[186,189] and an accuracy of 90% for the assessment of portal and splenic vein inva-

sion[178,190]. On the other hand, EUS does not appear to be accurate enough in assessing the invasion of SMA and superior mesenteric vein (SMV) with a NPV of 82% and sensitivity of only 50%[191,192].

In order to improve EUS performance in PC staging, recent studies have assessed the benefits of using parenteral contrast agents. This technique has shown 92% sensitivity, 100% specificity, 100% PPV, 86% NPV and 95% accuracy[193]. Although EUS is becoming a leading modality for staging and diagnosis of PC, drawbacks of this technique are the fact that it is invasive, highly operator dependent, costly and associated with a small risk of pancreatitis (0.85%)[194], bleeding and duodenal perforation.

CT: On contrast CT, PC appears as an ill-defined, hypoattenuating focal mass with dilatation of the upstream pancreatic and or biliary duct[174]. Optimum visualization of the pancreas requires imaging acquisition obtained during both arterial and portal phases[195]. Sensitivity and specificity of thin section triple phase helical CT is 77% and 100%, respectively, for lesions less than 2 cm[196]. In a multicentric trial, the diagnostic accuracy of CT for resectability was 73% with a PPV for non resectability of 90%[197].

With the advent of multi detector CT scanners (MDCT), the pancreas can be imaged at a very high spatial and temporal resolution[198,199]. The dual phase pancreatic protocol MDCT using 1 to 3 mm slice collimation is one of the most sensitive techniques for metastatic disease to the liver and peritoneum[186,200,201]. Recent studies have shown that MDCT has a NPV of 87% for tumor resectability compared to a NPV of 79% for conventional helical CT[202] and with an accuracy between 85% and 95%[203,204].

Images from MDCT can be used to visualize the biliary tree and normal vascular variants such as replaced hepatic arteries before surgical planning. Gangi *et al*[198] reported that pancreatic ductal dilatation in asymptomatic patients could be identified between 0 to 50 mo before PC diagnosis was confirmed. The sensitivity, specificity and accuracy of CT in the presence of hypo-attenuated pancreatic lesions, pancreatic ductal dilatation with cut-off, distal pancreatic atrophy, pancreatic contour abnormalities and common bile duct dilatation are reported in Table 6[205].

Despite these improvements, interpretation of the CT scan is quite challenging in the setting of pancreatitis forming mass effects[206] and in the presence of loco-regional lymph node involvement and small hepatic metastasis[207].

Magnetic resonance imaging-magnetic resonance cholangiopancreatography: In most institutions, MRI is performed when other imaging modalities provide insufficient data for the clinical staging of the tumor, or when treatment planning can not be based on the images obtained by other techniques. Several studies have shown that MRI is superior to CT for the detection and staging of PC (100% *vs* 94%, respectively)[208-211]. However, recent evidence has challenged this belief. The use of MRI-magnetic resonance cholangiopancreatography (MRCP) to better characterize PC is supported by a prospective analysis that compared these two modalities in patients with periampullary cancers[212]. MRI-MRCP was superior to CT in differentiating malignant from benign lesions (ROC = 0.96 *vs* 0.81, $P < 0.05$) and MRI-MRCP had better sensitivity (92% *vs* 76%), specificity (85% *vs* 69%), accuracy (90% *vs* 75%), PPV (95% *vs* 88%) and NPV (79% *vs* 50%) compared to CT. Another study confirmed the previous results with MRI-MRCP showing 97% sensitivity, 81% specificity and 89% accuracy[213].

Table 6 Sensitivity, specificity and accuracy of computed tomography findings in pancreatic cancer patients

CT finding	Sensitivity (%)	Specificity (%)	Accuracy (%)
Hypoattenuation	75	84	81
Ductal dilatation	50	78	70
Ductal interruption	45	82	70
Distal pancreatic atrophy	45	96	81
Pancreatic contour anomalies	15	92	70
CBD dilatation	5	92	67

CT: Computed tomography; CBD: Common bile duct.

On the other hand, other studies comparing gadolinium-enhanced MRI with MDCT have shown that MRI and CT had equivalent sensitivity and specificity (83%-85% *vs* 83% and 63% *vs* 63%-75%, respectively). Both techniques had good to excellent agreement between radiologists, although MRI had a superior agreement for the evaluation of distant metastases (inter-observer agreement between MRI and CT scan; 0.78 *vs* 0.59 $P = 0.1$)[214]. On the other hand, with the improvement in CT scan technology, recent studies have shown that MRI might have lower sensitivity in comparison to MDCT (82%-94% *vs* 100%)[215]. This was confirmed by a recent meta-analysis comparing the accuracy of several imaging modalities which showed that helical CT had superior sensitivity compared to MRI (91% *vs* 84%) and transabdominal US (91% *vs* 76%)[216]. Sensitivity for resectability of the tumor was equal for both MRI and helical CT (82% *vs* 81%, respectively)[216].

Positron emission tomography: ^{18}F-2fluoro-2-deoxy-D-glucose (FDG) accumulated by tumor cells provides positron emission tomography (PET) with the advantage of combining metabolic activity and imaging characteristics. Newly developed PET scanners can detect small PCs up to 7 mm in diameter and diagnose metastatic disease in about 40% of cases[217,218]. A Japanese study found that the overall sensitivity of PET-CT was superior to contrast CT (92% *vs* 88%) and that PET was better at detecting bone metastases (100% *vs* 12%). However, CT scanning was superior for the evaluation of vascular invasion (100% *vs* 22%), involvement of para aortic regional lymph nodes (78% *vs* 57%), identification of peritoneal dissemination (57% *vs* 42%) and hepatic metastases (73% *vs* 52%)[219]. Another Japanese study confirmed that PET had a sensitivity of 87%, a specificity of 67% and accuracy of 85%, and that tumors with metastatic

Table 7 Summary of the performance characteristics of imaging tests for the diagnosis of pancreatic cancer

Diagnostic modality	Author	Yr	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
US	Giovannini *et al*[176]	1994	48-95	40-91	92	100	46-64
	Böttger *et al*[177]	1998					
	Rösch *et al*[178]	1991					
	Niederau *et al*[179]	1992					
	Palazzo *et al*[180]	1993					
	Tanaka *et al*[231]	1996					
Doppler US	Candiani *et al*[232]	1998	50-94	80-100	79	88	81-95
	Casadei *et al*[184]	1998					
	Calculli *et al*[233]	2002					
EUS	Akahoshi *et al*[234]	1998	98	97	94	100	90
	Legmann *et al*[235]	1998					
Contrast enhanced US	Dietrich *et al*[185]	2008	90	100	100	86	93
CT	Bronstein *et al*[196]	2004	77	100	NA	NA	73
	Megibow *et al*[197]	1995					
MDCT	Park *et al*[214]	2009	83-91	63-75	80	87	85-95
	Vargas *et al*[202]	2004					
	Diehl *et al*[203]	1998					
	Schima *et al*[208]	2002					
MRI-MRCP	Andersson *et al*[212]	2005	83-92	63-85	95	79	89
PET	Maemura *et al*[217]	2006	87-100	67-77	94	100	85-95
	Delbeke *et al*[221]	1999					

PPV: Positive predictive value; NPV: Negative predictive value; US: Ultrasound; EUS: Endoscopic ultrasound; CT: Computed tomography; MDCT: Multi detector computed tomography; PET: Positron emission tomography; NA: Not applicable; MRI: Magnetic resonance imaging.

disease had significantly higher standardized uptake values [SUV = tissue concentration (millicuries/g)/injection dose (millicuries)/body weight (g)] than those without metastases[220]. PET had superior sensitivity (100% *vs* 65%), specificity (77% *vs* 61%), NPV (100% *vs* 31%), PPV (94% *vs* 87%) and accuracy (95% *vs* 65%) in an American study comparing PET-CT with a SUV cut off of 2.0 *vs* contrast CT[221]. A recent study enrolling 59 PC patients showed similar results, with 91% PPV and 64% NPV for PET-CT. One of the most interesting results was that the clinical management of patients undergoing PET was changed in 16% of cases deemed resectable after routine staging ($P = 0.031$) preventing unnecessary surgery because of distant metastases[222].

Diffuse uptake of FDG is frequent in pancreatitis in comparison to PC (53% *vs* 3%, $P < 0.001$), and therefore PET is extremely useful in distinguishing these two conditions in controversial cases[218,223]. Animal studies have shown that ^{11}C-acetate-PET appears to be superior to FDG PET for the detection of early PC and might be useful in differentiating inflammatory processes from malignancies as ^{11}C-acetate-PET is less affected by the presence of inflammation in human tissues[224].

Another very important characteristic of PET-CT is its ability to provide useful information on tumor viability, and this technique also allows monitoring of tumor response to treatment[217] and the metabolic features of PET help predict the prognosis as a SUV less than 3 appears to be a positive predictive factor[222,225-229].

Similar results were found by Zimny *et al*[230] who showed that better survival trends were noted in patients with PC and a SUV less than 6.0 in comparison to those with a higher SUV. Sensitivity and specificity of imaging modalities are summarized in Table 7.

STAGING

Pathological staging

In the 7th edition of the American Joint Committee on Cancer the different categories of PC are classified according to only one TNM staging system, even if neuroendocrine tumors have a different biology and a better prognosis than ductal carcinomas. Yet, the TNM system provides a reasonable discrimination and prognostic validity for these patients[236].

The TNM system classifies PC into 3 clinically important categories: (1) patients with Tis-T2 PC have localized cancer within the pancreas; (2) patients with T3 cancer have locally invasive disease; and (3) patients with T4 tumors have unresectable PC[237] (Table 8).

Prognostic features of PC include perineural and lymphovascular invasion, elevated serum CA19-9 levels and incomplete tumor resection. Therefore, gross and microscopic assessment of the resection margins is of major importance even if it is not included in the TNM staging system. Patients undergoing resections with grossly or microscopically positive margins have no survival benefits compared to individuals undergoing palliative chemo- radiation therapy alone.

Clinical staging

Surgery is the only chance of cure and the presence of negative resection margins of the primary tumor represent the strongest prognostic factor. Preoperative staging modalities include the combination of several imaging techniques such as CT scan, MRI, EUS, staging laparoscopy and laparoscopic ultrasound which aim to identify patients with resectable disease. There is consensus that patients with distant metastases (liver, lungs, peritoneum)

Table 8 American Joint Committee on Cancer staging of pancreatic cancer

AJCC 6th edition TNM staging system for pancreatic cancer		
TX	Primary tumor cannot be assessed	
T0	No evidence of primary tumor	
Tis	Carcinoma *in situ*	
T1	Tumor limited to the pancreas, 2 cm or less in greatest diameter	
T2	Tumor limited to the pancreas, greater than 2 cm at greatest diameter	
T3	Tumor extends beyond pancreas but no involvement of celiac axis or superior mestenteric artery	
T4	Tumor involves the celiac axis or the superior mestenteric artery (unresectable)	
NX	Regional nodes cannot be assessed	
N0	No regional lymph node metastasis	
N1	Regional lymph node metastasis	
MX	Distant metastasis cannot be assessed	
M0	No distant metastasis	
M1	Distant metastasis	
Stage grouping		
Stage 0	Tis N0 M0	Localized within pancreas
Stage ⅠA	T1 N0 M0	Localized within pancreas
Stage ⅠB	T2 N0 M0	Localized within pancreas
Stage ⅡA	T3 N0 M0	Locally invasive, resectable
Stage ⅡB	T1, 2, or 3 N1 M0	Locally invasive, resectable
Stage Ⅲ	T4 Any N M0	Locally advanced, unresectable
Stage Ⅳ	Any T Any N M1	Distant metastases

AJCC: American Joint Committee on Cancer.

or local invasion of the surrounding organs (stomach, colon, small bowel) are usually not surgical candidates.

The criteria for unresectability of PC include tumor encroachment (defined as tumor surrounding the vessel more than 180 degrees) of arteries such as the celiac artery, hepatic artery, superior mesenteric artery (SMA) or massive venous invasion with thrombosis. Portal or superior mesenteric venous invasion without thrombosis or obliteration of vessels can still be classified as resectable PC[204,238]. A recent study comparing the roles of EUS, CT, MRI and angiography in the assessment of PC staging and resectability has shown that CT scanning was the most accurate in assessing the stage of the tumor (73%), locoregional invasion (74%), vascular involvement (83%), distant metastases (88%), final TNM stage (46%) and overall tumor resectablity (83%)[239]. EUS appeared to be superior in detecting smaller tumors not visualized by CT. A decision analysis demonstrated that the best strategy to assess tumor resectability was based on CT as an initial test and the use of EUS to confirm the results of resectability by CT[221].

Laparoscopic staging

Diagnostic laparoscopy for PC was first introduced as a staging procedure in the late 1980s by Cuschieri *et al*[240] and Warshaw[241,242]. Staging laparoscopy is considered a simple, minimally invasive technique to identify radiographically occult distant metastatic disease and to prevent non-therapeutic laparotomies. Laparoscopic examination allows direct visualization of intra-abdominal contents and has been reported to identify hepatic and peritoneal metastases not shown by other modalities[243] as reported in some studies where 20%-48% of patients considered resectable by CT were found to be unresectable during surgery[244-246].

Diagnostic laparoscopy involves a general exploration of the abdominal surfaces including palpation of the liver with two instruments when necessary. The hilum of the liver is visualized, the foramen of Winslow is examined and periportal lymph nodes are biopsied when enlarged. The transverse colon and omentum are reflected cephalad and the base of the transverse mesocolon is examined with particular attention to the mesocolic vessels. The gastrocolic ligament/omentum is incised and the lesser sac is examined[247].

Laparoscopic ultrasonography (LUS) has been introduced as an additional procedure to increase the detection of intrahepatic metastases, identify enlarged and suspicious lymph nodes and to evaluate local growth in the vascular structures[248]. Some studies have demonstrated that LUS has improved the accuracy of predicting resectability up to 98%[249-251].

Despite these results, the routine use of staging laparoscopy and LUS in patients with radiographically resectable PC remains controversial as imaging modalities have significantly improved, thus reducing the risk of discovering non-resectable disease at the time of surgery. In addition, staging laparoscopy adds costs and it can be time consuming. Sustainers of staging laparoscopy are supported by a study by Kwon *et al*[250], which revealed that staging laparoscopy was able to detect unsuspected metastases and changed the surgical approach in 37% of patients even when using CT, MRI, ERCP and angiography for preoperative staging. Another study by Conlon *et al*[247], supported the use of staging laparoscopy as only 67 out of 115 patients (58%) with PC had resectable disease after completion of the laparoscopic examination. On the other hand, a more recent study from the same group at the Memorial Sloan-Kettering Cancer Center has shown that the yield of staging laparoscopy was only 8.4% when good imaging modalities were obtained at the referral center[252].

Based on the fact that minimally invasive approaches for the diagnosis of PC as well as radiological imaging techniques will continue to advance, the selective use of staging laparoscopy and LUS would play a role in cases where detection of unresectable disease is more likely. Factors which suggest a higher yield with diagnostic laparoscopy include a large primary tumor (diameter larger than 4 cm), a tumor in the body or tail of the pancreas, equivocal findings after imaging tests, severe weight loss, abdominal or back pain, hypoalbuminemia and significantly elevated tumor markers[240].

TREATMENT

Patients with suspected or confirmed diagnosis of PC should be assessed by a multidisciplinary team and stratified as resectable (stage Ⅰ or Ⅱ), borderline resectable (stage Ⅱa or Ⅱb), locally advanced unresectable (stage Ⅲ) or metastatic disease (stage Ⅳ). Treatment should be planned according to local expertise and established guidelines, as resectable and borderline patients should be referred to surgeons, unresectable and metastatic patients should be referred to medical and radiation oncologists and palliative care teams. A multidisciplinary approach to PC is necessary to improve the overall outcome of these patients, especially for borderline resectable or unresectable disease as neo-adjuvant chemo-radiation therapy may play a role in downstaging and the conversion to potentially curable disease[253,254].

SURGICAL THERAPY

Surgical treatment is the only potential cure for PC[255]. Although pancreatic surgery is considered challenging and technically demanding, improvements in surgical techniques and advances in perioperative supportive care have reduced the mortality rates to less than 5% in high-volume centers[256-258]. According to the United States Surveillance and Epidemiology End Results registries, the 5-year relative survival for the period between 1999 and 2006 was 22.5% for localized and 1.9% for metastasizing PC (Table 9)[259].

Because only 20% of patients with PC are candidates for radical resection at the time of diagnosis[260], accurate staging is important in identifying surgical candidates and sparing the risk and cost of surgery for patients who are affected by advanced disease[261]. Unresectable PC is commonly defined when there is tumor invasion of the SMA, inferior vena cava, aorta or celiac arteries; encasement or occlusion of the SMV-portal venous system or by distant metastasis (e.g. hepatic, extra-abdominal, peritoneum, omentum, lymph nodes outside the resection zone)[262]. An Italian study has recently demonstrated that the duration of symptoms (mainly jaundice and celiac pain) of more than 40 d, CA 19-9 levels above 200 U/mL and G3-G4 histological grade of the tumor are poor prognostic parameters, even if the disease is resectable by preoperative staging[263].

Table 9 Stage distribution of pancreatic cancer and 5-year relative survival by stage at diagnosis for 1999-2006, all races and both sexes (SEER registries)

Stage at diagnosis	Stage distribution (%)	5-yr relative survival (%)
Localized (confirmed to primary site)	8	22.5
Regional (spread to regional LNs)	26	8.8
Distant (cancer had metastasized)	53	1.9
Unknown (unstaged)	14	5

SEER: Surveillance Epidemiology and End Results.

Tumor of the head of pancreas

Preoperative biliary decompression *vs* immediate surgical resection: Obstructive jaundice is a common presentation for tumors located in the periampullary area or in the head of the pancreas. To reduce perioperative complications and mortality in patients with obstructive jaundice undergoing pancreaticoduodenectomy (PD), preoperative biliary drainage appears to have a positive impact supported by the findings of several observational studies[264-266]. On the other hand, several other non randomized studies failed to show any advantage of preoperative biliary decompression in these patients, as they developed a higher incidence of bacteriobilia and fungal colonization causing more wound infections, postoperative sepsis and longer hospital stay[267-270]. Two meta-analyses of randomized controlled trials and a systematic review of descriptive series have shown that the outcome of patients undergoing biliary decompression prior to PD was inferior to early surgery as they had higher rates of infectious complications and perioperative mortality[271,272]. These findings were confirmed by a recent multicenter randomized controlled study from the Netherlands which showed that the rates of serious complications were 39% for patients who underwent early surgical resection in comparison to 74% in the group that underwent pre-operative biliary decompression ($P < 0.001$)[264]. Similarly, surgical complications occurred in 37% of patients undergoing early resection in comparison to 47% for individuals who had preoperative biliary decompression. Although the difference did not reach statistical significance ($P = 0.14$), the overall mortality and hospital stay were comparable between the two groups[273].

During the last decade, there has been an increasing interest in treating patients with neo-adjuvant chemoradiotherapy to improve disease-free and overall survival in patients undergoing surgery. Although there are still no phase Ⅲ randomized controlled studies to support the use of this strategy, several phase Ⅱ randomized trials have shown that neo-adjuvant chemo and chemo-radiation therapy are relatively well tolerated, do not reduce the resectability rate and seem to increase the percentage of patients who undergo R0 resections[274-283]. For jaundiced PC patients, candidates for neo-adjuvant therapy must undergo biliary decompression to prevent liver decompensation and stent patency is required for several months. Currently, the only study assessing the outcome

of patients undergoing chemo-radiation therapy prior to PD has shown that plastic stents do not provide patency of the biliary system for long enough to complete the preoperative protocols. In fact, 55% of cases required unplanned repeat ERCP with stent exchange for recurrence of jaundice or ascending cholangitis[284]. For these patients, self expanding metallic stents should be used as the direct costs associated with repeating ERCP and hospital admissions for recurrent biliary obstruction and ascending cholangitis appear to be superior to the initial higher cost of using metallic stents[285].

Standard *vs* pylorus preserving PD: Walter Kausch first described PD in 1912[286], and Allan Whipple later popularized the procedure that bears his name[287]. The classic Whipple (CW) operation consists of an *en-bloc* removal of the pancreatic head, the duodenum, the common bile duct, the gall bladder and the distal portion of the stomach together with the adjacent lymph nodes[288]. This operation can lead to specific long-term complications such as early and late dumping syndrome, post-operative weight loss[289] and post-operative acid and bile reflux[290].

Pylorus preserving PD (PPPD) was first introduced by Watson in 1942[291], and the procedure was popularized by Traverso and Longmire in 1978[292]. Although it was originally described for the treatment of periampullary tumors, many surgeons nowadays perform PPPD for PC in the head of the pancreas. In order to retain a functioning pylorus, the stomach and the first 2 cm of the duodenum are preserved along with their neurovascular supply. The rationale behind preservation of the stomach is to improve long-term gastrointestinal function[293]. There is still some controversy as to which is the best surgical treatment for PC of the head of the pancreas. In comparison to CW, PPPD has the advantages of reduced operative time[294], less blood loss, better access to the biliary anastomosis for post-operative endoscopy in patients with recurrent biliary obstruction, improvement of post-operative weight gain and quality of life[295]. On the other hand, some series have reported that PPPD has a higher incidence of delayed gastric emptying[296,297]. Moreover, it has not been unequivocally shown that PPPD is oncologically equivalent to CW[298]. A number of RCTs and meta-analyses have demonstrated that both perioperative morbidity and long-term outcome are equal in CW and PPPD[263,299,300].

Pancreatic reconstruction: The most significant cause of morbidity and mortality after PD is the development of complications caused by leakage of pancreatic secretions and pancreatic fistulae observed in up to 20% in specialized centers[301,302]. The meticulous reconstruction of pancreatico-enteric continuity is the key to preventing pancreatic fistulae[303]. Pancreatico-jejunostomy and pancreatico-gastrostomy (PG) are the most commonly employed techniques for pancreaticoenteric reconstruction. PG was believed to be an easier technique and less prone to ischemia as a result of the close proximity between the stomach and the pancreatic stump and the presence of a better vascular supply in the stomach in comparison to the jejunum. However, RCTs have not demonstrated superiority of one technique over the other in terms of post-operative complication rates or incidence of pancreatic fistulae[304,305].

Tumor of the body/tail of pancreas

Distal pancreatectomy: Distal pancreatectomy is the surgical procedure of choice for PC of the body and tail of the pancreas. It entails resection of the portion of the pancreas extending to the left of the superior mesenteric vessels and not including the duodenum and the distal bile duct[306]. The spleen is conventionally removed in an *en-bloc* fashion[307]. However, splenic preservation could be accomplished without an increased rate of complications, operative time or the duration of post-operative hospital stay[295,308]. Several closure techniques have been introduced for the pancreatic remnant in an attempt to reduce pancreatic fistulae. They include hand-sewn suture techniques, staple closure techniques or a combination of both[309-312], ultrasonic dissection devices[313], pancreatico-enteric anastomosis[314], application of meshes, seromuscular[315] and gastric serosal patches[316], or sealing the pancreatic stump with fibrin glue[199].

Cancers of the body and tail of pancreas usually present at a later stage of the disease in comparison to PC of the head due to lack of early symptoms[317]. There are no survival differences between resections for equal TNM stage tumors of the head *vs* tumors of the body and tail as shown by a retrospective study that reported a 5-year survival of 17% after resection of the pancreatic head *vs* 15% for left-sided tumors in stage I cancers[318].

Laparoscopic pancreatic resection: Laparoscopic pancreatic surgery represents one of the most challenging abdominal operations[319,320]. Gagner and Pomp were the first to describe a laparoscopic duodeno-pancreatectomy in 1994[321]. Since then, the total number of laparoscopic duodeno-pancreatectomies has remained small due to technical difficulties associated with this operation[322]. A recent study from the Mayo clinic with 65 patients who underwent total laparoscopic PD (TLPD) outlined that TLPD is safe, feasible and its results appear to be comparable to the open approach[323] (Table 10).

Nevertheless, larger prospective studies are required in order to better assess the advantages of TLPD.

Laparoscopic distal pancreatic resection is currently the most frequently performed laparoscopic pancreatic surgery[327]. Most of the studies on distal laparoscopic pancreatectomy are case series with a relatively small number of patients[328]. Although recent studies have shown that laparoscopic distal pancreatectomy is feasible and safe[329-331], the morbidity, mortality and hospital stay are similar to those after open surgery[332]. This is probably due to the fact that morbidity after pancreatic surgery results from retroperitoneal dissection, length of the operation and pancreatic fistulae rather than the incision. In addition, a recent prospective observational study comparing 85 open *vs* 27 laparoscopic distal pancreatectomies has shown

Table 10 Published results on laparoscopic pancreaticoduodenectomies

Author	Yr	Patient No.	Morbidity (%)	Pancreatic fistula (%)	Mean hospital stay	Mortality (%)
Kendrick *et al*[323]	2010	62	42	18	7	1.6
Palanivelu *et al*[324]	2007	42	28.6	7.1	10.2	2.4
Dulucq *et al*[325]	2006	25	31.8	4.5	16.2	0
Pugliese *et al*[326]	2008	19	31.6	15.8	18	0

that the number of lymph nodes removed during the minimally invasive procedure was significantly inferior (mean number: 5.2) in comparison to the open approach (mean number: 9.4)[333]. These findings suggest that at this time there is a lack of evidence to support oncological equipoise between laparoscopic and open resections for PC.

Total pancreatectomy: Total pancreatectomy has been employed in selected patients with chronic pancreatitis[334], multifocal islet cell tumors or diffuse IPMN[335]. Total pancreatectomy for PC was initially proposed to avoid the risk of pancreatico-enteric leaks and to remove potential undetectable synchronous disease in other parts of the gland[336]. However, the indication of total pancreatectomy to avoid the risks of pancreatic fistulae is still controversial[337]. Improvement in operative techniques, advances in nutritional support, critical care and interventional radiology have significantly decreased the incidence of life-threatening sequels of pancreaticoenteric leaks[338]. In addition, the permanent endocrine insufficiency associated with total pancreatectomy impacts enormously on the quality of life and long-term outcome of these patients[339]. Some studies have demonstrated a significant increased risk of perioperative morbidity and mortality associated with total pancreatectomy compared with PD[318]. A recent study by Reddy *et al*[335] showed that long-term survival rates were equivalent after total pancreatectomy and PD (19.9% *vs* 18.5%), supporting the fact that there is no oncological benefit of total pancreatectomy *vs* a more limited resection in PC. Currently, total pancreatectomy should be performed in patients with PC if it is the only oncologically sound treatment option[335].

Vascular resections and extended lymphadenectomy: With the advancement in operative techniques and perioperative management of patients with PC, more radical surgical procedures with vascular resection and extended lymphadenectomy have been proposed for selected cases[340]. The results of extended vascular and lymphatic resections remain controversial.

The principal use of venous resection and reconstruction is to allow complete tumor clearance when precluded by tumor involvement of the superior mesenteric or portal vein, and when the surgeon expects to achieve a negative resection margin[341]. Post-operative morbidity and mortality rates following portal or superior mesenteric vein resections seem to be similar to those of patients with standard PD (42%-48.4% *vs* 47.1%, 3.2%-5.9% *vs* 2.5%, respectively)[342,343]. Another study showed that patients undergoing pancreatic resection with venous reconstruction (VR) had a median survival of 22 mo compared to 20 mo for those who had classic PD ($P = 0.25$)[344]. In another study, a slight survival benefit was noted in patients who did not require VR (33.5%) compared to those with VR (20%, $P = 0.18$), although this did not reach statistical significance[345].

Pancreatectomies with major arterial resections (common hepatic artery/celiac axis and superior mesenteric artery) have been reported in recent years with acceptable outcomes. Nevertheless, arterial reconstruction during pancreatectomies remains a challenging procedure with increased risk of complications compared to classic PD and PD with VR. In addition, most PCs with arterial invasion are for the majority, advanced tumors with distant lymph node involvement and metastases, and therefore indicated only in a very select group of patients[346]. Recent data on pancreatectomies requiring arterial resections at high volume tertiary centers have shown operative mortality rates of 4.3%[346], peri-operative mortality rates (60 d) of 17%[347], morbidity rates of 48%[348] and 3-year survival rates of 17%-23.1%, which are much higher than for classic PD[346,347].

It has been noted that lymph node involvement outside the standard PD specimens occurs in more than 30% of cases[349]. This has led to the evaluation of the need for a more extended lymph node dissection (ELND) in the surgical management of PC. To date, the definitions of a standard lymphadenectomy as well as ELND are still not very clear[341]. A number of Japanese studies have shown an increased survival rate in patients who have undergone ELND compared to conventional PD[350-352]. However, these studies were not randomized and their data were not validated by other centers[353].

The first RCT comparing standard PD and ELND was reported by Pedrazzoli *et al*[354] in 1998. In this study, standard lymph node dissection was defined as the removal of lymph nodes from the anterior and posterior pancreatoduodenal region, pyloric region, biliary duct, superior and inferior pancreatic head and body. In addition to the above, ELND included removal of lymph nodes from the hepatic hilum and along the aorta from the diaphragmatic hiatus to the inferior mesenteric artery and laterally to both renal hila, with circumferential clearance of the origin of the celiac trunk and SMA. This study showed no difference in morbidity, mortality or 4-year survival rates between the two groups.

Recently, a meta-analysis on standard PD and PD + ELND for PC patients showed comparable morbidity and mortality rates with a trend towards higher rates of delayed gastric emptying in the ELND group. The weighted

Table 11 Survival data after resection of pancreatic cancer

Author	Yr	Resection (*n*)	R0 resection (*n*)	Overall 5-yr survival (%)	R0 5-yr survival (%)	Median survival (mo)
Fatima *et al*[371]	2010	617	468	17.4	20	18
Kato *et al*[376]	2009	138	115	9.9	13.2	12.3
Raut *et al*[373]	2007	360	300	NA	NA	24.9
Cameron *et al*[258]	2006	1000	NA	18	23	33
Shimada *et al*[372]	2006	88	66	19	26	22
Howard *et al*[375]	2006	126	158	4	67	18
Moon *et al*[374]	2003	81	20	10.8	67.8	11.8

NA: Not applicable.

mean log hazard ratio for overall survival was 0.93 (CI: 0.77-1.13), revealing no significant outcome differences between the standard and extended procedure ($P = 0.480$) suggesting that ELND does not benefit overall survival and has a trend towards increased morbidity[355].

CLINICAL VOLUME AND OUTCOMES

During the last two decades, several large observational studies in the U.S., Canada and the Netherlands have shown that the institutional volume of pancreatic resections affects patients' outcomes. Higher perioperative morbidity, mortality and decreased use of multimodality therapy have been observed more frequently in low volume centers[356-363]. In 1993, Edge and colleagues reported that case load did not correlate with mortality after pancreatic resection[364]. However, surgeons who performed fewer than 4 resections per year had more complications. Recent studies have shown significant improvements in perioperative morbidity and mortality in patients undergoing pancreatic resections in high volume centers. For example, investigators at Memorial Sloan Kettering Cancer Center found that in a cohort of 1972 patients, high-volume centers defined as performing more than 40 cases per year in New York State had significantly less mortality (4% *vs* 12.3%) than low volume centers[356].

The definition of high and low volume varied among all these studies, but the findings were consistent and were confirmed by Birkmeyer *et al*[365] who showed that very low volume centers (0-1 procedure per year), low volume hospitals (1-2 procedures per year) and higher volume hospitals (more than 5 procedures per year) had significantly different mortality rates (16% and 12% *vs* 4% respectively; $P < 0.001$). The largest difference in operative mortality between very low volume (17.6%) and high volume (3.8%) centers is even more significant for PD when compared to other major surgeries as shown in a retrospective analysis of data from the national Medicare claims database and the Nationwide Inpatient Sample[257].

A recent study involving 301 033 patients with PC included in the National Cancer Database evaluated the treatment patterns of 1667 hospitals over a 19-year period[366]. During that time the pancreatectomy rate as well as the use of multimodality adjuvant therapy for patients with stage I and II disease increased significantly (pancreatectomy rate increased from 39.6% to 49.3%; $P < 0.001$, and the use of multimodality therapy increased from 26.8% to 38.7%; $P < 0.001$). Furthermore, patients were more likely to receive multimodality therapy at academic institutions, particularly those considered to be high volume hospitals. Despite these important advances, it appears that there is still a high percentage (71.4%) of patients with potentially resectable disease who are still not referred for surgical therapy as reported by Bilimoria *et al*[367]. These findings would suggest that a persistent nihilism of clinicians towards PC amd pancreatectomy may be the most significant correctable factor that contributes to the current poor long-term outcomes of PC.

ADJUVANT CHEMORADIATION THERAPY

Several single agent chemotherapeutic agents have been tried in the treatment of PC. 5-FU has been used in PC for more than 25 years with response rates of 8%-15%[368]. The addition of Leucovorin to 5-FU doubled the response rate to 26%, however, it showed no benefit in terms of survival[369]. The only chemotherapeutic agent that demonstrated prolonged survival in comparison to 5-FU and Leucovorin was Gemcitabine[370].

After pancreatic resection, the 5 year survival rate is only 20% or less as PC has a high loco-regional recurrence rate and a tendency towards early liver metastasis (Table 11)[258,371-376].

Based on these observations it appears necessary to employ adjuvant therapy in combination with surgical resection in order to improve survival. Only a few years ago there was no valid data on adjuvant chemoradiation therapy after curative surgical resection[377].

The first RCT that showed benefit from adjuvant chemoradiation therapy in comparison to surgery alone was the Gastrointestinal Tumor Study Group (GITSG) trial, where patients receiving 40 cGy followed by 5-FU showed a mean survival of 18 mo in comparison to 11 mo for those who received surgery alone ($P = 0.05$). The two- and five-year survival rates of the two groups were 43% *vs* 18% and 19% *vs* 0%, respectively[378].

The EORTC (European Organization for Research and Treatment of Cancer) study showed that patients undergoing chemoradiation therapy (5-FU protocol) had a median survival of 17.1 mo compared to 12.6 mo for the

controls (P = 0.099). The two- and five-year overall survival rates were 37% and 20% for the experimental arm and 23% and 10% for the control arm (P = NS)[379].

The European Study Group for PC 1 trial (ESPAC-1) compared four groups of patients who underwent pancreatic resection; (1) surgery alone; (2) 5-FU and Leucovorin adjuvant chemotherapy; (3) combination of adjuvant radiation therapy and 5-FU chemotherapy; and (4) adjuvant chemoradiation followed by chemotherapy[380]. In this study, the five-year survival rate for patients who received adjuvant chemotherapy was 21% compared to 8% for patients who did not (P = 0.009). Patients who underwent chemoradiation therapy had an inferior five-year survival rate (10% *vs* 20%) in comparison to patients who did not receive radiation (P = 0.05).

In 2006, the Radiation Therapy Oncology Group trial compared patients receiving adjuvant chemoradiation (5040 cGy in combination with continuous 5-FU) followed by 5-FU *vs* similar chemoradiation therapy followed by Gemcitabine. For patients affected by PC of the head, the arm treated with Gemcitabine had a superior median (18.8 mo *vs* 16.7 mo) and overall survival at 3 years [31% *vs* 21% (P = 0.047)], but with a higher incidence of toxicity (80% *vs* 60%)[381].

In 2007, a RCT conducted in Germany and Austria (CONKO-1 [Charite Onkologie Clinical Studies in GI Cancer 001]) compared patients undergoing R0 or R1 pancreatic resection alone *vs* resection followed by Gemcitabine-based chemotherapy. The median disease-free survival for patients treated with Gemcitabine was 13.9 mo *vs* 6.9 mo in the observation arm (P < 0.001), although there was no difference in the overall survival between the two groups (22 mo *vs* 20 mo)[382]. From the results of these studies, adjuvant chemotherapy has become the standard of care for patients who can tolerate the treatment after surgical resection.

NEOADJUVANT THERAPY

Neoadjuvant therapy is defined as the preoperative intervention aiming to convert unresectable PCs to resectable tumors or to increase the probability of complete microscopic tumor resection[383]. One of the limitations of the role of neoadjuvant therapy for PC is the fact that there is no standardized definition for tumor resectability and there is no data from randomized phase three trials on the benefit of neoadjuvant therapy. In addition, data from prospective and retrospective studies have several biases due to heterogeneity of inclusion and exclusion criteria, preoperative quality of imaging tests, and surgical pathology reports on lymph node involvement and resection margin status.

A recent systematic review[383] evaluating retrospective and prospective studies on neoadjuvant chemo and radiation therapy from 1966 to 2009 included a total of 111 studies and 4,394 patients. The results of this meta-analysis showed that the majority of patients were treated with Gemcitabine, 5-FU or oral analogue Mitomycin-c, and Platinum compounds. Patients undergoing neoadjuvant treatment received radiotherapy in the range of 24-63 Gy. The analysis showed that neoadjuvant treatment in patients with unresectable tumor was able to convert 33.2% of patients to resectable candidates, providing a median survival of 20.5 mo which was equivalent to patients undergoing resection followed by adjuvant therapy who had median survival of 20.1 to 23.6 mo. On the other hand, neoadjuvant therapy for patients with resectable cancer did not seem to improve overall outcome.

RADIATION THERAPY

Persistent loco-regional disease after pancreatic surgery is a major determinant of recurrence[384]. Although there is supportive evidence for the use of adjuvant chemotherapy[380,385], the role of adjuvant radiation remains unresolved. Generally it is believed that external-beam radiotherapy (EBRT) alone is a suboptimal treatment for locally advanced PC as most patients will die of systemic disease[386].

In the Mayo clinic clinical trial and the GITSG trial, patients who were randomized to receive EBRT only had a median survival of 5.3-6.3 mo which was inferior to EBRT plus 5-FU[387,388].

Among 210 patients who underwent surgical resection for PC [PD (73%), total and/or distal pancreatectomy (25%), Appleby procedure (2%)] followed by intraoperative electron beam radiotherapy (IOERT), some patients received a single fraction of IOERT alone (25 Gy), whereas others (30%) received additional EBRT and 54% received various forms of adjuvant chemotherapy. The study demonstrated excellent local control with the addition of IOERT (75%). Despite the benefit in local control, the overall median survival was similar to other studies with adjuvant chemotherapy or chemoradiation (19 mo)[389]. A combined study of extended resection and intraoperative radiation therapy (IORT) concluded that IORT contributed to local control; however, it provided no overall survival benefits (14.6% 5-year survival)[390].

In the United States, chemoradiation with concurrent 5-FU followed by Gemcitabine continues to represent the standard for adjuvant therapy of tumor of the pancreatic head. A direct comparison of chemo-radiation therapy and chemotherapy alone seems to be difficult to achieve and additive chemotherapy before or after chemo-radiation-therapy will have to be tested in randomized studies in order to determine the optimal sequencing[391].

PALLIATIVE MEASURES

Palliative treatment of patients with PC plays a very important role as 80% to 90% of newly diagnosed tumors are not resectable due to local invasion or presence of distal metastatic disease[392]. Median survival for patients with unresectable PC located in the head and body of the gland is approximately 7 mo, while for PC located in the tail median survival is significantly less [3 mo (P = 0.0002)], as they are usually diagnosed in more advanced stages[393]. For these patients, relief of symptoms secondary to gastric outlet obstruction, jaundice and pain are essential to

improve their quality of life and overall survival. In the past, surgical palliation was more common as the diagnosis of unresectable disease was frequently done in the operating room and patients underwent one or more of the following procedures: gastric bypass, hepatico-enteric decompression and celiac plexus neurolysis for pain relief during the same surgery. With the improvement in diagnostic imaging tests, the role of surgical staging has decreased as the vast majority of patients can be currently classified as suffering from unresectable disease by non-invasive modalities such as CT and MRI or by endoscopic US. Nevertheless, there are still controversies on the best palliative strategies for these patients as there is a lack of randomized controlled trials and abundant contrasting data from observational studies.

Gastro-duodenal decompression

There is still some controversy on the use of routine gastro-intestinal bypass for PC diagnosed as unresectable at the time of exploratory laparoscopy or laparotomy.

In a large observational study of 155 patients with unresectable PC staged by extended laparoscopy at the Memorial Sloan Kettering Cancer Center, only 4% of patients required surgical intervention for gastric outlet obstruction before their death: 2 patients required open gastro-jejunal anastomosis alone and 1 patient underwent a combined gastro and hepatico-jejunostomy a few days after laparoscopy[393]. In addition, 1 patient required a percutaneous endoscopic gastrostomy for palliation of gastric outlet obstruction a few weeks before demise. The authors concluded that the routine use of gastric bypass in patients with unresectable PC is not indicated. On the other hand, several other retrospective studies[394,395] have suggested that up to 25% of patients with unresectable PC would develop gastric outlet obstruction requiring surgical intervention.

A recent prospective randomized trial compared 44 patients who were found unresectable at the time of surgery and who underwent a retrocolic gastro-jejunostomy to 43 patients who did not[396]. The two groups had similar morbidity (32% *vs* 33%), mortality (0%) and hospital stay. On the other hand, patients who had gastric bypass did not develop any gastric outlet obstruction, while 19% of patients in the control group did ($P < 0.01$). Although this study would suggest that gastric bypass should be performed in all patients found unresectable at the time of surgery, the introduction of metallic self-expanding intestinal stents has changed the options for palliation.

A prospective multicenter cohort study of 51 patients with malignant gastric outlet obstruction treated with self-expandable metallic stents showed that in 98% of cases the stent was successfully deployed and that the median duration of patency was 10 mo. Only 14% of patients had stent dysfunction, and migration was observed in only 2% of cases[397]. Similar results were reported by another study from South Korea which showed a median stent patency of 385 d, and only 1% serious complications (gastrointestinal bleeding or perforation)[398]. Other observational studies have shown that compared with palliative surgery, stent placement provides a shorter hospital stay, earlier resumption of oral intake, fewer complications and lower hospital costs[399,400]. The only randomized controlled study that compared duodenal stent and laparoscopic gastrojejunostomy favored endoscopic therapy as it was associated with less discomfort, shorter hospital stay and improved physical health scores at 1 mo[401]. In this small study, only a third of patients were alive at 1 year and no cases of stent occlusion were observed. The two groups had similar overall survival supporting equipoise between endoscopic and surgical palliation. Nevertheless, surgical palliation can still play an important role when patients have a long life-expectancy, need biliary and gastric bypass in combination with celiac neurolysis for pain control.

Biliary decompression

The majority of PCs occur in the head of the pancreas and obstructive jaundice is one of the early symptoms for 50%-80% of patients[396]. In the past, staging laparotomy and biliary bypass were frequently performed for unresectable PC of the head[402,403]. During the last decades, the development of interventional radiology and endoscopy has allowed palliation of obstructive jaundice by the insertion of percutaneous or endoluminal stents with minimal morbidity and mortality. Currently, endoscopic biliary stenting is the treatment of choice for unresectable PC with obstructive jaundice. Percutaneous transhepatic stenting is reserved only for patients in whom endoscopic stenting has failed as it is associated with a higher complication rate than endoscopic palliation (61% *vs* 35%)[404,405]. High risk surgical patients are best managed by biliary stenting, however, it is still unclear whether palliative surgical biliary decompression is superior to other interventions for patients who are fit for surgery or who have a longer life expectancy. A European randomized controlled study comparing surgical biliary decompression *vs* endoscopic plastic stenting showed that both interventions were equally successful in palliating jaundice (95% *vs* 94%, respectively) and provided equal overall survival. Nevertheless, major complications (29% *vs* 11%) and procedure-related mortality (14% *vs* 3%) were significantly higher for surgical patients[406]. In addition, surgical decompression was more expensive than stenting, although recurrent biliary obstructions and late gastric bypasses were more common in patients undergoing endoscopic treatment even if that did not reach statistical significance. Similar results were reported in a more recent Brazilian study which found that endoscopic therapy with self-expandable metallic stents was more cost-effective than surgical decompression (US$2832 *vs* US$3821, $P = 0.031$) and provided better quality of life at 30 ($P = 0.04$) and 60 d ($P = 0.05$)[407]. The only available meta-analysis of randomized controlled studies comparing surgery with endoscopic stenting included only 3 studies where none tested the use of metallic self-expanding stents[408]. Although the reintervention rate was 3% (0%-16%) in surgically treated patients compared with 36% (28%-43%) in stented patients, because of the limited number of studies with a relatively small group of patients and heterogeneous quality, the authors

concluded that they could not identify which treatment was preferable.

The patency of biliary stents has greatly improved with the introduction of expandable metallic stents (EMS) as they offer a larger diameter for drainage and are associated with a lower occlusion rate than plastic stents[409,410]. The concurrent use of chemotherapeutic agents in patients palliated with SEMS was thought to increase the risk for ascending cholangitis. However, a Japanese retrospective study has demonstrated that the combination of SEMS and palliative chemotherapy for unresectable PC did not change the incidence of biliary infectious complications[411]. In patients with combined biliary and duodenal obstructions, concomitant biliary and duodenal stenting is now feasible and justified as the need to repeat endoscopic therapies is rarely required even in long-term survival patients[412].

Currently, surgical biliary bypass is advocated only for patients with obstructive jaundice who fail endoscopic or percutaneous stent placement.

Pain control

About 70% of patients with unresectable PC develop clinically important pain during their lives[413]. Pain is the main cause of the significant drop in quality and quantity of life of these patients and good palliation is necessary as pain incidence and severity increases with disease progression[414].

For the majority of patients, pain from PC can be managed with opioid analgesics. However, approximately one third of patients experience inadequate control of pain with oral analgesics alone[415]. For these patients, radiation therapy, chemotherapy and celiac plexus neurolysis have been used. Percutaneous neurolytic celiac plexus block with injection of 50%-100% ethyl alcohol under radiological guidance has become the most commonly recognized method of splanchnicectomy with a 70%-96% success rate[416]. The celiac plexus block has several advantages as it has been proven to ease pain without the side effects of opioids and can be administered intraoperatively, percutaneously, or by endoscopic ultrasonography. Recent studies have shown that endoscopic ultrasonography-guided neurolysis is effective and has minimal risk of the potentially serious complications associated with surgical or percutaneous approaches[417,418].

A recent double-blind randomized controlled study comparing patients treated with celiac plexus block *vs* systemic analgesic therapy showed that splanchnic neurolysis provided superior pain relief and quality of life scores, but overall opioid consumption, frequency of opioid adverse effects and overall survival did not reach statistical significance between the two groups[419]. For the majority of PC patients, pain is still controlled pharmacologically even if other modalities such as surgical thoracoscopic splanchnicectomy, epidural anesthesia, subcutaneous injection with octreotide, hypofractionated-accelerated radiotherapy and more recently photodynamic therapy have shown some temporary success[414,420-423].

Nutritional supportive care

The median survival of patients with unresectable PC is 33 wk and for advanced metastatic disease is only 10 wk[424]. About 90% of patients with PC have significant weight loss at the time of diagnosis and all of them develop progressive cachexia due to neoplastic metabolic derangements. Secondary events such as pancreatic exocrine insufficiency due to pancreatic duct obstruction, fat malabsorption due to biliary obstruction and poor oral caloric intake caused by nausea or gastric outlet obstruction are also responsible for the progressive weight loss. Even if weight loss has been found to have a prognostic effect on survival, most of the palliative care interventions for PC are directed at correcting biliary obstruction, gastric outlet obstruction and pain, and relatively little attention has been paid to interventions that can prevent or reduce the progressive weight loss of these patients[425]. Recently, a placebo-controlled trial comparing patients receiving enteric coated pancreatic enzyme supplements *vs* placebo showed that after 2 mo, patients receiving pancreatin had gained 1.2% of their body weight in comparison to controls who lost 3.7% ($P = 0.02$), and that they had higher daily total energy intake (8.4 MJ *vs* 6.6 MJ, $P = 0.04$)[424]. Although the Karnofsky performance status between the two groups was not different and survival analysis was not performed to determine if body weight gain translates into better prognosis, this study was the first to show an effective palliative strategy able to increase the intestinal absorptive function of patients who suffer from steatorrhea.

CONCLUSION

In recent decades, diagnostic modalities, and the surgical and palliative treatments of PC have clearly progressed although the overall prognosis has barely changed. The management of patients affected by PC is complex and requires expertise in many fields. Multidisciplinary teams are necessary to optimize the overall care, and palliative techniques have to be mastered as the majority of PCs are diagnosed in advanced stages. Better outcomes are reached if PC patients are appropriately referred to tertiary centers for assessment by surgical, medical and radiation oncologists, gastroenterologists, palliative care specialists and other dedicated health care providers. Despite recent progress, there is still a very limited ability to detect PC at an early stage, and there is a need for more studies to better understand genetic predisposing factors and to discover new markers that could assist physicians in this task. Randomized controlled studies are necessary to explore the role of neo-adjuvant therapies and new protocols for adjuvant strategies in patients undergoing pancreatic resection.

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ORIGINAL ARTICLE

Neuroprotective action of *Ginkgo biloba* on the enteric nervous system of diabetic rats

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Abstract

AIM: To investigate the effect of *Ginkgo biloba* extract on the enteric neurons in the small intestine of diabetic rats.

METHODS: Fifteen Wistar rats were divided into three groups: control group (C), diabetic group (D) and diabetic-treated (DT) daily with EGb 761 extract (50 mg/kg body weight) for 120 d. The enteric neurons were identified by the myosin-V immunohistochemical technique. The neuronal density and the cell body area were also analyzed.

RESULTS: There was a significant decrease in the neuronal population (myenteric plexus $P = 0.0351$; submucous plexus $P = 0.0217$) in both plexuses of the jejunum in group D when compared to group C. With regard to the ileum, there was a significant decrease ($P = 0.0117$) only in the myenteric plexus. The DT group showed preservation of the neuronal population in the jejunum submucous plexus and in the myenteric plexus in the ileum. The cell body area in group D increased significantly ($P = 0.0001$) in the myenteric plexus of both segments studied as well as in the ileum submucosal plexus, when compared to C. The treatment reduced ($P = 0.0001$) the cell body area of the submucosal neurons of both segments and the jejunum myenteric neurons.

CONCLUSION: The purified *Ginkgo biloba* extract has a neuroprotective effect on the jejunum submucous plexus and the myenteric plexus of the ileum of diabetic rats.

Key words: Diabetes mellitus; *Ginkgo biloba*; Myenteric plexus; Submucous plexus; Neuroprotection

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INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by high levels of glucose due to the lack of insulin and/or the inability of insulin to properly exercise its effects[1]. Long-term hyperglycemia induces morbid states in patients, resulting in macroangiopathy[2] complications, microangiopathy (retinopathy and nephropathy)[3] and neuropathies[4].

Neuropathy is the most common late complication in diabetic patients[5,6]. It compromises the sympathetic, parasympathetic and enteric nerves, causing a variety of abnormalities such as ulcerations of the lower limbs, sud-

den death by cardiac arrhythmia, gangrene, amputations, sexual dysfunction and gastrointestinal alterations[6,7].

The gastrointestinal tract is seriously affected by DM. Nearly 75% of diabetic patients may suffer with disorders such as late gastric emptying, vomiting, nausea, diarrhea, abdominal pain, swelling and constipation[8]. These disorders are usually correlated with enteric neuron lesions[9-12].

Oxidative stress plays an important role in the development and progression of diabetic neuropathy[13,14]. Hyperglycemia has been identified as the main cause in the development of oxidative stress by the production of reactive oxygen species (ROS) and reduction of endogenous antioxidants[15] due to auto-oxidation of blood glucose, excessive formation of AGEs (advanced glycation end products) and activation of the polyol pathway[13]. The excessive activation of the polyol pathway reduces the cytosolic NADPH, thus decreasing reduced glutathione (GSH), an important endogenous antioxidant. At the same time, this pathway produces an accumulation of sorbitol which causes cellular osmotic stress, also leading to oxidative stress[16].

ROS or free radicals such as superoxide anion (O_2), hydroxyl radical (OH) or intermediate species such as hydrogen peroxide (H_2O_2), damage all classes of cell macromolecular components and organelles (e.g. mitochondria, endoplasmic reticulum, proteins, *etc.*), which can lead to cell death. These free radicals also degrade the cell membrane phospholipids through a process called lipid peroxidation[17].

The use of antioxidants has beneficial effects in the treatment of diabetic complications[17-19]. *Ginkgo biloba* extract, obtained from *Ginkgo biloba* leaves, has medicinal properties and is one of the most sold natural supplements in the world. This extract has antioxidant activity and neuroprotective effect, inhibiting cell death[20,21]. Husstedt *et al*[22] noticed that treatment with *Ginkgo biloba* reduced symmetrical polyneuropathy when they analyzed clinical and neurophysiological parameters and the hemorheologic changes in patients with diabetes.

The immunohistochemical technique to identify protein myosin-V has been used to estimate the total neuronal population in different regions of the gastrointestinal tract[11,12]. This technique confers specificity in the identification of enteric neurons, because this protein is located in neuronal cytoplasm, allowing visualization of cell bodies and their projections[12].

Our aim was to analyze the effects of standardized extract of *Ginkgo biloba* (EGb 761) on neurons of the myenteric and submucous plexuses in the jejunum and ileum of streptozotocin-diabetic rats. To do so, a morphometric and quantitative study of enteric neurons after 120 d of treatment was carried out.

MATERIALS AND METHODS

Animals

Fifteen male *Wistar* rats (*Rattus norvegicus*) were used, obtained from the Central Vivarium of the Universidade Estadual de Maringá (UEM). The animal procedures described in this work were conducted in accordance with the ethical principles of the Brazilian Academy in Animal Experimentation (COBEA) and approved by the Ethics Committee in Animal Experimentation of UEM.

The weight of animals at the beginning of the experiment was 400 g, corresponding to an approximate age of 150 d. The animals were kept for 120 d in groups of five per box in a room with a light cycle of 12/12 h (7:00 to 19:00) and at constant room temperature of 21-22℃. They were fed with Nuvilab standard diet and water *ad libitum*.

Experimental design

The animals were divided into 3 experimental groups, each group comprised of 5 animals: control group (C) (normoglycemic); diabetic group (D); diabetic-treated with *Ginkgo biloba* extract (EGb 761) group (DT).

To induce diabetes the rats in groups D and DT were weighed and fasted for 16 h. Then, they were injected intravenously with streptozotocin (Sigma, St. Louis, MO, USA) at a dose of 35 mg/kg of body weight.

Blood glucose levels were determined after 7 d by the glucose oxidase method to confirm the disease onset. Only animals with blood glucose higher than 200 mg/dL were kept in groups D and DT.

Besides their normal diet, the DT group animals were treated daily by gavage with the *Gingko biloba* (EGb 761) extract (Tebonin, Altana Pharma, Jaguariúna, São Paulo, Brazil) at a dose of 50 mg/kg of body weight throughout the experiment.

Collection and processing of material

At the end of the 120-d trial period, all animals were anesthetized intraperitoneally with thiopental (40 mg/kg body weight) (Abbott Laboratories, Chicago, IL, USA). Blood was collected through cardiac puncture to assess the glycemia. After a laparotomy, the jejunum and ileum segments were collected. These segments were washed with 0.9% saline solution, the ends tied up and inflated with a fixative solution [periodate-lysine-paraformaldehyde (10 mmol/L sodium periodate, 75 mmol/L lysine, and 1% paraformaldehyde in 37 mmol/L phosphate buffer, pH 7.4)]. They were kept in vials containing the same solution for one and half hours. Thirty minutes later, two small holes were made near each end, and the fixative content was drained.

In order to improve the antibody tissue permeability, fragments of the jejunum and ileum were dehydrated in increasing series of alcohols (50%, 70%, 80%, 90%, 95%, 100% Ⅰ, 100% Ⅱ), cleared in xylol and rehydrated in decreasing series of alcohol up to 70%.

The dissection procedures were performed by cutting transversely the cylindrical segments of the jejunum and ileum, which were then opened longitudinally at the mesenteric insertion in order to obtain rectangular pieces. The procedure was carried out under a stereoscopy microscope and samples handled with watchmaker tweezers to obtain myenteric plexus membrane whole mounts. The

mucosa and submucosal tunica were removed from the myenteric plexus, while the external muscular layer was kept. The mucosa was removed from the submucosal plexus with the aid of a wooden spatula.

Immunohistochemistry of the myenteric and submucosal plexuses

The myenteric and submucous plexuses were stained by the anti-myosin-V immunohistochemical technique as described by Buttow *et al*[23]. The final concentration of antibody was 0.89 mg/mL. The dilution used was 1:1000 (v/v). The membranes were first immersed in a blocking solution of 0.1 mol/L PBS containing 2% bovine serum albumin (BSA) and 0.5% Triton X-100 and normal goat serum at a ratio of 1:50 (v/v) for 3 h. The material was incubated with primary antibody for 48 h at room temperature (RT); this was performed in a solution of 0.1 mol/L PBS containing 1% BSA and 0.1% Triton X-100 and normal goat serum in the proportion of 1:50 (v/v). After the incubation, the material was washed twice for 15 min with PBS solution 0.1 mol/L and Triton X-100 0.1% and then also washed twice in PBS 0.1 mol/L and Tween 20 at a concentration of 0.05% for 15 min. The whole-mounts were then incubated with anti-rabbit secondary antibody produced in goat, peroxidase-conjugated [ImmunoPure® Goat Anti-Rabbit IgG, (Fc), Peroxidase Conjugated, brand Pierce] in a blocking solution containing 0.1 mol/LPBS, 1% BSA and 0.05% Tween 20 for 24 h at RT. Normal goat serum at 1:50 (v/v) was also added to this blocking solution. The material was washed 4 times for 15 min in a solution of 0.1 mol/L PBS containing 0.05% Tween 20. The membranes were developed with the use of a diaminobenzidine solution (Sigma, St. Louis, MO, USA) for approximately 10 min at a concentration of 0.14 mg/mL. After developing, the material was mounted on histological slides with glycerol-gel (containing 50% glycerol, 0.07 g/mL gelatin in PBS, and 2 μL/mL phenol). The slides were then placed in refrigerator (4℃), in order to slowly dry the whole-mounts.

Density analysis of myosin-V immunoreactive neurons

Enteric neurons were counted on a BX 40 Olympus microscope under a 40 × lens. Forty microscopy fields, randomly selected, were counted for each preparation. The area of each field was 0.229 mm^2. The results were expressed in number of neurons per cm^2.

Morphometric analysis of myosin-V immunoreactive neurons

Images of the ganglia were taken and then measured with the aid of the image analysis software Image Pro-Plus 3.0.1 (Media Cybernetics, Silver Spring, MD, USA) to study the area of neurons in different groups. The area (μm^2) of 100 cell bodies per animal was measured, for a total of 500 neurons (5 animals per group). Neurons were classified into the class interval of 10 μm^2, and the percentage of each group was calculated for each interval.

Table 1 Final weight and glycemia in groups: control, diabetic and EGb 76-treated diabetic (mean ± SE)

Group	Final weight (g)	Blood glucose (mg/dL)
C	445.6 ± 63.04	78.97 ± 5.12
D	264.6 ± 22.88	253 ± 64.97
DT	308 ± 19.27	322 ± 20.42

n = 5/groups. C: Control; D: Diabetic; DT: EGb 76-treated diabetic.

Statistical analysis

To compare the parameters of the studied groups we used analysis of variance (ANOVA). When there was a significant difference we used Tukey's test. For this study we used the Prism software version 3.0. Results were considered significant when $P < 0.05$. The results were shown as mean ± SE, *n* indicating the number of samples in each group.

RESULTS

Streptozotocin caused diabetic syndrome onset in animal groups D and DT, as evidenced by the significant increase in blood glucose, as well as a significant reduction in body weight, when compared to group C (Table 1). Other typical symptoms of the disease (polyuria, polydipsia and polyphagia) were observed during the experimental period.

Neuronal density

There was a significant reduction ($P < 0.05$) in the neuronal density of myenteric neurons in the jejunum in group D when compared to C (Table 2). There was no significant difference in the DT group when compared to groups C and D. The neuronal density of submucosal neurons decreased significantly ($P < 0.05$) in group D when compared to C. No significant difference in the neuronal density was observed when group DT was compared to C (Table 2).

The neuronal density of myenteric neurons in the ileum decreased significantly ($P < 0.05$) in group D when compared to C (Table 3). No significant difference was seen when comparing group DT to C. There was no significant reduction in the neuronal density in the ileum submucous plexus when the three groups were compared (Table 3).

Areas of neuronal cell bodies

The results obtained with the measurements of 500 neurons per studied group were distributed according to the relative frequency of areas of neuronal cell bodies at intervals of 10 μm^2 (Figures 1 and 2). The cell body area in the jejunum ranged between 81.33 and 538.9 μm^2 for animals in group C; between 119.9 and 588.9 μm^2 in group D; and between 101.0 and 609.2 μm^2 in group DT. There were no significant differences in the mean areas of the jejunum myenteric neurons when comparing groups C and D. However, there was a significant reduction in the mean area ($P < 0.05$) of the DT group when compared to the other two groups (Table 2). The cell body area in the

Table 2 Neuronal density and mean area of cell bodies of myenteric and submucosal neurons in the jejunum of rat groups: control, diabetic and EGb 761-treated diabetic (mean ± SE)

Group	Myenteric plexus		Submucous plexus	
	Neuronal density (cm^2)	Mean area of cell body (μm^2)	Neuronal density (cm^2)	Mean area of cell body (μm^2)
C	15 884 ± 712.0	234.2 ± 88.10	12 602 ± 233.8	230.6 ± 62.89
D	13 483 ± 617.9	245.6 ± 77.19	11 383 ± 159.6	235.4 ± 67.99
DT	14 426 ± 301.2	218.2 ± 72.10	12 682 ± 353.4	216.2 ± 62.03

n = 5/myenteric plexus group; *n* = 3/submucous plexus group. C: Control; D: Diabetic; DT: EGb 76-treated diabetic.

Table 3 Neuronal density and mean area of cell bodies of myenteric and submucosal neurons in the ileum of rat groups: control, diabetic and EGb 761-treated diabetic (mean ± SE)

Group	Myenteric plexus		Submucous plexus	
	Neuronal density (cm²)	Mean area of cell body (μm²)	Neuronal density (cm²)	Mean area of cell body (μm²)
C	16 522 ± 625.5	232.7 ± 82.97	11 657 ± 403.9	210.0 ± 59.18
D	14 568 ± 424.7	251.4 ± 98.23	11 275 ± 281.9	231.3 ± 74.37
DT	16 884 ± 366.1	239.3 ± 81.19	11 943 ± 299.3	204.5 ± 57.36

n = 5/myenteric plexus group; *n* = 3/submucous plexus group. C: Control; D: Diabetic; DT: EGb 76-treated diabetic.

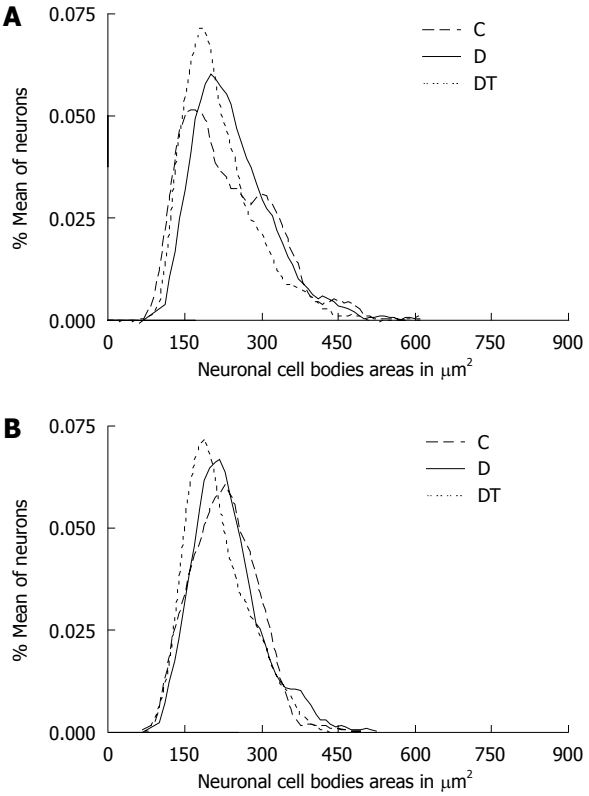

Figure 1 Neuronal behavior: area of cell body of myenteric (A) and submucosal (B) neurons, myosin-V immunoreactive in the jejunum, of control (C), diabetic (D) and diabetic-treated with EGb 761 (DT).

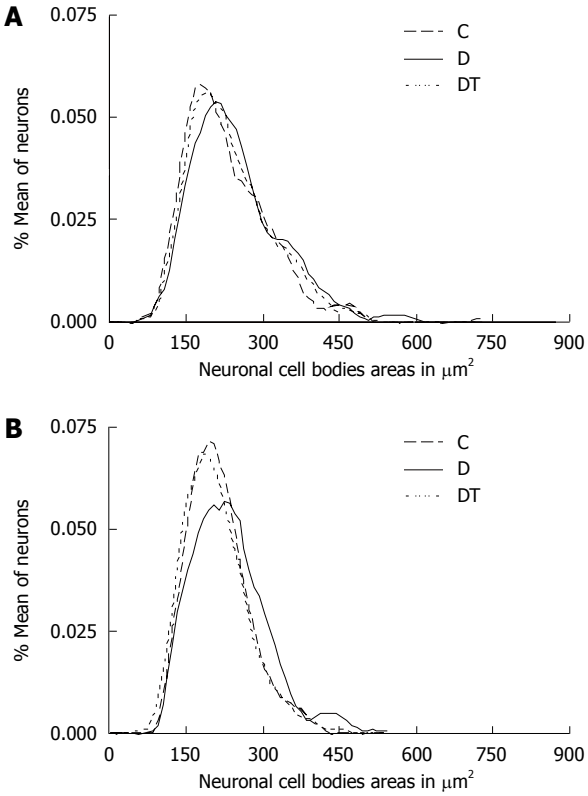

Figure 2 Neuronal behavior: area of cell body of myenteric (A) and submucosal (B) neurons, myosin-V immunoreactive in the ileum, of control (C), diabetic (D) and diabetic-treated with EGb 761 (DT).

submucosal neurons in the jejunum ranged between 106.1 and 474.4 μm^2 in group C, between 102.3 to 523.4 μm^2 in group D and between 91.73 to 401.1 μm^2 in group DT. There were no significant differences between the mean cell body areas in groups C and D ($P > 0.05$). However, there was a significant reduction ($P < 0.05$) in group DT when compared to groups C and D (Table 2).

The cell body area of myenteric neurons in the ileum ranged between 97.70 and 725.7 μm^2 in group C, between 101.5 and 595.5 μm^2 in group D, and between 96.32 and 512.9 μm^2 in group DT. There was a significant increase ($P < 0.05$) in group D when compared to C. No significant difference was observed when comparing group DT to groups C or D (Table 3). As for the ileum submucous

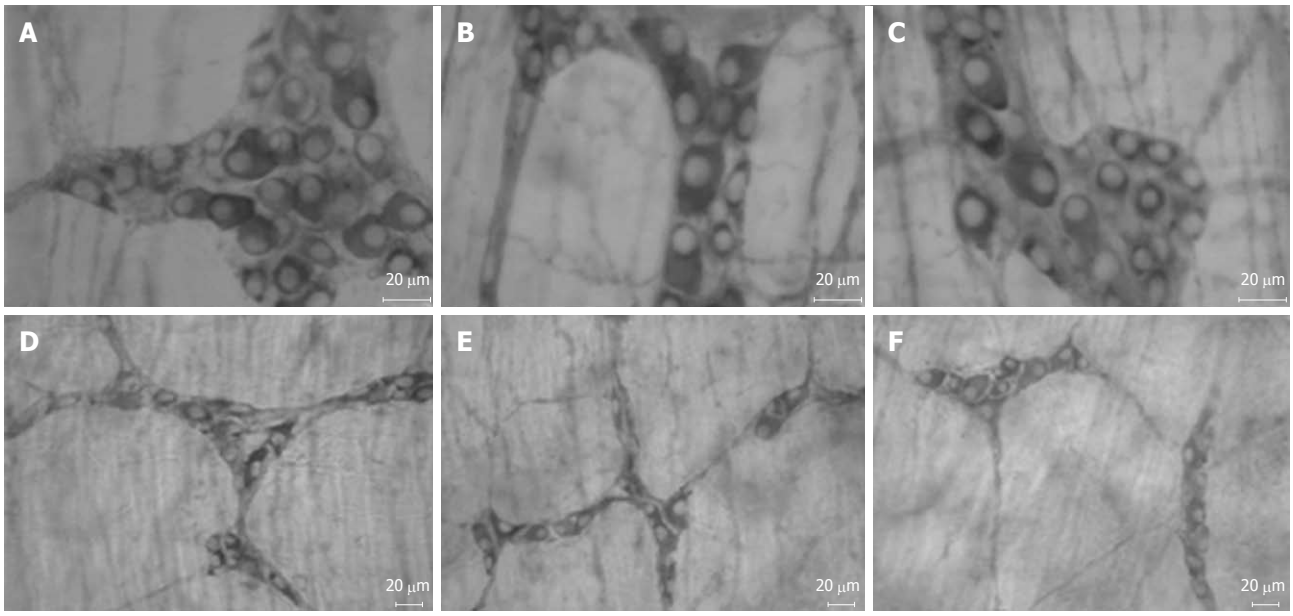

Figure 3 Myosin-V immunoreactive myenteric neurons in the jejunum (A-C) and myosin-V immunoreactive submucosal neurons in the jejunum (D-F). There is a significant reduction in the neuronal density in the myenteric (B) and submucous (E) plexus in group diabetic. The neuronal density in the submucous plexus (F) was preserved in group EGb 76-treated (DT) (F). There was a significant reduction in the neuronal cell body area in group DT of both plexuses (C and F).

plexus, the area ranged between 89.54 and 426.2 μm^2, between 99.52 and 534.0 μm^2 in group D, and between 72.77 and 435.0 μm^2 in group DT. There was a significant increase in the mean cell body area in group D ($P < 0.05$) when compared to C. The DT group showed no significant difference in mean cell body area when compared to group C (Table 3). In the submucous plexus, reduction in neuronal profile area was greater than in the myenteric plexus; the values in the submucous plexus just below those of the control group.

The distribution of the relative frequency of areas of cell bodies in the jejunum showed a displacement curve to the right in the myenteric plexus; thus showing a higher relative frequency of neurons at about 160 μm^2 in both plexuses (Figure 1). There was a similarity in the curves of groups C and DT in both plexuses in the ileum (Figure 2). Group D showed a displacement to the right in both plexuses.

DISCUSSION

Streptozotocin (STZ) is widely used in experimental animal models to induce DM. Its cellular action includes irreversible changes in genetic material causing lethal alterations in the metabolism of β cells[24]. There is a reduction in overall myenteric plexus neuron population in animal models with chronic STZ-diabetes[11,12,25,26]. There are no studies of changes caused by diabetes in the overall neuronal population of the submucous plexus. Our study showed that the 120-d treatment with purified *Ginkgo biloba* extract (EGb 761) has a neuroprotective effect on the ileum myenteric plexus and on the jejunum submucous plexus of STZ-diabetic rats.

Characteristic diabetic symptoms (polydipsia, polyuria and polyphagia) were observed in animals of D and DT groups. These data support the experimental model of streptozotocin-induced diabetes[27-29]. The immunohistochemical technique, anti-myosin-V (Figures 3 and 4), was used to assess the effect of *Ginkgo biloba* extract (EGb 761) on the enteric neuronal population. The protein myosin-V is present in cell bodies and projections of enteric neurons[30] and is being used as a pan-neuronal marker.

The reduction of the myenteric neuron density in the jejunum was 15.12% in group D when compared to C ($P < 0.05$). The submucosal neuron density was 9.61% lower in group D when compared to C ($P < 0.05$). A reduction of 11.83% in myenteric neuron density was observed in the ileum in group D when compared to C ($P < 0.05$). The submucosal neuron density in the ileum was similar among the three groups. Several authors report the reduction of myenteric neuron density in rats with STZ-diabetes in different regions of the gastrointestinal tract, including the cecum[31], ileum[11,26], jejunum[25] and proximal colon[12]. There are no studies in the submucosal plexus of the total neuronal population in STZ-diabetes models. Pereira *et al*[26] reported a 24% reduction in the number of myosin-V myenteric neurons in the ileum (after 120 d) of diabetic rats when compared to non-diabetic ones. De Freitas *et al*[25] observed a 37.9% neuronal loss of myosin-V myenteric neurons in the jejunum of diabetic rats when compared to non-diabetic animals, also after 120 d. These studies used 90-d-old animals at the beginning of the experiment and our study was carried out with 150-d-old rats, which may have contributed to the neuronal loss variation due to age.

The degenerative changes that affect the enteric nervous system seen in DM are due to metabolic disorders. High oxidative stress, resulting from the imbalance be-

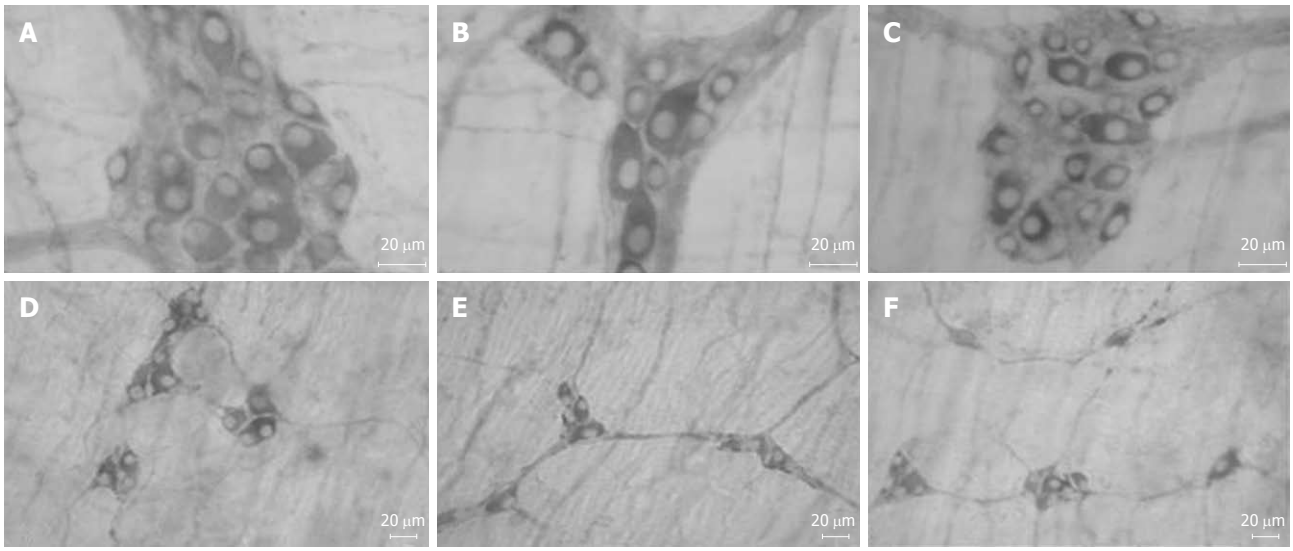

Figure 4 Myosin-V immunoreactive myenteric neurons in the ileum (A-C) and myosin-V immunoreactive submucosal neurons in the jejunum (D-F). There is a significant reduction in the neuronal density in the myenteric plexus (B), but the neuronal density was preserved in this plexus in group EGb 76-treated (DT) (C). There is a significant increase in the neuronal cell body area in group diabetic in the myenteric (B) and submucous (E) plexuses. There was a significant reduction in the neuronal cell body area in group DT in the submucous plexus (F).

tween ROS production and neutralization, is a well established mechanism of diabetic neuropathy pathogenesis and other complications[32,33]. The levels of endogenous and exogenous antioxidants are reduced in this condition. New studies have confirmed the destruction of endogenous antioxidants in peripheral nerves and the increased production of free radicals in the vasa nervorum[4].

Ginkgo biloba extract is widely used for its neuroprotective and antioxidant activity in several cardiovascular and neurologic disorders[34,35]. The *Ginkgo biloba* extract (EGb 761) was given at a daily dose of 50 mg/kg body weight for 120 d in this experiment. This standardized extract contains 24% flavonoid glycosides (quercetin, kaempferol, isorhamnetin) and 6% terpene lactones (ginkgolides, bilobalides). The EGb 761 extract components eliminate free radicals such as the hydroxyl radical and the superoxide anion[36]. Quercetin is a powerful antioxidant within the flavonoid family due to its molecular configuration which is capable of eliminating free radicals[37].

The myenteric neuronal density in the jejunum in the DT group was 9.17% lower when compared to C, though this reduction is not significant. On the other hand, the submucosal neuronal density in DT had very similar values to those of group C. The treatment with EGb 671 resulted in the preservation of the neuronal population in the ileum, represented by very similar values to those of the control group (Table 2), thus demonstrating a neuroprotective effect on this complex. The submucosal neuronal density in this segment was similar in all three groups. The *Ginkgo biloba* extract reduces the oxidative stress in diabetic rats by increasing the activity of antioxidant enzymes[38]. Wu *et al*[39] reported that this extract may be vital to postpone diabetic cataract, since their studies showed that, besides inhibiting aldose reductase activity, *Ginkgo biloba* also inhibits apoptosis induced by high glucose levels by reducing the Bax/Bcl2 ratio. This high ratio harms the mitochondria which release apoptosis-inducing proteins, such as the apoptosis-inducing factor, leading to the activation of caspase-3 *via* caspase 9. The myenteric plexus neuroprotection, seen only in the ileum, is similar to results in aging models[40] where 120-d treatment of rats with the same dose of *Ginkgo biloba* extract was more efficient in the ileum myenteric plexus than in the jejunum.

Few studies have been carried out in the submucous plexus due to the difficulty of dissection. Some authors have reported changes in neuronal subpopulations through the neurotransmitter immunoreactivity. Belai *et al*[41] observed an increase in VIP and neuropeptide Y immunoreactivity when analyzing the submucous plexus in the ileum of STZ-diabetic rats aged 8 and 16 wk. They also observed a reduction in calcitonin gene-related peptide (CGRP) immunoreactivity. However, no change in substance P immunoreactivity or dopamine beta hydroxylase was seen. VIP-ergic neurons of diabetic rats show increased immunoreactivity in the jejunum[42] and ileum[43] submucous plexus.

The mean cell body areas of myenteric neurons in the jejunum were similar in groups C and D. These results are similar to those observed by De Freitas *et al*[25], who did not observe an increase in the mean area of the cell body of immunoreactive myosin-V neurons in the jejunum of diabetic rats when compared to non-diabetic rats. The mean areas of cell bodies of submucosal neurons in the jejunum were similar in groups C and D. Studies on morphometric changes in the submucosal plexus caused by diabetic syndrome report an increase in the mean area of the cell body of neuronal subpopulations. Defani *et al*[42] observed an increase in the mean area of the cell body of submucous VIP-ergic neurons in the jejunum. The technique used to stain the total population showed no change

in the mean area of submucosal neurons in the jejunum. The mean area of the cell body of myenteric neurons in the ileum was 7.44% ($P < 0.05$) higher in group D than in group C in our study. This increase was also observed by Zanoni *et al*[11] and Pereira *et al*[26] in Wistar rats after a 120-d experimental period. The mean area of the body cell of submucosal neurons in the ileum showed a statistically significant increase of 9.2% ($P < 0.05$) in group D when compared to C. Zanoni *et al*[43] reported an increase in the mean area of the body cell of submucous VIP-ergic neurons in the ileum.

The increase in the neuronal cell body area in rats with chronic diabetes may be the result of neuronal edema[11]. The aldose reductase hyperactivity observed in diabetes is associated with increased levels of sorbitol[44] which increases the intracellular osmolarity, resulting in edema and neuronal lesions[43].

The EGb 761 treatment induced a reduction of 6.8% in the mean area of the cell body in the jejunum myenteric neurons in DT when compared to C ($P < 0.05$). The mean area of the cell body of submucosal neurons decreased 6.2% in group DT when compared to C ($P < 0.05$). The mean area of the cell body of myenteric and submucosal neurons in the ileum in DT was reduced to values similar to group C. Schneider *et al*[40] observed that the EGb 761 treatment reduced the mean area of myenteric neuronal cell bodies in the jejunum and ileum of aging rats. However, studies by Perez *et al*[45] in the large intestine treated with EGb 761 at a dose of 50 mg/kg of body weight observed that the EGb 761 extract promotes an increase in the mean area of myenteric neurons in rats in the aging process. These results show that the response to the use of antioxidants such as the *Gingko biloba* extract may be different according to the segment evaluated.

This study showed that treatment with *Ginkgo biloba* extract reduced the area of the cell body of myenteric and submucosal neurons in the jejunum and ileum of diabetic-treated rats (group DT) when compared to non-treated diabetic rats (group D). However, the reduction in the mean area of the cell body of myenteric neurons in the ileum was not significant. The inhibitory action of *Ginkgo biloba* on aldose redutase[19] enzyme activity may be responsible for the reduction in the mean area of neuronal cell bodies observed in rats treated with EGb 761 (DT group).

In conclusion, our results show that the 50 mg/kg of body weight dose of standardized *Ginkgo biloba* extract (EGb761) has a neuroprotective effect on the ileum myenteric plexus and on the jejunum submucous plexus of STZ-diabetic rats.

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COMMENTS

Background

Gingko biloba extract possesses various biological activities and has been shown to be useful in diabetes treatment. Oxidative stress has been known to play an important role in the development and progression of diabetes mellitus (DM), and reactive oxygen species (ROS) production is a direct consequence of hyperglycemia. Chronic hyperglycemia in diabetes is involved in direct neuronal damage caused by intracellular glucose which leads to altered neurotransmitter functions and reduced motor activity. Oxygen free radicals are also thought to play an important role in the diabetic and hypoxic condition of cells. Success of *Ginkgo biloba* application is determined by its main active substances, flavonoids (flavone glycosides, primarily composed of quercetin) and terpenoids (ginkgolides and bilobalides). *Ginkgo biloba* can improve hemodynamics, scavenge ROS, suppress platelet-activating factor (PAF) and relax vascular smooth muscle.

Research frontiers

Gastrointestinal (GI) afflictions are not normally life threatening but do profoundly affect quality of life. Diabetic patients experience a wide range of GI discomforts including nausea, vomiting, heartburn, diarrhea, constipation, abdominal pain and fecal incontinence. The high morbidity, high socioeconomic costs and lack of specific treatments are key factors that define the relevance of DM for human health and the importance of research on neuronal protective agents. Some studies provide a strong case for the application of *Ginkgo biloba* in diabetic nephropathy therapy.

Innovations and breakthroughs

Ginkgo biloba has been ascertained to be protective against DM. However, there has been little in the literature reporting on the protective effects of *Ginkgo biloba* on the enteric nervous system of the small intestine of streptozotocin-induced diabetic rats *in vivo*.

Applications

This study indicated that standardized extract of *Ginkgo biloba* (EGb 761) could improve antioxidant ability and protect the enteric nervous system of the small intestine of streptozotocin-induced diabetic rats *in vivo*. These biological activities have considerable potential in diabetes mellitus treatment.

Peer review

The authors investigated the effect of Ginkgo biloba extract on the enteric neurons on the small intestine of diabetic rats. They found purified Ginkgo biloba extract has a neuroprotective effect on the jejunum submucous plexus and the myenteric plexus of the ileum of diabetic rats. This is a well written paper.

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ORIGINAL ARTICLE

Outcome of non surgical hepatic decompression procedures in Egyptian patients with Budd-Chiari

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Abstract

AIM: To evaluate outcome of patients with Budd-Chiari syndrome after balloon angioplasty ± stenting or transjugular intrahepatic portosystemic shunt (TIPS).

METHODS: Twenty five patients with Budd-Chiari syndrome admitted to Ain Shams University Hospitals, Tropical Medicine Department were included. Twelve patients (48%) with short segment occlusion were candidates for angioplasty; with stenting in ten cases and without stenting in two. Thirteen patients (52%) had Transjugular Intrahepatic Portosystemic Shunt. Patients were followed up for 12-32 mo.

RESULTS: Patency rate in patients who underwent angioplasty ± stenting was 83.3% at one year and at end of follow up. The need of revision was 41.6% with one year survival of 100%, dropped to 91.6% at end of follow up. In patients who had Transjugular Intrahepatic Portosystemic Shunt, patency rate was 92.3% at one year, dropped to 84.6% at end of follow up. The need of revision was 38.4% with one year and end of follow up survival of 100%. Patients with patent shunts showed marked improvement compared to those with occluded shunts.

CONCLUSION: Morbidity and mortality following angioplasty ± stenting and TIPS are low with satisfactory outcome. Proper patient selection and management of shunt dysfunction are crucial in improvement.

Key words: Angioplasty; Stenting; Transjugular Intrahepatic portosystemic shunt; Patency rate

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INTRODUCTION

Budd-Chiari syndrome (BCS) results from hepatic venous outflow obstruction at any level, from hepatic venules to the right atrium[1]. If obstruction is due to endoluminal venous lesion like thrombosis, primary BCS is considered. In secondary BCS, the cause originates from neighboring structures like extrinsic compression or tumor invasion[2].

Imaging studies combined with clinical information

are often essential for reaching a definitive diagnosis[3].

The goals of treatment are to prevent extension of thrombosis in hepatic veins (HVs) and to alleviate venous obstruction in order to decrease hepatic congestion. Few patients respond to medical treatment (anticoagulation ± thrombolytic therapy, diuretics). However, most patients need intervention to restore the hepatic blood flow[4].

If there is a possibility of restoring hepatic venous outflow in one of the major HVs by balloon dilatation, recanalization, or stent insertion, then this is the procedure of choice as it is the most physiological method. However, in cases where blood flow cannot be restored or when the approach fails, transjugular intrahepatic portosystemic shunt (TIPS) is used as a decompressing non-surgical procedure[5].

MATERIALS AND METHODS

Study Design & Sampling: This prospective follow-up study was conducted on twenty five patients with confirmed diagnosis of primary BCS and eligible criteria for radiological intervention, who were presented to the Budd-Chiari Study Group and admitted to the Tropical Medicine Department, Ain Shams University Hospitals.

Patients were subjected to: (1) Complete Clinical Evaluation; and (2) Radiological Assessment, with special stress on the patency of HVs, portal vein and inferior vena cava (IVC) by abdominal Duplex/US. Abdominal MRI, MR venography or multislice CT scan were done to confirm diagnosis and to delineate vascular anatomy before intervention.

They were divided into two groups: (1) Patients with short segment occlusion of any of HVs who were candidates for angioplasty ± stenting; and (2) Patients with complete occlusion of all HVs who were candidates for TIPS.

Exclusion criteria: (1) Secondary BCS; (2) Retro or suprahepatic IVC obstruction; (3) Complete portal vein thrombosis; (4) Presence of comorbid etiology for liver disease in addition to BCS (e.g.: viral hepatitis); (5) Hepatocellular carcinoma; (6) Cardiac contraindications to TIPS (congestive heart failure and severe pulmonary hypertension); (7) Marked coagulopathy (INR > 5) and Thrombocytopenia (platelets < 20 000)[6]; (8) Biliary obstruction; and (9) Uncontrolled sepsis.

Details of the study and interventions were explained to recruited patients who signed a written consent form.

Pre-intervention assessment and preparation

Routine laboratory investigations and thrombophilia workup were done aiming at identification of etiology of BCS, in addition to assessment of liver disease severity.

Patients' general health was assessed according to WHO performance status scale[7]: 0: patient is fully active, able to carry on all pre-disease performance without restriction; 1: patient is restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g. light house work; 2: patient is ambulatory and capable of all self care but unable to carry out any work activities. Up and about more than 50% of waking hours; 3: patient is capable of only limited self care, confined to bed or chair more than 50% of waking hours; 4: patient cannot carry on any self care and totally confined to bed or chair.

Patients were classified as follows: According to Rotterdam prognostic classification[8] into 3 classes with scores according to the equation: 1.27 × encephalopathy + 1.04 × ascites + 0.72 × prothrombin time + 0.004 × bilirubin [Ascites and hepatic encephalopathy were scored as present (1) or absent (0) and prothrombin time as higher (1) or lower (0) than 2.3 INR. Bilirubin was included as a continuous variable]. Where Class Ⅰ (0-1.1): good prognosis; Class Ⅱ (1.1-1.5): intermediate prognosis and Class Ⅲ (> 1.5): poor prognosis.

According to Child-Pugh score into 3 classes (A, B and C)[9].

All patients started anticoagulation therapy when diagnosis of BCS was evident; in the form of low molecular weight heparin (LMWH) or unfractionated heparin. Then oral warfarin was added till INR reached its target (2-3), then continued on oral therapy alone after withdrawal of LMWH or unfractionated heparin.

Five days before procedure, oral anticoagulation therapy was stopped with administration of LMWH or unfractionated heparin only; to be stopped (6-12 h) before intervention in case of unfractionated heparin and (12-24 h) in case of LMWH to avoid intra or postoperative bleeding[10].

Antibiotic prophylaxis was administered for all patients (1-2 h) before intervention in the form of combination of ampicillin- sulbactam 1.5 gm IV and cefotaxime 1 gm IV[11].

Technical considerations

All procedures were performed in an angiographic interventional room with high resolution C-arm fluoroscopy, and digital subtraction angiography.

Interventions were done under general anesthesia.

All cases of TIPS or angioplasty with stenting had self expandable non covered metallic stents.

Post intervention management

Patients were admitted to hospital for 1 wk after procedure for early detection and management of any procedure-related complications and adjustment of anticoagulation.

Antibiotics regimen taken before procedure was continued for 5 d after.

Oral warfarin was introduced together with parental anticoagulation (LMWH after 24 h or unfractionated Heparin after 6 h) till INR reaches (2-3) then oral therapy was continued alone for life[12].

Duplex U/S was performed to detect shunt patency at days 1, 3, and 7 after the procedure.

Follow up

Patients were followed up clinically, by laboratory investigations (mainly liver profile and PT and PTT for monitoring of anticoagulation) and radiologically by duplex U/S.

Follow up after intervention was every three mo or when indicated (e.g.: clinical manifestations suggestive of angioplasty or TIPS dysfunction). Follow up was intended to be at least one year (Minimum: 12 mo, Maximum: 32 mo).

Aims of follow up were

(1) Assessment of patients' survival and shunt survival (i.e.; shunt patency and function) (at one year interval and at the end of follow up); (2) Description of procedures related complications and their management; and (3) Assessment of patients' improvement after intervention by comparison of clinical, laboratory and performance status criteria before intervention and one year after.

Statistical analysis

Descriptive statistics: (1) Quantitative data: mean, standard deviation (± SD); and (2) Qualitative data: frequency and percentage.

Analytical statistics: (1) Quantitative data: Wilcoxon Signed Ranks Test; and (2) Qualitative data: McNemar Test.

Levels of significance: (1) $P > 0.05$ = non significant (NS); (2) $P < 0.05$ = significant (S); (3) $P < 0.01$ = highly significant (HS); and (4) $P < 0.001$ = very highly significant (VHS).

Survival: (1) Patient Survival was *defined* as the duration between diagnosis of BCS, and patient death or loss to follow up. Survival rates were Kaplan-Meier estimates; (2) Shunt Survival was *defined* as the duration between shunt application, and shunt occlusion or loss to follow up. Survival rates were Kaplan-Meier estimates.

RESULTS

Descriptive data

This study was conducted on twenty five patients with BCS who underwent non surgical hepatic decompression procedures in the form of either angioplasty ± stenting or TIPS. They were 16 females (64%) and 9 males (36%) with a mean age of 28.28 ± 8.93 years (range 14-57 years). BCS was chronic form in 21 patients (84%), acute in three patients (12%), and fulminant in 1 patient (4%). When tested for underlying thrombophilia, 8 were negative (idiopathic), 4 primary antiphospholipid antibody syndrome (APS), 4 protein C deficiency, 3 Antithrombin Ⅲ deficiency, 1 myeloproliferative disorder, 1 combined protein C, S deficiency, 1 combined protein C, S, Antithrombin Ⅲ deficiency, 1 combined Antithrombin Ⅲ deficiency + factor V Leiden mutation (FVLM), 1 combined protein S deficiency + FVLM and 1 was primary APS + FVLM.

According to Child Classification, 5 patients (20%) were Child A, 16 (64%) were Child B and 4 (16%) were Child C. According to Rotterdam Classification, 7 patients (28%) were Class Ⅰ, 15 (60%) were Class Ⅱ and 3 (12%) were Class Ⅲ. The Performance status score was "0" in none of the patients, "1" in 4 patients (16%), "2" in 5 patients (20%), "3" in 11 patients (44%) and "4" in 5 patients (20%).

Table 1 Clinical manifestations and radiological criteria in studied patients

Findings	Patients, *n* (%)
Clinical manifestations	
Abdominal pain	23 (92)
Jaundice	9 (36)
Lower limb edema	10 (40)
Dilated veins over abdomen and trunk	5 (20)
Tender hepatomegaly	16 (64)
Ascites	24 (96)
Radiological criteria	
Hepatomegaly	24 (96)
Splenomegaly	22 (88)
Ascites	
Absent	1 (4)
Present	24 (96)
Liver mottling appearance	17 (68)
Intra hepatic collaterals	16 (64)
Caudate lobe hypertrophy	12 (48)
Hepatic Veins:Short segment occlusion	
RHV	2 (8)
MHV	7 (28)
LHV	5 (20)
Total occlusion	
RHV	23 (92)
MHV	18 (72)
LHV	20 (80)

Radiological criteria were obtained using duplex ultrasound, magnetic resonance venography and/or multislice computed tomography scan. RHV: Right hepatic vein; MHV: Middle hepatic vein; LHV: Left hepatic vein.

Pre-intervention clinical and investigational data

Clinical manifestations and baseline radiological criteria of studied patients using duplex U/S, MRV and/or Multislice CT scan are shown in Table 1.

Intervention details: The main indications for intervention in the studied patients were ascites associated with large esophageal varices; uncontrollable ascites only; large esophageal varices only and fulminant hepatic failure in 56%; 36%; 4% and 4% of patients respectively.

Twelve patients (48%) were candidates for angioplasty; of those; 10 patients (40%) had stenting (5; 20% in MHV, 4; 16% in LHV and 1; 4% in RHV) and 2 patients (8%) had angioplasty without stenting (1 patient in both LHV and MHV and the other patient in both RHV and MHV, where they shared a common short stenotic segment at their entrance into IVC).

Thirteen patients (52%) were candidates for TIPS.

The need of revision was 41.6% (5 out of 12 patients) in cases of angioplasty ± stenting and 38.4% (5 out of 13 patients) in cases of TIPS as shown in Table 2.

Table 2 Details of patients who needed revisions and their follow up (*n* = 10)

Patient	Intervention	Time of dysfunction	Action taken	No of revisions	1 yr patency	End of FUP patency
23 yr F	Angioplasty without stenting	Day 7 and Day 10	TIPS was done, occluded at day 10; then re-angioplasty was done[1]	2	Patent	Patent at 20th mo
27 yr M	Angioplasty and stenting	Day 7 and 2nd yr	Angioplasty was done-then angioplasty + thrombectomy	2	Patent	Patent at 24th mo
28 yr F	Angioplasty and stenting	4th mo	Angioplasty + local thrombolytic therapy	1	Patent	Patent at 12th mo
30 yr F	Angioplasty and stenting	1st, 4th, 6th and 9th mo	TIPS was done-then angioplasty (3 times)	4	Occluded at 9th mo	Occluded at 24th mo
28 yr M	Angioplasty and stenting	3rd mo and 14th mo	Angioplasty + stent was done-then mesoatrial shunt	1	Occluded at 1 yr	Dead[2] at 17th mo
27 yr F	TIPS	Day 1	(stent occlusion and migration to portal vein) - Re (TIPS)	1	Patent	Patent at 20th m
33 yr F	TIPS	Day 3	Angioplasty + thrombectomy + systemic thrombolytic therapy	1	Patent	Patent at 32nd mo
37 yr F	TIPS	Day 7 and 1st mo	Angioplasty (2 times)	2	Patent	Patent at 12th mo
27 yr M	TIPS	Day 7, 3rd and 8th mo	Angioplasty (3 times)	3	Patent	Occluded at 20th mo
17 yr M	TIPS	1st mo	Patient refused intervention	0	Occluded	Occluded at 12th mo

[1]Patient had angioplasty dysfunction at Day 7, so transjugular intrahepatic portosystemic shunt (TIPS) was done but was occluded at Day 10, so angioplasty of TIPS stent was done; [2]Cause of death: Intraperitoneal bleeding. Follow up period: Minimum (12 mo), Maximum (32 mo). F: Female; M: Male; yr: Years old; FUP: Follow up.

Table 3 Patient survival *n* (%)

	Angioplasty	TIPS	Total
One year			
Alive	12 (100)	13 (100)	25 (100)
Dead	0 (0)	0 (0)	0 (0)
End of follow up			
Alive	11 (91.6)	13 (100)	24 (96)
Dead	1 (8.4)	0 (0)	1 (4)

Because of death of one patient only out of 25; Kaplan–Meier curve couldn't be drawn for patient survival. TIPS: Transjugular intrahepatic portosystemic shunt.

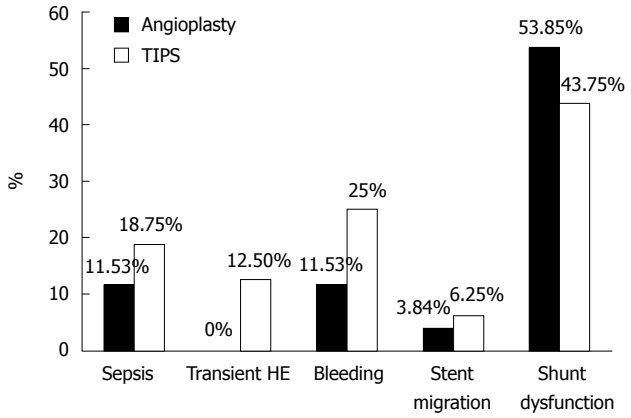

Figure 1 Procedure complications. Transient hepatic encephalopathy (HE): HE lasting 2-3 d after procedure with rapid response to treatment. Bleeding was either intra-peritoneal or hemobilia. TIPS: Transjugular intrahepatic portosystemic shunt.

Figure 1 shows frequency of all complications in total procedures done [Twenty six angioplasty ± stenting procedures (12 as primary intervention and 14 as a trial for maintenance of previously occluded angioplasty or TIPS) and 16 TIPS procedures (13 as primary intervention and 3 in patients with occluded stents following angioplasty in whom redilatation was not possible)].

In total procedures done (whether primary or revision procedures), the frequency of angioplasty dysfunction was 53.85% (14 out of 26 procedures) and the frequency of TIPS dysfunction was 43.75% (7 out of 16 procedures).

Statistical analysis

The mean duration of follow up was 20.04 ± 7.817 mo (ranging from 12-32 mo). One year survival rate was 100% for all patients and at the end of follow up survival rate was 96% due to death of one patient at the 17th mo of follow up as shown in Table 3.

Figure 2A shows patency rate in patients who underwent angioplasty ± stenting procedures; it was 11/12 (91.7%) at 9 mo (due to persistent shunt occlusion in one patient). Patency rate dropped to 10/12 (83.3%) at one year and continued till the end of follow up at 32 mo. (There was persistent shunt occlusion in 2 patients in spite of repeated revisions and optimal anticoagulation therapy).

Figure 2B shows patency rate in patients who had TIPS procedures; it was 12/13 (92.3%) at one year (due to persistent shunt occlusion in one patient despite repeated revisions). Patency rate dropped to 11/13 (84.6%) at 20 mo and this continued till the end of follow up at 32 mo (due to persistent shunt occlusion in another patient).

At one year of follow up, only three patients of 25 (12%) had occluded shunts. Patients with occluded shunts showed no improvement regarding their clinical manifestations, laboratory profile and performance status. On the contrary, patients with patent shunts (22 of 25; 88%) showed marked improvement as shown in Tables 4 and 5.

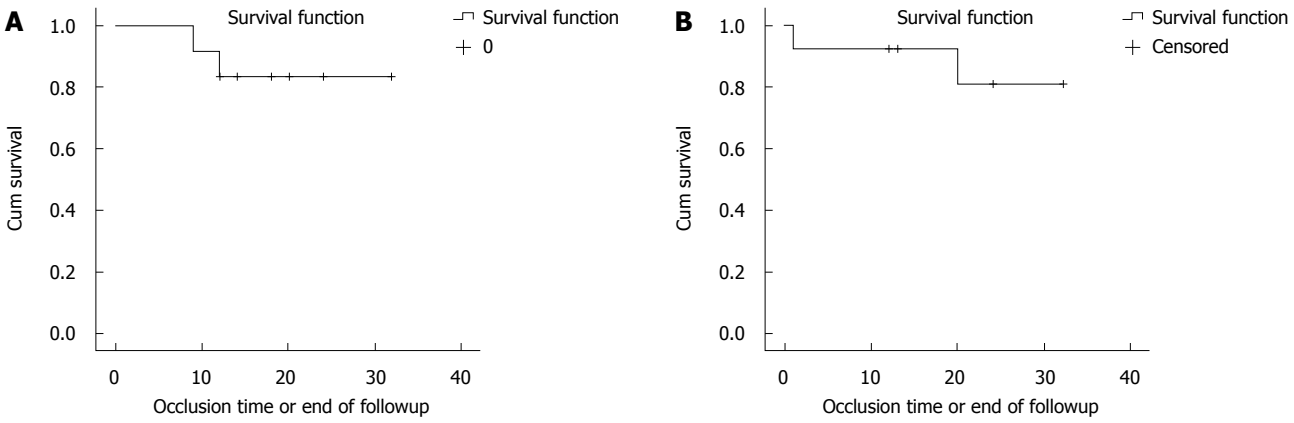

Figure 2 Patency rate in patients who underwent angioplasty ± stenting (A) and transjugular intrahepatic portosystemic shunt (B). A: Type of intervention: angioplasty ± stenting of hepatic veins, it was 91.7% at 9 mo and dropped to 83.3% at one year till the end of follow up at 32 mo; B: Type of intervention: transjugular intrahepatic portosystemic shunt, it was 92.3% at one year and dropped to 84.6% at 20 mo till the end of follow up at 32 mo.

Table 4 Clinical data of patients before and after intervention

	Before intervention		One year after intervention		*P* value	Sig
	+VE	-VE	+VE	-VE		
Patients with occluded shunts (*n* = 3)						
Abdominal pain	3	0	2	1	> 0.05	NS
Jaundice	1	2	0	3	> 0.05	NS
Lower limb edema	2	1	1	2	> 0.05	NS
Dilated veins	1	2	0	3	> 0.05	NS
Ascites	3	0	3	0	> 0.05	NS
Patients with patent shunts (*n* = 22)						
Abdominal pain	20	2	1	21	< 0.001	VHS
Jaundice	8	14	0	22	< 0.01	HS
Lower limb edema	8	14	1	21	< 0.05	S
Dilated veins	4	18	0	22	> 0.05	NS
Ascites	21	1	1	21	< 0.001	VHS

Sig: Significance; NS: Non significant; S: Significant; HS: Highly significant; VHS: Very highly significant; -VE: Negative; +VE: Positive.

DISCUSSION

This is the first study that addresses the short term outcome of interventional radiology procedures in management of Egyptian patients with BCS. In this study, 12 patients (48%) had short segment occlusion that enabled us to perform angioplasty with stenting in ten cases and without stenting in two cases. Thirteen patients (52%) were not suited for angioplasty and had TIPS.

According to Xu *et al*[13], short-term results of balloon angioplasty alone without stenting were excellent but the sustained patency rate was only 50% at two years after the procedure. In this study, one of the cases that had angioplasty alone was still having patent shunt at 24 mo after the procedure without any need for shunt revision; the other one had occluded shunt on the seventh day that necessitated re-intervention in the form of TIPS which was still patent at 20 mo after procedure.

Patency rate in patients who underwent angioplasty ± stenting procedures was 10/12 (83.3%) at one year and at the end of follow up due to persistent shunt occlusion in 2 patients in spite of repeated revisions and optimal anticoagulation therapy. This is a more or less satisfactory outcome; however it might have been influenced by the relatively short follow up period (ranging from 12 to 32 mo) as well as most of the patients having good or intermediate prognosis according to Rotterdam score. The need of revision in cases with angioplasty ± stenting was 41.6% (5 out of 12 cases). One year survival was 100% and at the end of follow up, survival dropped to 91.6% due to death of one patient who had occluded shunt after one year and was also referred for mesoatrial shunt due to occlusion of IVC.

Although angioplasty is considered a simple procedure; some complications were reported in the current study. Twenty six angioplasty ± stenting procedures have been done (12 procedures as primary intervention and 14 procedures as a trial for maintenance of previously occluded angioplasty or TIPS); of these procedures, angioplasty dysfunction was reported in 53.85%. This is consistent with Senzolo *et al*[14] who stated that although long-term patency rates can reach 80%-90% in angioplasty ± stenting procedures; angioplasty may later be required in 50% of these cases to overcome angioplasty dysfunction.

Table 5 Lab data and performance status of patients before and after intervention

	Before intervention		One year after intervention		*P* value	Sig
	mean	SD	mean	SD		
Patients with occluded shunts (*n* = 3)						
ALT (N = 7-40 IU/L)	70.33	75.070	29.66	24.66	> 0.05	NS
AST (N = 7-37 IU/L)	42	24.240	42.33	32.51	> 0.05	NS
Total bilirubin (N = 0.2-1.2 mg/dL)	2.9	2.940	1.26	0.832	> 0.05	NS
Direct bilirubin (N = 0-0.3 mg/dL)	1.53	1.560	0.53	0.577	> 0.05	NS
Albumin (N = 3.5-5.3 g/dL)	3.7	0.800	3.56	0.901	> 0.05	NS
Performance status	3.33	0.577	2.00	1.730	> 0.05	NS
Patients with patent shunts (*n* = 22)						
ALT (N = 7-40 IU/L)	66.95	117.265	26.45	8.528	< 0.05	S
AST (N = 7-37 IU/L)	53.95	33.832	32.22	9.586	< 0.01	HS
Total bilirubin (N = 0.2-1.2 mg/dL)	2.818	3.198	1.21	0.414	< 0.01	HS
Direct bilirubin (N = 0-0.3 mg/dL)	1.29	2.022	0.51	0.296	< 0.01	HS
Albumin (N = 3.5-5.3 g/dL)	3.5	0.475	3.93	0.576	< 0.01	HS
Performance status	2.59	1.007	0.18	0.664	< 0.001	VHS

N: Normal range; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Sig: Significance; NS: Non significant; S: Significant; HS: Highly significant; VHS: Very highly significant.

Table 6 Comparison of different transjugular intrahepatic portosystemic shunt studies in Budd-Chiari syndrome with the current study

Points of comparison	Mancuso *et al*[18]	Perelló *et al*[19]	Rössle *et al*[20]	Hernández-Guerra *et al*[21]	Current study
No. of patients	15	13	35	25 (9 covered stents)	13
Mean age in years (range)	40 (20-73)	36 (17-67)	43 (12-74)	40 (17-54)	29 (14-57)
Median child score	11	9	9	9	8
Acute, fulminant/chronic presentation	8/6	4/6	11/13	ND	2/11
Mean follow-up (mo)	24	48	37	20	18
Stent stenosis (%)	36	72	47	67 (19% covered stents)	38.4
Anticoagulation (%)	100	95	100	ND	100
Patients with acute presentation who died	4	ND	2	ND	0
Patients with chronic presentation who died	0	ND	1	ND	0
Death total (%)	30	10	9	0	0
Liver transplantation	0	1	2	0	0
Surgical portocaval shunt	0	2	0	0	0

ND: Not determined; Anticoagulation: Percent of patients who were adherent to anticoagulation therapy.

Stent migration, which is very rare, occurred in one angioplasty procedure (3.84%) where stent migrated to the heart just after insertion. However, no serious complications occurred and stent was embedded in the wall of right atrium and the patient was quite well.

Post procedure (angioplasty ± stenting) bleeding was encountered in 3 procedures (11.53%), 2 of which were intraperitoneal and one of which was hemobilia. All 3 cases were managed conservatively by temporary stoppage of anticoagulation and blood transfusion when indicated. This complication could be attributed to the application of a transhepatic approach in these procedures. Beckett and Olliff[5] stated that this approach has the merit of simplicity over a transjugular or transfemoral approach, as well as feasibility with major superior vena caval obstruction but with a potentially greater risk of bleeding.

Post procedure sepsis occurred in 3 procedures (11.53%) in spite of antibiotic prophylaxis with cefotaxime in combination with ampicillin-sulbactam. This could be due to infection from resistant organisms. According to McDermott *et al*[15], pathogens that precipitated infection after angioplasty and stent were *Staphylococcus aureus* and *S. epidermidis*, which were sensitive to cefazolin.

In this study, the results of angioplasty ± stenting agreed with Fisher *et al*[16] who stated that, with appropriate case selection, many patients with BCS caused by short length HV stenosis or occlusion may be managed successfully by angioplasty ± stenting with a good outcome following the procedure, provided that anticoagulation is maintained. According to the authors' comparative study between percutaneous angioplasty and operative shunt surgery; both groups had the same re-occlusion rate and both were related to suboptimal dose of anticoagulation.

In the current study, 13 patients (52%) were not candidates for angioplasty and underwent TIPS. The need for revision was 38.4% (compared to 41.6% in angioplasty ± stenting). One year and end of follow up survival rates following TIPS were 100%. This could be attributed to the relatively short follow up duration (ranging from 12 to 32 mo) and good selection of cases, as most of our patients had good or intermediate predictable prognosis according to Rotterdam score.

Patency rate in patients who had TIPS procedures was 12/13 (92.3%) at one year due to persistent shunt occlusion in one patient despite repeated revisions. At the end of follow up; patency rate dropped to 11/13 (84.6%) due to persistent shunt occlusion in another patient.

The results of the current study are much better than what had been reported by Valla[17], namely that secondary thrombosis or shunt dysfunction requiring revision occurs in about 70% of cases by 6 mo. However, the results of this study are more or less comparable to those reported by Senzolo *et al*[14] who stated that 36%-72% of patients needed reintervention after TIPS. The authors also reported a long-term patency rate of about 50% despite of routine anticoagulation therapy.

Comparison between the results of the current study, regarding TIPS, with other studies is shown in Table 6.

Sixteen TIPS procedures have been done throughout the current study (13 as primary intervention and 3 in patients with occluded stents following angioplasty in which predilatation was not possible).

Post TIPS sepsis occurred in 3 procedures (18.75%), in spite of prophylactic antibiotics. According to Dravid *et al*[22]; an infection rate of 13% following TIPS was reported.

According to Ryan *et al*[11], acute infection related to TIPS placement appears to be uncommon. Whether or not prophylactic antibiotics are of value remains undetermined. Options for prophylactic antibiotics for TIPS are: (1) no prophylaxis; (2) 1 g ceftriaxone single dose intravenously before procedure; and (3) 1.5-3 g ampicillin/sulbactam single dose intravenously before procedure. We adopted the third strategy successfully in combination with cefotaxime 1 gm IV and completed the course of antibiotics for five days after intervention.

Hepatic encephalopathy after TIPS occurred in 2 patients (12.5%) and was transient, lasting only for 2-3 d and responded well to anti hepatic encephalopathy measures.

Post procedure bleeding was encountered in 4 procedures (25%), 2 intraperitoneal and 2 hemobilia; all were managed conservatively with temporary stoppage of anticoagulation and blood transfusion if indicated.

In the current study, the overall 1 year shunt patency of all procedures (angioplasty ± stenting and TIPS) was 22/25 (88%) as 3 patients had occluded shunts in spite of repeated trials of dilatation and adherence to anticoagulation therapy. We compared clinical and laboratory characteristics before and after intervention in patients with patent shunts (22 patients) and in those with occluded shunts (3 patients) irrespective of the type of procedure performed. We observed that patients with occluded shunts showed no improvement compared to those with patent shunts even after multiple revisions in terms of clinical manifestations, laboratory profile and performance status.

These observations are consistent with Bachet *et al*[23] who concluded that, in patients with BCS treated with portosystemic shunting, shunt dysfunction has a major impact on morbidity and mortality and maintenance of shunt patency is of major importance for better long-term outcome.

In conclusion; Budd Chiari syndrome is a potentially life-threatening disorder that requires a multidisciplinary approach with hepatologist, hematologist, interventional radiologist and vascular surgeon. Morbidity and mortality following both angioplasty ± stenting and TIPS are low with satisfactory stent and patient survival. Proper selection of procedure candidates and maintenance of shunt patency by strict adherence to anticoagulation and early management of shunt dysfunction are crucial in clinical, laboratory and radiological improvement of BCS patients.

COMMENTS

Background

Budd-Chiari syndrome (BCS) results from hepatic venous outflow obstruction at any level from hepatic venules to the right atrium. Few patients respond to medical treatment (anticoagulation ± thrombolytic therapy, diuretics). However, most patients need intervention to restore the hepatic blood flow. Restoring outflow in one of the major hepatic veins by balloon dilatation ± stenting is the management of choice. When not possible or failed, Transjugular Intrahepatic Portosystemic Shunt is used.

Research frontiers

Follow up of patients after radiological intervention is crucial in order to assess patient improvement, shunt patency and function and to manage any procedure related complications. In this study, the authors demonstrate that morbidity and mortality following angioplasty ± stenting and transjugular intrahepatic portosystemic shunt (TIPS) are low with satisfactory outcome.

Innovations and breakthroughs

This is the first Egyptian study that addresses the short term outcome of interventional radiology procedures in management of BCS.

Applications

This study may represent a future strategy for good selection of procedure candidates, maintenance of shunt patency by strict adherence to anticoagulation and early management of shunt dysfunction which are all crucial in clinical, laboratory and radiological improvement of BCS patients.

Terminology

Angioplasty means balloon dilatation of hepatic vein; it may be with or without stent insertion. This procedure is performed in BCS patients with short segment stenosis or occlusion of the hepatic veins with significant patent segments. This approach will re-establish hepatic venous outflow *via* the physiological route. In cases where blood flow cannot be restored or where the approach fails (usually because the remaining patent veins are too small or have insufficient flow), Transjugular Intrahepatic Portosystemic Shunt is used; in which the shunt connects the hepatic vein to the portal vein to bypass the obstruction.

Peer review

The authors evaluated the outcome of patients with BCS after non surgical hepatic decompression procedures (either balloon angioplasty ± stenting or TIPS). It revealed that morbidity and mortality following both procedures are low with satisfactory stent and patient survival. Thus, proper selection of procedure candidates and maintenance of shunt patency by strict adherence to anticoagulation and early management of shunt dysfunction are crucial in clinical, laboratory and radiological improvement of those patients. Their results are excellent on managing a very challenging group of patients and their program should be commended for this outcome.

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ORIGINAL ARTICLE

Body mass index is associated with age-at-onset of HCV-infected hepatocellular carcinoma patients

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Abstract

AIM: To identify factors associated with the age at onset of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC).

METHODS: Five hundred and fifty-six consecutive patients positive for HCV antibody and treatment-naïve HCC diagnosed between 1995 and 2004 were analyzed. Patients were classified into three groups according to age at HCC onset: < 60 years (n = 79), 60-79 years (n = 439), or ⩾ 80 years (n = 38). Differences among groups in terms of sex, body mass index (BMI), lifestyle characteristics, and liver function were assessed. Factors associated with HCC onset in patients < 60 or ⩾ 80 years were analyzed by logistic regression analysis.

RESULTS: Significant differences emerged for sex, BMI, degree of smoking and alcohol consumption, mean bilirubin, alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (GGT) levels, prothrombin activity, and platelet counts. The mean BMI values of male patients > 60 years old were lower and mean BMI values of female patients < 60 years old were higher than those of the general Japanese population. BMI > 25 kg/m^2 [hazard ratio (HR), 1.8, P = 0.045], excessive alcohol consumption (HR, 2.5, P = 0.024), male sex (HR, 3.6, P = 0.002), and GGT levels > 50 IU/L (HR, 2.4, P = 0.014) were independently associated with HCC onset in patients < 60 years. Low ALT level was the only factor associated with HCC onset in patients aged ⩾ 80 years.

CONCLUSION: Increased BMI is associated with increased risk for early HCC development in HCV-infected patients. Achieving recommended BMI and reducing alcohol intake could help prevent hepatic carcinogenesis.

Key words: Age-at-onset; Hepatocellular carcinoma; Hepatitis C virus; Body mass index; Alcohol consumption; Sex difference

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most com-

mon cancer in men and the eighth most common cancer in women worldwide. The incidence and mortality associated with HCC have been reported to be increasing in countries in North America, Europe and Asia. Infection with hepatitis C virus (HCV) infection is likely to play an important role in the pathogenesis of HCC[1-3]. In Japan, over 70% of cases of HCC diagnosed in the last 20 years are related to HCV infection[3].

One report estimates that 3%-35% of patients progress to cirrhosis 25 years after infection with HCV and 1%-3% progress to HCC 30 years after infection[4]. However, the factors that influence the development of HCC in patients infected with HCV remain largely unknown. Previous studies have suggested that host factors, such as sex, alcohol consumption, smoking, diabetes mellitus, and obesity, are important risk factors for HCC[5-11]. In addition, recent studies have suggested that HCV infection causes insulin resistance and leads to oxidative stress, potentiating fibrosis and hepatic carcinogenesis[12-14].

Therefore, we hypothesized that obesity influences the time to onset of HCC related to HCV infection, which is reflected in the patient's age at onset. To test this hypothesis, we investigated the relationship between body mass index (BMI) and lifestyle factors and age at onset of HCC in HCV-infected patients.

MATERIALS AND METHODS

Study participants

The study was conducted in accordance with the Helsinki Declaration. Written informed consent on the use of clinical records for research purposes was obtained from all subjects.

From January 1995 to December 2004, 656 consecutive patients positive for HCV antibodies and diagnosed with HCC for the first time at Saga Medical School Hospital and Saga Prefectural Hospital, without prior HCC treatment, were recruited for this study. Patients were excluded from the study if they were positive for hepatitis B surface antigen (n = 8), were previously treated with interferon (n = 23), had uncontrolled ascites (n = 27), or had an advanced tumor stage accompanied by tumor thrombus in portal tract or extrahepatic metastasis (n = 42). The remaining 556 patients (351 men, 205 women), with a median age at HCC onset of 67.8 years (range, 41-92 years) were enrolled in this study.

Diagnosis and staging of HCC

Diagnosis of HCC was confirmed by combined ultrasonography and dynamic computed tomography (CT), dynamic magnetic resonance imaging, or CT during angiography, demonstrating a hypervascular contrast pattern of the nodule in the arterial phase and a hypovascular pattern in the portal phase. If the nodule contrast patterns were not consistent with those typical for HCC, a needle biopsy of the tumor was taken for pathological diagnosis.

Tumor stage was classified according to the 5th Edition of the General Rules for the Clinical and Pathological Study of Primary Liver Cancer, 2008, published by the Liver Cancer Study Group of Japan[15]. This classification system assumes three conditions: (1) tumor diameter of $\leqslant$ 2 cm; (2) a single tumor is present; and (3) no vascular invasion of the tumor. If all three conditions are met, the tumor is classified as stage Ⅰ; if two conditions are met, it is classified as stage Ⅱ; if only one condition is met, it is classified as stage Ⅲ; and if none of the conditions are met, it is classified as stage Ⅳ.

Exposure and laboratory data

At the time of HCC diagnosis, blood tests were performed and BMI was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Prothrombin activity and serum albumin and total bilirubin levels were measured and used to determine the Child-Pugh status. Blood samples were also used to measure alanine aminotransferase (ALT) and γ-glutamyl transpeptidase (GGT) levels and other liver function tests.

Patients were classified according to the World Health Organization (WHO) BMI criteria: underweight, BMI < 18.5 kg/m^2; normal weight, BMI 18.5-25 kg/m^2; overweight, BMI 25-30 kg/m^2; and obese, BMI $\geqslant$ 30 kg/m^2[16]. Diagnosis of diabetes mellitus was made either by reviewing medical history or by assessing glucose levels with fasting plasma glucose level of $\geqslant$ 7.0 mmol/L or a 2-h plasma glucose level of $\geqslant$ 11.1 mmol/L[17]. Patients were questioned by nurses about their smoking and drinking habits during the last 10 years. We defined heavy drinking as > 60 g of alcohol consumed per day and habitual smoking as > 20 pack years.

Statistical analysis

To identify factors associated with age at onset of HCC in patients with chronic hepatitis C, we compared clinical factors in two groups of patients; those aged < 60 years at HCC onset and those aged $\geqslant$ 80 years. We then analyzed risk factors affecting earlier (onset age < 60 years) and later (onset age $\geqslant$ 80 years) development of HCC in patients with chronic HCV.

We used the Kruskal-Wallis test or the χ^2 test to compare clinicopathological variables between three groups of patients. The differences in age at onset of HCC between the two groups stratified by BMI were analyzed by the Tukey-Kramer method. Univariate and multivariate logistic regression analyses were performed to identify factors associated with earlier or later onset of HCC.

Data processing and analysis were performed by using the SAS (SAS Institute Inc.). Two-tailed P values of < 0.05 were considered significant.

RESULTS

Patient characteristics

A histogram showing age at onset of HCC in 556 HCV-infected patients is depicted in Figure 1. The median age of patients was 67.8 years, with a nearly normal age distribution for the study population.

The clinical characteristics were categorized into three groups according to age at onset of HCC; < 60 years

Table 1 Clinical characteristics of patients classified with hepatocellular carcinoma occurrence age

Factors	Occurrence age of HCC (years old)			*P*
	< 60 (*n* = 79)	60-80 (*n* = 439)	⩾ 80 (*n* = 38)	
Sex				
Male/Female, *n*	70/9	264/175	17/21	< 0.0001[a]
BMI (kg/m^2)	23.8 ± 3.4	22.9 ± 3.4	21.8 ± 3.3	0.02[b]
< 25/25 <, *n*	50/29	325/114	32/6	0.039[a]
Diabetes mellitus				
With/without, *n*	16/63	86/353	2/36	0.088[a]
Smoking (pack years)				
< 20/⩾ 20, *n*	43/36	137/302	9/29	0.0001[a]
Alcohol consumption (g/d)				
< 60/⩾ 60, *n*	65/14	416/23	36/2	< 0.0001[a]
Tumor stage				
Ⅰ/Ⅱ/Ⅲ, *n*	21/33/25	116/196/127	11/16/11	0.981[a]
Child-Pugh class				
A/B/C, *n*	55/23/1	349/87/3	34/4/0	0.145[a]
Albumin (g/dL)	3.57 ± 0.53	3.64 ± 0.50	3.63 ± 0.41	0.586[b]
< 3.5/⩾ 3.5, *n*	30/49	151/288	14/24	0.433[a]
Total bilirubin (mg/dL)	1.23 ± 0.62	1.05 ± 0.55	0.80 ± 0.30	0.0002[b]
< 2.0/⩾ 2.0, *n*	70/9	413/26	38/0	0.047[a]
Prothrombin activity (%)	76.1 ± 16.6	79.9 ± 15.1	89.1 ± 12.2	0.0002[b]
< 70/⩾ 70, *n*	26/53	98/341	3/35	0.003[a]
Platelet count (× 10^4/μL)	10.4 ± 7.8	11.0 ± 5.5	13.1 ± 5.8	0.005[b]
< 10/⩾ 10, *n*	45/34	223/216	14/24	0.125[a]
ALT (IU/L)	78.3 ± 39.3	71.8 ± 44.1	41.7 ± 19.3	< 0.0001[b]
< 80/⩾ 80, *n*	47/32	288/151	36/2	0.0004[a]
GGT (IU/L)	123.7 ± 102.5	86.0 ± 86.6	54.6 ± 36.1	< 0.0001[b]
< 50/⩾ 50, *n*	15/64	173/266	20/18	0.0002[a]

Continuous variables are expressed as mean ± standard deviation. Statistical analysis was done using a: the χ^2 test or b: the Turkey-Kramer test. HCC: Hepatocellular carcinoma; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

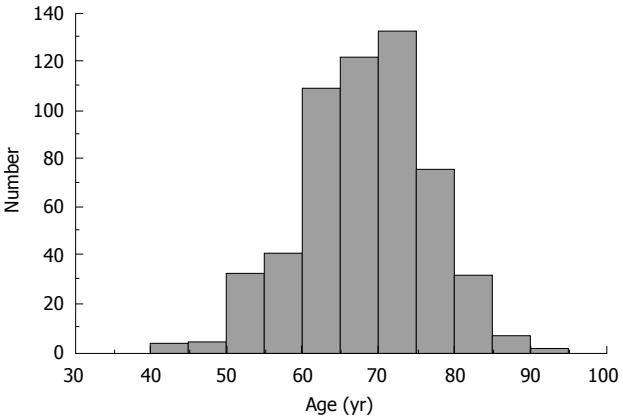

Figure 1 Histogram showing age at onset of hepatocellular carcinoma in hepatitis C virus-infected patients (*n* = 556). Median age, 67.8 years; range, 41-92 years.

(*n* = 79), 60-79 years (*n* = 439), and ⩾ 80 years (*n* = 38) (Table 1). Of those aged < 60 years, 88.6% were men, a much higher percentage than in those aged 60-79 years (60.1%) and those aged ⩾ 80 years (44.7%). In terms of BMI, the mean value increased, and the percentage of patients with BMI < 25 kg/m^2 decreased while that of patients with BMI > 25 increased with decreasing age at onset of HCC. However, this is a normal phenomenon in the general population. Therefore, we compared the mean BMI values according to the age at onset of HCC for patients in this study with BMI values of the general Japanese population in 2005 and 2006, which were published by the Ministry of Health, Labour and Welfare, Japan (http://www.mhlw.go.jp/). The mean BMI of male HCC patients aged > 60 years was lower whereas that of female HCC patients aged < 60 years was higher than those of the general population (Figure 2). This indicates that the association between BMI and age at onset of HCC observed in this study was affected by factors independent of natural aging. We found that there were significantly more heavy drinkers ($P < 0.0001$) and habitual smokers ($P = 0.0001$) among patients aged < 60 years, compared with the other two age groups. Although the three groups did not differ in terms of Child-Pugh status, total bilirubin, ALT, and GGT levels were higher, and prothrombin activity and platelet counts were lower in patients aged < 60 years at HCC onset. No differences emerged in terms of the prevalence of diabetes mellitus or the distribution of tumor stage among the three groups.

Factors associated with the development of HCC at < 60 years of age

We investigated risk factors associated with the development of HCC at a younger age (i.e. < 60 years of age) (Table 2). In univariate analysis, the following were found to be significant risk factors for earlier age at onset of HCC: male sex [hazard ratio (HR), 5.4; 95% CI, 2.65-11.12; $P < 0.0001$], BMI > 25 kg/m^2 (HR, 1.7; 95% CI, 1.04-2.85;

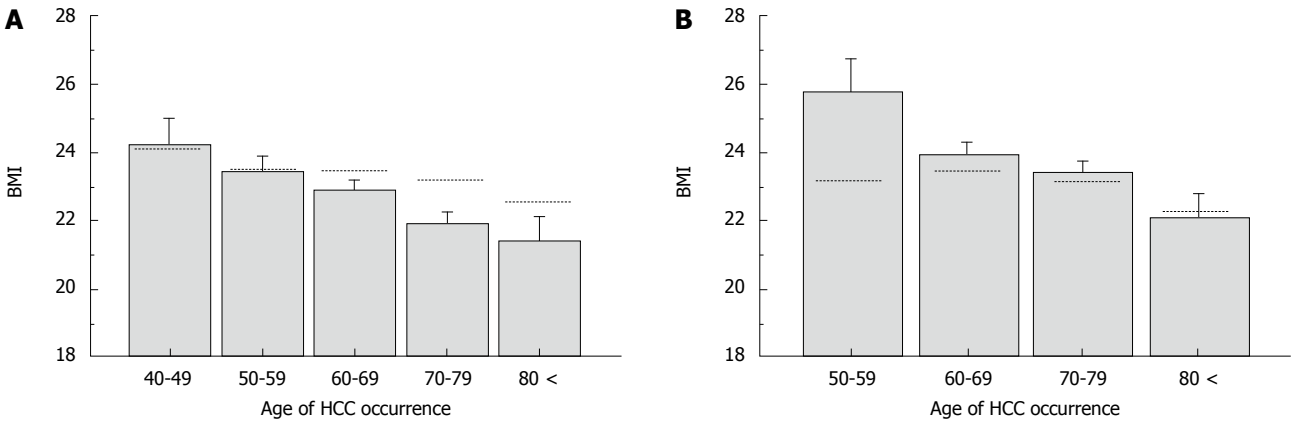

Figure 2 Mean body mass index in each age group at onset of hepatocellular carcinoma (A: Men; B: Women). The bars show the mean body mass index (BMI) ± SD in patients with hepatocellular carcinoma (HCC). The dashed lines show the mean BMI for the general Japanese population in 2005 and 2006, which was surveyed by the Ministry of Health, Labour and Welfare, Japan.

Table 2 Analysis of factors affecting development of hepatocellular carcinoma at younger age (under 60 yr old)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Sex						
Female	1			1		
Male	5.43	2.647-11.120	< 0.0001	3.58	1.580-8.133	0.002
BMI						
< 25	1			1		
⩾ 25	1.73	1.044-2.851	0.033	1.82	1.015-3.270	0.045
Diabetes mellitus						
Without	1			1		
With	1.12	0.619-2.037	0.703	1.00	0.516-1.952	0.991
Smoking (packs year)						
< 20	1			1		
⩾ 20	2.71	1.669-4.393	< 0.0001	1.64	1.904-2.991	0.104
Alcohol (g/d)						
< 60	1			1		
⩾ 60	3.89	1.926-7.874	0.0002	2.51	1.130-5.563	0.024
Total bilirubin (mg/dL)						
< 2.0	1			1		
⩾ 2.0	2.23	1.003-4.958	0.049	2.33	0.898-6.033	0.082
Prothrombin activity (%)						
⩾ 70	1			1		
< 70	1.91	1.111-3.262	0.019	1.60	0.859-2.987	0.139
Platelet (× 10^4/μL)						
⩾ 10	1			1		
< 10	1.34	0.829-2.166	0.232	1.60	0.877-2.886	0.118
ALT (IU/L)						
< 80	1			1		
⩾ 80	1.44	0.884-2.350	0.142	1.17	0.656-2.090	0.542
GGT (IU/L)						
< 50	1			1		
⩾ 50	3.24	1.731-6.053	0.0002	2.38	1.194-4.727	0.014

HR: Hazard ratio; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

P = 0.033), habitual smoking (HR, 2.7; 95% CI, 1.67-4.39; *P* < 0.0001), heavy drinking (HR, 3.9; 95% CI, 1.93-7.87; *P* = 0.0002), total bilirubin > 2.0 mg/dL (HR, 2.2; 95% CI, 1.00-4.96; *P* = 0.049), prothrombin activity > 70% (HR, 1.9; 95% CI, 1.11-3.26; *P* = 0.019), and GGT level > 50 IU/L (HR, 3.2; 95% CI, 1.73-6.05; *P* = 0.0002). In multivariate analysis, independent risk factors for earlier age at onset of HCC were male sex (HR, 3.6; 95% CI, 1.58-8.13; *P* = 0.002), BMI > 25 kg/m^2 (HR, 1.8; 95% CI, 1.015-3.270; *P* = 0.045), heavy drinking (HR, 2.5; 95% CI, 1.13-5.56; *P* = 0.024), and GGT > 50 IU/L (HR, 2.4; 95% CI, 1.19-4.73; *P* = 0.014).

Factors associated with the development of HCC at ⩾ 80 years of age

We also investigated factors associated with the develop-

Table 3 Analysis of factors affecting development of hepatocellular carcinoma at older age (over 80 yr old)

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Sex						
Female	1			1		
Male	0.45	0.229-0.867	0.017	0.47	0.200-1.119	0.089
BMI						
<25	1			1		
⩾ 25	0.49	0.201-1.201	0.119	0.48	0.174-1.321	0.155
Diabetes mellitus						
Without	1			1		
With	0.23	0.054-0.957	0.043	0.32	0.074-1.412	0.133
Smoking (packs year)						
< 20	1			1		
⩾ 20	0.58	0.270-1.258	0.169	0.81	0.306-2.164	0.680
Alcohol (g/d)						
< 60	1			1		
⩾ 60	0.72	0.167-3.118	0.663	0.45	0.056-3.606	0.451
Total bilirubin (mg/dL)						
< 2.0	1			1		
⩾ 2.0	1.00	-	0.97	1.00	-	0.98
Prothrombin activity (%)						
⩾ 70	1			1		
< 70	0.10	0.014-0.755	0.025	0.15	0.020-1.166	0.07
Platelet (× 10^4/μL)						
⩾ 10	1			1		
< 10	0.54	0.275-1.076	0.080	0.62	0.287-1.360	0.236
ALT (IU/L)						
< 80	1			1		
⩾ 80	0.10	0.024-0.427	0.002	0.13	0.030-0.569	0.007
GGT (IU/L)						
< 50	1			1		
⩾ 50	0.51	0.262-0.984	0.045	1.01	0.479-2.146	0.971

HR: Hazard ratio; BMI: Body mass index; ALT: Alanine aminotransferase; GGT: γ-glutamyl transpeptidase.

ment of HCC at an older age (i.e. ⩾ 80 years of age) (Table 3). In univariate analysis, the following were significantly and negatively associated with age at onset of HCC ⩾ 80 years: male sex (HR, 0.45; 95% CI, 0.23-0.87; $P = 0.017$), diabetes mellitus (HR, 0.23; 95% CI, 0.05-0.96; $P = 0.043$), prothrombin activity < 70% (HR, 0.1; 95% CI, 0.01-0.76; $P = 0.025$), ALT > 80 IU/L (HR, 0.1; 95% CI, 0.02-0.43; $P = 0.002$), and GGT > 50 IU/L (HR, 0.51; 95% CI, 0.26-0.98; $P = 0.045$). In multivariate analysis, ALT > 80 IU/L was the only independent factor associated with age at onset of HCC ⩾ 80 years (HR, 0.13; 95% CI, 0.03-0.57; $P = 0.007$).

Age at onset of HCC stratified by BMI in relation to sex or alcohol consumption

Differences in age at onset of HCC stratified by BMI were assessed in relation to sex or alcohol consumption. In men, age at onset decreased significantly with increasing BMI (mean age ± SD; underweight, 71.1 ± 7.4 years; normal weight, 67.0 ± 8.5 years; overweight, 63.6 ± 8.1 years; obese, 57.0 ± 7.0 years) (Figure 3A). Although a similar trend was noted in women, this was not significant (underweight, 73.6 ± 7.8 years; normal weight, 70.4 ± 7.0 years; overweight, 68.9 ± 6.4 years; obese, 67.0 ± 7.5 years) (Figure 3B).

Although an association between BMI and age at onset of HCC was found among non-heavy drinkers (Figure 4A), no association was found among heavy drinkers (Figure 4B).

DISCUSSION

The results of this study revealed that higher BMI, heavy alcohol consumption, male sex, and high GGT levels are independent risk factors for younger age at onset of HCC in patients with chronic HCV infection. This study confirms the previously reported risk factors for HCC and is the first to investigate the relationship between age and HCC development.

It seems plausible that the duration of HCV infection plays a role in the age at which cirrhosis progresses to HCC. However, Hamada *et al*[18] reported a significant negative correlation between the time from HCV infection to onset of HCC and the patient's age at the time of infection, and as a result, the onset of HCC was considered to occur in patients during their 60 s regardless of their age at time of infection. This indicates that factors other than duration of HCV infection may be associated with the age at onset of HCC in HCV-infected patients.

Recent studies have shown that HCV proteins, such

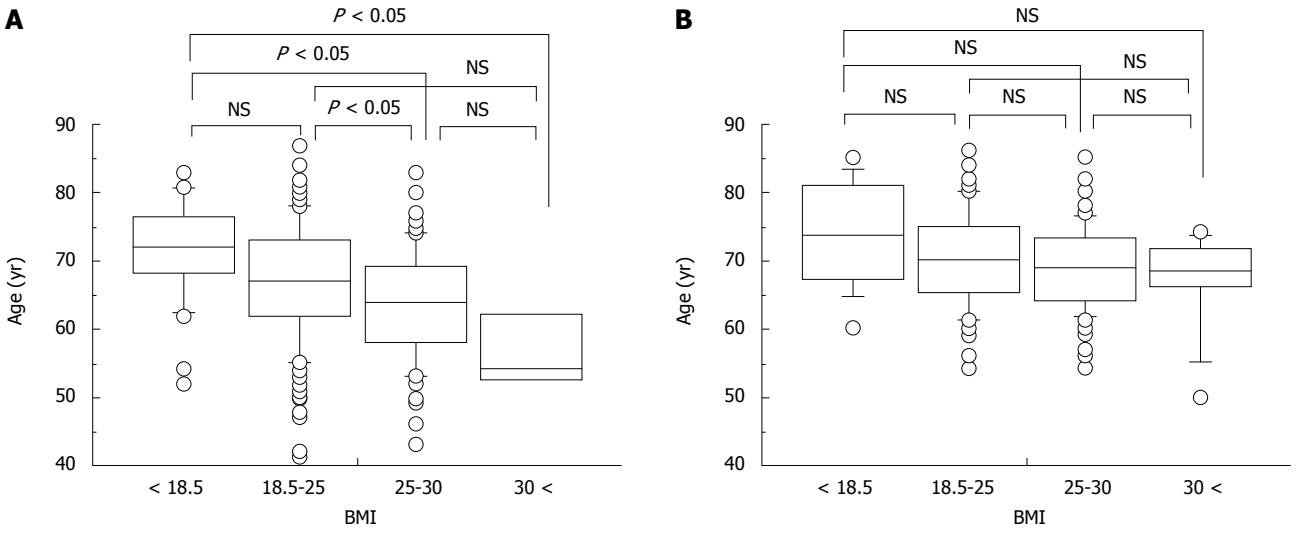

Figure 3 Differences in age at onset of hepatocellular carcinoma stratified by body mass index according to sex (A: Men; B: Women). Statistical analysis was performed using the Tukey-Kramer method. NS: Not significant; BMI: Body mass index.

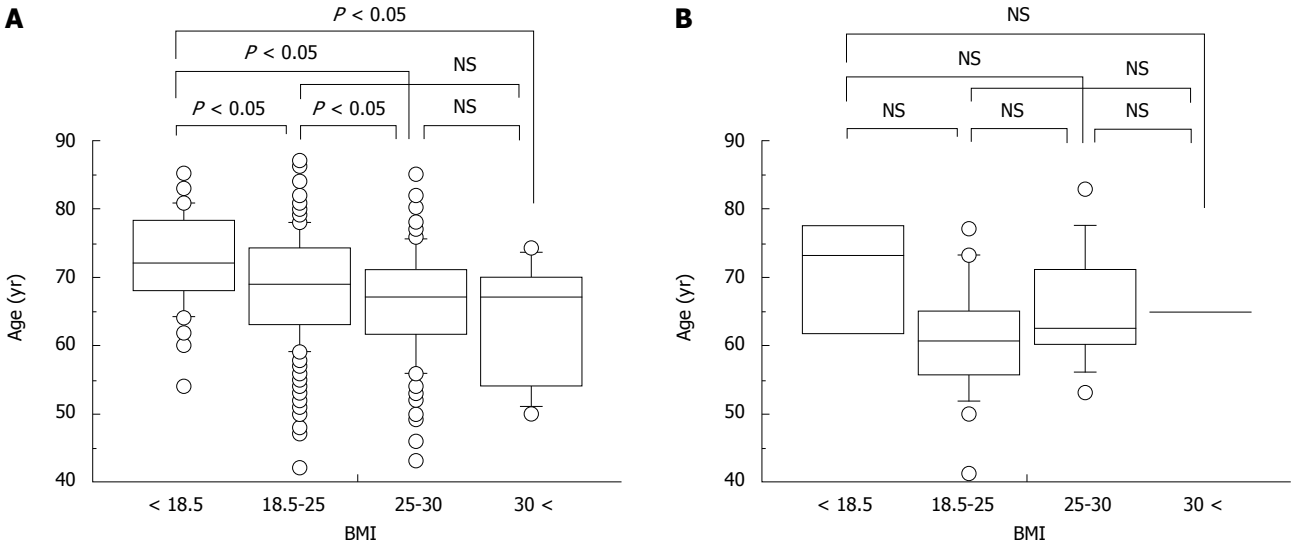

Figure 4 Differences in age at onset of hepatocellular carcinoma stratified by body mass index according to degree of alcohol consumption (A: Non-heavy drinkers < 60 g/d; B: Heavy drinkers ⩾ 60 g/d). Statistical analysis was performed using the Tukey-Kramer method. NS: Not significant; BMI: Body mass index.

as the core protein, cause oxidative damage by exposing the endoplasmic reticulum to oxidative stress[19-21]. Hepatic oxidative stress is strongly associated with increased risk for HCC in patients with chronic HCV[22]. Because oxidative stress is also caused by various host-related factors, it is expected to be influenced more strongly by host-related factors in HCV-infected patients than in those with HCV-negative liver disease. Indeed, we have previously reported that visceral fat accumulation was associated with greater insulin resistance in chronic HCV patients than in those with non-alcoholic fatty liver disease[23]. Therefore, it is plausible that the association between earlier onset of HCC and increased BMI is due to the generation of hepatic oxidative stress.

An interesting aspect of our results is that underweight patients, defined as those with a BMI of < 18.5 kg/m^2, tended to be older at HCC onset than patients within the normal weight range (BMI 18.5-25 kg/m^2). Recently, Ohki *et al*[11] reported that patients with a BMI < 18.5 kg/m^2 had the lowest risk of developing HCC due to chronic HCV infection among all BMI groups. In general, the mortality rate associated with cardiovascular disease or cancer is higher in underweight patients than in normal weight patients[24,25]. Clearly, a larger cohort study is needed to investigate whether leanness confers a protective effect against hepatocarcinogenesis in HCV-infected patients.

Excessive alcohol consumption is also known to exacerbate hepatic oxidative stress and evoke liver fibrosis or HCC[20,26]. In this study, there was no association between BMI and age at onset of HCC in heavy drinkers. We speculate that this group may include some patients who are malnourished and possibly losing weight.

Sex modulates the natural history of chronic liver disease. Previous studies have suggested that chronic HCV

infection progresses more rapidly in men than women, and that cirrhosis is predominately a disease of men and postmenopausal women[27]. Shimizu et al suggested that estrogens protect against oxidative stress in liver injury and hepatic fibrosis[28]. In this study, the effect of BMI on age at onset of HCC was more remarkable in men than women. We speculate two mechanisms to account for this difference: (1) estrogens mitigate oxidative stress or insulin resistance associated with obesity; and (2) subcutaneous fat accumulation is more dominant in obese women than visceral fat, which is known to produce several adipokines that cause insulin resistance[29].

In addition, we examined factors associated with onset of HCC at an older age (≥ 80 years). In this analysis, ALT level was the only independent factor associated with hepatocarcinogenesis in HCV-infected patients at an age ≥ 80 years. It is well known that ALT levels are associated with liver inflammation and fibrosis progression, and Ishiguro et al recently reported that elevated ALT levels were strongly associated with the incidence of HCC, regardless of hepatitis virus positivity, in a large population-based cohort study[30]. Therefore, lower ALT levels might indicate a slow course of progression of hepatic fibrosis or carcinogenesis.

A limitation of this study is that it was a cross-sectional observation, rather than a cohort follow-up study. Further studies are needed to confirm our results.

In conclusion, the results of the present study indicate that higher BMI, excessive alcohol consumption, and male sex are independent risk factors for onset of HCV-related HCC at an age of < 60 years. These results suggest that interventions to promote changes in the lifestyle of patients with chronic HCV may slow the progression of HCV infection to HCC.

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COMMENTS

Background

The incidence and mortality associated with hepatocellular carcinoma (HCC) have been increasing worldwide, and hepatitis C virus (HCV) infection plays an important role in the pathogenesis of HCC. However, the factors that influence the development of HCC in HCV-infected patients remain largely unknown. Previous studies have suggested that host factors, such as sex, alcohol consumption, smoking, diabetes mellitus, and obesity, are important risk factors for HCC. Meanwhile, it has been reported that HCV infection causes insulin resistance and leads to oxidative stress, potentiating fibrosis and hepatic carcinogenesis. Therefore, we hypothesized that body mass index (BMI) influences the onset age of HCC related to HCV infection.

Research frontiers

Many studies have indicated that obesity is an independent and a significant risk factor for HCC occurrence. Recently, several metabolic markers have been implicated in the development and progression of HCC.

Innovations and breakthroughs

This study indicated that higher BMI, heavy alcohol consumption, male sex, and high γ-glutamyl transpeptidase levels are independent risk factors for younger age at onset of HCV-related HCC. Interestingly, the underweight patients (BMI < 18.5 kg/m^2), tended to be older at HCC onset than patients within the normal weight range (BMI 18.5-25 kg/m^2).

Applications

The results of this study suggest that achieving an adequate body weight along with a reduction of alcohol intake in patients with chronic hepatitis C could help prevent hepatic carcinogenesis.

Peer review

The study was reasonably designed and well conducted, and the data support their conclusions.

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BRIEF ARTICLE

Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology

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Abstract

AIM: To examine the vitamin D status in patients with alcoholic cirrhosis compared to those with primary biliary cirrhosis.

METHODS: Our retrospective case series comprised 89 patients with alcoholic cirrhosis and 34 patients with primary biliary cirrhosis who visited our outpatient clinic in 2005 and underwent a serum vitamin D status assessment.

RESULTS: Among the patients with alcoholic cirrhosis, 85% had serum vitamin D levels below 50 nmol/L and 55% had levels below 25 nmol/L, as compared to 60% and 16% of the patients with primary biliary cirrhosis, respectively ($P < 0.001$). In both groups, serum vitamin D levels decreased with increasing liver disease severity, as determined by the Child-Pugh score.

CONCLUSION: Vitamin D deficiency in cirrhosis relates to liver dysfunction rather than aetiology, with lower levels of vitamin D in alcoholic cirrhosis than in primary biliary cirrhosis.

Key words: Alcoholic liver cirrhosis; Child-Pugh score; Primary biliary cirrhosis; Vitamin D deficiency

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INTRODUCTION

Patients with chronic liver disease have an increased risk for the development of osteoporosis and fractures, reduced muscle strength, an impaired inflammatory response, and malignancy[1-3]. These conditions have also been associated with vitamin D deficiency[4-6]. Vitamin D deficiency and osteomalacia have been described in chronic cholestatic liver disease, such as primary biliary cirrhosis (PBC)[7]. However, the frequency of vitamin D deficiency, specifically in alcoholic liver cirrhosis (ALC), has not been well described. The limited available data suggest that there is a high frequency of vitamin D deficiency in patients with chronic liver disease[8,9].

The main source of vitamin D in humans is the exposure of skin to sunlight. For further activation, vitamin D is hydroxylated in the liver to form 25-(OH) vitamin D

(25-OHD) and in the kidneys to form the active metabolite 1,25(OH)$_2$ vitamin D. The body stores of vitamin D are best reflected by the serum levels of 25-(OH)D[10].

The aim of the present study was to describe the serum vitamin D status in a retrospective case series of patients with ALC compared to those with PBC. Patients with PBC were considered a priori to demonstrate a high incidence of vitamin D deficiency.

MATERIALS AND METHODS

We collected data from the medical records of all patients with a diagnosis of PBC or ALC who visited our outpatient clinic in 2005. A total of 205 patients were identified: 58 had PBC, and 147 had ALC. The study population comprised patients for whom vitamin D measurements had been completed and for whom the Child-Pugh status could be assessed (34 and 89 patients, respectively). In patients who had undergone serial vitamin D measurements, the first blood sample collected in 2005 was used. The vitamin D status was defined according to the following levels of 25-(OH)D: severe deficiency: 0-12.5 nmol/L, deficiency: 12.5-25 nmol/L, insufficiency: 25-50 nmol/L, and vitamin D replete: > 50 nmol/L[11]. Data concerning previous and ongoing vitamin D supplementation were collected from the patients' medical records. To assess the severity of liver disease, the patients were scored according to the Child-Pugh classification. This score is based on the degree of encephalopathy, the presence of ascites, prothrombin time, and the serum levels of bilirubin, and albumin. The score ranges from 5 to 15 with increasing severity. Accordingly, the patients had either compensated liver disease (Class A, 5-6 points), moderate liver disease (Class B, 7-9 points), or severe liver disease (Class C, 10-15 points).

Techniques

Plasma 25(OH)D$_2$ and 25(OH)D$_3$ were analysed by isotope-dilution liquid chromatography-tandem mass spectrometry using an API3000 TM mass spectrometer (Applied Biosystems, Foster City, CA, USA) and a method adapted from Maunsell *et al*[12]. The interassay variation coefficients for plasma 25(OH)D$_2$ were 8.5% at 23.4 nmol/L and 8.0% at 64.4 nmol/L, and for plasma 25(OH)D$_3$ these values were 9.6% at 24.8 nmol/L and 8.1% at 47.7 nmol/L.

Statistics

Non-parametric statistics were used for the descriptions, and the Mann-Whitney *U* test was employed for comparisons between groups. The association between two variables was assessed by the contingency coefficient C, and statistical significance was determined using the χ^2 test.

RESULTS

In the patients with ALC, 18% had a severe vitamin D deficiency. In comparison, none of the patients with PBC had such a deficiency. Similarly, in a comparison of patients with ALC and PBC, vitamin D deficiency was identified in 37% *vs* 16% and vitamin D insufficiency was identified in 30% *vs* 41% of patients, respectively. Only 15% of patients with ALC were vitamin D replete in comparison to 40% of patients with PBC. The median 25-OHD blood concentration in ALC patients was 24 nmol/L, or 53% of the median serum level of 45 nmol/L in PBC patients ($P < 0.001$, Mann-Whitney *U* test) (Figure 1).

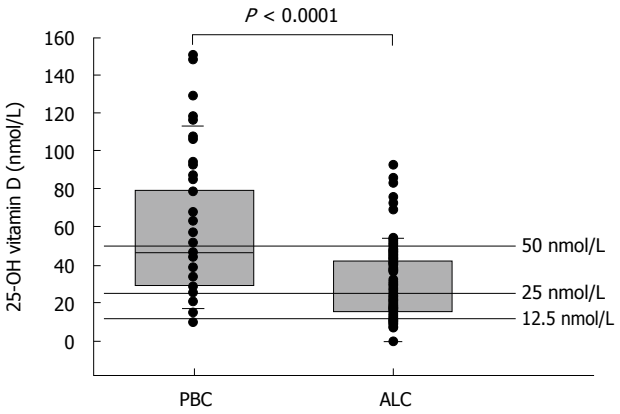

Figure 1 Vitamin D levels in the study group. Vitamin D levels in 37 patients with primary biliary cirrhosis and 89 patients with alcoholic liver cirrhosis. Patients with alcoholic liver cirrhosis demonstrated significantly lower overall vitamin D levels in comparison to patients with primary biliary cirrhosis ($P < 0.0001$, Mann-Whitney *U* test). PBC: Primary biliary cirrhosis; ALC: Alcoholic liver cirrhosis.

Table 1 Study group stratified according to the Child-Pugh class and the degree of vitamin D deficiency

Vitamin D (nmol/L)	Child-Pugh group		
	A	B	C
< 25	17	15	21
25-50	14	16	12
> 50	18	6	4

Four patients with ALC and 13 patients with PBC were receiving vitamin D supplementation at the time of blood sampling. Their vitamin D levels did not differ from those determined in patients who did not receive supplementation.

The distribution of Child-Pugh groups A, B, and C differed between ALC and PBC patients. Patients with ALC demonstrated more advanced disease (16 A, 36 B, and 37 C) compared to those with PBC (33 A, 1 B, and no C). In all the cirrhotic patients, there was an association between the Child-Pugh score and vitamin D status (contingency coefficient C = 0.29, $P < 0.05$, χ^2 test) (Table 1).

DISCUSSION

The vast majority (85%) of patients with ALC presented a compromised vitamin D status. The same was found in fewer than half of the patients with PBC (47%). This finding is in contrast to the standard clinical knowledge that vitamin D deficiency is expected in PBC. Further-

more, this marked vitamin D deficiency has never been demonstrated in a study population of this size.

Our study group included 60% of the cirrhotic patients who were seen at our clinic during 2005. This distribution does not introduce a selection bias because the vitamin D measurements were ordered without physician knowledge of the study purpose. Because the intensity of sunlight changes throughout the year, there might have been a seasonal difference in the vitamin D levels according to when the blood samples were drawn. However, patients were recruited throughout the year in both groups, and therefore, seasonal changes should not affect comparisons between the two groups.

The observed deficiency in vitamin D might be related to several causes: an impaired hepatic hydroxylation of vitamin D, dietary insufficiency, malabsorption, reduced hepatic production of vitamin D binding protein, and an impaired cutaneous production due to either reduced exposure to sunlight or jaundice[9,13]. The observation that the deficiency was less pronounced in PBC patients suggests that bile acid-related lipid malabsorption is not the only mechanism involved in vitamin D deficiency. It seems plausible that the mechanism of vitamin D deficiency is multifactorial and differs between the two groups of cirrhotic patients. When the results were stratified according to the Child-Pugh class, an association was observed between vitamin D deficiency and the severity of liver disease. This association has never been demonstrated in such a large study population. Thus, the better preservation of vitamin D status in patients with PBC might be ascribed to the diminished severity of their liver disease, as assessed by their Child-Pugh scores. Based on this finding, one could hypothesise that the risk for vitamin D deficiency or insufficiency might be influenced more by the degree of liver dysfunction than by the aetiology of the liver disease. However, our study was not designed to elucidate the exact mechanism underlying the vitamin D deficiency. The purpose of the study was to emphasise the importance of monitoring the vitamin D status in all patients with cirrhosis, especially those with ALC for whom nutritional status has been a relatively neglected area of study.

Our results imply that vitamin D deficiency is highly prevalent in patients with ALC. Because this was a retrospective study, we cannot extrapolate the results to the general population of cirrhotic patients. However, these results indicate that the frequency and severity of vitamin D deficiency in ALC patients warrant greater attention, similar to the usual clinical practice in patients with PBC.

Although 17 of the study patients received vitamin D supplementation, this supplementation was clearly insufficient, as their vitamin D concentrations remained low. Thus, it appears that the vitamin D deficiency in these patients should be treated with higher doses of vitamin D than that used in standard clinical practice for repletion.

The risk for bone disease in cirrhotic patients justifies the use of routine vitamin D therapy. Furthermore, the patients might also benefit from correction of their vitamin D status with respect to reduced muscle function, cancer risk, and immune impairment.

COMMENTS

Background

Patients with liver cirrhosis have an increased incidence of cancer, infections, osteoporosis, and decreased muscle strength. Vitamin D deficiency is associated with these complications in other patient groups and could be partially involved in the clinical complications related to cirrhosis.

Research frontiers

Vitamin D deficiency is a well reported complication in chronic cholestatic liver disease such as primary biliary cirrhosis. While the prevalence and treatment of this deficiency has been addressed in many articles over the last decades, little is known of the vitamin D status in alcoholic liver cirrhosis.

Innovations and breakthroughs

Recent studies imply that vitamin D deficiency is frequent in all patients with cirrhosis. The current study shows that vitamin D deficiency is more frequent and severe in patients with alcoholic liver cirrhosis than in patients with primary biliary cirrhosis. Furthermore, it indicates that the degree of liver dysfunction, rather than the aetiology of cirrhosis, dictates the risk of vitamin D deficiency.

Applications

This study emphasizes the importance of monitoring vitamin D levels in all patients with cirrhosis. However, further studies are needed to find the most favourable form of vitamin D supplementation for these patients.

Terminology

Primary biliary cirrhosis and alcoholic cirrhosis are two different diseases that cause cirrhosis of the liver. While primary biliary cirrhosis is a cholestatic, autoimmune disease, alcoholic liver cirrhosis is an alcohol-induced liver disease usually without cholestatic features. The Child-Pugh score assesses the prognosis in patients with cirrhosis and is also used to quantitate the degree of liver dysfunction.

Peer review

This brief article nicely demonstrated the association of the liver damage severity with the level of 25-hydroxy vitamin D. This is a very important report, as many doctors do not realize that liver damage could cause significant vitamin D deficiency.

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BRIEF ARTICLE

Natural orifice transluminal endoscopic wedge hepatic resection with a water-jet hybrid knife in a non-survival porcine model

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Abstract

AIM: To explore the feasibility of a water-jet hybrid knife to facilitate wedge hepatic resection using a natural orifice transluminal endoscopic surgery (NOTES) approach in a non-survival porcine model.

METHODS: The Erbe Jet2 water-jet system allows a needleless, tissue-selective hydro-dissection with a preselected pressure. Using this system, wedge hepatic resection was performed through three natural routes (trans-anal, trans-vaginal and trans-umbilical) in three female pigs weighing 35 kg under general anesthesia. Entry into the peritoneal cavity was *via* a 15-mm incision using a hook knife. The targeted liver segment was marked by an APC probe, followed by wedge hepatic resection performed using a water-jet hybrid knife with the aid of a 4-mm transparent distance soft cap mounted onto the tip of the endoscope for holding up the desired plane. The exposed vascular and ductal structures were clipped with Endoclips. Hemostasis was applied to the bleeding cut edges of the liver parenchyma by electrocautery. After the procedure, the incision site was left open, and the animal was euthanized followed by necropsy.

RESULTS: Using the Erbe Jet2 water-jet system, trans-anal and trans-vaginal wedge hepatic resection was successfully performed in two pigs without laparoscopic assistance. Trans-umbilical attempt failed due to an unstable operating platform. The incision for peritoneal entry took 1 min, and about 2 h was spent on excision of the liver tissue. The intra-operative blood loss ranged from 100 to 250 mL. Microscopically, the hydro-dissections were relatively precise and gentle, preserving most vessels.

CONCLUSION: The Erbe Jet2 water-jet system can safely accomplish non-anatomic wedge hepatic resection in NOTES, which deserves further studies to shorten the dissection time.

Key words: Natural orifice transluminal endoscopic surgery; Hepatic resection; Water-jet; Hybrid knife; Triangulation

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INTRODUCTION

Liver resection, a surgical procedure consisting of he-

patic parenchymal dissection as well as precise identification followed by control of intra/extra-hepatic vascular and biliary anatomy, is technically challenging due to the risk of massive bleeding during operation. Since excessive hemorrhage and subsequent blood transfusion are strongly associated with increased peri-operative morbidity and mortality, technical innovations have mainly focused on minimizing blood loss[1]. Besides inflow occlusion and low central pressure used to prevent bleeding from inflow vessels and hepatic veins in the transaction surface since the early 20th century, the development of specific devices for separating hepatic parenchyma, such as the ultrasonic dissector, water jet, Harmonic scalpel, Ligasure, and Tissue-Link dissecting sealer, has also contributed to bloodless transection. A meta-analysis[2] assessing the benefits and risks of current techniques of parenchymal transection showed that there were no significant differences in terms of the mortality, morbidity, markers of liver parenchymal injury or liver dysfunction in pairwise comparisons including cavitron ultrasound surgical aspirator, radiofrequency dissecting sealer, sharp dissection and hydro-jet. Among them, the water-jet dissector employs a pressurized jet of water to fragment the liver parenchyma tissue, with intact vascular and ductal structures, which can be ligated with staplers or clipped with titanium hemoclips, resulting in reduced blood loss, transfusion requirement, and biliary leak[3].

High-pressure water-jet dissection technology was originally developed in the steel and glass industries, where ultra-precise cutting and engraving were considered as professional demands[4]. Since introduced to medical application in 1982[5], this technology (Hydro-Jet®; ERBE, Tuebingen, Germany) has been successfully employed in open and laparoscopic operations, achieving favorable results in precise, controllable tissue-selective (indicating water-rich tissue such as liver parenchyma) dissection with excellent visualization and minimal injury to the surrounding fibrous structures (such as ductal and vessel systems with a high content of collagen and elastin)[6]. The above-mentioned Helix Hydro-Jet device with a rigid hand-held applicator is not designed with sufficient flexibility for natural orifice transluminal endoscopic surgery (NOTES) procedures, and can not be passed through a standard working channel of the current flexible endoscope because its outer-diameter is larger than the endoscopic operative channel. Now a new water-jet hybrid knife[7] incorporating with high-pressure water-jet and radiofrequency may overcome this drawback. It has a smaller size, being easy to handle, and showing more preciseness, with almost linear correlation of pressure and dissection depth, and less foaming compared with the precursor model Helix Hydro-Jet[5].

As is known, trans-luminal liver resection is technically demanding and its expansion has been lagged behind other NOTES procedures. Phee *et al*[8] demonstrated for the first time how a dexterous master and slave transluminal endoscopic robot could efficiently perform the wedge hepatic resection without laparoscopic assistance. Unfortunately, this technology is still an unexplored field in China. The aim of our study was to explore the safety and efficacy of a water-jet hybrid knife to facilitate wedge hepatic resection using a NOTES approach in a non-survival porcine model.

MATERIALS AND METHODS

Experimental design

This non-survival study evaluated the performance of the water-jet hybrid knife during NOTES procedure in a live porcine model. A pilot experiment in an isolated liver was conducted first, and followed by an open procedure in a 35-kg female porcine model. The formal study included three operations of wedge hepatic resection using NOTES and water-jet technology through three respective natural routes (trans-anal, trans-vaginal and trans-umbilical). The outcome measures were the time spent in performing a trans-visceral incision, the time spent in excising the liver segment, and the blood loss including oozing and brisk vascular hemorrhage, determined as blood accumulation in the suction device.

This study was conducted with prior approval by the Institutional Animal Care and Use Committee of Tongji University of China.

Experimental animal and instrument

Transluminal hepatic wedge hydro-dissection was performed in three 35-kg female pigs. The pigs were food deprived but allowed liquids for 24 h before the procedure. Urethral catheterization and warm saline enema were conducted immediately before surgery. The animals were then transferred to an operating table, and placed in supine position.

The water-jet hybrid knife (Erbe Elektromedizin) used in this study is a stainless-steel tube that incorporates a microcapillary with a diameter of 150 mm[7]. The flexible instrument has an outer diameter of 2.1 mm and a length of 2.20 mm so that it can pass through the operating channels (diameter, 2.8 and 3.7 mm) of a forward-viewing dual-channel therapeutic endoscope (GIF-2T160; Olympus Medical Systems Corporation, Tokyo, Japan). The hybrid knife can be used for hydro-dissection, rinsing blood clot and rinsing for a better endoscopic view by water-jet application, as well as coagulation by radiofrequency application. The foaming with the use of the hybrid-knife can be scavenged by the suction mechanism of the endoscope. In NOTES procedure, a 4-mm transparent distance soft cap was mounted onto the tip of the endoscope for holding up the desired surface, subsequently avoiding the deviation in the direction of the water-jet. However, it was not used in the previous open procedure, because distraction (with surgical retractors) could allow the water-jet hybrid knife to effectively dissect the tissue by exposing the base of the cutting plane.

Rau *et al*[6] found that a pressure of 30-40 bar was very effective to dissect normal human liver tissues, and the long-distance transmission attenuation was about 10%. Therefore, we set the pressure at 45 bar, which was proved to be effective in our pilot experiment and open operation.

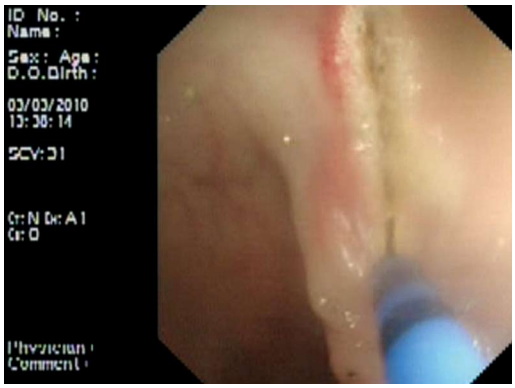

Figure 1 Colostomy on anterior wall of rectal junction and sigmoid colon. At the beginning of trans-annual natural orifice transluminal endoscopic surgery procedure, entry into the peritoneal cavity was *via* a 15-mm linear incision using the hook knife (cutting width set at 6 units and cutting interval set at 1 unit). The ideal access point was the junction of rectum and sigmoid colon at a distance of 15-20 cm away from the anus.

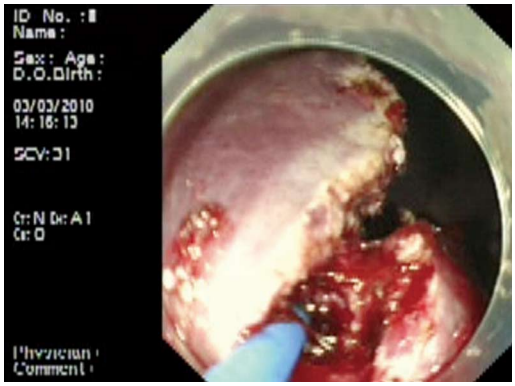

Figure 2 Hydro-dissection of liver segment in natural orifice transluminal endoscopic surgery procedure. Hepatic parenchyma dissection was performed using the water-jet hybrid knife kept away from the tissue in a no-touch fashion and perpendicular to but not tangentially against the predetermined surface, keeping in a smooth, reproducible, back-and-forth waving motion. A 4-mm transparent distance soft cap was mounted onto the tip of the endoscope for holding up the desired surface, subsequently avoiding the deviation in the direction of the water-jet.

Other instruments used were as follows: a flexible sterile overtube (MD48618, Sumitomo Bakelite, Tokyo, Japan), a transparent distance flat soft cap (D-201-13404, Olympus), a hook knife (KD-620LR, Olympus), endoscopic hemostatic forceps (FD-410LR, Olympus), endoclips (HX-610-135L OLYMPUS, Olympus), a foreign forcep (FQ-46L-1, Olympus), APC probe (argon plasma coagulation, APC) (ERBE Elektromedizin), and the modular VIO generator (VIO 300D; Erbe Elektromedizin, Tubingen, Germany).

Experimental procedure

Anesthesia was induced with 5% isoflurane administered intravenously. The animal was then intubated with endo-endotracheal tube, followed by general anesthesia with 1%-2% isoflurane. Throughout the operation, oxygen was administered to the animal at a flow rate according to oxygen saturation, and both pulse rate and oxygen saturation were monitored continuously using the pulse oximeter clamped to the animal tougue. Then normal saline enema was administered to each animal. Residual stool would be removed with aggressive washing, and suctioning during endoscopic inspection.

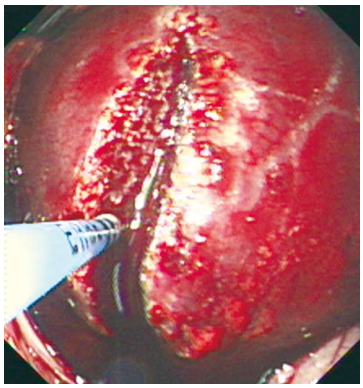

Figure 3 Hydro-dissection of liver segment in open procedure. Hepatic parenchyma dissection was performed using the water-jet hybrid knife in a similar natural orifice transluminal endoscopic surgery procedure, except that the 4-mm transparent distance soft cap was not used.

At the beginning of the procedure, entry into the peritoneal cavity was *via* a 15-mm linear incision made by the hook knife (a cutting width was set at 6 units and cutting interval was set at 1 unit). The ideal access point was the abdominal site 1 cm away from the umbilicus in trans-umbilical route, the bottom of the vagina in trans-vaginal route, the junction of rectum and sigmoid colon at a distance of 15-20 cm away from the anus in trans-anal route (Figure 1). Then the endoscope with a 4-mm transparent distance soft cap mounted onto the tip of the endoscope beforehand was passed through the access to reach the peritoneum using the air inflation mechanism of the endoscope.

After the target liver segment was identified, hepatic parenchymal dissection with the water-jet hybrid knife was performed in the following steps (Figure 2), which were generally similar to those in the previous open operation except the assistance of manual retraction (Figure 3). The range to be separated was marked by an APC probe. The Glisson's capsule was scored 2-3 mm deep along the demarcated plane of transaction with the hook knife. Then hepatic parenchyma dissection was performed using the water-jet hybrid knife kept away from the tissue in a no-touch fashion. The tip of the knife was perpendicular to but not tangentially against the predetermined surface (this was achieved with a 4-mm transparent distance soft cap mounted onto the tip of the endoscope for holding up the desired surface, subsequently avoiding the deviation in the direction of the water-jet). A smooth, reproducible, back-and-forth waving motion was used. Minor slow oozing from the cutting surface was controlled using the same knife, the hook knife or APC probe to initiate bursts of coagulation. Visible intra-hepatic vascular and ductal structures were clipped with endoscopic hemoclips. Once the liver segment was completely free and after checking for hemostasis, the incision was slightly enlarged, then an

Table 1 Comparisons of three routes for natural orifice transluminal endoscopic surgery procedure

Items	Trans-umbilical route	Trans-anal route	Trans-vaginal route
Access position	Visually inspected at para-umbilical region	Verified by finger pressing	Located by surrounding anatomic landmarks
Time to complete a trans-visceral incision		About 1 min	
Time to reach peritoneum		About 2 min	
Liver exposure	Antero-lateral segments could be easily detected, while posterosuperior segments were hard to be explored		
Working platform	Unstable		Relatively stable
Time to hydro-dissection	Abandoned 1 h later	2 h	2 h and 40 min
Size of resected liver segment	No resected specimen was obtained due to failure in trans-umbilical hepatic resection	50 mm × 25 mm × 5 mm	45 mm × 30 mm × 7 mm
Bile leak		Not found	
Blood loss	100 mL	200 mL	250 mL
Injury to surrounding organs		Not occurred	

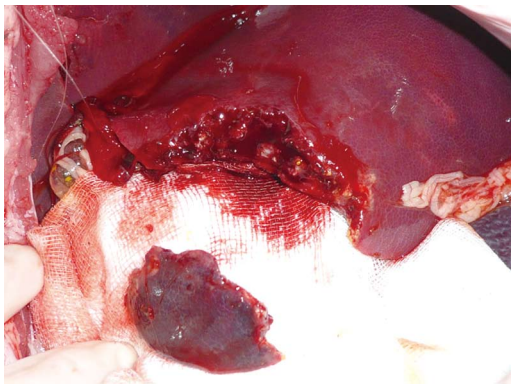

Figure 4 A resected liver segment compared with the reserved part. A resected liver segment was picked out with white gauze.

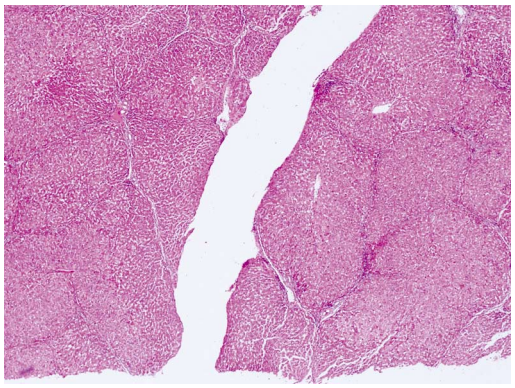

Figure 5 Microscopic findings of water-jet dissection in liver tissues (HE stain, × 40). A smooth and gentle cutting margin was presented. The cutting width at the bottom of the cut was similar to the dissection width at tissue surface, with little vessel damage.

endoscopic retrieval net was inserted through the endoscopic working channel and the specimen was introduced into the net and was retrieved intactly. After the procedure, the incision site was left open, and the animal was euthanized followed by necropsy.

Histopathological examination

Histologic examination was performed for all dissected specimens. The results were observed under microscope after hematoxylin and eosin staining based on the characteristics of the dissection margins, vessel preservation and dissection impact on the surrounding tissues. Thermal alterations such as edema and structural changes of different layers of the specimen were also microscopically analyzed.

RESULTS

It took 20 min to complete the excision of a liver segment 50 mm × 30 mm × 10 mm in size during the pilot experiment, and 45 min to complete the excision of a liver segment 45 mm × 25 mm × 10 mm in size during the open procedure. The blood loss was 100 mL in the open operation.

As for the NOTES procedure, using the Erbe Jet2 water-jet system, trans-anal and trans-vaginal wedge hepatic resections were successfully performed in two pigs without laparoscopic assistance. Trans-umbilical attempt failed due to an unstable operating platform. Each incision for peritoneal entry took 1 min, and 2 h was spent on excision of the liver tissue, indicating a hugely time-consuming part of the entire procedure. There was neither hemodynamic nor pulmonary instability throughout the NOTES procedure, and target visualization within the peritoneum was always kept clear. No untoward incident such as injury to surrounding organs occurred, and the whole intra-operative blood loss ranged from 100 to 250 mL. Parenchymal bleeding from resection could be adequately controlled by electrocautery with the hybrid knife itself, the hook knife or the APC probe (Table 1, Figure 4). Since all the exposed ductal structures were successfully clipped with Endoclips, no bile leak from the remnant liver occurred.

There were relatively smooth and precise cutting margins in all histological preparations. The cutting width at the bottom of the cut was similar to the dissection width at tissue surface, with little vessel damage (Figure 5). Some thermal alterations were obtained due to intra-operative electrocautery (Figure 6).

DISCUSSION

To the best of our knowledge, this is the first study in

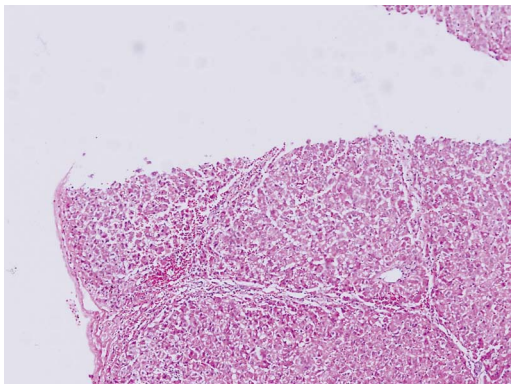

Figure 6 Thermal alterations due to intra-operative coagulation (HE stain, × 100). Removal of the liver capsule could be seen in an example of thermal damage.

a non-survival porcine model evaluating the feasibility and safety of wedge hepatic resection merely using a NOTES approach, Erbe Jet2 water-jet technology and endoscopic instrument.

Since first described by Kalloo *et al*[9], natural orifice transluminal endoscopic surgery (NOTES) has become the newest minimally invasive surgical procedure in contrast to open and laparoscopic technology. It involves passing flexible endoscopic systems through natural orifices (per-oral, trans-vaginal, trans-anal, trans-umbilical or trans-vesical routes), approaching target organs and performing intra-abdominal procedures. For the entry into the peritoneal cavity, a trans-luminal incision is mostly created by endoscopic needle knife followed by balloon dilation. However, in our study, it was achieved just in about 1 min *via* a hook knife, with the same desirable effect. The air-inflation mechanism of the endoscope was used to induce and maintain peritoneum, and the suction mechanism of the endoscope was used intermittently to avoid a high intra-abdominal pressure. Overall, there was neither hemodynamic nor pulmonary instability during NOTES, as described elsewhere[10].

Similar to laparoscopic liver resection, NOTES hepatic procedures must confront one and the same Achilles' heel, difficulty in obtaining hemostasis. Given the facts that protection of blood vessels is essential to minimize hemorrhage and blood transfusion, and smooth dissection margins might minimize adhesion formation[11], the water-jet hybrid knife was taken into consideration. Hydro-dissection was accomplished with the hybrid knife kept away from the tissue in a no-touch fashion and perpendicular to but not tangentially against the predetermined surface. Minor slow oozing from the cutting surface was controlled using the same knife, the hook knife or APC probe to initiate bursts of coagulation. Visible intra-hepatic vascular and ductal structures were clipped with endoscopic hemoclip. Certainly, the need for coagulation or clipping of individual vessels led to a prolonged operative time.

Current flexible endoscopes have significant limitations when used for complex therapeutic procedures. Stable platform and off-axis operation are often necessary for the NOTES. However, standard endoscopic shafts are too flexible and prone to looping, if these unfavorable factors caused the failure in transumbilical endoscopic hepatic resection. As for triangulation of endoscopically deployed instruments to approach the same target, internal double channels are small and in close proximity, producing parallelism and limiting possible triangulating interactions[12]. The operator interface parallelism does not allow satisfactory traction/countertraction for effective dissection of tissue and organs. To counteract the negative impact on dissection efficiency, a 4-mm transparent distance soft cap was mounted onto the tip of the endoscope for holding up the desired plane, subsequently avoiding the deviation in the direction of the water-jet. Unfortunately, its effect was limited due to the heavy weight of the porcine liver and the restricted field of view. As a result, excision of one piece of the same size from the porcine liver was more difficult in NOTES than in open procedure (more than 2 h was spent in NOTES, but only 45 min spent in open procedure).

Notably, non-anatomic wedge hepatic resection by a NOTES approach in either our or Phee's[8] study is still at a primary stage. As NOTES using current endoscopic instruments is technically difficult to realize pedicle control with an intrahepatic Glissonian approach[13], it is suitable only for superficial lesions of the liver mostly with the fine trabecular infrastructures and medium caliber structures. In order to achieve the same level of segment-based laparoscopic liver resection[14], advance in NOTES technology still has a long way to go.

In conclusion, the water-jet hybrid knife with the capacity of selective vessel-sparing tissue dissection can safely accomplish non-anatomic wedge hepatic resection through a NOTES approach. At the same time, its efficiency may be discounted by endoscopic deficiencies: lack of surgical triangulation, unstable operating platform as well as transmission attenuation caused by long distance and endoscopic looping. Although this technology is only at its beginning stage, as the old saying goes: well begun is half done.

ACKNOWLEDGMENTS

We thank Dr. Jiang-Fan Zhu for his editorial assistance.

COMMENTS

Background

Liver resection is technically challenging due to the risk of massive bleeding during operation. Since the early 20th century, the development of specific devices for separating hepatic parenchyma has contributed to bloodless transection. Furthermore, trans-luminal liver resection is technically demanding and its expansion has been lagged behind other natural orifice transluminal endoscopic surgery (NOTES) procedures.

Research frontiers

Phee described for the first time how a dexterous master and slave transluminal endoscopic robot could efficiently perform the wedge hepatic resection without laparoscopic assistance. This technology is still an unexplored field in China.

Innovations and breakthroughs

This is the first study to evaluate the feasibility and safety of non-anatomic wedge hepatic resection in a non-survival porcine model using a NOTES approach, Erbe Jet2 water-jet technology and endoscopic instruments. The

study demonstrated that the water-jet hybrid knife with the capacity of selective vessel-sparing tissue dissection can safely accomplish non-anatomic wedge hepatic resection through a NOTES approach.

Applications

Currently, non-anatomic wedge hepatic resection using NOTES approach and water-jet technology is suitable only for superficial lesions of the liver mostly with the fine trabecular infrastructures and medium caliber structures.

Terminology

High-pressure water-jet dissection technology was originally developed in the steel and glass industries, where ultra-precise cutting and engraving were considered as professional demands[4]. Since introduced to medical application in 1982[5], this technology has been successfully employed in open and laparoscopic operations, achieving favorable results in precise, controllable tissue-selective dissection with excellent visualization and minimal injury to the surrounding fibrous structures (such as ductal and vessel systems with a high content of collagen and elastin).

Peer review

This is the study in a non-survival porcine model evaluating the feasibility and safety of wedge hepatic resection by using pure NOTES approach.

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BRIEF ARTICLE

Upregulated CD133 expression in tumorigenesis of colon cancer cells

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Abstract

AIM: To analyze the upregulated CD133 expression in tumorigenesis of primary colon cancer cells.

METHODS: Upregulated CD133 expression in tumorigenesis of colorectal cancer cell lines (Lovo, Colo205, Caco-2, HCT116 and SW620) was analyzed by flow cytometry. Human colon cancer tissue samples were stained with anti-human CD133. SW620 cells were sorted according to the CD133 expression level measured by fluorescence-activated cell sorting. Spheroids of colorectal cancer cells were cultured with the hanging drop. Expression of CD133 and Lgr5 in spheroids of colorectal cancer cells and monolayer culture was detected by RT-qPCR. Spheroids of colorectal cancer cells were analyzed using anti-human CD133 with immunohistochemical staining.

RESULTS: CD133 antigen was expressed in colorectal cancer cell lines (Lovo, Colo205, Caco-2, HCT116 and SW620) as well as in primary and metastatic human colon cancer tissues. However, the CD133 was differently expressed in these cell lines and tissues. The expression levels of CD133 and Lgr5 were significantly higher in spheroids of parental, $CD133^{hi}$ and $CD133^{-}$ cells than in their monolayer culture at the mRNA level ($P < 0.05$). Immunohistochemical staining of spheroids of $CD133^{-}$ cells showed that CD133 was highly expressed in colorectal cancer cell lines.

CONCLUSION: Upregulated CD133 expression plays a role in tumorigenesis colorectal cancer cells, which may promote the expression of other critical genes that can drive tumorigenesis.

Key words: CD133; Colon cancer cells; Tumorigenesis; Cancer stem cells

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INTRODUCTION

CD133, also known as prominin-1, a transmembrane pentaspan protein, is originally described as a surface antigen specific for human hematopoietic stem and progenitor cells[1,2]. Later, CD133 is recognized as a stem cell marker for other normal tissues of brain[3], kidney[4], prostate[5], liver[6], pancreas[7], and skin[8]. It has been increasingly reported that CD133 is a marker of putative cancer stem cells (CSC) in brain tumor[9,10], prostate cancer[11], colon cancer[12-14], lung cancer[15], hepatocellular carcinoma[16], melanoma[17], ovarian cancer[18], and pancreatic cancer[19]. Accordingly, CD133 has been referred to as "the molecule of the moment"[20].

It has been recently shown that CD133 expression is broadly distributed in primary colon cancer cells including cancer stem cells, both $CD133^+$ and $CD133^-$ metastatic colon cancer cells initiate tumors[21-23]. However, whether CD133 expression plays a role in tumorigenesis of colorectal cancer cells is unknown.

In the present study, upregulated CD133 expression in several colorectal cancer cell lines as well as in human primary and metastatic colon cancer tissue samples was analyzed. SW620 cell line was sorted using CD133 antigen. Spheroids of parental, $CD133^-$ and $CD133^{hi}$ cells were cultured with the hanging drop. Expressions of CD133 and Lgr5 were detected in spheroids of colorectal cancer cells. CD133 was widely expressed in human colorectal cancer cell lines as well as in primary and metastatic colon cancer tissues and upregulated CD133 expression was detected in spheroids of colorectal cancer cells, indicating that upregulated CD133 expression may promote the expression of other critical genes that can drive tumorigenesis.

MATERIALS AND METHODS

Cell lines and cell culture and tissue samples

Human colorectal cancer cell lines (Lovo, Colo205, Caco-2, HCT116 and SW620) were cultured in RPMI1640 medium containing 10% fetal bovine serum (FBS), 2 mmol/L L-glutamine, 10 μmol/L thioglycerol, 12.5 U insulin, 0.5 mg hydrocostisone, and 30mg penicillin G/0.05 g streptomycin. Colorectal cancer cells were cultured at 37℃ in a humidified atmosphere containing 10% CO_2. CD133 expression was detected in formalin-fixed, paraffin-embedded primary and metastatic colorectal cancer tissue samples from Affiliated Sixth People's Hospital of Shanghai Jiaotong University. The study was approved by the Ethics Committee of Affiliated Sixth People's Hospital of Shanghai Jiaotong University.

Fluorescence-activated cell sorting

Single-cell suspensions were stained with antibodies against human CD133 (AC133, 1:40) and human CD133/1 and CD133/2(1:10, APC conjugated, Miltenyi Biotech, Germany). Dead cells, cell debris, doublets and aggregates were excluded by forward and side scattering and pulse-width gating. Colorectal cancer ells (1×10^5) were stained in an eppendorf tube. Primary antibody was incubated for 45 min on ice and second antibody (anti-mouse Alexa488, 1:400) was incubated for 30 min on ice in the dark. Flow cytometry analysis was carried out on a fluorescence-activated cell sorting (FACS) caliber (BD). Colorectal cancer ells (1×10^6) were prepared for sorting, stained with human CD133/1 (1:10, APC conjugated, Miltenyi Biotech) and 1 μg/mL propidium iodide (PI) to exclude dead cells during sorting. The cells were sorted using FACSAria (BD). Matched isotype antibodies were applied in parallel as controls.

Colon spheroids were culture with hanging drop

SW620 colorectal cancer cells and their sorted $CD133^-$ and $CD133^{hi}$ cells were prepared as a single cell suspension. The cells were counted and diluted in RPMI1640 containing 20% FBS and antibiotics to a concentration of 500 cells per 20 μL/drop in a sterile basin. The lid was lifted, inverted and placed on top of the dish containing 10 mL PBS. An 8-channel pipette was used to make rows of 20 μL drops on the up-turned inner surface of the tissue culture dish lid. The drops were incubated at 37℃ in an atmosphere containing 10% CO_2 for 10 d.

Immunohistochemistry

Frozen sections of the spheroids of colorectal cancer cells were fixed in acetone at -20℃ for 10 min and rehydrated in PBS. Endogenous peroxidase was inactivated by immersing the sections in 0.3% hydrogen peroxide for 20 min. The primary antibody for frozen sections of the spheroids of colorectal cancer cells and paraffin-embedded sections of colorectal cancer tissue samples was a mouse anti-human monoclonal CD133/2 (1:40, Miltenyi Biotech, Germany) and a rabbit anti-human polyclonal CD133 (1:100, Abcam, England), respectively. The sections were incubated overnight at 4℃ in a humidified chamber, then with biotinylated secondary antibody (VECTASTAIN ABC kit, Vector Laboratories) for 30 min at room temperature. Each section was incubated with the VECTASTAIN ABC reagent for 30 min at room temperature. The sections were developed using the DAB (Vector Laboratories) as the substrate and then counterstained with hematoxylin. The negative control was performed by incubating samples with PBS.

Quantification of CD133 expression by quantitative polymerase chain reaction

Total RNA was isolated from cultured colorectal cancer cells and their spheroids using the RNeasy extraction kit (GE Healthcare) and reverse transcribed using high-capacity cDNA reverse transcription kit (Applied Biosystems) according to their manufacturer's instructions, respectively. Relative quantitative polymerase chain reaction (PCR) was performed on a 7300 fast real-time PCR system (Applied Biosystems) using SYBR green PCR master mix (Applied Biosystems). The human-specific intron spanning primer pairs for CD133 were provided by QIAGEN (Catalog number: QT00075586). The sequences of primer pairs used for GAPDH and Lgr5 are CAATGACCCCTTCATTGACC (forward) and TGATGACAAGCTTCCCGTTC (reverse), and CTTCCAACCTCAGCGTCTTC (forward) and TTTCCCGCAAGACGTAACTC (reverse), respectively. PCR was performed for 1 cycle at 50℃ for 2 min and 1 cycle at 95℃ for 10 min, followed by 40 cycles at 95℃ for 15 s and 60℃ for 1 min. Specificity of PCR products was tested according to the dissociation curves. Relative values of transcripts were calculated using the equation: $2^{-\Delta\Delta Ct}$, where ΔCt is equal to the difference in threshold cycles for target and reference.

Statistical analysis

Results were expressed as mean ± SD for three repeated individual experiments in each group. Statistical analyses were conducted using the SPSS software (version 10.0).

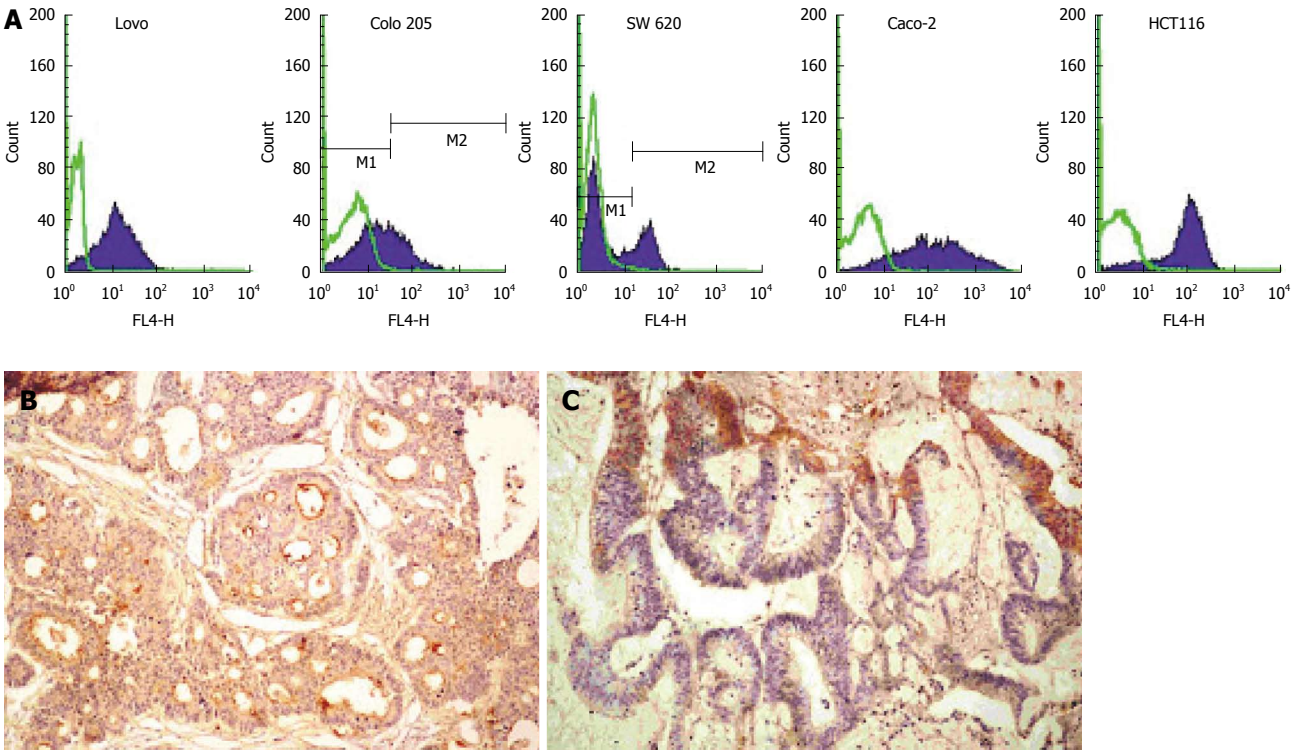

Figure 1 Fluorescence-activated cell sorting showing CD133 expression in different colorectal cancer cell lines (A), CD133 staining of human primary colorectal cancer tissue (B) and metastatic colorectal cancer tissue (C) (Original magnification × 100). Brown indicates positive staining.

Correlation between sample groups and molecular variables was assayed with paired *t* test. $P < 0.05$ was considered statistically significant.

RESULTS

CD133 expression in colon cancer cell lines and human colon cancer tissues

CD133 antigen was expressed in all colorectal cancer cell lines with a difference of 30%-95% (Figure 1A). CD133 in human colorectal cancer tissue samples was stained with polyclonal antibody. CD133 expression was detected in 18 of the 20 primary cancer tissue samples, exclusively on the membrane of the vast majority of colorectal cancer gland cells (Figure 1B), and in 9 of the 10 metastatic colorectal cancer tissue samples with positive staining in cytoplasm of cancer cells (Figure 1C).

CD133 expression in spheroids of sorted colorectal cancer cell subpopulations

To minimize the contamination between the sorted $CD133^{+}$ and $CD133^{-}$ cells, a high CD133 expression cell subpopulation ($CD133^{hi}$) and a $CD133^{-}$cell subpopulation sorted from the SW620 cells could be persistently passed. CD133 antigen was stably expressed in the monolayer culture (Figure 2A). To mimic the tumorigenesis of colorectal cancer cells *in vivo*, spheroids of the sorted cells were cultured with hanging drop. The parental, $CD133^{hi}$ and $CD133^{-}$ cells could grow into spheroids. CD133 expression was upregulated in spheroids of $CD133^{-}$ cells. Although the CD133 expression rate was not changed, the mean fluorescence intensity (MFI) was significantly increased in spheroids of $CD133^{hi}$ cells, and the CD133 expression rate and MFI were significantly increased in spheroids of parental cells detected by FACS assay (Figure 2B). Immunohistochemical staining of CD133 antigen was observed in spheroids of $CD133^{-}$ cells (Figure 2C). The CD133 gene expression level was significantly higher in spheroids of SW620, $CD133^{hi}$ and $CD133^{-}$ cells than in their monolayer culture at the mRNA level (4.224 ± 0.063 *vs* 2.680 ± 0.117, 3.653 ± 0.061 *vs* 1.325 ± 0.044, 8.746 ± 0.029 *vs* 3.761 ± 0.065, $P < 0.05$) (Figure 2D).

Lgr5 expression in spheroids of sorted colorectal cancer cell subpopulations

Lgr5 expression was analyzed by RT-qPCR in order to observe the role of the expression of other colon stem cell genes in tumorigenesis of colorectal cancer cells. The results showed that the Lgr5 expression level was significantly higher in spheroids of parental, $CD133^{hi}$ and $CD133^{-}$ cells than in their monolayer cells (5.942 ± 0.091 *vs* 4.003 ± 0.039, 6.611 ± 0.214 *vs* 3.645 ± 0.046, 5.910 ± 0.035 *vs* 3.903 ± 0.083, $P < 0.05$) (Figure 3).

DISCUSSION

Whether CD133 antigen can be used as a marker of colorectal cancer stem cells is still controversial. The focus is that CD133 expression is not restricted to just a small number of colorectal cancer cells. In this study, the CD133 expression was upregulated in colorectal cancer cell lines and primary or metastatic colorectal cancer tissue

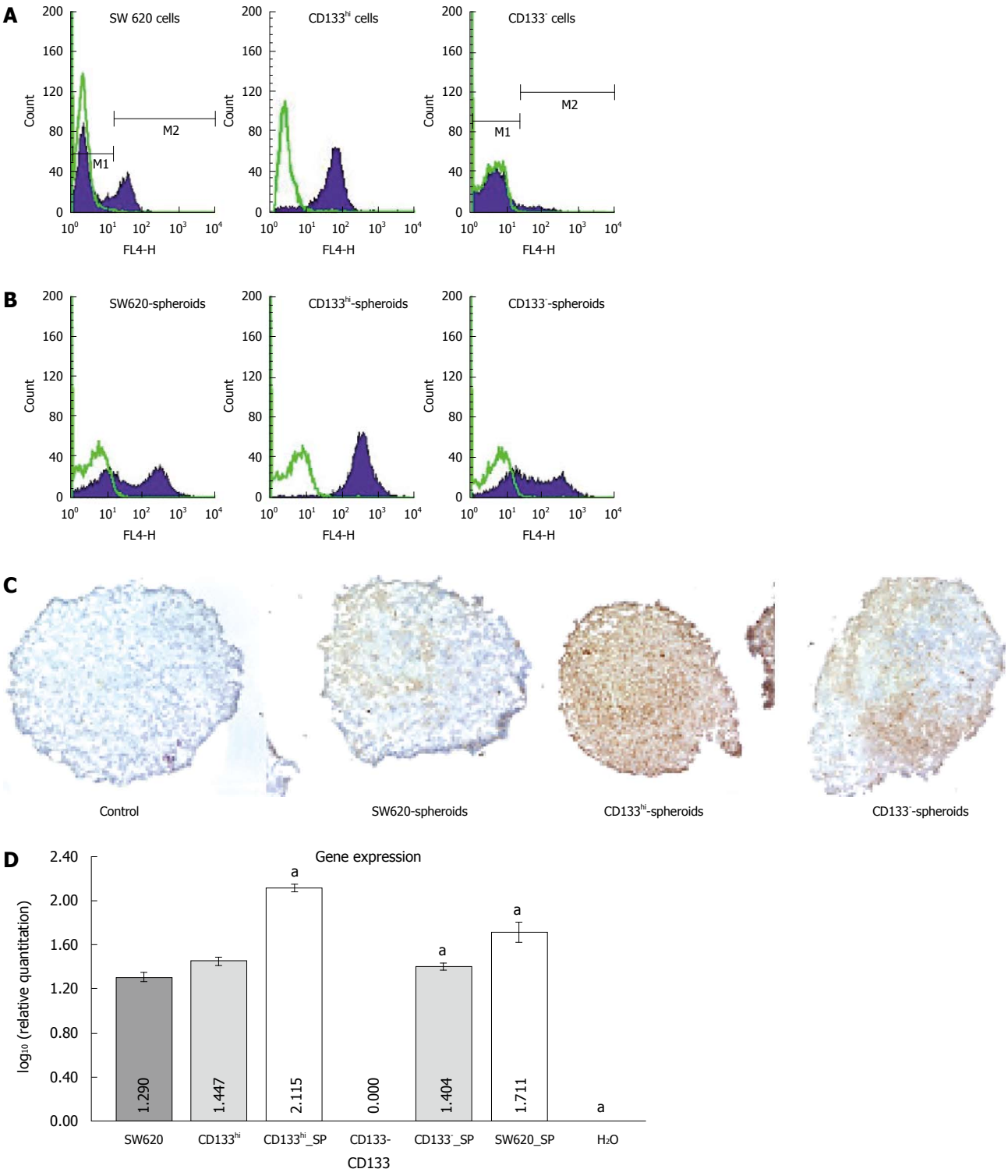

Figure 2 Fluorescence-activated cell sorting showing CD133 expression in SW620, CD133^{-} and CD133^{hi} cells (A) and in their spheroids (B), CD133 staining in spheroids of SW620, CD133^{-} and CD133^{hi} cells (original magnification × 100, brown indicates positive staining) (C), and reverse transcription-polymerase chain reaction showing CD133 expression in SW620, CD133^{-} and CD133^{hi} cells and their spheroids. $^{a}P < 0.05$ *vs* monolayer cells. SP: Spheroid.

samples, showing that CD133 antigen can be expressed in colorectal cancer cell lines with a difference of 30%-95%. CD133 expression was detected in 18 of the 20 primary colorectal cancer tissue samples, exclusively on the membrane of a large number of colorectal cancer gland cells, and in 9 of the 10 metastatic colorectal cancer tissue samples with a positive staining in cytoplasm of colorectal cancer cells, which is consistent with the reported findings[21-23]. The different CD133 expression levels in colorectal caner cell lines may be related to the different glycosylation to the mask specific epitopes of CD133 antigen in colorectal cancer cell differentiation[24]. Therefore, our data indicate that CD133 is commonly expressed in colorectal cancer cells.

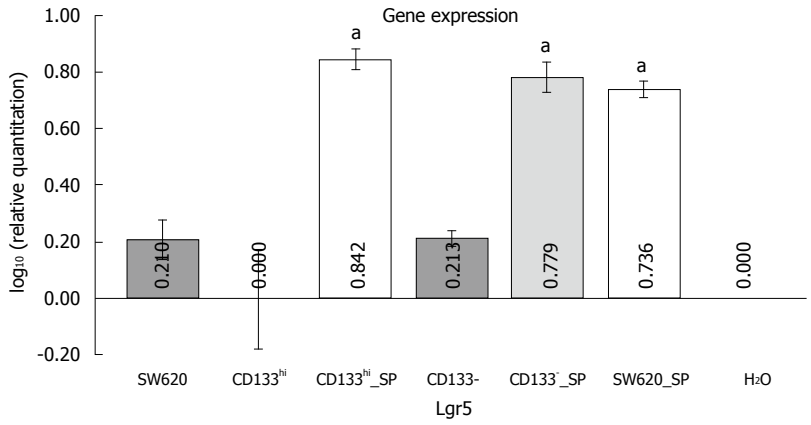

Figure 3 Quantitative reverse transcription-polymerase chain reaction showing Lgr5 expression in SW620, CD133$^-$ and CD133^{hi} cells and their spheroids. $^aP < 0.05$ *vs* monolayer cells. SP: Spheroid.

To investigate whether the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells, SW620 cell line containing two cell subpopulations (CD133^{hi}, CD133$^-$) was selected and sorted using CD133 antigen, the spheroids of parental, CD133^{hi} and CD133$^-$ cells were cultured with the hanging drop *in vitro*, which is based on the natural disposition of cells to aggregate without the need for polymer scaffolds such as matrigel, polyglycolic acid or microporous supports to achieve homogeneous multicellular tumor spheroids[25]. The spheroids represent a popular *in vitro* 3D tissue structure that mimics *in vivo* tumor tissue organization and microenvironment[26,27]. In the present study, CD133^{hi} and CD133$^-$ cells could be cultured into their spheroids, CD133 expression was upregulated in spheroids of CD133$^-$ cells. Although the CD133 expression was not changed, the mean fluorescence intensity (MFI) was significantly increased in spheroids of CD133^{hi} cells as detected by FACS assay. Immunohistochemical staining of CD133 antigen was observed in spheroids of CD133$^-$ cells, indicating that CD133 antigen expression is upregulated in spheroids of CD133$^-$ and CD133^{hi} cells. Further analysis revealed that the CD133 gene expression level was significantly higher in spheroids of SW620, CD133^{hi} and CD133$^-$ cells than in their monolayer culture at the mRNA level, suggesting that the upregulated expression of CD133 including protein and gene plays a role in tumorigenesis of colorectal cancer cells.

Since the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells, whether CD133 protein supports the growth of colorectal cancer is a subject that should be actively studied. As CD133 by itself may lack of a functional role in initiation of tumors and metastasis of human colorectal cancer[28,29], it has an impact on the survival of colorectal cancer patients[22,29]. It has been recently demonstrated that prominin 1 (also called CD133)-marked mouse intestinal stem cells are susceptible to neoplastic transformation[30], possibly due to the fact that upregulated CD133 expression may promote the expression of other critical genes that can drive tumorigenesis of colorectal cancer cells. In this study, the expression level of Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5), also known as Gpr49, a colon stem cell marker gene[31], was significantly higher in spheroids of parental, CD133^{hi} and CD133$^-$ cells than in their monolayer cells.

In conclusion, the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells, which may be related to the expression of other critical genes that can drive tumorigenesis of colorectal cancer cells. Further study is needed to confirm the present results *in vivo*.

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COMMENTS

Background

It has been recently shown that CD133 expression is broadly distributed in primary colorectal cancer cells, and not restricted to cancer stem cells. Whether the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells is unknown.

Research frontiers

It has been increasingly reported that CD133 is a marker of putative cancer stem cells (CSC) in some cancers. However, it has been recently shown that CD133 expression is broadly distributed in primary colon cancer cells and not restricted to cancer stem cells, and both CD133$^+$ and CD133$^-$metastatic colorectal cancer cells initiate tumors. Whether the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells is unknown. In this study, the upregulated CD133 expression was found to play a role in tumorigenesis of colorectal cancer cells.

Innovations and breakthroughs

Recent reports have shown that whether CD133 antigen can be used as a marker of colorectal cancer stem cells is controversial. This is the first study to report the role of upregulated CD133 expression in tumorigenesis of colorectal cancer cells. Furthermore, our *in vitro* studies suggested that the upregulated CD133 expression may promote the expression of other critical genes that can drive tumorigenesis of colorectal cancer cells.

Applications

Whether the upregulated CD133 expression plays a role in tumorigenesis of colorectal cancer cells was studied, the results may help to solve the controversy on CD133 antigen as a marker of colorectal cancer stem cells.

Terminology

CD133, also known as prominin-1, a transmembrane pentaspan protein, is originally described as a surface antigen specific for human hematopoietic stem

and progenitor cells. Lgr5 (leucine-rich-repeat-containing G-protein-coupled receptor 5), also known as Gpr49, is a colon stem cell marker gene.

Peer review

The authors detected the expression of CD133 in a panel of colorectal cancer cell lines and human colorectal cancer tissue samples. The expression of CD133 and Lgr5 in spheroids of the sorted colorectal cancer cell subpopulations suggests that the upregulated expression plays a role in tumorigenesis of colorectal cancer cells, which may promote the expression of other critical genes that can drive tumorigenesis. The results are interesting.

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BRIEF ARTICLE

Transplantation of microencapsulated umbilical-cord-blood-derived hepatic-like cells for treatment of hepatic failure

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Abstract

AIM: To investigate intraperitoneal transplantation of microencapsulated hepatic-like cells from human umbilical cord blood for treatment of hepatic failure in rats.

METHODS: $CD34^+$ cells in umbilical cord blood cells were isolated by magnetic cell sorting. In the *in vitro* experiment, sorted $CD34^+$ cells were amplified and induced into hepatic-like cells by culturing with a combination of fibroblast growth factor 4 and hepatocyte growth factor. Cultures without growth factor addition served as controls. mRNA and protein levels for hepatic-like cells were analyzed by reverse transcription-polymerase chain reaction, immunohistochemistry and immunofluorescence. In the *in vivo* experiment, the hepatic-like cells were encapsulated and transplanted into the abdominal cavity of acute hepatic failure (AHF) rats at 48 h after D-galactosamine induction of acute hepatic failure. Transplantation with PBS and unencapsulated hepatic-like cells served as controls. The mortality rate, hepatic pathological changes and serum biochemical indexes were determined. The morphology and structure of microcapsules in the greater omentum were observed.

RESULTS: Human albumin, alpha-fetoprotein and GATA-4 mRNA and albumin protein positive cells were found among cultured cells after 16 d. Albumin level in culture medium was significantly increased after culturing with growth factors in comparison with culturing without growth factor addition ($P < 0.01$). Compared with the unencapsulated group, the mortality rate of the encapsulated hepatic-like cell-transplanted group was significantly lower ($P < 0.05$). Serum biochemical parameters, alanine aminotransferase, aspartate aminotransferase and total bilirubin in the encapsulated group were significantly improvement compared with the PBS control group ($P < 0.01$). Pathological staining further supported these findings. At 1-2 wk post-transplantation, free microcapsules with a round clear structure and a smooth surface were observed in peritoneal lavage fluid, surviving cells inside microcapsules were found by trypan blue staining, but some fibrous tissue around microcapsules was also detected in the greater omentum of encapsulated group by hematoxylin and eosin staining.

CONCLUSION: Transplantation of microencapsulated hepatic-like cells derived from umbilical cord blood cells could preliminarily alleviate the symptoms of AHF rats.

Key words: Microencapsulation; Hepatic-like cells; Umbilical cord blood cells; CD34 antigen; Alginate; Acute hepatic failure

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INTRODUCTION

Substantial efforts have been made with regard to cell transplantation as an effective supporting system for hepatic failure and assisted therapies. However, immunological rejection has always been an important problem for cell transplantation. Alginate-poly-l-lysine-alginate (APA) microcapsules have proven to be effective in protecting enclosed target cells from immune rejection following transplantation into experimental animals, thereby eliminating the problems of immunosuppressive therapy[1-3].

Extensive studies have also been conducted on the core of this therapy, namely the cell sources. The investigated cells have included liver stem cells, embryonic stem cells, human umbilical cord blood (UCB) cells and bone marrow stem cells. Human UCB cells have some advantages that other cells do not have. The frequencies of UCB hematopoietic stem/progenitor cells exceed those from bone marrow and peripheral blood. In our previous study, we confirmed the differentiation of mononuclear cells (MNCs) from human UCB into hepatocytes in three different ways, namely co-culture with injured liver cells, growth factor-assisted culture, and MNC transplantation in animal models of liver injury[4]. In the present study, we found that $CD34^+$ cells derived from human UCB could be converted into hepatic-like cells that generate hepatocyte lineage cells. Furthermore, we encapsulated the hepatic-like cells using an alginate method and transplanted them into acute hepatic failure (AHF) rats to evaluate the effects of encapsulated hepatic-like cell transplantation.

MATERIALS AND METHODS

Isolation and identification of $CD34^+$ cells

UCB (more than 80 samples) from full-term deliveries were obtained from the Obstetrics Department of Peking University Shenzhen Hospital. UCB cells were harvested after written inform consent was obtained. The study protocol was approved by the Ethics Committee of Peking University Shenzhen Hospital. MNCs were isolated from the UCB samples by density-gradient centrifugation at 2000 r/min for 35 min using Ficoll-Hypaque (Huajing, Shanghai, China). $CD34^+$ subpopulations were isolated using a Miltenyi Direct CD34 Progenitor Cell Isolation Kit (Miltenyi Biotec, Bergisch Gladbach, Germany). The specific steps were as follows: (1) isolated MNCs were resuspended in a final volume of 300 μL of PBS that contained 5 g/L bovine serum albumin (BSA); (2) 100 μL of FcR Blocking Reagent and 100 μL of CD34 Micro Beads per 1×10^8 total cells were sequentially added, mixed well and incubated for 30 min in a refrigerator at 4℃; (3) cells were passed through a magnetic column twice and purified; and (4) $CD34^+$ cells were collected, resuspended in 100 μL PBS, incubated with 10 μL CD34-phycoerythrin for 10 min at 4℃ and identified by flow cytometry.

Table 1 Primers used for reverse transcription-polymerase chain reaction

Gene	Primer (5′-3′)		Amplicon (bp)
	Forward primer	Reverse primer	
ALB	CTTTCAAAGCATGGGCAGTAG	GCAGCAGCACGACAGAGTAA	411
GATA-4	ACCTGGGACTTGGAGGATAG	GACAAGGACATCTTGGGAAA	250
AFP	TGAGCACTGTTGCAGAGGAG	CTGAGACAGCAAGCTGAGGA	308

ALB: Albumin; AFP: α-fetoprotein.

Differentiation in vitro

Freshly isolated $CD34^+$ cells were primarily cultured in Dulbecco's modified Eagle's medium - low glucose (DMEM-LG, Gibco, Carlsbad, CA, USA), amplified for 3-5 d with a combination of 12.5 μg/mL thrombopoietin (TPO) (R&D Systems, Minneapolis, MN, USA), 50 ng/mL stem cell factor (SCF) (R&D Systems) and 50 ng/mL Flt-3 (R&D Systems); then induced into hepatic-like cells by culturing in DMEM-LG that contained 50 mL/L fetal bovine serum (Gibco), 100 U/mL penicillin, 100 μg/mL streptomycin, 4.7 μg/mL linoleic acid, 1 × insulin-transferrin-selenium and 1×10^{-4} mol/L L-ascorbic acid 2-P supplemented with 100 ng/mL fibroblast growth factor (FGF)4 (R&D Systems) and 20 ng/mL hepatocyte growth factor (HGF; Sigma, St. Louis, MO, USA). $CD34^+$ cells were incubated in 24-well plates at 37℃ in a 5% CO_2 atmosphere. Culture medium was replaced every 3 d. Cultured cells were collected after 8 and 16 d. Cultures without growth factors served as controls.

Total mRNA isolation and reverse transcription-polymerase chain reaction

Total mRNA was extracted from collected cells using Trizol (Mrcgene, Cincinnati, OH, USA). mRNA was reverse-transcribed and the resulting cDNA was amplified using the primer sets shown in Table 1 and a RobusT I reverse transcription-polymerase chain reaction (RT-PCR) Kit (Finnzymes, Espoo, Finland). Reverse transcriptase reaction was run at 48℃ for 45 min and PCR was initiated with pre-denaturation at 94℃ for 2 min, followed by 35 cycles of 30 s at 94℃, annealing at 58℃ for 30 s and extension at 72℃ for 30 s, with 72℃ for 7 min for final extension. The PCR products were separated on a 1.2% agarose gel.

Immunocytochemistry for $CD34^+$ cells

Cytospins prepared from cells were fixed with 4% paraformaldehyde and 0.15% picric acid in PBS at room temperature for 20 min, then permeabilized and blocked with 10% goat serum and 0.1% Triton X-100 in PBS at room temperature for 10 min. The cells were sequentially incu-

bated with a mouse anti-human albumin antibody (R&D Systems) for 30 min, a biotinylated peroxidase-conjugated secondary antibody (Zymed, South San Francisco, CA, USA) for 10 min, and diaminobenzidine for 10 min. Between the above steps, cells were washed with 0.1 mol/L PBS that contained 1 g/L BSA.

Albumin determination

Culture media were collected for the quantitative determination of human albumin by ELISA using a Human Albumin ELISA Kit (Alpha Diagnostics International, San Antonio, TX, USA) according to the manufacturer's instructions.

Cell encapsulation

Cells collected after 16 d induction were washed with PBS and resuspended in the alginate. The alginate-cell mixture was passed though a microcapsule generator and extruded into 40 mL 1.1% $CaCl_2$ solution. The airflow rate was adjusted for the regulation of the microcapsule diameter between 300 and 800 μm. The capsules and $CaCl_2$ solution were then transferred to 50-mL conical tubes. After removal of the supernatant, the capsules were gently mixed with the wash solution and allowed to settle for 2 min. Before transplantation, a few drops of encapsulated cells were placed on a slide, stained with 0.4% Trypan blue, covered with a cover glass and lightly pressed to force cells out of the microcapsules. Numbers of living cells were counted and expressed as percentages.

Induction of AHF and cell transplantation

Sprague-Dawley rats were purchased from the Experimental Animal Center of Southern Medical University (Guangzhou, China). The Scientific Committee at Peking University Shenzhen Hospital approved the use of animals for experimental purposes. Forty-eight hours before transplantation, the Sprague-Dawley rats (weight: 180-250 g) were intraperitoneally injected at 1.4 g/kg with a 10% D-galactosamine solution in normal saline. On the day of the experiment, microencapsulated cells at a density of 2×10^6 cells/mL were prepared and transplanted into the abdominal cavity of rats. Transplantation with PBS only or unencapsulated hepatocyte-like cells were performed for the establishment of control groups. As UCB samples are not delivered on the same day, animal experiments were carried out by batch and the transplantation of cells performed also on different days. The mortality rate, hepatic pathological changes and serum biochemical indexes were determined.

AHF rats grouping

We obtained total 135 AHF rats 48 h after injection of D-galactosamine. They were divided into three groups on the day of the transplantation. Namely, encapsulated group (transplantation with encapsulated hepatic-like cells, $n = 55$), unencapsulated group (transplantation with unencapsulated hepatic-like cells, $n = 40$), PBS group (transplantation with PBS, $n = 40$). Among these, 76 AHF rats were determined for hepatic pathological changes and serum biochemical indexes (encapsulated group, $n = 36$; unencapsulated group, $n = 20$; PBS group, $n = 20$). The remaining 59 rats were determined for mortality rate (encapsulated group, $n = 19$; unencapsulated group, $n = 20$; PBS group, $n = 20$).

Histology

The liver and greater omentum from all three groups were fixed in 4% buffered formaldehyde overnight. After paraffin embedding, 4-5-μm thick serial sections were stained with hematoxylin and eosin (HE) and observed under the light microscope.

Statistical analysis

Data were expressed as the mean ± SD. Mortality rate analysis was determined by Fisher's exact test. Serum biochemical index statistical analysis was performed by ANOVA using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Differences with P values < 0.05 were considered statistically significant.

RESULTS

Differentiation of $CD34^+$ cells into hepatic-like cells

Approximately 3×10^5-9×10^5/mL sorted cells were obtained using the CD34 immunomagnetic bead method, and 91% of them expressed CD34 by flow cytometry analysis (Figure 1). $CD34^+$ cells were firstly amplified 20-fold by a combination of TPO, SCF and Flt-3, and then they were cultured with HGF and FGF4. At 16 d, they developed larger volumes, richer cytoplasts, and binucleated structures, as observed under a Hoffman microscope (Figure 2). The RT-PCR showed no human albumin, α-fetoprotein (AFP) and GATA-4 mRNA expression in $CD34^+$ cells before the induction procedure. The expression of albumin and GATA-4 mRNA increased with the culture time after the addition of growth factors, whereas the amount of AFP mRNA expression peaked after 8 d and reduced at 16 d (Figure 3). Cells that expressed albumin and AFP were verified by immunocytochemical staining and ELISA (Figures 2 and 4). The percentage of albumin- and AFP-positive cells at 16 d was 30% and 24%, respectively. The albumin product in culture medium was significantly increased after culturing with HGF and FGF4 in comparison with control groups ($P < 0.01$).

Cell encapsulation and transplantation

The APA microencapsulation technique was used to encapsulate hepatic-like cells. The percentage of living cells was > 80%, as determined by trypan blue staining. The AHF animal model was successfully established using Sprague-Dawley rats by the injection of D-galactosamine. Pathological section of the AHF liver revealed that the structure of the hepatic lobules was destroyed and the hepatic cord was disordered, with large areas of denatured and necrotic hepatocytes, and infiltrating lymphocytes were found on the portal area at 48 h after injection. On the day of the experiment, microencapsulated cells at a density of 2×10^6 cells/mL were prepared and transplant-

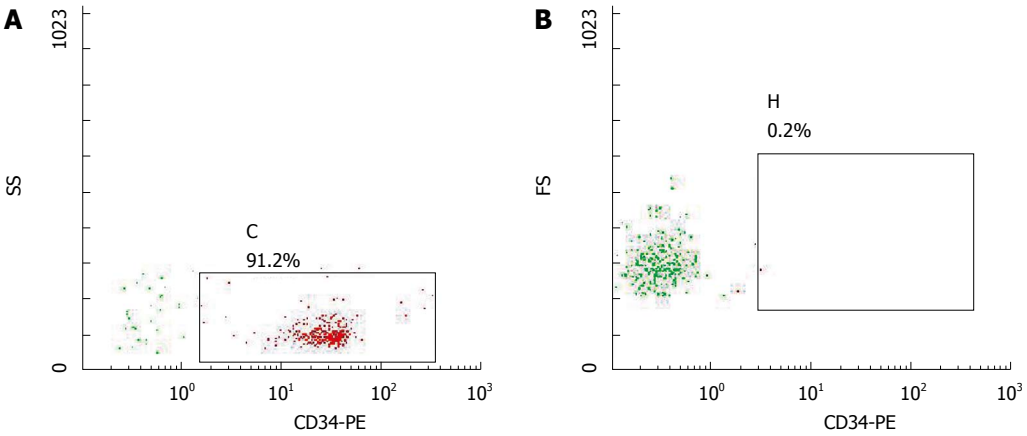

Figure 1 FACS determination of CD34$^+$ cells. A: Purity of CD34$^+$ cells; B: Homotypic control cells.

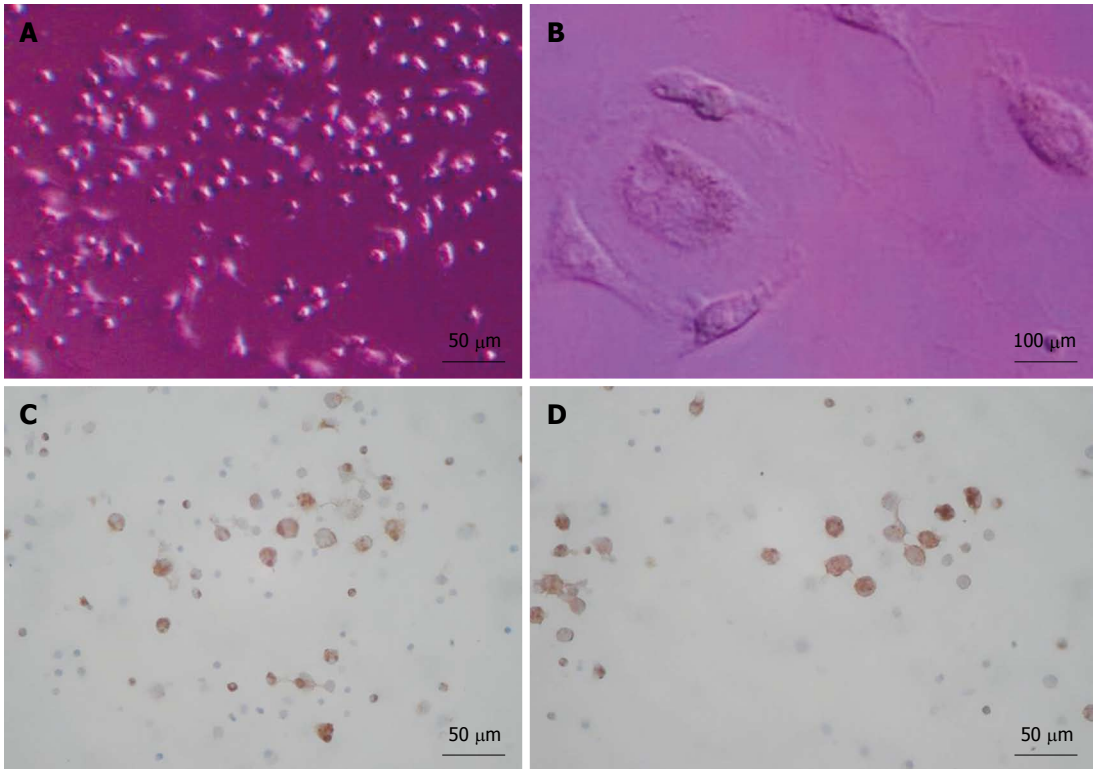

Figure 2 Cell culture and analyses. A: After 16 d; B: A binucleated cell; C, D: Positive staining for albumin (C) and α-fetoprotein (D) after 16 d of indction.

ed into the abdominal cavity of AHF rats. The mortality rate and hepatic pathological changes were determined. At 48 h after transplantation, HE staining of the encapsulated group revealed that the hepatic lobules were still intact; denaturation was the major change in hepatocytes and the area of necrosis nidus was small, and congestion and hemorrhage were almost undetectable (Figure 5). The mortality rate at 48 h after transplantation in three groups was 42.1% (encapsulated group), 65% (unencapsulated group) and 75% (PBS group), respectively. Compared with the unencapsulated group, the mortality rate of the encapsulated group was significantly lower ($P < 0.05$). In addition, the serum biochemical indexes of ALT, AST and total bilirubin in the microencapsulated group differed significantly from those in the PBS group ($P < 0.01$) at 48 h after transplantation, but there were no differences between the encapsulated and the unencapsulated group (Table 2). At 1-2 wk post-transplantation, free microcapsules with a round clear structure and a smooth surface were observed in peritoneal lavage fluid, surviving cells in microcapsules were found by trypan blue staining, but some fibrous tissues around microcapsules were also detected in the greater omentum of encapsulated group by HE staining (Figure 6).

DISCUSSION

With the continued increase in people with hepatic failure from cirrhosis and hepatocarcinoma, cell transplantation as an effective therapy is becoming a matter of concern

Table 2 Changes in serum biochemical indexes at different times

	48 h after injection D-GalN	48 h after transplantation			7 d after transplantation		
	All 3 groups	Encapsulated group	Unencapsulated group	PBS group	Encapsulated group	Unencapsulated group	PBS group
ALT (U/L)	3242.3 ± 2403.24	93.93 ± 63.45[b]	126.1 ± 54.35	245.9 ± 67.87	42.25 ± 11.86	45.07 ± 10.56	47.27 ± 11.08
AST (U/L)	4237.20 ± 1372.07	168.87 ± 89.33[b]	275.7 ± 52.74	439.7 ± 133.01	162.6 ± 54.29	124.52 ± 24.61	114.83 ± 16.50
TBIL (μmol/L)	5.57 ± 1.86	1.73 ± 1.01[a]	2.23 ± 1.98	3.50 ± 1.23	1.90 ± 0.52	2.72 ± 0.96	3.72 ± 1.18

Data are shown as means ± SD. [a]$P < 0.05$; [b]$P < 0.01$, in comparison with PBS group. TBIL: total bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

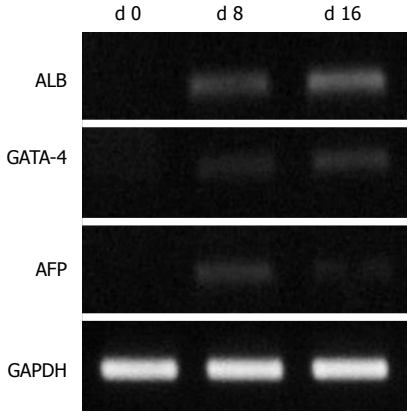

Figure 3 Reverse transcription-polymerase chain reaction analysis of umbilical cord blood CD34$^+$ cells cultured *in vitro* d 0, d 8 and d 16. ALB: Albumin; AFP: α-fetoprotein.

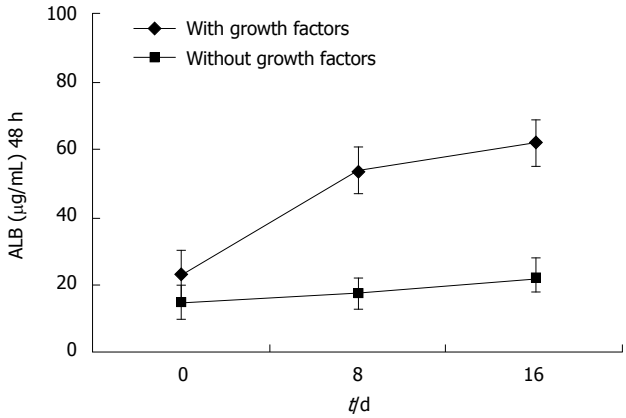

Figure 4 Determination of albumin expression by Enzyme-Linked Immunosorbent Assay. ALB: Albumin.

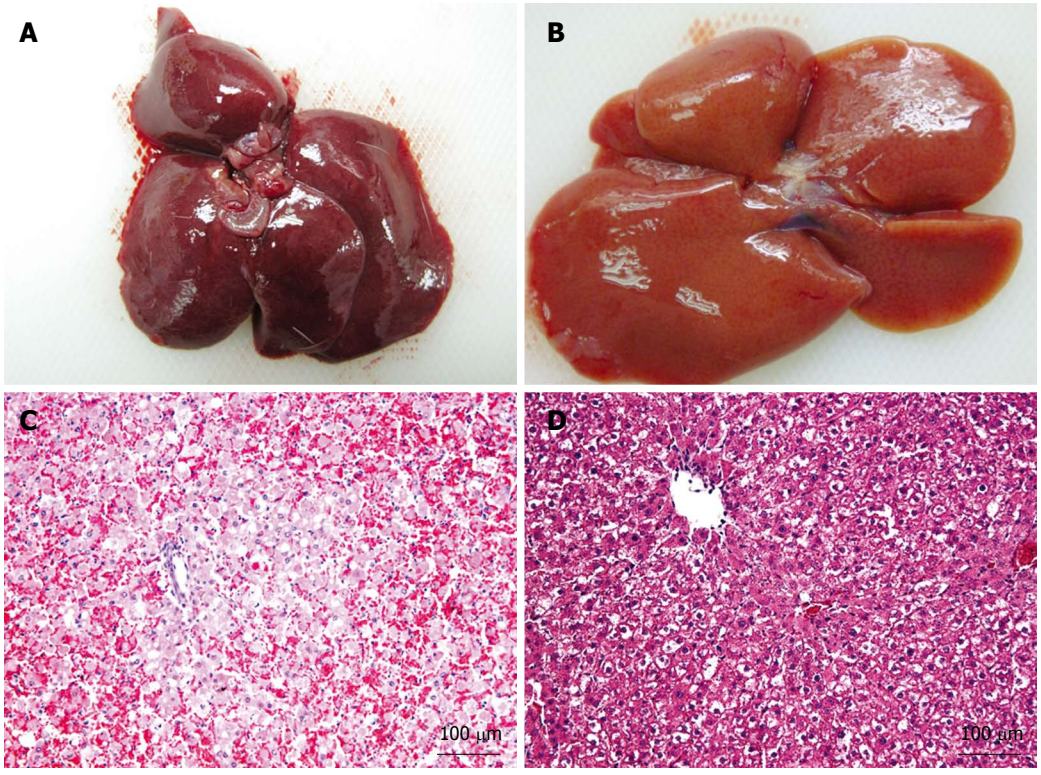

Figure 5 Pathological changes in the livers of acute hepatic failure rats. A: Liver at 48 h after injection of D-galactosamine; B: Liver at 48 h after microcapsule transplantation; C: HE staining of the liver shown in section (A); D: HE staining of the liver shown in section (B).

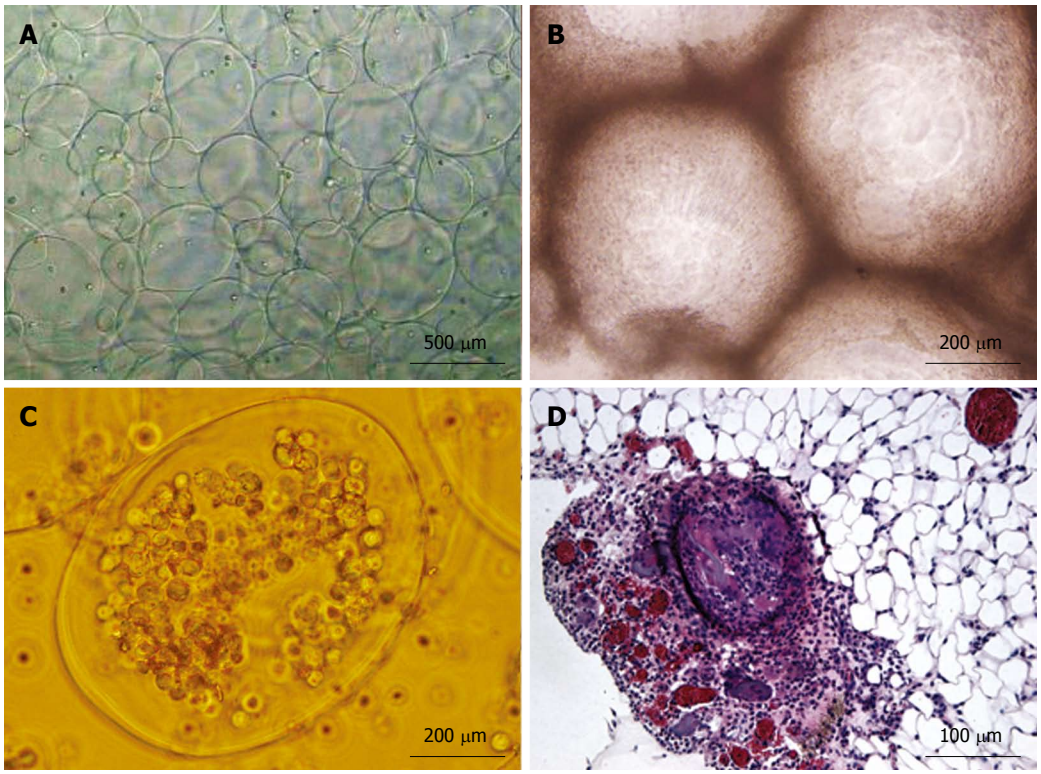

Figure 6 Encapsules observation. A: Microcapsules created by the Alginate-poly-l-lysine-alginate microencapsulation method; B: Microcapsule masses in the peritoneal lavage fluid; C: Free microcapsules in the peritoneal lavage fluid; D: HE staining shows microcapsules in the greater omentum.

for more scientists. Cell transplantation could offer metabolic support when liver function is damaged, and extend the waiting time for a liver donor[5,6]. Hepatic cell transplantation via the peritoneum or spleen has shown good prospects in clinical and animal experiments. However, the cell sources for transplantation and the requirement for long-term immunosuppression have caused stagnation in this field.

There have been some intriguing studies that have described adult stem cells displaying plasticity in recent years. These studies have led us to consider that using adult stem cells might cure diseases such as AHF[7,8]. Human UCB cells are enriched in hematopoietic stem/progenitor cells that exceed those in the bone marrow and peripheral blood. In comparison with bone marrow stem cells, UCB stem cells are even more immature and with lower immunogenicity. In our previous study, we confirmed that the conversion of UCB MNCs into hepatocytes by three different ways, namely co-culture with injured liver cells, growth-factor-assisted culture, and MNC transplantation in AHF animal models[4]. In the present study, we explored the possibility that $CD34^+$ cells derived from human UCB could be converted into hepatic-like cells. At present, the curative effect of hepatic-like cells derived from $CD34^+$ cells in the bone marrow has already been confirmed by *in vivo* animal experiments[9-12]. This showed that an AHF model was initially set up using immunodeficient mice, and $CD34^+$ cells enriched by immunobeads were injected through the tail vein or portal vein into the model animals. Expression of differentiation markers of donor cells in recipient livers at different times after transplantation was determined by fluorescence *in situ* hybridization, immunohistochemistry and molecular biological techniques. It was found that stress-induced signals, such as increased expression of stromal-cell-derived factor 1, matrix metalloproteinase-9 and HGF, recruits human $CD34^+$ progenitors with hematopoietic and/or hepatic-like potential to the liver of NOD/SCID mice[13]. Furthermore, another study has confirmed that FGF, leukemia inhibitory factor, SCF, HGF, FGF4 and oncostatin M contribute to the proliferation and/or differentiation of hepatic cells in different ways, and that combinations of these factors, especially HGF and FGF4, are necessary for human UCB cells to convert into albumin-producing cells[14].

With a combination of HGF and FGF4, we have established a 16-d culture system to induce $CD34^+$ cell differentiation. The culture system with HGF and FGF4 displays the capability to convert the $CD34^+$ cells from human UCB into cells with hepatocyte phenotypes, as confirmed by RT-PCR, immunohistochemical staining, and ELISA. Moreover, the positive ratio of albumin-containing cells by immunocytochemical staining was about 30%, which is consistent with the study of Kakinuma *et al*[14]. All these indicate that after proliferation and differentiation, we could obtain many transplantable hepatic-like cells.

Although the lower immunogenicity of UCB stem cells has advantages in heterogenic transplantation, untreated UCB cells can sometimes cause serious immune rejection. How to resolve this problem is therefore a key point for further studies. Microencapsulation offers a

possibility to overcome the difficulty. This technique uses microcapsules such as APA microcapsules to coat target cells or organs, and is beneficial for heterogenic transplantation because its biocompatible and semi-permeable membranes are capable of intercepting substances with molecular weights above 11×10^4. Since Lim *et al*[15] first presented the concept of bio-microcapsules in 1980, artificial cell microcapsules as an effective barrier system for immunoprotection have been successfully applied in diabetes, parkinsonism, spinal cord injury, and peripheral nerve regeneration[15,16].

Our study examined coated hepatic-like cells derived from UCB by the APA microencapsulation technique. The obtained microcapsules exhibited a good smooth surface and integrated appearance. Furthermore, living cells inside the microcapsules were > 80% as determined by trypan blue staining. The mortality rate of AHF rats transplanted with microencapsulated hepatic-like cells significantly decreased in comparison with AHF rats transplanted with unencapsulated cells. In addition, there were significantly better outcomes in serum biochemical indexes such as ALT, AST and total bilirubin in the encapsulated group than in the PBS group, but no differences were observed between the encapsulated and the unencapsulated groups. Liver pathological staining supported these findings. The reason why the latter two groups showed no difference requires further exploration, although it is possibly related to the lower number of encapsulated cells. There have been some studies to support the notion that microcapsules provide the encapsulated cells with a good living space, and can significantly increase their survival time, therefore, we could theoretically reduce the number of transplanted cells[17]. Our data suggest that the transplantation of microencapsulated hepatic-like cells could offer a metabolic support to AHF rats in the short term, but it is not sufficient to interrupt or repair the damage of the recipient hepatocytes.

In our study, the pathological staining clearly showed liver recovery at 7 d after induction of AHF with D-galactosamine. At 2 wk post-transplantation, the morphological form of free microcapsules could be observed in the peritoneal lavage fluid, and showed round clear structures and smooth surfaces, and some microcapsule fragments were observed as well. HE staining revealed that some microcapsules attached to the greater omentum exhibited lymphocyte invasion surrounded with fibrous tissues. Although transplantation of microencapsulated hepatic-like cells could preliminarily alleviate the symptoms of AHF rats, their short lifespan and varying stability are still problems for the further use of the technique. The improvement in the airflow encapsulation system might be considered to yield sufficient uniformity in the size of microcapsules[18].

Transplantation of microencapsulated cells could provide a temporary metabolic support to AHF patients and/or be a transitional treatment, because its mechanism is not only related to the immunosuppressive and substitution effects of the transplanted cells, but is also associated with liver repair promoted by the transplanted cells. This new approach could provide a potential alternative for severe liver diseases.

COMMENTS

Background

With the continued increase in people with hepatic failure from cirrhosis and hepatocarcinoma, cell transplantation could offer metabolic support when liver function is damaged, and extend the waiting time for a liver donor. However, the cell sources for transplantation and the requirement for long-term immunosuppression have caused stagnation in this field.

Research frontiers

Alginate-poly-l-lysine-alginate (APA) microcapsules have been proved effective in protecting enclosed target cells from immune rejection following transplantation into experimental animals. Many studies have been conducted on the cell sources such as liver stem cells, embryonic stem cells, umbilical cord blood (UCB) cells and bone marrow stem cells.

Innovations and breakthroughs

The research team led by Professor Yu has established an artificial cell microcapsules platform, which is based on APA microcapsule technology and stem cell differentiation, to study the therapeutic effects of intraperitoneal transplantation of microencapsulated hepatic-like cells derived from UCB cells on AHF in rats. The effective immunoprotectivity of artificial cell microcapsules has been observed in this study, which suggests that the transplantation of microencapsulated hepatic-like cells could offer a metabolic support to AHF rats in the short term, but it is not yet sufficient to interrupt or repair the damage of the recipient hepatocytes.

Applications

Transplantation of microencapsulated cells could provide a temporary metabolic support to AHF patients and/or be used as a transitional treatment. This new approach could provide a potential alternative for severe liver diseases.

Terminology

UCB was obtained from full-term deliveries at the Obstetrics Department of Peking University Shenzhen Hospital. Hepatic-like cells were induced from UCB $CD34^+$ cells by culturing with FGF4 and HGF. Alginate-poly-l-lysine-alginate microcapsules have biocompatibility and semi-permeable membranes, and can intercept substances with molecular weights > 1.1×10^5.

Peer review

Zhang *et al* reported that $CD34^+$ cells sorted from human UCB cells were cultured for 16 d in a specific medium and could differentiate into hepatocyte-like cells. When the hepatocyte-like cells were encapsulated by alginate and intraperitoneally transplanted into rats with galactosamine-induced AHF, the number of surviving rats increased compared to that of control rats at 2 d after transplantation. Although the differentiation of $CD34^+$ cells derived from UCB to hepatocyte-like cells has been reported, it is interesting to use the peritoneal injection of alginate-encapsulated hepatocyte-like cells for the alleviation of AHF. If the preserved UCB cells are used for the treatment of AHF and related diseases, it will be beneficial to the patients.

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BRIEF ARTICLE

Primary clear cell carcinoma in the liver: CT and MRI findings

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Abstract

AIM: To retrospectively analyze the computed tomography (CT) and magnetic resonance imaging (MRI) appearances of primary clear cell carcinoma of the liver (PCCCL) and compare the imaging appearances of PCCCL and common type hepatocellular carcinoma (CHCC) to determine whether any differences exist between the two groups.

METHODS: Twenty cases with pathologically proven PCCCL and 127 cases with CHCC in the Second Affiliated Hospital of Sun Yat-sen University were included in this study. CT or MRI images from these patients were retrospectively analyzed. The following imaging findings were reviewed: the presence of liver cirrhosis, tumor size, the enhancement pattern on dynamic contrast scanning, the presence of pseudo capsules, tumor rupture, portal vein thrombosis and lymph node metastasis.

RESULTS: Both PCCCL and CHCC were prone to occur in patients with liver cirrhosis, the association rate of liver cirrhosis was 80.0% and 78.7%, respectively (P > 0.05). The mean sizes of PCCCL and CHCC tumors were (7.28 ± 4.25) cm and (6.96 ± 3.98) cm, respectively. Small HCCs were found in 25.0% (5/20) of PCCCL and 19.7% (25/127) of CHCC cases. No significant differences in mean size and ratio of small HCCs were found between the two groups (P = 0.658 and 0.803, respectively). Compared with CHCC patients, PCCCL patients were more prone to form pseudo capsules (49.6% *vs* 75.0%, P = 0.034). Tumor rupture, typical HCC enhancement patterns and portal vein tumor thrombosis were detected in 15.0% (3/20), 72.2% (13/18) and 20.0% (4/20) of patients with PCCCL and 3.1% (4/127), 83.6% (97/116) and 17.3% (22/127) of patients with CHCC, respectively. There were no significant differences between the two groups (all P > 0.05). No patients with PCCCL and 2.4% (3/127) of patients with CHCC showed signs of lymph node metastasis (P > 0.05).

CONCLUSION: The imaging characteristics of PCCCL are similar to those of CHCC and could be useful for differentiating these from other liver tumors (such as hemangioma and hepatic metastases). PCCCLs are more prone than CHCCs to form pseudo capsules.

Key words: Clear cell carcinoma; Hepatocellular carcinoma; Pathology; Magnetic resonance imaging; Computed Tomography; X-ray

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. It can be classified according to its histological architecture or cytological features. HCC includes various cytological types; the less common ones are clear cell type, spindle cell type, giant cell type, small cell type and squamous cell type[1,2]. Primary clear cell carcinoma of the liver (PCCCL) is rare, with a frequency varying between 2.2% and 6.7% among HCCs reported in the published literatures[3,4]. Due to the accumulation of glycogens and/or fats, the PCCCL cell cytoplasm is clear to hematoxylin-eosin staining. PCCCL may pose a diagnostic dilemma even with histological evaluation because the morphology of PCCCL cells is similar to that of extrahepatic clear cell tumors, such as clear cell cancers of the kidneys, adrenal glands, ovaries, thyroid, endometrium, uterine cervix, and vagina[5,6]. PCCCLs should be differentiated from metastatic clear cell cancer because their treatment strategies and prognoses are quite different. The prognosis of PCCCL is generally considered better than that of the common type of HCC (CHCC)[3,7,8].

Computed tomography (CT) and magnetic resonance imaging (MRI) are important examinations for the detection and characterization of liver tumors[9,10]. To our knowledge, the imaging features of PCCCL have rarely been reported in the English literature[11]. The purpose of this study was to describe the CT and MRI findings of PCCCL and compare them to CHCC to determine whether any differences exist between the two groups.

MATERIALS AND METHODS

Patients

Between January 2005 and August 2009, a total of 570 patients with primary HCC underwent hepatectomy at the Second Affiliated Hospital of Sun Yat-sen University. Twenty (3.5%) of these patients had pathologically confirmed PCCCL. The participants of this study included 20 patients with PCCCL and 127 patients with CHCC (randomly selected from the other 550 cases of primary HCC). No patient had received preoperative treatment, such as interventional therapy or chemotherapy.

Of the 20 patients with PCCCL, 14 had right upper abdominal pain, two complained of fatigue and four were asymptomatic. All patients with PCCCL were positive for HBsAg, and two were positive for anti-hepatitis C virus-IgG. The serum concentration of α-fetoprotein (AFP) was 5.8-68 787.0 μg/L for PCCCL patients, with a median of 149.9 μg/L. Of the 20 patients with PCCCL, 17 were AFP-positive (> 25 μg/L).

Pathologic examinations were retrospectively reviewed by an experienced pathologist. According to diagnostic criteria generally accepted by pathologists in China, PCCCL was diagnosed when clear cells accounted for more than 50% of the tumor[1,3,4,12].

Imaging protocols

CT or MRI examinations were performed no more than 5 days before hepatectomy. Thirteen patients with PCCCL and 73 patients with CHCC underwent dynamic CT examination using a spiral CT scanner (HiSpeed NX/I; GE Medical Systems, Milwaukee, WI) or a multi-detector CT scanner (Sensation 64; Siemens Medical Solutions, Erlangen, Germany). The scan parameters were as follows: 5-7 mm slice thickness reconstructions, 120-kV, 220-400 mA current, 25 cm field of view, and 256 × 256 matrix. Scans began at the dome of the diaphragm and proceeded in a caudal direction. After pre-contrast CT scans, the patients underwent dynamic contrast-enhanced scans. A bolus injection of 80-100 mL of non-ionic contrast medium (Iopamidol, Bracco, Milano, Italy) with a concentration of 350 mg I/mL was given via the antecubital vein at a rate of 3.5 mL/s. Images of the hepatic arterial phase (HAP), portal venous phase (PVP) and equilibrium phase (EP) were obtained at 25 s, 70 s and 120 s, respectively, after the injection of contrast agent.

Seven patients with PCCCL and 54 patients with CHCC underwent MRI studies with a 1.5-T MR unit (Gyroscan Intera, Philips Medical System, Best, the Netherlands). Unenhanced MR images included T1-weighted images with a water-selective excitation technique (FFE, TR 218ms, TE 4.9 ms, flip angle of 80, one acquisition) and turbo spin-echo T2-weighted images with fat saturation (TR 1600 ms, TE 70 ms, TSE Factor 24, three acquisitions). Five patients with PCCCL and 43 patients with CHCC underwent dynamic contrast-enhanced MR scans using a high-resolution turbo spin-echo sequence (TR 5.3 ms, TE 1.4 ms, flip angle of 40, 3.0-mm slice thickness, no gap, one acquisition) via a power injector; contrast agent was administrated at a rate of 2.5 mL/sec. HAP, PVP and EP scans were obtained at 20, 60, and 110 s, respectively. The other 13 patients (2 with PCCCL and 11 with CHCC) received manual injections of gadopentetate dimeglumine (Magnevist, Bayer Schering, Berlin, Germany) at a dose of 0.1 mmol/kg; post-contrast T1-weighted images were obtained at PVP (60-80 s after injection) with the same scanning parameters as the pre-contrast T1W scan. Regardless of the technique employed, axial and coronal images were acquired with 5.0-mm slice thickness.

Image interpretation

The CT and MRI images were retrospectively analyzed by two radiologists who have 10 and 15 years of experience in diagnosing abdominal diseases. Neither radiologist was aware of the patients' clinicopathological data. Reviews were performed jointly and by consensus. The presence of liver cirrhosis, tumor size, the enhancement pattern on dynamic contrast scanning, the presence of pseudocapsule, tumor rupture, portal vein thrombus, and lymph node metastasis were recorded. A typical HCC enhancement pattern was defined as early enhancement at HAP and rapid contrast medium washout at PVP or EP with hypo-attenuation/intense signal or iso-attenuation/intense signal[9,10].

Statistical analysis

Differences in mean age and tumor size were assessed

with an independent-samples *t* test. Differences in the frequencies of liver cirrhosis, tumor capsule formation, tumor rupture, typical enhancement pattern, portal vein tumor thrombus and lymph node metastases between the two groups were compared using the Chi-squared test or Fischer's exact test. A *P* value of 0.05 or less was considered significant. Statistical analysis was performed using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, USA).

RESULTS

The male-to-female ratio was 4.0:1 in the PCCCL group and 6.1:1 in the CHCC group. The mean age was 52.00 ± 10.09 years (range, 29-66 years) in the PCCCL group and 51.82 ± 13.20 years (range, 19-83 years) in the CHCC group. There were no statistical differences between the two groups regarding sex or age ($P = 0.733$ and $P = 0.953$, respectively).

Table 1 summarizes the imaging features observed in patients with PCCCL and patients with CHCC. Both PCCCL and CHCC were prone to occur in patients with liver cirrhosis, with a rate of 80.0% and 78.7%, respectively. The mean sizes of PCCCLs and CHCCs were 7.28 ± 4.25 cm (range, 2.0-15.9 cm), and 6.96 ± 3.98 cm (range, 1.0-17.0 cm), respectively. Small HCCs with diameters ≤ 3.0 cm were found in 25.0% (5/20) of PCCCL cases and 19.7% (25/127) of CHCC cases. No statistically significant differences in mean size or ratio of small HCC were found between the two groups ($P = 0.658$ and 0.803, respectively). Compared with CHCCs, PCCCLs were more prone to form pseudo capsules, with a rate of 49.6% and 75.0%, respectively ($P = 0.034$). Pseudo capsules showed hypo-attenuation/intensity haloes on pre-contrast scans and rim enhancement after contrast administration (Figures 1 and 2).

A higher percentage of tumor rupture was found in patients with PCCCL (15.0%, 3/20) than in patients with CHCC (3.1%, 4/127); however, there was no significant difference between the two groups ($P > 0.05$). Of the 20 PCCCL cases, three showed tumor ruptures. The ruptured tumors were 15.9 cm, 10.9 cm and 9.3cm in diameter and were located at the periphery of the liver with protruding contours. Two cases presented as discontinuities of the liver surface on CT scan (Figure 1). The remaining case presented a local hematoma at the rupture site on MRI, which appeared as mixed iso-/hypo-intense signals on T1WI and hypo-intense signals on T2WI with no enhancement after injection of contrast agent.

Typical HCC enhancement patterns were noted in 72.2% (13/18) of PCCCLs and 83.6% (97/116) of CHCCs; however, no significant difference was found between the two groups ($P > 0.05$) (Figures 1 and 3). The other five PCCCL cases showed atypical CT features on dynamic scan: two cases showed minimal enhancement and remained hypo-attenuated at HAP and PVP, while the other three cases showed gradual contrast enhancement during the portal phase.

Four patients (20.0%) with PCCCL had portal vein tumor thrombosis: one located at the left branch of the portal vein, one at the right branch, and one at the right anterior branch and main portal vein. Compared with CHCC patients, PCCCL patients showed a slightly higher incidence of portal vein tumor thrombosis (17.3% and 20.0%, respectively); however, there was no significant difference between the two groups ($P > 0.05$). No PCCCL patients and 2.4% (3/127) CHCC patients showed sign of lymph node metastasis ($P > 0.05$).

Table 1 Characteristics of clear cell hepatocellular carcinoma in the liver

Parameters	PCCCL (n = 20)	CHCC (n = 127)	P value
Sex			0.733
Male	16	109	
Female	4	18	
Liver cirrhosis			1.000
Positive	16	100	
Negative	4	27	
Tumor diameter (cm)			0.803
≤ 3.0	5	25	
> 3.0	15	102	
Capsule formation			0.034
Positive	15	63	
Negative	5	64	
Rupture			0.053
Positive	3	4	
Negative	17	123	
Typical enhancement pattern			0.399
Positive	13	97	
Negative	5	19	
Portal vein tumor thrombus			1.000
Positive	4	22	
Negative	16	105	
Lymph node metastases			1.000
Positive	0	3	
Negative	20	124	

PCCCL: Primary clear cell carcinoma of the liver; CHCC: Common type of hepatocellular carcinoma.

DISCUSSION

PCCCL is a specific and rare subtype of primary HCC. The reported incidence of PCCCL is 0.4%-37%; inconsistent diagnostic criteria may be responsible for the variable reports[1,3,4,7,8,12,13]. Lai *et al*[7] suggested that the diagnosis of PCCCL could be made even when the proportion of clear cells was < 30%, while Buchanan *et al*[8] suggested that PCCCL should be diagnosed when the proportion of clear cells was > 30%. Most studies diagnosed PCCCL when the proportion of clear cells was > 50%[1,3,4,12]. Using this criteria, PCCCL only accounts for 2.2%-6.7% of all resectable HCCs in most reports[3,4]. Among the 570 cases of primary HCC resected in our hospital, only 3.5% patients had PCCCL. The clear cell development is presumed to involve metabolic disorders and abnormalities of sugar metabolism[14,15].

The clinicopathological presentations of PCCCL were different from those of CHCC. The rates of hepatitis C infection and capsule formation were higher in PCCCL patients than in those with CHCC; however, no remarkable differences in patients' age, sex, AFP-positive rate or

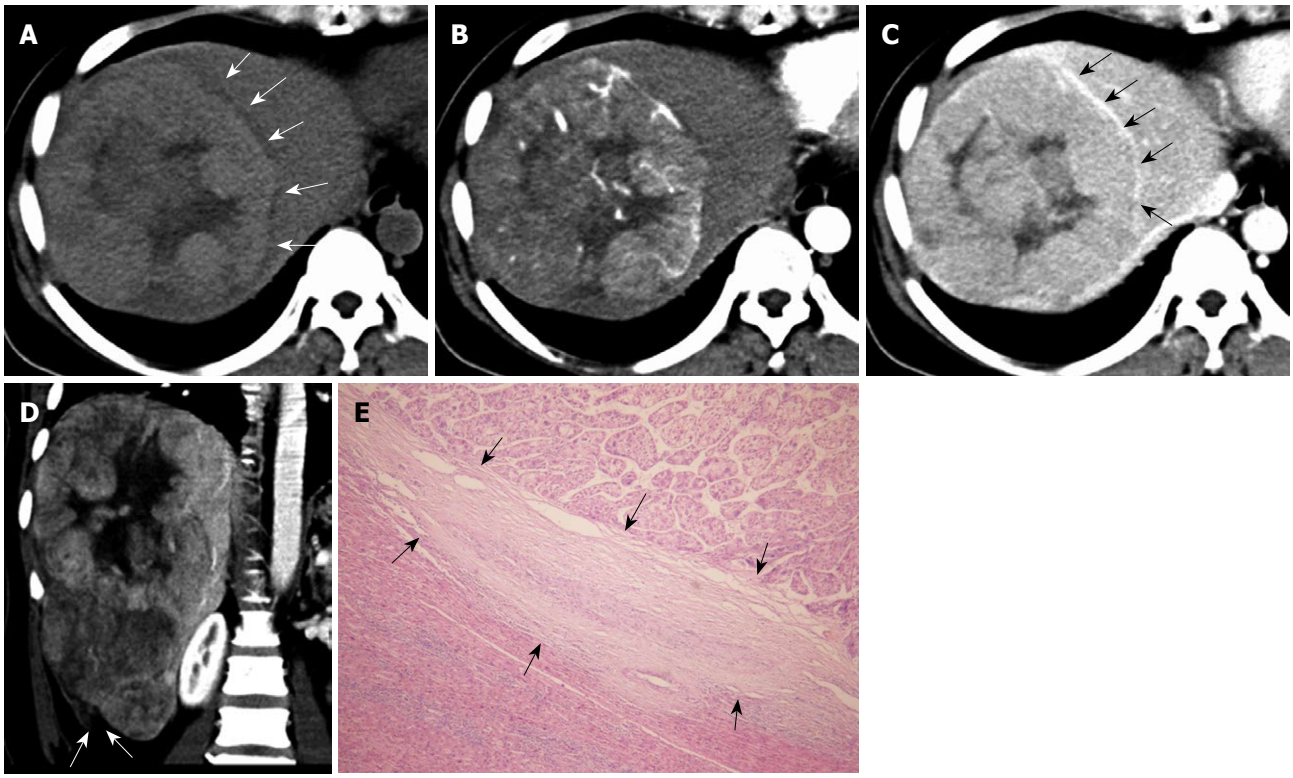

Figure 1 Primary clear cell carcinoma of the liver in a 47-year-old woman. A: On pre-contrast computed tomography scan, the mass shows slight hyper-attenuation with a hypo-attenuation halo (arrows); B: At hepatic arterial phase, the mass shows early enhancement; C: At the equilibrium phase, the mass presents hypo-attenuation with rim enhancement (arrows); D: At portal venous phase, the reconstructed coronal image shows the mass with a discontinuous liver capsule (arrows) at Segment Ⅵ, indicating tumor rupture, which was surgically confirmed; E: Pathologically, the mass shows a pseudocapsule (arrows) (HE, × 100).

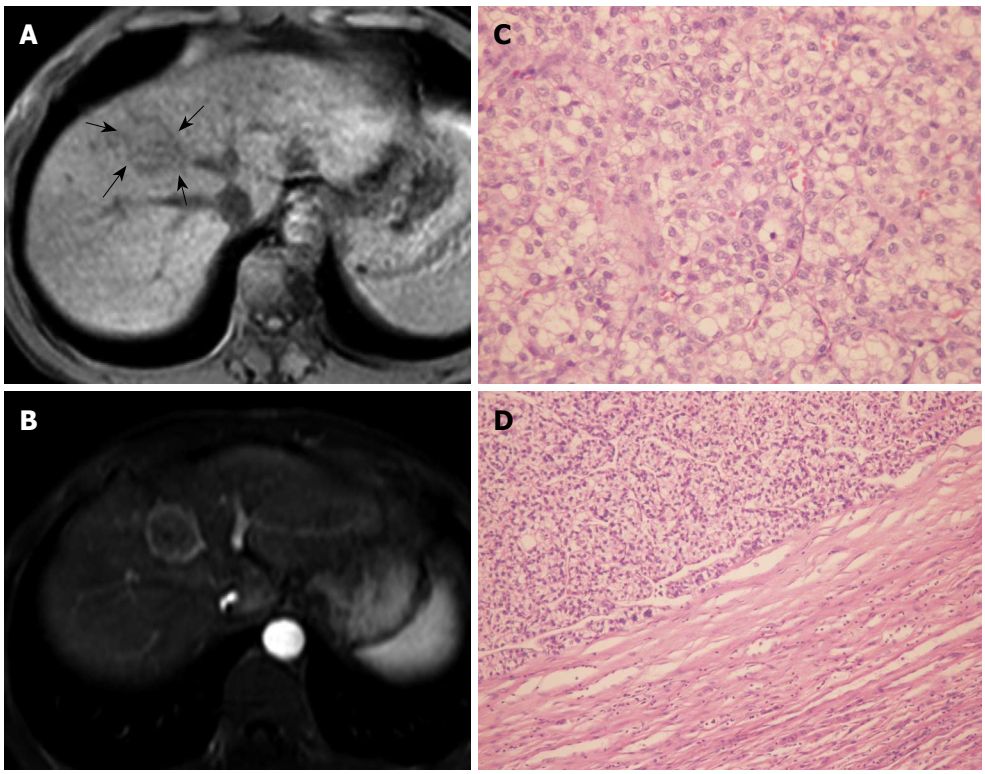

Figure 2 Primary clear cell carcinoma of the liver in a 29-year-old man. A: On T1WI, the mass shows slightly hypo-intense signals (arrows); B: At portal venous phase, the mass presents with rim enhancement (pseudocapsule); C: Pathologically, the mass is mainly composed of clear cells (HE, × 200); D: Pathologically, the mass shows a pseudocapsule (HE, × 100).

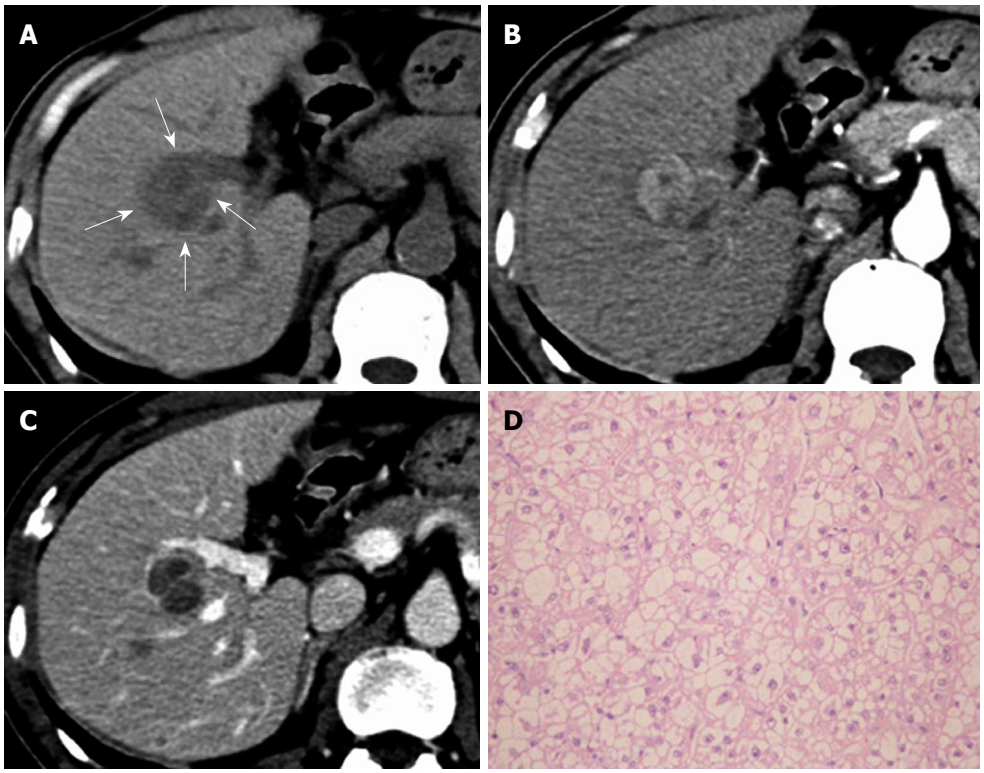

Figure 3 Primary clear cell carcinoma of the liver in a 62-year-old man. A: On pre-contrast computed tomography scan, the mass shows hypo-attenuation (arrows); B: At hepatic arterial phase, the mass shows early enhancement; C: At portal venous phase, the mass shows hypo-attenuation and thin rim enhancement (pseudocapsule); D: Microscopically, the mass is mainly composed of clear cells (HE, × 200).

the location, number, size and grade of tumors were observed between the two groups[3]. Both tumor types were prone to occur in patients with hepatitis B, mostly on the basis of liver cirrhosis[3]. PCCCL had a better prognosis than CHCC, mainly related to capsule formation, vascular invasion, preoperative liver function and clear cell proportion[3,4,12]. Surgical resection is an effective treatment for patients with PCCCL[3,4,7].

The presence of clear cells and fatty changes characterizes well-differentiated HCC in the early stage, and their ratio is presumed to decrease as the tumor enlarges[15]. In 1999, Monzawa *et al*[16] analyzed the pathologic and imaging changes of well-differentiated HCC; and found that some well-differentiated HCCs showed clear cell formation and/or fatty changes, which presented as high echo on ultrasound and hyper-intense signals on T1WI. However, in their study, the proportion of clear cells in the recruited HCC was less than 10%, or only 10%-50%, which did not meet the diagnosis criteria for PCCCL. In 2008, Takahashi *et al*[11] described CT, MR and angiographic findings of PCCCL in a woman with a normal liver. To our knowledge, no further research on the imaging manifestations of PCCCL has been conducted.

Pseudocapsule formation (consisting mainly of peritumoral hepatic sinusoids and/or fibrosis) is an important gross pathologic feature of HCC. Pseudocapsule indicates a relatively positive prognosis after tumor resection[17]. Liu *et al*[3] found a higher ratio of pseudocapsule formation in PCCCL than in CHCC microscopically (88.4% *vs* 68.0%, $P < 0.05$); and pseudocapsule formation might be related to a relatively lower degree of malignancy and a better prognosis for PCCCL. CT and MRI are reliable imaging examinations for the detection of HCC pseudo capsules. The pseudocapsule presents as rim enhancement on dynamic contrast scanning, and MRI is more sensitive than CT in identifying pseudocapsule[17-19]. Among the 20 cases of PCCCL in our study, 15 (75.0%) had pseudocapsule, all of which were confirmed pathologically. The percentage of pseudocapsule formation was higher in PCCCL patients than in CHCC patients ($P < 0.05$).

Because of hypervascular blood supply, typical HCC showed early enhancement at HAP, and rapid contrast medium washout at PVP or EP with hypo-attenuation/intense signal or iso-attenuation/intense signal[9,10]. Among the 18 PCCCL cases in our study that underwent dynamic contrast CT or MRI examination, 13 presented a typical HCC enhancement pattern, indicating that the tumor is rich of blood supply. The enhancement pattern of PCCCL is not different from that of CHCC ($P > 0.05$). This imaging characteristic may be useful in differentiating PCCCL from other liver tumors, such as hemangioma and hepatic metastases. The other five PCCCL cases presented atypical enhancement on dynamic CT scans: two cases showed minimal enhancement with hypo-attenuation at HAP and PVP, indicating hypovascularity, and three cases showed gradual contrast enhancement during the portal phase, which may be attributable to the difference in blood supply (such as existence of small arterioportal shunts), tumor differentiation or liver cirrhosis background[20,21].

Spontaneous rupture of HCC is usually life-threaten-

ing but relatively uncommon, with a reported incidence of 3%-15%[22]. CT is a valuable imaging technique for diagnosing HCC ruptures. The imaging findings include: discontinuity or disruption of the liver capsule adjacent to the liver mass and hematoma with hyper-attenuation at the rupture site. The enucleation sign is a specific sign for diagnosing HCC rupture[23,24]. To our knowledge, no report on PCCCL rupture is available for review. Among the 20 PCCCL cases in our study, only three had tumor rupture: two showed discontinuity of the liver capsule on CT scans, and the other showed a hematoma at the rupture site on MRI, with iso-/hypo-intense signals on T1WI and hypo-intense signals on T2WI.

Portal vein thrombosis, the characteristic growth pattern of HCC, occurs in 12.5%-39.7% of HCC patients[25]. Liu *et al*[3] reported that the microscopic vascular invasion rates are similar between PCCCL and CHCC (53.4% *vs* 65.0%, $P > 0.05$). In our study, the incidence of macroscopic portal vein tumor thrombus in PCCCL and CHCC detected on imaging examination was not significantly different ($P > 0.05$). Portal vein invasion was an independent risk factor for the prognosis of patients with PCCCL[12].

Chemical shift imaging is valuable for characterizing lesions with a mixture of water and fat[26]. Renal clear cell carcinomas usually contain fat, and present focal and diffused signal loss on chemical shift imaging. This imaging technique is helpful for differentiating renal clear cell carcinoma from other types of renal cancer[27,28]. The cell morphology of PCCCL is similar to that of renal clear cell carcinoma, with cytoplasmic accumulation of glycogens and/or fat. The signal reduction of HCC during chemical shift imaging may help identify intratumoral fatty components and confirm a diagnosis of PCCCL[2].

In summary, the imaging characteristics of PCCCL are similar to those of CHCC, including early enhancement and rapid washout of contrast agent on dynamic contrast scans, and presence of portal vein thrombus or tumor rupture. These imaging features may help differentiate PCCCL from other liver tumors, such as hemangioma and hepatic metastases. Pseudocapsule formation is more likely to occur in PCCCL than in CHCC and may be related to PCCCL's relatively lower degree of malignancy and better prognosis.

COMMENTS

Background

Primary clear cell carcinoma of the liver (PCCCL) is a specific and rare subtype of primary hepatocellular carcinoma (HCC), with a frequency varying between 2.2% and 6.7% among HCCs in the published literatures. PCCCL may pose a diagnostic dilemma even with histological sections because the morphology of PCCCL cells is similar to that of metastatic clear cell tumors. As a result of the paucity of cases, available data about its imaging findings are limited.

Research frontiers

Imaging modalities [computed tomography (CT) and magnetic resonance imaging (MRI)] are important for the detection and characterization of liver tumors. The imaging characteristics of common type hepatocellular carcinoma (CHCC) are well documented; for example, CHCC is usually associated with liver cirrhosis, typical enhancement pattern on dynamic contrast scanning (early enhancement at hepatic arterial phase and rapid contrast medium washout at portal venous phase or equilibrium phase) and the presence of pseudocapsule. However, the imaging features of PCCCL have not been unequivocally addressed. This study clarifies the CT or MRI findings of PCCCL.

Innovations and breakthroughs

The authors presented 20 surgically confirmed PCCCL cases and retrospectively analyzed their imaging findings. This study revealed that the imaging characteristics of PCCCL are similar to those of CHCC. PCCCLs are more likely to form pseudo capsules than CHCCs.

Applications

With a better understanding of the imaging features of PCCCL, further investigations should determine how to use imaging modalities, especially MRI, to differentiate PCCCL from CHCC or metastatic clear cell cancer. Chemical shift imaging with an MR scanner may help detect lipid component in the cytoplasm of clear cells in PCCCL.

Terminology

PCCCL is a rare variant of HCC. Due to the accumulation of large amounts of glycogen and/or lipids that are dissolved by routine histological processing (hematoxylin-eosin staining), the cytoplasm of PCCCL cells is clear. PCCCL can be diagnosed when the tumor cells are predominantly or wholly composed of clear cell cytoplasm (a proportion of clear cells > 50%). The prognosis of PCCCL is generally considered better than that of the CHCC.

Peer review

It is a well written paper, with interesting results.

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Meetings

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 4-5, 2011
13th Duesseldorf International Endoscopy Symposium, Duesseldorf, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011, Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
2nd International Congress on Abdominal Obesity, Buenos Aires, Brazil

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A whole-system strategic approach, Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine, Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-19, 2011
41. Kongress der Deutschen Gesellschaft für Endoskopie und Bildgebende Verfahren e.V., Munich, Germany

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform, Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 6-7, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 7-9, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 15-16, 2011
Falk Symposium 177, Endoscopy Live Berlin 2011 Intestinal Disease Meeting, Stauffenbergstr. 26, 10785 Berlin, Germany

April 18-22, 2011
Pediatric Emergency Medicine: Detection, Diagnosis and Developing Treatment Plans, Sarasota, FL 34234, United States

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 7-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course, Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV SPIGC, II ESYS, Napoli, Italy

June 14-16, 2011
International Scientific Conference on Probiotics and Prebiotics-IPC2011, Kosice, Slovakia

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 29-2, 2011
XI Congreso Interamericano de Pediatria "Monterrey 2011", Monterrey, Mexico

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
New Advances in Inflammatory Bowel Disease, La Jolla, CA 92093, United States

September 10-14, 2011
ICE 2011-International Congress of Endoscopy, Los Angeles Convention Center, 1201 South Figueroa Street Los Angeles, CA 90015, United States

September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
Cardiology & Gastroenterology | Tahiti 10 night CME Cruise, Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week, Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course, Washington, DC 20001, United States

November 11-12, 2011
Falk Symposium 180, IBD 2011: Progress and Future for Lifelong Management, ANA Interconti Hotel, 1-12-33 Akasaka, Minato-ku, Tokyo 107-0052, Japan

December 1-4, 2011
2011 Advances in Inflammatory Bowel Diseases/Crohn's & Colitis Foundation's Clinical & Research Conference, Hollywood, FL 34234, United States

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Acknowledgments

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Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixudiarrhoea. *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

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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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