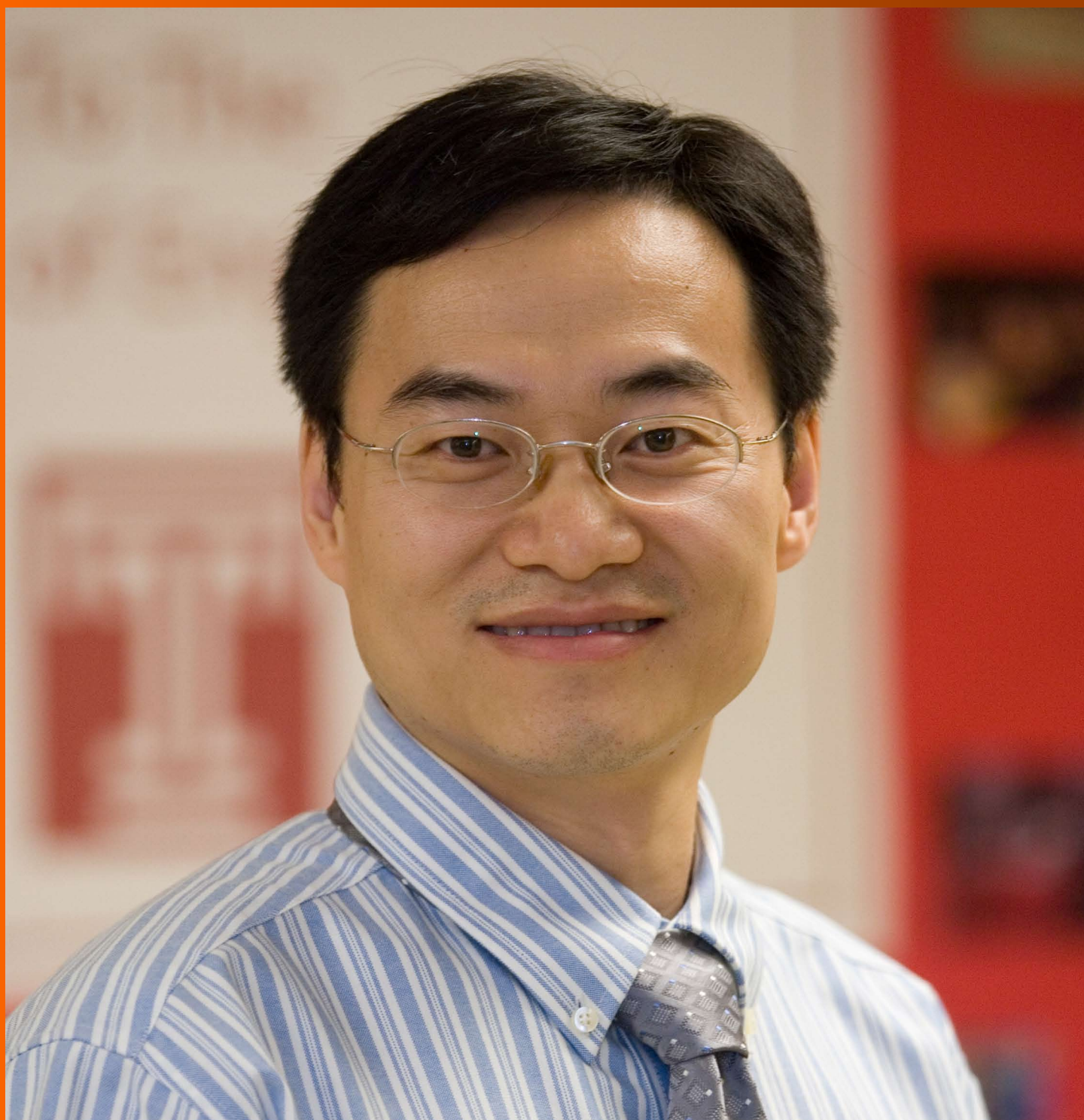


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Histone deacetylases, microRNA and leptin crosstalk in pancreatic cancer

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Abstract

Because pancreatic cancer (PC) historically has had poor prognosis and five year survival rates, it has been intensely investigated. Analysis of PC incidence and biology has shown a link between different risk factors such as smoking, alcoholism, and obesity and disease progression. Important factors affecting PC include the epigenomic changes driven by DNA methylation and histone acetylation, and actions of microRNA inducing oncogenic or tumor suppressor effects. Studies have identified markers whose dysregulation seem to play important roles in PC progression. PC markers involve classical histone deacetylases (HDAC), PC stem cell (PCSC), and leptin. In this review, we discuss the role of several PC biomarkers, and the potential crosstalk between HDAC, microRNA, and leptin in PC progression. Dysregulated expression of these molecules can increase proliferation, survival, PCSC, resistance to chemotherapy and tumor angiogenesis. The potential relationships between these molecules are further analyzed using data from The Cancer Genome Atlas and crosstalk pathways generated by the Pathway Studio Platform (Ariadne Genomics, Inc.). Oncogenic miRNA21 and tumor suppressor miRNA200 have been previously linked to leptin signaling. Preliminary analysis of PC biopsies and signaling crosstalk suggests that the main adipokine leptin could affect the expression of microRNA and HDAC in PC. Data analysis suggests that HDAC-microRNA-leptin signaling crosstalk may be a new target for PC therapy.

Key words: Pancreatic Cancer; MicroRNA; Histone

deacetylases; Pancreatic cancer stem cell markers; Leptin; Obesity

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Core tip: Pancreatic cancer has no targeted therapy. Obesity is a risk factor for pancreatic cancer, characterized by high levels of leptin. In this review, we discuss the potential crosstalk between histone deacetylases, microRNA, and leptin in disease progression. Crosstalk among these molecules increases proliferation, survival, cancer stem cells and resistance to chemotherapy. The potential relationships between these molecules are analyzed using data from the Cancer Genome Atlas and the Pathway Studio Platform. The crosstalk among these molecules could be a novel target for pancreatic cancer prevention or treatment, particularly in obese patients that show elevated levels of leptin.

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INTRODUCTION

Pancreatic cancer (PC) is a malignant disease, which is difficult to treat. It is a silent disease that can go undetected for long periods of time; however, when diagnosed, it is often in advanced stages (III or IV)^[1]. PC incidence and mortality rates vary across different racial/ethnic groups, with the highest rates found in African Americans, and the lowest in Asian Americans/Pacific Islanders. Moreover, PC incidence rate is higher in African Americans when compared to European Americans at every age^[1]. Risk factors for the development of PC include tobacco usage, continuous exposure to such chemicals as dyes and pesticides, family history, age, epigenetic changes, and obesity^[1-5]. The best outcomes from PC treatments are obtained after complete surgical resection, with no residual disease; this can improve 5 year survival, but only from 5% to about 20%-25%^[6]. Even patients who are eligible for surgical treatment with tumor free margins often experience recurrence and eventually require palliative treatment^[7,8]. Surgical resection of PC is performed on patients with locally advanced or borderline resectable tumors. Improved outcomes may be achieved with a multimodal approach, combining neo-adjuvant chemotherapy with radiation therapy and surgery. Adjuvant therapy includes 5-fluoruracil (5-FU) or capecitabine; gemcitabine induction followed by concomitant chemoradiation with either gemcitabine or 5-FU; FOLFIRINOX (an aggressive chemotherapeutic regimen, including several chemotherapeutic agents)

or gemcitabine-Nab paclitaxel (albumin bound) with or without subsequent chemoradiation. For patients with metastatic PC, the treatment options are very limited. It mainly consists of palliative care (pain and nutrition management), as well as chemotherapy. The chemotherapeutic regimens for metastatic PC (gemcitabine alone or in combination with other agents for example, FOLFIRINOX, Nab-paclitaxel) have only modest results, improving the survival of these patients by only a few months^[9,10].

Important factors affecting PC are the changes in the epigenome driven by DNA methylation and histone acetylation. Epigenetic changes are alterations in gene expression or cellular phenotype that occur without changes in the DNA sequence. Some of the epigenetic changes are DNA methylation and histone acetylation. This last process is characterized by the addition of acetyl groups to the lysine residues of the histones *via* histone acetyltransferases (HAT). Histone acetylation is essential to gene regulation, and is usually associated with the relaxed form of chromatin. Lysine residues can also be deacetylated by histone deacetylases (HDAC). These enzymes are involved in cancer progression by increasing proliferation, survival and resistance to chemotherapy of cancer cells as well as angiogenesis^[11-15].

The dysregulation of microRNAs is another factor involved in cancer progression^[16-18]. MicroRNAs (miRNA or miR) are noncoding endogenous RNAs that regulate protein expression. Accumulating data show important relationships between dysregulated miRNAs and cancer^[16-19]. The effect that miRNAs dysregulation has on the cancer cells determines whether these molecules are considered oncogenics or tumor suppressors. Oncogenic miRNAs promote cancer development through various signaling mechanisms while tumor suppressor miRNAs have contrary effects and their expression is decreased in cancer^[19,20]. There are many oncogenic microRNAs (*e.g.*, miR21) that have been reported to play a role in cancer progression^[20-23]. Furthermore, the decreased expression of tumor suppressor miR200 family has been associated with PC progression^[24,25].

Obesity is one the most observed risk factors for cancer progression. Obesity is a growing pandemic, and is associated with more than 100000 incidences of various cancers in the United States, particularly breast, colon, endometrium and PC^[26-28]. Obesity is characterized by the accumulation of excessive body fat, and a body mass index (BMI) value greater than 30. Obesity is also characterized by high levels of leptin, which has been consistently associated with many cancers, including PC^[29-33]. Preliminary analyses suggest that leptin could affect the expression of microRNA and HDAC in PC.

Because of the absence of targeted therapies for obese PC patients, there is a need to better understand the mechanisms behind the disease progression in order to develop better treatment strategies. Thus, in this review, we will discuss the potential relationships between HDAC, microRNA, cancer stem cells, and leptin

signaling in PC.

PC TYPES

There are two types of PC - those that comprise tumors arising from the endocrine pancreatic cells and those that arise from the exocrine pancreatic cells. Cancers of the endocrine pancreas are rare and represent less than 4% of all PC cases^[1]. Pancreatic Adenocarcinoma (PA) is the most common type of PC and usually begins in the ducts of the pancreatic glands. PC has been recently classified into four main subtypes based on their genomic analysis (*e.g.*, squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine or ADEX)^[34].

PC squamous subtype is characterized by four core genes programs involved in inflammation, hypoxia response, metabolic reprogramming, transforming growth factor- β (TGF- β) signaling, autophagy, and upregulated expression of TP63 Δ N and its target genes^[34]. The pancreatic progenitor subtype is characterized by the transcriptional networks of pancreatic and duodenal homeobox-1 (PDX1), motor neuron and pancreas homeobox-1 (MNX1), hepatocyte nuclear factor-4- α (HNF4A), hepatocyte nuclear factor-1- β (HNF1B), hepatocyte nuclear factor-1- α (HNF1A), forkhead box-A2 (FOXA2), forkhead box-A3 (FOXA3), and hairy and enhancer of split-1 (HES1) transcription factors^[34,35]. The immunogenic subtype of PC is characterized by changes in the programs of immune genes that include antigen presentation, CD4⁺ and CD8⁺ T cells, and toll-like receptor and B cell signaling pathways^[8]. ADEX is characterized by the upregulation of transcriptional networks of both exocrine and endocrines lineages that are important in later stages of pancreatic development and differentiation^[34].

In addition, some hereditary factors play roles in the development of PC. Individuals with a high risk of developing PC can be divided into underlying gene defect, like cyclin-dependent kinase Inhibitor 2A (CDKN2A), breast cancer gene 1 and 2 (BRCA1/2), partner and localizer of BRCA2 (PALB2), and serine/threonine kinase 11 (STK11) mutations^[35,36]. In a study performed by Vasen *et al.*^[36], the longterm outcome of prospective surveillance of a large group of CDKN2A/p216-Leiden carriers and, BRCA1/2 and PALB2 mutation carriers, and individuals at risk (IARs) for familial PC (FPC) was evaluated. The main goal of the study was to determine whether or not surveillance will lead to the detection of early stage PC or the detection of relevant precursors lesions (PRLs) as well as to assess if their program leads to improvements in prognosis^[36]. Based on the surveillance, it was determined that PRLs were more frequent in patients with FPC than those with CDKN2A/p216-Leiden mutation^[36]. The surveillance study also reveals that the resection of screen detected PC with CDKN2A/p216-Leiden mutation carriers was 75%, which is higher than that reported for patients with sporadic PC (15%-20%)^[36]. Overall, the study demonstrated that the surveillance of CDKN2A mutation carriers was successful

for the detection of PC at the resectable stage^[36].

PC BIOMARKERS

PC is generally diagnosed when approximately 30% of patients present a locally advanced disease^[1]. Because there is no effective treatment for advanced PC, this disease should be detected in the early stages when treatment could significantly increase the percentage of patients with five years of survival. The best way to PC early diagnosis would be *via* the usage of screening biomarkers with high specificity and sensitivity. Currently, the most established and used biomarker is CA19-9. However, CA19-9 detection is not highly specific for PC, as it can also be detected in colorectal cancer, stomach, and biliary epithelium and chronic pancreatitis^[1,37,38].

A vast array of other PC biomarkers has been investigated, but so far none are as yet widely used clinically. It has recently been shown that exosomes could potentially impact on the pathogenesis of PC through the modulation of tumor growth, microenvironment, and immune response. This suggests that exosomes could be used as biomarkers for PC^[39]. An additional PC marker could be the leptin receptor, OB-R, which has been detected in PC cell lines^[40]. Moreover, OB-R expression was positively correlated with the matrix metalloproteinase-13 (MMP-13) in human PC tissues. The increased expression of either OB-R or MMP-13 was significantly associated with lymph node metastasis; it also tends to be associated with the TNM stage in PC patients^[40].

Likewise, it has been proposed that the detection of PC cells in blood could be used as a surrogate for PC detection^[41,42]. Circulating tumor cells (CTC) could be related to metastatic and more aggressive PC disease, according to the results from an international multicenter randomized study that included 79 patients. A subgroup of PC patients was screened for CTCs before the start of the chemotherapy, and after two months of treatment. Overall, CTC detection was found in 11% of PC patients and associated with poor tumor differentiation ($P = 0.04$), and with shorter overall survival ($RR = 2.5$, $P = 0.01$). Therefore, CTC detection might be a new way to detect PC^[38].

HISTONE DEACETYLASES IN PC

HDAC play a major role in the regulation of gene expression *via* epigenetics changes. HDAC catalyze the removal of an acetyl group, which stimulates chromatin condensation, thus suppressing transcription. Currently, 18 HDAC family members have been identified in the human genome, which are grouped into four classes (I - IV)^[43]. HDAC are also classified into two major types: Sirtuins (SIRT) and classical HDAC. Classical HDAC include Classes I, II, and IV, whereas the sirtuins comprise Class III^[43,44] (Table 1). HDAC classes I, II, and IV are zinc dependent metalloproteins, while class III are nicotinamide adenine dinucleotide (NAD⁺) dependent

Table 1 Classification of classical histone deacetylases

Class	Members	Cellular localization	Function in cancer ^[13,14,45,46,104]	Substrates ^[43,44,104]
I	HDAC1	Nucleus	Proliferation, survival and resistance to chemoresistance	P53, E2F-1, Stat3, and androgen
	HDAC2	Nucleus	Proliferation and survival	Bcl-6, Stat3, YY-1, and glucocorticoid receptor
	HDAC3	Nucleus	Proliferation and anti-differentiation	GATA-1, RelA, Stat3, MEF2D, YY-1, and SHP
	HDAC8	Nucleus	Proliferation and anti-differentiation	ERRα, Inv (16), and CREB
II A	HDAC4	Nucleus/cytoplasm	Angiogenesis and anti-differentiation	GCMa, GATA-1, and HP-1
	HDAC5	Nucleus/cytoplasm	Anti-differentiation	Smad7, HP-1, and GCMa
	HDAC7	Nucleus/cytoplasm	Angiogenesis and migration	FLAG-1, and FLAG-2
	HDAC9	Nucleus/cytoplasm	Cell survival	ATDC (TRIM29)
II B	HDAC6	Cytoplasm	Angiogenesis and migration	Alpha-Tubulin, HSP-90, SHP, Smad7
	HDAC10	Cytoplasm	Angiogenesis	HSP90
IV	HDAC11	Nucleus/cytoplasm	Tumor immune response	OX40L

HDAC: Histone deacetylases.

enzymes^[43]. Class I HDAC family consists of HDAC 1, 2, 3, and 8. These enzymes are mainly located in the cellular nucleus. Class II HDAC family is divided into two groups - Classes II A and II B. These HDAC are mainly located in the cytoplasm, but can also be found in the nucleus, which is dependent on their phosphorylation status influencing their shuttle mechanism^[43,44]. Subclass II A HDAC family consists of HDAC 4, 5, 7, and 9; while subclass II B consists of HDAC 6 and 10. HDAC Class IV is only made of HDAC11 that is mainly located in the nucleus. Class III is composed of SIRT1, SIRT2, SIRT3, SIRT4, SIRT5, SIRT6, and SIRT7, which are located in the nucleus, cytoplasm, and mitochondria^[38]. Due to the role of HDAC in epigenetic regulations and their effect on chromatin structures, many studies have found them linked to cancer progression^[13,14,45,46]. The classical HDAC have been associated with cancer progression through the increase of proliferation, survival and resistance to chemotherapy of cancer cells, and angiogenesis. More studies suggest the roles of HDAC in PC progression. The use of HDAC inhibitors is a novel avenue toward targeted therapy for PC. Several HDAC inhibitors are currently under clinical trials for cancer-targeted treatment. However, currently there are only three FDA approved HDAC inhibitor drugs available [Vorinostat or Suberoylanilide Hydroxamic Acid (SAHA), Zolinetide, Romidepsin (Depsipeptide, ISTODAX), and Belinostat (Beleodaq)]^[47-50].

Vorinostat was the first FDA approved anti-HDAC drug^[47,49,51]. It is a hydroxamic acid based drug that inhibits Class I, II, IV HDAC by chelating the zinc cofactor. This drug shows apoptotic and anti-proliferative effects by modifying the expression of specific genes related to insulin-like growth factor-1 receptor signaling receptor (IGF-1R)^[47,49,52]. The second FDA approved HDAC inhibitor drug is Romidepsin, which was effective in phase II clinical trials when used with gemcitabine for treatment of advanced PC^[49,53-55]. The third FDA approved HDAC inhibitor drug is Belinostat. It showed a dose dependent growth inhibitory or pro-apoptotic effects promoting cell cycle arrest at the G0/G1 or S phase transition^[56-58]. Additionally, positive results in the treatment of PC have been reported with the use of benzamide derivative HDAC inhibitor (Class I HDAC inhibitor MGCD0103)

selective for Class I and IV HDAC^[59]. PC cell lines treated with MGCD0103 showed dose dependent growth arrest, apoptosis, and induction of p21, which mediated cell cycle arrest in G2/M phase^[59].

TUMOR SUPPRESSOR AND ONCOGENIC MIRNAS IN PC

MicroRNAs (miRNA or miR) are noncoding endogenous RNAs of 14-24 nucleotides that have the ability to regulate protein expression at the post-transcriptional level. Many studies have found strong correlations between dysregulated microRNAs and cancer^[17,18,60]. According to the effect that miRNAs dysregulation has on the cancer cells, these molecules are considered oncogenic or tumor suppressors. There are many oncogenic microRNAs, such as miR1290, miR24, miR134, miR146a, miR378, miR484, miR628-3p, miR1825^[61] and miR21^[20-23] that have been reported to play a role in cancer progression. It was reported that serum levels of miR1290 distinguished patients with low-stage PC from controls better than CA19-9 levels^[61]. Furthermore, decreased expressions of miRNA34^[62] and miR200^[20] family have been associated with PC progression.

A study found that oncogenic miR21 was expressed in the early stage of PA^[63]. Furthermore, knockdown of miR21 using lentiviral vectors inhibited cell proliferation in PC derived cell lines. In addition, miR21 was found to protect PC cell from apoptosis, and its knockdown resulted in the activation of mitochondrial pathway apoptosis *via* the downregulation of Bcl9 (a protein involved in Wnt Pathway), upregulation of Bax, and induction of Bim^[63]. Targeting miR21 *in vivo* strongly inhibited PC growth, which led to the suggestion that simultaneous standard gemcitabine chemotherapy combined with miR21 targeting could improve the prognosis of PC

MiRNA200 family consists of five members (miR200a, b, c and miR429, and miR141)^[64]. *In vitro* studies suggested that miR200c expression was related to low cancer invasion^[20]. MiRNA200 activities include inhibition of epithelial-mesenchymal-transformation (EMT), repression of cancer stem cell (CSC) self-renewal and differentiation,

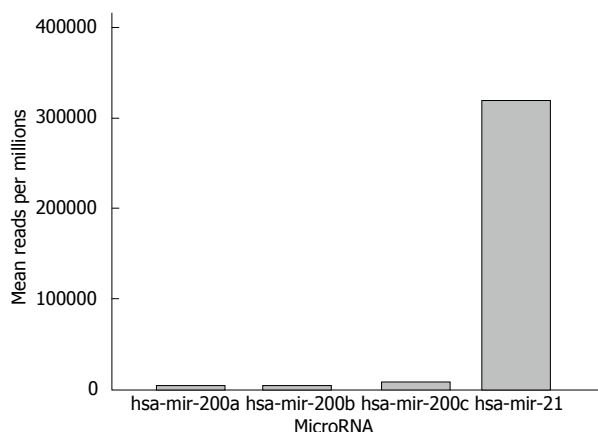


Figure 1 IlluminaHiSeq miRNA expression of tumors tissues biopsies from pancreatic cancer patients. The data sets used were generated from the the Cancer Genome Atlas Database^[71]. The oncogenic miR21 is highly expressed in PC while there is a low expression of tumor suppressor miR200a/b/c (pancreatic cancer samples $n = 45$).

modulation of cell division and apoptosis, and involvement in chemoresistance. High level of miR200c correlated to better survival rates^[20]; however, miR200a and miR200b, were hypomethylated and overexpressed in PC^[65]. It has been suggested that targeting miRNA200 upregulation could improve PC prognosis if used together with the chemotherapeutic drug gemcitabine^[20]. Indeed, treatment of PC cells with a curcumin analogue, CDF, improved gemcitabine effects by upregulating miR200 and downregulating miR-21 expression. These effects were found together with the downregulation of Akt, cyclooxygenase-2, prostaglandin E2, vascular endothelial growth factor, and nuclear factor- κ B DNA binding activity, and induction of PTEN^[66].

Transcriptor factor ZFH family (ZEB1 and ZEB2)^[67] represses the expression of epithelial genes. miR200 members increased Notch activation by ZEB1 that regulates the expression of Jagged1 and the mastermind-like coactivators Maml2 and Maml3. Moreover, in PC and breast cancer cells, decreased miR200 expression was associated with increased Jag1 and ZEB1 levels^[68]. Therefore, MiR200 inhibits EMT by interacting with ZEB1/2 and the Notch pathway, represses self-renewal and differentiation in CSCs, and is involved in the regulation of cell division and apoptosis^[64]. In turn, ZEB1 suppresses the expression of miR200 family, which inhibits the translation of ZEB1 mRNA, resulting in the double-negative ZEB/miR200 feedback loop^[69]. Additionally, in lung cancer Jagged2 inhibits the expression of miR200 family by induction of GATA transcription factors, which promotes tumor metastasis^[70].

We preliminarily analyzed PC biopsies using TCGA databank^[71]. Data analysis shows higher miR21 expression compared with miR200 in PC (Figure 1). These data suggest that progression of PC could be positively associated with upregulation of miR21 and downregulation of miR200.

PC STEM CELLS

PC is usually diagnosed in the advanced stages after distant metastasis has already occurred in most cases. PC shows high frequency of local relapse, even after surgical resection. Treatment of PC *via* surgery and chemotherapy has historically had little success. Patients still have a poor survival rate with chemoresistance and reoccurrence of the disease as significant factors. These features of PC could be related to the action of PC stem cell (PCSC)^[72]. Cancer stem cells are a small population that have the capacity to self-renew, and generate cells with identical tumorigenic potential that could also differentiate to form the bulk of the tumor cells, thereby contributing to the formation of heterogenic cellular composition of cancers^[55]. A highly tumorigenic PCSC population (CD24⁺CD44⁺ESA⁺) was described in PC for the first time by Li *et al.*^[73] in a xenograft human model. PCSC are resistant to chemotherapy and contribute to tumor initiation, growth and metastasis^[74]. PCSC were also identified as CD133⁺ population that is highly resistant to standard chemotherapy. A subgroup of these cells, CD133⁺CXCR4⁺ was found to be involved in metastasis^[75].

C-Met, also known as hepatocyte growth factor receptor (HGFR), is an oncogene involved in the progression of cancer. C-Met was earlier described as a potential PCSC marker^[75-77]. C-Met⁺ PCSC showed similar tumorigenic capacity as CD24⁺CD44⁺ESA⁺ population^[78-80]. C-Met is a heterodimer that consist of an extracellular α -chain bound through a disulphide bridge to a transmembrane β -chain^[79]. It is also a tyrosine kinase found in the cell membrane. HGF ligand binding to C-Met immunoglobulin like-domain induces C-Met dimerization, leading to autophosphorylation of the two tyrosine residues within the catalytic loop^[79]. Subsequently, further autophosphorylation of two more tyrosine residues occurs in the C-terminal of c-Met receptors, which provides the platform for the recruitment of other molecular factors and signal conveyors like Grb2-associated binding protein 1 (Gab1). This provides a binding site for such SH2-containing effectors as SHP2, PLC γ L, STAT3, Ras GTPase, and PI3K9^[79]. With the emerging evidence of c-Met as a stem cell marker, some studies were able to identify part of the c-Met cell population that also expresses CD44, CD24, CD133, and ALDH1^[78,81]. However, in a study c-Met⁺ PCSC produced tumors in 35% of cases when compared to PCSC CD133⁺ (16%) and CD44⁺ (25%) of cases^[78-80].

Notch signaling pathway is another factor that affects the maintenance of PCSC. Notch signaling pathway is a known regulator of the balance between cell self-renewal and cell differentiation. Abel *et al.*^[82] found that components of Notch signaling were upregulated in PCSC. Moreover, the inhibition of Notch signaling pathway with gamma secretase inhibitors or Hes1 shRNA in PCSC reduced the percentage of PCSC and their ability to form tumorspheres. Furthermore, these authors found that the activation of Notch signaling pathway

using an exogenous peptide ligand greatly increased the percentage of PCSC and formation of tumorspheres^[82].

Due to PCSC role in chemoresistance and disease recurrence, c-Met, CD44, CD24, CD133, and ALDH1 could potentially be used as biomarkers to detect PC progression. Moreover, developing therapies that would target PCSC markers could be adjuvant to the standard gemcitabine chemotherapy, which could improve PC survival rate^[72,78].

OBSESITY AND PC

Obesity is mainly the result of unhealthy diets and lifestyles, and has proven to be a contributing factor to higher risk and poor prognosis of cancer^[1,83,84]. Several studies have examined the impact that obesity has on the overall survival rate of PC patients^[84,85]. Some studies have determined that obesity in adulthood significantly shortened the overall survival of PC patients, whereas obesity at diagnosis was not associated with increased risk of death^[84,85]. In another study, Sandini *et al.*^[86] assessed whether the evaluation of different body compartments and their relationships were associated with the development of major postoperative complications after pancreatoduodenectomy for cancer. It was found that the prevalence of sarcopenia (loss of muscle tissue related to aging) was 24.2%. Overall, sarcopenic obesity^[86] and non-sarcopenic obesity^[87] are strong predictors of major complications after pancreatoduodenectomy for cancer.

Obese PC patients have the poorest prognosis, and often develop chemoresistance. Obesity is recognized as a co-morbidity factor to cancer and there is great interest in understanding the mechanism linking this condition and cancer. In this regard, a recent study has found that obesity promoted desmoplasia associated with accelerated PC growth and impaired delivery/efficacy of chemotherapeutics through reduced perfusion *in vivo*^[87]. Furthermore, the inhibition of angiotensin-II type-1 receptor (AT1) reversed obesity-augmented desmoplasia and PC growth and improved response to 5-FU chemotherapeutic *in vivo*^[87]. In addition, clinical studies have shown that excess weight alters PC micro-environment to augment the crosstalk between cancer associated adipocytes, tumor associated neutrophils, and pancreatic stellate cells, which subsequently lead to increased tumor progression and survival^[87].

LEPTIN AND PC

A potential link between obesity and PC could be the major adipokine leptin. A crosstalk between leptin and Notch (an embryonic signaling pathway altered in PC) has been reported in PC lines. Moreover, leptin induces PC tumorspheres formation and expansion of PCSC^[81,88]. Leptin is a small cytokine secreted by adipose tissue that is coded by the obese (*ob*) gene. Leptin has been the most studied adipokine since it was first cloned in 1994^[89]. Leptin is an adipokine that regulates appetite, energy intake and expenditure. Leptin plays many roles, some of

which involve regulation of glucose homeostasis, growth response, reproduction and immune response^[90]. The level of circulating leptin is proportional to total body fat. Obese patients exhibit high circulating levels of leptin due to leptin resistance^[91]. Leptin is a pleiotropic adipokine and pro-inflammatory molecule that belongs to the family of helical cytokines. It is structurally similar to interleukin (IL)-6, IL-12, IL-15, prolactin, GH, oncostatin M, and granulocyte CSF^[92].

Leptin receptor, OB-R, is a product of diabetic (*db*) gene that shows six alternatives spliced isoforms, including a long isoform (OB-RL, OB-Rb or LEPR) with full intracellular signaling capabilities, shorter isoforms with less biological activity (OB-Rs or OB-Ra) and a soluble isoform (OB-Re or sOB-R)^[93,94]. Both the long and short isoforms of OB-R are expressed in PC cell lines^[4]. Moreover, PC cells secreted leptin and expressed OB-R, which indicates a leptin autocrine/paracrine signaling loop could also affect tumor progression^[95]. The binding of leptin to OB-R activates a cascade of events that promotes tumor progression and cancer cell survival^[3,30,31]. Leptin binding to its receptor triggers an activation cascade of several canonical (JAK2/STAT3, MAPK, PI-3K/AKT1) and non-canonical signaling pathways (p38MAK, JNK and AMPK)^[29,88,96].

A nested case control study from three cohort studies of middle-aged adults showed that high pre-diagnostic circulating leptin concentrations were associated with an increased PC risk among those with longer follow-up^[97]. In another study, Mendonsa *et al.*^[4] showed the contribution of obesity and leptin to PC growth by using an *in vivo* orthotopic murine PC model. These studies revealed the increase of tumor growth in diet-induced obese mice when compared to lean mice.

We have recently showed that leptin and Notch crosstalk could influence PC progression. Our data suggest that a functional leptin-Notch axis affects PC progression and expansion of cancer stem cells (PCSC) in PC cell lines (BxPC-3, MiaPaCa-2, Panc-1, AsPC-1) and derived tumorspheres. Leptin treatment increased cell cycle progression and proliferation, and the expression of Notch receptors, ligands and targeted molecules (Notch1-4, DLL4, JAG1, Survivin and Hey2), PCSC markers (CD24/CD44/ESA, ALDH, CD133, Oct-4), ABCB1 protein, as well as tumorsphere formation. PC has no targeted therapy and is mainly treated with chemotherapy, whose efficiency could be decreased by leptin and Notch activities. Thus, the leptin-Notch axis could be a novel therapeutic target, particularly for obese PC patients^[95].

LEPTIN-HDAC-MICRORNA-CANCER STEM CELLS CROSSTALK

Resistance to leptin is observed in obese people who show high levels of leptin. Precise reasons explaining why some obese patients are leptin-resistant are not fully known. Some studies have suggested that leptin resistance could be due to abnormalities of the leptin molecule while others believe the resistance might be due to impairment of OB-R

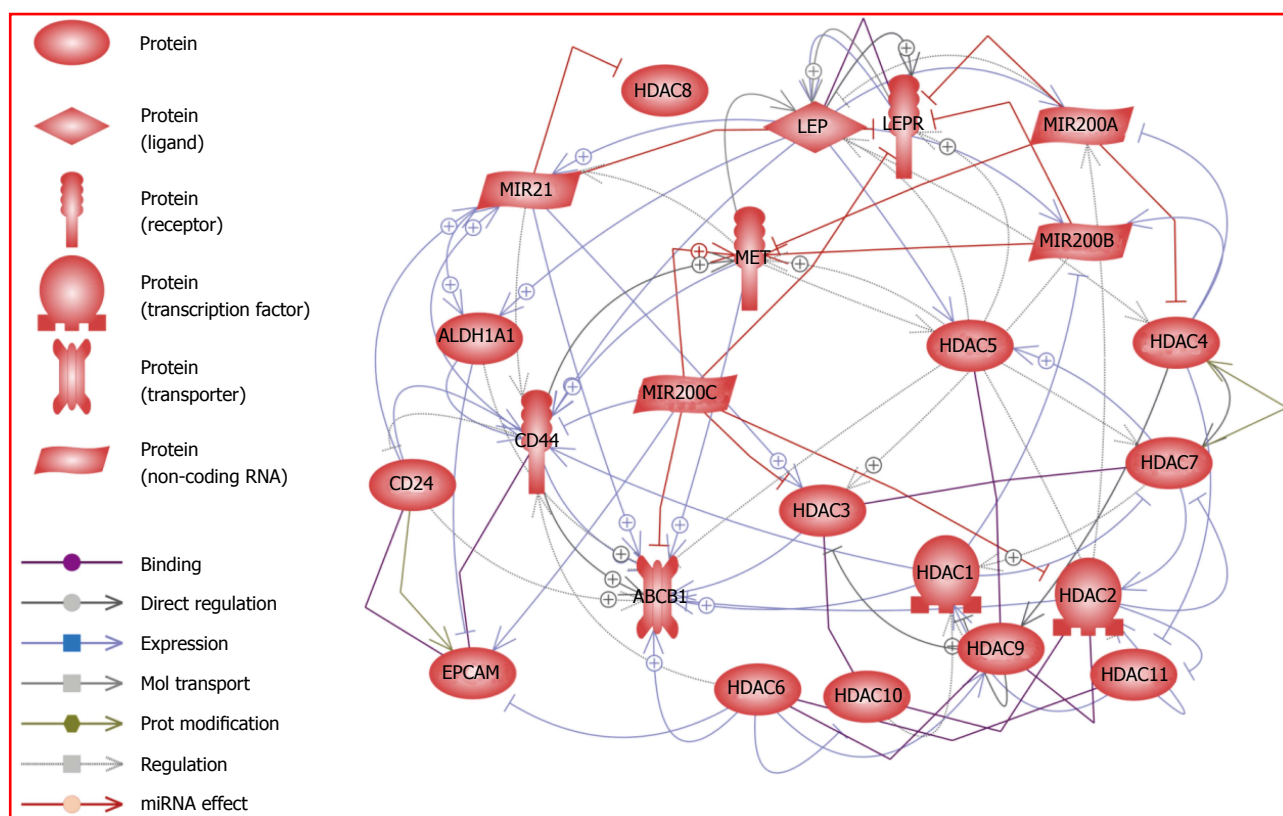


Figure 2 Potential crosstalk between leptin signaling, cancer stem cells, histone deacetylases and microRNA. Leptin and its receptor LEPR (OB-R) are involved in the regulation of PCSC markers, Classical HDAC, miR21, and miR200a/b/c. HDAC 1, 2, 3, 8: Histone Deacetylases Class I; HDAC 4, 5, 7, 9: Histone Deacetylases Class IIA; HDAC 6, 10: Histone Deacetylases Class IIB; HDAC11: Histone Deacetylase Class IV. MiR200a, miR200b, and miR200c: Tumor Suppressors MicroRNA; MiR21: Oncogenic MicroRNA. CD24, CD44, ALDH1A1, ABCB1, MET, and EPCAM: Pancreatic Cancer Stem Cell Markers. HGF: Hepatocyte growth factor cytokine; LEP: Leptin adipokine; LEPR: Leptin receptor. Data generated from Pathway Studio (Pathway Studio – web; Ariadne Genomics, Inc.). Genes were analyzed by Pathway Studio 11 software (Elsevier, Inc., Atlanta, GA, United States) for disease, cellular processes and miRNA interactions. Only genes that had a P value of 0.05 were reported in this study. Specific references supporting these relationships are shown in Supplemental Table 1. References found by Pathway Studio were exported into an Excel file, column D, that contains the PMID number for the citations.

function or deficient leptin transport. Leptin is a known proliferation factor for cancer^[3,29,31,96]. Analysis of data from Pathway Studio Platform shows that leptin signaling could promote PC through crosstalk mechanisms that involve PCSC, classical HDAC, oncogenic microRNA21, and tumor suppressor microRNA200a/b/c (Figure 2 and Supplemental Table 1).

A relationship between leptin signaling and miR21 in cutaneous wound healing was earlier reported^[98]. The expression of miR21 and miR200 was previously linked to leptin hypothalamic signaling^[16,99,100]. It was shown that the use of a pegylated leptin antagonist predisposed the rats to obesity and promoted leptin resistance in the both hypothalamus and liver. RT-PCR data from these studies showed that miR200 was upregulated in rats treated with leptin antagonist^[16]. Additionally, miR21 (oncogenic) and miR200 (tumor suppressor) have been shown to affect PC progression^[20,63]. The potential relationships between leptin signaling and miR21 and miR200a/b/c regulation in PC are shown in Figure 2. Leptin signaling is involved in the crosstalk to many important oncogenic and tumor suppressor molecules. Previous studies have determined that leptin increases the expression of PCSC markers ALDH1 and CD44. Leptin has also been

found to increase the expression of miR21 while the tumor suppressors miR200a, miR200b, and miR200c decrease the expression of OB-R. Interestingly, these tumor suppressors could also interact with some of PCSC markers (Met, ABCB1, CD44), which decrease their expression. In contrast, oncogenic miR21 increases the expression of ALDH1, ABCB1 and CD44 markers. With regard to the classical HDAC, only HDAC5 and HDAC4 were reported to be directly regulated by leptin signaling (Figure 2). However, leptin signaling could indirectly affect the expression of some of HDAC via microRNA or PCSC markers. Further analysis suggests that leptin increases the expression of miR21, which, in turn, could increase the expression of HDAC3. The combined action of these factors could promote cancer proliferation and the expression of an anti-differentiation phenotype (Supplemental Table 1).

Our published data show that leptin increased PCSC populations that correlated with growth of PC tumorspheres and resistance to gemcitabine^[88,95]. Furthermore, leptin induced PCSC populations (CD24⁺CD44⁺ESA⁺, CD133⁺, ALDH⁺) in MiaPaCa-2 PC cells. Additionally, in Panc-1 cells, leptin increased mostly CD133⁺ PCSC. Moreover, leptin increased ABCB1 (an ATP Binding Transporter Protein linked

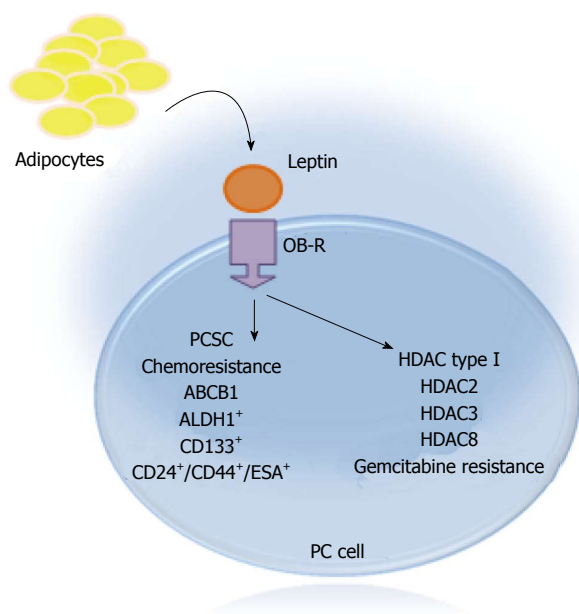


Figure 3 Leptin effects on pancreatic cancer stem cells and histone deacetylases in pancreatic cancer tumorspheres. Representative cartoon of the effects of leptin on PC cells *in vitro*. Leptin induced the expression of PCSC markers (CD24⁺/CD44⁺/ESA⁺, CD133⁺ and ALDH1⁺). Leptin also increased the levels of ABCB1 [P-glycoprotein 1 or multidrug resistance protein 1 (MDR1) or ATP-binding cassette sub-family B member 1], which is involved in chemoresistance. Additionally, leptin induced the expression of HDAC type I (HDAC 2, 3 and 8). Leptin attenuates the cytotoxic effects of gemcitabine on PC. PC cells were cultured in low attachment plates containing mammo cult media (Stem Cell Technol.), which allow the growth of tumorspheres. The tumorspheres were treated for 6 d with leptin (1.2 nmol/L), IONP-LPrA2 (a leptin antagonist bound to iron oxide nanoparticles; 0.0072 pmol/L), and gemcitabine (2 μ mol/L). PC viability, PCSC markers and HDAC expression were determined by flow cytometry. Experiments were repeated three times^[32,33,81,95].

to chemoresistance) expression in PC tumorspheres^[95]. These data suggest that leptin could play a role in the induction of PCSC and PC chemoresistance (Figure 3).

Several studies have found that classical HDAC are overexpressed in PC. Therefore, HDAC inhibition has become a potential target therapy for cancer^[101,102]. Intriguingly, high expression of HDAC Class I and II in PC could be associated with obesity. It was found that the hypothalamic expression of classical HDAC was increased in obese mice fed a high fat diet^[100,103]. Thus, it is possible that the increase in classical HDAC in obese mice could be related to leptin signaling. To initially explore the potential relationships between leptin signaling and HDAC, leptin effects on HDAC expression was preliminary determined in PC tumorspheres. Results from these experiments show that leptin increased the expression of HDAC3 and HDAC8 in BxPC-3 tumorspheres (Figure 3). Furthermore, preliminary data suggest that gemcitabine decreased the expression of HDAC2, HDAC3 and HDAC8 that was reverted by leptin. This suggests that leptin could affect the expression of HDAC in PC, which might be associated with chemoresistance.

CONCLUSION

PC is an aggressive disease commonly detected in its

late stages, continues to show poor prognosis, and has no targeted treatment. Surgical tumor removal is the best option to eliminate PC, but only in limited number of cases. Therefore, most PC patients are treated with chemotherapeutics, but survival rates have historically been poor. Obesity is a modifiable risk factor of PC that is characterized by inflammation and high levels of the adipokine leptin, which is a cancer proliferation factor that can also contribute to chemoresistance. Studies have identified that the dysregulation of HDAC, miR21, miR200, leptin, and PCSC could play important roles in PC progression. Previous reports showed that leptin signaling can induce PC proliferation, PCSC expand and regulate miR21, miR200, and HDAC levels. Moreover, the analysis of data from PC biopsies (Cancer Genome Atlas)^[71] showed inverse expression profiles for miRNA21 and miRNA200 that suggests these molecules could be involved in PC development. Furthermore, HDAC, miRNA21/200, and leptin could have complex signaling crosstalk, according to Pathway Studio analysis. Therefore, leptin, miR21, miR200 and HDAC could be involved in PC progression. Thus, the potential crosstalk among these molecules could be a novel target for PC prevention or treatment, particularly in obese patients who show elevated levels of leptin.

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Multidisciplinary approach of colorectal cancer liver metastases

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Abstract

Large bowel cancer is a worldwide public health challenge. More than one third of patients present an advanced stage of disease at diagnosis and the liver is the most common site of metastases. Selection criteria for early diagnosis, chemotherapy and surgery have been recently expanded. The definition of resectability remains unclear. The presence of metastases is the most significant prognostic factor. For this reason the surgical resection of hepatic metastases is the leading treatment. The most appropriate resection approach remains to be defined. The two step and simultaneous resection processes of both primary and metastases have comparable survival long-term outcomes. The advent of targeted biological chemotherapeutic agents and the development of loco-regional therapies (chemoembolization, thermal ablation, arterial infusion chemotherapy) contribute to extend favorable results. Standardized evidence-based protocols are missing, hence optimal management of hepatic metastases should be single patient tailored and decided by a multidisciplinary team. This article reviews the outcomes of resection, systemic and loco-regional therapies of liver metastases originating from large bowel cancer.

Key words: Colorectal cancer; Chemoembolization; Liver metastases; Hepatic resection; Colorectal cancer liver metastases; Chemotherapy; Arterial infusion chemotherapy; Radioembolization

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Core tip: Improvements of colorectal cancer liver

metastases (CRC-LM) treatment allows the down-staging of several patients. There is currently no agreement in the correct sequence of surgical resection of the primary cancer and metastatic disease. Surgical resection can be performed if the complete removal of cancer is achievable, leaving an adequate normal liver tissue. Neoadjuvant chemotherapy is widely accepted as primary therapy. Chemotherapy may lead to disease regression for unresectable CRC-LM, allowing resection and cure. The application of loco-regional therapies is increasing. They are recommended as third-line treatment for unresectable CRC-LM and have a palliative intent.

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INTRODUCTION

Colorectal cancer (CRC) is an increasing global health issue^[1,2] It is the most common gastro-intestinal tumor and the third most frequently diagnosed malignancy worldwide. It has a mortality rate of up to 10%^[1,2]. Most recent epidemiological data show more than 1.4 million newly diagnosed CRC each year^[1,2].

The liver is the most common site of CRC metastases with an incidence of 15%-20% at diagnosis. CRC patients have a > 50% probability of liver metastases development^[3]. The majority of CRC liver metastases (CRC-LM) were defined not resectable in the past century. Surgery methods are considerably improved nowadays, resulting in cure or survival increase. CRC-LM resection rates are also increased^[4]. Recent updating of resectability criteria of CRC-LM considerably improves outcomes, resulting in 5 and 10-year survival rates of 40% and 25% respectively^[5,6].

Notwithstanding these good outcomes, the recurrence rate one year after metastasis resection is 30% and a recent study on CRC-LM survival after resection shows a 5-year survival of 16%-71%^[7].

Neoadjuvant chemotherapy allow initially unresectable CRC-LM patients to have long term survival similar to those of resectable patients^[8-12]. Chemotherapy efficacy, in terms of tumor reduction, is strongly correlated to resectability^[10-13]. For this reason, chemotherapy associated to biological agents is increasingly used as resectability conversion of CRC-LM from unresectable to resectable. This method can efficiently increase downsizing rates^[14,15].

Candidate selection for resection is still difficult and several CRC-LM patients are never referred to hepatobiliary multidisciplinary group^[10,13]. For this reason CRC-LM patients need a multidisciplinary team for treatment decision. This team should include specialists from different disciplines: Oncology, surgery, radiology and radiotherapy. The purpose

of this review is to examine the current management of CRC-LM, in order to better define potential advantages and limitations of the several available treatments.

PERIOPERATIVE EVALUATION

The perioperative evaluation of a patient's global health and liver function is essential to reduce postoperative complications. A dedicated multidisciplinary team should assess co-morbidities and patient's performance status in order to decide a future treatment plan. Complete blood examination should be performed before surgery, to assess liver function [alanine aminotransferase (ALT), glutamic-oxalacetic transaminase (AST)], coagulation profile, bilirubin, creatinine and tumor markers, such as carcinoembryonic antigen (CEA).

Exclusion criteria for surgery include several factors to guarantee patient safety. They include advanced age, male gender, low serum albumin, presence of liver disease (hepatitis or alcoholic hepatitis), ascites, kidney or cardiologic impairment, bleeding syndromes, and chronic obstructive pulmonary disease^[16-18].

Morbidity and mortality after liver resection is often due to inadequate function of remnant liver, leading to liver failure. Morbidity and mortality rates are around 61% and 11%, respectively^[16,17].

The remnant liver cannot sustain metabolic, synthetic, and detoxifying functions if reduced below a critical liver volume^[18]. Liver volume is not the best index for liver functionality assessment^[16-20]. Patients with concomitant liver disease may have impaired liver regeneration capacity due to cirrhosis, steatosis, or jaundice obstruction^[20].

Most chemotherapeutic agents (5-fluorouracil, irinotecan, oxaliplatin) can result in hepatic damage and modification of liver regeneration^[11,19-22].

Morbidity and mortality after liver resection may be improved by measuring the intake of 99mTc mebrofenin of tumor-free liver in a pre-operative setting, in order to assess the risk of liver failure and liver failure-related mortality after partial liver resection^[17].

During liver regeneration induced by partial hepatectomy, normally quiescent hepatocytes start to replicate in order to restore the original liver. Several genes are involved in liver regeneration, including cytokine, growth factor and metabolic genes^[21]. Several studies show that recurrence and progression are directly proportional to the amount of liver resected^[22,23].

Neoadjuvant chemotherapy may induce hepatic changes, such as steatohepatitis, hepatic sinusoidal obstruction and periportal inflammation, negatively affecting patient outcome^[20,21] and increasing the risk of liver failure and death after major liver resection. A normal liver can bear an extensive resection. Severely compromised livers, on the contrary, cannot tolerate even a minor hepatectomy^[8,9,19]. For this reason, monitoring the functionality of surrounding tumor-free liver needs to be highly considered for selection of surgical method.

RADIOLOGICAL ASSESSMENT

CRC-LM radiological study is necessary for assessment of surgical resectability. This can be performed using any of these main radiological methods: Magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET) scan^[24]. Liver metastases can be detected as hypoattenuating lesions, when using contrast-enhanced normal or multidetector CT scans with a sensitivity rate of 85% and 90%, respectively^[25]. MRI performed with liver-specific contrast agents has > 90% sensitivity in cases of underlying liver disease (steatosis, cirrhosis) or very small lesions (< 1 cm). For this reason MRI is better than CT for metastasis detection^[26].

Specificity of CT, MRI and PET is very high: 95%, 93%, and 97% respectively. PET scan is useful to obtain whole body map, to identify extrahepatic disease (EHD) and to assess resectability^[24]. A recent study showed that the FDG PET scan is the best radiological modality for detecting CRC-LM. It can have high false negative rates in patients recently treated with chemotherapy^[17,18]. The association of CT to FDG PET scans is highly recommended because it improves the sensitivity up to 97%^[24].

Nowadays, also intraoperative ultrasound (IOUS) is a mandatory surgical tool to confirm preoperative investigations by CT or MRI and for detection of missed lesions^[27].

CRITERIA FOR RESECTABILITY

The preferred therapy for CRC-LM is surgery, providing up to 50% survival at five-years^[28]. Patient selection criteria for resectability are not standardized and still controversial in clinical practice. The American Hepato-Pancreato-Biliary Association (AHPBA) consensus on definition of resectability is currently accepted by most liver surgeons^[28,29]. Main CRC-LM resection criteria of AHPBA are: Presence of disease confined to the liver as identified after surgery of primitive cancer; disease in a single hepatic lobe; < 3 nodules; the largest size of nodules < 5 cm in diameter; margin FLR > 1 cm. According to these criteria, however, less than 10% of patients would be indicated for resection.

The classification of resectable disease is broader nowadays, increasing the number of resections^[30]. Current guidelines generally agree that resection should be performed for liver metastases only^[12,30,31], but hepatectomy and resections of concomitant extrahepatic disease are considered^[32]. The remaining liver must be undamaged and at least 20% or 25% of the whole hepatic volume, and have a full functional vascular and biliary in- and out- flow. In this case also multiple resection can be performed^[8,14,30]. The survival advantage of repeated resection is close to that after the surgery of primary hepatic disease^[33]. Hepatic resection safety depends on: Age of patients, performance score, and concomitant hepatic impairments. Resection is contraindicated when the following are observed: Non resectable extra hepatic tumor; wide involvement of parenchyma; or patient's poor

general conditions.

Possible prognostic factors of resection outcome of CRC-LM are: Age, sex, synchronous or metachronous hepatic metastases, tumor size, number and distribution of LM, primary tumor stage, extrahepatic distant metastases, surgical margin, type of primary hepatic tumor surgery and previous tumor pharmacological therapy, levels of tumor markers.

Fong *et al*^[34] report data from 1001 CRC-LM patients who were candidates for resection. These data led to the identification of seven criteria for worse prognosis prediction after resection. Five of these criteria are actually used for the Clinical Risk Score (CRS) that is a preoperative scoring system. These criteria are: Disease-free interval from primary to metastases < 12 mo; largest hepatic tumor > 5 cm in diameter; node-positivity; number of lesions > 1; and CA 19-9 > 200 ng/mL. Positive prognosis after surgery corresponds to a score < 2. Scores of 3-4 indicate that patients are candidates for resection followed by adjuvant therapy. Prognosis is poor when the score is five. The appropriateness of CRS is proved. CRS can predict patients' response and OS^[35].

A new method has been recently introduced in the CRC-LM resectability criteria assessment^[5]. Resection criteria are different. They depend less on the size, number, and location of the metastases. They give more importance to the volume and function of the future liver remnant (FLR), which should be > 25% estimated normal liver parenchyma or 30% in the presence of impaired liver function^[36]. Metastases are considered resectable if the excision of all metastatic lesions can be obtained with an adequate FLR^[37] and the presence of EHD is currently no longer considered as a contraindication^[5]. The new requirements for LM resection are: R0 resection achievement of intrahepatic and extra hepatic disease; adequate FLR; and > 2 adjacent liver segments to be spared with blood and bile inflow and outflow preservation^[31,37].

TIMING OF COLON AND LIVER RESECTION

The best sequence and timing of CRC-LM resection is still under debate and many options are available. The use of up front chemotherapy is increasing. Strong evidence is missing and there are currently no randomized controlled trials comparing the different approaches^[38].

The classic surgical method is "primary first", whose suggested sequence is to firstly resect the primary CRC, then to administer the chemotherapy and after 3-6 mo to eventually resect the LM. This approach is indicated for patients with advanced or symptomatic CRC, important comorbidities, or inadequate FLR. In cases of advanced CRC, indeed, the chemotherapy may be associated with high complication rates and the insurgence of disease progression may lead to unresectability^[39]. Any delay correlated to complications during surgery of CRC may also increase the risk of progression occurrence for some patients^[40,41]. A possible benefit of this method can be

Table 1 Recommendations for perioperative and conversion therapy (adapted from ESMO 2016^[110])

<p>Perioperative treatment</p> <p>It is defined by technical criteria for resection and prognostic considerations</p> <p>It may not be necessary in patients with clearly resectable disease and favourable prognosis, in this case upfront resection is justified</p> <p>It should administer FOLFOX or CAPOX to patients with resectable disease and unclear (probably unfavourable)</p> <p>Targeted agents should not be used in resectable patients with prognostic indication for perioperative treatment</p> <p>It should be considered when prognostic and resectability criteria are unclearly defined, and in patients with synchronous onset of metastases</p> <p>Adjuvant chemotherapy is not strongly indicated for patients with favourable oncological and surgical criteria, who did not receive any neoadjuvant chemotherapy</p> <p>Adjuvant chemotherapy is indicated for patients with unfavourable criteria</p> <p>Adjuvant treatment with FOLFOX or CAPOX is recommended for patients who have not received any previous chemotherapy, unless patients already received oxaliplatin-based adjuvant chemotherapy</p> <p>The choice of chemotherapy type should consider patients' clinical conditions and therapy preferences</p> <p>Conversion therapy</p> <p>A chemotherapy regimen leading to high response rates and/or a large tumour shrinkage is recommended for potentially resectable patients</p> <p>The best drug combination to use is still not clear because only few trials have addressed this issue:</p> <p>RAS wild-type patients may benefit from a cytotoxic doublet plus an epidermal growth factor receptors agents antibody (best benefit/risk), and from the combination of FOLFOXIRI plus bevacizumab and, to a lesser extent, from a cytotoxic doublet plus bevacizumab</p> <p>RAS mutant patients may benefit from a cytotoxic doublet plus bevacizumab or FOLFOXIRI plus bevacizumab</p> <p>Patients must be re-evaluated regularly (every 2-3 mo) to prevent the overtreatment of resectable patients</p>

the possibility to identify previously occult LM that may become visible during adjuvant chemotherapy. This allows avoidance of the morbidity of a liver resection.

Another surgical method is the "synchronous resection of LM and primary CRC". This approach can avoid delays in chemotherapy treatment that can be started earlier if no complications occur after surgery. The possible disadvantage of this method is the increased postoperative morbidity and mortality because of infection resulting from bacterial contamination of the surgical field^[36]. For this reason this approach is indicated for patients who can tolerate long operative times^[6].

The third available surgical method is the "alternative staged liver-first" approach that firstly resect the LM, then administer 3-6 cycles of chemotherapy, and at last resect the primary CRC. Adjuvant chemotherapy can be administered in between both procedures. Recent data report that this method is indicated for selected patients with advanced CRC-LM, and when neo-adjuvant and adjuvant chemotherapy may have better results^[9,12].

CHEMOTHERAPY FOR RESECTABLE CRC-LM

Neo-adjuvant chemotherapy

The utility of neoadjuvant chemotherapy for CRC-LM is unclear even if there is the tendency to use it frequently^[15]. There are many advantages of neo-adjuvant treatment such as increasing tumor sensitivity, downstaging large or multiple liver lesions, increasing resectability, and treating micrometastases^[8,9,11]. This therapy also allows better planning for the date of surgical resection.

On the other hand, neo-adjuvant chemotherapy can delay surgical treatment, which may be detrimental for patients, increasing the risk of disease progression^[12,15]. This chemotherapy can also induce liver toxicity, such as steatohepatitis, increasing postoperative mortality. It can also mask metastases on preoperative imaging, as is observed in 5%-25% of cases^[42].

Perioperative chemotherapy is widely used for patients with unresectable disease (Table 1 and Figure 1) with the purpose of reducing disease progression, which occurs in 50%-70% of patients after surgery^[3]. A multicentre randomized trial compared surgery alone with perioperative chemotherapy (6 cycles of preoperative and post operative FOLFOX4) in 364 unresectable CRC-LM patients. The results of this study showed no significant differences in five-year OS for the two groups; nevertheless, progression-free survival (PFS) increased by 7.3% at 3 years in the perioperative chemotherapy group^[43]. The rate of post-operative complications is also increased and is directly proportional to the length of therapy. For this reason, it is suggested that only 6 cycles of chemotherapy for no longer than 3 mo should be performed, in order to reduce toxicity^[28], especially for patients who need a major hepatectomy^[44].

Patients with more than 3 lesions, and tumor diameter greater than 3 cm are clearly indicated for this treatment. The surgery of lesions should be done 4-8 wk after the neo-adjuvant chemotherapy. In summary, the advantages of neo-adjuvant chemotherapy outnumber the disadvantages, and we are in favor of its utilization.

Adjuvant chemotherapy

The ultimate dilemma after complete CRC-LM resection is the rate of recurrence that is reported as high as 60% after complete surgical excision. Several studies show the benefits of adjuvant therapy such as FOLFOX4 (folinic acid, fluorouracil, and oxaliplatin), resulting in longer disease-free-survival (DFS)^[45] than liver resection alone.

Adjuvant chemotherapy also increases OS when compared to surgery alone, even if the difference is not statistically significant^[46,47].

The classic adjuvant chemotherapeutic drugs are: 5-fluorouracil/leucovorin (5-FU/LV), capecitabine, oxaliplatin and irinotecan^[47]. New molecular-targeted agents are now available. They include anti-angiogenic drugs (bevacizumab, regorafenib and aflibercept) and anti-

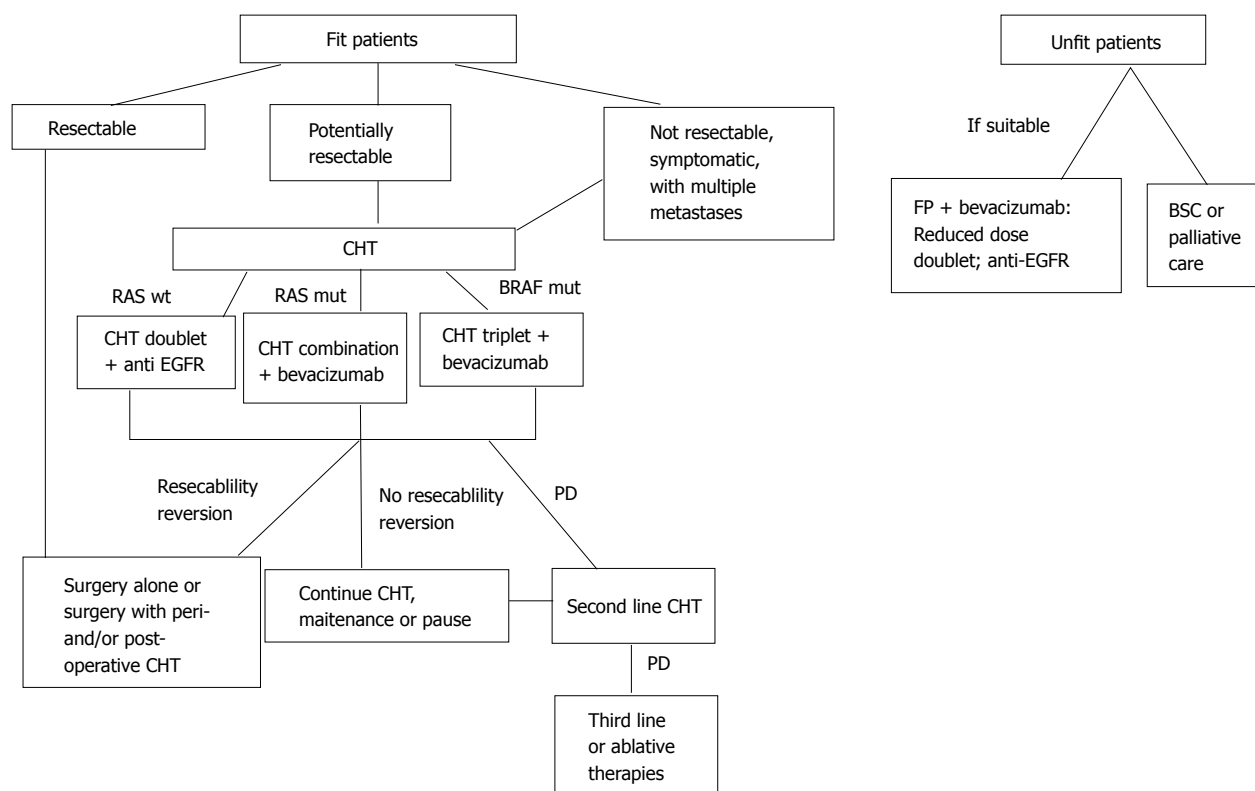


Figure 1 Treatment indications for fit and unfit colorectal cancer liver metastases patients. BSC: Best supportive care; CHT: Chemotherapy; EGFR: Epidermal growth factor receptors agents; mut: Mutated; FP: Fluoro pyrimidine. Adapted from ESMO 2016^[10].

epidermal growth factor receptors agents (anti-EGFR), such as cetuximab and panitumumab. These agents are widely used as adjuvant treatment without any evidence of clinical benefit^[48].

Adjuvant chemotherapy after metastasectomy is generally recommended by clinicians, even if the best regimen protocol is still unclear, and should be considered in a patient dependent manner^[24]. There, efficacy of adjuvant chemotherapy on OS for resectable CRC-LM is still under discussion^[45]. The National Comprehensive Cancer Network (NCCN) guidelines suggests the use of more than one chemotherapy line^[48]. Most study agree that 5-FU/LV with or without oxaliplatin should always be used as first-line^[47]. More recently, however, the use of combination therapy is increasing, and several combinations have emerged.

A recent study on FOLFIRI (5-FU/LV and irinotecan) vs 5FU/LV after R0 (complete resection) of CRC-LM does not report any difference in OS and median DFS. FOLFIRI improves DFS, but causes more frequent grade 3/4 toxic adverse events (47% vs 30%)^[49].

We suggest the use of adjuvant chemotherapy in patients with multiple lesions that are found in more than 3 liver segments, where the surgery, even if radical, may not be able to remove undetected tumor deposits.

CHEMOTHERAPY FOR UNRESECTABLE CRC-LM

Patients with unresectable CRC-LM from diagnosis

should receive chemotherapy in order to downstage the disease and allow the surgery (Figure 1 and Table 2).

About 70% of patients with CRLM are unresectable at diagnosis^[4]. They have a complicated disease, often requiring a combination of loco-regional therapy (chemoembolization, hepatic arterial infusion, ablation or radiation).

Perioperative chemotherapy is widely used also for unresectable CRC-LM, even if there is no proof of OS improvement^[50]. Systemic chemotherapy remains the first-line therapy. FOLFOXIRI followed by surgical resection has a 70.4% response rate, and 19% of patients obtain R0. OS at 5 and 8 years are 42% and 33% respectively, and 29% of patients are disease free at 5 years^[51].

Downstaging of unresectable CRC-LM ranges from 5% to 38%. This is due to multiple factors including disease extension, type and duration of chemotherapy^[51]. The purpose of the "conversion chemotherapy" in unresectable CRC-LM patients is to convert their disease to resectable, and is often the first line treatment. Standard regimens include FOLFIRI or FOLFOX that induce downstaging in 7%-40% of patients^[12]. Giacchetti's group reports that FOLFOX reduces the LM dimension by more than 50% in 59% of non-resectable CRC-LM, resulting in 38% of CR^[52]. FOLFOXIRI allows 36% of R0 in LM patients^[53]. The METHEP trial reports that FOLFIRINOX seems to be better therapy for CRC-LM than the others, bringing to resection 67% of cases with a survival > 48 mo. These results confirm that OS is greater for patients after R0 or

Table 2 Conversion rates in colorectal cancer liver metastases after perioperative chemotherapy

Trial name	Chemotherapy type	Control	n	KRAS status	Overall response	Conversion to resection	R0 resection
BEAT ^[61]	FOLFOX/XELOX/FOLFIRI or fluoropyrimidines + bevacizumab	No	1914	Not selected	NA	11.80%	NA
First BEAT ^[62]	FOLFOX/XELOX + bevacizumab	Placebo	1914	Not selected	38%	11.80%	6.3% vs 4.9%
OPUS ^[70]	FOLFOX + cetuximab	FOLFOX	233	Wilde type	61% vs 37%	9%	4.7% vs 2.4%
POCHER ^[72]	Chr IFLO + cetuximab	No	43	Wild type	79%	60%	25.70%
PRIME ^[77]	FOLFOX + panitumumab	FOLFOX	591	Wild type	57% vs 48%	31% vs 22%	29% vs 17%
CELIM ^[11]	FOLFOX6 + cetuximab	FOLFIRI + cetuximab	106	Wild type	68% vs 57%	43%	38% vs 30%
BOXER ^[63]	CAPOX + bevacizumab	No	47	Not selected	78%	40%	NA
Loupakis <i>et al</i> ^[55]	FOLFOXIRI + bevacizumab	FOLFIRI + bevacizumab	508	Not selected	65% vs 53%	15% vs 12%	NA
Ye <i>et al</i> ^[73]	FOLFIRI + cetuximab	FOLFOX + cetuximab	177	Wild type	57% vs 29%	26% vs 7%	NA
CRYSTAL ^[71]	FOLFIRI + cetuximab	FOLFIRI	599	Wilde type	47% vs 39%	16%	4.8% vs 1.7%
OLIVIA ^[79]	FOLFOXIRI + bevacizumab	FOLFOX + bevacizumab	80	Not selected	81% vs 62%	61% vs 49%	49% vs 23%

CAPOX, XELOX: Capecitabine-oxaliplatin; NA: Not available; Chr IFLO: Chronomodulated irinotecan, 5-fluorouracil, leucovorin, and oxalipatin; FOLFIRI: 5-fluorouracil, leucovorin and irinotecan; FOLFOX: 5-fluorouracil, leucovorin, and oxalipatin; FOLFOXIRI: 5-fluorouracil, leucovorin, oxalipatin and irinotecan.

R1 surgery, 65.2 mo vs 18.3 mo of not-operated or R2 patients^[54].

The use of bevacizumab is increasing for unresectable CRC-LM^[55,56], even if the benefits are extremely limited. A slight gain in response rate is observed when bevacizumab is associated with FOLFOXIRI as first line chemotherapy. The association of bevacizumab to first and second line chemotherapy for CRC-LM improves PFS^[57-60] and OS in some studies^[59,60]. Available data on the efficacy of bevacizumab associated to perioperative chemotherapy are limited. This may be due to concerns about possible complications in wound healing after resection^[61,62]. The Bevacizumab Expanded Access Trial reports good feasibility of LM surgery after first-line chemotherapy associated to bevacizumab, resulting in resection rates of 11.8% and 6% of R0^[63]. Bevacizumab association with FOLFOX, however, obtains higher resection rates (16.1%) than with FOLFIRI (9.7%), and higher R0 (6.3%) than FOLFOX plus placebo (4.9%) ($P = 0.24$)^[62]. Neoadjuvant capecitabine and oxaliplatin (CAPOX) plus bevacizumab resulted in 40% of CRC-LM resectability conversion^[63]. Loupakis *et al*^[55] report 64% of tumor response and 15% of rate of resection of CRC-LM after FOLFOXIRI plus bevacizumab, vs 53% and 12% respectively after FOLFIRI/bevacizumab.

Transarterial chemoembolization with irinotecan combined with FOLFOX plus bevacizumab chemotherapy results in a response rate of 78%, and allows resection of 35% of non resectable CRC-LM, offering a new cure option to these patients^[64].

A recent report by Stremtizer *et al*^[65] shows that mutated BRAF/RAS are correlated to a poor outcome after CRC-LM surgery. This is in agreement with the results of other 3 studies^[66-69]. These important evidences support the application of newer methods for the therapy of liver metastases, associating biological molecular aspects (biological resectability) to the other clinical and pathological indexes for the selection of good surgical candidates and the prediction of their outcomes.

Anti-EFGR agents such as cetuximab and panitu-

mumab are effective alone as well as in association with chemotherapy in CRC-LM that are RAS (both *KRAS* and *NRAS*) wild type^[69]. Some randomized trials report the effects of cetuximab for the therapy of unresectable CRC-LM. The OPUS trial^[70] showed that the association of FOLFOX-4 plus cetuximab as up front therapy doubled R0 (4.7%). The CRYSTAL study^[71] showed that the association of FOLFIRI plus cetuximab as up front therapy increased the R0 resection rate from 3.7% to 7.0%. The CELIM trial^[11] reported that neoadjuvant treatment with FOLFIRI plus cetuximab or FOLFOX6 resulted in 34% of R0 resections. Other studies also report that chemotherapy containing cetuximab significantly improves R0 in unresectable CRC-LM with *KRAS* wild-type^[72,73]. There are differences in resection rates among the above studies. Overall response rate is in the range 60%-79%, however, resection rates after chemotherapy/cetuximab are very variable (Table 2). These discrepancies may be due to the fact that the resection rate is defined and determined by clinical conditions of the patients and not by specialist oncologists in CRYSTAL and OPUS studies. Resection evaluation is done by a multidisciplinary team in the other trials.

The COIN^[74] and NORDIC VII^[75] trials report no advantage for the association of oxaliplatin based chemotherapy/cetuximab in first-line treatment of CRC-LM, independently from K-RAS status.

Resection rates of first-line FOLFIRI/panitumumab treatment of CRC-LM are 15% and 7% in the *KRAS* wild type (WT) and mutant groups respectively^[76]. FOLFOX4 plus panitumumab results in 32% of R0 resections vs 28% of those receiving only FOLFOX4^[77]. A post hoc analysis of the PRIME study on RAS WT (*KRAS*, *NRAS*) shows that panitumumab/FOLFOX can convert to resection 31% of initially unresectable CRC-LM patients and lead to 29% of R0 (Table 2)^[78]. A further analysis of PRIME trial also shows that *NRAS* mutations are indications of non-response to panitumumab^[77]. For this reason, it is extremely important to analyze other

types of mutations in the RAS gene to improve patient selection for anti EGFR therapy.

The OLIVIA trial studies FOLFOXIRI + bevacizumab vs mFOLFOX-6 + bevacizumab and reports an overall resection rate of 61% vs 49%, with R0 resection rates of 49% vs 23%^[79].

In conclusion “biologically directed” chemotherapy reduces the number and size of unresectable lesions. It also allows rescue of 15%-35% of patients, bringing them to surgery. These therapies are increasingly used worldwide.

EXTRA HEPATIC DISEASE

Extra hepatic disease (EHD) has a poor prognosis^[28]. Most common sites of EHD from CRC are lymph nodes, lungs, peritoneum, brain and bone. EHD is currently no longer a contraindication to metastasis resection, and patients after surgery have^[5] longer DFS and five-year-survival rates compared to those receiving only chemotherapy^[5,6].

OS after lymph node resection is different according to their site and number^[80]. Celiac or aorto-caval lymph node resections are associated with a worse outcome when compared to hepatic pedicle nodes, and mediastinal lymph nodes have a worse median survival than intra-thoracic ones^[80]. A high number of lymph nodes positive for metastases have also a poor outcome^[80].

In conclusion, the treatment of EHD is substantially palliative, aiming to improve the quality of life^[81].

LOCO-REGIONAL THERAPIES

Loco-regional therapies (Figure 2) are indicated for patients that are elderly, have a poor performance status, refusing surgery or chemotherapy, or refractory to chemotherapy. They also allow chemo-holidays with suspension of chemotherapy, and prolong the non-treatment period in between different chemotherapy lines. This reduces the treatment costs in respect to systemic chemotherapy.

In the last years new strategies have been developed in order to overcome several problems: High percentage of unresectable CRC-LM at diagnosis, high recurrence rates and presence of extensive disease. These methods increase the number of patients indicated for non surgical procedures.

Ablation techniques include radiofrequency ablation (RFA), Microwave ablation and external beam radiotherapy (EBRT). RFA is widely used and allows the application of extreme temperature to ablate the lesion with minimal toxicity (< 1%) in the surrounding liver tissue. RFA results in mortality and morbidity < 10% independently from the administration route^[82]. The “heat sink effect” is however a major disadvantage of RFA and may cause important hepatic or vascular injury. For this reason, RFA is not indicated for unresectable tumors, lesions near blood vessels or the diaphragm because of the high risk of perforation. Another disadvantage of RFA

is the recurrence rate that is higher when the tumor is > 3 cm or when treatment is delivered percutaneously^[82,83].

Microwave ablation uses high frequency microwave radiation to induce coagulation with necrosis of lesions. This method, however, is not well known and there are several concerns about its feasibility^[84]. Available data on this method show a 6% local recurrence rate^[85].

Improvements in imaging methods have increased the use of EBRT^[86]; that, however, has a low therapeutic window, and toxicity is still a major issue. EBRT is safe (at 60 Gy) and effective for liver tumors in general and in selected patients^[87,88].

Intra-arterial therapies: Hepatic artery infusion

Hepatic artery infusion (HAI) is indicated for patients with unresectable lesions when physicians want to associate an intra-arterial with an endovenous treatment.

The advantage of HAI is to minimize the toxicity to normal liver tissue, because the chemotherapeutic agents are injected directly to the tumor^[89]. Potential risks of this method are treatable complications related to catheter and pump placement, or life-threatening complications such as biliary sclerosis, hepatotoxicity and systemic toxicity. For this reason it should be performed by experienced hospitals^[89-91].

Intravenously 5-FU and intra hepatic artery oxaliplatin are successfully used^[92] for unresectable CRC-LM. Best results concerning survival and response rates are obtained with floxuridine based HAI^[93].

The comparison of OS between HAI therapy and systemic therapy alone (15.9 mo vs 12.4 mo) does not show any difference, however, there was a great response rate in favor of HIA (43% vs 18%)^[94].

In conclusion, HAI has interesting results; however it is a cumbersome method because it requires the implantation of an infusion pump.

Chemoembolization

Trans-arterial chemoembolization (TACE) is increasingly used for unresectable CRC-LM, improving survival and tumor response^[95]. TACE is indicated for unresectable CRC-LM as third line therapy, and allows the attainment of important palliative results.

The use of drug-eluting beads for TACE increases efficacy, while reducing adverse events due to systemic drug leakage or liver toxicity^[95-98]. The advantage of these beads is the direct delivery of toxic drugs inside the arterial capillary bed of the tumor, releasing the drug in a controlled manner. In this way the systemic exposure to toxic drugs is reduced, their local concentration is increased and a greater tissue necrosis than classic TACE with lipiodol is obtained^[99,100].

The indication for TACE is presence of multinodular LM, absence of extra hepatic disease, refractory to systemic chemotherapy^[101].

Recent reports show that TACE with irinotecan (DEBIRI) for the treatment of CRC-LM is effective, feasible and has limited side effects^[95-101]. Systemic chemotherapy (FOLFIRI) is compared to DEBIRI for

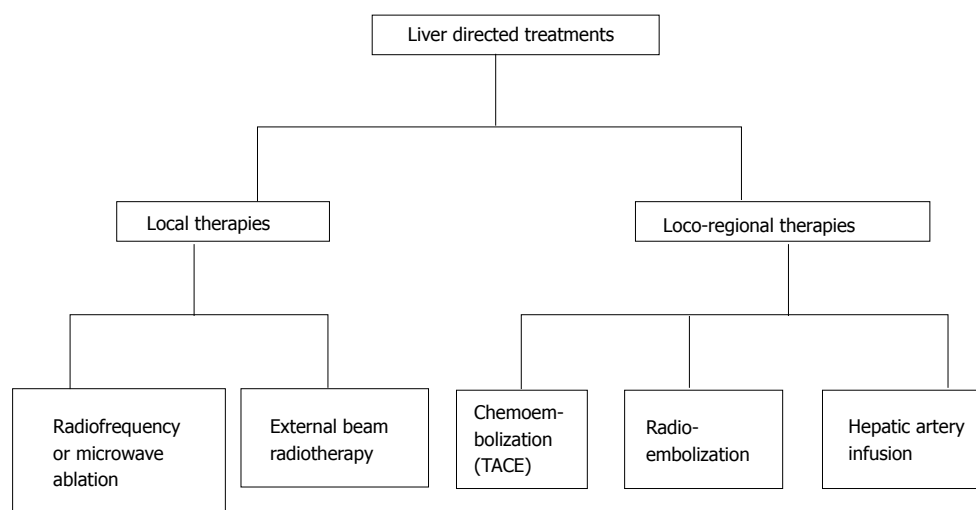


Figure 2 Liver directed treatments. TACE: Trans-arterial chemoembolization.

the therapy of refractory CRC-LM in some studies. This comparison shows that DEBIRI is statistically better than FOLFIRI in terms of OS, PFS, time to extra-hepatic progression, and quality of life^[95].

The association of cetuximab and TACE with irinotecan is an improvement in the treatment of CRC-LM, because these drugs are efficacious and have acceptable, and not cumulative, toxicities^[102].

The TACE methodology is constantly improving, in particular, the last innovation is the introduction of new embolics for drug delivery. Among the new types of microspheres there are polyethylene glycol (PEG) microspheres (LifePearls, Terumo), that are more resistant to stress and attrition. The advantages of these embolics are increased suspension time, better catheter deliverability and drug retention and release^[103].

In a recent study we show the data of TACE with PEG embolics for the treatment of 20 cases of non resectable liver tumors and metastases from colorectal carcinoma, breast cancer and uveal melanoma. Irinotecan and doxorubicin are used for PLC and LM respectively. More than 80% of cases respond to TACE patients. We observe 63% of CR, and 37% PR. The chemoembolization procedure is well tolerated by all the patients with only mild or moderate adverse events. These results indicate that PEG embolics-TACE is effective and tolerable for the therapy of hepatic primary and metastatic cancer^[103].

Radioembolization

In the last decade radioembolization (RE) with Yttrium 90 (Y90) has been widely used for the treatment of CRC-LM that are refractory to chemotherapy^[104]. Objective tumor response rates of RE are 33%-48% in second line^[105,106] and 10%-48% in third line^[107-109]. Survival and progression free survival are also improved after RE application as third line^[109]. RE with Y90 has, however, a low recommendation in the last ESMO guidelines^[110].

The treatment decision is very challenging for CRC-LM patients that are refractory to chemotherapy. Several

patients are unfit and have a biologically unfavorable progression often associated to comorbidities. Palliative care with chemo- or radio-embolization is indicated in these cases, in order to avoid too aggressive therapies.

MULTIDISCIPLINARY TEAM

The involvement of a multidisciplinary approach should be promoted in order to obtain the best CRC-LM management and outcomes, and to reduce peri-operative morbidity and mortality, prolonging OS and rising resection rates^[110,111].

For this reason, the multidisciplinary team management of CRC-LM is growing in most Western countries^[112]. The team includes different types of specialists including: Liver surgeons; interventional radiologists specialized in hepatobiliary disease; an oncologist; a pathologist; and a case manager nurse. They have to discuss each case to ensure resectability appropriateness and lead to down-staging wherever possible. The team should be consulted about the choice of chemotherapy combination and type of targeted agents and care to be used, timing of chemotherapy, and follow up.

Medical oncologists select the most active treatment for the shortest time combining chemotherapy to targeted drugs, in order to reduce tumor size without damaging the normal liver. The definition of the acceptable FRL should be performed by a radiologist and a liver surgeon. Repeating the resection is safe and effective, obtaining survival rates close to those after first resection^[112,113]. Finally the case manager nurse or the practitioner are important in patient's management, because they provide indications on the follow up and assistance.

CONCLUSION

Recent improvements of CRC-LM treatment allows the down-staging of several patients, resulting in increased number of patients cured or living with longer disease

control. There is currently no agreement about the correct sequence of surgical resection of the primary cancer and metastatic disease, however, the neoadjuvant chemotherapy is widely accepted as up front treatment.

Surgical resection can be performed if the complete removal of cancer is achievable leaving an adequate FRL. The use of adjuvant chemotherapy is highly suggested, even if standardized protocols are still unclear. The use of chemotherapy may lead to disease regression for unresectable CRC-LM, allowing resection and cure.

The application of loco-regional therapies is increasing, resulting in high tumor response, however, they are not recommended as first-line treatment in case of unresectable CRC-LM.

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Evolving role of Sorafenib in the management of hepatocellular carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is one of the most common malignant diseases worldwide and comes third

in cancer-related mortality. Although there is a broad spectrum of treatment options to choose from, only a few patients are eligible candidates to receive a curative therapy according to their stage of disease, and thus palliative treatment is implemented in the majority of the patients suffering from liver cancer. Sorafenib, a multikinase inhibitor, is the only currently approved agent for systemic therapy in patients with advanced stage HCC and early stage liver disease. It has been shown to improve the overall survival, but with various side effects, while its cost is not negligible. Sorafenib has been in the market for a decade and has set the stage for personalized targeted therapy. Its role during this time has ranged from monotherapy to neoadjuvant and adjuvant treatment with surgical resection, liver transplantation and chemoembolization or even in combination with other chemotherapeutic agents. In this review our aim is to highlight in depth the current position of Sorafenib in the armamentarium against HCC and how that has evolved over time in its use either as a single agent or in combination with other therapies.

Key words: Sorafenib; Hepatocellular carcinoma; Liver neoplasm; Multikinase inhibitor; Targeted therapy; Tumor angiogenesis; Signaling pathways; Adjuvant therapy; Liver cancer; Liver transplantation; Liver resection

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Core tip: Hepatocellular carcinoma (HCC) is an aggressive and invasive malignancy. Curative options, such as resection and liver transplantation, are limited to only a few patients, who are suitable candidates. Sorafenib is the only approved systemic treatment in HCC, especially for advanced tumor stage and early stage liver disease. Recent findings suggest that it may also be helpful in carefully selected decompensated patients. Its adjuvant role is yet to be proven with more promising results. The combination of Sorafenib with other chemotherapy agents has shown improved efficacy and safety. We aim

to present the evolution of Sorafenib's use over the last decade.

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INTRODUCTION

Hepatocellular carcinoma (HCC), the most common primary malignant neoplasm of the liver (85%-90%)^[1], is the sixth most frequent cancer in the world and the third cause of cancer-related^[2]. Cirrhosis is the stage of chronic liver disease characterized by disrupted architecture of the liver, therefore resulting in its dysfunction over the time. Regardless of the cause leading to cirrhosis, it is a major condition predisposing to a malignant transformation of the liver eventually leading to HCC^[3]. Nowadays, the incidence of HCC is increasing rapidly owing to the large number of people suffering from cirrhosis, mainly caused by hepatitis B and C virus infection, as well as due to longer survival among cirrhotic patients^[1].

Equally important to the presence and stage of cirrhosis, is the stage of the HCC, as any treatment that will follow will be in accordance to that. Specifically, surgical resection, ablation and liver transplantation are the only acceptable potentially curative options, but as it turns out, despite screening and frequent follow-ups, only 40%-60% of cirrhotic patients are diagnosed with very early or early stage HCC, therefore being eligible for curative treatment^[4,5]. Unfortunately, most patients are diagnosed with more advanced stage HCC, *i.e.*, portal vein invasion and/or extrahepatic spread or general symptoms attributed to cancer, unresponsive to such modalities. As a result alternative treatment combinations and algorithms including embolization, chemotherapy, radiotherapy, molecular target therapy or immunotherapy are constantly being generated in order to improve the overall survival (OS) of such patients^[4,6,7].

In particular, an aspect of systemic therapy tends to focus on an important characteristic of HCC, its angiogenesis, by developing antiangiogenic drugs that impede the formation of new blood vessels, thus inhibiting the proliferation and growth of the liver tumor^[8]. Sorafenib, an antiangiogenic drug, is the first and currently the only chemotherapeutic regimen approved as a palliative type of treatment in advanced stage HCC^[9]. This review describes the general characteristics of Sorafenib, its current place in the clinician's therapeutic armamentarium, as well as the clinical results of the evolving role of Sorafenib when combined or compared to other treatment modalities.

GENERAL PRINCIPLES

Molecular mechanisms

As stated above, HCC is a tumor with abundant vasculature and high heterogeneity, especially when it comes to the various signaling pathways involved^[10]. One of the key pathways involved in the growth and proliferation of HCC is the Raf/MEK/ERK mitogen-activated protein (MAP) kinase cascade, which shows particularly increased activity^[11]. This over-activation is mainly achieved by the combined action of hepatitis virus proteomics and growth factors, with platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) playing a critical role and highlighting the linkage between angiogenesis and HCC development^[12-14]. Sorafenib (Nexavar, BAY 43-9006), a biaryl urea, is an oral multikinase inhibitor of the serine/threonine-kinases (c-RAF and BRAF), therefore blocking the Raf/MEK/ERK pathway, and of the vascular endothelial growth factor receptor 2 (VEGFR2), VEGFR3, platelet-derived growth factor receptor (PDGFR), FLT3, Ret, and c-KIT^[15]. Moreover, it has been shown to result in apoptosis in various human tumor cell lines, independently of its involvement in the Raf/MEK/ERK pathway, by: (1) down-regulating an anti-apoptotic protein, the myeloid cell leukemia-1 (Mcl-1), member of the Bcl-2 family; and (2) inhibiting the phosphorylation of eukaryotic translation initiation factor 4E (eIF4E), which normally, when phosphorylated, promotes the expression of oncogenic genes^[16]. According to this rationale, Sorafenib is an effective drug against not only the tumor compartment, but also the formation of new vessels^[17,18]. Its mechanism of action is illustrated in Figure 1.

Sorafenib's history

This therapeutic action was firstly assessed in an uncontrolled phase 2 clinical trial of 137 patients with advanced and unresectable HCC, not having received any prior systemic therapy and with Child Pugh (CP) A or B cirrhosis^[19]. The dosage administered was 400 mg orally twice a day in 4-wk cycles with a partial response of 2.2%, a minor response of 5.8% and a 33.6% of the patients reporting non progressive disease for at least 16 wk. Some other major data reported were the 4.2-mo median time to progression (TTP) and the 9.2-mo OS, while CP A and B patients showed only negligible differences regarding the pharmacokinetics^[19].

Such positive results could not but be followed by the international phase 3, randomized, double-blind, placebo-controlled "Sorafenib HCC Assessment Randomized Protocol" (SHARP) clinical trial^[9]. For this purpose, 602 patients with advanced stage HCC, Eastern Cooperative Oncology Group (ECOG) performance status from 0 to 2, CP A liver disease and without any preceding systemic treatment, were randomized either for Sorafenib, same dosage as in phase 2, or for placebo. According to the data reported, Sorafenib resulted in a median OS of 10.7 mo vs the 7.9 mo of the placebo, as well as in a median

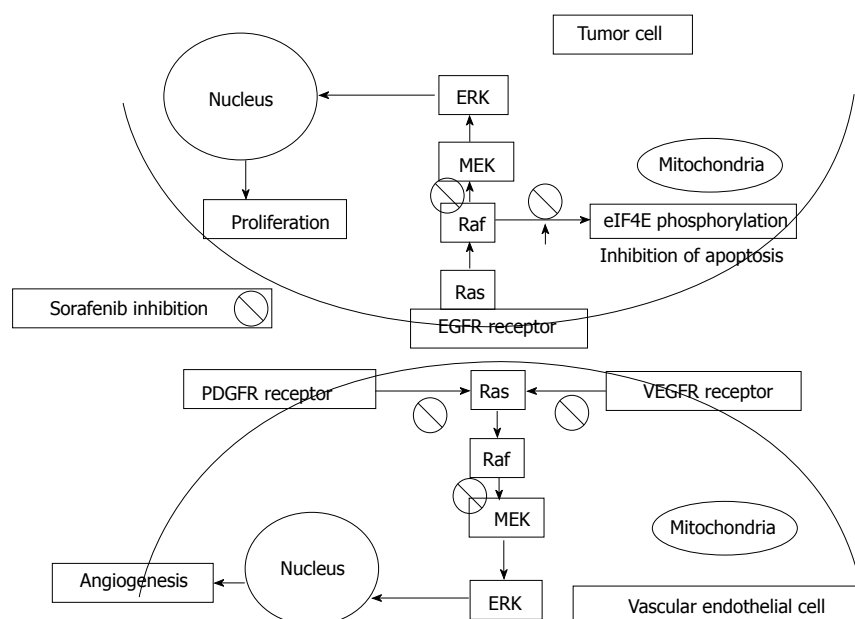


Figure 1 Sorafenib's mechanism of action. In tumor cells sorafenib blocks the Raf/MEK/ERK cascade and can lead to apoptosis through various mechanisms, such as inhibition of eukaryotic translation initiation factor 4E phosphorylation. In vascular endothelial cells, it inhibits receptor tyrosine kinases, such as VEGFR and PDGFR. PDGFR: Platelet-derived growth factor receptor; VEGFR: Vascular endothelial growth factor receptor.

TTP of 24 wk compared to 12 wk of the placebo. Also, although the median TTP based on radiologic findings was 5.5 mo in the Sorafenib arm compared to 2.8 mo in the placebo arm, there was again no complete response, while the partial response was limited^[9]. In spite of the positive clinical effects and the improvement in OS, Sorafenib was assessed within the frontiers of advanced stage HCC, but very early stage liver disease. This leads to many questions regarding its potential place in the treatment of patients with both advanced HCC and liver disease.

Adverse effects

On the other hand, nobody claimed that Sorafenib was harmless. The SHARP trial, as a phase 3 study, except for the effectiveness, also reported details about some possible adverse effects, which were more frequent in the Sorafenib group compared to the placebo one (80% vs 52%, respectively). The most commonly described toxicities were grade 1 and 2 regarding the severity, *i.e.*, weight loss, anorexia, diarrhea, changes in voice, hand-foot skin reaction, rash or desquamation and hair loss^[9]. Some of these toxicities led to drug discontinuation (Sorafenib 11% vs placebo 5%)^[9]. Another important study, the Sorafenib Italian Assessment (SOFIA) trial, showed that intervening by down-dosing at the appropriate time might be beneficial regarding an improved toxicity-tolerance rate and an increased OS^[20].

Moreover, significant findings from the routine clinical practice were presented by Sacco *et al.*^[21], who stated that when Sorafenib is administered early at a low dose, especially in patients characterized as high-risk, it may be easier to render the patients compliant to the continuation of the therapy and for the drug to be well-tolerated. As a

result, Sorafenib may induce some harmful events, mostly minor, which can be better tolerated by adjusting the dosage.

FOOD AND DRUG ADMINISTRATION APPROVAL

According to the European Association for the Study of the Liver (EASL) - European Organisation for Research and Treatment of Cancer (EORTC) guidelines (2012), Sorafenib is currently the only standard systemic treatment for HCC^[6]. Its use is approved since 2007 upon the publication of the results of two studies: (1) the SHARP trial^[9], conducted in the United States of America and Europe; and (2) the Sorafenib Asia-Pacific (Sorafenib-AP) trial^[22], conducted in South Korea, China and Taiwan, which both showed an increased OS and a reduced risk of mortality in patients treated with Sorafenib. However, the aforementioned guidelines^[6] highlight that Sorafenib is recommended only in patients with early stage liver disease - Child-Pugh A - and advanced stage HCC - Barcelona - Clinic Liver Cancer (BCLC) stage C - or as an adjuvant therapy combined with loco-regional treatment options. Sorafenib's current place in the treatment algorithm, in accordance with the BCLC staging system for HCC, is presented in Figure 2^[4,23].

MONOTHERAPY

As mentioned above, the results of systemic monotherapy with Sorafenib were encouraging according to a phase 2 trial^[19] and two phase 3 trials (SHARP^[9] and Sorafenib-AP^[22]). There was general agreement that Sorafenib has a great impact in increasing the OS, even though in the

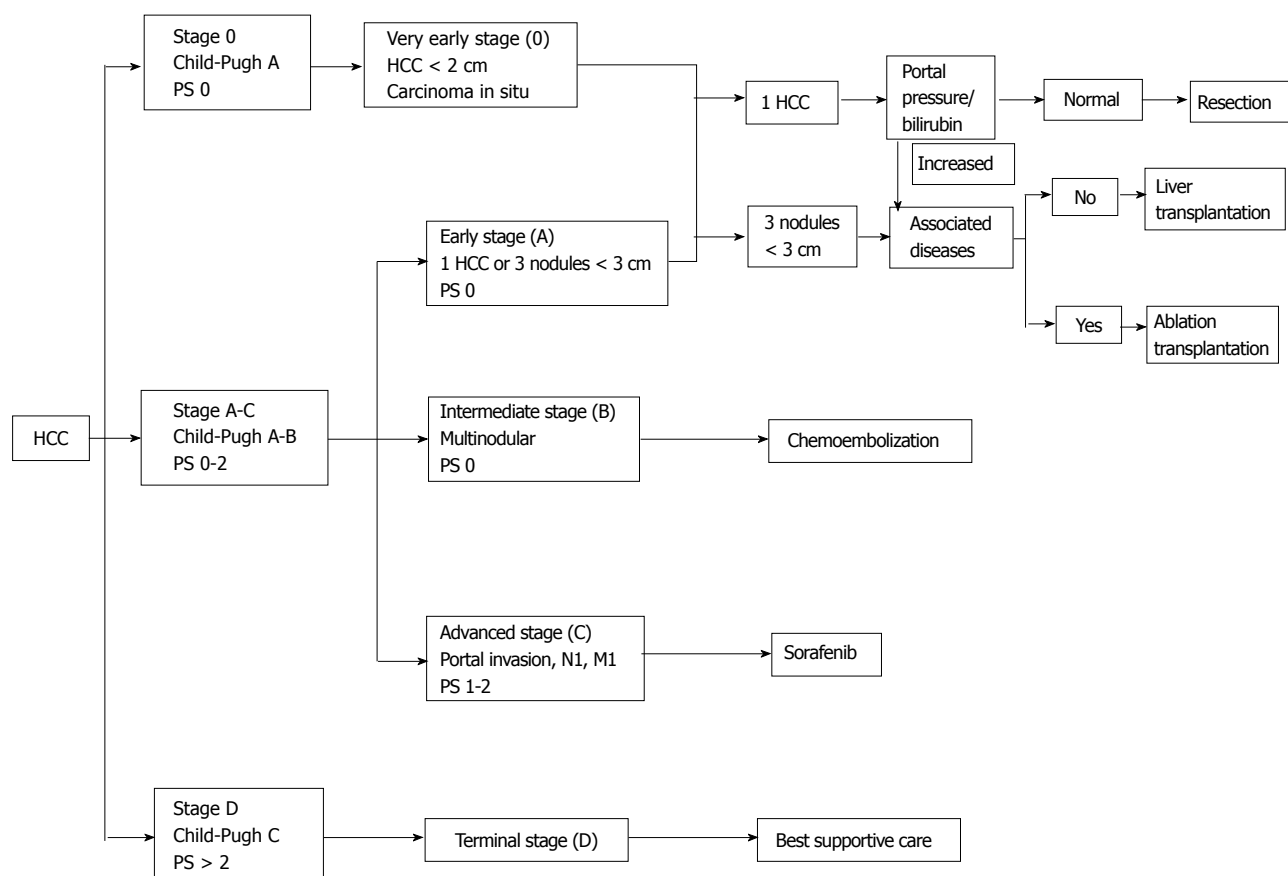


Figure 2 Barcelona clinic liver cancer staging system and treatment algorithm. PS: Performance status; N: Nodules; M: Metastases; HCC: Hepatocellular carcinoma.

phase 2 study 28% of the patients, who had CP B cirrhosis, showed a shorter median OS of 3.2 mo and could tolerate the treatment for only 1.8 mo. Also the incidence of ascites, encephalopathy and advanced hyperbilirubinemia is higher in advanced liver disease^[24]. Interestingly, a phase 1 study, assessing the use of Sorafenib in patients with higher Child-Pugh class, underlined its link with the dose-limiting rises in serum bilirubin concentration^[25]. Therefore, treatment guidelines^[7] recommend taking bilirubin into consideration when adjusting the dose of Sorafenib. In addition, a post-marketing trial (GIDEON)^[26] has shown equivalent results regarding safety and dosing strategy regardless of the Child-Pugh score. On the other hand, several studies evaluating the role of Sorafenib among the different stages of liver function reserve, reported a decreased response in advanced CP class, while liver-specific toxicities were independent of the liver cirrhosis stage^[27-29].

On the whole, a systematic review has shown that in a male elderly population with advanced HCC and CP A cirrhosis, Sorafenib monotherapy can yield a statistically significant, yet clinically insignificant, increase in OS, time to tumor progression and disease control rate^[30]. Besides, the cumulative data underline the decrease response of HBV-infected patients when compared to HCV, while patients with worse level of cirrhosis tend to display a more prominent Sorafenib-driven toxicity^[30].

A study published in 2017 analysing the SEER-

Medicare database, reported that elderly patients with advanced stage HCC may survive longer if treated with Sorafenib vs placebo (150.5 d vs 62 d, respectively), while the most remarkable factor associated with increased mortality was treatment taking place in an urban setting, although this survival effect was found to be neither prolonged, nor cost-effective in decompensated patients^[31]. Currently, a randomized controlled phase 3 study - the B Child Patient-Optimization of Sorafenib Treatment (BOOST) study - is ongoing so as to evaluate the safety and efficacy of Sorafenib in CP B patients and is going to provide helpful information regarding the treatment of patients with decompensated disease^[32]. However, reality is that for most patients Sorafenib is only one of the treatments that they receive, thus rendering it essential to review the adjuvant role of Sorafenib within the spectrum of other therapies.

SORAFENIB AND SURGICAL RESECTION

Currently, surgical resection remains the treatment of choice for HCC, when it is associated with solitary masses and the hepatic remnant can maintain liver function^[6]. Recently, there has been great interest concerning the down-staging of advanced HCC in order to make surgical resection even more efficient. One way to accomplish that is by taking advantage of Sorafenib's use as a

neoadjuvant treatment. In fact, a study has reported the incidence of Sorafenib-driven tumor necrosis, when used pre-operatively, therefore making resection an applicable treatment modality for a previously unresectable HCC tumor^[33]. Moreover, the use of Sorafenib before surgery was not found to lead to any intra- or post-operative side-effects^[34].

However, it is unclear whether Sorafenib could also be efficacious as an adjuvant therapy post-operatively. Specifically, a phase 3 study (STORM) evaluating its use after resection or ablation showed that Sorafenib is not superior to placebo when it comes to OS, recurrence-free survival or time to recurrence^[35]. Unfortunately, many patients enrolled in this study could not tolerate the standard dose used^[35]. These results are against incorporating Sorafenib in the guidelines as an appropriate adjuvant treatment option after resection^[6].

SORAFENIB AND LIVER TRANSPLANTATION

Another curative treatment, especially for patients within the Milan criteria is orthotopic liver transplantation^[36]. The challenges involved in liver transplantation, such as graft availability, have led to the increased use of grafts, including split grafts or those from living donors or from marginal donors. However, sometimes the delay between joining the waiting list and actually having a liver transplant may be quite significant, leading to patients dropping off the list^[37]. Consequently, those patients with HCC waiting for a liver donor for at least six months are recommended to receive the so called "bridging therapy", which mainly consists of locoregional treatment approaches, such as radiofrequency ablation (RFA) or transarterial chemoembolization (TACE)^[6]. The rationale of "bridging" is entirely understandable when trying to prevent tumor progression in cirrhotic patients with HCC, who patiently wait a suitable donor organ to become available. An alternative strategy is down-staging of HCC patients outside the Milan criteria, in an effort to make them eligible for transplantation. A legitimate question is whether Sorafenib has an adjuvant role in this endeavor.

This neoadjuvant use of Sorafenib for down-staging comes with little evidence not demonstrating any significant advantages, even though some cases seem to accomplish reduction in the tumor boarder, down-staging and therefore allowing the patient to be added to the waiting list^[38,39]. All-in-all, Sorafenib has shown a safe profile, when used before transplantation, with insignificant post-operative negative events^[40,41]. Besides, the Sorafenib-driven hypoxia, because of its antiangiogenic effects, is thought to result in alterations in molecular mechanisms and growth factors, thus allowing the tumor to develop resistance and become more invasive or even metastatic^[42]. Until more convincing data is reported from large clinical trials, the use of Sorafenib in this setting should be limited to investigational protocols.

On the other hand, the post-operative adjuvant

use of Sorafenib has proven to be inefficient (STORM trial)^[35], but when it comes to post-transplantation, results may be different. Specifically, a lot of studies agree with the fact that the use of Sorafenib, either concomitantly with mammalian target of rapamycin (mTOR) inhibitors or without them, can improve the survival when used for recurrent disease after liver transplantation, with the disadvantage of some drug-induced toxicity leading to a decrease in the dosage or even cessation of treatment^[43-50]. As a matter of fact, Sorafenib has also resulted in complete remission of recurrent HCC after liver transplantation^[51]. In general its use in this setting is thought to be safe^[52].

Alltogether, current evidence is not favorable regarding the adjuvant use of sorafenib either pre- or post-transplantation and more research on this particular topic needs to take place, especially in the form of randomized controlled trials^[53].

SORAFENIB AND LOCOREGIONAL THERAPIES

Current guidelines suggest the implementation of transarterial TACE in patients with intermediate stage HCC, consisting of multiple nodules, presenting without symptoms, invasion of the vessels or metastases and without advanced liver disease^[6]. Although TACE can be helpful and efficient in this particular group of patients by improving survival^[4], it is classified as a palliative option because it cannot achieve complete necrosis of the tumor and is associated with increased recurrent disease and tumor proliferation^[54]. This tumor growth is also promoted by the ischemic area appearing after treatment with TACE, and owes its existence to the overexpression of certain growth factors, with VEGF playing a major role^[55,56]. VEGF's place in this equation lies on the side of tumor progression and metastasis and thus Sorafenib can be the ideal agent to deal with this process and impede angiogenesis, while simultaneously supplementing the promising action of TACE by eliminating the possibilities of future proliferation or recurrence^[57].

Some phase 2 studies^[58,59] evaluating the concurrent use of TACE and Sorafenib in patients with HCC not amenable to resection have shown a fairly safe profile for this combination with encouraging results regarding the efficacy and toxicity. When this duet was compared to TACE plus placebo in intermediate stage HCC on the background of HCV infection, it greatly improved time to tumor progression, without any unforeseen adverse events^[60]. The comparison mentioned above was also assessed in a meta-analysis of six studies (1254 patients) reassuring that TACE plus sorafenib in either intermediate or advanced stage HCC patients can increase OS, time to tumor progression, as well as objective response rate, while the risk of side effects is also high^[61]. Other recent meta-analyses, however, evaluating the marriage of Sorafenib and TACE for unresectable HCC showed an improvement in time to tumor progression, but not in

OS^[62,63].

It should also be mentioned that Sorafenib has been assessed in combination with drug-eluting beads (DEB)-TACE, an alternative method of delivering regional chemotherapy with minimal systemic exposure, for the management of both intermediate and advanced stage HCC. The results proved the increased efficacy and safety of this strategy^[57].

Another important issue is that of the time of TACE and Sorafenib administration, for which three different options have been suggested: (1) TACE is followed by antiangiogenic therapy; (2) continuous antiangiogenic treatment interrupted only for the moment of TACE administration; and (3) continuous antiangiogenic therapy with no interruption at the moment of TACE administration^[64]. Although the first two options are superior regarding the risk of bleeding, which is reduced, the third eliminates the possibilities of VEGF increase after TACE.

In general, it appears that Sorafenib plus TACE can lead to improved clinical results, especially regarding the intermediate stage HCC, mainly consisting of a highly heterogeneous group of patients for whom the overall approach is still to be defined based on several ongoing studies^[57,65].

SORAFENIB AND OTHER CHEMOTHERAPEUTIC DRUGS

Sorafenib and hypoxia-inducible factor-1 α inhibitors

Locoregional treatment modalities can be efficient when it comes to HCC, but up to a point. Radiofrequency and microwave ablation trigger hypoxia and consequently hypoxia-induced angiogenesis, thus increasing the possibility of HCC recurrence. This process is primarily mediated by the hypoxia-inducible factor (HIF)-1 α /vascular endothelial growth factor-A (VEGF-A) pathway, which can be impeded by Sorafenib^[66]. Therefore, Sorafenib has been shown to limit the tumor's invasive nature *in vitro*, a result of the cobalt chloride's increase of the expression of HIF-1 α , and to reduce proliferation and promote apoptosis in HCC cells^[66].

2-Methoxyestradiol (2ME2), an inactive end product of estrogen metabolism, has recently been proven to have an antitumor effect by inhibiting proliferation and angiogenesis and by promoting apoptosis in many cancer types and especially in HCC^[67]. The most important mechanism 2ME2 acts is through the inhibition of HIF-1 and the down-regulation of the HIF-driven VEGF expression^[68]. It has been shown that 2ME2 comes up with synergistic effects in combination with Sorafenib in accordance to HCC suppression and antiangiogenesis, effects mostly driven by HIF-1 and -2 deregulation^[69].

Sorafenib and mTOR inhibitors

mTOR, a protein kinase, plays a key role in cell growth, proliferation, angiogenesis and metabolism in several cancers, including HCC^[70]. It represents the target of rapamycin and its analogues, as well as Everolimus

and Sirolimus, which present with an antitumor profile through the down-regulation of hypoxia-inducible factor, thus resulting in low VEGF and PDGF expression.

Everolimus has been evaluated in a phase 1/2 study in patients with advanced stage HCC, who were previously treated with systemic therapy, and has shown encouraging results in terms of tolerability and efficacy^[71]. However, when Everolimus was combined with Sorafenib, again in a phase 1 trial, so that its maximum tolerated dose (MTD) could be determined, the results were disappointing regarding its efficacy in the MTD^[72]. In addition, a randomized clinical trial (EVOLVE-1)^[73] assessing the use of Everolimus in patients with advanced HCC, who presented with tumor progression during or after taking Sorafenib or who showed limited tolerability towards Sorafenib, reported no increase in OS.

On the other hand, the significant immunosuppressive role of mTOR inhibitors has been used in combination with Sorafenib vs Sorafenib alone in cases of post-transplantation late recurrent HCC, thus highlighting the broadening of the horizons in the treatment options against HCC towards the direction of personalized molecular targeted therapy^[74]. Besides, cohort studies^[46,49] assessing the combination of Sorafenib and mTOR inhibitors in the same disease context showed improved survival, but with some serious adverse events.

As a result, it is suggested that further studies are carried out, so as to evaluate the combination of mTOR inhibitors with Sorafenib in terms of achieving the maximum possible synergy and the minimum possible toxicity overlap.

Sorafenib and PI3K/AKT inhibitors

Despite the blockade of the Raf/MEK/ERK cascade by Sorafenib, HCC has remarkable compensation through the over-expression of several other pro-survival pathways. The phosphoinositide 3-kinase (PI3K)/AKT pathway comes into play here as one of those and data state that it can render the tumor less susceptible to Sorafenib^[75]. Thus, synergy may result from the combination of Sorafenib with a PI3K/AKT inhibitor, such as PKI-587 which simultaneously blocks the mTOR pathway, and this significant additive inhibitory effect has been proven in liver cancer stem cell patterns^[76].

Sorafenib and WNT/ β -catenin inhibitors

The complexity of the molecular mechanisms involved in the multistep process of tumor growth in HCC has been shown to incorporate mutations in the Wnt/ β -catenin pathway as well^[77]. Therefore, it is possible that the Wnt/ β -catenin pathway represents a novel target for systemic treatment in HCC and as such it may also show an additive effect when used concurrently with Sorafenib. Indeed, not only has Sorafenib been able to down-regulate this pathway in different models^[78], but also FH535, a Wnt/ β -catenin inhibitor, was found to impede tumor growth of HCC and hepatoblastoma^[79,80]. Moreover, when Sorafenib was combined with FH535, their synergistic

effect on inhibiting the proliferation of HCC was more significant^[81,82].

Sorafenib and MEK inhibitors

The MAPK/ERK kinases (MEK) 1 and 2 can be consequently activated if a Ras mutation shows up, as they are found downstream in the RAS cascade, the activation of which can therefore provide proliferative and anti-apoptotic capabilities to the tumor. This “vertical” type of inhibition totally differs from the “parallel” blockade previously described in the mTOR inhibition, in which two unconnected cascades are concurrently inhibited^[83]. Interestingly, MEK inhibitors, such as Refametinib (BAY 869766) which is an allosteric MEK 1/2 inhibitor, have proven their efficacy in preclinical HCC models^[84]. When combined with Sorafenib in a phase 2 trial, Refametinib was found efficacious, especially in case of Ras mutations, and was well-tolerated^[85].

Sorafenib and JAK/STAT inhibitors

The Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathway plays an important part as a signal transduction cascade, with several proteins of the STAT family participating in cell growth, immunity and survival^[86]. The one with the most significant role in oncogenesis is STAT3^[87]. This STAT3 protein is key in modulating sensitization of HCC in recombinant tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), an antitumor drug with encouraging efficacy^[88]. When Sorafenib was combined with TRAIL, it decreased the expression of STAT3 and proteins involved in its actions, thus rendering, the previously resistant to TRAIL, HCC susceptible to TRAIL-induced apoptosis^[88]. Besides, Sorafenib targets STAT3 in a kinase-independent manner in patients with HCC^[88,89].

In addition, the SH2 domain-containing tyrosine phosphatases family (SHP-1 and SHP-2), which are included in the family of protein tyrosine phosphatases (PTP), consist of two Src Homology (SH) 2 domains just as their name indicates^[90]. These phosphatases dephosphorylate STAT3, leading to a significant decrease in its activation^[91] and as a result they represent a potential target for systemic treatment of HCC. In fact, SHP-1 is a target of Sorafenib and through conformational modifications and signaling pathways, in which STAT3 is also involved, Sorafenib can also exhibit its anti-HCC effect^[92]. However, we have already experienced the evolution of Sorafenib through its derivatives, such as SC-43 and SC-40, which are potent SHP-1 agonists and have proven to be superior to Sorafenib for the management of HCC^[92]. Another novel derivative of Sorafenib, SC-59, when combined with radiotherapy has also shown to be superior to Sorafenib for treating HCC and its actions are mediated through STAT3 inhibition^[93]. Last but not least, the synergistic combination of Sorafenib with SC-43, through their SHP-1 agonist effects, has been found efficacious, as it decreased tumor size and improved survival in preclinical models^[94].

Sorafenib and phytochemicals

Data from preclinical models indicate that dietary phytochemicals with anti-inflammatory, antioxidant and anti-neoplastic characteristics may reduce the risk of HCC.

Curcumin is a yellow polyphenol derived from turmeric and has been shown to be protective against HCC caused by aflatoxins in mice^[95]. Due to its solubility issues, polymeric nanoparticle formations of curcumin (NFC) have been developed and it is reported that the use of NFC alone or in combination with Sorafenib presents with remarkable findings regarding the suppression of tumor proliferation and invasiveness of HCC, as well as that of lung metastases^[96].

Resveratrol is also a dietary polyphenol, mostly present in grapes, berries, peanuts and red wine, and has appeared as a promising chemopreventive agent against liver cancer^[97]. The combination of Resveratrol and Sorafenib can lead to apoptosis and reduced tumor growth in HCC mice by fighting the diverted metabolic phenotype of aerobic glycolysis^[98].

Indole-3-carbinol (I3C), found in cruciferous vegetables, is also one of the phytochemicals that have recently emerged with antineoplastic and antiangiogenic properties^[99]. Specifically, its combined use with sorafenib has shown synergy by increasing the latter's cytotoxicity and antiangiogenic properties, by promoting cell cycle arrest and apoptosis, as well as by reducing the expression of p-Akt, HIF-1 α , VEGF and EGFR in HCC cells^[99].

Regorafenib: A new era

Several antiangiogenic drugs with the same antiangiogenic capabilities as Sorafenib have been developed over time for the management of HCC, mostly as second-line systemic therapy agents.

In case of failure to respond to Sorafenib, patients with HCC can be treated with another multikinase inhibitor, Regorafenib^[100]. The addition of a fluorine atom in the central phenyl ring of Sorafenib transforms Regorafenib into an agent with increased potency^[101]. A phase 2 study evaluating Regorafenib for intermediate or advanced HCC in patients that had previously received Sorafenib reported encouraging results, such as an OS of 13.8 mo, a safety profile similar to Sorafenib and no deaths attributed to Regorafenib^[102]. Recently, in July 2016, at the ESMO World Congress on Gastrointestinal Cancer in Barcelona findings from a phase 3 trial (RESORCE, NCT01774344) assessing Regorafenib in HCC patients, who received prior therapy with Sorafenib, exhibited a remarkable increase in median OS for those treated with Regorafenib vs those receiving placebo as a second-line agent after radiologic progression under Sorafenib (10.6 vs 7.8)^[103].

Almost a decade has passed with numerous clinicians and scientists getting negative results in trials for systemic therapy in HCC patients, while the RESORCE trial is the only one after the SHARP trial to come forward with positive findings. The most important causes of those negative results are: (1) the heterogeneity among the

HCC patients recruited and the lack of selection criteria based on molecular patterns; and (2) the imbalance between adverse events and tolerable dosage vs anticancer efficiency and drug potency of the tested agents. Current advances in medicine and biology will improve our knowledge regarding the different and complex molecular mechanisms and driving mutations involved in this vast heterogeneity of this unique and multidimensional type of cancer and will guide us towards the right direction of conducting successful trials in the near future^[104].

CONCLUSION

Sorafenib represents a type of medicinal revolution, therefore making antiangiogenesis drugs a feasible choice when it comes to dealing with cancer and opening the road for personalized targeted therapy. Currently, Sorafenib is the only accepted treatment for systemic therapy, as it has shown to increase the OS in patients suffering from advanced HCC, but with liver disease of early stage with tolerable adverse effects. Recently, studies show that Sorafenib is also safe in patients with advanced liver disease as well, but neither adequately efficient, nor cost-effective. Thus, ongoing studies (*i.e.*, BOOST trial) are going to define its role in decompensated population in the future and up until then, patient selection in patients treated with Sorafenib is critical.

All-in-all, Sorafenib has evolved through time by being evaluated in several treatment protocols either as a neoadjuvant or as an adjuvant agent. Its use prior to or after liver transplantation has demonstrated a range of some minor advantages to even complete remission of recurrence, while preserving an acceptable safety profile. Still, a lot of research is needed in this field, as Sorafenib's role post-resection was not that much promising, while its combination with TACE showed encouraging results. Overall, understanding the molecular mechanisms of HCC and Sorafenib, as well as those resulting from the implementation of other treatment methods, will guide us to the future development of combinations involving Sorafenib, agents with higher efficacy that derive from Sorafenib or even second-line agents that will complement the therapeutic role that Sorafenib could not accomplish by itself.

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Magnetic resonance imaging for diagnosis and neoadjuvant treatment evaluation in locally advanced rectal cancer: A pictorial review

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(MRI) is the primary method for staging rectal cancer. MRI is highly accurate in the primary staging of rectal cancer; however, it has not proven to be effective in re-staging, especially in complete response evaluation after neoadjuvant therapy. Neoadjuvant chemoradiotherapy produces many changes in rectal tumors and on adjacent area, as a result, local tumor extent may not be accurately determined. However, adding diffusion-weighted sequences to the standard approach can improve diagnostic accuracy. In this pictorial review, an overview of the situation of MRI in the staging and re-staging of rectal cancer is exhibited as a pictorial assay. An experience- and literature-based discussion of limitations and difficulties in interpretation are also presented.

Key words: Rectal cancer; Locally advanced; Magnetic resonance imaging; Staging; Neoadjuvant treatment

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Core tip: Accurate staging and circumferential resection margin evaluation significantly impacts determining optimal treatment scheme. Preoperative magnetic resonance imaging (MRI) is highly accurate; however, it has yet to be proved as effective in re-staging. The adding of diffusion-weighted sequences to standard T2-weighted MRI can positively affect its diagnostic accuracy.

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Abstract

High-resolution pelvic magnetic resonance imaging

INTRODUCTION

Multimodal treatment of rectal cancer, with the combination

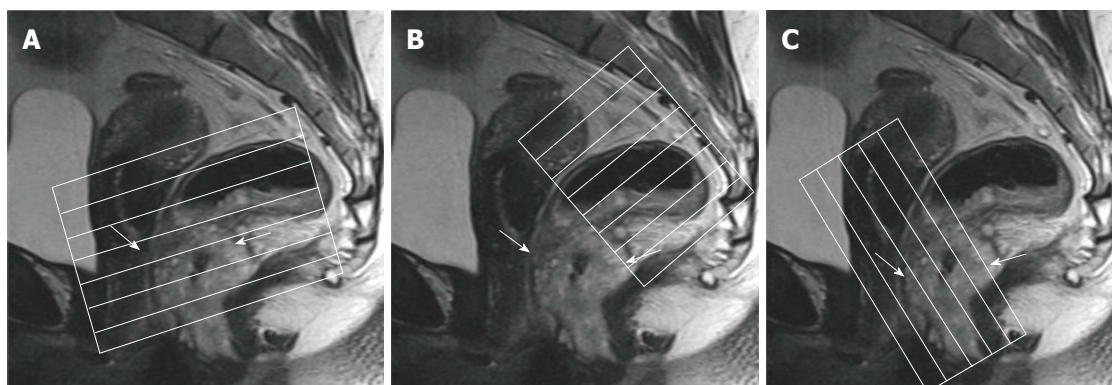


Figure 1 Magnetic resonance imaging planes. T2-weighted sagittal images are used to determine the longitudinal tumor axis in order to angle the axial and coronal planes. A: Oblique axial plane is obtained perpendicular to the rectal wall at the level of the rectal mass; B: Oblique axial plane is angled perpendicular to the pelvic floor, used to cover lymph node drainage territory; C: Coronal plane is angled parallel to the anal canal for imaging of low rectal tumors. Rectal tumor is indicated by arrows.

of preoperative (neoadjuvant) chemoradiotherapy (CRT) followed by surgery increases local control in locally advanced cancers and has become the standard approach to such rectal cancers^[1-5].

High-resolution pelvic magnetic resonance imaging (MRI) is the primary method for evaluation in rectal cancer^[6-10]. When applied according to the optimal protocols, high-resolution MRI accurately determining patients regarding neoadjuvant CRT requirement^[11]. Moreover, assessing treatment response in tumors using MRI also predicts probable survival outcomes, and could be used in the future to further adjust treatment according to the patients' response^[12]. In recurrent rectal cancer, MRI enables the depiction of the extent of tumor growth, and can establish the resectability of disease^[13,14].

MRI has not met expectations in re-staging, especially in complete response evaluation after neoadjuvant CRT because of post-therapeutic fibrosis and inflammation^[15-19]. However, adding functional MR sequences such as dynamic contrast-enhanced and diffusion-weighted sequences to the standard approach can improve diagnostic accuracy of MRI^[20-23].

In this pictorial review, we present a synopsis of the current standing of MRI in the staging and re-staging of rectal cancer. We also present an experience- and literature-based discussion of limitations and difficulties in interpretation.

MRI TECHNIQUE

Rectal MRI should be performed with pelvic phased-array coils. Rectal MRI using this technique provides overall assessment of the rectal wall layers with high-spatial-resolution and benefits from a large field of view^[15,24].

PATIENT PREPARATION

Routine rectal filling using endoluminal contrast agents such as ultrasonography gel is discouraged^[24] because this can distend of the rectum and compress the mesorectal fat, which may result in overestimation of

fascial involvement and interfere with assessment of mesorectal nodes^[25].

Bowel preparation is generally not necessary before the examination, but spasmolytics can be used when excessive fecal matter is visible on the planning images^[15,24]. For this purpose, a dose of 40 mg butylscopolamine is used intramuscularly unless contraindicated, immediately prior to placing the patient on the MRI table.

IMAGING PROTOCOL

Standard MR rectal protocols must at least include 2D T2-weighted sequences in sagittal, axial, and oblique coronal planes with 1-3 mm slice thicknesses. Sagittal sequences are used to identify the longitudinal tumor axis such that axial and coronal planes may be angled as perpendicular and parallel to the tumor axis as possible, respectively. Coronal planes must be angled in line with the anal canal for low tumors in order to evaluate the relation to the anal complex and pelvic floor muscles^[15,24,26] (Figure 1). Axial images are useful for evaluation of the tumor and its relationship with the intestinal wall, mesorectal fascia (MRF), and the adjacent pelvic tissue. Sagittal images are useful for the assessment of the tumor height and length and its relationship with peritoneum and other adjacent tissue.

In addition to T2-weighted sequences, diffusion-weighted imaging (DWI) sequences are recommended for inclusion in restaging protocols. DWI provides no additional benefit in primary staging; however, evidence is accumulating suggesting that it increases the diagnostic capability of MRI in the assessment of therapy response (yT-stage) after CRT^[24]. DWI also helps T2-weighted fast-spinecho (FSE) sequences to distinguish patients having good vs poor response^[20-23]. However, there is not adequate proof for supporting the usage of DWI for primary T-staging and lymph node assessment^[27].

ANATOMIC LANDMARKS

The rectum is approximately 15 cm in length from the anal verge, which is the lowest part of the anal canal.

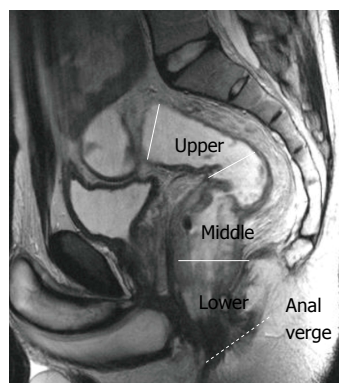


Figure 2 Rectal segments. T2-weighted sagittal image shows rectal segments: Lower, < 5 cm; middle, 5-10 cm; upper, > 10 cm from the anal verge.

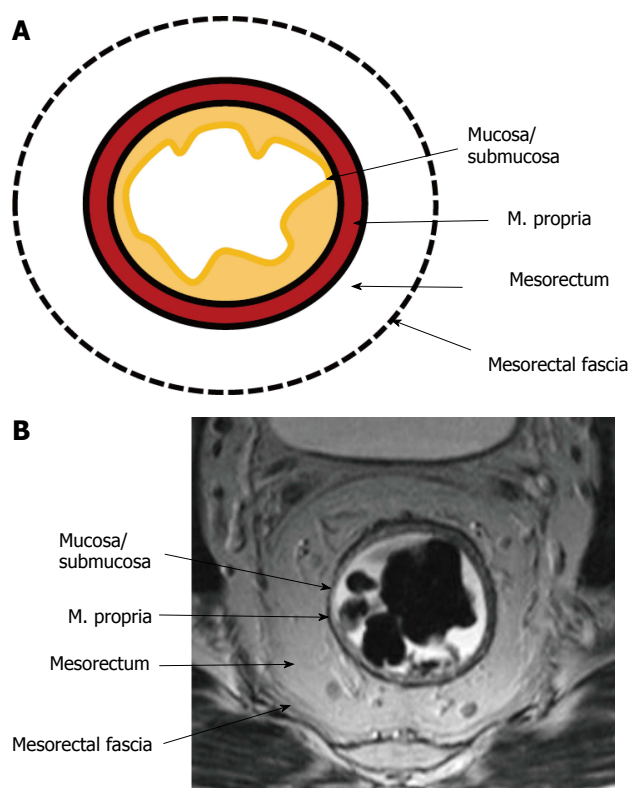


Figure 3 Normal rectal wall anatomy of higher and middle rectum. Schematic (A) and T2-weighted axial magnetic resonance imaging (B) presentation. The internal hyperintense layer represents the mucosa and submucosa (no distinction is possible between in two layers); the medial hypointense layer and external hyperintense area represent the muscularis propria and the mesorectum, respectively. Mesorectal fascia is seen thin hypointense layer enveloping the mesorectum (arrows).

The rectum has traditionally been divided into three segments according to the distance from the anal verge: Upper (> 10 cm), middle (5-10 cm), and lower (< 5 cm)^[27,28] (Figure 2).

The upper and middle rectal walls consist of three separate layers that can be distinguished in MRI. T2-weighted MRI sequences are the best for visualizing rectal wall anatomy. The internal hyperintense layer represents the mucosa and submucosa (no distinction is possible between in two layers); the medial hypointense

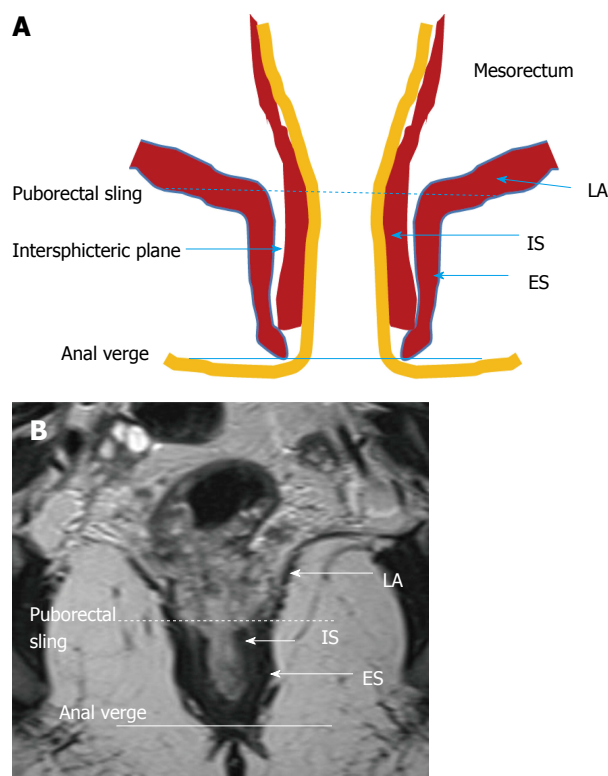


Figure 4 Normal anatomy of lower rectum. Schematic (A) and coronal plane T2-weighted (B) magnetic resonance imaging presentation. Puborectal sling, the upper portion of the puborectal muscle displaying the uppermost portion of the anal canal (intermittent line). Anal verge is the lowermost portion of the anal canal (line). LA: Levator ani muscle; IS: Internal sphincter; ES: External sphincter.

layer and external hyperintense area represent the muscularis propria and the mesorectum, respectively^[15,29] (Figure 3).

The puborectal sling constitutes the upper limit of the anal canal. The inner muscular wall of the anal canal comprises the internal sphincter, which is the direct continuation of the circular layer of the muscularis propria of the rectum. The outer muscular wall of the anal canal is cranially composed of the puborectal muscle and caudally of the external sphincter^[15,26] (Figure 4).

The puborectal sling constitutes the upper limit of the anal canal. The internal sphincter (the internal muscular wall) of the anal canal is consisted of the direct continuity of the muscularis propria circular layer of the rectum. The external muscular wall of the anal canal is formed by the puborectal muscle in cranially and the external sphincter in caudally^[15,26] (Figure 4).

The peritoneal reflection covers the anterior wall of the upper rectum; the risk of peritoneal perforation in upper rectal tumors is high^[27]. The peritoneal reflection can be easily displayed on sagittal and axial high-resolution T2-weighted images. In sagittal images, it can be depicted whereon upper pole of the seminal vesicles in men and at the uterocervical angle in women^[15]. The evaluation of the peritoneal invasion is very important in staging, because rectal tumor is staged as T4a in the presence of peritoneal invasion (Figure 5).

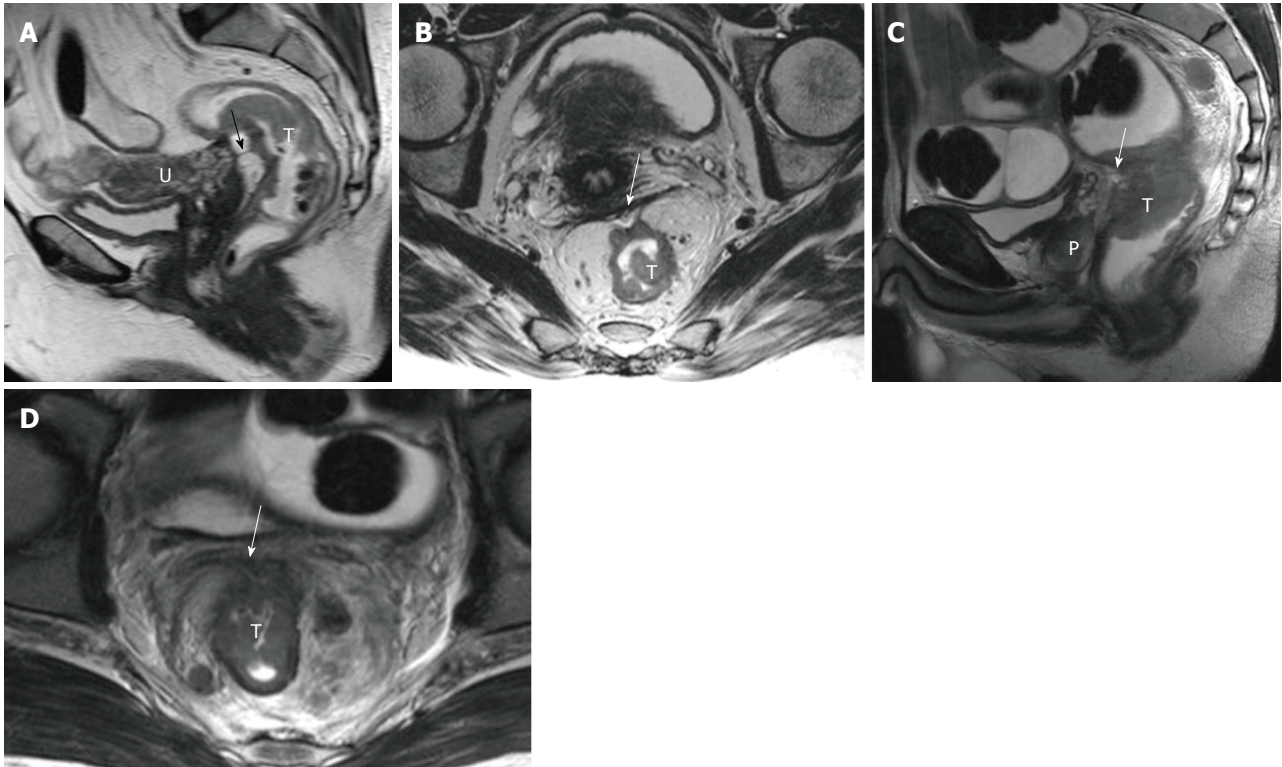


Figure 5 Periton invasion in female (A and B) and male (C and D) patients with T4a rectal tumors. On sagittal T2-weighted images, periton is seen as a hypointense linear structure in front of the tumor (arrows in A, C). On axial T2-weighted images, the peritoneum has a V shape and attaches onto the anterior aspect of the rectal cancer (arrows in B and D). T: Tumor; U: Uterus; P: Prostate.

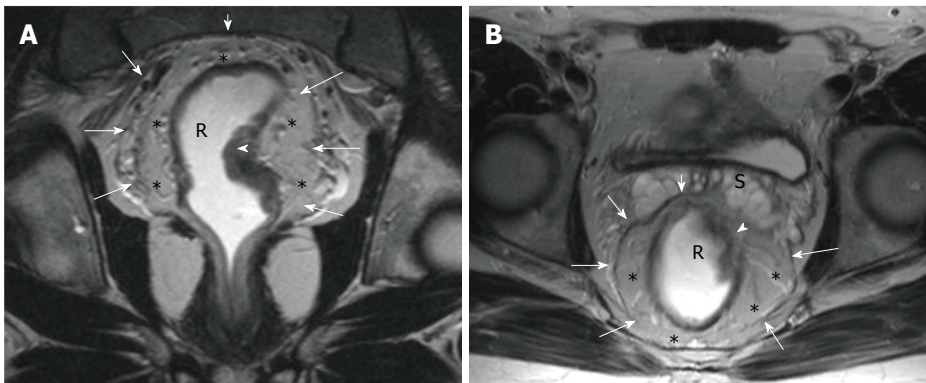


Figure 6 Magnetic resonance imaging anatomy of mesorectum and mesorectal fascia. On T2-weighted (A) axial and (B) coronal plane magnetic resonance images, mesorectal fascia (arrows) is seen as a thin, low-signal intensity layer enveloping the mesorectal fatty tissue (*) and rectum in a male patient with rectal carcinoma.

The middle rectum, which lies below the peritoneal reflection, is completely surrounded by mesorectal fatty tissue which is called the mesorectum. Mesorectum is encircled by the MRF which constitutes the circumferential resection margin (CRM)^[26-29]. The MRF can be seen as a thin, low-signal intensity envelop which surrounds the rectum and mesorectum (Figure 6). MRF tapers downward at the lower rectal level^[26]. The MRF is easily seen in posterolateral views, although it is difficult to distinguish it from Denonvilliers' fascia in the anterior wall^[30].

PRIMARY STAGING OF RECTAL CANCER

Tumor height and length

Tumor height and length should be routinely reported because outcomes and surgical management are affected by the location of the tumor^[24].

The distance and length are measured on a line drawn on the sagittal MR images. For tumor localization, the distance of the lowest portion of the tumor from the anal verge is measured. Rectal tumors are classified as high, middle or low when their most caudal border

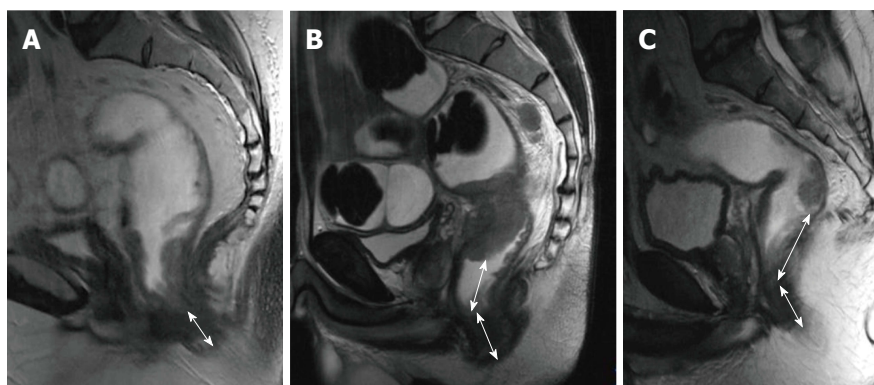


Figure 7 Rectal tumor levels. T2-weighted sagittal images in different patients with rectal carcinoma show distance from the anal verge (double-headed arrows) in (A) low rectal, (B) midrectal, and (C) upper rectal tumors (low rectal tumor, < 5 cm; midrectal, 5-10 cm; upper rectal, > 10 cm).

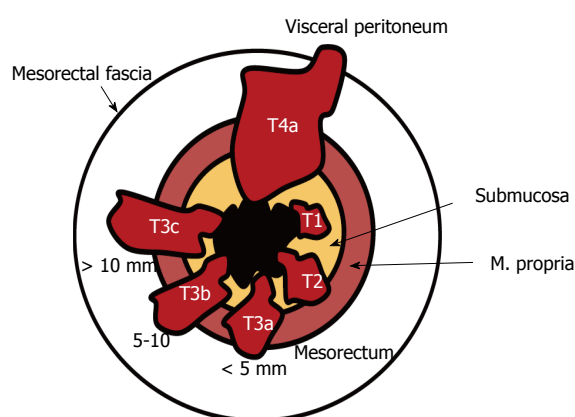


Figure 8 Rectal tumor T staging. The American Joint Committee on Cancer suggested an optional stratification of T3 tumors based on the extramural depth of invasion: Less than 5 mm, T3a; 5-10 mm, T3b; and more than 10 mm, T3c (adapted from ref. [27]: Nougaret S, Reinhold C, Mikhael HW, Rouanet P, Bibeau F, Brown G. The use of MR imaging in treatment planning for patients with rectal carcinoma: have you checked the "DISTANCE"? *Radiology* 2013; **268**: 330-344).

is > 10 cm, 5-10 cm, or < 5 cm from the anal verge, respectively^[15] (Figure 7).

T staging for middle and high tumors

On T2-weighted imaging, the muscularis propria is seen as a hypointense line between the hyperintense mesorectal fat and the inner submucosa and mucosa, which show intermediate to mild hyperintensity. The signal intensity of a rectal tumor on T2-weighted images is typically intermediate between the signal intensity of the muscularis propria and mucosa (Figure 8).

T1 tumors are confined to the submucosa; T2 tumors extend into, but not beyond, the muscularis propria. The differentiation of T1 tumors from T2 tumors on MRI is usually not reliable without an endorectal coil or endorectal ultrasound, and tumors should generally be staged as T1/T2^[15]. A tumor is staged as T3 when it extends beyond the muscularis propria and strands the mesorectal fat. Disruption of the muscularis propria because of penetrating vessels should not be overstaged as T3 (Figures 8 and 9).

The extramural depth of invasion refers to extension

of tumor beyond the muscularis propria^[31]. The American Joint Committee on Cancer suggested an optional stratification of T3 tumors based on the extramural depth of invasion: Less than 5 mm, T3a; 5-10 mm, T3b; and more than 10 mm, T3c^[32]. An extramural depth of invasion of less than 5 mm presents a significantly higher survival rate, and these early T3 tumors may be adequately managed with surgery alone and have a prognosis comparable to that of tumors characterized as T1/T2^[33]. T4 tumors extend onto the surface of the visceral peritoneum or an adjacent structure (Table 1, Figures 8 and 10).

Distance to the mesorectal fascia

For T3 tumors, the shortest distance between the most penetrating parts of the tumor and the MRF should be measured^[34,35]. The distance to the MRF is a critical local prognostic factor for rectal cancer^[36,37]. A tumor-MRF distance of more than 1 mm is a reliable predictor for negative margins after TME^[38]. In the presence of satellite nodules such as tumor deposits, lymph nodes or extramural vascular invasion (EMVI), the shortest distance between the nodules and the MRF should also be reported^[15] (Figures 11 and 12).

EMVI

EMVI is associated with local and distant recurrence and poor survival^[39]. It is defined as the presence of malignant cells within blood vessels located beyond the muscularis propria in the mesorectal fat. EMVI is suggested when vessels close to the tumor are obviously irregular or expanded by tumoral signal intensity^[39] (Figure 13).

The assessment of EMVI is a routine component of MR evaluation for primary staging; however, for restaging, there is no agreement as to whether evaluation of EMVI remains beneficial^[24].

T staging for low tumors

A specific T staging system is used to identify tumors and its circumferential resection margin (CRM)^[40] (Table 1, Figures 14 and 15).

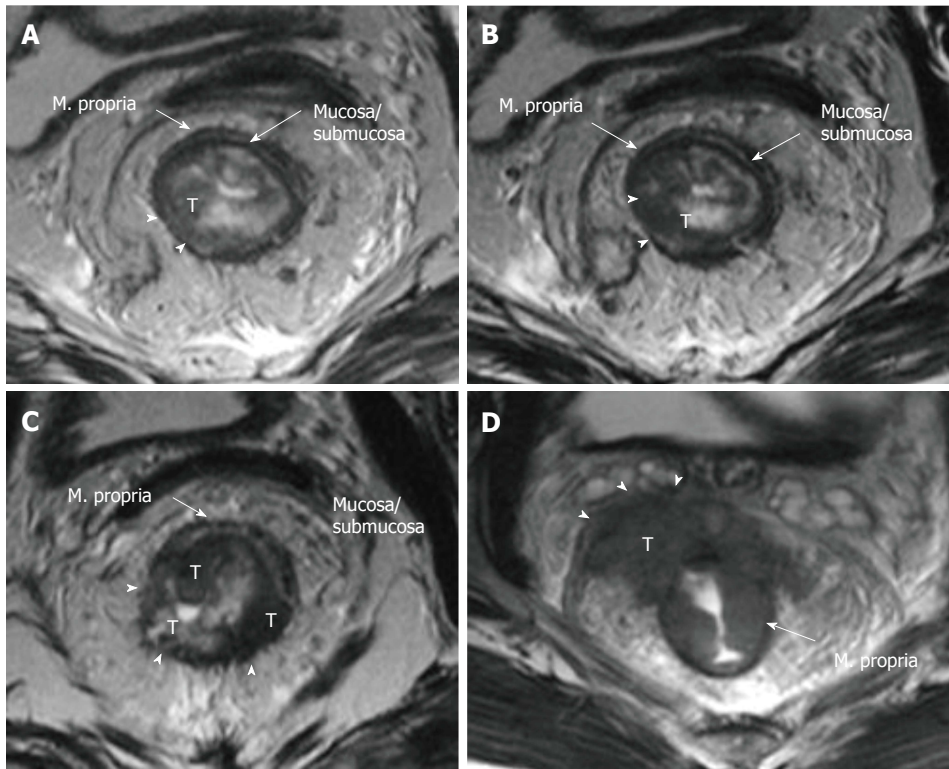


Figure 9 Rectal cancer T staging on magnetic resonance imaging. T2-weighted axial images showing rectal carcinomas with different T stages. A: T1 tumor is confined to the submucosa, has not entered the muscularis propria (arrowheads); B: T2 tumor extends into, but not beyond, the muscularis propria (arrowheads); C: T3 tumor extends beyond the muscularis propria and strands into mesorectal fat (arrowheads); D: T4a tumor invades the visceral peritoneum (arrowheads). T: Tumor.

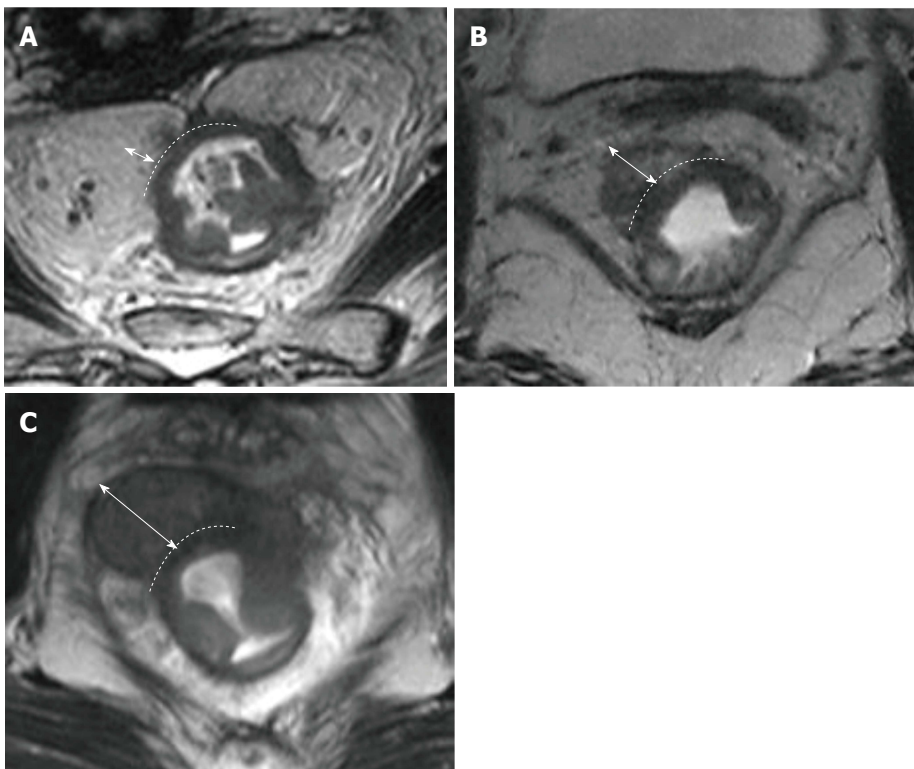


Figure 10 Stratification of T3 tumors on magnetic resonance imaging. T2-weighted axial magnetic resonance images in different patients with T3 rectal carcinoma showing extension of the tumor beyond the muscularis propria (double-headed arrows). The distance A: Less than 5 mm, T3a; B: 5-10 mm, T3b; and C: More than 10 mm, T3c.

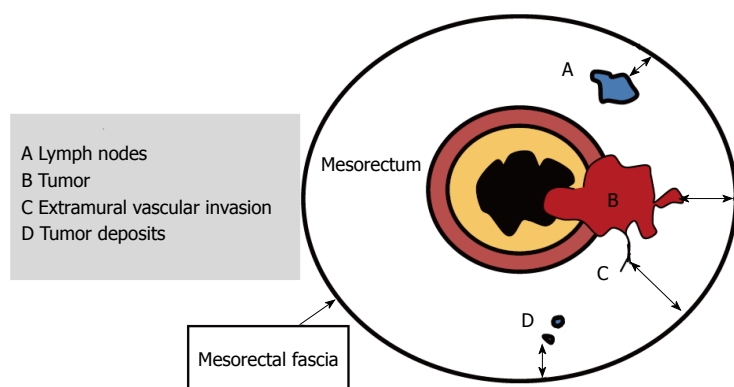


Figure 11 Schematic representation of positive resection margin. For T3 tumors, the shortest distance between the most penetrating parts of the tumor and the MRF is measured (double-headed arrows). A tumor mesorectal fascia distance of more than 1 mm is a reliable predictor for negative margins. In the presence of satellite nodules such as tumor deposits, lymph nodes or EMVI the shortest distance between the nodules and the MRF should also be reported (Adapted from ref. [27]: Nougaret S, Reinhold C, Mikhael HW, Rouanet P, Bibeau F, Brown G. The use of MR imaging in treatment planning for patients with rectal carcinoma: have you checked the "DISTANCE"? *Radiology* 2013; **268**: 330-344). EMVI: Extramural vascular invasion; MRF: Mesorectal fascia.

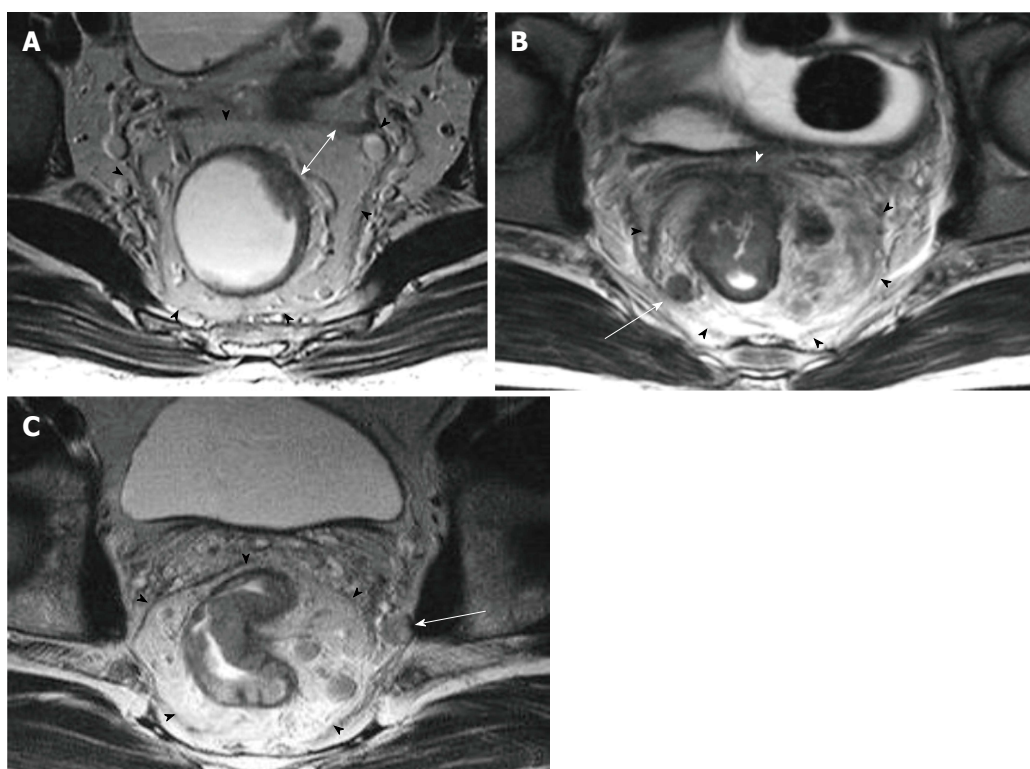


Figure 12 Distance to mesorectal fascia and mesorectal fascia invasion in different patients on T2-weighted axial images. A: T3a tumor is far away from the mesorectal fascia (double-headed arrow); B: T4a tumor (white arrowhead) and a suspicious mesorectal lymph node (arrow) are abutting the mesorectal fascia; C: Rectal tumor is lying > 1 mm from the mesorectal fascia; however, a suspicious lymph node, located out of the mesorectal fascia, is lying within < 1 mm of the mesorectal fascia (arrow). Mesorectal fascia is indicated with black arrowheads.

N-staging

Staging of nodes is very important for planning preoperative treatment^[41]. In the TNM system, disease involving only the regional nodes, including the mesorectal and internal iliac nodes, accounts for the N stage (Table 1); involvement of other nodes is regarded as metastasis^[38].

Mesorectal nodes are often the first and the most commonly involved group of nodes. Nodal metastases are usually within the proximal 5 cm of the tumor^[41].

Extramural nodes (iliac, superior rectal or inferior mesenteric nodes) are generally involved in locally advanced cancers^[42]. Low rectal tumors can also spread superficial inguinal nodes and imply poor prognosis^[43].

Node size is the usual criterion in nodal staging using MRI. Lymph nodes are usually considered pathologic when their short axis is longer than 0.5 cm; however, no

optimal cut-off threshold exists for involved nodes^[24]. The inclusion of morphologic features such as round shape, irregular contour, and nonhomogeneous signal intensity to a size cutoff increases the accuracy of MR^[44]. Although DW MRI is not accurate enough for characterizing nodes, it may be useful for locating them^[45] (Figure 16).

RESTAGING AFTER NEOADJUVANT TREATMENT

Neoadjuvant CRT provides downstaging and downsizing along with improvement in less extensive surgery, decreased local recurrence, and general survival^[12,46]. Tumor restaging involves correlating the posttreatment images with the pretreatment images with respect to

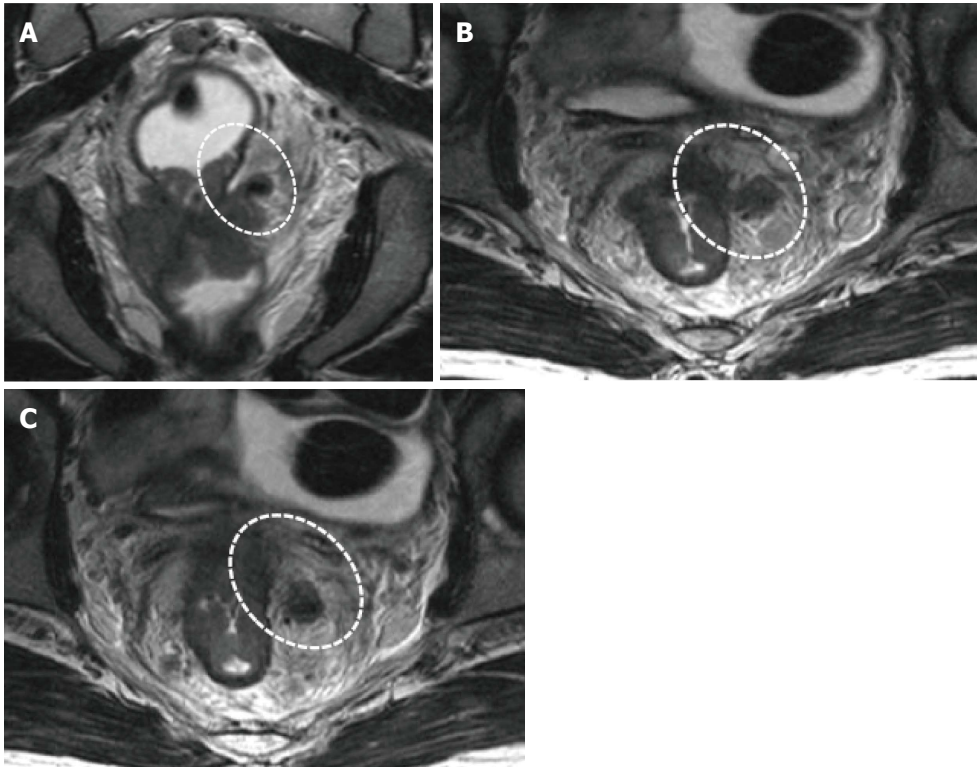


Figure 13 Extramural vascular invasion. T2-weighted (A) coronal and (B and C) serial axial magnetic resonance images in the same patient with T4a rectal cancer showing an irregular and expanded vessel insert to the tumor with tumoral signal intensity (circles).

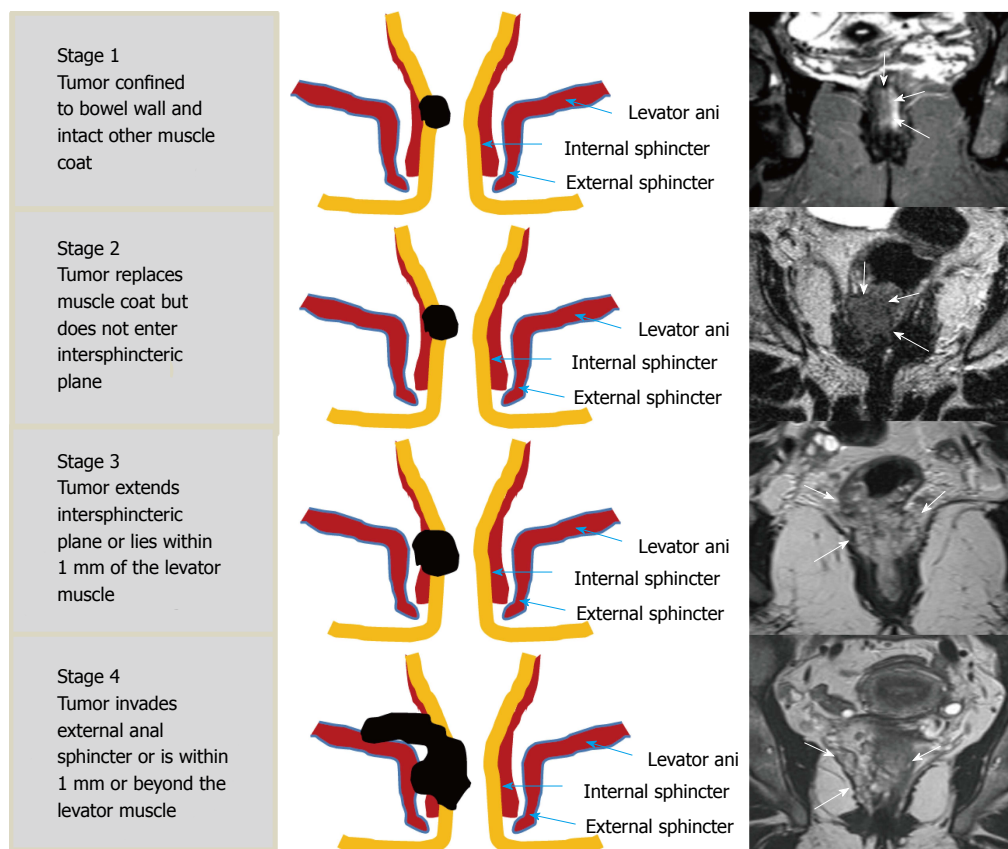


Figure 14 Schematic and high-spatial-resolution coronal T2-weighted magnetic resonance images for each stage according to the low rectal cancer. Rectal tumors in different patients are indicated with arrows on magnetic resonance images (Adapted from ref. [27]: Nougaret S, Reinhold C, Mikhael HW, Rouanet P, Bibeau F, Brown G. The use of MR imaging in treatment planning for patients with rectal carcinoma: have you checked the “DISTANCE”? *Radiology* 2013; **268**: 330-344).

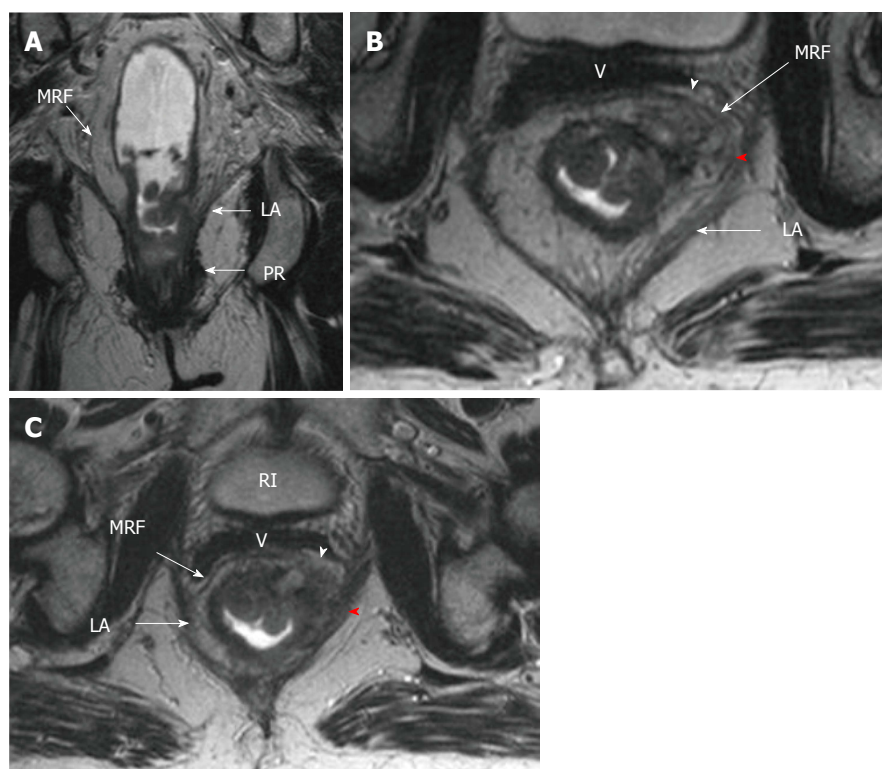


Figure 15 Stage 4 low rectal cancer. On T2-weighted (A) coronal (B, C) serial axial magnetic resonance images, rectal cancer showing invasion of levator ani (red arrowheads) and mesorectal fascia (white arrowhead). LA: Levator ani; PR: Puborectalis; MRF: Mesorectal fascia; BL: Bladder; V: Vagina.

Table 1 Staging systems for rectal cancer

Stage	MRI findings
T stage for middle and high tumors ¹	
T1	Tumor signal intensity is confined to the submucosal layer
T2	Tumor signal intensity extends into the muscle layer, with loss of the interface between the submucosa and circular muscle layer
T3	Tumor signal intensity extends through the muscle layer into the perirectal fat, with obliteration of the interface between muscle and perirectal fat
T3a	Tumor < 5 mm into the perirectal fat
T3b	Tumor 5-10 mm into the perirectal fat
T3c	Tumor > 10 mm into the perirectal fat
T4a	Tumor signal intensity extends to surface of visceral peritoneum
T4b	Tumor signal intensity extends into an adjacent structure or viscus
T stage for low tumors ²	
T1	Tumor signal intensity confined to bowel wall, outer muscle coat intact
T2	Tumor signal intensity replaces muscle coat but does not enter intersphincteric plane
T3	Tumor signal intensity extends intersphincteric plane or lies within 1 mm of levator muscle
T4	Tumor signal intensity extends external anal sphincter or is within 1 mm or beyond levator muscle with/without adjacent organ invasion
N stage	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1-3 regional lymph nodes
N2	Metastasis in > 3 regional lymph nodes

¹Adapted from ref. [32]: Edge SB, Byrd DR, Compton CC. AJCC cancer staging handbook: from the AJCC cancer staging manual, 7th ed. New York, NY: Springer, 2010: 718; ²Adapted from ref. [40]: Taylor FG, Swift RI, Blomqvist L, Brown G. A systematic approach to the interpretation of preoperative staging MRI for rectal cancer. *AJR* 2008; **191**: 1827-1835. MRI: Magnetic resonance imaging.

all the elements assessed in the initial staging, and necessitates image acquisition with almost the same protocol and on the same planes.

T staging

Post-CRT restaging using conventional MR sequences is less accurate than primary staging, especially when

confirming complete response (yT0), mostly because it is difficult to distinguish fibrosis, edema and normal mucosa from small foci of residual tumor^[46-48]. As such, a normal, two-layered rectal wall after CRT is indicative of complete response, whereas residual fibrosis indicates either residual tumor or complete response (Figure 17). In practice, areas of fibrosis have very low signal intensity

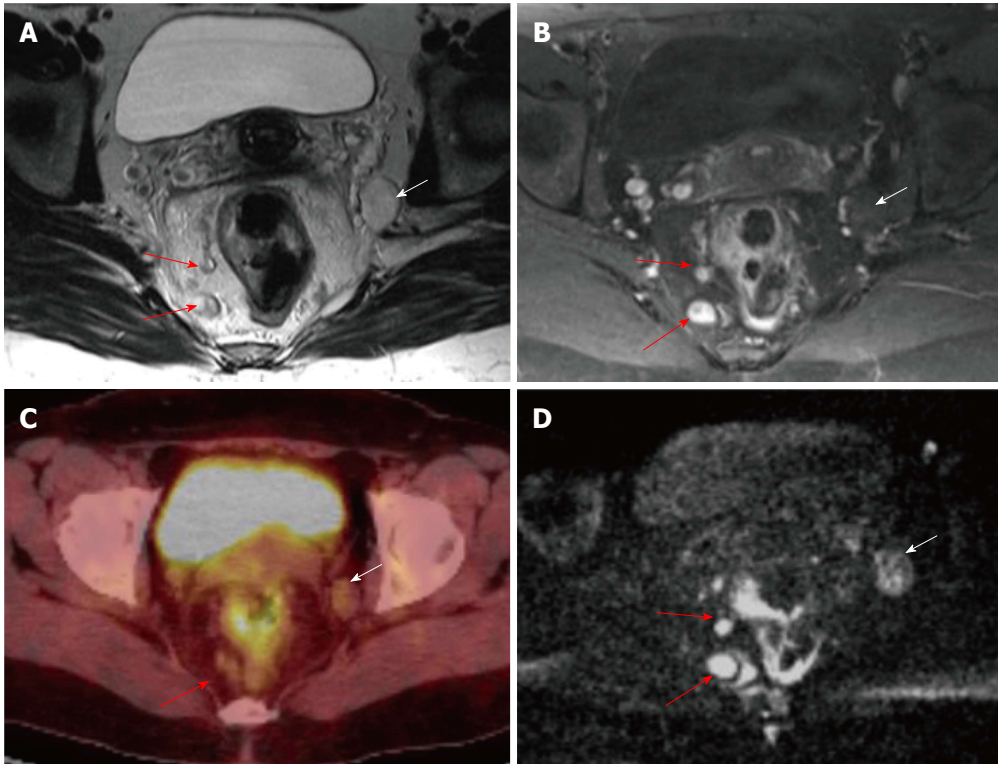


Figure 16 Mesorectal and extramesorectal lymph node involvement in rectal cancer. A: T2-weighted; B: T1-weighted contrast-enhanced axial MR images; C: ^{18}F -FDG PET-CT; D: DWI showing suspicious lymph nodes in mesorectal (red arrows) and extramesorectal areas (white areas). On DWI, extramesorectal lymph node is more remarkable than T2W and contrast-enhanced T1W sequences. DWI: Diffusion-weighted imaging; ^{18}F -FDG PET-CT: ^{18}F -fluorodeoxyglucose positron emission tomography-computed tomography.

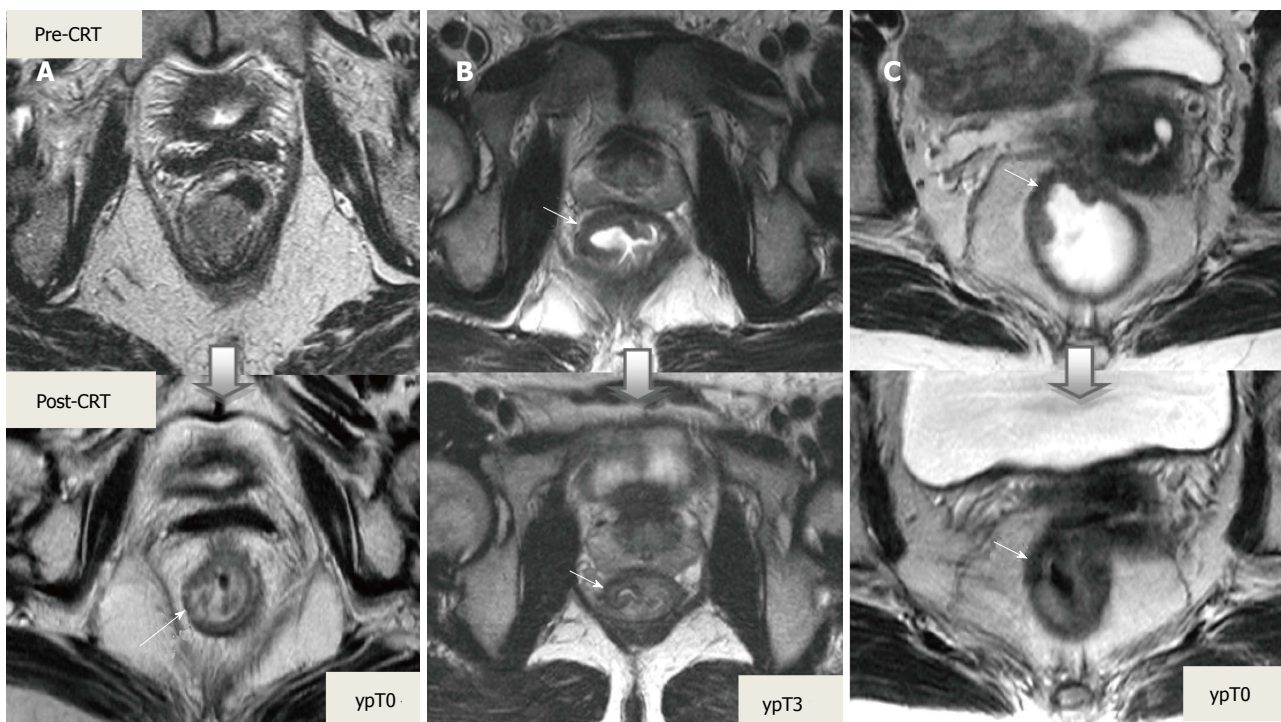


Figure 17 Tumor restaging after neoadjuvant chemoradiotherapy. On T2-weighted MR images in different patients showing baseline and post-CRT images on upper and lower series, respectively. A: In ypT0 rectal tumor, posttreatment axial image shows a normal, two-layered rectal wall (arrow), corresponding to complete response; B: In ypT3 rectal tumor, posttreatment axial image shows normal, two-layered rectal wall (arrow). This is an example for false-negative MR assessment of complete tumor regression; C: In ypT0 rectal tumor, posttreatment axial image shows thick, fibrotic low signal intensity scar (arrow) in pretreatment T3 tumor area. CRT: Chemoradiotherapy.

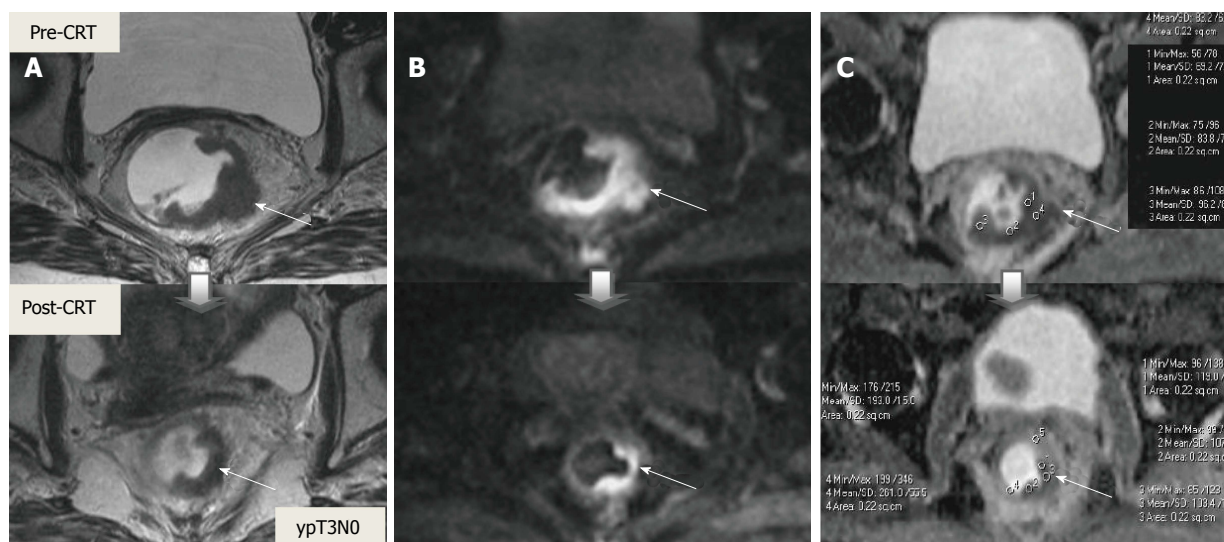


Figure 18 Post-chemoradiotherapy restaging using diffusion-weighted imaging in ypT3 rectal tumor. On T2-weighted (A), DW (B) and ADC (C) images in the same patient, baseline and post-CRT images are shown on upper and lower series, respectively. A: Posttreatment T2-weighted axial image shows semiannular infiltrating tumor, compatible with a residual T3 tumor (arrow); B: Posttreatment DW; C: ADC images delineate high and low signal-intensity corresponding to the tumor, respectively (arrow). Pre- and post-treatment mean ADC values are $0.68\text{--}0.72$, $1.22\text{--}1.44 \times 10^{-3} \text{ mm}^2/\text{s}$, respectively, in the tumor area. Post-therapy ADC increase is compatible with therapy response. CRT: Chemoradiotherapy.

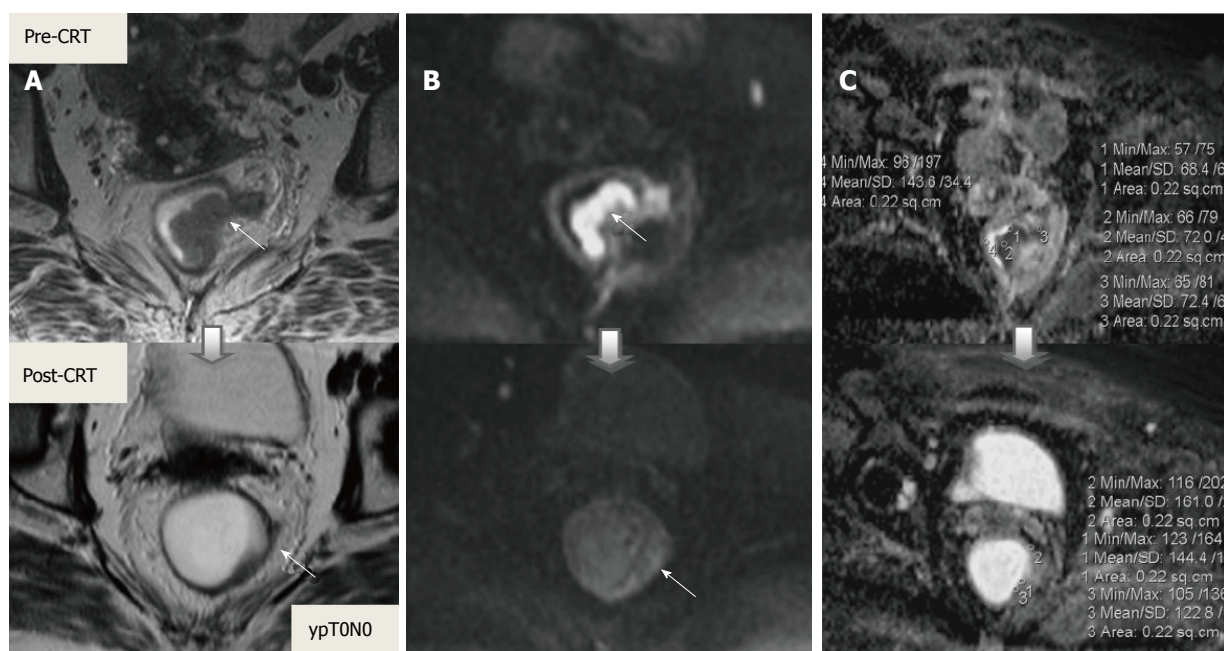


Figure 19 Post-chemoradiotherapy restaging using diffusion-weighted imaging in ypT0 rectal tumor. On T2-weighted (A), DW (B) and ADC (C) images in the same patient, baseline and post-CRT images are shown on upper and lower series, respectively. A: Posttreatment T2-weighted axial image shows a thick wall of low-signal-intensity fibrosis in the previous rectal tumor area (arrow). It is difficult to determine whether this area contains tumor cells or completely devoid of tumor cells (complete response); B: On posttreatment DW image (B-800), there is no diffusion signal in previous tumor area (arrows), compatible with complete response. In this case, DWI allows the correct differentiation of viable tumor from fibrosis; C: ADC images show post-therapy mean ADC increase ($0.70 \times 10^{-3} \text{ mm}^2/\text{s}$ vs $1.40 \times 10^{-3} \text{ mm}^2/\text{s}$) compatible with therapy response, but does not allow prediction of complete response. DWI: Diffusion-weighted imaging; CRT: Chemoradiotherapy.

on post-CRT T2-weighted MRI, in contrast, areas of residual tumor have intermediate signal-intensity^[46]. Careful review of high-resolution images and DWI can enable distinction of small residual tumor within fibrosis (Figure 18).

In addition to morphologic findings, DWI can provide

functional information that can be correlated with changes at the cellular level in response to treatment. After CRT, the decrease in cellularity and development of fibrosis or necrosis in responders results in an increase in diffusion, which decreases diffusion signal intensity in diffusion-weighted images and increases ADC values

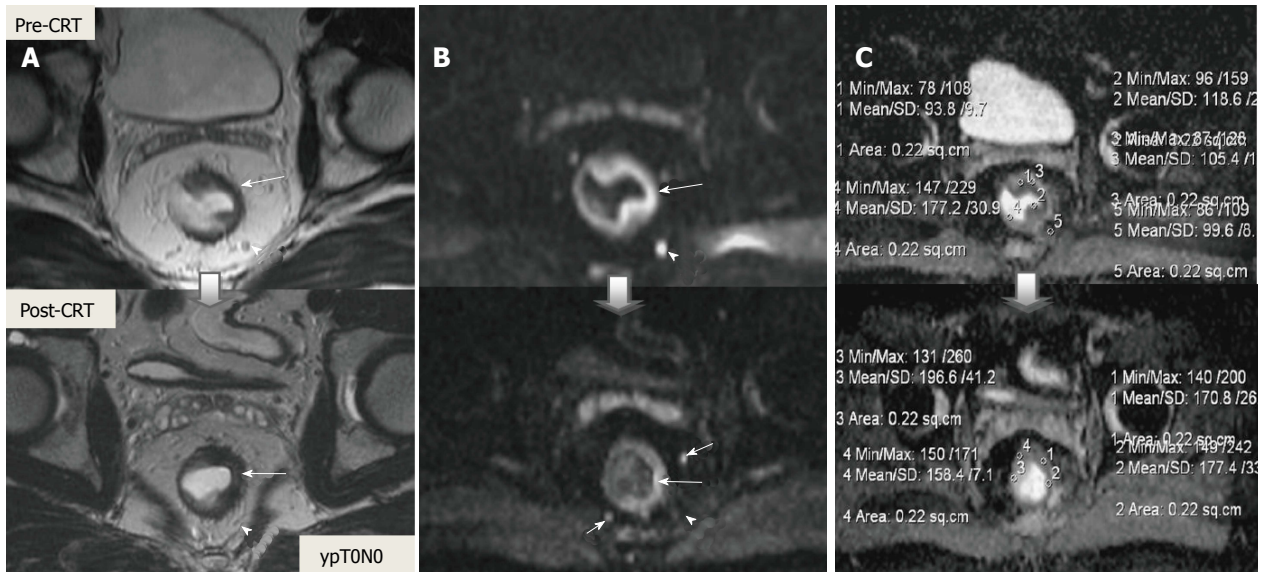


Figure 20 Post-chemoradiotherapy restaging using diffusion-weighted imaging in ypT0 rectal tumor. On T2-weighted (A), DW (B) and ADC (C) images in the same patient, baseline and post-CRT images are shown on upper and lower series, respectively. A: Posttreatment T2-weighted axial image shows a thick wall of low-signal-intensity fibrosis and areas suspicious for residual tumor have intermediate signal-intensity in the previous rectal tumor area (long arrow); B: Posttreatment DW images delineate a small foci of intermediate and low signal-intensity, respectively, compatible with residual tumor (long arrow); C: ADC images show post-therapy mean ADC increase ($1.05 \times 10^{-3} \text{ mm}^2/\text{s}$ vs $1.80 \times 10^{-3} \text{ mm}^2/\text{s}$), compatible with therapy response, but not with complete response. The suspicious mesorectal lymph node (arrowheads) is invisible on T2 and DWI after CRT, but the other two are still visible (short arrows). This case is an example for false-positive tumor and lymph node response evaluation of DWI. DWI: Diffusion-weighted imaging; CRT: Chemoradiotherapy.

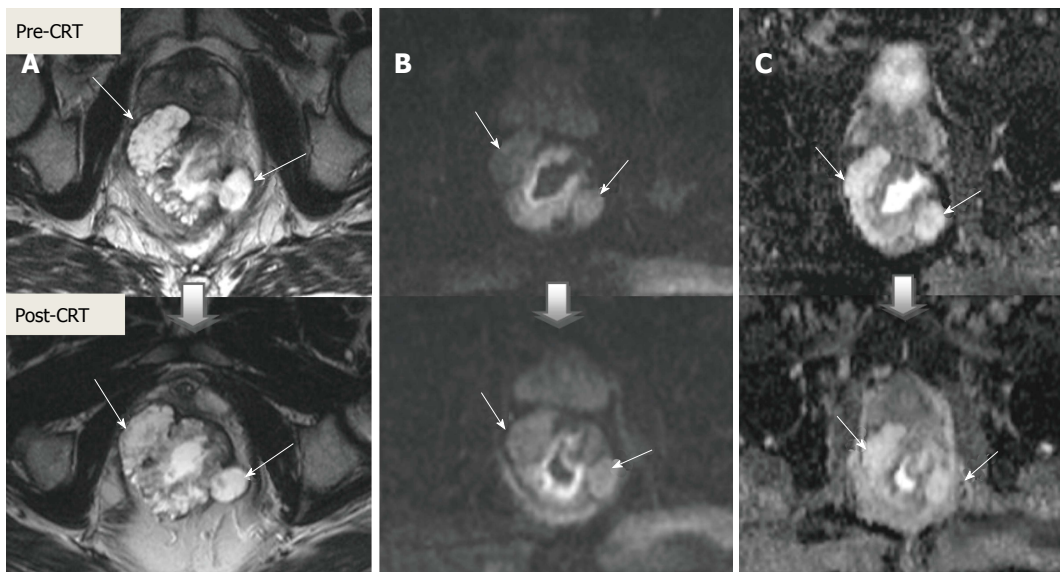


Figure 21 Mucinous adenocarcinoma. A: T2; B: Diffusion-weighted; C: ADC images in the same patient, baseline and post-CRT images are shown on upper and lower series, respectively. The mucinous tumor exhibits hyperintensity on T2, diffusion, and ADC images before and after treatment regardless of their response to treatment. Pre- and post-treatment ADC values are $1.70 \times 10^{-3} \text{ mm}^2/\text{s}$ and $2.10 \times 10^{-3} \text{ mm}^2/\text{s}$, respectively. Their response to CRT cannot be assessed using diffusion-weighted imaging. CRT: Chemoradiotherapy.

and ADC signal intensity in ADC images^[20,23] (Figures 18 and 19). Although DWI can differentiate viable tumor from fibrosis and good and bad response, it does not allow for predicting complete response^[19] (Figure 20). Moreover, the response of mucinous tumors to CRT cannot be assessed using DWI because they exhibit ADC hyperintensity even before treatment (Figure 21).

DISTANCE TO THE MESORECTAL FASCIA

CRM is considered uninvolved if a tumor free margin is seen at least 1 mm from MRF after CRT. This finding has strong negative predictive value (98%) of MR imaging for CRM involvement, whereas it has low positive

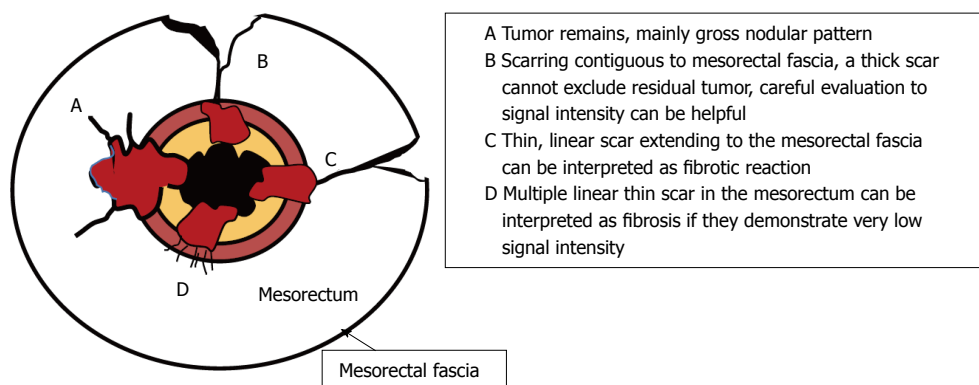


Figure 22 Schematic representation of effects of chemoradiotherapy on a rectal tumor and circumferential resection margins. Adapted from ref. [27]: Nougaret S, Reinhold C, Mikhael HW, Rouanet P, Bibeau F, Brown G. The use of MR imaging in treatment planning for patients with rectal carcinoma: have you checked the "DISTANCE"? *Radiology* 2013; **268**: 330-344.

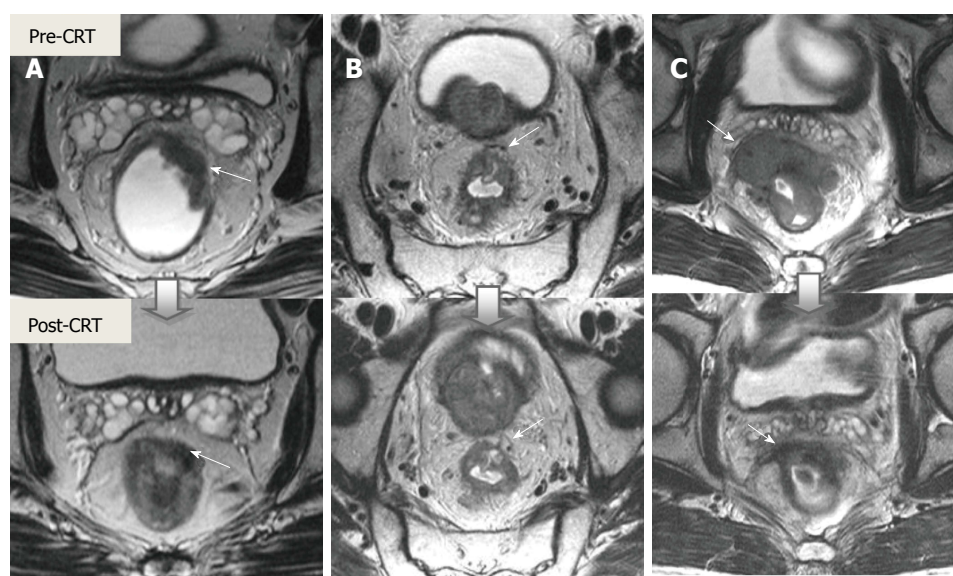


Figure 23 The effects of chemoradiotherapy on a rectal tumor and circumferential resection margins. T2-weighted axial magnetic resonance images in different patients show baseline and post-CRT images on upper and lower series, respectively. A: Overstaging due to thick, hypointense tissue infiltration at the mesorectal fascia (arrow) in ypT2 rectal tumor with no MRF invasion; B: In ypT3 rectal tumor with no MRF invasion, thick fibrous retractions of the tumor, suspicious for CRM positivity (arrow); C: Rectal mass is markedly shrunk with low-signal-intensity tissue infiltration at the mesorectal fascia (arrow). At surgery, there was tumor invasion of the mesorectal fascia. CRM: Circumferential resection margins; MRF: Mesorectal fascia; CRT: Chemoradiotherapy.

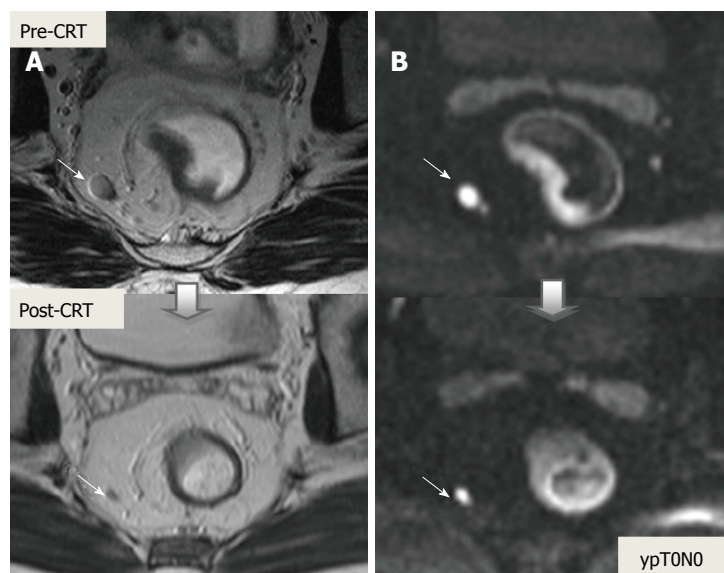


Figure 24 On diffusion-weighted imaging, false-positive mesorectal lymph node evaluation after chemoradiotherapy in ypT0N0 rectal cancer. A: T2-weighted axial magnetic resonance images show significant diminution in nodal size after chemoradiotherapy, compatible with negative lymph node (arrows); B: Diffusion-weighted images, high diffusion signal continues after treatment in the perirectal lymph node, compatible with positive lymph node (arrows). CRT: Chemoradiotherapy.

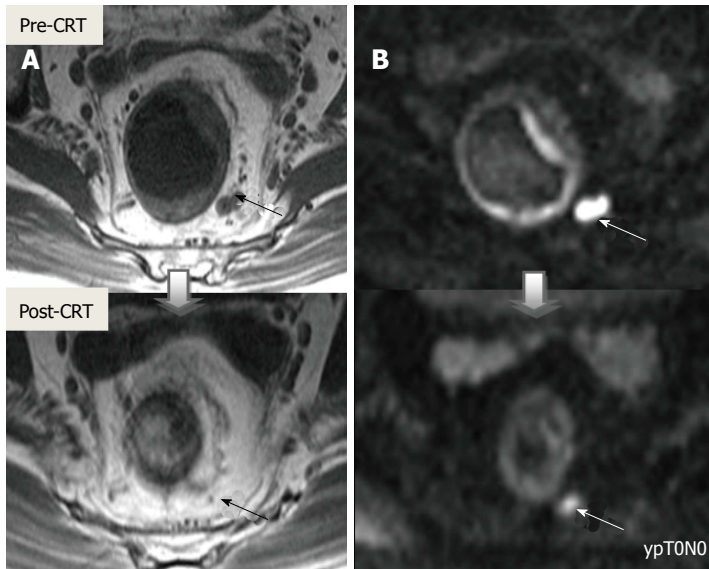


Figure 25 On diffusion-weighted imaging, false-positive mesorectal lymph node after chemoradiotherapy in ypT0N0 rectal cancer. A: T2-weighted axial images show significant diminution in nodal size, compatible with complete response; B: The continuation of high diffusion signal intensity on residual fibrotic lymph node incorrectly corresponds to a metastatic lymph node (arrows). CRT: Chemoradiotherapy.

predictive value^[49]. In some rectal tumor, however, CRT results in a markedly reduction tumor volume, but also in retraction of pre-existing contacts with MRF. It is difficult to determine whether this area contains tumor cells or completely devoid of tumor cells^[50] (Figures 22 and 23).

N-staging

After CRT, nodal size (short axis diameter) is more reliable for nodal re-staging. It is difficult to differentiate a metastatic lymph node from a healthy lymph node with irradiation changes using morphologic criteria or DWI; therefore, lymph node restaging often results in overstaging^[27,50] (Figures 24 and 25).

The accuracy of MRI for restaging is generally lower than the accuracy of MRI for initial staging, mainly owing to overstaging of nodal disease, failure to differentiate tumoral infiltration or residual tumor from desmoplastic reaction or radiation fibrosis^[50]. According to recent meta-analysis results, MRI accuracy was variable for restaging rectal cancer after neoadjuvant treatment; however, significantly better results were achieved when DWI was used or with experienced observers. The authors also reported that MRI could be used for evaluating CRM staging, but nodal staging remained a challenge^[51].

CONCLUSION

Using high-resolution MRI, standardizing image acquisition techniques and interpretation of images, comparative evaluation of pre- and post-CRT MR images, adding DWI to the standard approach, and importantly, experience and awareness of the limitations can improve diagnostic accuracy of MRI for re-staging.

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Immunotherapy in pancreatic cancer: Unleash its potential through novel combinations

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Abstract

Pancreatic cancer is the third leading cause of cancer mortality in both men and women in the United States, with poor response to current standard of care, short progression-free and overall survival. Immunotherapies that target cytotoxic T lymphocyte antigen-4, programmed cell death protein-1, and programmed death-ligand 1 checkpoints have shown remarkable activities in several cancers such as melanoma, renal cell carcinoma, and non-small cell lung cancer due to high numbers of somatic mutations, combined with cytotoxic T-cell responses. However, single checkpoint blockade was ineffective in pancreatic cancer, highlighting the challenges including the poor antigenicity, a dense desmoplastic stroma, and a largely immunosuppressive microenvironment. In this review, we will summarize available clinical results and ongoing efforts of combining immune checkpoint therapies with other treatment modalities such as chemotherapy, radiotherapy, and targeted therapy. These combination therapies hold promise in unleashing the potential of immunotherapy in pancreatic cancer to achieve better and more durable clinical responses by enhancing cytotoxic T-cell responses.

Key words: Immunotherapy; Pancreatic cancer; Anti-programmed cell death protein-1; Anti-programmed cell death protein-ligand1; Anti-cytotoxic T lymphocyte antigen-4; Single therapy; Combination therapies; Radiation therapy; GVAX; CRS-207; CD40 agonist

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Core tip: Pancreatic cancer is the third leading cause of cancer mortality in both men and women in the United States. Pancreatic cancer is one of nonimmunogenic

cancers that lacks of optimal treatments especially from immunotherapy prospective. Therefore, combining immune checkpoint therapies with other treatment modalities in pancreatic cancer will be the best strategy to achieve better and more durable clinical responses by enhancing cytotoxic T-cell responses.

Guo S, Contratto M, Miller G, Leichman L, Wu J. Immunotherapy in pancreatic cancer: Unleash its potential through novel combinations. *World J Clin Oncol* 2017; 8(3): 230-240 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/230.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.230>

INTRODUCTION

Pancreatic cancer is the third leading cause of cancer mortality in both men and women in the United States^[1]. The vast majority of patients with pancreatic cancer are diagnosed with advanced disease, and there has been a lack of optimal treatment option as the cancer is highly refractory to standard chemotherapy. Recently, two chemotherapy regimens, FOLFIRINOX and gemcitabine plus albumin-bound paclitaxel (nab-paclitaxel), have emerged as the standard of care for metastatic pancreatic cancer. These two regimens showed improved overall and progression-free survival (PFS) compared to gemcitabine alone in two phase III randomized controlled trials^[2,3]. Nevertheless, only up to 30% of patients showed response to either of these two regimens. The median PFS and overall survival (OS) remain poor, under 6 and 12 mo, respectively. Thus, there is still an urgent need to develop therapies that deliver more effective and durable clinical responses.

RELEVANCE OF IMMUNITY TO PANCREATIC CANCER

Observations in human disease and murine modeling has suggested that pancreatic cancer is almost invariably associated with a robust inflammatory infiltrate which can have divergent influences on disease progression by either combating cancer growth *via* antigen-restricted tumoricidal immune responses or by promoting tumor progression *via* induction of immune suppression (Figure 1)^[4-6]. For example, cluster of differentiation 8 (CD8⁺) and T-helper type 1 cells (Th1)-polarized cluster of differentiation 4 (CD4⁺) T cells mediate antitumor effects in murine models of pancreatic cancer and are associated with increased survival in patients with pancreatic cancer^[7-10]. Conversely, we recently reported that T-helper type 2 cells (Th2)-polarized CD4⁺ T cells promote pancreatic cancer progression in mice and intra-tumoral CD4⁺ Th2 cells infiltrates correlate with reduced survival in human disease^[7-9,11-13]. Similarly, Foxp3⁺ T-regulatory cells (Tregs) facilitate tumor immune escape in pancreatic cancer^[14]. Myeloid cells can influence T cells differentiation and

cytotoxicity in pancreatic cancer. We reported that tumor-infiltrating myeloid-derived suppressor cells (MDSCs) negate cytotoxic CD8⁺ T cells anti-tumor responses, accelerates pancreatic cancer growth and metastasis^[8,15-17]. Similar to T cells, macrophages also have cell types with different properties such as classically activated (M1) macrophages induce immunogenic responses, whereas alternatively activated (M2) macrophages have permissive influences on tumor growth by recruiting Tregs and Th2 cells^[18]. However, the drivers of immunosuppressive cell differentiation in pancreatic cancer are based on comprehensive understanding of regulation of the balance between immunogenic and immune-suppressive T cell populations.

THE EMERGENCE OF CHECKPOINT IMMUNOTHERAPY

The last few years witnessed a paradigm shift in cancer treatment strategy incorporating immunotherapy. Unprecedented clinical success has been observed for therapies targeting two major checkpoints of T cell response (Figure 2): Cytotoxic T lymphocyte antigen-4 (CTLA-4) and programmed cell death protein-1 (PD-1). Both checkpoints are expressed on activated T cells, but they act in distinct pathways. CTLA-4 blocks the essential cluster differentiation 28 (CD28) costimulation by competing and depleting the ligand of CD28 (B7-1 and B7-2) on antigen presenting cells (APCs). On the other hand, PD-1 interferes with the signaling pathways mediated by the T cell receptor and serves as a more distal block of T cell response by binding to its ligands (programmed death-ligand 1 (PD-L1) and programmed death-ligand 2 (PD-L2) which are present on many cell types including tumors cells^[19].

Monoclonal antibodies targeting CTLA-4 or PD-1 have shown durable clinical responses and prolonged OS in patients with melanoma, a highly immunogenic cancer. While single agent PD-1/PD-L1 inhibitors demonstrate impressive clinical benefits in many cancers such as non small cell lung cancer (NSCLC), renal cell carcinoma, bladder cancer, and Hodgkin's lymphoma^[20-29]. These results have led to FDA approval of Ipilimumab (anti-CTLA-4) in 2011 in melanoma^[30]. PD-1 inhibitors such as pembrolizumab and nivolumab were approved later in melanoma as well^[23,28,29]. PD-1 inhibitors (nivolumab and pembrolizumab), along with PD-L1 inhibitors such as atezolizumab have been approved in NSCLC, another example of immunogenic cancer^[21,22,24,29]. The activity of CTLA-4 and PD-L1 inhibitors are being explored in pancreatic cancer as well^[22,31].

EVIDENCE OF MINIMAL ACTIVITY OF SINGLE AGENT CHECKPOINT IMMUNOTHERAPY IN PANCREATIC CANCER

In early clinical trials single agent therapy with anti-CTLA-4

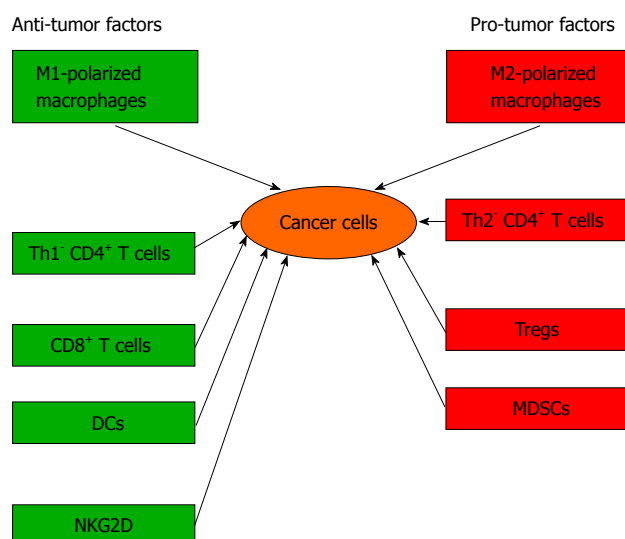


Figure 1 Anti-tumor and pro-tumor factors. Anti-tumor factors: M1 (classically activated macrophages), Th1CD4⁺ T cells (T-helper type 1-cluster differentiation 4 T cells), CD8⁺ T cells, DC (dendritic cells), NKG2D (natural killer group 2 member). Pro-tumor factors: M2 (alternatively activated macrophages), Th2CD4⁺ T cells (T-helper type 2-cluster differentiation 4 T cells) Th2, Tregs (T-regulatory cells), and MDSCs (myeloid-derived suppressor cells).

or anti-PD-1/anti-PD-1 pathway (anti-PD-L1) alone were largely ineffective in pancreatic cancer^[22,31,32]. In a single-arm phase II study, Ipilimumab failed to induce tumor response in patients with advanced pancreatic cancer^[32]. Similarly, single agent BMS-936559, an anti-PD-L1 monoclonal antibody, did not show any activity in 14 patients with advanced pancreatic cancer in a phase I study^[22].

POTENTIAL BARRIERS THAT HINDER EFFICACY OF IMMUNOTHERAPY

The efficacy of immunotherapy in pancreatic cancer is handicapped by small number of cumulative mutational load that can lead to expression of non-self-antigens, or “neoantigens” which are recognized by the immune system as foreign. Cancers with higher number of mutational load are associated with more neoantigens that are easier to be recognized by the immune system, compared to cancer with lower number of mutational load^[33-35]. There are 3 major barriers for the utility of immunotherapy in pancreatic cancer. First, the mutational load in pancreatic cancer is very low as compared with melanoma and lung cancers^[36,37]. Second, pancreatic cancer features a largely immunosuppressive microenvironment, characterized by a dense desmoplastic reaction with prominent infiltration of tumorigenic macrophages and myeloid derived suppressor cells (MDSCs)^[38]. Third, there are very few infiltrating T cells in the microenvironment of pancreatic cancer, therefore could not provide sufficient T cell responses. Pancreatic cancer creates a nonimmunogenic (or “cold”) tumor microenvironment, limiting the activity of immune

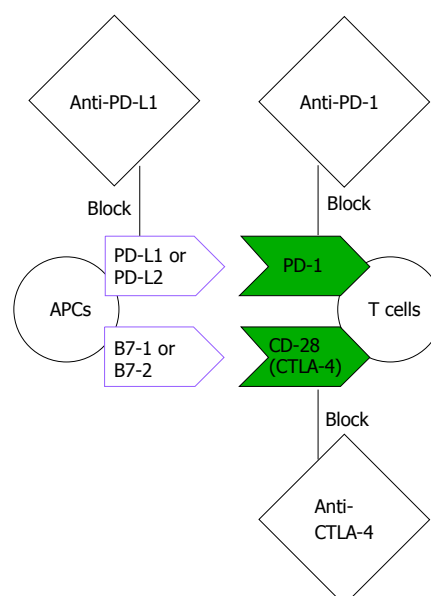


Figure 2 Immunotherapy basics. Anti-PD-L1 inhibit PD-L1 (programmed cell death-ligand 1) binding to PD-1 (Programmed cell death protein-1). Anti-PD-1 inhibit PD-1 on T-cell that binds to PD-L1 or PD-L2 (programmed cell death ligand-2) on APC (antigen presenting cell). Anti-CTLA-4 (anti-cytotoxic T lymphocyte antigen 4) inhibit CD28 (cluster differentiation 28) on T cell that binds to B7-1 or B7-2 (ligand of CD28) on APC.

checkpoint therapies^[31].

EVIDENCE OF T CELL IMMUNITY

On the other hand, there is still evidence of T cell-mediated immunity in pancreatic cancer. An analysis of resected surgical samples of pancreatic cancer patients has shown that higher levels of CD4⁺ and CD8⁺ tumor infiltrating T cells are associated with better prognosis^[10]. In addition, since immunosuppression occurs early during tumorigenesis as shown in Pdx1^{Cre};Kras^{G12D};Tp53^{R172H} (KPC) mouse model, the tumor cells may have been shielded from immune pressure, thus preserving their sensitivity to T cell attack^[38].

In addition, downstream signals are also critical in the T cell immune responses. Interferon-gamma (IFN- γ) promotes inhibition of melanoma cell growth and induces apoptosis of tumor cells by regulating T-cells responses^[39-44]. Immune checkpoint inhibitors increase production of IFN- γ from T-cell^[45-50]. However its effect will be suboptimal if there is a defect in the IFN- γ pathway^[51]. Studies in patients with melanoma showed that a defect in the IFN- γ pathway can lead to resistance to anti-CTLA4 and anti-PD-1 therapies^[51,52]. Several genomic biomarkers of IFN- γ pathways such as interferon gamma receptor 1, janus kinase 1 (JAK1), and JAK2 have been identified in melanoma patients with good response to immune checkpoint therapies^[41-43,51,52]. On the other hand, genes such as suppressor of cytokine signaling 1 (SOCS1) and protein inhibitor of activated signal transducer and activator of transcription 4 (PIAS4) have

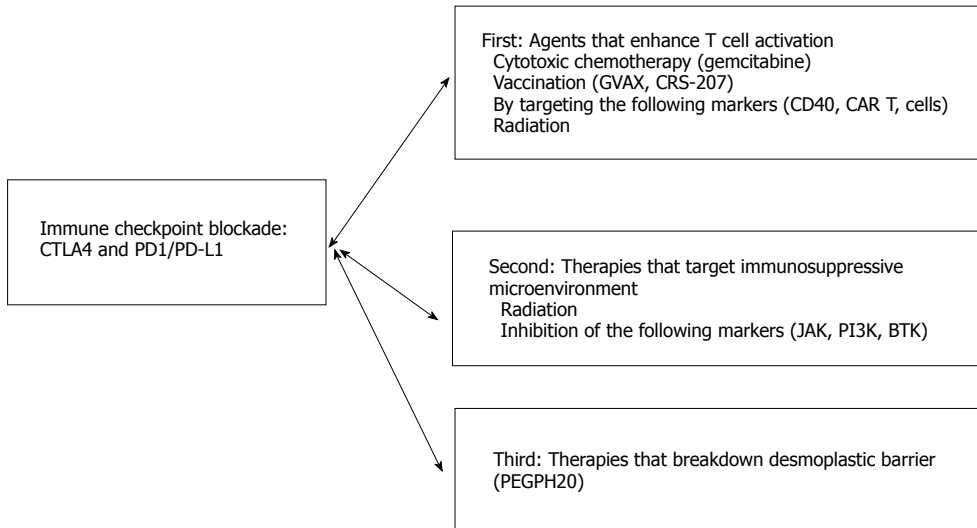


Figure 3 Searching for the optimal combination to maximize the potential of immune checkpoint blockade for the treatment of pancreatic cancer. CTLA-4: Cytotoxic T lymphocyte antigen-4; PD-1: Programmed cell death protein-1; PD-L1: Programmed death ligand-1; CD40: Cluster differentiation 40; CAR T cells: Chimeric antigen receptor T cells; PI3K: Phosphoinositide-3-kinase; BTK: Bruton tyrosine kinase; JAK: Janus kinase; PEGPH20: Pegylated hyaluronidase.

demonstrated the opposite effects by inhibiting IFN- γ signaling pathway^[51,53,54].

STRATEGIES OF TURNING ON THE ACTIVITY OF IMMUNOTHERAPY

Thus, the incorporation of additional therapies that can turn a “cold” tumor microenvironment into a “hot” one presents an important strategy to elicit clinical activity of immune checkpoint therapies. These additional therapies mainly fall into three categories (Figure 3): First, therapies that enhance tumor antigen presentation to help T cell priming/activation; second, therapies that modulate tumor microenvironment to relieve immunosuppression. Third, therapies which breakdown the desmoplastic barrier surrounding pancreatic cancer to bring infiltrating T cells. Below we will summarize the combination therapies that have already been assessed clinically and provide future directions of new combinations that may hold promise.

FIRST (ENHANCE T CELL ACTIVATION)

Immune checkpoint therapy + chemotherapy

Gemcitabine is one of the backbone chemotherapy agents for the treatment of pancreatic cancer. It has been suggested that gemcitabine is not immunosuppressive in pancreatic cancer patients and may be able to enhance naïve T cells activation^[55]. Combination of gemcitabine and immune checkpoint blockade has been evaluated for their potential synergistic activity.

Gemcitabine plus CTLA-4 blockade: A phase I clinical study evaluated the combination of gemcitabine and an anti-CTLA-4 antibody (tremelimumab) in treatment naïve patients with metastatic pancreatic cancer. This combination showed a tolerable side effect. Among 28

out of 34 evaluable patients, 2 achieved partial response (PR) and 7 showed stable disease (SD) for > 10 wk^[4]. In another ongoing phase Ib study of unresectable pancreatic cancer, preliminary results showed that, among 11 evaluable patients (out of 13 enrolled), ipilimumab and gemcitabine resulted in 2 PR and 5 SD^[56,57].

Gemcitabine plus PD-1/PD-L1 blockade: An immunohistochemistry analysis has shown that positive PD-L1 expression in resected pancreatic cancer was correlated with worse OS^[58]. In a mouse model of pancreatic cancer, combining gemcitabine with either anti-PD-1 or anti-PD-L1 antibody enhanced tumor infiltration of CD8⁺ T cells and resulted in complete responses in treated mice^[58]. A clinical pilot study of combination of gemcitabine and anti-PD-1 antibody has closed to enrollment (NCT01313416).

Immune checkpoint therapy + cancer vaccines

The most extensively studied pancreatic cancer vaccine is GVAX. GVAX is a whole cell vaccine composed of irradiated, allogeneic pancreatic tumor cells genetically engineered to secrete granulocyte macrophage-colony stimulating factor (GM-CSF), a cytokine that stimulates dendritic cell activation and T cell priming. When used as part of adjuvant therapy in the post-resection setting, GVAX was able to induce pancreatic cancer specific CD8⁺ T cell expansion as shown in a phase II study^[59]. Also, when used as neoadjuvant and adjuvant therapy, GVAX and low dose cyclophosphamide (an alkylating agent with an ability to deplete Tregs) resulted in formation of intratumoral tertiary lymphoid aggregates and T cell infiltration, suggesting the ability of GVAX in the conversion of pancreatic cancer from a “non-immunogenic” into an “immunogenic” state^[60].

GVAX plus CTLA-4 blockade: In a small phase Ib

study, GVAX in combination with anti-CTLA-4 antibody ipilimumab was evaluated in 30 patients with advanced, refractory pancreatic cancer that were previously treated with gemcitabine-based chemotherapy. Compared to ipilimumab alone, the combination therapy resulted in improved survival (27% vs 7% at 1 year). Also, a longer survival was associated with an increase in peak mesothelin-specific T cells and a larger T cell repertoire (the percentage of mesothelin peptides for which enhanced T-cell responses were measured), indicating a positive role of T cell response^[61].

GVAX plus PD-1/PD-L1 blockade: Detailed analysis of lymphoid aggregates formed after GVAX therapy revealed elevated expression of PD-L1 on monocytes/macrophages^[60,62], suggesting the potential benefit of targeting PD-1/PD-L1 checkpoint. This concept was supported by experiments in a pancreatic cancer mouse model, where the combination of GVAX and an anti-PD-1 antibody resulted in better survival than anti-PD-1 antibody alone, and this activity was correlated with increased CD8⁺ T cells and elevated IFN- γ production in the tumor microenvironment^[62]. Currently, a randomized clinical study (NCT02451982) is ongoing to evaluating GVAX with or without anti-PD-1 antibody (nivolumab) as neoadjuvant and adjuvant treatment in patients with resectable pancreatic cancer.

GVAX and CRS-207 plus PD-1/PD-L1 blockade: CRS-207 is a bacterial vaccine composed of live-attenuated, double deleted *Listeria monocytogenes* expressing human mesothelin, an antigen commonly overexpressed in pancreatic cancer cells. CRS-207 can induce robust innate as well as mesothelin-specific adaptive immune response, therefore allowing for a "boost" to the immune response initiated by GVAX. In a randomized, phase II study, GVAX prime followed by CRS-207 boost resulted in prolonged OS compared to GVAX alone in patients with metastatic, refractory pancreatic cancer. This study also showed that mesothelin-specific CD8⁺ T cell response was correlated with better survival^[63,64]. On the basis of these findings, a randomized phase II study (NCT02243371) was to evaluate whether adding anti-PD-1 therapy (nivolumab) will further enhance the activity of this prime-boost strategy^[65]. This study has closed to enrollment.

In a phase IIb study (NCT02004262) in refractory and metastatic pancreatic cancer, 303 patients were randomized between GVAX and CRS-207 (arm A), only CRS-207 (arm B), and single agent chemotherapy (arm C)^[66]. No OS advantage was seen in arm A when compared to arm C^[66]. A large number of patient drop out prior to treatment was observed in both arm A and C (40% versus 60%, respectively), indicating the challenge of therapeutic benefit in refractory pancreatic cancer. It also hints that these patients in the refractory setting may be too sick to benefit from immunotherapy due to rapid deterioration of disease.

Immune checkpoint therapy + agents enhancing T cell immunity

CD40 agonist: CD40 is a member of the tumor necrosis factor receptor family. Ligation of CD40 can occur on dendritic or B cells, or at CD40 ligand (CD154) on activated T cells, such effect can enhance T cell immunity^[67]. In a 22 patients series with unresectable pancreatic cancer, a CD40 agonist (CP-870, 893) and gemcitabine led to an encouraging clinical response^[7,11]. Rather unexpectedly, it showed that tumor infiltration by macrophages played a larger role for depletion of tumor stroma and killing of tumor cells^[7]. In a more recent study in the KPC mouse model, however, the use of CD40 agonist monoclonal antibody (mAb) with gemcitabine and nab-paclitaxel induced macrophage-independent T cell immunity. This study also found that CD40 agonist in addition to chemotherapy was able to sensitize the tumors to anti-CTLA-4 and/or anti-PD-1 therapies, leading to tumor regression and improved survival^[31]. A recent study using an orthotopic pancreatic cancer mouse model also demonstrated tumor regression and enhanced immune response with the combination of CD40 agonist antibody with gemcitabine/Nab-paclitaxel^[68]. It is yet to be seen whether these pre-clinical results can translate into clinical benefits.

CAR T cells: Autologous T cells genetically engineered to express a chimeric antigen receptor (CAR) have been developed to trigger cancer-specific T cell immunity and have shown impressive activity in acute lymphoblastic leukemia^[69]. For the treatment of pancreatic cancer, the CARs are engineered to recognize mesothelin, a specific membrane protein antigen overexpressed on pancreatic cancer cells. Mesothelin-specific CAR T cells are currently under phase I clinical evaluation, with preliminary results suggesting acceptable safety profiles and potential clinical activity against advanced pancreatic cancer. This study demonstrated that 2 out of 6 patients achieved SD and one patient with liver metastasis at baseline showed no fluorodeoxyglucose (FDG) uptake within 1 mo of treatment^[12,70,71]. Therefore, CAR T cells represent another treatment modality to combine with immune checkpoint therapies.

Immune checkpoint therapy + radiotherapy

The effects of radiotherapy (RT) on the immunology of pancreatic cancer have not been intensively studied. However, work in other cancers has suggested that RT should be considered an immune adjuvant as evidenced by radiotherapy (RT) induced enhancement of both innate and adaptive immunity. For example, the immunogenicity of dendritic cells (DCs) is reportedly improved by RT-induced necrotic tumor cell release of high mobility group box 1 protein (HMGB1) which ligates toll-like receptor 4 (TLR4) and toll-like receptor 9 (TLR9) on DCs. Such events promote DCs' cellular maturation and enhance their antigen processing capabilities^[72]. Another consequence of RT-induced necrotic cell death is the translocation of calreticulin from the endoplasmic reticulum to the

plasma membrane which facilitates assembly of major histocompatibility-1 (MHC I)-peptide complexes. Calreticulin also enhances DCs cross presentation of antigens to cytotoxic T lymphocytes. In addition to upregulating the antigen-presentation machinery in DCs, RT can reportedly enhance immunogenicity by inducing the release of tumor antigens, upregulating the expression of T-cell co-activating ligands, and sensitizing tumor cells to antigen-independent cell death *via* the Fas receptor^[72]. RT is further thought to augment diverse aspects of T cell immunity *via* adenosine triphosphate release from apoptotic cells which induces secretion of Interleukin-1-beta (IL-1 β). A consequence of this cascade is T helper1 (Th1) polarization of antigen-restricted CD4⁺ T cell responses and activation of cytotoxic T cells. Additionally, activation of cytotoxic T cells can be further activated by irradiation, *via* natural killer group 2 member D (NKG2D) receptor on cytotoxic T cells. NKG2D receptor can be induced in a stress event such as DNA damage which can be achieved by RT^[72]. Therefore, ionizing radiation can result in "immunogenic cell death", in which the dying tumor cells trigger "danger signals" (a signal of releasing HMGB1 and binding to TLR4 and TLR9 on DCs to process the antigen) to boost T cell activation^[72,73].

SECOND (TARGETING IMMUNO-SUPPRESSIVE MICROENVIRONMENT)

As described earlier, an important barrier to the success of immunotherapy in pancreatic cancer is an immunosuppressive tumor microenvironment, enriched with immunosuppressive cells such as tumor associated macrophages (TAMs) and MDSCs. In animal models of pancreatic cancer, blockade of immunosuppressive MDSCs could promote antitumor T-cell responses and block protumor macrophage responses^[6,74-76]. Therefore, drugs that block these immunosuppressive cells in the tumor microenvironment represent attractive strategies to sensitize pancreatic cancer to immune checkpoint therapies.

Immune checkpoint therapy + radiotherapy

RT's theoretical potential ability to convert the tumor microenvironment from a "cold" to a "hot" state suggests the opportunity of RT combination with immune checkpoint therapy. In the KPC pancreatic mouse model, any combination of immune checkpoint inhibitor with RT substantially increased OS, when compared to anti-CTLA-4 antibody or anti-PD-L1 antibody alone without RT. In particular, the triple therapy (RT + CTLA-4 antibody + PD-1 antibody) resulted in the highest response rate and longest OS among any of the immunotherapy group as single therapy or in combinations^[77].

However, our recent preclinical studies on RT in pancreatic cancer suggest caution as we found that RT induced the programming and recruitment of immunosuppressive M2-like macrophages which lead to the expansion of tumor promoting Th2-polarized CD4⁺ T

cells and Tregs. We also found that combining RT with either macrophage neutralization or M-CSF blockade resulted in synergistic efficacy in mice model, suggesting another treatment strategy for pancreatic cancer utilizing RT combining with colony stimulating factor-1 receptor inhibitor^[76,78].

So far there have been no published clinical results on RT plus checkpoint blockade for the treatment of pancreatic cancer. Currently, an open-label, three-cohort, multi-institutional phase Ib study is ongoing at New York University (NCT02868632) to assess stereotactic body radiation therapy (SBRT) in combination with either MEDI4736 (an anti-PD-L1 antibody) alone, tremelimumab (an anti-CTLA4 antibody) alone, or the combination of MEDI4736 and tremelimumab in patients with unresectable/locally advanced previously untreated pancreatic cancer. A study with similar design that tests the combination of radiation with checkpoint blockade in second line setting is also ongoing (NCT02311361).

Immune checkpoint therapy + therapies targeting immunosuppressive microenvironment

JAK inhibitors: The Janus kinase (JAK) and its downstream factor signal transducer and activator of transcription (STAT) are important mediators of signaling pathways initiated from cytokine and growth factor receptors. Excessive JAK/STAT signaling can lead to production and release of inflammatory cytokines, promote recruitment, expansion of MDSCs and Tregs which induce an immunosuppressive tumor microenvironment^[79]. Also, JAK/STAT pathway has been shown to induce the expression of PD-L1 on cells in the tumor microenvironment^[14,80]. In pre-clinical studies, JAK inhibitors led to decreased numbers of Tregs, TAMs and MDSCs, with enhanced number of activity of CD4⁺ and CD8⁺ T cells^[18]. The study of JAK inhibitor Ruxolitinib and capecitabine for the treatment of advanced pancreatic cancer has closed to enrollment (JANUS study; NCT02117479)^[81].

PI3K inhibitors: Phosphoinositide-3-kinase (PI3K) is a family of lipid kinases that catalyze the production of second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3), which leads to activation of downstream kinases. PI3K was known to play an important role in signaling pathways in B cells, which were found to contribute to an immunosuppressive microenvironment that dampens T cell immunity^[82]. Inactivation of PI3K was associated with a decrease in Tregs and MDSCs and an increase in CD8⁺ cytotoxic T cell activity, indicating a role of PI3K in regulating tumor microenvironment^[5]. PI3K inhibitors could shift immunosuppressive microenvironment in pancreatic cancer into a more immunogenic one. Therefore PI3K inhibitors could help potentiate the activity of immune checkpoint inhibitors.

BTK inhibitors: BTK is a cytoplasmic, Tec family tyrosine kinase important in B-lymphocyte development, differentiation, and signaling. In pancreatic cancer, the BTK

inhibitor (ibrutinib) was shown to inhibit mast cells, and as a result, to reduce fibrosis in the tumor microenvironment both in a KPC mouse model and patient-derived xenograft^[83]. Ibrutinib was also known to inhibit interleukin-2-inducible T-cell kinase (ITK), an important enzyme for the survival of Th2 cells; thus ibrutinib may be able to shift the balance away from the Th2 cells protumor response and toward the Th1 cells antitumor immune responses. A phase I/II clinical study assessing ibrutinib in combination with anti-PD-L1 antibody MEDI4736 in relapsed or refractory solid tumors, including pancreatic cancer has closed to enrollment (NCT02403271)^[84].

THIRD APPROACH (BREAKDOWN DESMOPLASTIC BARRIER)

Strategy that targets the desmoplastic stroma

PEGPH20: In pancreatic cancer, high levels of hyaluronan in the extracellular matrix contribute to a high interstitial pressure in the tumor stroma, leading to vascular compression and hypoperfusion. Pegylated hyaluronidase PEGPH20 is an enzyme that can degrade hyaluronan, and has been shown in a KPC mouse model to deplete hyaluronan in the tumor stroma and enhance the activity of gemcitabine^[85]. In a phase I (28 patients) and a phase II (135 patients) studies, patients with previously untreated advanced pancreatic cancer, PEGPH20 along with chemotherapy (gemcitabine, or gemcitabine/nab-paclitaxel) resulted in good tumor response and PFS, but only in patients with high levels of hyaluronan^[15,86]. Therefore, in pancreatic cancers with high levels of hyaluronan, PEGPH20 therapy may allow more effective T cell infiltration and enhance the activity of immune checkpoint therapies.

CONCLUSION

Both challenges and opportunities exist for the development of effective immunotherapy for pancreatic cancer. Given that single agent therapies against CLTA-4 or PD-1 or PD-L1 immune checkpoint were largely ineffective in pancreatic cancer, ongoing investigations and future directions lie in the field of combination therapies, where additional treatment modalities may unleash durable antitumor immune responses by enhancing tumor-specific T cell activation and antagonizing the immunosuppressive microenvironment in pancreatic cancer.

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Current state and controversies in fertility preservation in women with breast cancer

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cancer under the age of 45 annually in the United States. Because an increasing number of young women delay childbearing to later life for various reasons, a growing population of women experience breast cancer before completing childbearing. In this context, preservation of fertility potential of breast cancer survivors has become an essential concept in modern cancer care. In this review, we will outline the currently available fertility preservation options for women with breast cancer of reproductive age, discuss the controversy behind hormonal suppression for gonadal protection against chemotherapy and highlight the importance of timely referral by cancer care providers.

Key words: Fertility preservation; Female breast cancer; Cryopreservation; Oocyte; Embryo; Ovarian suppression; Gonadotropin-releasing hormone agonist; Letrozole; Ovarian tissue cryopreservation

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Core tip: Field of fertility preservation has experienced remarkable advances within the last 20 years. As a result, young cancer survivors have numerous options to maintain an important aspect of their quality of life, fertility. In this article we review the current state and controversies in fertility preservation. The article should be an important resource for professionals who take care of young women with breast cancer and other malignancies.

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Abstract

On average, over 25000 women are diagnosed with breast

INTRODUCTION

Breast cancer is the most common malignancy in women

and on average more than 25000 women are diagnosed with breast cancer before reaching the age of 45 years, each year in the United States^[1]. Early diagnosis by virtue of significant advances in detection, and newly developed treatment strategies have remarkably improved the course of breast malignancies. According to the National Cancer Institute, 5-year-survival rate for the women under age 45 was estimated to be as high as 88%-98.5% in 2011^[2].

While survivorship rates have dramatically increased in women with breast cancer, an important issue related to reproductive function has emerged. Most women with breast cancer are likely to undergo systemic adjuvant or neo-adjuvant chemotherapy with gonadotoxic side effects. As a consequence, preserving fertility potential has become an essential concept in the management of young cancer survivors. Fertility preservation has emerged from this concept as a new and dynamic discipline where oncology and reproductive medicine intersect.

In this review, we aimed to highlight the importance of fertility preservation as a part of routine care for breast cancer patients of childbearing age and outline the key fertility preservation options along with still experimental but promising therapeutic procedures.

COUNSELING FOR FERTILITY PRESERVATION

Because of the trend for having children in later reproductive ages, the number of women who experience breast cancer before completing childbearing is growing. Coupled with the increased survival rates and the growing healthy survivor population, fertility preservation has become an important component of cancer care and the maintenance of quality of life for survivors^[3].

American Society of Clinical Oncology (ASCO) and American Society of Reproductive Medicine (ASRM) guidelines for fertility preservation in cancer patients strongly recommend that oncologist should inform their patients about the potential negative effects of chemotherapy on fertility before the initiation of the planned treatment and promptly refer patients to reproductive specialist to discuss the risk of ovarian damage and currently available fertility preservation options^[4,5]. However, less than half of the oncologists in the United States always or often refer their cancer patients with fertility-related questions to fertility preservation specialist^[6].

It should be stressed that providing timely and accurate information for women of reproductive age with breast cancer is critical for the preservation of future fertility chances before complete loss of the limited and irreplaceable ovarian reserve due to chemotherapy. We have previously shown that early referral of breast cancer patients, especially before breast surgery results in larger number of oocytes and embryos being cryopreserved and less time to the initiation of chemotherapy^[7].

IMPACT OF CANCER TREATMENT ON OVARIAN RESERVE

Modern chemotherapeutic agents that are in use for breast cancer treatment can have a spectrum of ovarian toxicity, depending on the class of the agent, age of the patient, and the cumulative dose^[8]. We have shown that the most gonadotoxic agents are those that mainly target oocyte genome causing DNA double strand breaks (DBSs)^[9]. Under normal circumstances, DNA repair mechanisms are capable of maintaining genomic integrity, however, at the level of severe DNA damage due to genotoxic agents, those repair mechanisms remain insufficient. The severe DNA damage consequently leads to apoptotic death^[9]. Ovarian reserve is made up of about 1 million primordial follicle oocytes at birth, and this number is reduced to approximately 500000 at the onset of puberty. These numbers are reduced to about 25000 at age 37 and nearly exhausted at menopause. Because primordial follicles cannot be regenerated, any chemotherapeutic agent that induces DNA breaks in primordial follicle oocyte will result in apoptotic death and cause irreversible reduction in ovarian reserve^[9].

Among all gonadotoxic agents, those belong to the alkylating category such as cyclophosphamide, are the most gonadotoxic agents^[10]. Because alkylating agents are non cell-cycle specific chemical compounds and hence can target and damage resting primordial follicles that constitute ovarian reserve^[9,10].

The risk of chemotherapy-induced ovarian damage has been investigated in numerous clinical studies. Unfortunately, menstruation was used as the surrogate for ovarian function and fertility in the majority of the past studies^[11]. However, return of menses is a poor surrogate for reproductive potential, and ovarian reserve might be severely diminished despite the resumption of regular menses^[12,13]. In this context, it is reported that after treatment with CMF protocol (cyclophosphamide/methotrexate/5-fluorouracil) 20%-70% of women younger than age 40 experienced amenorrhea^[14]. Comparing CMF protocol to the AC protocol (doxorubicin/cyclophosphamide), significantly lower rates of amenorrhea (69% vs 34%, respectively) have been reported with the AC protocol^[15]. This finding is most likely related to a lower cumulative dose of cyclophosphamide reached with AC regimen. When a taxane administered in combination with AC (AC-T), it did not significantly increase the risk of amenorrhea compared with standard AC regimen^[16]. Tables 1 and 2 summarize chemotherapeutic agents that are commonly used in breast cancer treatment and their potential impact on ovarian function^[15-19].

Patient age at the time of chemotherapy inversely correlates with the likelihood of post-chemotherapy amenorrhea. In women with breast cancer, while the incidence of chemotherapy-induced amenorrhea was

Table 1 The risk of infertility and mechanism of damage associated with chemotherapeutic agents that are commonly used in breast cancer treatment

Chemotherapeutic agent	Class	Mechanism of action	Cell cycle effect	Risk of infertility
Cyclophosphamide	Alkylating agent	DNA cross-link formation and double strand breaks that result in inhibition of DNA function and synthesis leading to cellular apoptosis	Cell cycle non-specific	High risk
Doxorubicin Epirubicin	Anthracyclines	Inhibition of DNA synthesis and function due to inactivation of DNA topoisomerase II, free oxygen radical formation and induction of DNA double-strand breaks	Cell cycle non-specific	Medium risk
Carboplatin	Platinum analog	Inhibition of DNA synthesis and function <i>via</i> intra- and interstrand DNA cross-link formation by covalent binding to genome	Cell cycle non-specific	Medium risk
Paclitaxel Docetaxel	Taxanes	Inhibition of mitotic division by binding to microtubules with enhancement of tubulin polymerization	M phase	Low risk
Methotrexate	Antimetabolites	Inhibition de novo purine nucleotide synthesis by inactivation of dihydrofolate reductase	S phase	Low risk
5-fluorouracil		Inhibition of DNA synthesis and function via inactivation of Thymidylate synthase and alteration in RNA processing	S phase	Low risk
Trastuzumab	Monoclonal antibodies	Blockage of Human epidermal growth factor receptor 2 subdomain IV, antibody dependent cellular toxicity	NA	Low or no risk
Pertuzumab		Blockage of Human epidermal growth factor receptor 2 subdomain II, antibody dependent cellular toxicity		

Table 2 Common adjuvant chemotherapy regimens for breast cancer and their impact of fertility

Chemotherapy regimen	Risk of amenorrhea or infertility	
	Age ≤ 35 yr	Age > 35 yr
CMF	4%-40%	80%-100%
CEF	47%	80%-100%
CAF	No data	30%
AC	13.90%	68.20%
AC-T	9%-13%	65%-67%
AC-TH	0-14%	56%-67%

A: Doxorubicin; C: Cyclophosphamide; E: Epirubicin; F: 5-Fluorouracil; H: Trastuzumab; M: Methotrexate; T: Paclitaxel.

reported as 15%-40% under the age of 30, this incidence dramatically increases to 49%-100% for women older than 40 years of age^[20]. The reason for this age-related difference is the fact that younger women have a larger ovarian reserve. Our previous studies indicated that on average, gonadotoxic chemotherapy regimens result in the loss of approximately 10 years worth of ovarian reserve^[21]. Though both younger and older women would lose follicles, gonadotoxic chemotherapy is more likely to push older women over the threshold for menopause as they have lower reserve to begin with. However, regardless of age, females of all ages, including children, are expected to experience early menopause after exposure to gonadotoxic chemotherapy agents. Therefore fertility preservation and completion of family building as early as possible, is critical regardless of the age at chemotherapy exposure in most instances^[22].

GONADOTROPIN-RELEASING HORMONE ANALOGS AND OVARIAN PROTECTION

There has been an ongoing controversy regarding the

role of ovarian suppression in cancer patients using gonadotropin-releasing hormone (GnRH) analogs in order to protect ovaries from chemotherapy-induced damage^[23].

The biggest concern regarding the effectiveness of ovarian suppression is that primordial follicles that constitute the ovarian reserve are quiescent and do not express gonadotropin or GnRH receptors^[24,25]. Thus, any change in gonadotropin or GnRH serum levels has no plausible direct or indirect effect on primordial follicles (Figure 1). Furthermore, we have shown that gonadotoxic agents induce primordial follicle death *via* inducing DNA double strand breaks in oocytes in a non-cell cycle dependent fashion, hence there is no mechanism for ovarian suppression by GnRHa to prevent chemotherapy-induced DNA damage^[9,26]. It should be recognized that GnRHa induces a hormonal state similar to prepubertal stage, and if ovarian suppression were to be protective, children of prepubertal age would be resistant to gonadotoxic effects of chemotherapy, which is shown to be not to be the case^[27].

While some studies in women with breast cancer, which used menstruation as a marker, suggested some benefit in restoration of menstruation post-chemotherapy, these studies were marred by numerous weaknesses^[28-30]. These include the utility of self-reported menstrual status, a highly unreliable surrogate for fertility, lack of placebo control (instead of GnRHa) or blinding, and lack of correction for the difference in desire to conceive between study and control groups^[31].

Use of amenorrhea as the sign of ovarian failure is also key weakness in trials of GnRHa for ovarian protection. Especially for breast cancer patients, chemotherapy often induces occult ovarian insufficiency that most frequently presents as irregular or even normal appearing periods rather than amenorrhea. When the serum anti-Müllerian Hormone (AMH), which is the most reliable quantitative

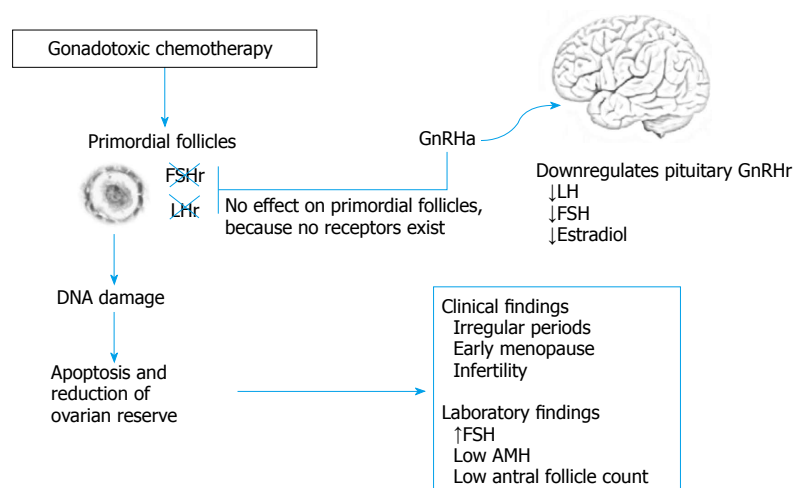


Figure 1 Impact of gonadotoxic chemotherapy and gonadotropin-releasing hormone analog on ovarian reserve and function. Gonadotoxic chemotherapy reduces ovarian reserve, which is made up of resting and hormone-insensitive primordial follicles, by induction of DNA damage and apoptotic death. GnRHa reduces pituitary GnRH production and, as a result, blocks the release of FSH and LH from the pituitary, which in turn results in the cessation of late-stage follicle development. Because primordial follicles do not have FSH, LH, or GnRH receptors, GnRHa cannot have a direct influence on ovarian reserve. AMH: Anti-Müllerian hormone; FSH: Follicle-stimulating hormone; FSHr: FSH receptor; LH: Luteinizing hormone; LHr: LH receptor; GnRH: Gonadotropin-releasing hormone; GnRHa: GnRH receptor. Oktay *et al.* *J Clin Oncol* 2016; **34**: 2563-2565, used with permission.

biomarker for ovarian reserve or appropriate criteria with serum FSH levels for defining ovarian failure was used, none of the studies showed fertility preservation benefit from GnRHa treatment^[32-34].

Given the contradictory results and ovarian biological facts, the use of GnRHa for the prevention of ovaries from chemotherapy damage is still controversial and cannot be recommended as an effective method of fertility preservation.

OVARIAN RESERVE IN WOMEN WITH BRCA MUTATIONS

Most hereditary breast cancers are associated with germline mutations in *BRCA1* and *BRCA2* genes. BRCA genes are members of the ataxia-telangiectasia-mutated (ATM)-mediated DNA damage signaling pathway and are essential for DNA double-strand break (DSB) repair^[35]. In addition to the increased risk for multiple malignancies, several clinical and experimental studies showed an association between BRCA mutations and diminished ovarian reserve^[26,36-41]. While performing ovarian stimulation in women with breast cancer by using aromatase inhibitors for fertility preservation, we found significantly lower ovarian response rates in BRCA mutation carriers particularly, among those with BRCA1 mutations^[36]. In another important study, authors reported that unaffected women with BRCA mutation experience menopause 3-4 years earlier than healthy controls^[38]. Recently, our laboratory showed that in BRCA1 mutant mice there is increased age-related accumulation of DNA double strand breaks in primordial follicle oocytes and the ovarian reserve is significantly lower. These BRCA1 mutant mice also showed reduced litter size and poor embryo development. These findings clearly indicate a biological connection between BRCA mutations, DNA repair and reproductive function. In the same study, we also showed that affected women with BRCA1 mutations had lower serum AMH levels compared to controls. Interestingly we did not find these differences in either BRCA2 mutant mice or affected women

with BRCA mutations^[26]. Confirming our findings in a prospective study, Philips *et al.*^[41] found 25% lower AMH concentrations on average in BRCA1 carriers compared to non-carriers. There was no significant association between the BRCA2 mutation status and the AMH levels.

Given the accumulating evidence that the ovarian reserve may be lower in women with BRCA mutations, it is possible that these women are more prone to chemotherapy-induced loss of ovarian reserve and ovarian insufficiency. However this is yet to be shown in prospective clinical trials. Nevertheless, while counseling women with BRCA mutations on fertility preservation, the possibility of higher risk of chemo-induced infertility should not be omitted.

FERTILITY PRESERVATION OPTIONS FOR BREAST CANCER PATIENTS

Embryo cryopreservation after *in vitro* fertilization (IVF) is currently considered as an established fertility preservation option, which offers the best chance of livebirth for women with a partner or single women who elect to use donor sperm. Numerous studies have demonstrated up to 60% clinical pregnancy rates and around 34% livebirth rates after transfer of frozen-thawed embryos in infertility patients with mean age of 35.1 ± 4.03 , which is comparable to fresh embryo transfer^[42,43]. When preimplantation genetic screening utilized, the livebirth rates can increase up to 77% after transfer of euploid frozen-thawed embryos^[44]. In women with breast cancer with the mean age of 35.8 ± 4.1 , we have shown a livebirth rate of 45%, which appeared to be superior to those undergoing frozen embryo transfer for infertility^[45].

Cryopreservation of mature or immature oocytes is another fertility preservation option for women without a partner and those not wishing to use donor sperm due to legal, ethical or religious considerations. Mature oocytes can be effectively cryopreserved using a vitrification method and the success rates of post-thaw fertilization and pregnancy rates have approached those with

Table 3 Fertility Preservation options for reproductive age women with breast cancer

Fertility preservation option	Current status	Advantages	Disadvantages
Embryo Cryopreservation	Established	Highest cumulative pregnancy rates	Requires about two weeks delay in the initiation of cancer treatment Requires hormonal stimulation for oocyte retrieval Requires <i>in vitro</i> fertilization with male partner or donor sperm
Oocyte Cryopreservation	Established	No need for male partner or sperm donor	Requires about two weeks delay in the initiation of cancer treatment Requires hormonal stimulation for oocyte retrieval
Ovarian Tissue Cryopreservation and Transplantation	Currently experimental, may change as success rates are rising	No need for hormonal stimulation No need to significantly delay in the initiation of chemotherapy No need for male partner or sperm donor	Requires outpatient laparoscopic surgery for ovarian tissue harvesting and subsequent transplantation

fresh oocytes in young patients, though success rates with frozen embryos may still be better^[46,47]. Oocyte cryopreservation success rates vary depending on age, number of oocytes frozen and the freezing protocol. In a recent individual patient data meta-analysis we calculated these success rates^[48] (An interactive online success rate estimator can be found online at <http://fertilitypreservation.org/index.php/probability-calc>).

Based on an individual patient meta-analysis encompassing thaw cycles with frozen oocytes, we have calculated the age-based success rates for oocyte cryopreservation. An interactive online egg freezing success rate estimator can be found at this link: <http://fertilitypreservation.org/index.php/probability-calc>, and can be useful in patient counseling.

Immature oocytes can be obtained from patients without undergoing ovarian stimulation due to dearth of time and also at the time of ovarian tissue harvesting for fertility preservation. After retrieval, immature oocyte may be cryopreserved before or after undergoing *in vitro* maturation (IVM) process^[49]. Lee *et al.*^[50] suggested performing IVM for immature oocytes before cryopreservation rather than post-thaw as they observed significantly higher maturation and survival rates with that approach. Although IVM is still an experimental fertility preservation method and limited to a number of fertility centers, this method has recently resulted in live births^[51].

Embryo and oocyte cryopreservation methods are widely used and currently considered as established methods of fertility preservation. However, typically 10-14 d of controlled ovarian stimulation is needed to obtain mature oocytes (Table 3).

When there is insufficient time for ovarian stimulation, the only available strategy other than immature oocyte retrieval and IVM for women with breast cancer is ovarian tissue harvesting and cryopreservation for future transplantation. Since the first report of ovarian transplantation with cryopreserved tissue by our group, there have been more than 80 livebirths with over 30% of livebirth rate after ovarian transplantation^[52,53]. Some have

raised the concern of reintroducing malignant cells back into the body along with ovarian tissue. However, studies showed no evidence of malignant cells in cryopreserved ovarian tissues from non-metastatic breast cancer patients and those with bone and soft tissue tumors^[54-56].

CONTROLLED OVARIAN STIMULATION PROTOCOLS

The major issue associated with the conventional ovarian stimulation protocols is elevated circulating estradiol levels due to the development of large number of follicle at once. Therefore, conventional stimulation protocols are considered unsafe in women with estrogen-sensitive breast cancer.

Although oocytes can be retrieved from ovaries without performing ovarian stimulation (natural cycle IVF), this strategy typically does not provide more than one oocyte per cycle and yield an embryo in only 60% of cycles^[57]. On the other hand, use of tamoxifen alone for ovulation induction showed better results in mature oocyte and embryo yield compared to natural cycle IVF^[58]. Tamoxifen may also be used in combination with low dose gonadotropins for IVF, resulting in increase multiple mature oocytes and embryos^[59].

While reducing the circulating estrogen levels, aromatase inhibitors induce the secretion of endogenous FSH by releasing the hypothalamic-pituitary axis from estrogenic negative feedback^[60]. We showed that letrozole in combination with gonadotropins can produce comparable outcomes to conventional IVF while providing significantly lower estradiol levels and decreased gonadotropin requirements^[45]. We also showed that pregnancy outcomes after ovarian stimulation with letrozole protocol in premenopausal breast cancer patients before adjuvant chemotherapy were similar to a non-cancer population^[60]. Moreover, after short and mid-term follow up letrozole-gonadotropin protocol was associated with disease free survival rates^[61].

One of the concerns related with ovarian stimulation

before adjuvant or neo-adjuvant chemotherapy is the delay in the initiation of breast cancer treatment. However, studies have shown that initiation of chemotherapy can be delayed up to 12 wk after breast surgery without any adverse effect on survival and recurrence rates^[62,63].

Another concern is that letrozole protocol is that it is a teratogenic agent if used during pregnancy. However, in the setting of fertility preservation, embryos are never exposed to letrozole as the fertilization takes place *in vitro* and the resultant embryos are cryopreserved for later use. Additionally, it has been reported that there was no difference in congenital malformation and chromosomal abnormality rates among children born after ovarian stimulation with clomiphene or letrozole for infertility^[64].

PREGNANCY AFTER BREAST CANCER

Patients in the decision process for fertility preservation treatments frequently question the safety of pregnancy after completion of cancer treatment. Based on the current evidence, pregnancy after breast cancer is not associated with increased risk of adverse outcomes^[65]. In general, patients are advised to delay pregnancy at least 2 years after diagnosis, as the risk of recurrence is highest in this time frame. In the case of ER-positive breast cancer, pregnancy is contraindicated during tamoxifen treatment because of teratogenicity. For breast cancer survivors who do not want to delay childbearing for the completion of tamoxifen treatment or for those with other medical contraindications, gestational surrogacy may be a suitable option to utilize their frozen eggs or embryos in the future^[10,65].

CONCLUSION

Fertility preservation has become a crucial part of survivorship and an important aspect of comprehensive cancer care. Fortunately, there are several well-established treatment options including embryo and oocyte cryopreservation and safer ovarian stimulation protocols. Moreover, there are emerging experimental methods such as ovarian tissue cryopreservation and transplantation and IVM, which are showing promise. To maximize the utility of these available options and avoid significant delays in the initiation of chemotherapy, timely referral to fertility preservation counseling should be an integral part of the care of young women with breast cancer.

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Biological mesh reconstruction of the pelvic floor following abdominoperineal excision for cancer: A review

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Abstract

Extralevator abdominoperineal excision and pelvic exenteration are mutilating operations that leave wide perineal wounds. Such large wounds are prone to infection and perineal herniation, and their closure is a major concern to most surgeons. Different approaches to the perineal repair exist, varying from primary or mesh closure to myocutaneous flaps. Each technique has its own associated advantages and potential complications and the ideal approach is still debated. In the present study, we reviewed the current literature and our own local data regarding the use of biological mesh for perineal wound closure. Current evidence suggests that the use of biological mesh carries an acceptable risk of wound complications compared to primary closure and is similar to flap reconstruction. In addition, the rate of perineal hernia is lower in early follow-up, while long-term hernia occurrence appears to be similar between the different techniques. Finally, it is an easy and quick reconstruction method. Although more expensive than primary closure, the cost associated with the use of a biological mesh is at least equal, if not less, than flap reconstruction.

Key words: Biological mesh; Rectal cancer; Pelvic exenteration; Abdominoperineal resection; Primary perineal wound closure; Perineal wound infection; Perineal hernia

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Core tip: Current literature regarding the use of biological mesh reconstruction after pelvic exenteration and extralevator abdominoperineal excision is scarce. However, it does suggest that the use of biological mesh has a lower short-term perineal hernia rate, but is probably not superior to other approaches with regards to perineal wound complications.

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F. Biological mesh reconstruction of the pelvic floor following abdominoperineal excision for cancer: A review. *World J Clin Oncol* 2017; 8(3): 249-254 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/249.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.249>

INTRODUCTION

Pelvic exenteration (PE) and extralevator abdominoperineal excision (ELAPE) are mutilating operations, leaving a large perineal incision. ELAPE for low rectal cancer was introduced to decrease the rate of positive resection margins and specimen perforation occurring during conventional abdominoperineal resection (cAPR)^[1,2]. In a recent retrospective study, Stelzner *et al*^[3] showed that the 5-year recurrence rate was 5.9% in the ELAPE group vs 18.2% in the cAPR group ($P = 0.153$). However, other units have not been able to reproduce such results^[4], nor could they demonstrate a statistically significant superiority of ELAPE in terms of CRM positivity and bowel perforation. Furthermore, they reported comparable perineal complication rates for the two APR approaches.

Vivid discussions continue to fuel the debate regarding the pros and cons of ELAPE. Overall, it is well accepted that larger wounds are independent risk factors for perineal wound complications. The combination of neoadjuvant chemoradiotherapy and ELAPE almost doubles the rate of perineal wound complications (31% for ELAPE vs 18% for cAPR)^[5]. While new techniques and approaches have attempted to reduce the size of the perineal incision (and therefore reduce the risk of wound complications)^[6], optimal management of perineal defects is still under investigation. The options include primary closure, myocutaneous flaps, and mesh reconstruction, including the use of a biological mesh.

We aimed to evaluate the outcomes of perineal reconstruction with biological mesh following ELAPE and PE in our center and to review the current literature.

CURRENT STATUS

Perineal wound complications are a major concern following PE and ELAPE leading to increased morbidity, longer hospital stay, and delayed chemotherapy. Different reconstruction methods are currently used in practice with the aim of reducing the rates of wound complications and avoiding perineal herniation.

Risk factors for major perineal wound complications following APR are well known: Preoperative radiotherapy, patients with anal cancer, flap reconstruction, tumor size, obesity, and diabetes^[7]. Minor wound complications appear more commonly in patients with inflammatory bowel disease or anal cancer than in those with rectal cancer^[8].

Most patients with locally advanced rectal cancer, recurrent rectal cancer, and recurrent or persistent squamous cell carcinoma receive neoadjuvant radio-

chemotherapy or radiotherapy alone^[9,10]. The poor healing ability of irradiated wounds has been attributed to local endarteritis and damaged fibroblasts^[11]. It has been clearly demonstrated that preoperative radiotherapy increases the rate of major wound complications^[5,12]. For example, Aldulaymi *et al*^[13] reported a significantly increased risk of major perineal wound complications in patients undergoing APR for rectal cancer with primary closure of the perineum (26% in non-irradiated vs 71% in irradiated patients). Chadwick *et al*^[14] found that the risk of developing a wound complication was 10 times higher after previous irradiation. This substantial problem with wound healing calls for the need to consider alternative closure techniques of the perineum.

Different methods have been described ranging from direct/primary closure to mesh reconstruction, gluteal and rectus abdominis flaps or combinations of these techniques. Currently, there is no consensus on which is the most ideal technique^[15]. The vertical rectus abdominis flap (VRAM) is indicated to bring non-irradiated tissue into the perineal defect^[16]. After VRAM, perineal wound complications have been reported to range from 0% to 28%^[17-20]. The use of laparoscopy for the abdominal part of the resection is almost impossible because of the donor site. In addition, in cases of PE (with a right sided urostomy and left sided end colostomy), VRAM is often contra-indicated. A potential solution is the use of a wet double-barreled colostomy^[21].

Other myocutaneous flaps can potentially be used, such as the gracilis flap and the gluteus maximus flap, which have a perineal wound complication rate of 12%^[22] and 10%^[2] respectively. However, these flaps are typically smaller than the VRAM flap and unlikely to provide adequate cover of large defects.

In addition, authors argue that myocutaneous flaps carry significant risks of donor site morbidity, flap necrosis, prolonged operative time, and usually require co-ordination with plastic surgeons^[2,23,24]. Mesh reconstruction is another technique, which has attracted a lot of interest in the last few years, especially with the adoption of ELAPE. Briefly, the biological mesh is sutured directly to the pelvic side wall (Figures 1-3). The size of the mesh is adapted to the size of the defect. A perineal drain is routinely left at the end of the procedure, in order to avoid a perineal collection.

Both allogenic and xenogenic biological meshes are available for the reconstruction of the perineum. These types of meshes were initially used for abdominal wall reconstructions^[25,26]. The allogenic mesh is predominantly made of human acellular dermis (e.g., HADM® Ruinuo, Qingyuanweiyue Bio-Tissue Engineering Ltd, Beijing, China) as used by Han *et al*^[27,28]. The xenogenic mesh consists of bovine pericardium or porcine dermis and intestinal mucosa. Similar to Musters *et al*^[29] in the BIOPEX-study, we used the Strattice® mesh (LifeCell, Acelity Company, Branchburg, NJ) which is composed of non-reticulated porcine dermis. Jensen *et al*^[30] and Christensen *et al*^[24] used the Permacol® mesh (Tissue Science Laboratories plc, Covington, GA, United States) derived from reticulated

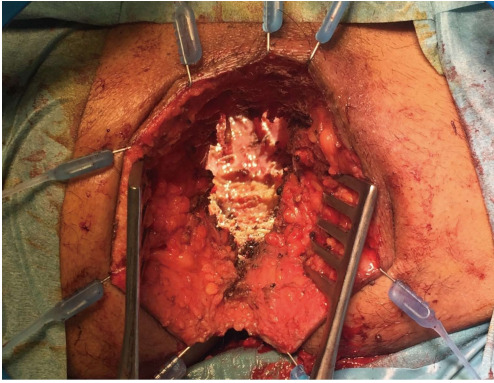


Figure 1 Perineal view before reconstruction in pelvic exenteration patient.

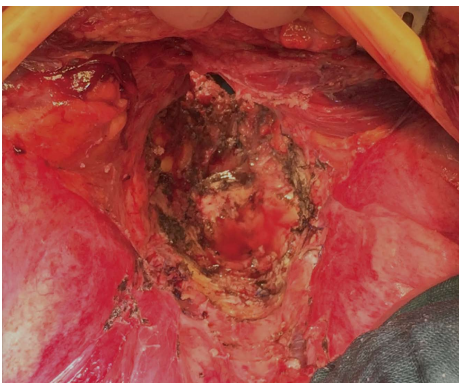


Figure 2 Abdominal view before reconstruction in pelvic exenteration patient.

porcine dermis^[24,30]. Surgisis® Biodesign™ (Cook Medical, Bloomington, IN, United States) created using porcine intestinal mucosa was used by Peacock *et al*^[31] for their pelvic reconstruction.

Reconstruction using a mesh is relatively simpler and faster compared to flap reconstruction^[24]. When considering cost, meshes are expensive, especially if biological. However, with a potentially shorter operative time and length of hospital stay, overall costs can be controlled and even reduced in comparison to VRAM-flaps^[32]. Biological meshes also have the advantage of being absorbable and can be used in infected environments^[33].

On the other hand, perineal mesh reconstruction is not without its risks. Internal hernias following mesh repair have been reported. Melich *et al*^[34] described resecting ischemic small bowel loops incarcerated in a pelvic hernia along the mesh in three patients. Jensen *et al*^[30] reported a hole in the biological mesh in a patient with an infected perineal wound, who subsequently required mesh removal. These reports clearly raise concerns and highlight the risk of small bowel incarceration and necrosis associated with the use of a perineal mesh.

Table 1 summarizes the largest studies focusing on the use of biological mesh for perineal reconstruction. Interestingly, only one mesh was removed^[30]. The overall safety profile appears to be good.

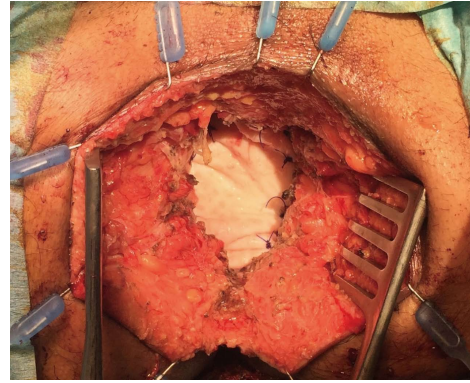


Figure 3 Perineal view after reconstruction using a biological mesh.

Perineal wound complications

The clinical consequences of perineal wound complications are wide and range from a simple redness of the skin to a persistent perineal fistula, and perineal sepsis. Perineal wound complications are often subdivided into two subgroups: Early and delayed wound dehiscence. The delayed (> 4 wk) perineal healing can occur in approximately 25% of cases. Importantly, up to 50% of these cases will develop long-term and persistent perineal symptoms such as pain, chronic sinus, sitting disability or tension between buttocks. All of which can seriously impact the patient's quality of life. Delayed perineal healing may therefore be a risk factor for persistent symptoms providing yet another reason why surgeons must strive to identify the best repair method possible^[35,36].

Primary closure leads to perineal wound complications in 18%-34%^[27,29,37]. Moreover, one third of patients after PE will develop perineal wound dehiscence^[38]. As a corollary, persistent presacral sinus was found in 10% of the patients following APR^[39].

As mentioned in Table 1, 17%-37% of patients with biological mesh presented some degree of perineal wound dehiscence/infection. A Danish retrospective study reported that 15% of patients with biological mesh had a surgical re-intervention for perineal infection. In addition, 21% of the patients had a perineal fistula with 9% requiring surgical excision^[30]. Similarly, Peacock *et al*^[31] reported an overall perineal wound complication rate of 32%. Vacuum assisted wound therapy and surgical debridement were needed in up to 9% of cases.

Christensen *et al*^[24] compared gluteal flap reconstruction with biological mesh repair. Seventeen percent of patients in the mesh group had a wound infection compared to 6% in the flap group ($P = 0.26$). At 3 mo, all wounds healed with one persistent sinus in each group^[24].

Han *et al*^[28] found similar results and subsequently conducted a randomized controlled trial evaluating ELAPE vs cAPR. Interestingly, in the ELAPE group, patients had biological mesh reconstruction. Overall, the perineal wound infection rate (11.4%) after ELAPE was lower than in the cAPR group where 18.8% of patients developed a perineal complication. However, seromas were more frequent in the mesh group (11.4% vs 0%)^[27].

Table 1 Perineal reconstruction with biological mesh

Ref.	Study type	Operation	No. of patients	Average age (median years)	Perineal complications (%)	Surgical perineal debridement n	Perineal hernias	Follow up	Comments
Musters BIOPEX-study 2016 ^[29]	RCT	ELAPE	50	65	37% overall perineal wound complications	4% surgical drainage of perineal abscess, 6% percutaneous drainage of perineal abscess	13% at 1 yr	12 mo	
Jensen <i>et al</i> ^[30] , 2014	Cohort, prospective	ELAPE	53	NR	21% perineal fistula, 7.5% superficial perineal abscess, 7.5% deep perineal abscess	5 (9%) fistulectomy, 8 (15%) surgical debridements	5.60%	Median 36 mo	1 mesh removed (infection), 1 mesh failure (hole) replacement of a new mesh
Christensen <i>et al</i> ^[24] , 2011	Cohort, retrospective	ELAPE	24	69.7	17%, with one fistula after 3 mo	0	0	Median 1.7 yr	-
Han <i>et al</i> ^[28] , 2010	Cohort, retrospective	ELAPE	12	68	16% infection, 8% seroma	0	NR	Median 8 mo	-
Han <i>et al</i> ^[27] , 2012	Derived from RCT	ELAPE	32	68	11.4% wound infections 11% seroma	NR	14%	NR	-
Peacock <i>et al</i> ^[31] , 2014	Cohort, prospective	ELAPE	34	62	32% overall; 9% superficial wound infections, 14% perineal fistula; 9% perineal abscess	3 (9%) surgical debridement/VAC therapy	0	Median 21 mo	-
Schiltz present study	Cohort, retrospective	ELAPE + PE	11	63	Overall 27% wound infections with 1 superficial	2 (18%) surgical debridement	0	Mean 18 mo	-

NR: Not reported; ELAPE: Extralevator abdominoperineal excision; PE: Pelvic exenteration.

Seroma formation can be problematic, pushing most of the authors to recommend the routine use of a perineal drain.

Adding to the present literature, we conducted a retrospective study of our local data. From January 2012 to December 2015, all patients undergoing ELAPE or PE with biological mesh reconstruction were analyzed. Eleven patients were found; all of whom had preoperative radiochemotherapy. Overall, perineal complications were found in 3 (27%) of the patients. In 2 (18%) patients, perineal abscesses were surgically drained and treated with a vacuum assisted wound closure system. One superficial wound infection was treated conservatively. No meshes were removed.

The relatively poor quality of the available studies in the literature remains an issue. These are mainly retrospective or simple cohort studies designed to analyze oncological outcomes. Very few of them focus specifically on perineal complications. Additionally, the severity and grading system of wound complications can differ between reports, and thus it is difficult to draw definitive conclusions.

The only multicenter randomized controlled trial focusing on perineal reconstruction using biological mesh after ELAPE, the BIOPEX study^[29], was recently published. Patients were randomized into two groups, one with perineal mesh reconstruction and the other with

primary closure only (control group). Regular blinded wound follow-up, using the Southampton wound healing score, did not show a significant difference between the two groups at 30 d. In the control group, 34% of perineal wound complications occurred vs 37% in the mesh group ($P = 0.7177$). At 12-mo follow-up, the healing rates did not differ between groups (52% vs 54%). Omentoplasty or use of perineal drains did not affect the results in this study^[29].

In summary, current evidence suggests that biological mesh reconstruction does not appear to reduce the risk of perineal wound complications. Results are similar between primary closure, flap and biological mesh.

Perineal hernia

The incidence of perineal hernia after APR ranges from 0.6% to 27% in the literature^[5,29,40], occurring on average 8 to 22 mo after surgery^[41,42] (Table 1). Such a wide range can partly be explained by the definition of a perineal hernia itself. Indeed, a clinical hernia is quite different from an asymptomatic radiologically identified perineal hernia. Smoking and chemoradiotherapy are well reported risk factors^[42].

Given that recurrence rates following perineal hernia repair are high (up to 37%), prevention is certainly the best strategy^[15]. Perineal hernia occurs significantly less often after biological mesh reconstruction (0%) than

following gluteal flap surgery (21%) ($P < 0.01$)^[24]. Thus suggesting that biological mesh repair can be a good option in order to avoid herniation.

The BIOPEX-study showed that 13% of perineal hernias (diagnosed on CT scan) occurred after biological mesh repair vs 27% in the primary closure group at one-year follow-up ($P = 0.036$)^[29]. The hernias occurred nearer the end of the 12-mo follow-up in the mesh group. The long-term follow-up results are still pending. Interestingly, this delay in the hernia presentation is also described in patients without mesh reconstruction. However, this seems to occur after a median of 8 mo^[41]. A possible explanation is that perineal hernias occur later in the mesh group due to the slower degradation of the biological mesh^[43].

In our own data, no perineal hernia was found, neither clinically or radiologically, even after a mean follow-up of 18 mo.

Overall, biological mesh seems to protect, at least in early follow up, from the occurrence of perineal hernias in comparison to flap reconstruction or primary closure.

CONCLUSION

Perineal reconstruction following ELAPE, APE or PE remains a major problem and challenge. No ideal solution currently exists but various approaches have been attempted with more or less success. Primary closure remains the most frequent technique, carrying a significant risk of perineal hernia formation. On the other hand, the use of flap or mesh reconstruction could help reduce the risk of herniation. Biological mesh appears to be a valid option, at least in terms of hernia prevention, which can be reduced by up to 50%.

Yet, the role of mesh reconstruction in reducing wound infections is less clear. Whilst perineal infection is frequent in irradiated patients, the use of biological mesh seems logical, even if the evidence is scarce to draw definitive recommendations. On the other hand, perineal wound infection remains frequent and a perineal drain should be routinely used.

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Potential prognostic biomarkers in pancreatic juice of resectable pancreatic ductal adenocarcinoma

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Abstract

Despite potentially curative surgery pancreatic cancer has a dismal prognosis. Serum cancer antigen 19-9 (CA 19-9) correlates with tumor burden, resectability and survival in patients with pancreatic ductal adenocarcinoma. Identification of novel biomarkers may facilitate early diagnosis of pancreatic cancer and improve survival.

Pancreatic juice is a rich source of cancer-specific proteins rendering it a promising tool for identifying biomarkers. Recent proteomic and microRNA expression analyses have identified several biomarkers of potential diagnostic and prognostic value. Tumor markers CA 19-9 and carcinoembryonic antigen (CEA) are widely used in the characterization of premalignant and malignant lesions of the pancreas. Elevated level of CEA in bile is a marker for malignancy and a predictor of hepatic recurrence. The potential value of CA 19-9, CEA and lactate dehydrogenase as prognostic biomarkers in pancreatic juice and bile is unknown. Specimens of pancreatic juice and bile can be readily collected during surgical resection of the tumor. Profiling of pancreatic juice and bile to identify novel prognostic biomarkers may improve selection of patients for adjuvant therapy with a favorable impact on overall survival in patients diagnosed with pancreatic cancer.

Key words: Prognostic biomarkers; Pancreatic juice; Bile; Pancreatic adenocarcinoma; Surgery

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Core tip: Pancreatic juice is a rich source of cancer-specific proteins rendering it a promising tool for identifying novel biomarkers in pancreatic ductal adenocarcinoma. Recent proteomic and microRNA expression analyses have identified several diagnostic and prognostic biomarkers. Elevated carcinoembryonic antigen (CEA) in bile is a marker of malignancy and a predictor of hepatic recurrence. The potential of cancer antigen 19-9, CEA and lactate dehydrogenase as prognostic biomarkers in pancreatic juice and bile is unknown. Specimens of pancreatic juice and bile can be readily collected during pancreatic resection. Profiling of pancreatic juice and bile to identify novel biomarkers may facilitate early diagnosis and improve selection of patients for adjuvant therapy.

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INTRODUCTION

Pancreatic cancer is the fourth leading cause of cancer-related mortality in the United States. Despite improvements in adjuvant therapy and identification of novel biomarkers, pancreatic cancer continues to have a dismal prognosis^[1]. Pancreatic ductal adenocarcinoma (PDAC) is one of the few cancers for which incidence and mortality rates have changed very little over the past three decades. Surgery is the only potentially curative treatment for prolongation of survival and the use of adjuvant therapy following curative surgery significantly improves 5-year survival^[2-4].

Pathologic stage of the tumor is the major determinant of survival after curative resection for PDAC^[1]. Serum levels of cancer antigen 19-9 (CA 19-9), carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) correlate with the extent of disease and are predictive of survival^[5-13]. Serum CA 19-9 correlates with tumor burden, resectability and overall survival. Low preoperative serum CA 19-9, postoperative decline and level < 200 U/mL are independent predictors of survival^[5]. Identification of novel diagnostic and prognostic biomarkers in the serum, tissue, bile and pancreatic juice of patients with PDAC may improve early diagnosis and selection of patients for adjuvant therapy.

BIOMARKERS OF PANCREATIC ADENOCARCINOMA

CA 19-9 and CEA in PDAC are well-characterized serum and tissue biomarkers of diagnostic and prognostic value. Recent proteomic and microRNA (miRNA) expression analyses have identified several biomarkers of potential value in the early diagnosis of PDAC and improvement in patient selection for aggressive treatment protocols. Comparative proteomic profiling of tumor and nontumor pancreas samples in patients with PDAC identified a new prognostic biomarker prolargin (PRELP)^[14]. Survival analysis demonstrated a significant correlation of protein abundance of PRELP with postoperative survival confirming its value as a candidate prognostic biomarker. Pancreatic juice is a rich source of cancer-specific proteins rendering it a promising tool for identifying novel biomarkers. Additional sources of biomarkers including serum, tumor tissue, pancreatic juice, bile and other body fluids have revealed distinct biomarker patterns in PDAC. These data suggest that analysis of pancreatic juice and bile samples collected at the time of a surgical resection may identify prognostic biomarkers of value in PDAC. Biomarkers may be used to stratify patients based

on prognosis and those who will benefit from intensive neoadjuvant protocols or adjuvant hepatic artery infusion therapy.

BIOMARKERS IN PANCREATIC JUICE

Diagnostic biomarkers

During the development of PDAC, malignant ductal cells preferentially shed into the ductal lumen, making pancreatic juice a rich source of cancer-specific proteins. CA 19-9 expression is demonstrated in 90% patients with pancreatic head adenocarcinoma compared to 11%-62% perampullary cancers of duodenal, ampullary or distal bile duct origin^[15]. Overexpression of CA 19-9 and CEA in PDAC is shown in Figures 1-4 and it correlates with a higher histologic grade^[15,16]. Elevation of CEA level and presence of *K-ras* mutation in pancreatic juice is a strong predictor of PDAC^[17]. Increased levels of CA 19-9 and CEA in pancreatic juice are predictive of malignant transformation in benign intraductal papillary mucinous neoplasm (IPMN)^[18-21]. Immunohistochemical staining of CEA is strongly positive in invasive IPMN and correlates with the grade of cellular atypia^[21,22].

In a comparison of the levels of CA 19-9 in the serum and pancreatic juice of patients with PDAC, the authors reported elevated levels in the pancreatic juice of all patients with normal levels in the sera of several patients^[23]. Tumor marker levels are predictive of tumor burden with the level in pancreatic juice correlating with the local tumor and serum level with the systemic burden of disease. This may explain the elevation of tumor markers in pancreatic juice with normal serum levels in patients with malignant IPMN.

Genetic and epigenetic markers such as mutant *K-ras*, *p53* mutations, DNA methylation alterations, mitochondrial DNA mutations and miRNAs in pancreatic juice are under evaluation for their role in distinguishing benign pancreatic pathology or chronic pancreatitis from preinvasive pancreatic neoplasia, IPMN and pancreatic intraepithelial neoplasia (PanIN)^[24-33]. Mass spectrometry proteomics of pancreatic juice collected at the time of surgical resection of the tumor suggested distinct proteomic signatures for PDAC^[34]. CEA and S100 calcium-binding protein P (S100P) concentrations in duodenal juice were significantly higher in PDAC than the benign conditions and may serve as a useful screening test for the detection of PDAC^[35,36]. Immunohistochemical expression of human telomerase reverse transcriptase (hTERT) in preoperative pancreatic juice samples was detectable in 84% PDAC and 88% malignant IPMN and the accuracy of diagnosing PDAC improved when combined with cytology^[30,37]. Proteomic analysis of pancreatic juice from patients with PDAC demonstrated three up-regulated proteins, matrix metalloproteinase-9 (MMP-9), oncogene DJ1 (DJ-1) and alpha-1B-glycoprotein precursor (AIBG) indicative of their potential as diagnostic biomarkers in PDAC^[38]. Accurate peripheral markers of PDAC are lacking and select miRNAs identified in plasma and bile demonstrated excellent

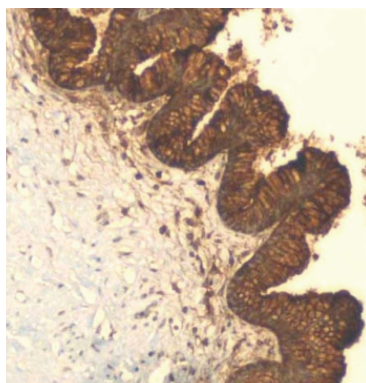


Figure 1 Pancreatic ductal adenocarcinoma with overexpression of carcinoembryonic antigen. The neoplastic cells demonstrate strong cytoplasmic and membranous staining (100 ×). Courtesy, Department of Pathology, Temple University Hospital, Philadelphia, PA, United States.

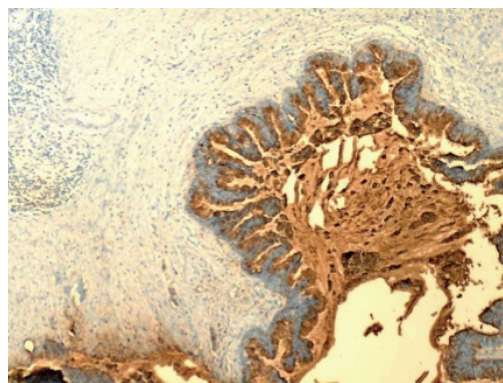


Figure 3 Pancreatic ductal adenocarcinoma with overexpression of cancer antigen 19-9. The neoplastic cells demonstrate strong cytoplasmic staining (100 ×). Courtesy, Department of Pathology, Temple University Hospital, Philadelphia, PA, United States.

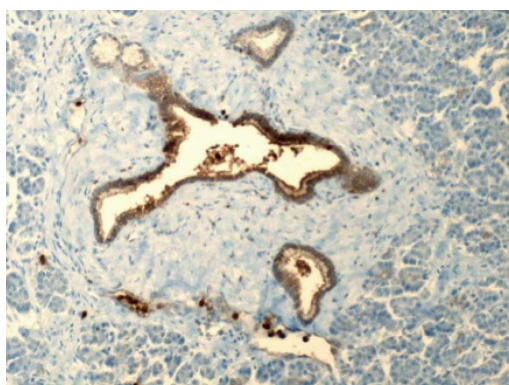


Figure 2 Benign pancreatic ducts and acini with weak staining of the ductal cells for carcinoembryonic antigen (200 ×). Courtesy, Department of Pathology, Temple University Hospital, Philadelphia, PA, United States.

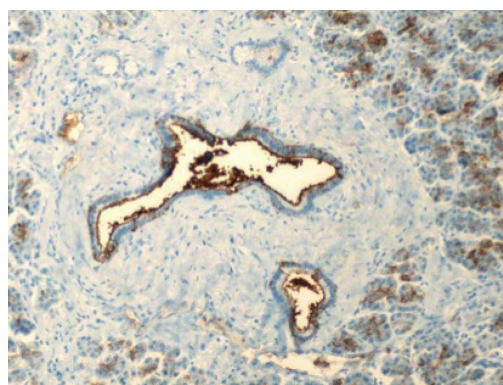


Figure 4 Benign pancreatic ducts and acini with weak staining of the ductal cells for cancer antigen 19-9 (200 ×). Courtesy, Department of Pathology, Temple University Hospital, Philadelphia, PA, United States.

accuracy in distinguishing PDAC from benign conditions^[33]. These data highlight the potential value of biomarkers from various biological sources in the early diagnosis of pancreatic cancer.

Prognostic biomarkers

Normal pancreatic juice contains multiple proteins and administration of secretin alters the concentration but not the spectrum of these proteins^[39]. The proteome of pancreatic juice in patients with PDAC is markedly altered^[39,40]. The proteome of the pancreas after surgical resection contains regenerative and immunomodulatory factors which vary depending on neoadjuvant therapy, history of smoking and vary over time to stimulate restoration of organ function^[41]. Profiling of miRNAs in pancreatic juice of patients with PDAC demonstrated higher contents of miR-205 and miR-210 correlating with lymph node metastasis and diminished survival demonstrating their potential value as candidate biomarkers of disease progression and prognosis^[42]. Assay of Adna-9 in pancreaticobiliary secretions and PDAC tumor demonstrated its potential value as a candidate biomarker for diagnosis and prognostication^[43]. Elevated

level of S100A8 or A9 in pancreatic ductal fluid, a near absence of pancreatic enzymes and high level of mucins (MUC1, 2, 5AC, 5B, 6 and 13) were predictors of poor survival suggesting that pancreatic ductal fluid is a promising tool for identifying prognostic biomarkers^[34].

BIOMARKERS IN BILE

Diagnostic and prognostic value

Intraoperative samples of bile from gallbladder in patients with pancreaticobiliary diseases demonstrated significantly higher levels of CA 19-9 in malignancy and correlated with the tumor burden^[44]. Biliary CEA > 10 ng/mL in patients undergoing a curative surgery for colorectal cancer is a strong predictor of hepatic recurrence suggesting that it is a marker for occult liver metastases^[45,46]. Liver is the site of first recurrence in 50% patients following curative surgery for PDAC^[47]. The use of adjuvant liver-directed therapy including hepatic artery infusion chemotherapy (HAI) significantly decreases the incidence of liver metastases with a trend towards improvement in cumulative survival^[48,49]. Prediction of the site of early recurrence can impact choice of the optimal modality for adjuvant therapy

Table 1 Potential biomarkers in the pancreatic juice and bile of patients with pancreatic adenocarcinoma

Body fluid	Biomarker
Pancreatic ductal fluid	CA 19-9 CEA K-ras p53 mutations DNA methylation alterations Mitochondrial DNA mutations S100 calcium-binding protein P (S100P) Human telomerase reverse transcriptase Matrix metalloproteinase-9 Oncogene DJ1 Alpha-1B-glycoprotein precursor MicroRNA- miR-205, miR-210 Adnab-9 S100A8 or A9 Mucins MUC1, 2, 5AC, 5B, 6 and 13
Bile	CEA Mac-2-binding protein MUC4 Vascular endothelial growth factor

CA 19-9: Cancer antigen 19-9; CEA: Carcinoembryonic antigen.

following curative surgery for PDAC.

Novel diagnostic biliary biomarkers for biliary tract cancer include Mac-2-binding protein (Mac-2BP) identified in bile using tandem mass spectrometry^[50]. Alterations in epithelial mucin expression has identified MUC4 in pancreatic juice as a diagnostic and prognostic marker for pancreatic cancer, biliary MUC4 as a diagnostic biomarker and serum MUC5A as a sensitive diagnostic marker correlating negatively with survival in biliary tract cancer^[34,51]. Elevated levels of biliary vascular endothelial growth factor (VEGF-1) distinguishes patients with pancreatic cancer from other etiologies of biliary stricture^[52]. Potential biomarkers in the pancreatic juice and bile of patients with pancreatic adenocarcinoma are shown in Table 1. These preliminary data demonstrating the diagnostic and prognostic value of biliary markers in cancer require prospective evaluation and validation in large scale multicenter studies.

SELECTION OF BIOMARKERS

Choice of the optimal biomarker

Biomarkers obtained from readily accessible biological materials *via* non-invasive procedures minimize downstream investigations and costs^[53]. Pancreatic juice and/or bile is readily collected during the course of a pancreatic resection for PDAC. In contrast to the recently identified biomarkers requiring further investigation prior to recommendation for clinical use, CA 19-9 and CEA are widely used and validated markers of diagnostic and prognostic value in PDAC and pre-neoplastic lesions of the pancreas^[54]. However, the prognostic value of CA 19-9, CEA and LDH levels in the pancreatic juice and bile of patients with PDAC has not been evaluated. Standardized laboratory protocols are available for the assay of CA 19-9

and CEA in body fluids rendering them optimal biomarkers in the evaluation of patients with PDAC.

CONCLUSION

Pancreatic juice is a rich source of cancer-specific proteins rendering it a promising tool for identifying novel prognostic biomarkers in PDAC. Elevated level of CEA in bile is a marker for malignancy and a predictor of hepatic recurrence. CA 19-9, CEA and LDH are widely used in clinical practice as diagnostic markers of pancreatic cancer however, the prognostic value of their levels in pancreatic juice and bile is unknown. Specimens of pancreatic juice and bile can be readily obtained during surgical resection of the tumor and analyzed according to well-established laboratory protocols for assays of CA 19-9, CEA and LDH to evaluate their prognostic value. Profiling of pancreatic juice and bile to identify biomarkers may improve early diagnosis and selection of patients for the optimal adjuvant therapeutic modality.

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Case Control Study

Levels of neutrophil gelatinase-associated lipocalin in patients with head and neck squamous cell carcinoma in Indian population from Haryana state

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Author contributions: Verma M and Dahiya K designed the research, interpreted the results and drafted the manuscript; Verma M and Bansal A analyzed the samples; Soni A, Dhankhar R and Bansal A helped in statistical analysis, critical revision and final approval of the article; Ghalaut VS and Kaushal V helped in designing the results, making critical revisions and final approval of the article.

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Abstract

AIM

To study the levels of neutrophil gelatinase associated lipocalin (NGAL) in head and neck squamous cell carcinoma (HNSCC).

METHODS

This was a non randomized case control study conducted at Department of Biochemistry, in collaboration with Regional Cancer Center over a period of one year. The study population included 50 adult newly diagnosed HNSCC patients reporting in outpatient department at Regional Cancer Center and compared with 50 healthy controls. NGAL was estimated by ELISA technique. Student *t* test and χ^2 test were applied for comparison of means of study groups. Correlations between groups were analyzed using Pearson correlation coefficient (*r*) formula.

RESULTS

Patients with HNSCC exhibited significantly increased levels of NGAL (*P* < 0.05) as compared to healthy controls

(978.88 ± 261.39 ng/mL *vs* 34.83 ± 7.59 ng/mL). Out of 50, 26 patients (52%) were in stage IV, 21 (42%) in stage III, 1 (2%) patient in stage II and 2 (4%) patients were in stage I. Metastasis was absent in 98% patients and mean NGAL levels were highest in these patients but *P* value was not significant. Mean NGAL levels were highest in stage IV [1041.54 ± 222.15 ng/mL (stage IV) *vs* 1040 ± 0.00 ng/mL (stage I); 900 ± 0.00 ng/mL (stage II) and 1031.90 ± 202.55 ng/mL (stage III)] and χ^2 test was highly significant (*P* < 0.001). Thirty-six patients (72%) were having moderately differentiated HNSCC and mean NGAL levels were maximum in patients with well differentiated HNSCC (1164 ± 315.64 ng/mL *vs* 1013.33 ± 161.19 ng/mL in moderately differentiated and 890 ± 11.55 ng/mL in poorly differentiated) and the results were also highly significant (*P* < 0.001, χ^2 test).

CONCLUSION

The present work demonstrates a potential role of NGAL as cancer biomarker and its use in monitoring the HNSCC progression.

Key words: Neutrophil gelatinase associated lipocalin; Head and neck squamous cell carcinoma; Metastasis; Biomarker; Lipocalin 2

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Core tip: Neutrophil gelatinase associated lipocalin might play a significant role as a biomarker in head and neck squamous cell carcinoma.

Verma M, Dahiya K, Soni A, Dhankhar R, Ghalaut VS, Bansal A, Kaushal V. Levels of neutrophil gelatinase-associated lipocalin in patients with head and neck squamous cell carcinoma in Indian population from Haryana state. *World J Clin Oncol* 2017; 8(3): 261-265 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/261.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.261>

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, affecting 600000 new patients each year. The molecular alterations observed in HNSCC are mainly due to oncogene activation and tumor suppressor gene inactivation, leading to dysregulation of cell proliferation. These alterations include gene amplification and over expression of oncogenes such as ras, myc, epidermal growth factor receptor (EGFR) and cyclin D1 and mutations and deletions leading to p16 and TP53 tumor suppressor genes inactivation^[1].

Neutrophil gelatinase associated lipocalin (NGAL) is a small molecule of 178 amino acids that belongs to the superfamily of lipocalins, which are proteins specialized in binding and transporting small hydrophobic molecules.

It is also known as lipocalin 2, protumorigenic protein. Increased expression of NGAL was first identified in SV 40 tumour virus infected quiescent mouse primary kidney cells. It plays a significant role in inducing tumour progression and chemoresistance in cancer cells. It acts mainly as a biomarker of kidney injury but now a day it has emerged as a biomarker for several benign and malignant diseases^[2]. Lipocalin 2 is thought to be an acute phase protein^[3], the expression of which is upregulated in epithelial cells under diverse inflammatory conditions including appendicitis, inflammatory bowel disease and diverticulitis^[4]. Lipocalins affect cellular proliferation and differentiation and may be involved in the development of carcinomas^[5].

Levels of lipocalin 2 is upregulated or downregulated in different cancers, *i.e.*, lung, colon, pancreas, breast, prostate, *etc*^[4,6-8]. But studies are scarce in head and neck carcinoma particularly with respect to its involvement in invasion and metastasis. Five year survival rate of HNSCC is only 50%^[9]. Early diagnosis can improve the outcome. So, there is an urgent need for the development of novel biomarkers for timely diagnosis of this fatal disease. Therefore this study was planned to estimate NGAL levels in HNSCC.

MATERIALS AND METHODS

This was a non randomized case control study conducted at Department of Biochemistry, in collaboration with Regional Cancer Center over a period of one year from September 2013 to September 2014. A total of 50 newly diagnosed patients were selected for the study. Group A consisted of 50 HNSCC patients and 50 apparently healthy age and sex matched volunteers acted as controls (Group B).

After taking care of all ethical issues, 12 h fasting venous blood samples without application of tourniquet were collected aseptically from median antecubital vein. Serum was separated and stored at -20 °C till analysis. Serum-NGAL was measured using ELISA (Bioporto, Gentofte, Denmark)^[10].

Data was subjected to appropriate statistical analysis. Values are shown in the text, tables and figures as mean ± SD. Student *t* test and χ^2 test were applied for comparison of means of study groups. A value of *P* < 0.05 was considered significant. Correlations between groups were analyzed using Pearson's correlation coefficient (*r*) formula.

RESULTS

The mean age of the patients in group A was 57.40 ± 11.33 years (35-95 years), while in group B was 40.01 ± 10.8 years (37-90 years). Out of 50 patients, 40 were males and 10 were females in group A while there were 38 males and 12 females in group B. Both the groups were statistically comparable in age and gender distribution. The biochemical parameters are shown in Table 1. Serum NGAL levels were significantly raised

Table 1 Biochemical parameters in head and neck squamous cell carcinoma patients and healthy controls

Parameter	HNSCC patients	Healthy controls
<i>n</i>	50	50
Age (yr)	57.40 ± 12.61	40.01 ± 10.8
Gender (M:F)	40:10	38:12
Smoker:non-smoker	40:10	12:38
Serum NGAL (ng/mL)	^a 978.88 ± 261.39	34.83 ± 7.59

^aSignificant; all values are in mean ± SD. HNSCC: Head and neck squamous cell carcinoma; NGAL: Neutrophil gelatinase-associated lipocalin.

in HNSCC patients ($P < 0.05$) as compared to controls (978.88 ± 261.39 ng/mL vs 34.83 ± 7.59 ng/mL) (Figure 1).

Out of 50, 26 patients (52%) were in stage IV, 21(42%) in stage III, 1 (2%) patient in stage II and 2 (4%) patients were in stage I. Relation between NGAL and various study variables is shown in Table 2. Mean NGAL levels were highest in stage IV and χ^2 test was highly significant ($P < 0.001$). Metastasis was absent in 49 patients (98%). Only 1 patient (2%) was having metastasis of unknown origin in head and neck area with occult primary. Results were not significant but mean NGAL levels were highest in patients with no metastasis. Ratio of smokers to non smokers in HNSCC patients was 40:10 while in controls it was 12:38. Smoking history seemed to have no effect on systemic levels of NGAL as $P = 0.097$. Thirty six patients (72%) were having moderately differentiated HNSCC and mean NGAL levels were maximum in patients with well differentiated HNSCC and the results were also highly significant ($P < 0.001$, χ^2 test).

In our study larynx was the most common site involved (38%), other sites being base of tongue (36%), tonsils (22%) and cheek mucosa (4%). Serum NGAL values were significantly higher in patients with base of tongue involvement ($P < 0.001$, χ^2 test).

DISCUSSION

Various studies have shown different levels of NGAL levels in many carcinomas, *i.e.*, lung, colon, pancreas, breast, prostate, *etc.* But very few reports are available for head and neck cancer. A significant increase was observed in serum NGAL levels in HNSCC patients as compared to healthy controls ($P < 0.05$). Moreover, levels were significantly raised in stage IV ($P < 0.001$) and well differentiated HNSCC carcinoma patients ($P < 0.001$). Metastasis was absent in 98% patients and mean NGAL levels were highest in these patients showing anti-metastatic effect of NGAL. Majority of patients in our set up usually present in advanced stage of the disease due to lack of awareness or resources or both.

Previous studies have shown increased levels of NGAL in adenocarcinoma of lung, colon, pancreas and decreased levels in renal cell carcinomas and prostate

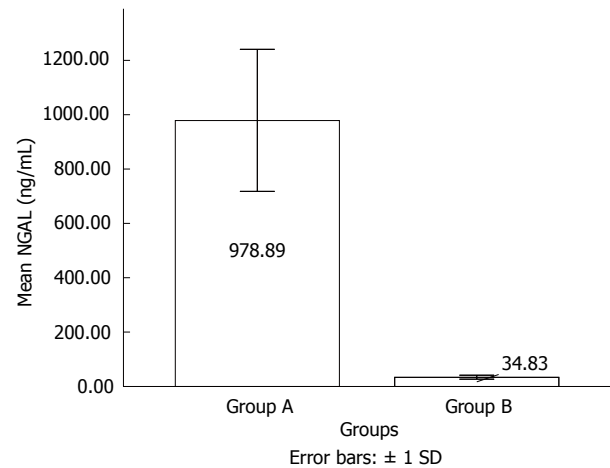


Figure 1 Mean serum neutrophil gelatinase-associated lipocalin levels in head and neck squamous cell carcinoma patients (group A) and healthy controls (group B). NGAL: Neutrophil gelatinase-associated lipocalin.

cancers^[11]. Role of NGAL in cancer is controversial. NGAL has been shown to have a pro-tumoral effect in breast, stomach and esophagus cancer. Some studies show that NGAL exert an anti-tumoral and anti-metastatic effect in ovarian and pancreatic cancers similar to present study^[12]. These increased levels may be due to increased synthesis induced by factors promoting the development of carcinoma. NGAL and pro-matrix metalloprotein-9 (MMP-9) bind to integral membrane proteins on tumour cells and leads to pro or anti tumour effect on growth, migration, invasion, survival and angiogenesis depending on the type of cancer^[13]. *In vitro* studies in human breast and lung cancer cells have revealed that NGAL expression is significantly up regulated in response to multiple apoptosis inducing agents in an attempt to survive the apoptotic stimulus rather than a pro-apoptotic response^[14].

Metastasis was absent in 49 patients and an over expression of NGAL, as obvious by increased levels in all these. Only 1 patient was having metastasis of unknown origin in head and neck area. We assumed the primary center being head and neck site. So, it is assumed that over expressed NGAL levels are responsible for reduction in distant metastasis of HNSCC cells as seen in many other cancers also though nothing can be concluded as sample size being very small^[15]. The mechanisms by which NGAL regulates tumor metastasis are still unclear. Down-regulation of epithelial proteins and the induction of mesenchymal proteins (EMT) enhance the metastatic potential of epithelial tumors.

Lipocalin 2 is an epithelial inducer, which stimulates the epithelial phenotype in ras transformed cells and reverses their metastatic potential^[16]. NGAL has siderophore chelating capacity. It binds to intracellular iron and this complex then interacts with NGAL-R (NGAL receptor) leading to internalization of complex. After entering cytoplasm, it releases iron leading to iron accumulation and regulating specific iron-dependent genetic pathways. These events induce proliferation and

Table 2 Correlation between neutrophil gelatinase-associated lipocalin levels and various study variables

	<i>n</i>	Serum NGAL (mean \pm SD)
Differentiation		
Well differentiated	10	¹ 1164 \pm 315.64
Moderately differentiated	36	1013.33 \pm 161.19
Poorly differentiated	4	890 \pm 11.55
Site		
Base of tongue	18	¹ 1072.22 \pm 251.39
Larynx	19	1037.89 \pm 217.35
Tonsil	11	947.27 \pm 50.02
Cheeks	2	1120 \pm 0.00
Metastasis		
No	49	1036.73 \pm 206.64
Yes	1	880 \pm 0.00
Staging		
I	2	1040 \pm 0.00
II	1	900 \pm 0.00
III	21	1031.90 \pm 202.55
IV	26	¹ 1041.54 \pm 222.15
Smoking		
Smoker	40	1051 \pm 223.57
Non-smoker	10	964 \pm 84.22

¹Highly significant. All values are in mean \pm SD. NGAL: Neutrophil gelatinase-associated lipocalin.

epithelial transformation^[17]. It inhibits focal adhesion kinase phosphorylation and vascular endothelial growth factor synthesis leading to decreased cell adhesion, invasion and angiogenesis^[12]. NGAL also suppresses c-Jun N-terminal kinase (JNK) and phosphoinositide 3-kinase (PI3)/AKT signalling pathways, thereby, decreasing metastasis in HNSCC^[13].

Lipocalin 2 levels were significantly higher in well differentiated carcinoma as compared to moderately and poorly differentiated HNSCC ($P < 0.001$). Similar results have been reported by another study in which it was shown by immuno-histochemical examinations that NGAL expression was strongly up-regulated in well-differentiated oral squamous cell carcinoma (OSCC) tissues and moderately to weakly up-regulated in moderately to poorly differentiated OSCC tissues as compared to normal mucosa and leukoplakia showing very weak expressions. Western blot analysis showed positive correlation of NGAL expression levels with cell morphology patterns and loss of E-cadherin^[18]. So, NGAL levels may be raised as an effect or cause of tumor. NGAL may act as biomarker for diagnosis of HNSCC and for assessment of its severity.

Some limitations of our study include small sample size, lack of follow up and monitoring of the effect of treatment on NGAL outcome that will make part of our future project.

Our analysis demonstrates a potential role of NGAL as cancer biomarker which may be useful in monitoring the HNSCC progression. Many drugs which induce lipocalin 2 can be of therapeutic benefit in HNSCC to prevent metastasis and further spread.

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COMMENTS

Background

Neutrophil gelatinase-associated lipocalin (NGAL), plays a significant role in generating innate immune response and safeguards against bacterial infections by sequestering iron. Recently, it has emerged as a biomarker for several benign and malignant diseases with its differential expression pattern.

Research frontiers

As NGAL has anti-tumoral and anti-metastatic effect, it could be a new and effective biomarker for monitoring the progression of disease in head and neck squamous cell carcinoma (HNSCC).

Innovations and breakthroughs

NGAL was initially defined as a useful bacteriostatic agent actively against bacteria. Later on it was found that it acts as a biomarker of kidney injury but now a day it has emerged as a biomarker for several benign and malignant diseases. This study describes for the first time the increased levels of NGAL and its association with anti-metastatic effect in HNSCC.

Applications

The goal of treatment in HNSCC is mainly surgery, radiotherapy and chemotherapy. Biomarkers like NGAL having significant diagnostic and prognostic significance and with specific molecular target are demand of newer treatment modalities to increase the survival of patients.

Peer-review

In this study, the authors examined the levels of NGAL in patients with HNSCC in an Indian population. Overall, the methodology of the study is adequate, and the findings are clinically and scientifically relevant.

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Recurrence-free survival as a putative surrogate for overall survival in phase III trials of curative-intent treatment of colorectal liver metastases: Systematic review

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Abstract

AIM

To verify whether recurrence-free survival (RFS) surrogates overall survival (OS) in phase III trials for resectable colorectal liver metastases (CRLM).

METHODS

MEDLINE, EMBASE, and Scopus databases were consulted. Eligible studies were phase III trials testing any type of systemic therapy (neoadjuvant, adjuvant or perioperative) added to surgery in patients with resectable CRLM. A linear regression model based on hazard ratios (HR) of OS and RFS was performed.

RESULTS

Of 3059 studies, 5 phase III trials (1162 patients) were included for analyses. A linear regression weighted by each trial was used to estimate the association between each HR and RFS. The originated formula was: OS HR = $(0.93 \times \text{RFS HR}) + 0.14$; with RFS 95%CI (0.48-1.38), with $P = 0.007$.

CONCLUSION

This association suggests that RFS could work as a putative surrogate endpoint of OS in this population, avoiding bigger, longer and more resource-consuming trials. The OS could be assumed based on RFS and our model could be useful to better estimate sample size calculations of phase III trials of CRLM aiming for OS.

Key words: Colorectal liver metastases; Surgery; Chemotherapy; Clinical trial; Long-term outcomes; Surrogate endpoints

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Core tip: This study addresses a systematic review of curative-intent treatment of colorectal liver metastasis looking for oncologic outcomes. We describe the association between overall survival (OS) and recurrence free survival in the setting of resectable colorectal liver metastases (CRLM). It suggests that recurrence free survival could work as a putative surrogate of OS in this population, avoiding bigger, longer and more resource-consuming trials. We do believe that our model can be useful to better estimate sample size calculations of superiority phase III trials of CRLM aiming for OS.

Araujo RLC, Herman P, Riechelmann RP. Recurrence-free survival as a putative surrogate for overall survival in phase III trials of curative-intent treatment of colorectal liver metastases: Systematic review. *World J Clin Oncol* 2017; 8(3): 266-272. Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/266.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.266>

INTRODUCTION

Randomized clinical trials (RCT) represent a high level of evidence and a mainstream analysis of oncologic outcomes. However, they involve a time-consuming methodology with inherent high costs. Moreover, trials using overall survival (OS) as their primary endpoint in patients with slow progressive malignancies, such as colorectal cancer, must have longer follow-up for events to arise and thus properly evaluate potential differences in OS. In turn, long-term follow up increases cost associated with image and laboratory tests, salaries of research coordinators, pharmacists and research nurses, investigators fees, medications, etc. Therefore, such trials claim for fundings that are not always provided by governmental agencies or by pharmaceutical companies. For example, Emanuel *et al*^[1] reviewed the cost of conducting clinical trials and demonstrated that monitoring and treating 20 patients in a 12-mo randomized placebo-controlled trial of a new chemotherapeutic agent cost more than United States \$ 6900 per enrolled subject in an industry-sponsored trial.

In order to reduce the cost and time to conduct RCT, investigators have looked at surrogate endpoints of OS, such as progression free survival (PFS) and recurrence free survival (RFS), as measures of clinical benefit in cancer trials. Gains in RFS associating chemotherapy to surgery vs surgery alone for initially resectable colorectal liver metastases (CRLM) have been demonstrated by phase III trials^[2-4]. While surgery plus chemotherapy has not been associated with improvements in OS in phase

III trials, it has been suggested by a meta-analysis of published data^[5]. However, it is unknown whether RFS can substitute, and if so to which extent, OS in RCT of CRLM. In this regard we hypothesized that if gains in RFS predicted gains in OS, trials of new drugs in the setting of CRLM could use RFS as a surrogate endpoint, and thus expedite drug development. The objectives of this systematic review were to evaluate RCT of curative-intent treatment to resectable CRLM and to verify whether RFS surrogates OS in phase III trials for this population.

MATERIALS AND METHODS

Eligibility criteria

Type of studies: All published RCTs with curative-intent treatment for initially resectable CRLM were evaluated; curative-intent therapies were surgery alone vs associated systemic cancer-directed therapy. Two considerations were made to assume curative-intent treatment: Patients were not treated for conversion therapy because they were already resectable at the time of study enrollment and removal of all macroscopic disease (no residual disease). No language restriction was applied. Studies with extra-hepatic disease were generally excluded, but when extra-hepatic disease was present in no more than 5% they were accepted. Studies using regional chemotherapy or presenting initially unresectable disease were also excluded. For situations in which two studies from the same institution were identified, the most recent or the most informative study was selected unless different periods were evaluated or the data of overlapping patients could be subtracted.

Type of interventions: Only treatments with curative-intent treatment for initially resectable hepatic lesions were evaluated. However, any standardized description of resectable disease was used to define this group of patients, as they were defined according to clinic-radiological evaluation of each tumor board of their respective authors' institutions. Any additional systemic treatment was considered as the following: Adjuvant chemotherapy (surgery followed by systemic therapy), neoadjuvant chemotherapy (preoperative chemotherapy followed by surgery), perioperative chemotherapy (preoperative chemotherapy followed by surgery and postoperative chemotherapy), and targeted agents at any perioperative period. This study did not discriminate between type of liver resection or surgical techniques because all of them were procedures with curative-intent. The study also did not aim to compare types of systemic therapies or times of its administration.

Type of outcome measure: The primary end point of the study was to describe the association between OS and RFS in the setting of resectable CRLM. Calculation of OS was based on survivorship status (deceased or alive) at the last follow-up visit as reported by RCT. Calculation of RFS was based on the first detected recurrence or the last follow-up visit without recurrence. Start time

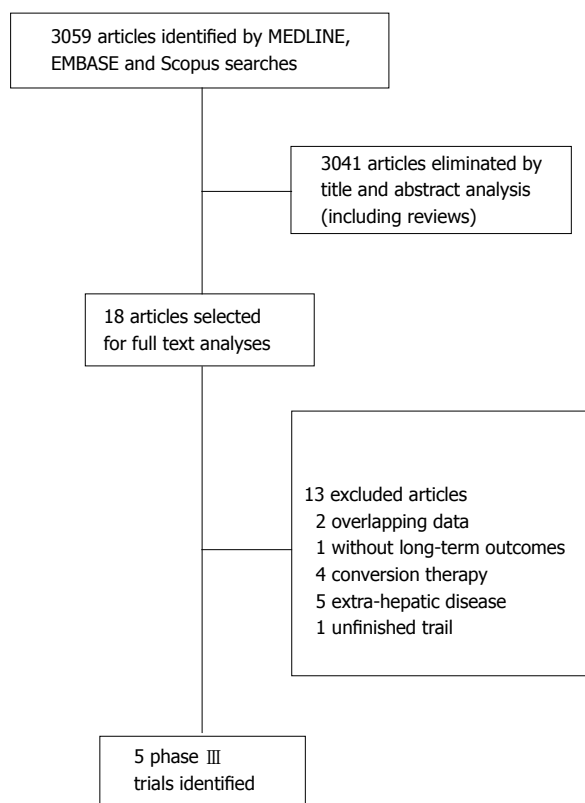


Figure 1 Flowchart of search and article selection process.

was counted as defined by each study. Imaging tests were mostly performed at 3-mo intervals until disease recurrence, as defined by RCT. The terminology chosen was RFS since all patients did not present any residual macroscopic disease after curative-intent treatment. We counted reappearance of disease and/or liver-only disease as recurrence.

Search: The MEDLINE, EMBASE, and Scopus databases were searched using the mesh terms (“colorectal liver metastases” or “colorectal liver metastasis”) and (surgery or surgical or chemotherapy or “drug therapy” or “Antineoplastic Agents”) and (Clinical Trial or Comparative Study or Randomized Controlled Trial). They were filtered from January 1990 to February 2015 and only for studies in humans.

Data collection process: Relevant data were extracted independently from all the studies by two reviewers (Raphael LC Araujo and Rachel P Riechelmann) and included study features, population characteristics, and data needed for quality assessment. For the purpose of this study, only OS and RFS were extracted according to the description provided by the authors.

Quality assessment: The RCTs were evaluated by individual components based on the Cochrane Risk-of-Bias Tool (version 5.1.0). The qualitative evaluation was performed and discriminated for each RCT. This study was performed according to the recommendations of the preferred reporting items for systematic reviews and

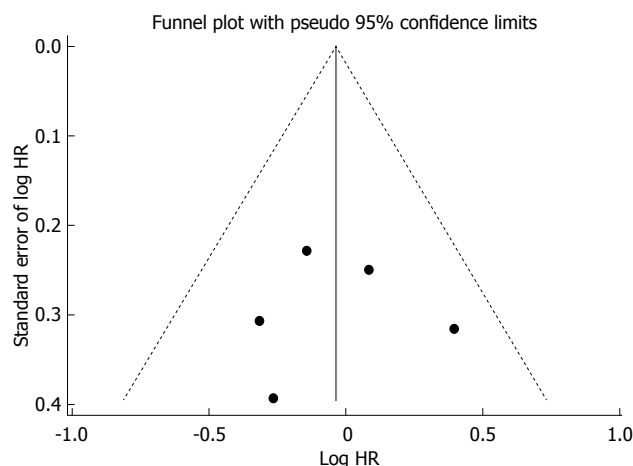


Figure 2 Funnel plots of randomized clinical trials comparing surgery alone or with additional chemotherapy for the treatment of patients with potentially resectable colorectal liver metastases. The HR was fit for overall survival. HR: Hazard ratio.

meta-analysis (PRISMA) statement. We used Begg’s funnel plot as an analytic tool to detect publication bias^[6,7].

Statistical analysis

Linear regression was performed to examine the association between HR for both outcomes. Demographics were demonstrated as percentages as appropriate. Survival probabilities were estimated by cited hazard ratios (HR) accordingly to each published study. The graph of linear regression was based on linear prediction of OS HR according to RFS HR, along with a 95%CI based on the mean. For all tests, statistical significance was defined by a two-sided *P* value lower than 0.05. All analyses were performed by STATA 13 statistical software (StataCorp, College Station, TX, United States).

RESULTS

We identified five RCT addressing curative-intent treatment with surgery alone or with systemic therapy for initially resectable CRLM. They were selected among 3059 articles. The flowchart of selection process is summarized in a flow diagram in Figure 1.

This systematic review was made properly to the *PRISMA* Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analysis). Additionally, the Cochrane Risk-of-Bias Tool was used to qualitative evaluation of RCTs, and it is described in Table 1. As frequently seen in surgical trials, the difficulty concealing the allocation of patients and blinding in the randomization between chemotherapy and surgery first could not be granted. However, it was not considered as a drawback neither affecting outcomes directly nor compromising the primary endpoint of our review. No publication bias was demonstrated using Begg’s funnel plot as depicted in Figure 2.

Only five phase III trials were accepted for this review, and all of them were looking for initially resectable CRLM^[2-4,8,9]. Comparative distributions of accessible

Table 1 Quality assessment of selected randomized clinical trials evaluated by Cochrane Risk-of-Bias Tool (Risk of bias per study)

Criteria	Langer	Portier	Nordlinger	Ychou	Primrose
Random sequence generation	Unclear	Low	Low	Low	Low
Allocation concealments	Low	Low	Low	Low	Low
Blinding of participants and personnel ¹	Low	Low	Unclear	Low	Low
Blinding of outcome assessment ^{1,2}	Low	Low	Low	Low	Low
Incomplete outcome data	Unclear	Low	Low	Unclear	Low
Selective reporting	Low	Low	Low	Low	Low
Other bias	Unclear	Low	Low	Low	Low

¹Blinding is not possible; ²Implementation of a protocol for postoperative management was considered the best alternative.

Table 2 Comparative distribution of accessible baseline characteristics of patients among studies included in the systematic review

Characteristics	Langer <i>n</i> = 107 (%)		Portier <i>n</i> = 171 (%)		Nordlinger <i>n</i> = 364 (%)		Ychou <i>n</i> = 306 (%)		Primrose ¹ <i>n</i> = 257 (%)	
	Surg <i>n</i> = 55	S + C <i>n</i> = 52	Surg <i>n</i> = 85	S + C <i>n</i> = 86	Surg <i>n</i> = 182	S + C <i>n</i> = 182	S + 5-FU <i>n</i> = 153	S + FOLFIRI <i>n</i> = 153	S + C <i>n</i> = 128	S + C + Cetuximab <i>n</i> = 129
Median age	60	63.5	63	63	62	64	61	63	64	63
Gender (male)	65.4	65.4	62.4	53.5	63	70	65.4	58.8	63	71
Primary site (rectum)	30.9	26.9	40	40.7	37	46	26.1	28.8	-	-
DFI ≤ 12 mo	38.2	34.6	74.1	74.4	24	27	62.3	61.4	-	-
Node-positive primary	45.4	50	50.6	44.3	57	55	-	-	-	-
No. of lesions > 1	32.7	36.5	30.1	31.4	52	51	35.9	36	-	-
Largest met ≥ 5 cm	-	-	-	-	-	-	-	-	-	-
Chemotherapy	5-FU		5-FU		FOLFOX		5-FU	FOLFIRI	5-FU + OX or 5-FU + Cap or FOLFIRI	5-FU + OX or 5-FU + Cap or + Cetuximab

¹*n* of eligible patients = 257, although only 234 patients had response rates analyzed; -: Represents data not assessable by authors; Surg: Surgery only; S + C: Surgery with additional chemotherapy; 5-FU: 5-fluorouracil based; FOLFOX: Fluorouracil, leucovorin, and oxaliplatin; FOLFIRI: Fluorouracil, leucovorin, and irinotecan.

baseline characteristics in the studies are depicted in Table 2. Three of them compared surgery alone vs surgery plus chemotherapy, and two RCT compared surgery plus chemotherapy on both arms, with one of them testing the addition of cetuximab, a monoclonal antibody against epidermal growth factors receptor (EGFR). Four studies had RFS as their primary endpoint. Only Langer *et al.*^[2] pursued OS as primary endpoint, but they failed to show a significant difference with postoperative chemotherapy. A total of 1162 patients (per protocol) were included in this pooled analysis. Most of them were male, the median age ranged from 60 to 64 years old, most presented colon as their primary site and with a single hepatic lesion. Comparisons of original planned and analyzed design of RCT are demonstrated in Table 3.

A linear regression was used to fit a predict model for OS based on RFS and using HR values. The assumption of linearity was based on this formula: OS HR = (0.93 × RFS HR) + 0.14; with RFS 95%CI (0.48-1.38); standard error of 0.14, and *P* = 0.007. HR for RFS and OS, the originals and those assumed by the formula above are described in Table 4 and depicted in Figure 3 (the intention-to-treat analysis from Nordlinger *et al.*^[3] was used).

DISCUSSION

This systematic review and pooled analyses of published RCT of resectable CRLM demonstrates that RFS can be considered a surrogate endpoint for OS in this setting. We found a linear association between RFS gains, as measured by HR, and OS increments. This finding has numerous implications for future trial designs of new cancer-directed therapies added to curative-intent hepatic resection of CRLM.

The practice of evidence-based medicine (EBM) has been in vogue in the last 30 years. Sackett *et al.*^[10] categorized levels of evidence according to quality of study designs, ranging from expert opinion (level V - the lowest level) to RCT (level I - highest level). While RCT represent the best way to deliver evidence-based medicine, over the last decades, their costs have skyrocketed, what may limit national fundings and consequently, demand for-profit sponsorship^[11]. This is particular relevant for clinical cancer research^[12]. The cost of RCT, including cancer trials, can be split in fixed (trial administration, hospital facilities, personnel training, equipment, infrastructure, etc.), variable (randomization, recruitment cost, patient

Table 3 Comparison of original planned and analyzed design of randomized clinical trials with patients who underwent surgery and additional chemotherapy for initially resectable colorectal liver metastases

Studies by author	Initial design			No. of patients				Chemotherapy			%	Median FU		RFS		OS	
	Primary endpoint	Planned HR	Type of analyses	Planned	Accrued	ITT enrolled	PP (weight)	Regimen	Std Arm	Exp Arm		Std Arm	Exp Arm	Std Arm	Exp Arm	Std Arm	Exp Arm
Langer	OS	NR	PP	NR	129	129	107 (9)	Adj	0	5-FU × 6	100%	NR	NR	20	39	43	53
Portier	RFS	20% abs dif 2 yr ¹	ITT	200	173	171	171 (15)	Adj	0	5-FU × 6	100%	87.4	87.4	17.6	24.4	46.4	62.1
Nordlinger	RFS	0.714	Both	NR	364	364	342 (29)	Periop	0	FOLFOX × 12	93%	8.7 yr	8.7 yr	20	12.5	54.3	61.3
Ychou	RFS	NR	PP	420	321	321	306 (26)	Adj	1	FOLFIRI × 6	100%	42.4	41.7	21.6	24.7	72% at 3-yr	73% at 3-yr
Primrose	RFS	0.68	ITT	268	272	257	236 (20)	Periop	FOL-FOX	Cetux + FOLFIRI (70%)	85% (Chemo) 82% (Cetux)	21.1	19.8	14.1	20.5	39.1	NR

¹Absence of difference at 2-year. HR: Hazard ratio; OS: Overall survival; RFS: Recurrence free survival; ITT: Intention to treat; PP: Per protocol; Std: Standard; Exp: Exposed; Adj: Adjuvant; Periop: Perioperative; 5-FU: 5-Fluorouracil; FOLFOX: 5-FU + Leucovorin + Oxaliplatin; FOLFIRI: 5-FU + Leucovorin + irinotecan; Cetux: Cetuximab; Chemo: Chemotherapy; NR: Non-reported.

Table 4 Comparison of original hazard ratio and those from linear regression formula

Studies (by author)	n total (weight %)	RFS			OS			Assumption OS HR
		HR	95%CI		HR	95%CI		
Langer	107 (9)	0.78	0.46	1.31	0.77	0.42	1.4	0.87
Portier	171 (15)	0.66	0.45	0.96	0.73	0.48	1.1	0.75
Nordlinger ¹	342 (29)	0.78	0.61	0.99	0.87	0.66	1.14	0.87
Ychou	306 (26)	0.89	0.66	1.19	1.09	0.72	1.64	0.97
Primrose	236 (20)	1.48	1.04	2.12	1.49	0.86	2.6	1.52

¹Intention-to-treat analysis. HR: Hazard ratio; OS: Overall survival; RFS: Recurrence free survival.

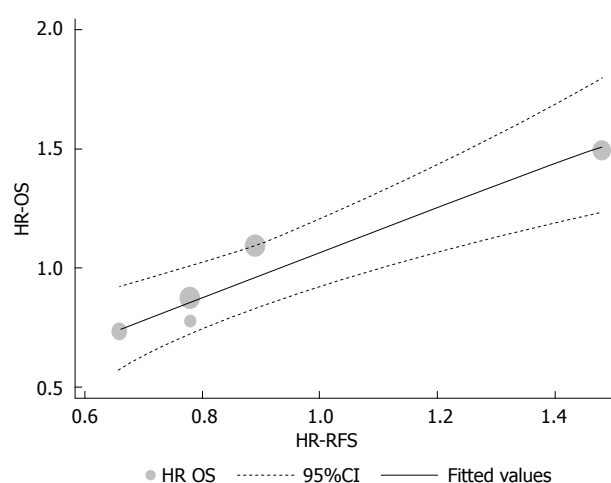


Figure 3 Linear prediction of overall survival according to recurrence free survival. The sizes of dots are proportional to weight of each study. The linear regression was based on linear prediction of OS HR according to RFS HR, along with a 95%CI based on the mean. RFS: Recurrence-free survival; OS: Overall survival; HR: Hazard ratio.

track and follow-up) and indirect costs (hospital overhead, public relations and networking, and legal consultancy)^[11]. In this context, expensive new cancer drugs can cost to society in two different ways: Firstly, it costs directly

to payers; and secondly, their high prices preclude new trials to compare their effectiveness against effective but cheaper alternatives^[13]. Looking for oncologic outcomes in resectable CRLM, RCT can be even more expensive since OS is usually a required primary endpoint. For example, it is clear that slow-progressive tumors demand longer follow-up (more than 5 years to reach median OS, e.g.,) in phase III trials than those just looking for RFS. Therefore, there are several pros and cons of utilizing OS as a primary endpoint for a cancer RCT. The first issue with the measurement of OS is that the curative-intent treatment works as just first-line therapy; when disease progresses, the patient can still undergo further lines of systematic therapy or R0 surgery, what contaminates and dilutes eventual OS gains from first line. This phenomenon can sometimes be overcome by planning trials with large samples aimed to look for small statistical OS benefits. This in turn, increases the cost of conducting RCT in oncology. The argument in favor of using only OS as the primary endpoint in cancer RCT is that survival is a hard endpoint, not subjected to measurement biases. On the other hand, those in favor of surrogate endpoints for OS, such as PFS and RFS, highlight benefits in terms of faster trial results, less cost and perceived clinical benefit by patients and physicians. We argue that while both OS and surrogates endpoints can be used depending

on the scenario, surrogates endpoints, if mathematically demonstrated, are useful tools to expedite clinical research and avoid unrealistically large and expensive trials, and also to early identify and stop enrollment into futile trials.

PFS has been demonstrated to be a surrogate endpoint for OS in treatments for metastatic^[14,15] as well as for early stage colorectal cancer. In the adjuvant, *i.e.*, curative setting, Sargent *et al*^[16] pooled individual data of 18 RCT (20898 patients) for early stage colorectal cancer, showing that gains in disease free survival predicted for gains in OS. Our study resembles the results of the Sargent *et al*^[16] because we selected studies with a population more inclined to be cured since patients presented potentially resectable CRLM and underwent curative-intent treatment. In RFS, likewise OS, recurrence is a hard endpoint that is not subject to measurement bias, although it is dependent on the intervals of radiological evaluation.

In this review four studies were powered to RFS but not OS^[3,4,8,9]. Although Langer *et al*^[2] investigated OS as the primary endpoint, it failed to show any benefits of adjuvant chemotherapy compared to surgery alone. Nordlinger *et al*^[17] reported the long-term outcomes with median follow-up of 8.5 years, which also did not find differences in OS. We recently published a systematic review and meta-analysis concerning also surgery alone vs surgery plus chemotherapy, and we found a relative increasing of 23% in OS at 5 years^[5]. However, it was only possible using published data from both randomized and non-randomized trials. One may think that all these negative trials for OS suggest that larger trials would still be necessary to detect small differences in OS. We argue that this is unrealistic and that surrogates endpoints such as RFS should replace OS in RCT of CRLM.

Our study has some limitations. Despite our extensive search, only five studies were suitable to our analysis and it was conducted based on published instead of individual data. As most patients presented low volume disease, our model likely reflect CRLM patients with better prognosis and might not be generalizable to settings of bulky or conversion CRLM. Moreover, as expected, part of those patients will recur but they will still be candidate to rescue treatments (chemotherapy with or without surgery or radiofrequency ablation)^[18,19]. For these reasons, we do not consider our formula useful for individual estimative of OS in clinical practice. The patterns of recurrence are heterogeneous and our correlations could not address such questions since they were not addressed in the original trials. The limitations of our study are those inherent of systematic reviews. And because of that, we think predictive value of RFS demonstrated by our model should be externally tested in future studies. However, we attempted to search as wide as possible, and moreover, we did not detect publication bias. Another limitation to our study and to all others in the field of CRLM is that colorectal cancer is a heterogeneous disease, with patients presenting variable outcomes even when following similar treatments.

Recently, colorectal cancer has been molecularly classified as four distinct prognostic subgroups: CMS1

(microsatellite instability and immune activation features, better prognosis), CMS2 (epithelial, with marked WNT and MYC signaling activation), CMS3 (metabolic dysregulation) and CMS4 (mesenchymal features, worse outcomes)^[20]. It is clear that while perioperative benefits patients with resectable CRLM, many relapse and are not cured. Hence is crucial to properly identify the patients who are more likely to be cured or not by hepatectomy. Once this is done, the surrogacy of RFS on OS will have to be reevaluated according to treatments tailored to each of these molecular subgroups.

Based on RCT, it seems that chemotherapy should always be offered as additional treatment to curative-intention liver resections, increasing RFS, and likely OS^[5]. However, given the lack of evidence on OS gain by RCT, we foresee that surgical trials of systemic treatment for CRLM may prefer OS as their main endpoint. We think such approach should be revisited since larger sample than those already used would be necessary. Based on this systematic review and pooled analysis, we suggest RFS as surrogate of OS for phase III trials comprising patients with resectable CRLM could be used.

In summary, this study demonstrates a linear prediction of OS based on RFS of RCT of patients with resectable CRLM who were managed by curative-intent surgery and systemic therapy. This association suggests that RFS could work as a putative surrogate of OS in this population, avoiding bigger, longer and more resource-consuming trials. Our model can be useful to better estimate sample size calculations of superiority phase III trials of CRLM aiming for OS. However, future RCT should test this model to externally validate its efficiency.

ACKNOWLEDGMENTS

The statistical methods of this study were reviewed by Marcos Alves Lima, biostatistician from Epidemiology and Biostatistics Center, Institute of Learning and Research, at Barretos Cancer Hospital.

COMMENTS

Background

Gains in recurrence-free survival (RFS) for resectable colorectal liver metastases (CRLM) have been demonstrated by phase III trials, but have not been associated with improvements in overall survival (OS). This systematic review verified whether RFS surrogates OS in phase III trials for resectable CRLM.

Research frontiers

Although OS is considered the most appropriate outcome sought in oncology clinical trials, its use is not always feasible in trials of curative-intent treatment of CRLM. Most studies have evaluated RFS as their primary endpoint and none of them had demonstrated benefit in OS, except for a meta-analysis of published randomized trials. The study hypothesized a linear correlation between RFS and OS for this population after a systematic review of literature.

Application

This study addresses an alternative option for analyses of oncologic outcomes in patients who have undergone a curative-intent treatment for CRLM. The linearity identified suggests a corresponding comportment between RFS and

OS. The authors' model could be useful to calculate the sample size for new trials in this field.

Innovations and breakthroughs

The use of modern chemotherapies and surgical resection for CRLM has made the comportment of this disease change into a more indolent profile, with many patients achieving long-term survival. Therefore clinical trials looking for OS as their primary endpoint are associated with long follow up times and high cost. The present study proposes a paradigm change in oncology clinical research because it sought to investigate another outcome which associated with patient benefit, RFS, that may be used in future research to avoid resource-consuming trials.

Peer-review

The paper is well written, properly designed, and comprehensive.

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Robot-assisted laparoscopic vs open gastrectomy for gastric cancer: Systematic review and meta-analysis

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Author contributions: Caruso S conceived the design of the study, directed the acquisition of data, the analysis and interpretation of data; De Franco L and Franceschini F performed the literature search, the acquisition of data, the analysis and interpretation of data; Patriti A, Roviello F, Ceccarelli G and Coratti A contributed to the critical appraisal of the work, revising the article critically for important intellectual content and supervising the interpretation of data for final approval.

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Abstract

AIM

To evaluate the potential effectiveness of robot-assisted gastrectomy (RAG) in comparison to open gastrectomy (OG) for gastric cancer patients.

METHODS

A comprehensive systematic literature search using PubMed, EMBASE, and the Cochrane Library was carried out to identify studies comparing RAG and OG in gastric cancer. Participants of any age and sex were considered for inclusion in comparative studies of the two techniques independently from type of gastrectomy. A meta-analysis of short-term perioperative outcomes was performed to evaluate whether RAG is equivalent to OG. The primary outcome measures were set for estimated blood loss, operative time, conversion rate, morbidity, and hospital stay. Secondary among postoperative complications, wound infection, bleeding and anastomotic leakage were also analysed.

RESULTS

A total of 6 articles, 5 retrospective and 1 randomized controlled study, involving 6123 patients overall, with 689 (11.3%) cases submitted to RAG and 5434 (88.7%) to OG, satisfied the eligibility criteria and were included in the meta-analysis. RAG was associated with longer operation time than OG (weighted mean difference 72.20 min; $P < 0.001$), but with reduction in blood loss and shorter hospital stay (weighted mean difference -166.83 mL and -1.97 d respectively; $P < 0.001$). No differences were found with respect to overall postoperative complications ($P = 0.65$), wound infection ($P = 0.35$), bleeding ($P = 0.65$), and anastomotic leakage ($P = 0.06$). The postoperative mortality rates were similar between the two groups. With respect to oncological outcomes, no statistical differences among the number of harvested lymph nodes were found (weighted mean difference -1.12; $P = 0.10$).

CONCLUSION

RAG seems to be a technically valid alternative to OG for performing radical gastrectomy in gastric cancer resulting in safe complications.

Key words: Robot-assisted gastrectomy; Gastric resection; Open gastrectomy; Gastric cancer

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Core tip: We took into consideration how safe and efficient robot-assisted gastrectomy (RAG) is compared to open gastrectomy (OG) for gastric cancer *via* systematic review and meta-analysis. The available studies to date and the analysis of pooled data extracted from these showed that RAG is safe and feasible, making it possible to obtain lower blood loss related to surgery and a more rapid patient recovery. At the same time similar lymph node dissection between the two techniques were revealed. We can reasonably expect that the innovative robotic technique could represent a valid alternative with potential benefit to equal oncological adequacy with respect to OG.

Caruso S, Patrì A, Roviello F, De Franco L, Franceschini F, Ceccarelli G, Coratti A. Robot-assisted laparoscopic vs open gastrectomy for gastric cancer: Systematic review and meta-analysis. *World J Clin Oncol* 2017; 8(3): 273-284 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/273.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.273>

INTRODUCTION

Since its introduction by Kitano *et al*^[1] in 1994, laparoscopy has been increasingly used for the treatment of gastric cancer. During this period of time, a number of works have shown laparoscopic gastrectomy (LG) to be a feasible option in treating gastric cancer and level III studies provided the evidence that laparoscopic assisted distal

gastrectomy (LADG) leads to better results in the short-term than conventionally performed open gastrectomy (OG) for early gastric cancer^[2-5]. Due to the high incidence of gastric cancer and the extremely high levels of expertise achieved by Asian surgeons, LG is now a routine procedure for early gastric cancer in eastern states^[6]. On the other hand, laparoscopic surgery did not meet the same widespread requirements for the management of advanced gastric cancer, mainly due to the technical difficulties posed by the D2 lymphadenectomy and the intestinal reconstruction after total gastrectomy. Concerns regarding oncological adequacy, in particular for potential inadequate lymphadenectomy and long-term outcomes^[6,7], make LG for advanced gastric cancer still questionable. Therefore, a significant proportion of patients with advanced stage disease are still treated with OG, especially in Western countries. In an effort to overcome the technical disadvantages of laparoscopic technique, robotic surgery has been introduced and it has gradually spread worldwide. Robotic systems have three-dimensional (3D) high-resolution imaging, tremor filter, and internal articulated endoscopic wrist (EndoWrist™ System), which lead to significant improvements in visibility and manipulation with respect to conventional laparoscopy. With this advanced equipment, robot-assisted gastrectomy (RAG) has been advocated to give a global advantage over the traditional laparoscopic approach, particularly in performing the D2 lymphadenectomy and facilitating complex reconstruction after gastrectomy^[8-10]. A variety of reports have demonstrated the safety and feasibility of this technique^[6]. However, so far most of the reports derives from not large, retrospective, non-randomized studies.

In order to achieve a confirmed acceptance, an innovative technique with minimum invasiveness absolutely has to show that it is not disadvantageous to oncologic result. As LG still has not reached a comprehensive validation for the treatment of all (advanced) gastric cancer, the introduction of robotic surgery can represent a fair cue of advancement potentially able to make the laparoscopic technique more oncologically adequate, and so to increase its use as alternative procedure to the conventional open approach. Yet, to date a mere handful of trials have shown high quality comparative analysis of RAG vs OG in the treatment of gastric cancer, and most of these studies have been limited to small sample size and a single institution design. To overcome these limitations, we performed a systematic review and meta-analysis which can increase the statistical power of short-term results available so far on these two techniques. Thus, relevant trials comparing the safety and efficacy of RAG vs OG in treating gastric cancer were analyzed, to verify if at present there is actual evidence of an advantage to the introduction of this new minimally invasive technique with respect to the validated open procedure. Positive results could represent the preliminary impulse to potentiate the robotic tool which, by overcoming some intrinsic limits of the conventional laparoscopic method, might increase the use and acceptance of the minimally invasive procedure for gastric cancer in the future.

MATERIALS AND METHODS

Literature search

A comprehensive systematic literature search was conducted using PubMed, EMBASE, and the Cochrane Library to identify relevant articles comparing RAG vs OG for the treatment of gastric cancer published up to December 2016. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines were adopted for performing and reporting meta-analysis data^[11]. No restriction was set for type and date of publication, and for age and sex of participants. Article language was limited to English. The following terms were used for the search strategy: "Robot" or "robotic" or "robot-assisted" or "robotic-assisted" and "open" in combination with "gastrectomy" or "gastric resection" or "gastric cancer" or "stomach cancer" or "gastric carcinoma". Either free-text and medical subject heading (MeSH) searches were used for keywords. The search was further broadened by extensive cross-checking of all reference in the retrieved articles fulfilling the inclusion criteria in order to identify eventual additional non indexed literature. Discrepancies in the search were resolved by consensus discussion among the entire author group. All relevant texts, tables and figures were reviewed for data extraction.

Study selection

Two authors (FF and LDF) independently screened the primary data from the studies identified in the electronic search. The initial assessed data included authors, titles and abstract. Then, the following inclusion criteria were set for inclusion the studies in the meta-analysis: (1) trials comparing robotic and OG for gastric adenocarcinoma, independently from the type of gastrectomy (distal gastrectomy, proximal gastrectomy and total gastrectomy) and tumour stage (early or advanced gastric cancer); and (2) Studies reporting at least one of the perioperative outcome measures among the following: Operative blood loss, operative time, numbers of harvested lymph nodes, postoperative complication rate, postoperative mortality, and hospital stay (interval from operation to discharge).

The following exclusion criteria were set: (1) duplicate studies; (2) non-comparative studies; (3) if publications are reviews, conference abstracts, letters, comments or case reports; (4) non-relevant topic papers or when all the reported appropriate outcomes were not included; (5) studies where it was not possible to extract or calculate data of interest from the published results; and (6) if more than one study was reported by the same institute, the most recent work or that containing more complete data was selected.

Primary relevant data from the original included studies were independently extracted and summarized by the same two authors. In addition, in terms of postoperative complications, anastomotic leakage, bleeding, as well as wound infection were also analyzed when reported. Any

disagreement was resolved by consensus among the author group.

Quality assessment

The modified Newcastle-Ottawa Scale was used to assess the methodological quality of retrospective non randomized studies^[12]. The scale consists of eight multiple-choice questions assessed essentially on three major categories: Patient selection, comparability (of cases and controls in case-control studies, of cohorts in cohort studies), and the assessment of the outcome (in case-control studies) or exposure (in cohort studies)^[12]. The number of possible answers per question ranges from 2 to 5. High-quality responses earn a star, totaling up to nine stars (the comparability question earns up to two stars).

The quality of randomized clinical trials was assessed using Jadad's scoring system^[13]. The Jadad's scale, widely validated for reporting randomized controlled trials quality, assess a score (ranging 0 to 6) on the base of three major elements: randomization (0-2 points), blinding (0-2 points) and patients withdrawal (0-1 point).

Risk of bias

Assessment for potential publication bias was analysed through drawing of funnel plots which were inspected for asymmetry for all outcome measures and evaluated by the Begg's^[14] and Egger's tests^[15].

Statistical analysis

The statistical analysis was performed using Review Manager software version 5.3 (RevMan 5.3, Cochrane Collaboration, Oxford, United Kingdom). Weighted mean differences (WMD) and odds ratios (OR) were used as a summary measure of efficacy for continuous and dichotomous variables respectively. A 95%CI was reported.

Statistical heterogeneity among the studies was evaluated using the χ^2 test and according to the Higgins' I^2 statistic^[16]. I^2 values of 0-25%, 25%-50% and > 50% were considered as indicative of homogeneity, moderate heterogeneity and high heterogeneity, respectively^[17]. To estimate the pooled WMD or OR, the inverse variance method with fixed-effects model was applied when no or moderate heterogeneity was detected among studies ($I^2 < 50\%$) according to Mantel-Haenszel method^[18], whereas the random-effect model was used for analysis when I^2 was greater than 50% (DerSimonian and Laird method)^[19]. WMD was pooled by using the inverse variance model. The Z test was used to determine the pooled WMD or OR. Sensitivity analyses and funnel plots were assumed to investigate potential publication bias.

Funnel plot asymmetry, which reflects the presence of publication bias in the studies, was assessed using Begg's and Egger's tests. Begg and Mazumdar's rank correlation tests the rank correlation (Kendall's tau) between the standardized effect size and the variances (or standard errors) of these effects^[14]; the Egger's linear

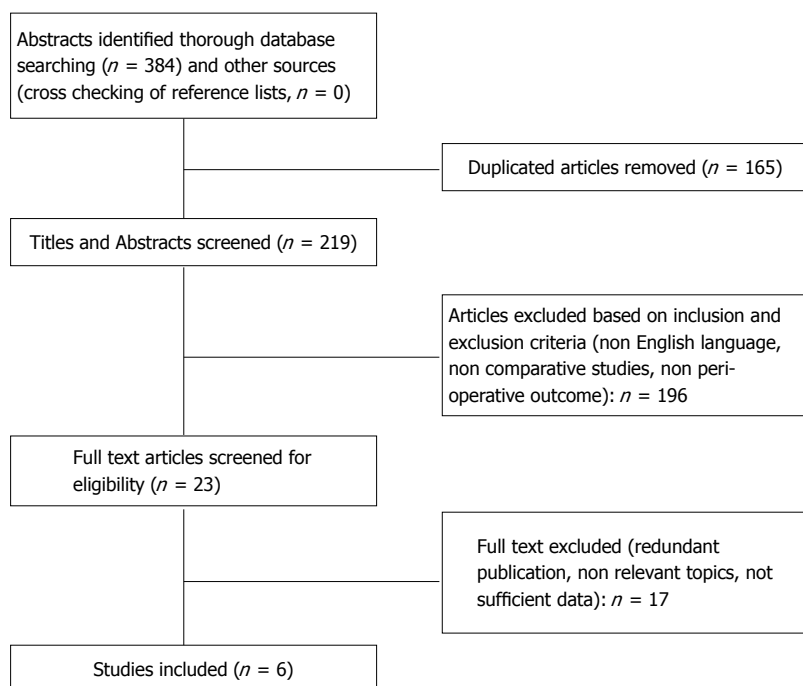


Figure 1 Flow chart of study selection.

regression method^[20] quantify the bias captured by the funnel plot. P value < 0.05 were considered to indicate statistical significance.

RESULTS

Study selection

The literature search yielded a total of 384 articles (Figure 1). After elimination of duplicates ($n = 165$), the remaining 219 titles and abstracts were reviewed. Based on the methodological inclusion and exclusion criteria, 196 studies were excluded: 115 did not compare techniques, 21 were non English studies, 60 were review articles, letters, case reports or comment. The full text of the remaining 23 articles were reviewed; of these, 1 was excluded because it was a redundant and lower level series, 11 contained non relevant topics, 5 because it was impossible to retrieve or calculate data of interest. Finally, a total of 6 articles^[21-26] (South Korea 2, China 2, Italy 1, Romania 1) were considered eligible for inclusion in the meta-analysis (Figure 1).

Only one of these studies was a randomized controlled trial^[26], while the others were retrospective non-randomized trials. The same two authors extracted the number and characteristics of patients of both the RAG and OG groups, which globally included 6.123 patients.

Huang *et al*^[23] did not provide in their original papers data regarding means and standard deviations of peri-operative outcome, which instead were expressed as medians and ranges. This additional initially unpublished information was retrieved from a previous meta-analysis^[27], in which the data was obtained by contacting the authors.

The baseline characteristic, quality assessment and main perioperative data of the included studies were listed in Tables 1 and 2.

Operative time

All included studies^[21-26] reported a significantly longer operation time of the RAG group than OG (Table 2). The meta-analysis of pooled data (Figure 2A) confirmed the result showing a significantly lower operative time in the group of OG compared to RAG group (WMD: 72.20 min, 95%CI: 48.82 to 105.13 min, $P < 0.001$). There was significant heterogeneity ($I^2 = 85\%$) (Figure 2A).

Estimated blood loss

All the included studies reported the mean intra-operative related to surgery estimated blood loss. A concordant result of statistical significantly lower blood loss volume in the RAG group than in the OG group (Table 2) was reported. The pool meta-analyzed data confirmed that blood loss was notably less in the RAG group as opposed to OG (WMD: -166.83 mL, 95%CI: -205.18 to -65.80 mL, $P < 0.001$) with a significant heterogeneity between studies ($I^2 = 82\%$) (Figure 2B).

Harvested lymph nodes

The mean number of harvested lymph nodes was reported in all studies (Table 2). The pooled data from the included studies showed that the two groups did not differ significantly in the number of harvested lymph nodes (WMD = -1.12; 95%CI: -2.31 to 0.58; $P = 0.10$), with low heterogeneity between studies ($I^2 = 25\%$) (Figure 2C).

Postoperative hospital stay: All the 6 included studies reported the length of hospital stay, showing in agreement a statistically significant reduction in favour of the RAG group compared to OG (Table 2). The meta-analysis of combined data confirmed the result, showing shorter postoperative hospital stay in the RAG group

Table 1 Baseline characteristics of include studies and quality assessment

Ref.	Year	Country	Type of study	Total patients (n)	Group	n	Sex (M/F)	P value	Age (mean ± SD)	P value	BMI (mean ± SD)	P value	Quality assessment
Kim <i>et al</i> ^[21]	2010	South Korea	Retrospective clinical trial	28	RAG	16	10/6	NS	53.8 ± 15.6	NS	21.3 ± 3.4	> 0.05	6 stars ¹
					OG	12	9/3		56.0 ± 12.4		25.2 ± 1.9		
Caruso <i>et al</i> ^[22]	2011	Italy	Retrospective clinical trial	149	RAG	29	18/11	NS	64.8 ± 12.4	NS	27 ± 3	NS	6 stars ¹
					OG	120	65/55		65.1 ± 11		28 ± 4		
Huang <i>et al</i> ^[23]	2012	China	Retrospective clinical trial	625	RAG	39	19/20	< 0.05	65.1 ± 15.9	NS	24.2 ± 3.7	NS	5 stars ¹
					OG	586	406/180		67.9 ± 30.1		23.7 ± 3.6		
Kim <i>et al</i> ^[24]	2012	South Korea	Retrospective clinical trial	4978	RAG	436	265/171	NS	54.2 ± 12.5	< 0.05	23.6 ± 3.1	NS	5 stars ¹
					OG	4542	3008/1534		57.7 ± 11.8		23.8 ± 8.0		
Procopiuc <i>et al</i> ^[25]	2015	Romania	Retrospective clinical trial	47	RAG	18	13/5	NS	59.1 ± 13.7	NS	26.0 ± 3.24	NS	6 Stars ¹
					OG	29	21/8		60.1 ± 12.4		24.8 ± 4.58		
Wang <i>et al</i> ^[26]	2016	China	Randomized clinical trial	296	RAG	151	109/42	NS	57.5 ± 12.7	NS	22.1 ± 2.9	NS	3 points ²
					OG	145	89/56		55.9 ± 13.1		21.3 ± 2.5		

¹According to the NOS (Newcastle-Ottawa Scale) classification; ²According to Jadad's scale for reporting randomized controlled trials. RAG: Robot-assisted gastrectomy; OG: Open gastrectomy; NS: Not statistically significant.

Table 2 Main perioperative data of the included studies

Ref.	Open conversion (%)	Group	Operation time (min ± SD) ¹	P value	Blood loss (mL ± SD) ¹	P value	Harvested nodes (n ± SD) ¹	P value	Morbidity (%)	P value	Mortality (%)	P value	Hospital stay (d ± SD) ¹	P value
Kim <i>et al</i> ^[21]	0	RAG	259.2 ± 38.9	< 0.05	30.3 ± 15.1	< 0.05	41.1 ± 10.9	NS	0	NS	0	NS	5.1 ± 0.3	< 0.05
		OG	126.7 ± 24.1		78.8 ± 74.1		43.3 ± 10.4		20		0		6.7 ± 1.4	
Caruso <i>et al</i> ^[22]	0	RAG	290 ± 67	< 0.05	197.6 ± 202.1	< 0.05	28.0 ± 11.2	NS	10.3 ²	NS	0	NS	9.6 ± 2.8	< 0.05
		OG	222 ± 94		386.1 ± 95.5		31.7 ± 15.6		10.0 ²		3.3		13.4 ± 8.5	
Huang <i>et al</i> ^[23]	NR	RAG	415.9 ± 101.2	< 0.05	93.9 ± 89	< 0.05	32 ± 13.7	NS	15.4	NS	1.4	NS	11.3 ± 14.4	< 0.05
		OG	331.8 ± 92.9		192 ± 193		34 ± 14.8		14.7		2.6		16.5 ± 13.6	
Kim <i>et al</i> ^[24]	NR	RAG	226 ± 54	< 0.05	85 ± 160	< 0.05	40.2 ± 15.5	NS	10.1	NS	0.5	NS	7.5	< 0.05
		OG	158 ± 52		192 ± 193		40.5 ± 16.6		10.7		0.5		10.2	
Procopiuc <i>et al</i> ^[25]	0	RAG	320.8 ± 85.1	< 0.05	208.2 ± 139.8	< 0.05	22.0 ± 8.9	NS	11.1 ²	NS	0	NS	8.1 ± 2.0	< 0.05
		OG	243.3 ± 57.9		564.6 ± 468.4		25.2 ± 9.0		20.7 ²		0		11.4 ± 2.9	
Wang <i>et al</i> ^[26]	1.9 ³	RAG	242.7 ± 43.8	< 0.05	94.2 ± 51.5	< 0.05	29.1 ± 6.7	NS	9.3	NS	0	NS	5.7 ± 2.3	< 0.05
		OG	192.4 ± 31.5		152.8 ± 94.2		30.1 ± 7.2		10.3		0		6.4 ± 2.5	

¹Mean value; ²Major complications rate base on Clavien-Dindo classification ≥ 3 , such as anastomotic and duodenal leakage; ³Rate of patients excluded from the study analysis. RAG: Robot-assisted gastrectomy; OG: Open gastrectomy; NS: Not statistically significant difference.

compared to OG (Figure 2D). The robotic approach reduced the postoperative stay by a mean of 1.97 d (WMD = -1.97; 95%CI: -2.47 to -1.18 d; $P < 0.001$). Although there was a significant heterogeneity among the studies ($I^2 = 55\%$) (Figure 2D).

Postoperative complications: Short-term postoperative complications were recorded in all analyzed studies. The meta-analysis did not significantly differ in the overall postoperative complication rate of the two groups (OR = 0.95, 95%CI: 0.60-1.34, $P = 0.65$) with low heterogeneity ($I^2 = 12\%$) (Figure 3A).

Five out of 6 studies^[22-26] reported the incidence by group of the following subtype of early postoperative complications: wound infection, bleeding and anastomotic leakage. The meta-analysis of pooled data regarding these complications showed no difference between the two groups (respectively: Wound infection, OR = 1.48, 95%CI: 0.86-3.12, $P = 0.35$, $I^2 = 10\%$; bleeding, OR = 1.10, 95%CI: 0.40-4.49, $P = 0.65$, $I^2 = 0\%$; anastomotic

leakage OR = 1.74, 95%CI: 0.99-3.05, $P = 0.06$, $I^2 = 0\%$) (Figure 3B-D).

Three studies out of 6^[22-24] reported postoperative mortality rate value ranging from 0.5% to 3.3%, without statistically significant differences between the robotic and open procedures, while the rest of the studies^[21,25,26] did not detect any case of mortality related to both surgical techniques (Table 2). A meta-analysis of pooled data was therefore considered unnecessary, as 50% of studies did not report any event of mortality in both groups and the data are insufficient to calculate an objective OR, thus the combined data reflected the evident equality of mortality rates among RAG and OG groups.

Publication bias

A standard-error based funnel plot using fix effect size between RAG and OG was constructed for morbidity (Figure 4). The overall postoperative complication rate of all the studies lay within the limits of 95%CIs with just a slight asymmetry, indicating no serious publication

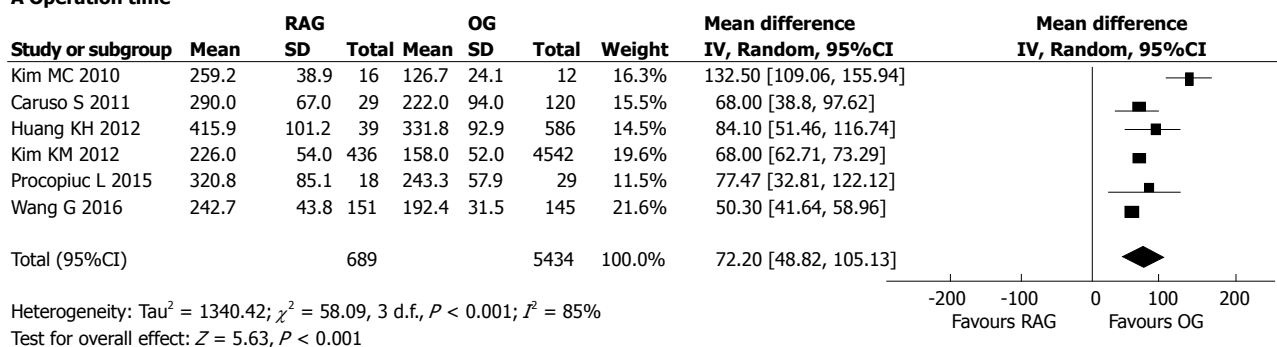
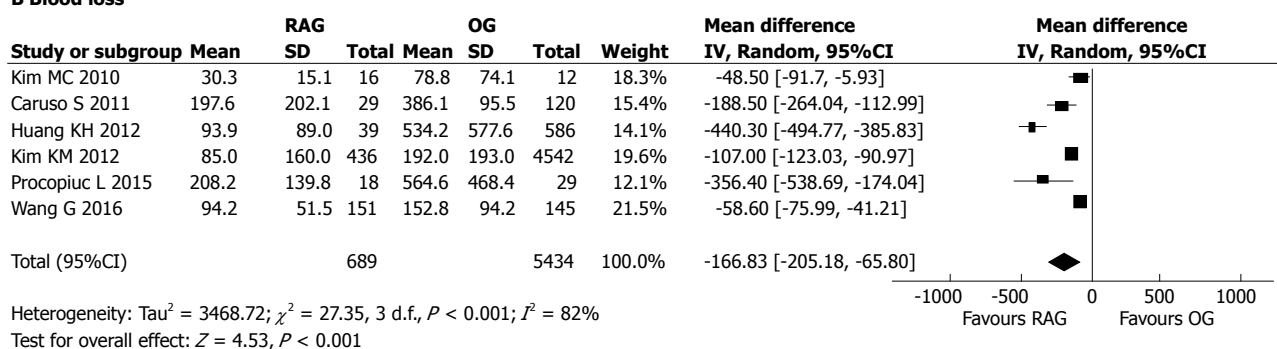
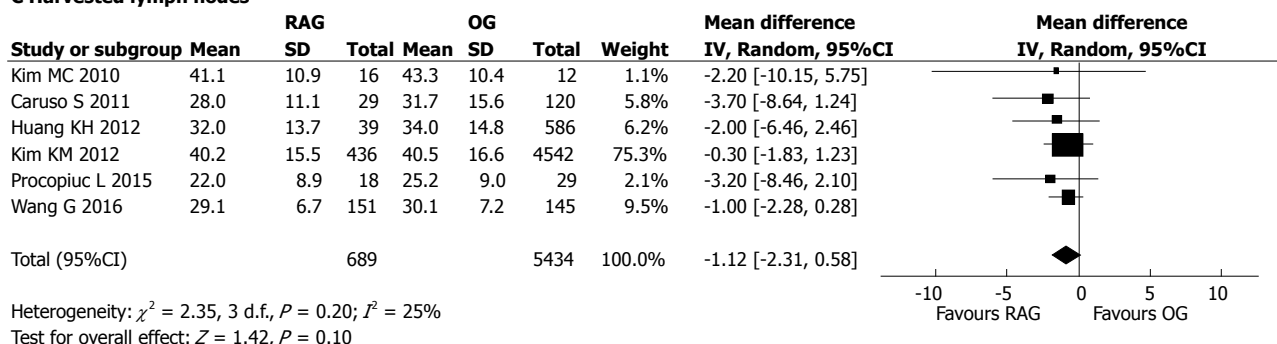
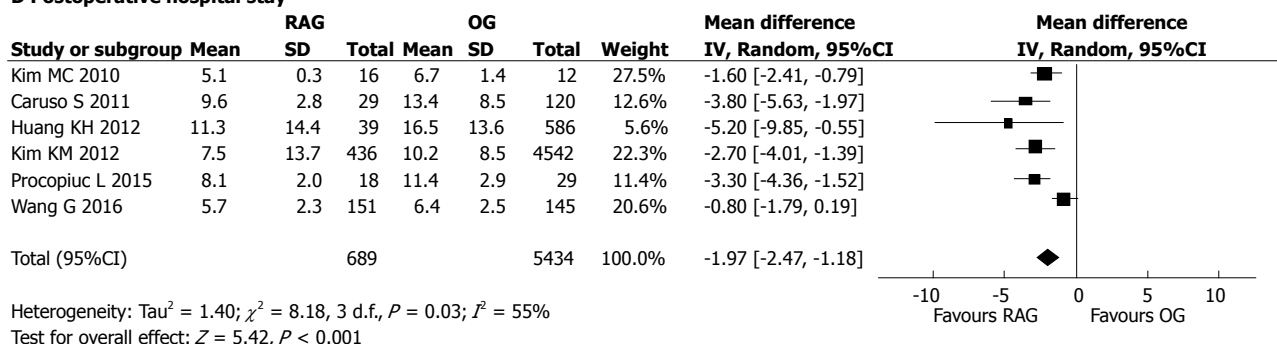
A Operation time**B Blood loss****C Harvested lymph nodes****D Postoperative hospital stay**

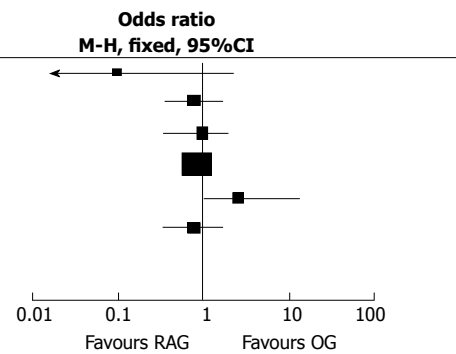
Figure 2 Forest plot showing the meta-analysis of pooled data on robot-assisted gastrectomy vs open gastrectomy. A: Operation time; B: Estimated blood loss; C: Harvested lymph nodes; D: Postoperative hospital stay. RAG: Robot-assisted gastrectomy; OG: Open gastrectomy.

biases. No evidence of publication bias was revealed among the studies from statistical tests for any primary

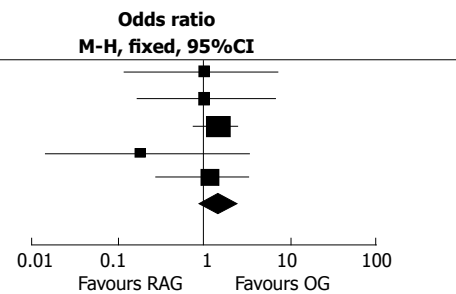
outcomes (Begg's test all $P > 0.10$; Egger's test all $P > 0.10$).

A Overall postoperative complication rate

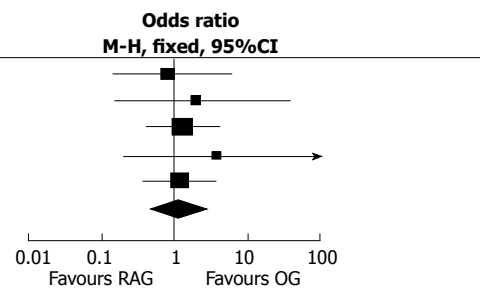
Study or subgroup	Events	RAG		OG		Weight	Odds ratio M-H, fixed, 95%CI
		Total	Events	Total	Events		
Kim MC 2010	0	16	2	12	12	4.1%	0.13 [0.01, 2.92]
Caruso S 2011	12	29	51	120	120	9.1%	0.96 [0.42, 2.17]
Huang KH 2012	6	39	86	586	586	7.5%	1.06 [0.43, 2.60]
Kim KM 2012	44	436	487	4542	4542	60.5%	0.93 [0.67, 1.29]
Procopiuc L 2015	11	18	9	29	29	7.8%	3.49 [1.02, 11.97]
Wang G 2016	15	151	14	145	145	11.0%	0.97 [0.45, 2.08]
Total (95%CI)		689		5434		100.0%	0.95 [0.60, 1.34]
Total events	88		649				
Heterogeneity: $\chi^2 = 2.58$, 3 d.f., $P = 0.72$; $I^2 = 12\%$							
Test for overall effect: $Z = 0.44$, $P = 0.65$							

**B Wound infection**

Study or subgroup	Events	RAG		OG		Weight	Odds ratio M-H, fixed, 95%CI
		Total	Events	Total	Events		
Caruso S 2011	1	29	4	120	120	14.2%	1.04 [0.11, 9.63]
Huang KH 2012	1	39	14	586	586	15.4%	1.08 [0.14, 8.40]
Kim KM 2012	14	436	93	4542	4542	39.6%	1.59 [0.90, 2.81]
Procopiuc L 2015	2	18	1	29	29	10.1%	0.28 [0.02, 3.40]
Wang G 2016	3	151	4	145	145	20.7%	1.29 [0.28, 5.85]
Total (95%CI)		673		5422		100.0%	1.48 [0.86, 3.12]
Total events	21		116				
Heterogeneity: $\chi^2 = 2.70$, 2 d.f., $P = 0.52$; $I^2 = 10\%$							
Test for overall effect: $Z = 0.88$, $P = 0.35$							

**C Bleeding**

Study or subgroup	Events	RAG		OG		Weight	Odds ratio M-H, fixed, 95%CI
		Total	Events	Total	Events		
Caruso S 2011	1	29	5	120	120	22.3%	0.82 [0.09, 7.31]
Huang KH 2012	0	39	3	586	586	7.4%	2.11 [0.11, 41.57]
Kim KM 2012	2	436	16	4542	4542	32.8%	1.30 [0.30, 5.69]
Procopiuc L 2015	1	18	0	29	29	10.3%	5.06 [0.20, 131.05]
Wang G 2016	1	151	1	145	145	27.2%	1.29 [0.28, 5.85]
Total (95%CI)		673		5422		100.0%	1.10 [0.40, 4.49]
Total events	5		25				
Heterogeneity: $\chi^2 = 0.73$, 2 d.f., $P = 0.86$; $I^2 = 0\%$							
Test for overall effect: $Z = 0.35$, $P = 0.65$							

**D Anastomotic leakage**

Study or subgroup	Events	RAG		OG		Weight	Odds ratio M-H, fixed, 95%CI
		Total	Events	Total	Events		
Caruso S 2011	1	29	7	120	120	13.3%	0.58 [0.07, 4.88]
Huang KH 2012	3	39	27	586	586	15.4%	1.73 [0.50, 5.96]
Kim KM 2012	10	436	51	4542	4542	46.5%	2.07 [1.04, 4.10]
Procopiuc L 2015	2	18	1	29	29	6.3%	3.50 [0.29, 41.70]
Wang G 2016	4	151	3	145	145	18.5%	0.71 [0.16, 3.25]
Total (95%CI)		673		5422		100.0%	1.74 [0.99, 3.05]
Total events	20		89				
Heterogeneity: $\chi^2 = 1.58$, 2 d.f., $P = 0.68$; $I^2 = 0\%$							
Test for overall effect: $Z = 1.95$, $P = 0.06$							

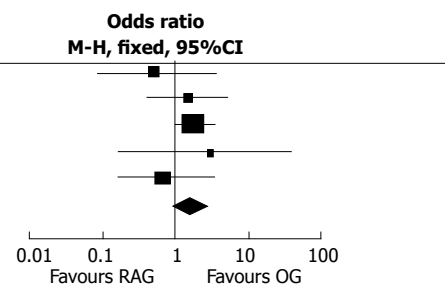


Figure 3 Forest plot showing the meta-analysis of postoperative complications between robot-assisted gastrectomy and open gastrectomy. A: Overall postoperative complications; B: Wound infection; C: Bleeding; D: Anastomotic leakage. RAG: Robot-assisted gastrectomy; OG: Open gastrectomy.

Sensitivity analysis: Sensitivity analysis was performed by excluding the study with the lowest quality score and the smallest sample size^[21]. All variables were conducted for sensitivity analysis. The results were not affected by sensitivity analysis as shown in Table 3.

DISCUSSION

Procedures which offer minimum invasiveness would

present a perfectly acceptable alternative to open surgery, with better short-term results, if it were possible to respect oncologic criteria to the same degree as the open approach, and if there were no compromising effect on long-term survival. Even though long-term survival is one of the major oncologically prominent issue, lymph node metastasis has long been seen as the element which most significantly predicts recurrence and therefore survival in patients suffering from gastric

Table 3 Sensitivity analysis of outcomes

Outcomes	No. of studies	Patients		WMD/OR	Analysis model	95%CI	P value	Heterogeneity	
		RAG	OG					I ² (%)	P value
Operative time (min)	5 ^[22-26]	673	5422	60.12	Random	41.31, 98.06	< 0.00001	80	0.41
Estimated blood loss (mL)	5 ^[22-26]	673	5422	-193.78	Random	-215.77, -72.13	< 0.0001	72	0.007
Harvested lymph nodes	5 ^[22-26]	673	5422	-1.05	Random	-2.01, 0.39	0.35	0	0.12
Overall postoperative complication	5 ^[22-26]	673	5422	0.92	Fixed	0.61, 1.36	0.6	12	0.72
Postoperative hospital stay	5 ^[22-26]	673	5422	-2.57	135.8 ± 133.9	-2.68, -1.56	< 0.001	0	0.54

RAG: Robot-assisted gastrectomy; OG: Open gastrectomy; WMD: Weighted mean difference; OR: Odds ratio.

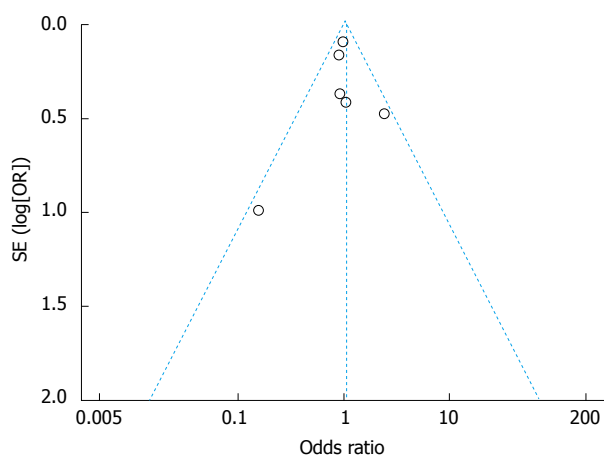


Figure 4 Funnel plot for results from each study comparing overall morbidity between robot-assisted gastrectomy and open gastrectomy. OR: Odds ratio; SE: Standard error.

cancer^[28]. Thus, the amount of harvested lymph nodes is an accurate reflection of whether gastric resection for adenocarcinoma is an adequate option, and can be used as indicator of oncological adequacy when no long follow-up times are available.

Total and distal gastrectomy with D2 lymphadenectomy node is the recommended surgical procedure for most resectable gastric cancer patients^[29]. LG with lymph node dissection has developed as a minimally invasive surgery for gastric cancer over the last two decades and it has been utilized principally for early gastric cancer. Some randomized studies and meta-analysis showed that LG with limited lymph node dissection for patients with early-stage gastric lesion provided oncologic results which were not inferior compared to OG, with however improved short-term outcomes^[2-5].

In contrast, a handful of trials, which all contained not large cohort of patients, outline the safety of laparoscopic assisted distal and total gastrectomy with D2 lymph node dissection in advanced-stage of gastric cancer. Several meta-analysis regarding this issue have been recently published. However, the outcomes were contradictory, especially regarding postoperative complications and the amount of harvested lymph nodes^[30-32].

Thus, although LADG has been widely developed for early gastric cancer, the global effectiveness in therapeutic terms of LG still has not been extensively

looked into with regards to the treatment of advanced-stage of gastric cancer. Although a totally LG with extended D2 lymphadenectomy has been demonstrated to be feasible by several authors^[33-36], owing to the intrinsic difficulty of execution, oncologic concerns still exist regarding the possibility of performing a D2 lymphadenectomy radically and suitably. Indeed, the meta-analysis of the randomized controlled trials (RCTs) demonstrates that whenever results on LADG is gathered from advanced gastric cancers together with the early stage the same extent of lymph node dissection as in traditional surgery could not be guaranteed^[37,38].

Although laparoscopic sub-D2 lymphadenectomy may be seen as suitable for nearly all early gastric cancer in which lymph node metastases rarely occur (2%-20% of cases)^[37], and so far is routine in Asia^[39], the same cannot be said about advanced gastric cancer and so LG cannot be advised as a standard approach for all patients with gastric cancer.

With the development of technology, the introduction of a robotic tool as a useful adjunctive method to assist laparoscopy has gradually increased the use of minimally invasive procedures in several fields of surgery. For the treatment of gastric cancer, RAG has been widely demonstrated to be feasible and safe in many studies^[8,40-49]. Robotic surgery is progressively becoming an attractive option for surgeons, in particular because it may overcome some intrinsic limitations of conventional laparoscopy, in particular for the D2 lymphadenectomy, expanding the application of minimally invasive procedures. In fact, this technique has certain indisputable advantages, such as high definition 3D imaging, improved dexterity enabled by the endowristed movements, tremors filtration, motion scaling, stereoscopic visualization, which are particular useful when precise dissection is needed, such as during the lymphadenectomy along major abdominal vessels (gastric, gastroepiploic, common hepatic, and celiac artery lymph nodes). Thus, as long as drawbacks of the LG technique exist, the introduction of new innovative technologies, such as robotic gastrectomy, are desirable. In fact, the median number of retrieved nodes, reported by many authors through the use of robotic system for D2 lymphadenectomy, is not dissimilar to that of traditional open technique, and in several instances even superior to laparoscopy^[27,40,41,50-57].

However, significant limitations exist in the inter-

pretation of data available so far regarding the comparison of RAG with respect to OG, as a result of the shortage of randomized trials, the restricted amount of observational and comparative studies of high quality, the small sample sizes so far, and the shortened length of follow-up. Therefore, there has been difficulty in drawing final conclusions regarding the superiority of one approach over another.

A meta-analysis is a suitable way to widen the source of evidence. Evaluating pooled data among the most relevant studies is a quantitative method that may increase the statistical power of otherwise poorly consistent results and may resolve some controversy of evidence.

Robotic surgery is a technical innovation which improves the effectiveness of laparoscopic technique, which is used through the same laparoscopic way as a non independent adjunctive tool. Thus, we strictly limit the research by focusing exclusively on RAG with the intent to evaluate the real merit of the addition of robotic assistance to laparoscopy over the traditional OG for gastric cancer, performing a comprehensive systematic review and meta-analysis. Such a way of conducting the trial will provide a more objective appraisal of the effectiveness of RAG in gastric cancer patients, in order to confirm the single-institute promising results in favour of this innovative technique to date reported. This could represent the preliminary cue in support of the increasingly widespread view which considers robotics to be a completion of laparoscopy, making it possible to fill the existing performance gap with respect to OG.

Six studies, of which 5 retrospective clinical trials and 1 RCT, involving 6123 patients with 689 (11.3%) cases of RAG and 5434 (88.7%) of OG, were considered eligible for inclusion in this meta-analysis.

The results show globally that RAG provided short-term results which can be compared to OG, with outcomes which can be considered as satisfactory with regards to perioperative results and oncological effectiveness.

The operation time was significantly longer with RAG than OG ($P < 0.001$). The greater length of robotic surgery is principally due to the additional time for set-up and docking of the robotic system^[58]. Nevertheless, it should be noted that the time of operation notably diminished as surgical experience increased and the robotic procedure was standardized^[8,47,59].

An advantageous lower blood loss and shorter hospital stay were revealed in favor of RAG, that can be principally due to globally lesser surgical damage than OG. The robotic system enables a meticulous and precise dissection in a magnified vision, which minimizes the risk of bleeding. Moreover, the technical advancement of the robotic device, which is provided by a high definition 3D stereoscopic vision, enabling a better detection of vascular structures and allowing to easier inspect the bleeding occurring intra abdominally with tremor filtration and stable haemostatic strain provided with the robotic instrument.

No statistical difference was observed between RAG

and OG in terms of postoperative complication rate ($P = 0.65$), and also specifically referring to subcategories of complications, such as wound infection, bleeding, anastomotic leakage. In particular regarding the most feared adverse event after gastric cancer, the rate of anastomotic leakage is comparable to that reported by previous studies^[60,61], ranging from 1% to 10%, and the rate among pooled data was 2.97% (20/673) for RAG and 1.64% (89/5422) for OG ($P = 0.06$).

Analysis of the pooled data revealed that the number of harvested lymph nodes was similar between RAG and OG. The feeling is that the technically advantageous properties of robotic surgery can easily and safely execute an effective, and oncologically adequate lymphadenectomy^[62,63]. In particular, the meticulous dissection, together with the high 3D definition image and dexterity provided by the robotic system, seems to make the lymph node dissection safely feasible in difficult lymphatic stations around major vessels or in difficult area^[6], with less blood loss^[6,38].

The main limitation of this meta-analysis is that it does not resolve certain heterogeneity of the included studies, such as in terms of baseline characteristics of patients, type of gastrectomy, stage of disease, details of surgery, difference in reporting perioperative outcomes. For example, in the study of Kim *et al.*^[21] the body mass index (BMI) of the RAG group was significantly lower than that of the open ($P = 0.0004$). Huang *et al.*^[23] included patients in the robotic group which were associated with female predominance and were reconstructed mainly by Roux-en-Y anastomosis. In the study of Kim *et al.*^[24], the patients of RAG group were significantly younger than OG. Kim *et al.*^[24] and Huang *et al.*^[23] reported in their series a significantly higher proportion ($P < 0.001$) of advanced gastric cancers in the OG gastrectomy group with respect to the RAG group, that would suggest a corresponding higher number of lymph nodes retrieved in advanced stages than in early stages. Effectively, that reflects a trend of a higher amount of lymph nodes dissected with the open procedure than with the robotic technique, both in the single institute reports and in the pooling data meta-analysis, however this difference did not reach a statistical significance. Globally, this result suggests that RAG, even if applied in a greater proportion of early gastric cancer than OG, guarantees an adequate removal of lymph nodes, similar to that of OG in a larger amount of advanced gastric cancer. Since it was difficult to match baseline characters in all selected studies, the meta-analytic method planned the use of a random effected model to evaluate these parameters. However, high heterogeneity still existed in terms of operation time, blood loss and postoperative hospital stay, which the meta-analysis cannot completely resolve.

However, the meta-analytic method can represent a valid preliminary analysis of the global framework of these data, eventually susceptible to a sub-set analysis of more homogeneous groups. Two previous meta-analysis^[27,57], comparing RAG with conventional laparoscopy and OG, conducted a subgroup analysis

matched for some of these parameters, such as the extent of lymphadenectomy, type of gastrectomy (total or subtotal), and blood loss. However, the final results were substantially equal to the pooled data here presented in our meta-analysis. Moreover, although sensitivity analysis using matched data should reduce some of these potential bias, it cannot eliminate all of them and essentially it was impossible to match patient characteristics in all studies. For example, robotic procedures included the initial learning period, which may have resulted in an unequal surgical quality comparison. Moreover, most of the studies had small sample sizes with fewer than 50 RAG procedures and one single high-volume centre (Kim *et al.*^[24]) contributed more than half of the total number of RAG; this uneven distribution in the number of patients contributed to heterogeneity.

An advantage of our meta-analysis with respect to previous ones is that it included, even if only one, RCT and presently it is the most up to date work with the largest sample size comparing RAG and OG.

In conclusion, RAG seems to offer a viable option to OG in treating gastric cancer patients. It allows the reduction of the estimated blood loss and the length of postoperative stay with respect to OG with, at the same time, a comparable oncologically adequate lymphadenectomy. The longer operative time did not seem to affect the patient's recovery, with equal postoperative complications rate, risk of bleeding, wound infection and anastomotic leakage compared to open procedure.

Moreover, by overcoming some of the intrinsic limits of conventional laparoscopy, robotic gastrectomy probably represents the most promising technological innovation able to fill the gap still existing between laparoscopy and traditional open approach, particularly in the performance of D2 lymphadenectomy.

That could make LG when assisted with the robotic tool more oncologically adequate and then more widespread, so as to maintain and expand the well-known advantages of a minimally invasive surgery with respect to the open procedure.

Future research should be directed towards comparing RAG to OG, to delineating significantly quantifiable advantages between the two techniques, also in terms of cost analysis, especially in well-designed prospective randomized controlled trials. Finally, as a result of a lacking adequate follow-up and a small amount of high quality studies, it is too soon to formulate certain conclusive opinions.

COMMENTS

Background

Robot-assisted gastrectomy (RAG) is an innovative technique which improves the effectiveness of traditional laparoscopy, making it possible to overcome some of its typical limits. Several reports have demonstrated that this new procedure is technically feasible and safe, but no consensus is available in literature yet about the potential benefit of this technique with respect to the traditional open procedure.

Research frontiers

Minimally invasive surgery has progressively improved and spread, because it

offers a number of patient benefits compared to open surgery. Future research will be directed towards innovative techniques which could further minimize the surgical invasiveness for patients, so as to improve postoperative outcomes. From this point of view, RAG appears to be a promising advancement of minimally invasive surgery, and will probably continue to be increasingly used in the treatment of gastric cancer.

Innovations and breakthroughs

Here the authors presented the meta-analysis of pooled data originating from the systematic review of relevant studies which compared short-term outcomes between RAG and open gastrectomy. Presently, this is the most up to date and largest clinical work comparing the effectiveness of these two techniques, and the only one that included a randomized controlled trial.

Applications

The present work elucidates the current scientific evidence concerning the hypothesized beneficial application of RAG in gastric cancer patients.

Peer-review

This paper is a meta-analysis of 6 reports comparing the outcomes of robot-assisted laparoscopic gastrectomy for early gastric cancer with open gastrectomy, with favourable results for the former group. The information is important and needs to be made known. It is well written.

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Bilateral diffuse grade 5 radiation pneumonitis after intensity modulated radiation therapy for localized lung cancer

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Abstract

We are reporting a case of fatal radiation pneumonitis that developed six months following chemoradiation for limited stage small cell lung cancer. The patient was a 67-year-old man with a past medical history of Hashimoto's thyroiditis and remote suspicion for CREST, neither of which were active in the years leading up to treatment. He received 6600 cGy delivered in 200 cGy daily fractions *via* intensity modulated radiation therapy with concurrent cisplatin/etoposide followed by additional chemotherapy with dose-reduced cisplatin/etoposide and carboplatin/etoposide and then received prophylactic cranial irradiation. The subsequent months were notable for progressively worsening episodes of respiratory compromise despite administration of prolonged steroids and he ultimately expired. Imaging demonstrated bilateral interstitial and airspace opacities. Autopsy findings were consistent with pneumonitis secondary to chemoradiation as well as lymphangitic spread of small cell carcinoma. The process was diffuse bilaterally although his radiation was delivered focally to the right lung and mediastinum.

Key words: Radiation; Pneumonitis; Small cell lung cancer; Intensity modulated radiation therapy

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Core tip: Radiation pneumonitis is an uncommon but serious complication from radiation therapy which can on rare occasions be fatal. This report not only documents the details of such a case but also includes pathologic confirmation and computed tomography images. Although the radiation field was limited to the right lung and mediastinum, the process was also noted to be bilateral and diffuse.

Osborn VW, Leaf A, Lee A, Garay E, Safdieh J, Schwartz D,

Schreiber D. Bilateral diffuse grade 5 radiation pneumonitis after intensity modulated radiation therapy for localized lung cancer. *World J Clin Oncol* 2017; 8(3): 285-288. Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/285.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.285>

INTRODUCTION

Pneumonitis is an inflammatory lung reaction marked by dyspnea, cough, and occasional fever. It can occur following radiation therapy as a result of cytokine production^[1,2], and patients are at increased risk of developing pneumonitis if they have a history of chronic lung disease or smoking^[3], or if they received concurrent chemotherapy^[3,4]. Rarely, it can be fatal. In the following case report we examine a patient who developed fatal pneumonitis six months after receiving concurrent chemoradiation for small cell lung cancer (SCLC).

CASE REPORT

A 67-year-old man with a 40 pack-year smoking history initially presented with chills and a productive cough and was given antibiotics for presumed pneumonia. When his condition did not improve, a computed tomography (CT) of the chest was performed and revealed a large right hilar mass with extensive mediastinal adenopathy as well as surrounding infiltrate and atelectasis. Bronchial brushings and a right hilar node FNA were consistent with SCLC. The remainder of the workup, including brain magnetic resonance imaging (MRI), bone scan and positron emission tomography (PET)-CT, was negative for distant metastatic disease, establishing a diagnosis of limited stage (LS) disease. His medical history was significant for numerous coexisting medical conditions including a remote history of suspected but unconfirmed connective tissue disorder (CREST), colitis, esophagitis, duodenitis, livedo reticularis, Hashimoto's thyroiditis, multinodular goiter, arthritis, glaucoma, hypertension, multifocal motor neuropathy and atrioventricular (AV) nodal reentry tract for which he had undergone AV nodal ablation. Of note, neither the Hashimoto's nor CREST were active for multiple years leading up to his diagnosis of SCLC. The latter diagnosis had been suspected by the Rheumatology Service but after a negative workup, he was discharged from their clinic.

After completion of staging, he was advised to undergo definitive chemoradiation. He was also advised to re-establish follow up with the Rheumatology Service, but declined. After a detailed discussion of the potential for increased risk of complications from radiation with an underlying connective tissue disorder, he elected to proceed. He was treated with intensity modulated radiation therapy to the right lung and mediastinum in 33 daily fractions of 200 cGy to a total dose of 6600 cGy with two cycles of concurrent cisplatin (cis) and etoposide. After 3000 cGy, another CT was performed to

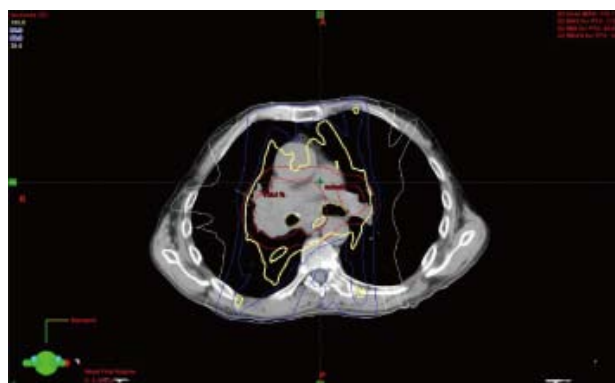


Figure 1 Intensity modulated radiation therapy radiation plan. The yellow line represents the 100% isodose line, blue lines represent the 90% and 50% isodose lines, and the white line represents the 20% isodose line. The red lines represent the gross tumor and planning treatment volumes (GTV and PTV).

allow for decrease in treatment field after initial response. RT was completed in 8 wk and 1 d. A representative image from his intensity modulated radiation therapy (IMRT) radiation plan is presented in (Figure 1). His treatment course was complicated by pancytopenia (for which he received filgrastim and one unit of packed red blood cells), as well as dysphagia and odynophagia. He received two cycles of chemotherapy during the radiation and two cycles in the adjuvant setting after concurrent chemotherapy and radiation therapy, though the last three cycles were dose-reduced because of hematologic toxicities. During chemotherapy he was treated for clostridium difficile colitis and was briefly admitted for generalized weakness. Approximately three months after completion of thoracic RT, he received prophylactic cranial irradiation (PCI) which was given as 10 fractions of 250 cGy.

During PCI, he required admission due to inability to tolerate daily travel. Shortly after completion of PCI he developed recurrent clostridium difficile colitis and within weeks of completion of PCI he was readmitted and remained hospitalized for two months. While admitted, he experienced episodes of hypoxemic respiratory failure requiring repeated use of a nonrebreather and for which he underwent intubation twice. Chest imaging demonstrated development of worsening bilateral interstitial and airspace opacities (Figure 2). He was aggressively treated with broad spectrum antibiotics and high dose steroids. Eventually he developed tachycardia, respiratory distress, hypotension and suspected disseminated intravascular coagulation. In accordance with his family's wishes he underwent palliative extubation and expired shortly thereafter.

An autopsy was performed and the report described extensive, diffuse, bilateral alveolar damage consistent with post-radiation changes, as well as small cell carcinoma in multiple foci within septal capillaries and contiguous alveolar spaces.

DISCUSSION

Radiation pneumonitis is an uncommon complication of

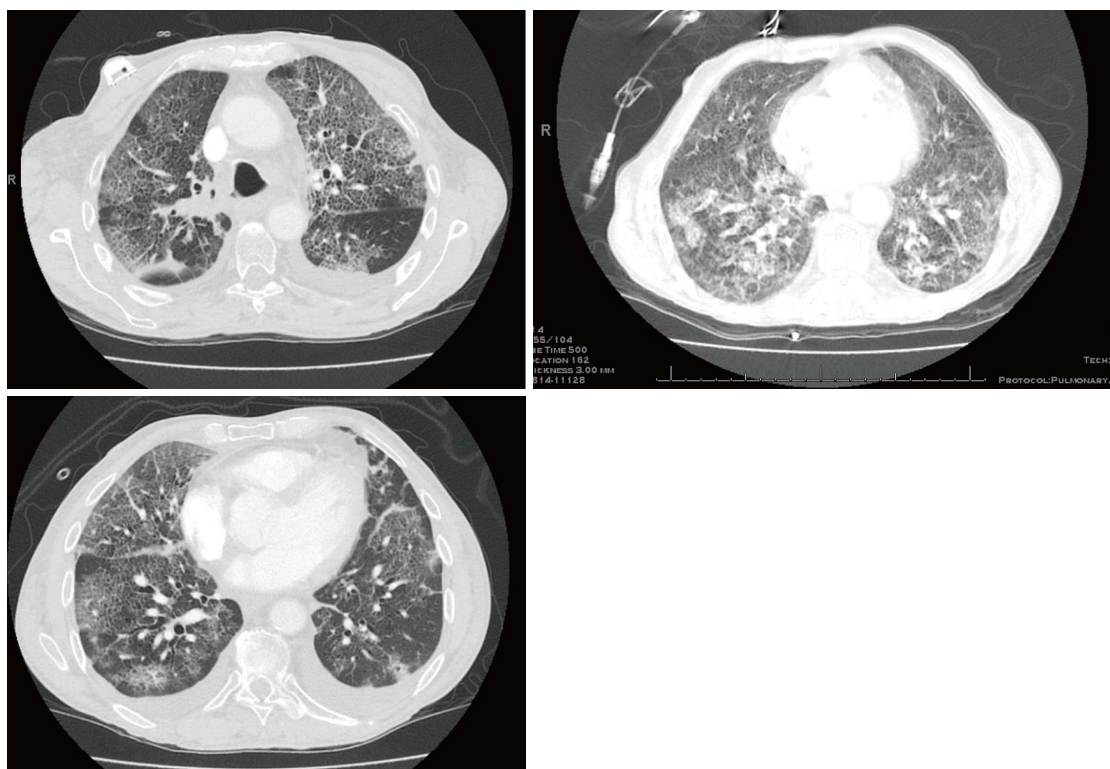


Figure 2 Chest computed tomography scan images demonstrating bilateral interstitial and airspace opacities.

chemoradiation for lung cancer but one which can be fatal in almost 2% of patients^[4]. It has been previously been described as having two types of presentations: "Classical" vs "sporadic". The former is attributed to local cytokine production within the radiated field, while the latter is likened to a hypersensitivity reaction and can be out of proportion to volume irradiated or manifest its effects outside of the treated field. It has even been proposed that the majority of patients develop subclinical lymphocytic alveolitis following lung radiation, but that acute pneumonitis only develops in the fraction that have some genetic or environmental predisposition^[5]. Our literature search did not reveal any specific associations between connective tissue disorders and pneumonitis, however in the event that our patient did have a true diagnosis of a connective tissue disorder, one could postulate that it could have served as such a predisposing factor for him.

Although certain radiation dose parameters have also been found to be associated with increased risk for radiation pneumonitis, including mean lung dose (MLD), volume of lung receiving 20 Gy (V20) and possibly 5 Gy (V5), this patient's parameters were within recommendations. His MLD was 1822 cGy, V20 28%, and V5 69.5%. Qualitative Analysis of Normal Tissue Effects in the Clinic (QUANTEC) guidelines, as well as others, indicate that mean lung dose of 13 Gy results in a 10% rate of symptomatic pneumonitis, MLD of 20 Gy results in 20% risk, and V20 of $\leq 30\%$ -31% keeps the risk below 20%^[6,7]. The current Radiation Therapy Oncology Group protocols recommend V20 not to exceed 40% and MLD of no more than 20 Gy^[8]. Not only did our

patient's plan meet all of the recommended criteria, it was essentially unilateral, targeted at the right hilar mass and mediastinum. His presentation is therefore more consistent with the development of "sporadic" radiation pneumonitis, given that his ultimate condition was spatially diffuse and out of proportion to what would have been expected from the doses received by his normal tissues.

Further complicating this patient's condition was the presence of lymphangitic spread of tumor which may have contributed to compromise of the patient's lung function. Additionally, he had a history of both a possible CREST and autoimmune disease (Hashimoto's Thyroiditis). Connective tissue disorders have been described as potential predisposing factors for increased toxicity from radiation therapy, and the mechanism of sporadic radiation pneumonitis itself is in some ways analogous to an autoimmune reaction with cytokine-mediated destruction^[9]. However in this case the autoimmune diseases had not been active for years and the collagen vascular disease, though suspected, had not been officially diagnosed, so it is difficult to evaluate whether the patient's toxicity could be attributed to these medical issues.

This case is notable for striking imaging findings of diffuse interstitial and alveolar processes (Figure 2) as well as pathologic confirmation of diagnosis of a rare complication from radiation for lung cancer. Limitations are akin to those of any case report, in that it is anecdotal. The patient had multiple processes occurring in the lungs as determined by autopsy, including lymphangitic spread of tumor as well as pneumonia so the fatal respiratory

failure may not be entirely attributable to radiation pneumonitis. Furthermore, the patient received concurrent chemotherapy and additional cycles of chemotherapy after radiation which may have resulted in its own toxicity.

This is a case report of grade 5 radiation pneumonitis in a patient with a potential history of connective tissue disease and/or autoimmune disease who also developed lymphangitic spread of tumor. Standard of care chemoradiation was provided to this patient and all of the radiation dose parameters were well within commonly accepted ranges. Furthermore, connective tissue disorder diagnosis was in question and autoimmune disorder was not active. Despite appropriate precautions, he still developed fatal pneumonitis. Further research is needed to develop a better understanding of the interplay of all of these factors.

COMMENTS

Case characteristics

This is a case report of grade 5 radiation pneumonitis in a patient with a potential history of connective tissue disease and/or autoimmune disease who also developed lymphangitic spread of tumor after receiving chemoradiation with intensity modulated radiation therapy (IMRT) technique for limited stage small cell lung cancer.

Clinical diagnosis

Grade 5 radiation pneumonitis and lymphangitic spread of tumor developed after chemoradiation for small cell lung cancer.

Differential diagnosis

Differential included pneumonitis, lymphangitic spread of tumor, pneumonia, or other interstitial and/or airspace disease.

Imaging diagnosis

Chest X-ray and computed tomography showed worsening bilateral interstitial and airspace opacities.

Pathological diagnosis

Autopsy examination of lung tissue demonstrated extensive, diffuse, bilateral alveolar damage consistent with post-radiation changes, as well as small cell carcinoma in multiple foci within septal capillaries and contiguous alveolar spaces.

Treatment

Initial therapy consisted of IMRT radiation therapy with concurrent and adjuvant chemotherapy. For his pneumonitis, he was treated with steroids, antibiotics, non-invasive and later mechanical ventilation.

Experiences and lessons

Standard of care chemoradiation was provided to this patient and all of the radiation

dose parameters were well within commonly accepted ranges. Furthermore connective tissue disorder diagnosis was in question and autoimmune disorder was not active. Despite appropriate precautions, he still developed fatal pneumonitis in addition to lymphangitic tumor spread. Further research is needed to develop a better understanding of the interplay of all of these factors.

Peer-review

The authors present a case report showing a patient with a fatal radiation pneumonitis 6 mo after radiation for limited stage of small cell lung cancer. The article is well explained and implemented.

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Prostatic adenocarcinoma oncocytic variant: Case report and literature review

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Abstract

The oncocytic variant of prostatic adenocarcinoma is exceptionally rare with only 4 cases reported in the English literature. Little is known about the clinical behavior of this variant of prostatic adenocarcinoma, because of the exceptionally low number of reported cases. The 2016 World Health Organization Classification of Tumors of Prostate does not recognize the oncocytic variant, again likely related to the exceptional paucity of reported cases. Here, we report the fifth case of the oncocytic variant of acinar type prostatic adenocarcinoma in an asymptomatic 64-year-old Caucasian American male with elevated serum prostate specific antigen (7.33 ng/mL; normal range 0-4.00 ng/mL) during routine blood screening for diabetes mellitus. At subsequent transrectal prostate biopsy, the right side of prostate was infiltrated by adenocarcinoma with tumor cells forming variably differentiated glands, including some poorly differentiated. Tumor cell nuclear: cytoplasmic ratio was low, with small to intermediate sized vesicular nuclei and only rare discernable small nucleoli. Cellular cytoplasm was characteristically granular pink with sharply defined cell membranes. Positive AMACR (P504S) epithelial immunohistochemical staining and absence of staining for prostatic basal cells confirmed the tumor to be primary prostatic adenocarcinoma. AMACR immunohistochemical staining was also helpful with accurate grading of the tumor due to the difficulty of differentiating tumor cells from residual prostate myocytes at routine hematoxylin and eosin (HE) staining. This new case adds to the exceptionally small number of previously reported cases of the oncocytic variant of primary prostatic adenocarcinoma. It also highlights the difficulty associated with Gleason scoring of the oncocytic variant by routine HE evaluation and the usefulness of AMACR (P504S) immunostaining for accurate grading of prostatic adenocarcinoma in the oncocytic variant.

Key words: Prostate; Adenocarcinoma; Clinical behavior; Oncocytic; Gleason; Prognosis

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Core tip: The oncocytic variant of prostatic adenocarcinoma is exceptionally rare with only 4 cases reported so far. Through reporting this new case, the oncocytic variant is being highlighted and challenges associated with its accurate diagnosis and staging discussed. The use of immunohistochemistry to confirm prostatic origin of this tumor for accurate grading of this lesion is also highlighted. It is also postulated that the tumor cells may be difficult to locate for their presence and organization at hematoxylin and eosin evaluation, potentially resulting in inaccurate grading of the tumor, the tumor likely behaves no different from the usual/typical variant of acinar-type adenocarcinoma if appropriately graded.

Klaimont MM, Zafar N. Prostatic adenocarcinoma, oncocytic variant: Case report and literature review. *World J Clin Oncol* 2017; 8(3): 289-292 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/289.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.289>

INTRODUCTION

Prostatic adenocarcinoma is a common malignancy, however, the 2016 World Health Organization (WHO) Classification of Prostatic Tumors^[1] does not mention the oncocytic variant of acinar-type adenocarcinoma, likely due to the very small number of reported cases in the literature. There is a paucity of data concerning the clinical behavior of this variant compared to the traditionally established varieties of acinar-type prostate adenocarcinoma. Accordingly, there is a critical need for more cases of the oncocytic variant to be reported, for it to be added to a future WHO classification, and to identify a variable clinical behavior from the usual variant, if that is indeed the case.

CASE REPORT

The 64-year-old Caucasian male with past medical history of hypertension, hyperlipidemia, type-2 diabetes, and otherwise asymptomatic, was also found at routine screening to have an elevated total serum prostate-specific antigen (PSA) of 7.33 ng/mL (range 0-4.0 ng/mL). Review of systems was unremarkable. He denied tobacco use and reported occasional alcohol use. Family history was unremarkable for genitourinary malignancy. Digital rectal exam indicated irregular prostate borders with a single indurated nodule on the right. The patient subsequently underwent transrectal prostate biopsy which revealed a right-sided prostate adenocarcinoma, the left-side being unremarkable. Patient was discharged after biopsy and elected to undergo targeted cryoablation of the prostate at an outside institution.

Histology

At routine hematoxylin and eosin (HE) histology, the right side of the prostate contained a poorly delineated malignancy with tumor cells arranged in vague glandular forms, as well as apparent cords, and possibly some single cells (Figure 1A). The cells had a low nuclear:cytoplasmic ratio, small to intermediate sized vesicular nuclei with only very rare prominent nucleoli, granular amphophilic to acidophilic cytoplasm, and sharp cell membranes (Figure 1B). It was difficult to reliably differentiate tumor cells from residual prostatic myocytes because of overlapping cytomorphology and staining quality (Figure 1B). Routine ABC immunohistochemistry with AMACR (P504S) and prostate basal cell markers (PIN4) was very helpful to confirm this cancer to be primary to prostate, and for accurate Gleason scoring of the tumor, as it clearly demonstrated the absence of single tumor cells and extensive gland formation, mostly discrete, with some tumor cells merging into more solid structures (1C and 1D). The tumor was assigned Gleason score 3 + 4 (20%) = 7, present in all 6/6 cores, 25% of total biopsy, with perineural invasion, but no vascular invasion. Biopsies of the left prostate were negative for malignancy.

DISCUSSION

Oncocytic tumors of prostate are exceedingly rare. The first reported case was an oncocytoma in an 87-year-old man who underwent transurethral resection for prostatic hypertrophy^[2]. While the tumor cells were immunoreactive with cytochrome-oxidase, they were not reactive with PSA.

In 1992, Ordóñez *et al*^[3] reported the first case of prostatic carcinoma with oncocytic features in a 63-year-old patient who presented with inguinal lymph node metastasis and an unknown primary, with a normal serum PSA. The tumor cells had finely granular cytoplasm, which ultrastructural examination showed to contain numerous mitochondria. The cells were immunoreactive with PSA and prostatic acid phosphatase. Subsequent prostatectomy confirmed primary oncocytic adenocarcinoma of the prostate. The authors postulated that the reason for oncocytic transformation may involve possible mitochondrial dysfunction in the cancer cell of origin, resulting in the proliferation of an oncocytic cancer cell type.

Pinto *et al*^[4] reported the 2nd case of primary carcinoma of prostate with diffuse oncocytic changes in a 66-year-old patient, who presented with a retro-ocular tumor and a PSA level of 100 ng/mL. Digital rectal exam indicated prostatic enlargement, which was subsequently biopsied, and the retro-ocular metastasis was also resected. Both the tumor sites contained identical poorly differentiated oncocytic tumor cells with strong immunoreactivity for PSA. This patient also had hyperdense metastatic lesions in various bony sites. The authors postulated that the prognosis of these very rare oncocytic tumors is no different from the usual prostatic acinar carcinoma and is more related to the tumor differentiation (Gleason

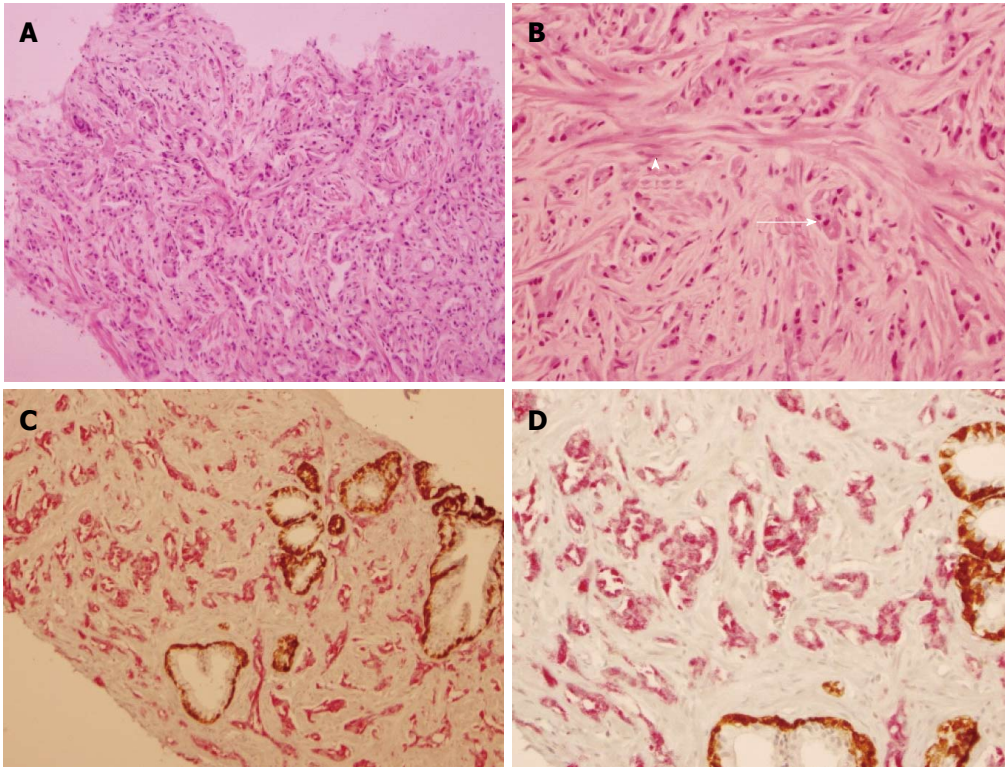


Figure 1 Oncocytic variant of prostatic adenocarcinoma: Hematoxylin and eosin and Immunohistochemical evaluation. A: Low power view of tumor, showing the tumor cells arranged in glandular and loose epithelial clusters (100 \times); B: High power view of tumor in glandular formations (arrow) and spindled residual prostate myocytes (arrowhead) (200 \times); C: PIN4 immunohistochemical staining identifies tumor (in red) and benign prostatic glands with residual basal cells (in brown) (100 \times); D: PIN4 immunohistochemical staining high power (200 \times) view with tumor (in red) and benign prostatic glands with residual basal cells (in brown).

scoring). We agree with this opinion, though we feel this is still anecdotal because of insufficient experience with this tumor variant.

Fiandrino *et al*^[5] reported the third case of prostatic adenocarcinoma with oncocytic features in a 72-year-old patient who presented with dysuria and prostate enlargement. The patient underwent prostatectomy which revealed prostatic adenocarcinoma with oncocytic features involving the entire tumor mass. Capsular infiltration and perineural invasion were also present. Based on extensive gland fusion, it was assigned a Gleason score of 8 (4 + 4) involving 60% of the prostate (both lobes). At immunohistochemistry, the oncocytic tumor cells were strongly positive for PSA and prostatic acid phosphatase (PSAP). Cells also stained positive for antimitochondrial antibody which demonstrated granular cytoplasmic reactivity in tumor cells but not normal glands. Ultrastructural evaluation was performed and similarly demonstrated a high mitochondrial density in tumor cells compared to the adjacent parenchyma.

Khadim *et al*^[6] reported the most recent case of oncocytic variant of prostatic adenocarcinoma in a 57-year-old, who presented with urinary urgency, hesitancy, increased frequency, poor stream, enlarged firm prostate at digital rectal examination and a markedly elevated PSA level of 40 ng/mL. At transurethral resection, the entire prostatic tumor comprised of oncocytic cells, arranged in solid sheets, with round to ovoid hyperchromatic nuclei and granular eosinophilic cytoplasm and PSA immunoreactivity. Gleason

score of 5 + 4 = 9 was assigned, involving 80% of the tissue sampled and without perineural or lymphovascular invasion. No follow-up was provided.

Gilloteaux *et al*^[7] have reported a peculiar, rare oncocyte-like cell in prostatic carcinoma (DU145) cell line, with a small nucleus and with cytoplasm almost entirely filled with often distorted mitochondria. It is enticing to speculate if this might be the cell-type which gives rise to the oncocytic variant of prostatic adenocarcinoma.

In summary, the reasons for presenting this case are multiple, foremost to add to the very limited literature on this variant of prostatic adenocarcinoma and to highlight the challenge of optimal Gleason scoring at HE assessment only. Our calculated Gleason score prior to AMACR (P504) staining was 4 + 5 = 9 because of the presence of poorly differentiated glands and perceived numerous single eosinophilic cells, with only rare well-formed glands. Our final Gleason score was 3 + 4 (20%) = 7, as AMACR (P504S) staining confirmed the absence of single tumor cells in the biopsy and the presence of numerous glands, mostly well-formed, with rare additional distorted and merged tumor glands. We believe the overestimation of Gleason score at HE is related to the difficulty of differentiating residual benign myocytes from tumor cells because of the overlapping cytomorphology and staining characteristics. We feel that AMACR (P504S) staining is critically important for optimal assessment of tumor differentiation and Gleason scoring of the oncocytic variant of prostatic adenocarcinoma. The non-recognition

of this cancer variant in the 2016 WHO classification of tumors of prostate, among other known variants of classic acinar type prostatic adenocarcinoma^[8], is likely related to the exceptionally low number of reported cases, most likely related to very low incidence. Variants of conventional prostate cancer (pseudohyperplastic, foamy gland, hypernephroid, atrophic, microcystic, with Paneth cell-like changes, with collagenous micronodules, with glomeruloid formations, and oncocytic) do not have any known prognostic significance and are graded according to the Gleason system. The prognosis and clinical behavior of the oncocytic variant, therefore, is also likely to be related to the degree of tumor differentiation and clinical staging, and not morphologic variation.

COMMENTS

Case characteristics

A 64-year-old Caucasian male with a serum prostate-specific antigen level of 7.33 ng/mL (range 0-4.0 ng/mL).

Clinical diagnosis

Prostatic enlargement.

Differential diagnosis

Prostatic carcinoma, prostatitis, prostatic hypertrophy.

Pathologic diagnosis

Primary adenocarcinoma of prostate, oncocytic variant, Gleason score 3 + 4 (20%) = 7, 6/6 cores, 20% of total tissue involved on the right, with peri-neural invasion.

Treatment

The patient opted for cryoablation of the prostate at another facility. No further follow-up is available at this point.

Peer-review

This is an interesting case report of a very rare tumor.

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Pancreatic neuroendocrine tumor Grade 1 patients followed up without surgery: Case series

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Author contributions: Sugimoto M designed and performed the research and wrote the paper; Takagi T and Suzuki R designed the research and supervised the report; Konno N designed the research and contributed to the analysis; Asama H, Watanabe K, Nakamura J, Kikuchi H, Waragai Y and Takasumi M provided clinical advice; Kawana S and Hashimoto Y provided histopathological advice; Hikichi T and Ohira H supervised the report.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Fukushima Medical University Hospital.

Informed consent statement: Patients were not required to give informed consent for the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent. For full disclosure, the details of the study are published on the home page of Fukushima Medical University.

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Abstract

Among the three grades of neuroendocrine tumors (NETs), the prognosis for Grade 1 (G1) with surgery is very good. Therefore, we evaluated the prognoses of pancreatic NET (PNET) G1 patients without surgery. A total of 8 patients who were diagnosed with NET G1, with an observation period of more than 6 mo until surgery or without surgery, were recruited. The patients who underwent surgery were ultimately diagnosed using specimens obtained during the surgery, whereas the patients who did not undergo surgery were diagnosed using specimens obtained by endoscopic ultrasonography-guided fine needle aspiration. Overall, we mainly evaluated the observation period and tumor growth. The observation period for the five cases

with surgery ranged from 6-80 mo, and tumor growth was observed in one case. In contrast, the observation period for the three cases without surgery ranged from 17-54 mo, and tumor growth was not observed. Therefore, although the first-choice treatment for NETs is surgery, our experience includes certain NET G1 patients who were followed up without surgery.

Key words: Pancreatic neuroendocrine tumors; Metastasis; Neuroendocrine tumors Grade 1; Follow-up; Surgery

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Core tip: We evaluated the prognoses of pancreatic neuroendocrine tumor Grade 1 (NET G1) patients without surgery. A total of 8 patients who were diagnosed with NET G1, with an observation period of more than 6 mo until surgery or without surgery, were recruited. The observation period for the five cases with surgery ranged from 6-80 mo, and tumor growth was observed in one case. In contrast, the observation period for the three cases without surgery ranged from 17-54 mo, and tumor growth was not observed. Our experience thus includes certain NET G1 patients who were followed up without surgery.

Sugimoto M, Takagi T, Suzuki R, Konno N, Asama H, Watanabe K, Nakamura J, Kikuchi H, Waragai Y, Takasumi M, Kawana S, Hashimoto Y, Hikichi T, Ohira H. Pancreatic neuroendocrine tumor Grade 1 patients followed up without surgery: Case series. *World J Clin Oncol* 2017; 8(3): 293-299 Available from: URL: <http://www.wjgnet.com/2218-4333/full/v8/i3/293.htm> DOI: <http://dx.doi.org/10.5306/wjco.v8.i3.293>

INTRODUCTION

Neuroendocrine tumors (NETs) of the digestive organs are classified as Grade 1 (G1) or Grade 2 (G2) or as neuroendocrine carcinoma (NEC) by the World Health Organization (WHO) 2010 classification, which is based on cellular proliferative potential (Ki-67 index and the mitotic count)^[1]. Generally speaking, pancreatic NETs (PNETs) are a rare condition, accounting for only 2%-5% of pancreatic tumors^[2]. However, reports about PNETs have been increasing in direct proportion to more detailed diagnostic imaging.

Among the three grades of NETs, the prognosis for G1 is very good. It has been reported that the two-year progression-free survival rate for NET G1 is 92%^[3] and that the two-year survival rate is 100%^[4]. Five-year survival was reported to be 55.7% by Zeng *et al*^[5] and 82.6% by Yang *et al*^[6]. In other reports, however, the five-year survival rate was 90% or more^[4,7-10].

Regarding PNET treatment, the National Comprehensive Cancer Network^[11], the North American Neuroendocrine Tumor Society^[12], and the European Neuroendocrine Tumor Society^[13] have established guidelines. The first-choice

treatment is surgery for all grades of PNETs if the lesions are resectable.

Regarding diagnosing NETs before surgery, the efficacy of endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) has been reported^[14-17]. As mentioned above, the first-choice treatment for resectable PNETs is surgery. However, if a patient is diagnosed with NET G1 based on the Ki-67 index of an EUS-FNA specimen, there is a possibility that the patient will not agree to surgery because of a good prognosis.

Accordingly, we examined the following two topics in this report: (1) the prognoses of NET G1 diagnosed by EUS-FNA without surgery; and (2) the tumor growth of NET G1 from diagnosis until surgery.

CASE REPORT

A total of 34 patients were diagnosed with PNETs from February 2001 to December 2015. Among these patients, 21 underwent measurement of the Ki-67 index using specimens obtained by EUS-FNA or surgery (Figure 1). Thirteen patients were diagnosed with NET G1, seven patients were diagnosed with NET G2, and one patient was diagnosed with NEC. We recommended surgery for the NET patients, regardless of their WHO 2010 classification. However, if a patient did not agree to surgery, we only performed a follow-up. We focused on eight NET G1 patients who waited for surgery for no less than six months or who were followed up for no less than six months without surgery. The observation period was defined as no less than 6 mo based on a report on everolimus by Yao *et al*^[18]. In that report, the length of progression-free survival of the placebo group was 5.4 mo.

The patients who underwent surgery were ultimately diagnosed using specimens obtained during surgery, and the patients who did not undergo surgery were diagnosed using specimens obtained by EUS-FNA. UCT260, GF-UCT240-AL5, or GF-UC240P (Olympus Medical Systems, Tokyo, Japan), was used as the echoendoscope, and EU-ME1 or EU-ME2 (Olympus Medical Systems, Tokyo, Japan) was used as the ultrasonography diagnostic device. EchoTip 19 or 22 or 25G (Cook Medical Inc., NC, United States), and EZ Shot 22G (Olympus Medical Systems) and Expect 22G (Boston Scientific, MA, United States) were used as the aspiration needles.

All patients underwent echoendoscope insertion under sedation with midazolam. After we drew the target on the monitor and checked that no blood flow was present in the aspiration line, we punctured the target, passing through the gastric or duodenal wall. We excluded the stylet of the needle and connected a syringe with 10-20 mL negative pressure to the edge of the needle. We then moved the needle back and forth 20 times within the lesion. In particular, we moved the needle to multiple locations within the target (this has been reported as the "fanning method")^[19]. After we terminated the negative pressure, we removed the needle. The EUS-FNA sample was then placed on a glass slide, and the specimen was

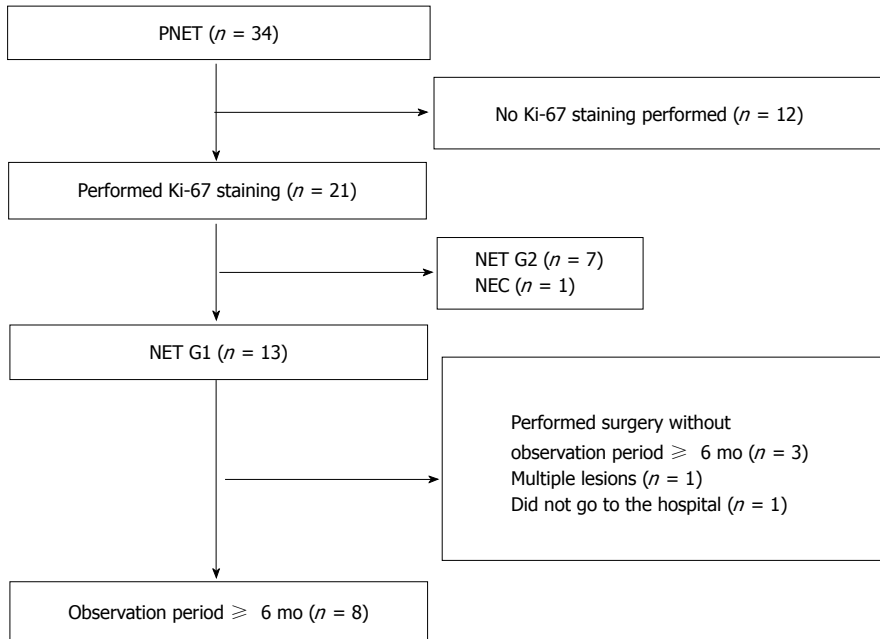


Figure 1 The characteristics of pancreatic neuroendocrine tumor patients at our hospital. A total of 34 patients were diagnosed with PNETs. Among these patients, 21 had specimens that underwent Ki-67 immunostaining. There were 13 PNET G1 patients, and the observation period was more than six months long for eight PNET G1 patients. PNET: Pancreatic neuroendocrine tumor; G1: Grade 1.

preserved in 15% formalin for histological diagnosis. All other samples were stained using Cyto-Quick. We observed the samples to assess whether a sufficient number of cells were sampled (rapid on-site cytological evaluation, or ROSE)^[20]. If a sample was sufficient, we halted the EUS-FNA; if a sample was not sufficient, we performed another aspiration. The samples obtained for histological diagnosis were stained with hematoxylin and eosin and were also immunostained for the following: Ki-67, chromogranin, synaptophysin (DAKO, Glostrup, Denmark), and CD56 (ZYMED, Carlsbad, CA, United States). The grades of the PNET cases were determined based on the Ki-67 index outlined in the WHO 2010 classification. The grades of the specimens obtained during surgery were also determined based on the Ki-67 index and the mitotic count, as defined in the WHO 2010 classification.

We reviewed each patient's characteristics (sex, age, initial tumor size, and location of the tumor), the method of diagnosis (EUS-FNA or surgery), the Ki-67 index, the mitotic count, whether the patient was functional or not, tumor marker levels, observation period, and tumor growth. The observation period was determined as the number of months from tumor discrimination by abdominal echo or computed tomography (CT) until the tumors were resected. For the patients without surgery, the observation period was determined as the number of months from tumor discrimination by abdominal echo or CT until the tumors were recognized by a final abdominal echo or CT. The patients specifically underwent dynamic CT or abdominal echo approximately 2 times per year, performed by an attending physician.

The age range of the patients was 41-81 years, and

the patient group included two males and six females (Table 1). The initial major tumor axes ranged from 3-40 mm. The locations of the tumors were the pancreatic head ($n = 3$), pancreatic body ($n = 3$), and pancreatic tail ($n = 2$). Five patients underwent surgery, and three patients did not but did undergo EUS-FNA. The Ki-67 index ranged from 0.4%-1.3% (five patients did not undergo precise measurement, but their index was < 2.0%). The mitotic count of the specimens obtained during surgery was 0-2/10 HPFs. Three patients were functional (1 with a growth hormone-producing tumors, 1 with a glucagonoma, and 1 with an insulinoma). AFP, NSE, CEA or CA19-9 was also measured, but these tumor markers were not elevated in any of the patients.

The observation periods ranged from 6-80 mo for patients 1-4. Only patient 2 was observed to exhibit tumor growth (Figure 2). In contrast, the observation periods for the three cases without surgery ranged from 17-54 mo, and all three cases did not show tumor growth. Among these three cases, one case is shown in Figure 3.

DISCUSSION

In this report, we examined whether we could follow up NET G1 without surgery. Among eight patients who were observed before surgery for no less than six months or who did not undergo surgery for at least six months, tumor growth was observed in one patient.

As described above, the prognoses of the NET G1 were very good. However, the data were relevant to prognoses only after surgery. Sadot *et al.*^[21] reported the prognoses of 104 PNET patients who were diagnosed pathologically

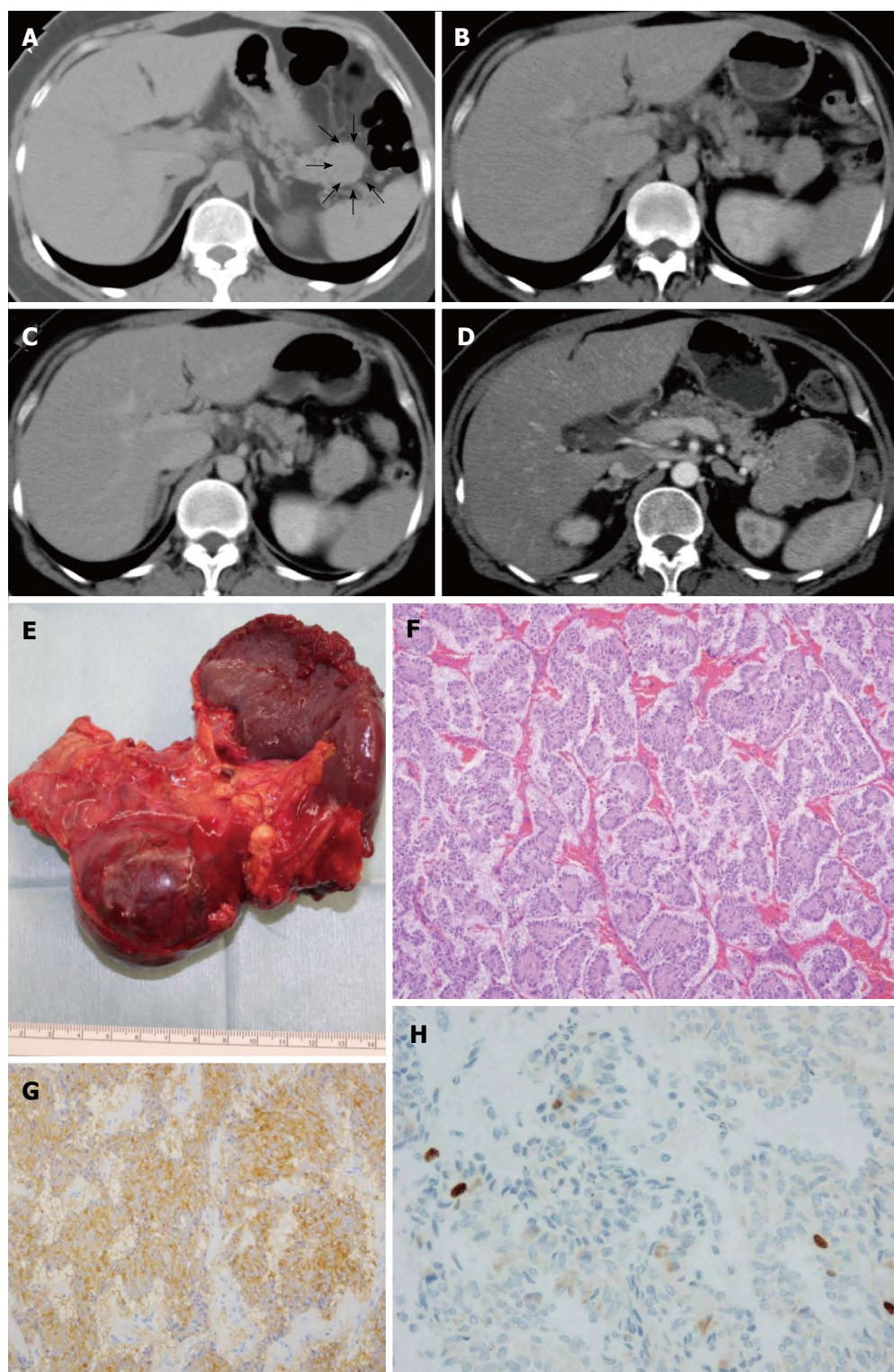


Figure 2 The patient who exhibited growth of the pancreatic neuroendocrine tumor. A: Abdominal CT. Initial CT indicated a PNET. The lesion was identified in the pancreatic tail. The diameter of the PNET was 34 mm (arrow); B: The lesion grew slightly after 11 mo; C: The lesion grew further after 29 mo; D: The diameter of the tumor became larger than 70 mm after 79 mo; E: The patient underwent distal pancreatectomy after 80 mo; F: Hematoxylin and eosin stain ($\times 100$). Tumor cells formed ribbon-like lines; G: Chromogranin A staining ($\times 200$). Tumor cells were chromogranin A positive; H: The Ki-67 index was 0.9%, with tumor grade G1 ($\times 200$). PNET: Pancreatic neuroendocrine tumor; CT: Computed tomography.

or by imaging. In that report, the diameters of all PNET lesions were smaller than 3.0 cm. Among the patients, 26 did not undergo surgery; those without surgery who were only followed up did not exhibit tumor growth or

metastases to other organs. Though cases diagnosed by only imaging were included in that report, certain PNET patients could be followed up without surgery. Additionally, Shin *et al*^[22] reported 72 gastroenteropancreatic NET cases

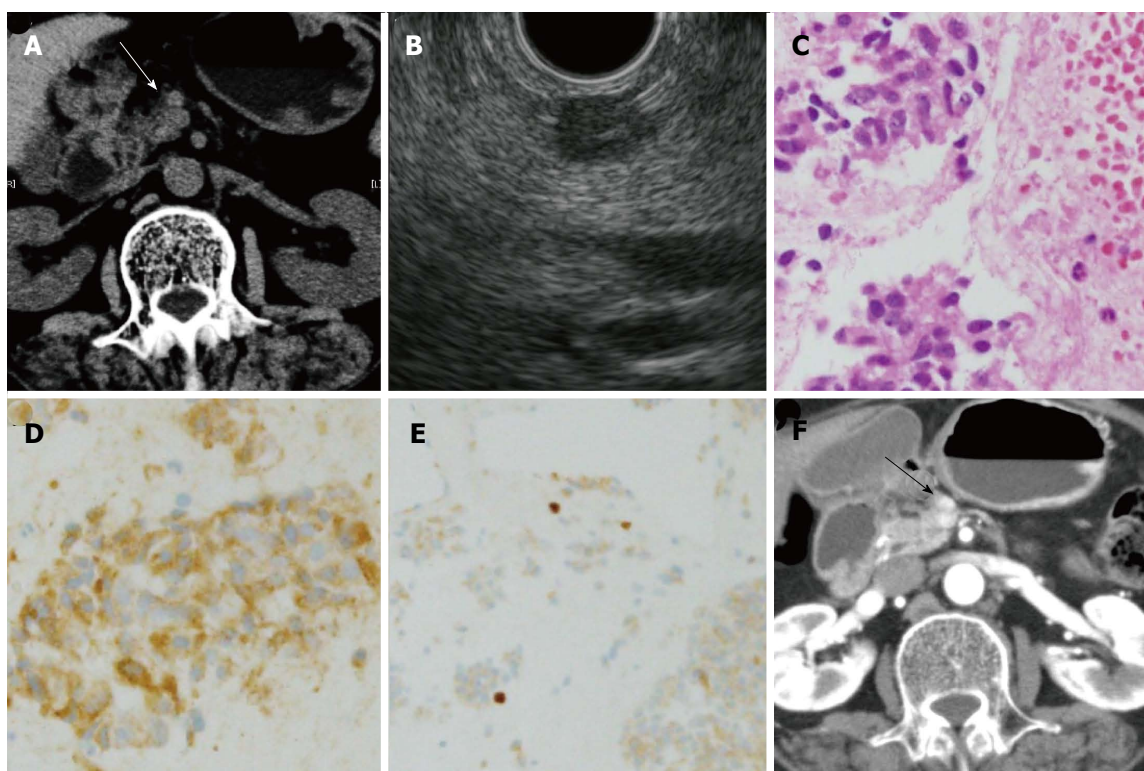


Figure 3 Pancreatic neuroendocrine tumor case followed up without surgery. A: Abdominal CT. A tumor was recognized in the pancreatic body. The diameter of the lesion was 8 mm; B: Endoscopic ultrasonography. The tumor was recognized as a low echoic lesion. A 22G needle was inserted into the tumor; C: Hematoxylin and eosin stain ($\times 400$). Spindle-shaped tumor cells with ellipsoidal nuclei formed fascicles; D: Chromogranin A staining ($\times 400$). Tumor cells were chromogranin A-positive; E: The Ki-67 index was $< 1.0\%$ ($\times 200$), with tumor grade G1; F: Abdominal CT. The tumor did not grow after 54 mo. PNET: Pancreatic neuroendocrine tumor; CT: Computed tomography.

with liver metastases. Among these cases, 12 were NET G1 (17%). Zerbi *et al.*^[23] reported that 16.1% of NET G1 showed metastases to the lymph nodes and that 12.6% of NET G1 showed liver metastases. In addition, Gaujoux *et al.*^[24] reported 20 PNET G1 cases with liver metastases. In the present report, one case exhibited tumor growth in the observation period. Therefore, we have to follow up NET G1 while taking the risk factors for metastases and tumor growth into consideration.

What are the specific risk factors for NETs? In the past reports, nonfunction and symptoms such as abdominal pain, weight loss, and jaundice were reported to be risk factors for liver metastases. Moreover, Tao *et al.*^[25] reported that elevated tumor markers (AFP, CEA, CA125, CA19-9) were predictive factors for liver metastases or lymph node metastases, and Jiang *et al.*^[26] reported that a tumor diameter larger than 25 mm was a risk factor for lymph node metastases. In the present report, the lesion diameters of 3 cases were larger than 25 mm; the patients were numbered 2, 4, and 5 (Table 1). Though patients 4 and 5 underwent surgery six months after diagnosis, the lesion of patient 2 grew from 34 to 76 mm in diameter. Though past studies involved not only NET G1 but also other grades of NETs, the risk factors cited in these past reports were considered to be important to determine follow-up without surgery.

In this report, there were certain limitations. First, the research was retrospectively performed at a single

institution, and a small number of patients were included. More patients will be needed for more conclusive research. Second, the followed-up patients were diagnosed only by EUS-FNA. However, a high accordance rate between specimens obtained during surgery and specimens obtained by EUS-FNA was reported in past studies^[14-17], and for NET G1, the accordance rate between specimens obtained during surgery and specimens obtained by EUS-FNA was 92.3% (36/39)^[14-17] (Larghi, 2012 #59). We believe that we relatively correctly judged the grading based on the Ki-67 index of NET G1. Third, mitotic counts were not measured in EUS-FNA specimens. Therefore, surgery is desirable as a treatment for NETs. Fourth, we did not measure several of the tumor markers described above. Rossi *et al.*^[27] reported the efficacy of plasma chromogranin A as a predictive factor for NET progression; this should be studied further in the future.

The first-choice treatment for NETs is absolutely surgery. However, our experience includes certain patients who were followed up without surgery because of a lack of consent for surgery.

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Table 1 Prognoses of pancreatic neuroendocrine tumor Grade 1 patients

	Sex	Age (yr)	Initial size (mm)	Location of tumor	Method of final diagnosis	Ki-67 index (%)	Mitotic count (/10 HPFs)	Function	Elevated tumor markers	Observation period (mo)	Tumor growth (mm)
1	F	79	19	Body	Surgery	< 2.0	0	No	No	6	No
2	F	41	34	Tail	Surgery	0.9	2	Yes	No	80	76
3	M	69	3	Body	Surgery	< 2.0	0	Yes	No	15	No
4	M	55	40	Head	Surgery	< 1.0	0	Yes	No	6	No
5	F	73	32	Head	Surgery	1.3	0	No	No	9	No
6	F	81	4	Head	EUS-FNA	< 1.0	Difficult	No	No	22	No
7	F	64	8	Tail	EUS-FNA	0.4	Difficult	No	No	17	No
8	F	70	8	Body	EUS-FNA	< 1.0	Difficult	No	No	54	No

M: Male; F: Female; EUS-FNA: Endoscopic ultrasonography-guided fine needle aspiration.

University Hospital as well as American Journal Experts, an English-language proofreading company.

COMMENTS

Case characteristics

Pancreatic neuroendocrine tumor Grade 1 (PNET G1) patients who were followed for more than six months before surgery or who were followed up without surgery for more than six months.

Clinical diagnosis

PNETs were diagnosed using specimens obtained by endoscopic ultrasonography-guided fine needle aspiration (EUS-FNA) or obtained during surgery.

Differential diagnosis

Metastatic pancreatic tumors, accessory spleen, acinar cell carcinoma, paraganglioma.

Laboratory diagnosis

All tumor markers were not elevated.

Imaging diagnosis

PNETs are pancreatic tumors that are strongly enhanced on contrast-enhanced computed tomography.

Pathological diagnosis

Spindle-shaped tumor cells were observed. The tumor cells formed funicular lines and were positive for immunostaining of chromogranin A.

Treatment

Surgery or follow-up.

Related reports

The prognosis of PNET G1 is very good. However, certain PNET G1 patients exhibit metastases. Therefore, the first-choice treatment for resectable NETs is surgery.

Term explanation

EUS: A technique in which an echoendoscope is used to enable observation of the chest and abdominal organs, namely, the esophagus, stomach or duodenum; EUS-FNA: A technique used to obtain specimens from chest and abdominal lesions by aspiration under EUS guidance.

Experiences and lessons

The gold standard of treatment for NET G1 is surgery. However, if patients are diagnosed with NET G1 by EUS-FNA, there is a possibility that the patients will not agree to surgery. In fact, certain NET G1 patients did not agree to surgery

in the current case series, so the authors only followed up these patients. If we only follow up PNET G1 patients, the authors have to be careful about certain risk factors for metastasis of the PNETs.

Peer-review

This is an interesting paper on whether patients with G1 pancreatic NET can be followed without surgery using a case series of patients.

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Target migration from re-inflation of adjacent atelectasis during lung stereotactic body radiotherapy

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Abstract

Stereotactic body radiotherapy (SBRT) is a widely accepted option for the treatment of medically inoperable early-stage non-small cell lung cancer (NSCLC). Herein, we highlight the importance of interfraction image guidance during SBRT. We describe a case of early-stage NSCLC associated with segmental atelectasis that translocated 15 mm anteroinferiorly due to re-expansion of the adjacent segmental atelectasis following the first fraction. The case exemplifies the importance of cross-sectional image-guided radiotherapy that shows the intended target, as opposed to aligning based on rigid anatomy alone, especially in cases associated with potentially "volatile" anatomic areas.

Key words: Radiation therapy; Stereotactic body radiation therapy; Non-small cell lung cancer; Image-guided radiation therapy; Stereotactic ablative radiation therapy

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Core tip: This is a case of early-stage non-small cell lung cancer associated with segmental atelectasis that translocated owing to re-expansion of the adjacent segmental atelectasis following the first fraction. There are image-guidance systems that register solely based on rigid (bony) anatomy and others that also show soft tissue; if the former would have been used, the translocated target would have been missed. The case exemplifies the importance of cross-sectional image-guided radiotherapy that shows the intended target, as opposed to aligning based on rigid anatomy alone, in cases associated with

potentially “volatile” anatomic areas.

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INTRODUCTION

Among other indications, stereotactic body radiotherapy (SBRT) plays an important role in the treatment of early-stage non-small cell lung cancer (NSCLC), chiefly in medically inoperable candidates, or if patients refuse surgery^[1-7]. It is well-known that target and respiratory motion management is critical, and that spatial uncertainty in SBRT can be caused by both internal motion with respiration and set-up errors. Therefore, image-guided radiotherapy (IGRT) before each treatment is strongly recommended for SBRT; IGRT can confirm that the gross tumor is consistently located within the pre-defined treatment volume.

SBRT may utilize one of two IGRT subtypes: Systems that rely on rigid bony anatomy, and those that provide soft tissue discrimination. For the first type, two in-room systems are specifically designed for stereotactic treatments and are widely used: CyberKnife (Accuray Inc., Sunnyvale, CA, United States) and ExacTrac (BrainLabAG, Feldkirchen, Germany). These systems use orthogonal kilovoltage (kV) electronic 2-D radiographs, generated by X-ray tubes combined with flat panel detectors, to align and verify the patient treatment position - usually according to bony anatomy. Though providing better soft-tissue contrast than its MV counterparts, 2D kV IGRT systems largely consider bony landmarks for registration instead of the internal target alignment, except in cases with bulky thoracic tumors or implanted fiducial markers. For the second type, good soft tissue cross-sectional visibility is provided by 3D images. Some of these systems use kV cone-beam computed tomography (CBCT) generated by linear-accelerator (LINAC)-integrated systems such as On-Board-Imager (Varian Medical Systems, Palo Alto, CA, United States) and X-ray-Volume-Imaging (Elekta Oncology Systems, Crawley, United Kingdom). Alternatively, kV computed tomography (CT) can also be implemented by CT-on-Rails (Siemens, Erlangen, Germany). These systems offer 3D images and soft-tissue-based target verification without fiducials for small lung lesions treated with SBRT^[8]. The most common application of this IGRT scheme is a two-step verification process with an initial bony registration followed by a soft-tissue target alignment^[9,10].

Though most studies report localization accuracy improvements of 3D-vs-2D on the order of a few millimeters for lung SBRT^[11-13], we describe a patient with an

early-stage NSCLC which translocated after the first fraction of SBRT owing to re-expansion of segmental atelectasis. We further discuss the role of IGRT systems that align to bone vs soft-tissue in detection and management of the resulting misalignment.

CASE REPORT

A 72-year-old man presented with a nonproductive cough; computed tomography (CT) scan showed a 2.5-cm right lower lobe nodule. He had a 50-year history of smoking and used 4 L of nighttime oxygen (ECOG performance status 3). On auscultation, he had diminished breath sounds; pulmonary function tests showed an FEV1 (forced expiratory volume, 1 s) 41% of the predicted value and a DLCO (diffusion capacity of carbon-monoxide) 29% of the predicted value. Subsequent positron emission tomography (PET) scan showed no other hypermetabolic foci. Needle biopsy revealed poorly-differentiated lung adenocarcinoma.

He was not a surgical candidate owing to poor pulmonary function, and was appropriate for SBRT. On CT simulation including a four-dimensional (4D) and free-breathing CT, new distal segmental atelectasis was noted near the nodule (Figure 1A). The target was delineated as an internal target volume (ITV) based on maximum intensity projection (MIP) generated by 4DCT simulation, with an additional 5 mm expansion to create the planning target volume (PTV). He then started Volumetric Modulated Arc Therapy to the right lower lobe PTV at a dose of 5000 cGy in 5 daily fractions. Daily kV CBCT was used for IGRT on a TrueBeamSTx LINAC. After the first dose, the tumor was found to have translocated 15mm anteroinferiorly due to re-expansion of segmental atelectasis as demonstrated by the kV CBCT prior to the second fraction. Of note, the movement was significant enough that it was only partially covered by the PTV. He underwent re-simulation, which confirmed the geometric target migration (Figure 1B and C). SBRT was re-adjusted for the new target location after migration. For subsequent fractions, daily kV CBCTs validated tumor position within the ITV; the remainder of therapy was completed uneventfully. Post-treatment CT showed resolution of disease at 4 mo post-SBRT.

DISCUSSION

Although surgery is currently the principal option for early-stage NSCLC patients that are medically operable, stereotactic body radiotherapy (SBRT) has emerged as the option of choice for patients who are medically inoperable. SBRT is a technique that administers high doses of radiation to the target while minimizing the dose to surrounding normal tissues. Reports show high local tumor control rates upwards of 90%, with severe toxicities well under 10%^[14]. This has made it a favorable option for medically inoperable patients with stage I NSCLC, endorsed by both the National Comprehensive Cancer Network (NCCN) Guidelines

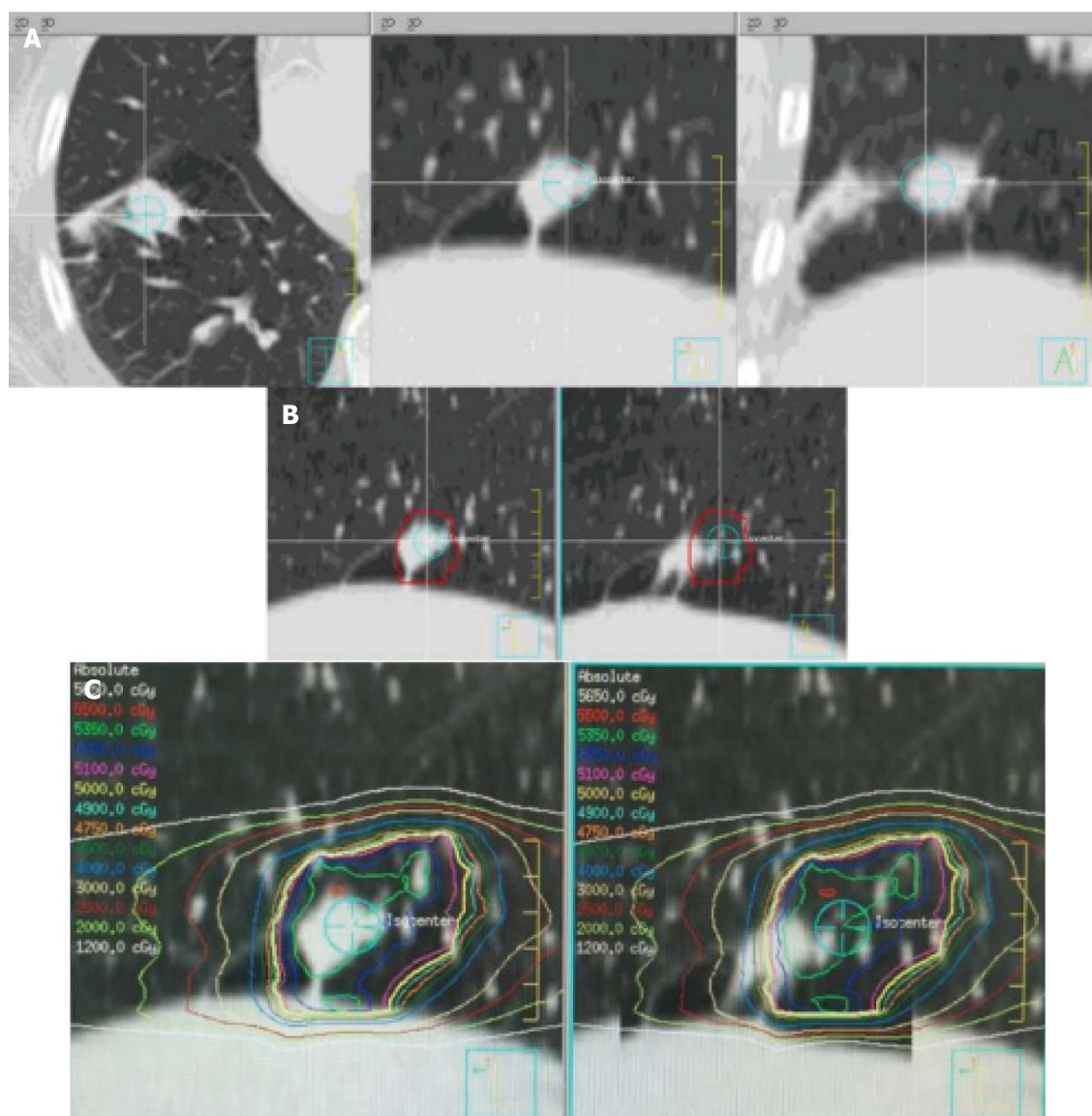


Figure 1 Computed tomography simulation including a four-dimensional and free-breathing computed tomography. A: Computed tomography (CT) images at simulation (left to right; axial, sagittal, and coronal views) showing the tumor, treatment isocenter, and the adjacent segmental atelectasis (best pictured on coronal image, lateral to isocenter); B: Left panel shows the tumor enclosed within the planning target volume (PTV) on the initial simulation CT; right panel demonstrates the translocation as compared with the original PTV on the re-CT after the first fraction. Sagittal views are shown in both panels; C: Dose distribution of initial (left) and translocated (right) tumor with isodose line values provided on left. Sagittal views are shown in both panels.

and the European Society of Medical Oncology (ESMO) Clinical Practice Guidelines^[15].

However, accounting for target and respiratory motion presents a challenge to proper delivery. Hence, in order to verify target location with high accuracy, high-fidelity IGRT is considered essential to SBRT. In our case report, the target translocated 15 mm anteriorly and inferiorly due to re-expansion of adjacent atelectasis after the first fraction. Without soft tissue discrimination in cross-sectional imaging, 2D IGRT based on bony anatomy only would have resulted in systematically missing the tumor in the remaining 4 fractions. These advantages of 3D IGRT are highlighted in anatomic areas liable to changes in morphology.

There have been several studies describing the utility of IGRT in SBRT. A study was carried out to evaluate

the potential of image guidance, gating and real-time tumor tracking to improve accuracy in pulmonary SBRT. It illustrated that CBCT-based IGRT for pre-treatment verification of the target position and online correction of errors reduced safety margins most effectively in pulmonary SBRT^[16]. Another recent study illustrated that application of continuous monitoring and intra-fraction target position correction during treatment improved the target coverage for patients in prostate SBRT. Without these IGRT techniques, intra-fractional motion would have significantly altered coverage in about 10% of patients^[17]. These studies have demonstrated that inter-fractional, and possibly even intra-fractional IGRT, can improve SBRT delivery. We advocate for increased use of cross-sectional imaging IGRT with soft tissue definition, especially in cases of tumors near potentially "anatomically volatile" areas.

While such large translocations such as reported here may be unlikely, intra-fractional real-time tumor tracking may provide additional benefit. An ionization radiation-free system using thoracic transducers and radiofrequency tracking using the Calypso system is under development (Varian Medical Systems, Palo Alto, CA, United States).

In summary, image guidance is a prerequisite for SBRT delivery, but 2D IGRT systems that solely align patients based on rigid bony anatomy may be notably inadequate in some cases. Instead, the use of imaging that provides cross-sectional soft tissue anatomical information to verify the target may prevent systematic misses from changes in target position.

COMMENTS

Case characteristics

A 72-year-old man of stage I non-small cell lung cancer associated with segmental atelectasis that translocated owing to re-expansion of the adjacent segmental atelectasis following the first dose of stereotactic body radiation therapy (SBRT).

Clinical diagnosis

Lung re-expansion of segmental atelectasis.

Differential diagnosis

Diminished breath sounds, pulmonary function, positron emission tomography scan and needle biopsy.

Laboratory diagnosis

The patient was not a surgical candidate owing to poor pulmonary function, and was appropriate for SBRT.

Imaging diagnosis

Re-simulation with high resolution computed tomography (CT) and image comparison using ridged image registration of primary CT simulation images confirmed geographic moves of the tumor due to re-expansion of an adjacent pulmonary atelectasis.

Pathological diagnosis

Needle biopsy showed poorly-differentiated lung adenocarcinoma.

Treatment

Daily kV cone-beam computed tomography was used for IGRT during SBRT.

Related reports

Related reports have demonstrated that inter-fractional, and possibly even intra-fractional IGRT, can improve SBRT delivery.

Term explanation

Non-small cell lung cancer is a deadly disease that may threaten people's life.

Experiences and lessons

Image guidance is extremely important for SBRT delivery.

Peer-review

This is an interesting case report worthy for publication. The authors reported on an early-stage lung tumor undergoing SBRT, translocating outside of the PTV after re-inflation of nearby atelectasis. The case herein presented highlight the risks of relying on IGRT system based on rigid anatomy alone. The manuscript is original, well-written and summarized in very explanatory figures.

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