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Circular RNAs in hepatocellular carcinoma: Recent advances

Zhao-Shan Niu, Wen-Hong Wang

Abstract
Circular RNAs (circRNAs) have covalently closed loop structures at both ends, exhibiting characteristics dissimilar to those of linear RNAs. Emerging evidence suggests that aberrantly expressed circRNAs play crucial roles in hepatocellular carcinoma (HCC) by affecting the proliferation, apoptosis and invasive capacity of HCC cells. Certain circRNAs may be used as biomarkers to diagnose and predict the prognosis of HCC. Therefore, circRNAs are expected to become novel biomarkers and therapeutic targets for HCC. Herein, we briefly review the characteristics and biological functions of circRNAs, focusing on their roles in HCC to provide new insights for the early diagnosis and targeted therapy of HCC.

Key Words: Hepatocellular carcinoma; Circular RNAs; Function; Diagnosis; Biomarkers; Targeted therapy

Core Tip: Current studies have shown that aberrantly expressed circular RNAs (circRNAs) play crucial roles in hepatocellular carcinoma (HCC) by affecting the proliferation, apoptosis and invasive capacity of HCC cells. Certain circRNAs may be used as potential biomarkers to diagnose and predict the prognosis of HCC. Therefore, circRNAs are expected to become novel biomarkers and therapeutic targets for HCC. Herein, we briefly review the characteristics and biological functions of circRNAs, focusing on their roles in HCC to provide new insights for the early diagnosis and targeted therapy of HCC.
INTRODUCTION

Early hepatocellular carcinoma (HCC) usually lacks specific symptoms, and most patients have missed the opportunity for effective treatment because they are diagnosed at middle-to-advanced stages. The emergence of novel therapeutic strategies for HCC, such as immunotherapy and molecularly targeted therapies[1], can prolong the survival of HCC patients. Unfortunately, patients with advanced HCC are prone to metastasis and recurrence, and long-term prognosis remains poor[2]. Therefore, identifying new biomarkers for early diagnosis and effective therapeutic targets of HCC is critical.

Circular RNAs (circRNAs) are covalently closed loops generated by the back splicing of precursor mRNA (premRNA) molecules, which exist widely in mammalian cells and are characterized by stability, conservative evolution, and cell or tissue specificity. These characteristics endow circRNAs with many biological functions, such as acting as microRNA (miRNA) sponges, regulating the transcription of parental genes, binding RNA binding proteins (RBPs), and encoding proteins and peptides[3]. CircRNAs exert their biological functions mainly at the epigenetic, transcriptional and posttranscriptional levels[4,5]. Dysregulated circRNAs play crucial roles in various diseases, particularly with respect to the occurrence and development of tumors and tumor proliferation, apoptosis and metastasis[6-8]. Currently, aberrantly expressed circRNAs are closely associated with the proliferation, cell cycle, apoptosis, migration, epithelial-mesenchymal transition (EMT), invasion, metastasis, cancer stem cells (CSCs), glycolysis, microvascular invasion (MVI), angiogenesis, immune surveillance, immune escape, chemoresistance, and immunotherapy resistance of HCC. Thus, circRNAs may be promising biomarkers for the diagnosis and prognosis of HCC as well as effective therapeutic targets. Herein, we briefly review the characteristics and biological functions of circRNAs, focusing on their roles in HCC to provide new insights into the early diagnosis and targeted therapy of HCC.

CHARACTERISTICS, CATEGORIES AND GENERATION OF CIRCRNAS

Characteristics of circRNAs

Most circRNAs have the following characteristics: (1) High abundance: The abundance of circRNA expression varies greatly; in some cases, the abundance of circRNAs exceeds 10 times that of their linear RNA counterparts[9]; (2) Stability: The stability of circRNAs is 2.5-5 times higher than that of linear transcripts because the unique covalently closed loop of circRNAs lacks 3’ and 5’ ends, resulting in the absence of ribonuclease binding targets; therefore, circRNAs are not easily degraded[10]; (3) Conservation: CircRNAs are widely present in different species and are evolutionarily conserved. Some studies suggest that most circRNAs in different species are evolutionarily conserved, while a few are not conserved[11]; and (4) Specificity: CircRNAs have tissue and cell specificity, with differential expression in different stages of oncogenic and disease progression[12].

Categories and generation of circRNAs

CircRNAs are categorized into four classes based on their origins: Exon circRNAs (ecircRNAs), intron circRNAs (icRNAs), exon-ciRNAs (EicRNAs), and intergenic circRNAs[13] (Figure 1). EcircRNAs are predominant and are mainly located in the cytoplasm. IcRNAs and EicRNAs are located in the nucleus. The generation mechanism of circRNAs is very complex and has not yet been understood. Current studies have shown that the cyclization of circRNAs is principally driven by intron pairing, RBPs or transcription factors and lariat[14].

Intron pairing-driven cyclization or “direct back splicing” is the most common cyclization mode of ecircRNA and EicRNA, where the special premRNA containing ALU repeats is sheared to form ecircRNA after reverse base complementary pairing[11]. Lariat-driven cyclization or “exon skipping” connects exons at both ends through donor and acceptor sites provided by spliceosomes to form lariat selective splicing to generate ecircRNA[11]. In RBP-driven cyclization, RBPs bound to the complementary sequences on both sides of the intron of premRNA interact with each other to form a circular structure and promote the terminal connection at both ends of the head and tail to form ecircRNA[15]. EicRNAs can be formed if introns are retained between exons during the above three mechanisms[16]. Self-cyclization of introns: When pre-RNA has a 7 nt guanine (G)- and uracil (U)-rich sequence near an exon and an 11 nt cytosine (C)-rich sequence near another exon, the introns escape branching and degradation during the splicing reaction to produce an intron lariat structure and cyclize to form a stable ciRNA[17].
BIOLOGICAL FUNCTIONS OF CIRCRNAS

CircRNAs serve in regulatory roles in different biological behaviors through different mechanisms, including acting as sponges of miRNAs, interacting with RBPs, and regulating gene transcription and translation (Figure 2). A recent review analyzed the functions of circRNAs in HCC, of which acting as miRNA sponges accounted for 79.6%[18].

Acting as miRNA sponges

As molecular sponges of miRNAs, circRNAs harbor many miRNA binding sites, which can competitively bind to and restrain the activity of miRNAs[19], thereby regulating the expression of downstream target genes posttranscriptionally. Currently, clinical studies have mainly focused on circRNAs as miRNA molecular sponges[20]. Compared with other types of competing endogenous RNAs, circRNAs have the following advantages. First, circRNAs are not easily degraded by RNA enzymes (RNase or RNA exonucleases)[21,22], which makes the circRNA structure stable and enables the possibility to stably inhibit the performance of miRNA function, with a stronger adsorption capacity for miRNAs than linear mRNAs and long noncoding RNAs. Second, existing studies have shown that the majority of circRNAs are highly expressed and that they can contain substantial miRNA response elements in a single molecule[23-25]; therefore, circRNAs are able to instantly bind or release large amounts of miRNAs to efficiently exert their regulatory roles. For example, cirs-7, also known as CDR1as, is a circRNA containing more than 70 miR-7 binding sites[26], which can bind to miR-7 and act downstream of its mRNA. This molecular axis is widely expressed in various malignancies, including oral squamous cell carcinoma and lung cancer[27,28]. In addition, circRNAs may store and transport miRNAs[29]. For example, CDR1as has both miR-7 and miR-671 binding sites[30], and CDR1as first binds to miR-7 and is transported to subcellular locations, where CDR1as is then degraded by miR-671 to eventually release miR-7[26].

It is worth noting that only circRNAs meeting specific stoichiometric requirements can act as endogenous miRNA sponges, where the abundance of circRNAs as miRNA sponges must match that of miRNAs[31]. Thus, circRNAs as miRNA sponges may not be a universal phenomenon, but one unique to some circRNAs. Only ecircRNAs can act as miRNA sponges, while EciRNAs and ciRNAs contain few miRNA binding sites that are relatively scattered; thus, EciRNA and ciRNA may lack the miRNA sponge action ability possessed by ecircRNA[17]. The dysregulation of the circRNA-miRNA-mRNA axis, whether manifesting a promoting or inhibitory role, has been confirmed in many cancers. However, the specific biological mechanism of the circRNA-miRNA-mRNA axis in the occurrence and development of tumors and whether molecular targeted therapy can be improved by intervention in this approach remain to be further studied.

Regulating parental gene transcription

Although most circRNAs are located in the cytoplasm, a fraction exists in the nucleus and participate in regulating RNA transcription. CiRNAs are abundantly expressed in the nucleus and interact with...
phosphorylated RNA polymerase II to change its transcriptional activity, thereby playing a role in transcriptional regulation [32]. For example, a circRNA (ci-ankrd52), derived from the intron of the ankyrin repeat domain 52 gene, can enhance the expression of its parent gene ankrin52 by interacting with the RNA polymerase II elongation complex [17]. ElciRNAs are intron-preserving circRNAs located near the promoter of their parent genes and bind to RNA polymerase II to improve transcription efficiency by interacting with the 5' splicing site preserved in introns, thereby promoting the expression of their parent genes [33]. Interestingly, ElciRNAs can act as RBPs sponges, like ecircRNAs, and regulate parental gene expression [34]. Additionally, circRNAs can also regulate the expression of parent genes through epigenetic modification. Recently, it has been found that certain circRNAs have N6-methyladenosine (m6A) modifications, and these circRNAs will affect the stability of the parent gene [35].

**Interacting with RBPs**

RBPs are an important class of proteins that participate in posttranscriptional regulation. RBPs interact with circRNAs and play a role in circRNA splicing, replication, stabilization, and localization. The combination of RBPs and circRNAs fulfills roles mainly in the following two ways: (1) RBPs are involved in the action of ceRNA: CircRNAs serve as miRNA “sponges” to modulate mRNA translation, and the potential of these “sponges” is higher than that of their linear counterparts because RBPs participate in the miRNA competition process [36]; and (2) CircRNAs competitively bind to RBPs: CircRNAs play biological roles by binding to RBPs through their specific sequence binding sites [37]. Here we present the most extensively studied RBP, human antigen R (HuR), as an example. HuR, as an RBP, can bind guanylate-rich elements in the 3’ untranslated region (UTR) to prevent mRNA from being degraded and accomplishes the function of stabilizing RNA structure [38, 39]. HuR is widely expressed in eukaryotic tissues [40], and circE2F2 binds to HuR and enhances the stability of the mRNA of the HuR target gene E2F2 [41]. In contrast, circRHOBTB3 binds to HuR and reduces the stability of the mRNA of HuR target gene PTBP1 [42]. In addition, circBACH1 can bind to HuR, facilitate HuR translocation to the cytoplasm and inhibit p27 translation [43].

**Encoding proteins and peptides**

CircRNAs were previously considered to be noncoding RNAs that cannot be translated into proteins. However, emerging evidence suggests that circRNAs can also be translated into proteins and peptides [44-46]. Some circRNAs initiate protein translation by binding to ribosomes via the internal ribosome
entry site (IRES) sequence or after modifying m^6A in the 5'UTR\cite{45,47}. In addition, some circRNAs with an open reading frame (ORF) can initiate small proteins or micropeptides\cite{48}. The 40S subunit of eukaryotic ribosomes binds to circRNA and directly initiates \textit{in vitro} translation\cite{49}. Furthermore, unlike other noncoding RNAs, a few ecircRNAs in the cytoplasm can be translated into functional proteins\cite{11}. Thus, the elements required for circRNA translation are IRES and an m^6A sequence or ORF. Although circRNAs have translation ability, the translation efficiency is not high because of the influence of their special ring structure, and the functions of circRNA translation products (proteins and peptides) must be further explored.

**ROLE OF CIRCRNAS IN HCC**

Recent studies have confirmed the different critical roles of aberrantly expressed circRNAs in HCC (Figure 3). Here, we summarize the roles of certain circRNAs in HCC (Table 1).

**Proliferation, cell cycle and apoptosis**

Aberrant cell cycle regulation, uncontrolled cell proliferation and blocked apoptosis are considered the main causes of malignant tumors. Accumulating studies have highlighted the important regulatory roles of circRNAs in HCC proliferation, the cell cycle and apoptosis, among which oncogenic circRNAs accelerate HCC proliferation and suppress cell cycle arrest and apoptosis. For example, circRNA ZFR serves as an oncogene to facilitate the proliferative ability of HCC by upregulating mitogen-activated protein kinase kinase1 (MAP2K1), a promoter of tumor cell proliferation\cite{50,51}. Similarly, c-Myc, a promoter of cell proliferation\cite{52}, and hsa_circ_0091581, as an oncogene, facilitates the proliferation of HCC cells by promoting c-Myc expression through sponging miR-526b\cite{53}. Furthermore, TXNDC5, a promoter of tumor cell proliferation and survival\cite{54}, and circ_0000517, an oncogene in HCC, promotes tumor growth and inhibits cell cycle arrest and apoptosis by upregulating TXNDC5 through sponging miR-1296–5p\cite{55}. Conversely, the roles of tumor suppressive circRNAs are opposite those of oncogenic circRNAs. For example, MAPK14, a suppressor of cell proliferation in HCC cells\cite{56}, and circSETD3, a tumor suppressor of HCC, enhances MAPK14 expression by sponging miR-421 in HCC, thereby inhibiting proliferation and inducing G1/S arrest\cite{57}. Similarly, exosomal circ-0051443, another tumor suppressor of HCC, upregulates the expression of BR1-associated kinase 1, a regulator of cell death, by sponging miR-331-3p, stimulating apoptosis and impeding the cell cycle\cite{58,59}. The above findings reveal the importance of circRNAs in regulating HCC cell proliferation, the cell cycle and apoptosis.

**Migration, EMT, invasion, and metastasis**

EMT is an important phenomenon in the occurrence and development of tumors and can promote the migration, infiltration and metastasis of tumor cells. Invasion and metastasis of tumor cells are the main characteristics of malignant tumors and together constitute the primary cause of death in patients with malignant tumors. Elucidating their molecular mechanisms will help to develop effective interventions for cancer. Recently, many circRNAs have been reported to regulate the progression of HCC cells by affecting migration, EMT, invasion and metastasis. For example, circ-101368 promotes high-mobility group (HMG) box 1 protein/advanced glycation end products signaling by sponging miR-200a, facilitating HCC cell migration\cite{60}. Additionally, circ-CCND1 enhances HMGA2 expression by sponging miR-497-5p, thus promoting HCC proliferation, migration and invasion\cite{61}. Similarly,
<table>
<thead>
<tr>
<th>circRNAs</th>
<th>Dysregulation</th>
<th>Mechanism by competitively binding miRNAs/RBP or m^6^A modification/mRNA braking</th>
<th>Targets/signaling pathways</th>
<th>Biological functions</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>circRNA ZFR</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>MAP2K1</td>
<td>Promotes HCC proliferation</td>
<td>Cedric et al[50]</td>
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<td></td>
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<td>miR-3619-5p</td>
<td>CTNNB1 Wnt/β-catenin pathway</td>
<td>Promotes HCC proliferation and EMT</td>
<td>Tan et al[59]</td>
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<td>hsa_circ_0091581</td>
<td>Up-regulated</td>
<td>miR-526b</td>
<td>c-Myc</td>
<td>Promotes HCC proliferation</td>
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<td>circ_0000517</td>
<td>Up-regulated</td>
<td>miR-1296-5p</td>
<td>TXNDC5</td>
<td>Promotes HCC growth and inhibits cell cycle arrest and apoptosis</td>
<td>Zang et al[55]</td>
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<td></td>
<td></td>
<td>miR-326</td>
<td>SMAD6</td>
<td>Promotes HCC invasion and metastasis</td>
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<td>circSETD3</td>
<td>Down-regulated</td>
<td>miR-421</td>
<td>MAPK14</td>
<td>Inhibits HCC proliferation and induces G1/S arrest</td>
<td>Xu et al[57]</td>
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<td>Exosomal circ-0051443</td>
<td>Down-regulated</td>
<td>miR-331-3p</td>
<td>BAK1</td>
<td>Promotes HCC cell apoptosis and arrests the cell cycle</td>
<td>Chen et al[58]</td>
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<td>circRNA_101368</td>
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<td>miR-200a</td>
<td>HMG1B/RAGE pathway</td>
<td>Promotes HCC cell migration</td>
<td>Li et al[60]</td>
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<td>circ-CCND1</td>
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<td>HMGA2</td>
<td>Promotes HCC proliferation, migration and invasion</td>
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<td>circRNA-103809</td>
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<td>miR-377-3p</td>
<td>FGFR1/ERK</td>
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<td>Zhan et al[64]</td>
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<td>PAX5</td>
<td>Promotes self-renewal of HCC stem cells</td>
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<td>RBP: FMRP</td>
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<td>m^6^A-METTL3</td>
<td>HULC and Cbf5</td>
<td>Inhibits malignant differentiation of human liver CSCs</td>
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<td>FOXK1</td>
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<td>HMGA2</td>
<td>Promotes HCC glycolysis</td>
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<td>miR-188-5p</td>
<td>HK2</td>
<td>Enhances HCC glycolysis</td>
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<td>ALX4</td>
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<td>circRNA-ITCH</td>
<td>Down-regulated</td>
<td>miR-7 or miR-214</td>
<td>c-myc and cyclinD1/Wnt/β-catenin</td>
<td>Inhibits HCC proliferation and apoptosis</td>
<td>Yang et al[103]</td>
</tr>
<tr>
<td>circ-0003418</td>
<td>Down-regulated</td>
<td>miR-7 and miR-383</td>
<td>Wnt/β-catenin pathway</td>
<td>Increases HCC sensitivity to cisplatin</td>
<td>Chen et al[104]</td>
</tr>
<tr>
<td>circ-IGFIR</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>P3K/AKT pathway</td>
<td>Promotes HCC cell proliferation</td>
<td>Fu et al[106]</td>
</tr>
<tr>
<td>hsa_circ_0079299</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>CCNBIPI3K/Akt/mTOR pathway</td>
<td>Inhibits HCC growth</td>
<td>Zheng et al[107]</td>
</tr>
<tr>
<td>circSOD2</td>
<td>Up-regulated</td>
<td>miR-502-5p</td>
<td>DNMT3A JAK2/STAT3 pathway</td>
<td>Promotes HCC growth, cell migration and cell cycle progression</td>
<td>Zhao et al[108]</td>
</tr>
<tr>
<td>circ_0004913</td>
<td>Down-regulated</td>
<td>miR-184</td>
<td>HAMP JAK2/STAT3/Akt pathway</td>
<td>Inhibits HCC proliferation, migration, invasion, EMT and glycolysis</td>
<td>Wu et al[109]</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td></td>
<td>Predicts better prognosis of HCC</td>
<td>Li et al[150]</td>
</tr>
<tr>
<td>circ_0031242</td>
<td>Up-regulated</td>
<td>miR-924</td>
<td>POU3F2</td>
<td>Enhances HCC resistance to cisplatin</td>
<td>Fan et al[112]</td>
</tr>
<tr>
<td>circARNT2</td>
<td>Up-regulated</td>
<td>miR-155-5p</td>
<td>PDK1</td>
<td>Promotes HCC resistance to cisplatin</td>
<td>Li et al[115]</td>
</tr>
<tr>
<td>circ-G004213</td>
<td>Down-regulated</td>
<td>miR-513b-5p</td>
<td>PRPF39</td>
<td>Facilitates HCC sensitivity to cisplatin</td>
<td>Qin et al[117]</td>
</tr>
<tr>
<td>circUBE2D2</td>
<td>Up-regulated</td>
<td>miR-889-3p</td>
<td>LDHA</td>
<td>Promotes HCC resistance to sorafenib</td>
<td>Huang et al[121]</td>
</tr>
<tr>
<td>circFN1</td>
<td>Up-regulated</td>
<td>miR-1205</td>
<td>E2F1</td>
<td>Facilitates HCC resistance to sorafenib</td>
<td>Yang et al[122]</td>
</tr>
<tr>
<td>circRNA-SORE</td>
<td>Up-regulated</td>
<td>RBP-YBX1</td>
<td>AKT, Raf1, ERK, c-Myc, and TGF-β</td>
<td>Promotes HCC resistance to sorafenib</td>
<td>Xu et al[124]</td>
</tr>
<tr>
<td>circMEMO1</td>
<td>Down-regulated</td>
<td>miR-106b-5p</td>
<td>TCF21</td>
<td>Increases HCC sensitivity to sorafenib</td>
<td>Dong et al[126]</td>
</tr>
<tr>
<td>circUHRF1</td>
<td>Up-regulated</td>
<td>miR-449C-5p</td>
<td>TIM-3</td>
<td>Promotes HCC resistance to PD1 immunotherapy</td>
<td>Zhang et al[131]</td>
</tr>
<tr>
<td>CircRNA</td>
<td>Regulation</td>
<td>miRNA</td>
<td>Function</td>
<td>Reference</td>
<td></td>
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<td>---------</td>
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<td></td>
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<tr>
<td>circMET</td>
<td>Up-regulated</td>
<td>miR-30-5p</td>
<td>Snail/DPP4/CXCL10 axis</td>
<td>Promotes HCC resistance to PD1 immunotherapy</td>
<td></td>
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<tr>
<td>Exosomal circ_0070396</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Serves as a biomarker of early diagnosis of HCC</td>
<td></td>
</tr>
<tr>
<td>circ_104075</td>
<td>Up-regulated</td>
<td>miR-582-3p</td>
<td>YAP</td>
<td>Serves as a biomarker of early diagnosis of HCC</td>
<td></td>
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<tr>
<td>has_circ_00224 and hsa_circ_00520</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Serves as biomarkers of early diagnosis of HCC with HCV infection</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0000976</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Serves as biomarkers of early diagnosis of HCC with HBV infection</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_007750</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0139897</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0091579</td>
<td>Up-regulated</td>
<td>N/A</td>
<td>GPC3</td>
<td>Predicts poorer prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0000798</td>
<td>Up-regulated</td>
<td>miR-646</td>
<td>N/A</td>
<td>Predicts poorer prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>has_circ_0002677</td>
<td>Up-regulated</td>
<td>miR-326, miR-532-5p</td>
<td>MAPK1</td>
<td>Predicts poorer prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>circ-ZNF652</td>
<td>Up-regulated</td>
<td>miR-203/miR-502-5p</td>
<td>Snail-mediated EMT</td>
<td>Predicts poorer prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0001649</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>circSETD3</td>
<td>Down-regulated</td>
<td>miR-421</td>
<td>MMP1</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0036683</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
<tr>
<td>hsa_circ_0000986</td>
<td>Down-regulated</td>
<td>N/A</td>
<td>N/A</td>
<td>Predicts better prognosis of HCC</td>
<td></td>
</tr>
</tbody>
</table>

HCC: Hepatocellular carcinoma; ceRNA: Competitive endogenous RNA; CircRNAs: Circular RNAs; miRNAs: MicroRNAs; RBPs: RNA binding proteins; m6A: N6-methyladenosine; EMT: Epithelial-mesenchymal transition; MAP2K1: Mitogen-activated protein kinase 1; CTNNB1: Beta-catenin; 1; Wnt/beta-catenin; Wingless/beta-catenin; TXNDC5: Thioredoxin domain-containing 5; SMAD6: SMAD family member 6; MAPK14: Mitogen-activated protein kinase 14; MV1: Microvascular invasion; BAK1: BCL2-asssociated kinase 1; HMG2B1/RAGE: High-mobility group box 1 protein/advanced glycation end products; HMG2A2: High mobility group A2; PLAGL2: Phosphoinositide-3-kinase regulatory subunit 3; SERBP1: SERPINE1 mRNA binding protein 1; FGFRI/ERK: Fibroblast growth factor receptor 1/extracellular signal-regulated kinase; PLAKGL2: PLAG1 like zinc finger 2; MMP2: Matrix metalloproteinase 2; PAX5: Paired box protein 5; AUF1: AU-rich binding factor 1; FMRP: Fragile X mental retardation protein; CCAR1: Cell division cycle and apoptosis regulator 1; METTL3: Methyltransferase-like 3; HULC: Highly upregulated in liver cancer; HIF-1alpha: Hypoxia inducible factor-1 alpha; H1N1: Hematological and neurological expressed 1; ULBP1: UL16-binding protein 1; TET1: Ten-eleven translocation 1; ICAM-1: Intercellular adhesion molecule-1; RTKN2: Rhethin 2; DKK3: Dickkopf-3; P3KAK: Phosphoinositide-3-kinase/protein kinase B; CCNB1: Cyclin B1; DNM3TA: DNA methyltransferase 3a; JAK/STAT: Janus kinases/signal transducer and activator of transcription; JAK2/STAT3: Janus kinase 2/signal transducers and activators of transcription; HAMP: Hepcidin; POUM2: POU class 3 homeobox 2; PDK1: Pyruvate dehydrogenase kinase 1; PRP39: PremRNA splicing factor 39; LDHA: Lactate dehydrogenase A; E2F1: E2F transcription factor 1; YBX1: Y-box-binding protein 1; Raf1: Proto-oncogene serine/threonine-protein kinase-1; ERK: Extracellular signal-regulated kinase; TGF-β1: Transforming growth factor beta 1; TCF21: Transcription factor 21; TIM-3: T cell immunoglobulin and mucin domain 3; PD1: Programmed cell death protein 1; DPP4: Dipeptidyl peptidase 4; CXCL10: Chemokine C-X-C motif ligand 10; Yap: Yes-associated protein; HCV: Hepatitis C virus; HBV: Hepatitis B virus; GPC3: Glypican-3 protein; MAPK1: Mitogen-activated protein kinase 1; MMP1: Matrix metalloproteinase 1; MAPK1: Mitogen-activated protein kinase 1; AFP: Alpha fetoprotein; AFP-L3: Alpha-fetoprotein variants; DCN: Des-carboxy prothrombin; OS: Overall survival; RFS: Recurrence-free survival; PFS: Progression-free survival; N/A: Not applicable.
critical for regulating HCC migration, EMT, invasion and metastasis.

**CSCs**
CSCs are considered the root cause of tumor occurrence, invasion, metastasis, recurrence, and resistance to radiotherapy and chemotherapy because of their self-renewal ability, sustained proliferation potential and therapeutic resistance. circRNAs and tumor stem cells are closely related to cancer. For example, the high expression of circ-MALAT1 in HCC CSC samples mediated by RBP AU-rich binding factor 1 is closely associated with the regeneration of HCC CSCs. Mechanistically, circ-MALAT1 blocks paired box protein 5 mRNA translation on the ribosome and forms a trimer with the ribosome and mRNA to facilitate self-renewal of CSCs. This blocking mechanism is known as “circRNA braking” and has become another posttranscriptional regulatory mechanism in addition to the function of circRNA sponges.[69] Additionally, circZKSCAN1 inhibits HCC stem cell activity by mediating the function of fragile X mental retardation protein (FMRP). Regarding the mechanism, circZKSCAN1 competes with FMRP, which serves as RBP, for the target gene cell division cycle and apoptosis regulator 1 (CCAR1), thereby inactivating the Wingless (Wnt) pathway.[70] Similarly, circMEG3 inhibits malignant differentiation of CSCs by restraining highly upregulated in liver cancer and centromere-binding factor 5 in HCC CSCs.[71] The above findings indicate that circRNAs may provide novel treatment strategies for HCC by targeting CSCs.

**Glycolysis**
Aberrant glucose metabolism is the most prominent feature of tumor metabolism. In recent years, numerous studies have shown that circRNAs regulate glucose metabolism, among which oncogenic circRNAs promote glycolysis in HCC cells. For example, Forkhead box K1 (FOXK1) is an inducer of aerobic glycolysis,[72] and circ-PRKCI promotes HCC glycolysis by enhancing FOXK1 expression by sponging miR-1294 and miR-186-5p.[73] Similarly, HMG2 promotes HCC tumor growth and metastasis,[74] and circZFR promotes glycolysis in HCC cells by inhibiting miR-375 and increasing HMG2 expression.[75] Furthermore, PKM2 serves as a mediator of aerobic glycolysis of cancer cells [76], and circMAT2B facilitates HCC glycolysis by strengthening PKM2 expression by acting as a sponge of miR-338-3p.[77] Hexokinase 2 (HK2) is also a regulator of aerobic glycolysis in HCC.[78], and circ-PRMT5 promotes HCC glycolysis by sponging miR-188-5p to increase HK2 expression.[79] In contrast, tumor suppressive circRNAs impede HCC glycolysis. For example, aristless-like homeobox 4 (ALX4) inhibits HCC proliferation and invasion,[80] and circ_0001445, a tumor suppressor, enhances ALX4 expression by sponging miR-942-5p, thus inhibiting HCC glycolysis.[81] Collectively, circRNAs have become important regulatory factors in glycolysis in HCC cells, but the specific mechanism of their regulation of metabolism remains to be elucidated. Considering the characteristics of circRNAs in regulating glycolysis in HCC cells, it is possible to interfere with the abnormal expression of downstream genes and some key action sites of specific circRNAs, thereby altering the metabolic pathways of HCC cells and opening up novel therapeutic approaches for HCC.

**MVI**
MVI is a characteristic of HCC and an independent risk factor affecting the prognosis of HCC patients. The exact mechanism by which MVI occurs in HCC has not been fully elucidated. Emerging evidence suggests that circRNAs play important roles in the MVI process of HCC. For example, ciRS-7 (Cdr1as), an oncogene in HCC,[82] facilitates HCC MVI by competitively inhibiting miR-7 and interfering with the PI3Kdelta catalytic p110delta/ribosomal protein S6 kinase/mammalian target of rapamycin (mTOR) pathway.[83] Conversely, the downregulation of hsa_circ_0068669, a tumor suppressor, is correlated with HCC MVI.[84] Similarly, low expression of circSETD3, another tumor suppressor, in HCC is associated with the existence of MVI.[85] In summary, circRNAs are associated with the occurrence of MVI in HCC and can be used as indicators for the early detection of MVI and clinical intervention to reduce recurrence and improve the survival rate of patients with HCC.

**Angiogenesis**
HCC is a solid tumor rich in blood vessels with obvious vascular hyperplasia and vascular abnormalities in HCC. Tumor angiogenesis refers to tumor-induced capillary angiogenesis and the formation of microcirculation networks within the tumor. Tumor angiogenesis is responsible for HCC proliferation, invasion and metastasis. Nevertheless, the regulatory mechanism underlying HCC angiogenesis is unclear, although multiple studies have found that circRNAs can regulate angiogenesis. For example, circCRIM1 can promote HCC angiogenesis by upregulating SKP2 expression via sponging miR-378a-3p [86]. Additionally, hsa-circ-0046600 affects malignant angiogenesis in HCC cells by sponging miR-640 to facilitate the expression of hypoxia inducible factor-1α, a promoter of angiogenesis.[87,88] Similarly, hsa_circ_0000092 facilitates HCC angiogenesis by competitively binding to miR-338-3p to elevate the expression of hematological and neurological expressed 1, a promoter of tumor growth and invasion.[89, 90] Furthermore, circGFA1 promotes the angiogenic activity of HCC by binding to miR-149[91]. Taken together, the above findings confirm that circRNAs play an essential role in HCC angiogenesis, thus contributing to clarification of the regulatory mechanism of HCC angiogenesis and highlighting the
usefulness of circRNAs in targeted therapy for HCC angiogenesis.

**Immune surveillance and immune escape**

Abnormal circRNAs may act as tumor antigens in immunocytes to activate antitumor immunity[92]. Natural killer (NK) cells play a pivotal role in tumor immune surveillance. CircAR5F91 increases the cytotoxicity of NK cells by elevating UL16-binding protein 1 in HCC, thereby enhancing innate immune surveillance[93].

The immune system monitors and kills tumor cells through specific and nonspecific pathways. When malignant cells appear in the body, the immune system recognizes and eliminates these cells specifically through the immune mechanism to resist the occurrence and development of tumors. However, in some cases, malignant cells can escape the recognition and attack of the immune system through various mechanisms to achieve immune escape in order to survive and proliferate in the body[94]. Current studies have shown that circRNAs play a critical role in tumor immune escape, which is closely associated with drug resistance and tumor recurrence[95]. For example, the low expression of tumor suppressive circTRIM33-12 promotes the immune escape ability of HCC cells by upregulating ten-eleven translocation 1 expression through sponging miR-191[96]. Similarly, hsa_circ0007456, another tumor suppressor, shows low expression in HCC and can promote tumor immune escape by regulating the expression of intercellular adhesion molecule-1 by sponging miR-6852-3p[97]. These findings indicate that circRNAs that regulate immune escape are promising immunotherapeutic targets for HCC.

**Modulating the malignant progression of HCC by mediating signaling pathways**

Various circRNAs mediate the Wnt/beta-catenin (Wnt/β-catenin), phosphoinositide-3-kinase/protein kinase B (PI3K/Akt) or Janus kinase 2/signal transducers and activators of transcription (JAK2/Stat3) pathways by sponging miRNAs to modulate the malignant progression of HCC. In addition to circRNA-miRNA regulation, no study has investigated circRNAs modulating these signaling pathways through direct regulation of processes such as gene transcription and protein translation.

**Wnt/β-catenin pathway:** Aberrant activation of this pathway is prevalent in HCC occurrence and progression, and this is considered the most frequently activated carcinogenic pathway in HCC[98]. Emerging evidence suggests that circRNAs affect the malignant progression of HCC by mediating the Wnt/β-catenin pathway, among which oncogenic circRNAs can promote HCC progression by triggering the Wnt/β-catenin pathway. For example, circZFR upregulates beta-catenin 1 and activates the Wnt/β-catenin pathway by sponging miR-3619-5p to promote the proliferation and EMT of HCC cells[99]. Similarly, hsa_circ_104348 facilitates HCC proliferation, migration, and invasion by sponging miR-187-3p to elevate rhoetin 2 expression and activate the Wnt/β-catenin pathway[100]. In particular, circβ-catenin, an oncogenic circRNA in HCC, facilitates HCC cell growth by activating the Wnt/β-catenin pathway[101]. Instead, tumor suppressive circRNAs can restrain HCC progression by inhibiting the Wnt/β-catenin pathway. For example, hsa_circ_0004018 enhances Dickkopf-3 expression and inhibits the Wnt/β-catenin pathway by sponging miR-626, thereby restraining HCC proliferation and migration[102]. Similarly, circRNA-ITCH restrains the Wnt/β-catenin pathway and decreases c-myc and cyclin D1 expression by sponging miR-7 or miR-214, thereby inhibiting HCC proliferation and apoptosis[103]. Intriguingly, circRNA-ITCH inhibits the Wnt/β-catenin pathway[104].

**PI3K/Akt/mTOR pathway:** Aberrant activation of this pathway frequently occurs in HCC and is closely related to HCC growth[105], invasion and metastasis. Current studies support that circRNAs mediate the PI3K/Akt or PI3K/AKT/mTOR pathway to modulate HCC progression. For example, circ-insulin-like growth factor 1 receptor promotes HCC cell proliferation by activating the PI3K/AKT pathway[106]. Additionally, the overexpression of tumor-suppressive hsa_circ_0079299 inhibits HCC growth and retards cell cycle progression partly by modulating the PI3K/Akt/mTOR pathway[107].

**JAK2/STAT3 pathway:** As a signal transduction pathway stimulated by cytokines, activation of the JAK/STAT pathway is closely related to tumor cell proliferation, apoptosis and differentiation. The JAK2/STAT3 pathway, an important component of the JAK/STAT pathway, is activated in diverse malignant tumors, including HCC. For example, circSOD2 enhances DNA methyltransferase 3A expression and activates the JAK2/STAT3 pathway by sponging miR-502-5p, thereby promoting the growth, migration and cell cycle progression of HCC cells[108]. Additionally, CIRC_0004913 upregulates hepcidin expression and inhibits the JAK2/STAT3/Akt pathway by sponging miR-184 and suppressing HCC proliferation, migration, invasion, EMT and glycolysis[109]. Taken together, the above findings demonstrate that circRNAs modulate the malignant progression of HCC by mediating signaling pathways, such as the Wnt/β-catenin, PI3K/Akt/mTOR and JAK2/Stat3 pathways. These pathway-associated circRNAs may serve as novel therapeutic targets in HCC.

**Chemo-resistance**

Chemotherapy is a comprehensive treatment for advanced HCC, although the drug resistance of HCC cells considerably limits its efficacy. Multidrug resistance is the principal factor leading to the failure of
chemotherapy for HCC, and its mechanism is extremely complex. Therefore, clarifying the mechanisms of drug resistance to improve the drug resistance of patients with HCC is critical. Recent evidence has prioritized the importance of abnormally expressed circRNAs in the chemotherapy resistance of HCC.

**Cisplatin resistance:** Cisplatin is one of the few most common chemotherapy drugs used to treat HCC. However, thus far, the drug resistance of HCC cells during chemotherapy has been revealed to be the main factor affecting chemotherapy failure[110,111]. Therefore, how to control the occurrence of cisplatin resistance in HCC cells and improve drug sensitivity and therapeutic effects are critical to prolonging the survival of patients with advanced HCC. Current studies have confirmed that circRNAs impact HCC cisplatin resistance. For example, circ_0031242 enhances cisplatin resistance in HCC by sponging miR-924 to enhance the expression of POU class 3 homeobox 2, a promoter of tumor progression and metastasis[112,113]. Additionally, pyruvate dehydrogenase kinase 1 (PDK1), a glycolytic enzyme, is closely associated with chemotherapy resistance[114]. As an oncogene, circARNT2 promotes cisplatin resistance in HCC cells, an activity mechanistically achieved by upregulating PDK1 through sponging miR-155-5p[115]. Analogously, PRPF39 is closely associated with cisplatin sensitivity[116], circ-G004213 promotes HCC cisplatin sensitivity by sponging miR-513b-5p to increase PRPR39 expression[117].

**Sorafenib resistance:** Sorafenib is an oral multikinase multitarget inhibitor and an important targeted therapy for advanced HCC[118]. However, sorafenib resistance is a common problem in clinical applications, substantially limiting its application[119]. The mechanism leading to sorafenib resistance remains incompletely understood. Therefore, further research on the possible mechanisms of sorafenib resistance and reducing its resistance are crucial for the treatment of HCC. CircRNAs also affect sorafenib resistance in HCC. For example, overexpression of lactate dehydrogenase A (LDHA), an oncogene, facilitates cancer cell invasion and metastasis[120]. CircUBE2D2 promotes sorafenib resistance to HCC, possibly because of the upregulation of LDHA by sponging miR-889-3p[121]. Additionally, circFN1 contributes to sorafenib resistance in HCC cells by elevating the expression of E2F1, a transcription factor associated with cancer chemotherapy resistance, by acting as a miR-1205 sponge[122,123]. Analogously, circRNA-SORE induces sorafenib resistance in HCC by binding to Y-box-binding protein 1, a regulator of EMT in cancer cells[124,125]. In particular, circMEMO1 promotes the sensitivity of HCC to sorafenib by upregulating transcription factor 21 (TCF21) expression by sponging miR-106b-5p[126].

Although the existing evidence partially reveals the critical role of circRNAs in HCC chemotherapy resistance, it suggests that circRNAs associated with chemotherapy resistance offer potential value in predicting and monitoring the efficacy of HCC and even reversing chemotherapy resistance. However, further clinical samples and in vivo experiments are needed to validate the relevant molecular mechanisms involved.

**Immunotherapy resistance**

Immunotherapy is currently an effective therapeutic modality for advanced HCC. Immunotherapy enhances antigen presentation, activates the immune response and improves the immunosuppressive status of the tumor microenvironment in different ways, thus improving survival benefits. However, increasing clinical evidence indicates that only 20%-30% of patients treated with programmed death 1 (PD1) and programmed death-ligand 1 are sensitive to immunotherapy, and 70%-80% of patients show an ineffective response because of drug resistance[127]. Therefore, further exploration and understanding of the mechanism of immunotherapy resistance may provide important insight to guide clinical practice. T cell immunoglobulin and mucin domain 3 (TIM-3) is an immunoregulatory receptor that binds to NK cell-dominated ligands in tumor cells and the microenvironment to inhibit NK cell-mediated antitumor immunity in various cancers, including HCC[128-130]. CircUHRF1, an exosome-derived from HCC, upregulates TIM-3 expression in NK cells by sponging miR-449C-5p in patients' resistant to PD1 immunotherapy, leading to NK cell dysfunction and driving HCC resistance to PD1 [131]. Additionally, circMET is an oncogene in the chromosome 7q21-7q31 region, and the amplification of this region is considered to be related to HCC prognosis[132]. CircMET overexpression induces the development and immune tolerance of HCC through the miR-30-5p/Snail/dipeptidyl peptidase (DPP) 4/chemokine C-X-C ligand (CXCL) 10 axis, while DPP4 inhibitors such as sitagliptin block the progression of the pathway, which can enhance the efficacy of PD1 inhibitors in the treatment of HCC [133]. Taken together, the above findings demonstrate that circRNAs participate in regulating HCC immunotherapy resistance, and that intervention by circRNAs may be an effective means to improve the immunotherapy tolerance of HCC cells.

**BIOMARKERS FOR HCC DIAGNOSIS AND PROGNOSIS**

CircRNAs are characterized by high abundance, stability and conservatism. CircRNAs are not easily degraded by RNA enzymes and stably exist in human tissues, serum, saliva and urine. Additionally, the expression profiles of circRNAs in HCC patients are significantly different from those of normal
controls. Thus, abnormally expressed circRNAs may be utilized as biomarkers to diagnose and predict the prognosis of HCC patients[134-136].

**Biomarkers of the early diagnosis of HCC**

There are certain limitations of commonly used clinical diagnostic markers for HCC, such as alphafetoprotein (AFP), AFP variants (AFP-L3) and Des-carboxy prothrombin (DCP), and only approximately 1/3 of patients can be diagnosed early[137,138]. The high mortality rate of HCC indicates that exploring new biomarkers for the early diagnosis of HCC is the most reliable strategy to improve the survival rate of HCC patients.

Emerging evidence thus far supports the possibility of utilizing circRNAs as ideal biomarkers to diagnose HCC. For example, exosome CIRC_0070396 has better diagnostic accuracy than AFP with respect to HCC patients[139]. Analogously, the sensitivity (96.0%) and specificity (98.3%) of serum circ_104075 to predict HCC are higher than those of AFP, DCP and AFP-L3, indicating the possibility of employing circ_104075 as an effective serum biomarker for HCC diagnosis[140]. Additionally, compared with AFP, hsa_circ_00224 and hsa_circ_00520 show higher sensitivity and specificity in diagnosing HCC patients with hepatitis C virus infection[141]. Furthermore, the accuracy of plasma hsa_circ_0000976, hsa_circ_0007750, and hsa_circ_0139897 is superior to AFP in diagnosing HCC patients with hepatitis B virus infection[142].

Although the existing evidence supports the feasibility of using specific circRNAs as noninvasive circulating diagnostic biomarkers for the early detection and screening of HCC, further analysis of their sensitivity and specificity and suitable patient populations is warranted. The pathogenesis of HCC is extremely complex and varies among ethnic and regional populations, and circRNAs that can be used as biomarkers in single-center studies may not be applicable to other ethnic and regional populations. Therefore, multicenter trials and large-scale studies are required to verify the performance of serum or plasma circRNAs as biomarkers. Additionally, it is necessary to establish accepted standards, unified detection and analysis methods and to use a rigorous experimental design with the best clinical samples to determine universally representative and practical diagnostic circRNA molecules.

**Prognostic biomarkers of HCC**

Because of the delay in diagnosis and the high rates of postoperative recurrence and metastasis, the prognosis of HCC patients remains poor[143]. Therefore, exploring more effective HCC markers for prognosis assessment is crucial. Existing evidence has shown the feasibility of circRNAs as biomarkers to predict HCC prognosis. Among these circRNAs, oncogenic circRNAs are associated with worse overall survival (OS) or worse OS and recurrence-free survival (RFS). For example, high expression of hsa_circ_0091579 or circ_0000798 is correlated with shorter OS of HCC patients[144,145]. Similarly, high expression of circ_0000267 or circ_0139897 is closely related to shorter OS in HCC patients[146,147]. Additionally, high circ-ZNF652 (hsa_circ_0003258) expression indicates shorter OS and RFS of HCC patients[148]. Conversely, tumor suppressive circRNAs are associated with better OS and RFS or better OS and progression-free survival (PFS). For example, high expression of hsa_circ_001649 or circ_0014913 signifies longer OS in HCC patients[149,150]. Furthermore, high circSETD3 or hsa_circ_0036683 expression indicates better OS and RFS in HCC patients[151]. Moreover, high hsa_circ_005986 expression implies better OS and PFS in HCC patients[152]. The above findings support the feasibility of the use of circRNAs as biomarkers for predicting HCC prognosis.

**CONCLUSION**

In conclusion, circRNAs play important roles in HCC and are expected to be ideal diagnostic biomarkers and therapeutic targets for HCC. However, problems persist that must be solved. First, determining the exact mechanism underlying certain circRNAs in pathogenesis is challenging because of the different nomenclatures of circRNAs, mechanisms of action and tumorigenicities. Second, current studies on circRNAs mainly focus on the function of circRNAs as molecular sponges. We should further explore the biological functions of circRNAs, such as regulating the transcription of parental genes, binding RBPs, and encoding proteins and peptides, in the context of the malignant behavior of HCC. Third, some studies have only investigated circRNA expression in HCC cell lines without detection in clinical samples, and the clinical value of such circRNAs is uncertain. Fourth, most of the studies only knocked down the expression level of circRNAs but did not perform reverse verification by overexpression of circRNAs. Fifth, presently, studies on the pathogenesis of circRNAs in HCC remain in the preliminary stage. The pathogenesis of HCC is complex and heterogeneous, and the disease states of different HCC patients may involve different primary pathogenetic pathways and pathogenic molecules. Exploring the pathogenesis of a certain class of HCC patients with stronger homogeneity at the beginning of the experimental design is crucial to obtain more reproducible conclusions. In summary, we must improve these issues to better clarify the roles and mechanisms of circRNAs in HCC so that circRNAs can become useful diagnostic indicators and therapeutic targets for HCC.
FOOTNOTES

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Practical considerations for colorectal cancer screening in older adults

Dana Gornick, Anusri Kadakuntla, Alexa Trovato, Rebecca Stetzer, Micheal Tadros

**Abstract**

Recent guidelines recommend that colorectal cancer (CRC) screening after age 75 be considered on an individualized basis, and discourage screening for people over 85 due to competing causes of mortality. Given the heterogeneity in the health of older individuals, and lack of data within current guidelines for personalized CRC screening approaches, there remains a need for a clearer framework to inform clinical decision-making. A revision of the current approach to CRC screening in older adults is even more compelling given the improvements in CRC treatment, post-treatment survival, and increasing life expectancy in the population. In this review, we aim to examine the personalization of CRC screening cessation based on specific factors influencing life and health expectancy such as comorbidity, frailty, and cognitive status. We will also review screening modalities and endoscopic technique for minimizing risk, the risks of screening unique to older adults, and CRC treatment outcomes in older patients, in order to provide important information to aid CRC screening decisions for this age group. This review article offers a unique approach to this topic from both the gastroenterologist and geriatrician perspective by reviewing the use of specific clinical assessment tools, and addressing technical aspects of screening colonoscopy and periprocedural management to mitigate screening-related complications.

**Key Words:** Colorectal cancer; Colonoscopy; Cancer screening; Early detection of cancer; Aged; Elderly

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Core Tip: Clinical guidelines do not recommend colorectal cancer (CRC) screening after age 75. Given the improvements in CRC treatment and post-treatment survival, and increasing life expectancy in the population, the current approach to CRC screening in older adults needs to be revised. This review examines the personalization of CRC screening in older individuals based on specific factors influencing life and health expectancy. Screening modalities, techniques, and risks are also discussed.

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INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in both men and women in the United States, with almost 150000 total cases in 2020[1]. CRC is predominantly a disease of older adults, as the risk increases markedly with age[2]. CRC incidence is 59.5 per 100000 for individuals aged 50 to 54 years, but increases to 197.5 per 100000 for adults aged 75 to 79 years[1]. The relative survival rate for CRC is 64% at 5 years following diagnosis and 58% at 10 years. The most important predictor of CRC survival is stage at diagnosis. The 5-year survival rate is 90% for patients diagnosed with localized-stage disease, but declines to 71% and 14% for those diagnosed with regional and distant stages, respectively[1]. The percentage of CRC deaths is highest among people aged 65 to 74 years of age at 24.2%. 23.6% of CRC deaths occur among people aged 75 to 84 years, and 20.3% among people 84 years and older[3].

CRC screening is an opportunity to both decrease incidence and increase survival by detection and removal of growths at early stages. Recent guidelines recommend that CRC screening after age 75 be considered on an individualized basis, and that providers engage in shared decision making for screening individuals in this category[4-6]. CRC screening is discouraged for people over 85 due to competing causes of mortality[5,6]. Although these guidelines provide a general framework for screening older individuals, the evidence provided is not robust, as most of the studies in older patients referenced by the guidelines are either microsimulation, meta-analysis, or studies with few patients above 80 years of age[4,7,8]. Guidelines acknowledge that few empirical data exist on when best to stop offering screening, yet continue to use 75 years and 85 years as screening upper age limits. Given the heterogeneity in the health of older individuals and lack of data for personalized CRC screening approaches, there remains a need for a clearer framework to inform clinical decision-making. While CRC diagnoses and mortality are still highest in older adults, treatment of CRC has evolved dramatically, with studies showing improved survival in older patients. The current US population has over 13 million individuals between ages 75 and 85, and is expected to increase to over 28 million by 2050[9,10].

The expansion of this age group coupled with increasing life expectancy requires reevaluation of the current approach to CRC screening decisions in the older population.

Currently, for CRC, there is lack of consistency in best practices to screen older individuals. Some studies indicate that screening is underutilized in older individuals and those with comorbidities[11,12]; other studies indicate that this population may be more likely to undergo screening, potentially due to frequent contact with the healthcare system[11-13]. Nevertheless, this inconsistency in the application of consensus guidelines to clinical practice indicates that knowledge gaps persist when deciding which older patients should be offered CRC screening.

A basic tenet of cancer screening is that the benefits of screening should outweigh the harms. This necessitates a reliable means for assessing the risks and estimating if a person has enough of a life and health expectancy to realize screening benefit. CRC screening is associated with short-term risks, including complications from colonoscopy, overdiagnosis, and treatment of tumors that may not have led to symptoms. Appropriate utilization of screening in older adults requires a balance between short-term screening risks and long-term benefits of early detection. Another important consideration in recommending screening is patient preference, as a patient’s values may dictate willingness to be screened.

In this review, we will examine the personalization of CRC screening in older individuals based on specific factors influencing life and health expectancy. This includes age, frailty, cognitive status, and disease burden, as these factors more accurately reflect life expectancy rather than age alone. We will also review the risks of screening unique to older adults, screening modalities and endoscopic technique for minimizing risk, and CRC treatment outcomes in older patients, in order to provide important information to aid CRC screening decisions for this age group (Figure 1). This review article offers a unique approach to this topic from both the gastroenterologist and geriatrician perspective by reviewing the use of specific clinical assessment tools, and addressing technical aspects of screening colonoscopy and periprocedural management to mitigate screening-related complications.
Figure 1 Practical considerations for colorectal cancer screening in older adults.

**FACTORS FOR DECIDING WHO TO SCREEN**

Guidelines currently recommend that patients with reduced life expectancy should not be screened for CRC[4-6]; however, they do not provide a method to estimate life expectancy in the clinical setting[10, 14] (Table 1). A consistent method to estimate life expectancy is critical to determine the patient population who would benefit from screening. Randomized trials have suggested that the mortality difference between screened and unscreened patients becomes noticeable only after 5 or more years after screening[15,16]. Similarly, the benefits of polypectomy are delayed by 7–10 years after screening has occurred[17] and, thus, screening is of limited benefit for those with lower life expectancy. Conversely, patients with a longer life expectancy could gain more years of life from screening.

The research regarding CRC screening in older individuals is limited, many studies either do not take factors beyond age into account, or were conducted in patients younger than 75 years. The lack of high quality data that are stratified by more than age is significant. For example, Lin et al[8] found that patients aged 80 years or older had a mean extension of life expectancy with screening colonoscopy of only 0.13 years, compared to an average gain of 0.85 years for patients 50 to 54 years-old, a 6.5 fold difference. However, this study only took age into account, and did not consider comorbidity, frailty, or any other modifying factors that affect life expectancy. The conclusion that older age is associated with less years gained from screening may or may not be applicable to a given older individual, depending on their overall health and functional status.

When considering screening an older patient, chronological age must be supplemented. Factors such as comorbid status, frailty, cognitive function, and patient priorities[18,19] are important tools for doing so (Figure 2).

**Comorbidity**

Comorbidity status has a strong influence on life expectancy in older adults[20]. A study by DuGoff et al [21] evaluating chronic conditions and life expectancy found that the average marginal decline in life expectancy is 1.8 years with each additional chronic condition. The study found that a 67-year-old individual with no chronic conditions will live on average 22.6 additional years, while a 67-year-old individual with 5 chronic conditions will live 7.7 fewer years, and for greater than 10 chronic conditions, 17.6 fewer years[21].

Another study by Cho et al[20] evaluating Medicare claims data and the Charlson Comorbidity Index (CCI) found that persons with higher levels of comorbidity had shorter life expectancies, whereas those with no comorbid conditions, including oldest adults, had favorable life expectancies relative to an average person of the same chronological age. The study found substantial variation between estimated life expectancies for healthy persons without comorbidity and those with high levels of comorbidity, or with specific conditions, such as diabetes, CHF, and COPD. Details about the CCI are summarized in Table 2.
Table 1 Comments on colorectal cancer screening across professional organizations

<table>
<thead>
<tr>
<th>Professional organization</th>
<th>Recommended ages for screening</th>
<th>Other considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>American College of Gastroenterology (2021)</td>
<td>50-75</td>
<td>Screening after age 75 should be considered on an individualized basis; providers must engage in shared decision making</td>
</tr>
<tr>
<td>United States Preventive Services Task Force (2021)</td>
<td>45-75</td>
<td>Screening adults aged 76-85 should be conducted on an individualized basis; do not screen adults age 86 years and above</td>
</tr>
<tr>
<td>United States Multi-Society Task Force on Colorectal Cancer (2021)</td>
<td>45-75</td>
<td>Consider discontinuation when persons up to date with screening, who have prior negative screening reach age 75 or have &lt; 10 yr of life expectancy. Persons without prior screening should be considered for screening up to age 85, depending on age and comorbidities.</td>
</tr>
<tr>
<td>Canadian Task Force on Preventive Health Care (2016)</td>
<td>50-74</td>
<td>Recommend not screening adults aged 75 yr and older. (Weak recommendation; low-quality evidence)</td>
</tr>
<tr>
<td>American College of Physicians (2019)</td>
<td>50-75</td>
<td>Discontinue screening in average-risk adults older than 75 yr or in adults with a life expectancy of 10 yr or less</td>
</tr>
<tr>
<td>American Cancer Society (2018)</td>
<td>45-75</td>
<td>Screening adults aged 76-85 should be conducted on an individualized basis; screening discouraged above age 85</td>
</tr>
</tbody>
</table>

Adapted from Ref. [14].

Table 2 Charlson comorbidity index conditions and scoring

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 50, 50-59, 60-69, 70-79, 80+</td>
<td>0, +1, +2, +3, +4</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>CHF</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>PVD</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>CVA or TIA</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>Dementia</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>COPD</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>Connective tissue disease</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>Peptic ulcer disease</td>
<td>Yes/no</td>
<td>+1</td>
</tr>
<tr>
<td>Liver disease</td>
<td>None/mild/severe</td>
<td>+1 (mild), +3 (moderate-severe)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>None or diet controlled / uncomplicated/ end-organ damage</td>
<td>+1 (uncomplicated), +2 (end-organ damage)</td>
</tr>
<tr>
<td>Hemiplegia</td>
<td>Yes/no</td>
<td>+2</td>
</tr>
<tr>
<td>CKD</td>
<td>Yes/no</td>
<td>+2</td>
</tr>
<tr>
<td>Solid tumor</td>
<td>None/localized/metastatic</td>
<td>+2 (local), +6 (metastatic)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Yes/no</td>
<td>+2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>Yes/no</td>
<td>+2</td>
</tr>
<tr>
<td>AIDS</td>
<td>Yes/no</td>
<td>+6</td>
</tr>
</tbody>
</table>

CHF: Congestive heart failure; PVD: Peripheral vascular disease; CVA: Cerebrovascular accident; TIA: Transient ischemic attack; COPD: Chronic obstructive pulmonary disorder; CKD: Chronic kidney disease; AIDS: Acquired immunodeficiency syndrome.

The total number of chronic illnesses as well as specific comorbid conditions should also be considered prior to initiating a screening colonoscopy. A systematic review by Søgaard et al.[22] found that there is a 1.2- to 4.8-fold higher 5-year mortality for CRC patients with comorbidity versus without comorbidity. The total number of chronic illnesses as well as specific comorbid conditions should also be considered prior to initiating a screening colonoscopy. Even if a patient’s comorbid conditions do not specifically affect the procedure, a larger number of comorbidities may indicate decreased ability to compensate for and recover from stresses. These comorbidities, combined with normal physiologic changes of aging, create a state of homeostenosis, or decreased physiologic reserve[23]. This renders
patients at higher risk for complications from even low-risk procedures, such as screening colonoscopy.

Coexisting chronic illness is associated with a substantial reduction in life expectancy after diagnosis of early-stage CRC[22]. Screening colonoscopies may also put these patients at higher risk of complications[23]. This, along with the decrease in life expectancy associated with comorbid conditions in general, should be considered when deciding whether to screen older persons.

Frailty
Frailty is a reflection of homeostasis. It is a state of vulnerability to stressors and increased risk of adverse health outcomes due to multisystem decline in physiologic reserve and function[24]. Clinically, it presents as slow gait speed, exhaustion, weight loss, and decreased grip strength and physical activity. The presence of frailty has been shown to be predictive of falls, worsening mobility, ADL disability, hospitalization, and death. It provides important information about functional status and longevity[24,25]. Cancer patients defined as being functionally dependent according to the validated instrument of daily living were found to have a 2- to 3-fold increased risk of postoperative morbidity compared with those defined as independent[26]. In the perioperative setting, the presence of frailty has been shown to independently predict postoperative complications, length of stay, likelihood of discharge to subacute nursing facilities, and mortality at 30 d and 6 mo[27].

Given that frailty has been associated with poor survival, evaluating an individual’s degree of frailty is critical when making decisions to continue CRC screening[19]. There are various published frailty scoring tools, but none have been established as the standard or best method in this setting. The most well-known is the Fried score, which measures specific criteria for unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and low physical activity[24]. However, the details needed for the Fried and many other frailty scores are often time-consuming and may require special training or tools beyond the scope of a typical outpatient office[28].

Other frailty and risk assessment tools, such as gait speed, have been evaluated and validated. Of these, the authors feel the most accessible tool in the primary care or preoperative setting is the clinical frailty scale (CFS). The CFS has been developed for rapid frailty screening without the need for specific geriatric expertise or functional testing and has been validated against the very detailed Canadian Study of Health and Aging 70-item frailty index[29]. A recent multicenter, prospective study evaluated CFS score and postoperative complications in CRC patients over the age of 80. The study found that postoperative complications were significantly more common in patients with a CFS score of 3 and above. Interestingly, age was not found to affect postoperative outcomes[30]. Details about how to apply and interpret the CFS are provided in Figure 3.

ePrognosis
ePrognosis is a web-based tool that facilitates shared decision-making among clinicians and patients about colorectal and breast cancer screening. It uses a point system that assigns points based on health behaviors, disease burden, and function that are most highly associated with 10 years chance of death. Its calculator is based on data collected from The National Health Interview Survey and the Health and Retirement Survey. After data are collected, a pictograph representation of the risks versus benefits of
Cognitive status

Although not a normal part of aging, cognitive decline and dementia are common geriatric syndromes that may result in loss of functional status. Low ADL score and suspected dementia are associated with increased mortality in older individuals\(^1\). One study by Katz \textit{et al}\(^2\) found that increases in senile cognitive decline (SCD) were associated with higher risk of mortality, as were dementia and amnestic mild cognitive impairment. The study posited that the association of SCD with mortality may be due to the association of SCD with clinical cognitive status. Another study by Lv \textit{et al}\(^3\) found that faster cognitive decline was associated with higher mortality, independent of initial cognitive function, especially among those aged 65-79 years and those with normal cognitive function at baseline. This association indicates the practical significance of monitoring cognitive change in older adults, and for cancer screening purposes, provides another basis for informed screening decisions based on life expectancy.

Cognitive evaluation provides insight about the patient’s baseline functioning, ability to understand discussion of the medical issues and ability to follow instructions. Screening for cognitive impairment can be accomplished with the Mini-Cog. The Mini-Cog is a simple cognitive screening tool that is relatively uninfluenced by level of education or language. It has three steps: 3-word registration, clock drawing, and 3-word recall, and is scored on a scale of 0-5 based on number of words recalled and accuracy of the clock\(^4\). It may reveal previously undetected cognitive concerns, as well as inform risk assessment. This is significant in the context of minor procedures such as screening colonoscopy, as even mild cognitive impairment confers a higher risk of delirium with anesthesia\(^5\). Furthermore, if cognitive impairment is detected it will be important to ensure an adequate support system to help review pre- and post-procedure instructions\(^6\).

Another necessary component of cognitive evaluation is confirming medical decision-making capacity\(^7\). Even individuals with mild dementia can have capacity to make medical decisions, which may include decisions about screening and treatment. As these decisions necessitate weighing risks and benefits of screening, it is essential to ensure patients understand the pros and cons, as well as implications of a positive screening test. Achieving this involves conducting a capacity evaluation. The MacArthur Competence Assessment Tool-Treatment is a tool which assesses the ability to understand,
appreciate, reason, and express a choice[37]. Details about how to assess medical decision-making capacity are outlined in Figure 4.

Given that cognitive decline and functional dependence are associated with increased mortality in older patients, overall functional status and medical capacity must be taken into consideration when making CRC screening decisions.

**Personal choices**
Screening decisions must be patient-centered, and should involve a discussion of patient priorities. This begins with exploring which health outcomes are most important. For some older adults, this may be longevity or surviving to a specific family event. For others, the priority may be maintaining independence, or avoiding worry and having peace of mind. Others may prioritize minimizing interventions and time spent at doctors’ offices or avoiding pain. Within the context of a patient’s health-priority outcomes, the clinician can better assist in making individual screening decisions[19].

For a patient that prefers to minimize medical intervention, but has a life expectancy exceeding ten years, a discussion may involve stool-based screening as an initial consideration, with plans to follow up a positive test with colonoscopy. For a patient that prefers to minimize pain, and has a life expectancy of only a few years, a discussion of screening cessation would be appropriate. Discussing patient health priorities allows the clinician to aid the patient in realistic goal-setting and guide decision-making.

**RISK FACTORS FOR DEVELOPING CRC**
There are both modifiable and non-modifiable risk factors for developing CRC, including lifestyle, a history of certain medical conditions, family history, and prior screening[1].

In the United States, more than half of all CRCs are attributable to lifestyle factors, such as unhealthy diet, sedentary lifestyle, heavy alcohol consumption, and smoking[38]. Numerous studies have shown that people with healthy lifestyle behaviors have a 27% to 52% lower risk of CRC compared to those without these behaviors[39].

Certain conditions that increase risk include a history of type 2 diabetes, personal history of CRC, or history of other cancer types due to the carcinogenic effects of some cancer treatments[1].

People with a first-degree relative who has been diagnosed with CRC have 2 to 4 times the risk of developing the disease compared to people without this family history[40]. A history of CRC among more distant relatives also increases risk, as does a family history of adenomas[41]. However, the rate of CRC in patients 80 years and above with a family history is significantly lower than that in younger age groups with a family history of CRC[42]. This study by Miller et al[42] found that family history should not be used as an inclusionary criterion for CRC screening in the 80 years and above age group for this reason.

Another risk factor to consider is screening history. Individuals with prior negative screening history may have reduced risk, whereas unscreened individuals are at a higher risk for CRC than adequately screened patients. Screening should be initiated for those who have never been screened, even after the age of 75 years for individuals without significant comorbidities[43].

Previous colonoscopy findings are also important when considering risk, as a personal history of advanced adenomatous polyps, especially multiple or large polyps, increases the risk of CRC[44,45]. Additionally, polyp shape and histology are significant[44,45]. Hyperplastic polyps have a relatively low risk for malignancy, and have little to no risk when found in the left colon[46]. Inflammatory polyps, or pseudopolyps, are benign and generally do not carry the risk of developing into CRC. Sessile serrated lesions frequently exhibit dysplasia, and disproportionally contribute to interval CRCs[47]. Villous adenomas also carry a high risk of malignancy[47].

Studies have found that individuals with advanced adenomas (≥ 10 mm, high-grade dysplasia, or tubulovillous or villous histology) and large (≥ 10 mm) serrated polyps have a significantly higher risk of CRC as compared to those without adenomas[44,45,48,49]. This is important to consider when factoring in an individual’s previous colonoscopy findings.

**OUTCOMES**

**Colonoscopy outcomes in older adults**
It has been shown that colonoscopies decrease both morbidity and mortality related to CRC. In particular, a meta-analysis identified nearly 1.5 million patients from observational studies that evaluated CRC incidence and mortality[50]. Pooled analysis of this data showed that colonoscopies are associated with a 61% relative risk reduction in CRC incidence (RR = 0.39; 95%CI: 0.26-0.60) and a 61% reduction in CRC mortality (RR = 0.39; 95%CI: 0.35-0.43) in patients who underwent a colonoscopy with non-malignant findings.
There have been recent studies assessing performing colonoscopies in older patients. A nationwide retrospective data analysis of the Department of Veterans Affairs electronic medical record identified United States male veterans at least 80 years of age that underwent a colonoscopy. They found that of nearly 81,946 patients who received a colonoscopy, 9,365 were diagnosed with CRC\[51\]. Of the total patients that received a colonoscopy, they found that 13% that were diagnosed with CRC were 80-84 years old, 10.2% were 85-90 years old, and 12.6% were over 90 years old. Overall, their findings suggest that there is a large number of colonoscopies being performed in patients who are at least 80 years of age and that CRC is diagnosed in both octogenarians and nonagenarians in spite of guidelines suggesting cessation of screening at 75 years of age. Other studies have shown that with older patients in particular, colonoscopies prove to be beneficial. In two large, prospective cohort studies, continuation of screening lower endoscopy after 75 years of age, regardless of screening history, was associated with reduced risk of CRC incidence and mortality\[52\]. These findings raise the importance of considering other factors besides age when deciding to screen a patient for CRC.

**Adverse events associated with colonoscopy in older adults**

Adverse effects and complications from a colonoscopy, while rare, are still something to consider when recommending colonoscopy for older patients. Studies have suggested that the benefits of endoscopy in older adults may be compromised due to a higher risk of inadequate bowel preparation, lower procedural completion rates, and higher complication rates such as perforation, bleeding, and cardiovascular adverse events\[52,53\]. However, these risks are associated with comorbidity\[52\]. A population-based matched cohort study assessed adverse events in outpatient colonoscopies among a random 5% sample of Medicare beneficiaries age 66-95 years of age from 2001-2005\[54\]. This study found that rates of adverse events after colonoscopy increased with age, and that patients having a polypectomy had a high risk for all adverse events compared to the matched group as well as the screening and diagnostic colonoscopy groups. This study also found that comorbid conditions increased the risk of adverse events such as history of stroke, COPD, atrial fibrillation, or CHF. Overall, this study did find the risk for adverse events to be low, with a risk of 0.6 per 1000 procedures for perforation and a risk of 2.1 per 1000 procedures for gastrointestinal bleeding for the screening group and 8.7 per 1000 procedures for the polypectomy group\[54\]. Other studies demonstrated that colonoscopies are safe in older patients with overall low complication rates, however these rates are still higher than they are in younger patients\[55\]. A systematic review found that patients 80 years of age and older experienced higher rates of cumulative gastrointestinal (GI) adverse events (incidence rate ratio 1.7; 95%CI: 1.5–1.9) and had a greater risk of perforation (incidence rate ratio 1.6, 95%CI: 1.2–2.1) compared with younger patients undergoing colonoscopy\[53\]. A large, population-based retrospective cohort study found the overall risk of post-colonoscopy complications to be twice as high in patients aged 75 years or older compared with that of patients aged 50 to 74 years at 30 d post procedure. While advanced age was associated with increased risk of post-colonoscopy complications, baseline comorbidities were independently associated with complications, regardless of age. Specific independent risk factors for post-colonoscopy complications were the presence of anemia, cardiac arrhythmia, heart failure, hypertension, chronic kidney disease, smoking history, liver disease, and obesity\[52\]. A retrospective study evaluated the complication rates of colonoscopies in patients > 90 years old compared to those 75-79 years old age\[56\]. Interestingly, this study found that while the complication rates in patients who
were at least 90 years of age were higher, the yield for advanced neoplasia (28.4% vs 6.4% of controls, \( P < 0.001 \)) as well as any neoplasia (\( P < 0.001 \) vs controls) were higher in this age group as well. The majority of the post-colonoscopy complications were cardiopulmonary events. This study sheds light on the importance of carefully judging the benefits and risks of performing a colonoscopy in oldest older patients.

**Outcomes of foregoing colonoscopy**

Despite some of the risks that the procedure poses in older patients, the continuation of screening endoscopy among many adults older than 75 years prevents CRC incidence and death, especially among those who do not have significant comorbidities. It is important to assess an individual’s risks for CRC in order to decide whether pursuing a screening colonoscopy is the best choice as outcomes of not performing a colonoscopy include very advanced metastatic disease. A retrospective cohort study assessed data in Saudi Arabia, where there is no population-based CRC screening, to see if there is any association between sex and metastatic CRC adjusting for patient covariates. They found over just one thousand CRC patients, and found that female patients were 20% more likely than males to present with a metastatic tumor (\( \text{RR} = 1.61, 95\% \text{CI}: 1.04-1.38 \))[57]. Their reasoning for this discrepancy was that women had less access to healthcare compared to men. Another study performed at Massachusetts General Hospital assessed outcome differences in women of screening age presenting for surgical treatment for CRC[58]. This study found that unscreened women had a significantly higher risk for having high-grade tumors (\( \text{RR} = 1.61 \)), lymph node metastasis (\( \text{RR} = 1.36 \)), and distant metastasis on pathology (\( \text{RR} = 2.26 \)). Overall, the findings in these studies suggest that patients who do not receive proper screening are at increased risk of advanced, metastatic disease. Given that there is an increase in incidence of CRC in patients beyond 75 years of age, this is an important factor to consider.

**CRC surgery outcomes**

While older studies have found that older patients have worse CRC surgical outcomes and survival than their younger counterparts[59], recent studies have shown otherwise[60-67]. Improvements in perioperative care, surgical techniques and the introduction of multimodal treatments have made surgery feasible for the majority of patients, including many older cancer patients[26]. These perioperative improvements include risk assessments, pre-habilitation, fast-track programs, medication list review, and patient education, and optimize the procedure[68].

More recent studies have been published that evaluate the feasibility, safety and advantages of the laparoscopic approach for colorectal cancer in older patients. These have supported that minimally invasive surgery in older CRC patients reduces overall mortality and morbidity when compared to a laparotomy, and correlates with shorter hospital stays and faster functional recovery. Furthermore, it has been clearly demonstrated that postoperative outcomes in advanced age do not significantly differ from those of younger CRC patients[60-67]. One study found that intraoperative and postoperative complications were equally distributed among CRC patients over 75 years of age and younger patients [60]. Virk et al[69] found that a substantial number of octogenarians undergo surgery for treatment of stages 0-2 of CRC and have comparable 5–10-year survival (25.45%) with their younger counterparts (34.40% and 30.86% in ages 60-69 and 70–79). This study concluded that despite a statistically significant difference between age groups, the survival rates were surprisingly similar from a clinical perspective, and show that CRC surgery should still be part of the conversation in octogenarians[68].

Additionally, Flynn et al performed a single institution, retrospective cohort study of patients who underwent resection of CRC from 2010-2018 and concluded that resection of CRC in patients over the age of 85 is safe and effective and that age alone is not a sufficient reason to withhold surgical treatment in this patient cohort[67]. The study also found that both the short-term post-operative outcomes as well as 30-d mortality was similar among patients who received open surgery versus laparoscopic intervention.

Another important point is that comorbidity may play a more significant part in CRC treatment outcomes than age alone. Patients with greater comorbid medical conditions have lower survival rates after an initial diagnosis of CRC, poorer survival after chemotherapy and prolonged hospitalizations as a consequence of their CRC[33].

Various studies have demonstrated the association between Charlson Comorbidity Index (CCI) and poor CRC surgical outcomes. Zingmond et al[70] found that, of 56621 CRC patients undergoing tumor resection, those with a higher CCI were significantly associated with postoperative complications. Tan et al[71] showed that the CCI was an independent predictor of morbidity in a population of 121 octogenarians undergoing CRC surgery. Ouellette et al[72] demonstrated that CCI was associated with a longer length of stay, perioperative mortality, and overall mortality in 239 CRC patients. Please see Table 2 for more details about the CCI.

CRC surgery in the aged was previously considered high risk. Recent studies have shown that this is not necessarily the case, while advances in minimally invasive surgical technique and perioperative management have lowered the risk of postoperative complications and improved recovery.
PRACTICAL CONSIDERATIONS FOR SCREENING

Modality

Screening can be done via direct visualization methods such as colonoscopy, CT colonography, colon capsule, or flexible sigmoidoscopy, or via stool-based tests such as guaiac-based fecal occult blood test, FIT, or multitargeted stool DNA test, or via serology test such as SEPT9 DNA test[4,73]. A stool-based test such as FIT may be offered as an initial, non-invasive screening method. However, stool-based tests should not be used in older patients that are unable to undergo a follow up colonoscopy in the event that the test is positive[4]. CT colonography is another non-invasive screening technique that offers visualization that stool based tests do not. Nevertheless, it still requires bowel preparation, exposes patients to ionizing radiation, and requires follow-up colonoscopy for positive findings[4]. There is also risk of incidental findings, as CT colonography provides images of the entire abdomen and pelvis. Studies report extracolonic incidental findings in 15%-69% of imaging, and the risk likely increases with age[74]. These findings may result in unnecessary testing, burdening the patient with more cost, radiation exposure, and mental distress[74].

Colonoscopy is the gold standard, as it has the highest sensitivity among available screening modalities[75], and offers both detection and treatment of precancerous lesions at the time of the procedure. The regional distribution of CRC changes with age, with proximal colon cancer being more common in older patients, and distal colon cancer predominating in younger patients[76,77]. This is significant when considering screening modalities in older patients, as sigmoidoscopy cannot reach the proximal colon. Therefore, colonoscopy is the superior direct visualization method in older patients.

All screening tests other than colonoscopy are two-step tests. A limitation of non-colonoscopy-based CRC screening tests is that a positive test requires a follow-up colonoscopy. This must be kept in mind when deciding whether to screen an older patient, regardless of the initial screening modality. On the other hand, a major advantage of stool-based screening as a first step is that invasive screening colonoscopy is limited to those individuals with a higher pretest probability of cancer. This is important in the balance of risks and benefits, as the potential benefit of screening colonoscopy is higher in individuals with a positive FIT or other stool-based screening method.

Prevention and management of colonoscopy-associated risks in older patients

Screening colonoscopy carries the risk of major adverse events such as perforation, bleeding and cardiopulmonary complications. In addition to risks of the procedure, there are risks associated with the bowel preparation and sedation required for the colonoscopy. These risks are affected by age and influenced by additional factors such as comorbid conditions[19,52,53]. When considering colonoscopy for older individuals, caution should be taken during every step including the pre-procedure preparation, the analgesia and sedation, and the procedure itself[78] (Table 3).

During the pre-procedure preparation of a colonoscopy, bowel preparation is essential and obtained either through polyethylene glycol-based solutions or sodium phosphate solutions[78]. Sodium phosphate has an osmotic mechanism of action, which results in fluid shifts and electrolyte abnormalities such as hyperphosphatemia, hypernatremia, and hypokalemia[78]. The geriatric populations, especially those with renal or cardiac disease, are more sensitive to these changes and should therefore avoid sodium phosphate solutions[78]. Older individuals are at greater risk of dehydration as well, which can commonly occur in any bowel preparation. This can be managed with adequate hydration throughout the pre-procedure preparation[78]. Another concern throughout this process is the large volume of oral preparations required for adequate colonic preparation. Older patients may not be able to consume such a large volume and are therefore at risk for poor colonic preparation[78]. This can have major consequences including failure to complete the procedure, missed lesions, and prolonged procedure time with increased risk for complications[79]. Safe and adequate bowel preparation is very important in planning colonoscopies for older patients.

Age-related effects on the body also put the geriatric population at higher risk for adverse effects from sedation. GI colonoscopies are generally performed under conscious sedation with numerous guidelines published for monitoring during the procedure[78]. Physiologic changes such as decreased arterial oxygenation, delayed or blunted cardiorespiratory stimulation in response to the body’s requirements, increased risk of aspiration, and reduced hepatic and renal clearance mechanisms are factors that make the choice of sedation more difficult when managing an older adult[78,80]. Due to increased sensitivity to effects of sedatives, it is recommended to administer fewer agents at a slower rate and lower dose for safe sedation during colonoscopies[78]. Endoscopies without sedation are not commonly performed in the United States, but are an option for higher risk patients[19]. In older patients with multiple comorbid medical conditions, there is greater risk of experiencing an adverse event if the colonoscopy is performed under general anesthesia[53,81]. Minimizing sedation during procedures reduces risk for anesthesia-related adverse events. During the procedure, standard monitoring procedures should be followed during procedures in the geriatric population. Maintaining a low threshold for beginning supplemental oxygen therapy is important as older patients are at higher risk of oxygen desaturation[82]. Another serious adverse event is delirium. Older adults are at an increased risk for experiencing delirium related to sedation, and this risk is further increased in older
patients with underlying cognitive impairment and polypharmacy[68]. Delirium is a poor prognostic factor for survival among hospitalized older[83], and is associated with nursing home placement within the year after surgery[84].

In addition to the risks of bowel preparation and sedation, the screening colonoscopy procedure is also carries risk of perforation, bleeding, and cardiopulmonary complications. Of all adverse events associated with colonoscopy, the greatest risk associated with age is perforation. Studies show that older patients have a 30% higher risk of experiencing a perforation than younger patients undergoing colonoscopy and a 14-fold higher risk of having a perforation than patients of the same age who do not undergo the procedure[53]. Bowel perforation can be a surgical emergency and puts the patient at significant risk of life-threatening infection. Older patients are at an increased risk of perforation due to the higher prevalence of diverticulosis, tortuosity of the intestines, inadequate bowel preparation, and post-surgical adhesions or strictures, which threaten the integrity of the tissue and make the procedure more technically challenging[78,80,85,86]. Furthermore, there is a higher risk of mortality associated with perforation in older individuals[19,87]. For this reason, it is critical to weigh the risk of complications and comorbid conditions when considering colonoscopy in older patients.

**Endoscopic technique**

Endoscopists use a variety of techniques during colonoscopies to effectively screen for CRC. The most widely used techniques include air insufflation, carbon dioxide insufflation, water exchange, and water immersion[88]. Air insufflation, the oldest colonoscopy technique, allows for distension of colonic lumen and visualization during colonoscopy[88,89]. However, air insufflation results in unabsorbed air in the colon and prolonged bowel distension and subsequently can cause symptoms of pain and nausea[89]. This can also increase risk of postprocedural bowel ischemia and spasms, and patients may require unnecessary hospitalization or testing to rule out colonic perforation[89]. The risks of air insufflation are important to consider in older individuals as they are at higher risk of complications.

Air insufflation was followed by introduction of carbon dioxide insufflation[88]. Numerous studies have demonstrated reduction in abdominal pain during and following the completion of colonoscopy with carbon dioxide insufflation compared to air insufflation[89]. The reduction in pain is likely related to faster absorption of carbon dioxide by intestinal mucosa compared to air. The rapid absorption was confirmed early on by a study that looked at abdominal radiographs at 1 h[89]. The benefits of the reabsorption of carbon dioxide include less bowel distension, improved abdominal pain, and decreased interference in colonic mucosal blood flow[89]. However, concerns have been raised related to increased blood carbon dioxide concentrations. One study showed end-tidal carbon dioxide was increased in both carbon dioxide and air insufflation groups, suggesting this was likely related to hypoventilation from sedation rather than carbon dioxide insufflation[90]. Further studies are still required to determine risk of hypercapnia from carbon dioxide insufflation.

More recently, newer water-assisted methods including water immersion and water exchange have been used to reduce colonic spasms, facilitate cecal intubation, and lower patient discomfort and need for sedation[91]. Water immersion involves removing infused water during withdrawal while water exchange involves removing infused water during insertion[92]. The water immersion technique was initially used for patients with severe diverticulosis, as the water opened and straightened the sigmoid colon to distinguish the real lumen[91]. As older individuals are commonly affected by diverticulosis,

<table>
<thead>
<tr>
<th>Risk</th>
<th>Associated problems</th>
<th>Mitigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perforation</td>
<td>Bleed, infection, necrotic bowel</td>
<td>Endoscopic technique (carbon dioxide insufflation, use of pediatric endoscopic equipment, careful navigation of diverticular disease, adequate bowel preparation</td>
</tr>
<tr>
<td>Bleeding</td>
<td>Post-polypectomy bleed</td>
<td>Hemoclip placement for bleeding prevention when appropriate, diluted epinephrine injection, use of detachable snare, thermal coagulation</td>
</tr>
<tr>
<td>Cardiovascular event</td>
<td>Arrhythmia</td>
<td>Medication review, screen for high-risk medications, confirm dosing appropriate for renal function; adjustment of anesthesia</td>
</tr>
<tr>
<td>Anticoagulation therapy interruption</td>
<td>Risk of thrombosis, MI, CVA</td>
<td>Liaise with prescribing physician; avoid colonoscopy during high-risk period; avoid interruption if possible</td>
</tr>
<tr>
<td>Delirium</td>
<td>Cognitive impairment</td>
<td>Risk assessment; optimize medication list, avoid holding medications with withdrawal potential on morning of procedure</td>
</tr>
<tr>
<td>Medication interaction</td>
<td>Polypharmacy increases sensitivity to anesthesia</td>
<td>Medication review; adjustment of anesthesia</td>
</tr>
<tr>
<td>Dehydration</td>
<td>Electrolyte disturbances</td>
<td>Appropriate counseling prior to colonoscopy prep; caretaker supervision to ensure patient safety during prep</td>
</tr>
</tbody>
</table>

MI: Myocardial infarction; CVA: Cerebrovascular accident.
utilizing this technique can allow for efficient completion of the procedure. Another study that investigated the results of water-assisted colonoscopies without sedation showed that this technique resulted in less reported abdominal pain and faster cecal intubation compared to air insufflation[91]. Older individuals with more comorbidities are at high risk for adverse events from sedation; this population may therefore benefit from a water-assisted colonoscopy performed with no sedation. In addition, minimizing sedation allows patients to indicate pain during the procedure, which can be indicative of risk of perforation[91,93]. Both the water immersion and water exchange techniques allow for decreased pain with insertion and completion of even difficult colonoscopies, with the advantages more pronounced in the water exchange technique[92]. Water exchange is also associated with increase in adenoma detection rate[92]. The benefits of these newer colonoscopy techniques may allow for feasible procedures in older individuals with decreased pain and risk of adverse events.

Management of anticoagulation

Over 6 million patients in the United States receive long-term anticoagulation therapy for the prevention of thromboembolism due to atrial fibrillation, mechanical heart-valve prosthesis, or venous thromboembolism[94]. Atrial fibrillation specifically affects over 33 million people worldwide and the prevalence is increasing with the aging population[95]. Older individuals with atrial fibrillation are at higher risk of stroke, as evidenced by the CHA₂DS₂-VASc score[95,96]. The CHA₂DS₂-VASc score calculates stroke risk in patients with atrial fibrillation and takes age into account, with 2 points added for individuals older than 75 and 1 point for individuals between 65-74[96]. Those at high risk are commonly started on anticoagulation, which may be a concern if a colonoscopy is required.

When considering colonoscopy in patients on anticoagulation, multiple factors need to be taken into consideration. This includes the patient’s risk of thromboembolism, the bleeding risk of the procedure, the features of the anticoagulant the patient is taking, and the urgency of the procedure[97,98]. Regarding bleeding risk of the procedure, diagnostic colonoscopy, mucosal biopsy sampling, and polypectomy less than 1 cm have low risk of bleeding[99]. However, procedures that involve an endoscopic treatment are at higher risk of bleeding; this includes polypectomy greater than or equal to 1 cm, tumor ablation, and endoscopic submucosal dissection[99]. The severity of the bleeding risk after polypectomy is between 0.3% to 10% depending on factors such as polyp size, location, morphology, resection technique, and cautery used[99]. Based on this, providers should consider the likelihood of a patient having polyps when deciding how to manage the anticoagulation. Providers may consider a colonoscopy and biopsy on anticoagulation if there is low likelihood of polyps but should hold the medication if large polyps are suspected.

While the risks and benefits of stopping anticoagulation prior to colonoscopy need to be weighed for every patient, it is of utmost importance in the older population. With older individuals, they may have more comorbidities with higher risk of thromboembolism or stroke with stopping anticoagulation. If a patient is still in their high-risk window of thromboembolism, colonoscopy may be delayed until this period has passed. It is important for physicians to exercise a considerable amount of clinical judgement and consult with prescribing providers as necessary.

CONCLUSION

Screening extends life by detecting disease at an earlier stage, when there may be better chance of survival after treatment than in the absence of screening. The current approach to CRC screening in older individuals, in which the decision to offer screening is often based primarily on age, is inefficient, resulting in underuse of screening for some and overuse of screening for others.

Life and health expectancy should be used to inform cancer screening guidelines to ensure that patients live long enough to benefit from early detection. There is considerable heterogeneity of life expectancy beyond what described by chronological age, and subjective estimation of life expectancy has been shown to be inaccurate. Therefore, maximizing the potential benefits of CRC screening while minimizing potential harms requires attention to comorbidity, frailty, cognitive status, and patient preferences. The Charlson Comorbidity Index, Clinical Frailty Score, and Mini-Cog are helpful tools for evaluating these elements. Screening history and previous colonoscopy findings are also important to take into consideration when evaluating CRC risk.

Despite some of the risks that colonoscopy poses in older patients, the continuation of screening endoscopy among many adults older than 75 years prevents CRC incidence and death, especially among those who do not have significant comorbidities. Recent studies also show that fit older patients fair similarly to younger patients undergoing CRC treatment, while advances in laparoscopic surgical technique and adjuvant therapies have improved outcomes for CRC patients as a whole.

Colonoscopy-associated risks can be mitigated in older patients by conducting thorough medication review, coordinating with the prescribing provider, educating the patient about bowel preparation, adjusting sedation, and using endoscopic techniques that minimize perforation and bleed risk.

Altogether, this review emphasizes the importance of patient assessment, with chronological age being just one aspect of that evaluation, and highlights important considerations for CRC screening in
older patients that age-based screening guidance may overlook.

FOOTNOTES

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Fibrolamellar hepatocellular carcinoma: A rare but unpleasant event

Walaa Abdelhamed, Mohamed El-Kassas

Abstract

Fibrolamellar carcinoma (FLC) is a rare variant of hepatocellular carcinoma (HCC), comprising 1%–9% of all HCCs. FLC is a poorly understood malignancy, which seems to be more prevalent in young patients with no underlying liver diseases. The term “fibrolamellar” is derived from thick fibrous collagen bands surrounding the tumor cells. Unlike HCC, cirrhosis and viral hepatitis infection are not predisposing to FLC, and it is not associated with elevations in serum alpha-fetoprotein. FLC patients often present with vague abdominal pain, nausea, malaise, and weight loss. Most cases present at an advanced stage at the time of initial diagnosis. However, curative treatment options can still be offered to up to 70% of patients. Surgery (resection/liver transplantation) is the mainstay of treatment and the only potentially curative option. FLCs have been less chemo-responsive than the conventional HCC, however, in advanced cases, multi-modality treatments can be effective. Recent advances in molecular studies of FLC have found a unique DNAJB1–PRKACA fusion transcript in most of the cases studied. The review aims to describe clinical characteristics, diagnostic methods, and therapeutic modalities for this rare tumor to raise awareness among clinicians and surgeons.

Key Words: Fibrolamellar carcinoma; Hepatocellular carcinoma; Hepatitis; Cirrhosis; Viral hepatitis infection

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Core Tip: Fibrolamellar hepatocellular carcinoma (FLC) is a rare liver cancer that displays unique features in behavior and clinical findings from conventional hepatocellular carcinoma (HCC). No certain underlying trigger is detected in FLC. Alpha-fetoprotein levels are normal, unlike in traditional HCC. Surgery (resection/liver transplantation) is the current mainstay of treatment and remains the only curative option. FLCs have been less chemo-responsive than the conventional HCC. Controlled trials evaluating checkpoint inhibitors in FLC are lacking. In this review, we collect and summarize current evidence and clinical experience of conversion therapy, highlight remaining problems and challenges for further research.

INTRODUCTION

Fibrolamellar carcinoma (FLC) is an uncommon liver cancer with behaviour and clinical findings that vary significantly from ordinary hepatocellular carcinoma (HCC)[1]. It comprises 1%–9% of all HCCs, according to the Surveillance, Epidemiology, and End Results SEER database[2]. Edmonson first described FLC in 1956 as an adult type of liver cancer in a 14-year-old female without a background of liver affection[3]. FLC receives its name from the histologically distinct intra tumoral lamellar collagen bands observed between large polygonal cells with abundant eosinophilic cytoplasm, large vesiculated nuclei, and large nucleoli[4]. The majority of FLC patients are in their second or third decade[5,6]. It often affects patients between (10–35 years of age) with no primary liver disease[6,7]. No certain underlying trigger is detected in FLC. Less than 10% of patients with FLC have cirrhotic liver morphology[8]. Unlike HCC, cirrhosis and viral hepatitis infection are not predisposing to FLC, and it is typically not associated with elevations in serum alpha-fetoprotein[9,10]. More than half of patients with FLC are Caucasians, while more than 80% of HCC patients are Caucasians[6]. Tumour markers are increased in less than 10% of affected patient and have no role in the assessment or diagnosis of FLC[11,12].

Most cases with FLC cases are advanced at the time of diagnosis; however, up to 70% of patients may still be treated with curative therapy. The current cornerstone therapy (resection/liver transplantation) is still the sole possibly curative approach[13]. Chemotherapy was utilized as a complimentary treatment before and after postoperative resection. However, because of the low frequency of FLC, no randomized controlled trial (RCT) has explained the most successful regimens[14]. Still, no neo-adjuvant/adjuvant systemic therapies have been reported to improve survival in patients with resected FLC[14]. Therefore, chemotherapeutic agents like gemcitabine, cisplatin, 5-fluorouracil, interferon, and oxaliplatin have been tried and have demonstrated various degrees of response[15,16].

EPIDEMIOLOGICAL FACTS

Due to the rarity of this tumor, exact estimates of its occurrence across nations are difficult to come by. FLCs account for less than 1% of primary liver tumors in the United States and 5.8% of liver tumors in Mexico[17]. Incidence rates, on the other hand, are very consistent over the world[6,18]. FLC affects a younger group, with a median age of 21 years, compared to HCC, affecting people between the ages of 14 and 33. The vast majority of cases (64%) are discovered before the age of 40[6]. A bimodal age distribution has been described, with incidence peaks between the ages of 10 and 30, and a second peak between the ages of 60 and 69 years[19,20]. Most research show that both sexes are equally affected, however a few have shown a slight male predominance (Male: Female = 1.7:1)[6,18].

Another interesting study showed a that female gender was more prevalent in the FLC group than in the traditional HCC group (60% vs 37%)[6]. This was also seen in the SEER study, where the authors discovered that FLC had a larger percentage of females (51.5% vs 26.3%)[6].

Furthermore, the United States, Mexico, Sweden, Saudi Arabia, Thailand, France, Canada, South Africa, Japan, South Korea, India, Taiwan, and the United Kingdom have all reported a comparable incidence of FLC. This could exclude the strong association between race and ethnicity with FLC risk [21-24].
PATHOGENESIS OF FLC

The etiology of FLC remains uncertain. It typically occurs in normal livers without a clear background of liver fibrosis or cirrhosis[25]. Unlike HCC, which are usually found in the presence of cirrhosis or chronic hepatitis[26], FLC has been reported to occur in association with focal nodular hyperplasia (FNH), a benign form of liver tumors[27,28]. Pathologically, both have a central stellate scar which appears on imaging studies, and copper accumulation upon histological examination[28,29]. Hepatitis B viral proteins or DNA have been found in FLC on rare occasions, although this seems to be by coincidence given the enormous global frequency of chronic hepatitis B infection, and there is no evidence to identify hepatitis B as an etiological agent[30-33]. Similarly, FLCs have arisen in women who use oral contraceptives, although the link seems to be coincidental[34].

PATHOLOGICAL PICTURE OF FLC

FLC tumors are big, yellow/tan, hypervascular, well-circumscribed lumps in otherwise normal liver parenchyma with patches of necrosis[35]. A central stellate scar and conspicuous fibrous tissue may be seen in up to 75% of tumors[35]. Histological examination generally reveals well-differentiated big polygonal tumor cells with eosinophilic hyaline cytoplasmic particles. Large polygonal or spindle-shaped tumor cells with highly eosinophilic cytoplasm due to numerous mitochondria and conspicuous nuclei grouped in cords surrounded by lamellated collagen fibres describe FLC microscopically[30,36,37].

Usually, there is no cirrhosis in the surrounding liver parenchyma, although mononuclear cells and lymphocytes imply nonspecific inflammation[37]. Electron microscopy often reveals an increase in mitochondrial number, a pathogenic characteristic unique to FLC[30]. FLC immunohistochemistry has several characteristics with HCC, such as staining positive for hepatocyte paraffin[38]. Unlike HCC, however, FLC stains are negative for alpha-fetoprotein and significantly positive for CK7 and epithelial membrane antigen, both of which are indicators of biliary differentiation (CK19 and Ep CAM)[39,40]. Additionally, FLC exhibits the stem cell markers CD133 and CD44[41]. FLC also stains for epithelial growth factor receptor and transforming growth factor-beta more often and diffusely than classic HCC[41,42]. FLC may be distinguished from normal liver parenchyma and HCC thanks to genetic differences revealed. Honeyman and his colleagues found that a 400-kb deletion on chromosome 19 leads in a functional DNAJB1-PRKACA chimeric transcript in 100% of FLC tumors examined, further

DIAGNOSTIC APPROACH TO FLC

Clinical presentation

Patients frequently report with a variety of symptoms and signs, ranging from discomfort to a liver tumour discovered during a clinical examination for another indication[11,45]. Symptoms commonly seen with the conventional HCC are not seen in FLC[24,46]. FLC patients often complain of nonspecific abdominal pain, nausea, abdominal fullness, malaise, and weight loss[30]. A palpable abdominal mass or hepatomegaly with or without right upper quadrant pain and jaundice due to biliary obstruction[47-49], male gynecomastia[50], fulminant liver failure[2,51-53], recurrent deep venous thrombosis[54], encephalopathy[55], lower limb thrombophlebitis[56], anemia[57], ascites[58], and hypoglycemia[59]. Hepatic transaminases and alkaline phosphatase levels are usually normal or slightly elevated[30,60,61]. Common characteristics of FLC upon presentation are featured in Figure 1.

Tumor markers

Alpha-fetoprotein levels are predominately normal, unlike in traditional HCC[62]. Several case reports have described increased levels of blood transcobalamin I (haptocorrin) and vitamin B12 binding capacity[63,64]. To assess their diagnostic function, however, further research is needed. Although serum neurotensin levels have been reported to be high with FLC, this test was not enough sensitive or specific to be used for diagnosing FLC[25,65,66]. Des-gamma carboxy prothrombin is elevated in FLC and conventional HCC, which is less useful[66].

Imaging

Ultrasound: FLCs exhibit a wide variety of sonographic characteristics and usually appear as well-defined masses with varying echogenicity[67].

Contrast-enhanced computed tomography: FLCs often appear on computed tomography as large heterogeneous well-defined lesions (80%–100%) with a lobulated contour. Calcification and core stellate scarring, as well as tumour necrosis, are found in 65%–70% of cases[39]. In the arterial phase, more than
80\% of patients have increased contrast avidity, which reflects the tumors’ primary blood supply. In the venous phase, half of these masses enhance similarly to the background liver, one-third of these tumors show increased contrast avidity, and approximately two-thirds of these tumors enhance similarly to the background liver in the delayed phase, making differentiation difficult\cite{5}. The hepatic hilum and hepatoduodenal ligament are the most prevalent sites for nodal metastatic lesions, accounting for up to 50\% of cases\cite{7,35,68}. On imaging, distant FLC metastasis, particularly to the lungs, peritoneum, and adrenal gland, has been recorded in 20\%–30\% of patients\cite{6,68}.

**Magnetic resonance imaging:** On Magnetic resonance imaging, FLC is hypointense on T1-weighted images and hyperintense on T2-weighted images with no intrallesional fat. Unlike the FNH, the fibrous central scar is hypointense on both T1 and T2-weighted imaging\cite{69}. When using Gadolinium as a contrast agent, the enhancement pattern is similar to that of a CT scan, with heterogeneous contrast enhancement on the arterial phase and isointense or hypointense contrast enhancement on the portal venous and delayed phases\cite{70}.

**Nuclear medicine**
Nuclear medicine imaging may help diagnose FLC in certain cases\cite{71}. On delayed phase pictures, these tumors demonstrate enhanced absorption of 99 mTc-labeled RBCs during the arterial phase and washout. They also seem photopenic when 99 mTc-sulfur colloid scanning is performed\cite{71}. Although the relevance of 18FDG PET/CT in FLC is unknown, it may be beneficial for primary staging and restaging in recurring cases\cite{72}.

**Role of biopsy**
Histologic appearances are the most objective and have widely accepted differences between FLC and HCC\cite{73,74}. So, histologic confirmation is needed to diagnose FLC with certainty\cite{26}. Core biopsy is recommended over fine-needle aspiration for percutaneous biopsy because malignant hepatocytes may be aspirated without the distinctive fibrotic lamellae, resulting in a diagnosis of HCC rather than FLC\cite{75}.
**LINES OF TREATMENT**

**Surgical resection**
Surgical resection is the ideal treatment option that could carry an advantageous prognosis[14]. Over 70% of patients need a major hepatectomy (i.e., semi hepatectomy or extended hepatectomy), with a median tumor size of 10.5 cm. Around 24% of patients undergo partial or minor hepatectomy[76-78]. When compared to older patients, young patients (under 40 years old) had a higher likelihood of resection[6]. Resected patients have 26%–76% postoperative survivals at five years with a median survival of 32–174 mo[7,79-81]. Patients undergoing resection had an overall survival rate of 58.2% (44%–70%) according to a SEER database analysis[6]. Recurrence occurs in a large number of patients (more than two-thirds)[1]. Disease recurrence after complete surgical resection is high, ranging from 33% to 100%[19,77,81,82]. The median time for recurrences to occur ranges between 10 and 33 mo, which is obviously short[7,35,80,83]. While recurrence of the disease after over five years postoperative is a rare event[81,84]. The significant recurrence rate after surgery may come as a surprise, particularly given that these patients were treated at highly skilled hepatobiliiary facilities. These patients, on the other hand, were often in late stages, with large primary tumors and lymph node metastases, both of which have been recognized as poor prognostic indicators[81-83]. Surgical resection may also be beneficial for patients with recurrent illness. Yamashita et al[79] found that 86% of patients had recurrent illness following resection in their investigations. Surgical excision of recurrent FLC was linked to a longer median overall survival of 122 mo, compared to 37 mo without surgery.

FLC recurrence occurred in all patients after first surgery in Maniaci et al[83]’s analysis of ten patients, with a median time to recurrence of 2.2 years, and seven patients were surgically handled, with a median survival of 4.7 years and an OS of 48% at five years. Patients who are not surgical candidates, on the other hand, have few therapy alternatives, with a median overall survival of less than 12 mo[7,84,85].

**Liver transplantation**
A curative alternative with transplantation has comparable survival rates to transplanted classical HCC in unresectable FLC[86]. Liver transplantation should be regarded a 3-year survival rate if 75%–80% of the liver is transplanted[80]. Because HCC is more prevalent than FLC, and regional lymph node metastases (a relative contraindication to transplant) is more likely in FLC (42.2%) compared to HCC (22.2%), liver transplantation is considerably more typically demonstrated for HCC than FLC[76].

**Systemic chemotherapy**
Because of the limited incidence of FLC, no available RCT has shown the most effective chemotherapeutic option. It’s worth noting that no neo-adjuvant/adjuvant systemic therapy have yet been found to increase survival in patients with excised FLC[14]. Furthermore, FLC is not normally sensitive to chemotherapy; nevertheless, platinum-based chemotherapy regimens and combination regimens, including interferon alpha-2b, have been utilized successfully[83,85].

A full or partial response has been observed in five out of eight patients treated with fluorouracil plus recombinant interferon alpha-2b in a Phase II trial[87,88]. Gemcitabine, cisplatin, 5-flourouracil, interferon, and oxaliplatin are examples of agents that must be taken and have varied degrees of response[16]. Better results have been seen with combined treatment regimens that involve surgery, chemotherapy, and radiation[83]. Furthermore, percutaneous radioembolization has been used to reduce the size of the tumor prior to surgical excision[89]. One of the targeted therapies that have shown efficacy in treating HCC, sorafenib, was evaluated in cases with FLC but has shown limited efficiency[16].

**Radiation therapy**
Because FLC is not frequently responsive to systemic chemotherapy, only a few cases of FLC treated with radiation treatment have been reported[83,90]. Radiation treatment was utilized to treat unresectable primary tumors[90], to convert unresectable to resectable tumors[91], and to treat metastases or relapses[83] in these reports. One report found that employing targeted internal radiation treatment with Yttrium-90 resulted in a substantial FLC response, enabled the patient to undergo curative surgical resection[89]. Using 40 Gy in ten parts over 13 d, one case report demonstrated an 85% reduction in tumor volume of FLC metastases[92]. Three patients achieved objective partial responses, six patients had tumour volume stability, and one patient had early progression in a separate retrospective analysis of 10 patients with unresectable metastatic cancer treated with external beam radiation in addition to chemotherapy[90].

**Recent developments**
In the IMbrave150 study, the combination of atezolizumab and bevacizumab improved survival and considerably delayed deterioration, lowering the chance of death by 42% compared to sorafenib monotherapy in the treatment of patients with unresectable classical cancer (HCC). Patients with FLC, on the other hand, were not included in this study[93]. Another research found that three cases with...
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metastatic FLC progressed after 2–3 mo of initiating PD-L1 antibodies, one of them was treated with pembrolizumab and the other two with nivolumab[16]. Checkpoint inhibitors have been shown to be effective in the treatment of melanoma, lung cancer, renal cell carcinoma, and head and neck cancers [94], and they seem to be a viable therapeutic strategy in HCC[95,96]. Several tumor features seem to encourage a response to checkpoint inhibitors, including tumor-inherent genomic instability and a high mutational burden, both of which are linked to increased overall survival[94,97]. In a Phase II trial of advanced HCC, checkpoint inhibitors showed acceptable efficacy[39]. However, there are no controlled studies testing checkpoint inhibitors in FLC, and case reports are few and contradictory[14,16]. FLC’s molecular characterization has recently identified potential targets such as the mTOR pathway and Aurora A kinase. Despite the positive findings of mTOR inhibition in sporadic cases[98], no encouraging results from controlled studies have been revealed to date[99].

DNAJB1-PRKACA: In conventional HCC and cholangiocellular cancers, DNAJB1-PRKACA rearrangements are absent[100]. In primary hepatocellular neoplastic processes, DNAJB1-PRKACA and PRKACA rearrangement detection using a break-apart fluorescence in situ hybridization probe or a polymerase chain reaction provides both sensitive and specific elucidation[100]. Introducing the DNAJB1-PRKACA fusion gene into wild-type mice resulted in hepatic tumors in mice with characteristics similar to human FLC, according to Engelholm et al.[101]. The kinase activity of PRKACA, the catalytic subunit of protein kinase A (PKA), has been shown in this newly characterized predominant fusion protein[43,102,103]. This fusion is not unique to FLC, since it has been shown in other cancers[104]. However, significant levels of DNAJB1-PRKACA protein expression (amplified in over 70% of FLC) compared to a normal liver or HCC[103] make DNAJB1-PRKACA a promising therapeutic target. Because PKA regulates so many oncogenic signaling pathways[105,106], kinase inhibitors that bind at the active region of the PKA catalytic subunit may simultaneously target many oncogenic proteins. There are no known clinical studies utilizing such inhibitors against FLC[107].

mTOR: The first randomized Phase II clinical trial for FLC had three arms: The mTOR inhibitor everolimus, estrogen-deprivation therapy with leuprolide plus letrozole, and everolimus plus estrogen-deprivation therapy. This study was discontinued due to a lack of improvement in progression-free survival among the three study arms[108]. The mammalian target of rapamycin (mTOR) is an intracellular protein kinase expressed in mammalian cells and is important for the development of many cancers[109]. When this route is disrupted, mTOR is activated, resulting in enhanced cell proliferation, angiogenesis, and apoptosis evasion[110].

For low and intermediate-grade neuroendocrine tumors, the mTOR inhibitor everolimus coupled with octreotide is helpful. The majority of patients had a partial response or stable disease, with a small percentage having tumor progression[111].

OUTCOME AND PROGNOSTIC FACTORS IN FLC

Despite the fact that FLC patients frequently have advanced illness, around 50% to 84% of them are surgically treatable and have a five-year survival rate of up to 76%. FLC patients tend to have a better prognosis than HCC patients, who have a far poorer prognosis, with a 5-year survival rate of just 6.8%[112]. Those with FLC, on the other hand, do not have a better prognosis and do not react to therapy any differently than patients with HCC in non-cirrhotic livers at the same stage of disease[113-116]. The apparent superior result reported in FLC might be due to the lack of liver cirrhosis, as well as the disease’s indolent character and younger age, which allows for intensive surgical treatment[6,7,113,117].

Tumor stage, number and size of tumors, vascular invasion, regional lymph node metastases, extrahepatic disease, non-white race, and female gender have all been linked to poor surgical outcomes[7,78,81,82]. However, it seems that the tumor’s early stage at the time of treatment is the most important driver of prognosis. Patients with stage I–III illness had a better prognosis than those with stage IV disease[7,84,118,119], and sometimes, this difference attains statistical significance[81,84,119].

CONCLUSION

FLC is a rare liver cancer and this relative rarity makes data collection and clinical research protocol designing difficult. Collaboration between international institutes and societies in conducting large scale global research addressing epidemiologic aspects of FLC is needed. No predictive standards have been elucidated for FLC. Unfortunately, non-surgical options for FLC patients remain limited. Experimental animal studies may be needed to better understand FLC’s pathogenesis and molecular genetics. Evidence supporting systemic therapies in FLC is scarce, further research is required on the chemotherapeutic compounds used, including cisplatin, epirubicin, 5-fluorouracil. There is a need to expand our understanding of the molecular underpinnings of FLC and outline the current knowledge gaps to reach a consensus regarding effective treatment modalities.
FOOTNOTES

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Can dietary flavonoids be useful in the personalized treatment of colorectal cancer?

Cristina Pereira-Wilson

**Abstract**

Activating mutations in the oncogenes KRAS, BRAF and PI3K define molecular colorectal cancer (CRC) subtypes because they play key roles in promoting CRC development and in determining the efficacy of chemotherapeutic agents such as 5-fluorouracil and anti-epidermal growth factor receptor monoclonal antibodies. Survival of patients with cancers displaying these molecular profiles is low. Given the limited efficacy of therapeutic strategies for CRC presenting mutational activations in mitogen-activated protein kinase and/or PI3K pathways, developing combination therapies with natural flavonoids or other phytochemicals with demonstrated effects on these pathways (and little or no toxic effects) may constitute a valuable path forward. Much has been published on the anticancer effects of dietary phytochemicals. However, even an exhaustive characterization of potential beneficial effects produced by *in vitro* studies cannot be extrapolated to effects in humans. So far, the available data constitute a good starting point. Published results show quercetin and curcumin as possibly the best candidates to be further explored in the context of adjuvant CRC therapy either as part of dietary prescriptions or as purified compounds in combination regimens with the drugs currently used in CRC treatment. Clinical trial data is still largely missing and is urgently needed to verify relevant effects and for the development of more personalized treatment approaches.

**Key Words:** Colorectal cancer; Personalized treatment; Quercetin; Curcumin; KRAS; BRAF

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Core Tip: Given the limited efficacy of therapeutic strategies for colorectal cancer presenting mutational activations in mitogen-activated protein kinase and/or PI3K pathways, developing combination therapies with natural flavonoids with demonstrated effects on these pathways may constitute a valuable path forward. Published results show quercetin and curcumin as possibly the best candidates to be further explored in the context of adjuvant colorectal cancer therapy either as part of dietary prescriptions or as purified compounds in combination treatment. Clinical trial data is still largely missing and is urgently needed to verify relevant effects and for the development of more personalized treatment approaches.

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INTRODUCTION

Colorectal cancer (CRC) is the fourth most common cause of cancer related death worldwide and accounts for 9% and 10% of all new cancer diagnoses per year in women and men, respectively[1].

Wide variation in geographical distribution of CRC exists with incidence rates in Australia and New Zealand being 10-fold higher than in Western Africa[1]. The highest mortality rates are, however, registered in Central and Eastern Europe[1]. Both CRC incidence and mortality are associated with higher income countries with westernized lifestyles, and CRC rates are expected to increase by about 60% over the next 15 years[1].

Lifestyle and dietary choices play an important role in CRC development. Smoking and excessive alcohol consumption also increase CRC risk[1]. There is strong evidence that being physically inactive, overweight or obese and consuming red or processed meat increases the risk of developing CRC. There is also moderate evidence that low consumption of non-starchy vegetables and fruits might increase the risk of CRC. On the other hand, consuming foods containing dietary fiber decreases CRC risk[1].

The relevance of lifestyle and dietary choices to CRC risk are reflected in the fact that only 5%-10% of CRC cases have a known hereditary cause. These hereditary types of CRC are classified as Familial Adenomatous Polyposis and Hereditary Non-Polyposis Colorectal Cancer (or Lynch syndrome) and are attributed to germline mutation in the APC gene or in DNA repair genes, respectively. A further 20% of cases have a family history of CRC (but no known germline mutations), and the remaining 70% of CRC cases are sporadic (caused by gene defects that are not germline mutations)[2,3]. In addition to dietary and lifestyle factors, patients with irritable bowel disease syndrome are at significantly higher risk of developing CRC[3].

CRC progression takes place over many years through a series of stages that go from lesions in single cells of the epithelium to benign tumors to malignant invasive carcinomas. The evolution from a precursor lesion to CRC is estimated to take 10-15 years.

Accumulation of mutations due to loss of genomic stability is an important driver of CRC development. Chromosomal instability or a mutator phenotype involving DNA repair defects or aberrant DNA methylation have been identified as major mechanisms in CRC development[2,3].

Chromosomal instability may cause the loss of a wild-type copy or inactivating mutations in tumor suppressor genes such as APC or TP53[2,3]. APC mutations are the most common early mutations in CRC, present in up to 85% of cases. APC germline mutations (cause of Familial Adenomatous Polyposis) or APC somatic mutations or deletions are found in most cases of CRC. TP53 mutations are the second most common in CRC, present in up to 55% of cases, and inactivate the p53 pathway, which compromises cell-cycle arrest and cell death pathways and has strong implications on decreased responsiveness to therapy[2,3]. Other relevant and frequently mutated genes in CRC are the oncogenes KRAS and BRAF, which activate proliferative and antiapoptotic pathways[2,3].

DIET AND CRC RISK

Factors that prevent DNA lesions, induce DNA repair at the cancer initiation stages or modulate the rate of tumor growth and invasiveness have potential in cancer preventive strategies. Many food constituents have been described that exert cancer preventing effects due to their role as antioxidants or to their effects on the activity of genotoxic metabolizing enzymes. Multiple reports have been published on the anticancer potential of plant foods and individual phytochemicals. Molecular targets for dietary constituents that may be responsible for their cancer chemopreventive effects have been identified. This has been the subject of intense research and major results compiled in several recent reviews[4-7]. Green tea, cruciferous vegetables, red grapes, turmeric, garlic, soybeans, apples and citrus fruits are examples
of plant foods with established anticancer properties[4].

In spite of the recognized health benefits of the intake of dietary fruits and vegetables, the World Cancer Research Fund/American Institute of Cancer Research report[1] does not classify the evidence gathered from human studies as being convincingly strong with regard to reduced CRC risk. It is possible that the explanation for this is that effects vary according to CRC molecular type and that this precludes generalized conclusions.

In a large pooled analysis by Hidaka et al[8] of associations by CRC molecular type, the authors found that higher fruit intake was associated with lower risk of BRAF mutated CRC and the traditional adenoma-CRC pathway[8]. However, most other epidemiologic studies investigating the association of different molecular CRC subtypes and intake of fruits, vegetables and fiber ingestion have been inconclusive.

With regard to the individual food chemical constituents responsible for the beneficial effects, much attention has been given to polyphenols, and in particular to flavonoids, due in part to their strong antioxidant activity, repeatedly demonstrated in vitro and animal studies.

Fruits and vegetables are rich sources of flavonoids and other polyphenols (Table 1). These compounds, flavonoids in particular, are potent antioxidants, have anti-inflammatory properties and have been extensively studied with regard to their potential as anticancer dietary constituents[4,5,7].

The effects of human dietary intake of flavonoids on decreased risk of CRC have been the subject of much fewer reports. Procyanidin (oligomeric forms of catechin and epicatechin) and isoflavone ingestion was found by He and Sun[9] in population studies to decreased CRC risk, while total flavonoid intake was not found to associate with decreases in CRC risk[9].

In another report, Chang et al[6] found that high intake of flavonols, flavones and anthocyanidins may decrease CRC risk. However, Djuric et al[10] corroborated that total flavonoid intake did not associate with CRC risk nor did total flavanones or flavan-3-ols. High variability between the studies in this meta-analysis precluded any further conclusions[6].

Previously, Djuric et al[10] found that quercetin (Q) decreased risk of proximal CRC but lost its effect if tea consumption was high. Importantly, the authors found that increased intake of Q was associated with increased risk of distal colon cancer when total fruit intake was low[10]. On the other hand, APC mutations were found to be associated with alcohol and red and processed meat consumption[11].

CRC TREATMENT

Endoscopic or surgical lesion removal is the best curative strategy for CRC[3]. In order to increase treatment efficacy, adjuvant fluoropyrimidine based [5-fluorouracil (5-FU) or capecitabine] chemotherapy combined with oxaliplatin and/or irinotecan is also used[3].

Another recent group of drugs known as “biologics” are increasingly used in the treatment of CRC. These are monoclonal antibodies such as bevacizumab, an anti-vascular endothelial growth factor (VEGF) antibody that targets angiogenesis, or cetuximab and panitumumab, anti-epidermal growth factor receptor (EGFR) antibodies that target proliferative signals mediated by EGFR[3,12] (Figure 1).

FOLFOX (leucovorin, 5-FU and oxaliplatin) or FOLFIRI (leucovorin, 5-FU and irinotecan), frequently combined with EGFR or VEGF receptor inhibitors are the most commonly utilized chemotherapeutic regimes in CRC treatment[12].

Under normal conditions, upon ligand binding to EGFR or the VEGF receptor, both the mitogen-activated protein kinase (MAPK) (RAS/RAF/MEK/ERK) and the PI3K/AKT/mTOR phosphorylation cascades will be activated, leading to cell proliferation and survival[3,13]. Although EGFR is overexpressed in 60%-80% of CRC tumors (which makes it a good anticancer target) favoring the proliferative and antiapoptotic activity of the MAPK and PI3K pathways[14], efficacy of anti-EGFR drugs (and to a smaller extent also anti-VEGF receptor) decreases dramatically in the presence of KRAS and particularly BRAF mutations[3,12,13] due to their overriding downstream effects on the signaling pathways (Figure 1).

Mutations in the oncogene KRAS occur in 37% of CRCs and constitutively activate proliferative and antiapoptotic pathways such as the MAPK and the PI3K pathway. Mutations in the oncogene BRAF occur in 13% of CRCs activate the MAPK pathway downstream of KRAS but not the PI3K pathway[2,15]. KRAS mutations do not co-occur with BRAF mutations in CRC[13,16]. Activating mutations of PI3K are present in 15%-20% of CRC and loss of PTEN, an inhibitor of the PI3K pathway, also contribute to increased activity of the PI3K/AKT pathway[3,13].

Therefore, mutations in the key players KRAS, BRAF and PI3K promote CRC development, define molecular subtypes and are relevant for the efficacy of chemotherapeutic agents such as anti-EGFR monoclonal antibodies[2,3,12,14,15].

In addition, targeted anti-EGFR therapy with cetuximab or panitumumab often leads to secondary resistance through selection of resistant cells even in patients wildtype for EGFR, KRAS and BRAF[13,14]. More recently, regorafenib has shown to be of use as third-line treatment for KRAS and BRAF mutated tumors[15,17] (Figure 1).
Table 1  Fruits and vegetables are rich sources of flavonoids and other polyphenols

<table>
<thead>
<tr>
<th>Class of polyphenol</th>
<th>Representative compounds</th>
<th>Food sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonol(^1)</td>
<td>(+)-Catechin; (-)-Epicatechin Epigallocatechin gallate</td>
<td>Cocoa, green tea</td>
</tr>
<tr>
<td>Flavone(^2)</td>
<td>Luteolin, Apigenin, Chrysin</td>
<td>Parsley, red peppers</td>
</tr>
<tr>
<td>Flavonol(^3)</td>
<td>Quercetin, Rutin, Kaempferol</td>
<td>Onions, broccoli, apples</td>
</tr>
<tr>
<td>Flavanone(^2)</td>
<td>Naringin, Naringenin, Hesperidin</td>
<td>Citrus fruits</td>
</tr>
<tr>
<td>Isoflavones(^3)</td>
<td>Genistein, Daidzein</td>
<td>Soybean</td>
</tr>
<tr>
<td>Anthocyanidin(^1)</td>
<td>Cyanidin, Delphinidin, Pelargonidin</td>
<td>Blueberries, raspberries</td>
</tr>
<tr>
<td>Curcuminoid</td>
<td>Curcumin</td>
<td>Turmeric</td>
</tr>
<tr>
<td>Stilbene</td>
<td>Resveratrol</td>
<td>Red grapes</td>
</tr>
</tbody>
</table>

\(^1\)Subclasses of flavonoids.

Figure 1 Signaling pathways and molecular targets of anti-colorectal cancer chemotherapeutic drugs and of dietary phytochemical constituents. Generated with BioRender.com. EGFR: Epidermal growth factor receptor; EGCG: Epigallocatechin-3-gallate; VEGFR: Vascular endothelial growth factor receptor.

Genetic profiling to determine the presence of EGFR, KRAS or BRAF mutation is, therefore, important to predict treatment outcome, although other unknown factors play important roles in a patient’s response to therapy[14]. Targeted chemotherapy tailored to improve patient response with minimal side effects remains a goal, but much work remains in order to achieve it since the 5-year survival rate of CRC patients is still less than 15% for those diagnosed in advanced stages of the disease with metastatic CRC[14].

Therapeutic strategies to treat tumors with mutations in these key genes remain limited, and improving treatment efficacy for these patients constitutes a pressing clinical need. Despite this, dietary and other plant derived phytochemicals remain an untapped resource despite the large body of evidence of their acting on the relevant molecular targets.

CHEMOTHERAPEUTIC POTENTIAL OF DIETARY PHYTOCHEMICALS

Several compounds have been shown to target the MAPK and/or the PI3K pathways. Epigallocatechin-3-gallate is a green tea polyphenol also present in chocolate. Epigallocatechin-3-gallate has been shown to inhibit EGFR signaling and ERK1 (MAPK pathway) and PI3K/AKT activation, with effects on cell proliferation and survival[14,18].
Curcumin, another natural polyphenol present in turmeric, has also been shown to decrease EGFR gene expression thereby decreasing ERK-mediated signaling and gene expression[14]. In addition, curcumin suppressed the PI3K/Akt pathway in vitro with induction of apoptosis[19,20] and decreased NF-kB activation by traditional chemotherapeutic drugs thereby contributing to overcoming treatment resistance[21]. Inhibitory effects on PI3K/AKT and NF-kB seem to be involved in sensitization to treatments with 5-FU and capecitabine[22,23].

In other studies, curcumin was shown to produce a similar effect to that of the MEK inhibitor U0126 and to synergistically enhance the efficacy of the multi-kinase inhibitor regorafenib in HCT116 cells[16,24]. New delivery formulations that address curcumin’s low bioavailability look promising and will increase the compound’s clinical use[21].

Q, is a flavonoid particularly abundant in onions, apples and broccoli. The health benefits of Q have been widely reported and include antioxidant, anti-inflammatory and cancer chemopreventive activity. Recent reviews of Q cancer chemopreventive mechanisms have been published by Kashyap et al[25] and Rather and Bhagat[26]. These include scavenging of reactive oxygen species and induction of antioxidant defenses, modulation of signaling pathways resulting in decreased cell proliferation and increased apoptotic cell death, cytochrome P450 enzyme activity modulation, induction of Nrf2-mediated phase II enzymes and inhibitory effects on inflammatory markers such as iNOS, COX2, IL-6 and TNF-alpha among others. Q has also been shown to decrease signaling through the MAPK and PI3K pathways as well as improve the response to chemotherapeutic drugs[25-29].

In studies with human CRC-derived cell lines harboring either KRAS or BRAF mutations, it is possible to test the impact of individual compounds (or complex plant extracts) on the activity of pathways caused by that particular mutation thereby identifying the subset of patients that would benefit the most from strategies involving that particular flavonoid. Q and luteolin (L) are two of the best studied flavonoids and two of the most abundant in plant foods. They have demonstrated anti-CRC activity in numerous in vitro and in vivo studies[28].

In a study using the human CRC cells line HCT15, (harboring a KRAS mutation and wild-type BRAF) and CO115 (harboring a BRAF mutation and wild-type KRAS) the authors showed that both Q and L decreased MAPK activity in HCT15 but not in CO115 cells[27]. It seems, therefore, that the BRAF mutation present in CO115 overrides the inhibitory effects of Q and L on MAPK pathway activity. In addition, Q and L decreased KRAS total protein expression but had no effect on total BRAF expression levels. This is an indication that Q and L could benefit MAPK dependent therapeutic effects in KRAS-mutated but not be as effective in BRAF-mutated tumors[27]. Q and several of its analogs are also known inhibitors of PI3K activity[25-29].

Crosstalk between MAPK and PI3K/AKT suggests that levels of PI3K pathway activity may also be affected in KRAS-mutated cells and contribute to the overall effects on cell proliferation and apoptotic cell death. However, PI3K activity may also be regulated independently of its upstream regulator KRAS.

Interestingly in the Xavier et al[27] study was the fact that the specific MEK inhibitor PD-98059 (MEK a downstream link of both KRAS and BRAF in the MAPK pathway) significantly reduced phosphor-ERK levels but did not significantly decrease cell proliferation or induce apoptotic cell death[27]. Also wortmanin, a reference PI3K inhibitor, decreased phosphor-AKT levels, an indication of pathway inhibition, without producing significant effects on proliferation or apoptotic cell death. Of relevance here, in the comparison between the effects of the reference compounds PD-98059 and wortmanin and Q or L is the fact that the flavonoids had significant effects on cell proliferation and cell death, a clear indication that the multitarget nature of the flavonoids’ actions was beneficial and not detrimental to the overall effect[27].

In another study by Yang et al[30], Q induced apoptosis in KRAS-mutated cells in a c-Jun N-terminal kinase activation-dependent way. Both the G15D and G12V mutations of KRAS rendered CRC cells more sensitive to Q than KRAS wild-type cells[30].

**COMBINATION OF FLAVONOIDS PLUS CHEMOTHERAPEUTIC AGENTS**

Studies where flavonoids are tested in combination with chemotherapeutical drugs also demonstrate possible enhancement of therapeutic effects and consequent benefits of co-administration regimens. The majority of studies involving the role of phytochemicals in combination with chemotherapeutic drugs, use 5-FU and address the involvement of p53 in the response, due to the key role of p53 in apoptosis induction and treatment sensitivity[29,31-39]. Tumor progression and low treatment efficacy is strongly dependent on successful evasion of cell death due to mutations in TP53[31], present in 35%-55% of CRC cases.

In addition to p53 status, CRC displaying DNA mismatch repair defects and microsatellite instability show lower sensitivity to 5-FU and poorer treatment outcome. In this scenario of increased treatment resistance, Q was tested, and the dependence on p53 status for the cell’s response to 5-FU was determined[29]. Q significantly increased apoptotic cell death in response to 5-FU in p53 wild-type CO115 and HCT116 comparatively to effects on HCT15 p53-mutated cells. P53 silencing in CO115
completely abrogated the apoptotic effects of Q + 5-FU. This p53 dependence was further corroborated in the isogenic HCT116 p53-/− cells, where both the effects of the drug alone and of the combination Q + 5-FU were lost. This constituted a clear indication that wild-type p53 was required for Q enhancement of 5-FU apoptotic effects and suggested that tumors displaying wild-type p53 are more likely to benefit from combination treatment of 5-FU with Q than tumors harboring inactivating mutations of p53[29].

Resveratrol, on the other hand, increased 5-FU-induced apoptosis in a p53-independent manner in both HCT116 wild-type p53 and p53-deficient cells. Resveratrol also decreased MAPK and PI3K/AKT signaling and upregulated miR-34a expression, in a new mechanism of inhibition of HCT116 proliferation induced by the combination of resveratrol + 5-FU[40]. Upregulated miR-34a expression was also involved in chemosensitization of HCT116 and HT-29 to oxaliplatin by resveratrol[41].

Combination exposures to FOLFOX and curcumin showed reduction of tumor explant cell survival due to enhanced antiproliferative effects[42,43]. Information on the anti-CRC effects of curcumin including clinical trials involving curcumin are the subject of a recent publication[21]. Molecular targets for curcumin include MAPK and PI3K pathways and decreases in chemotherapy induction of NF-κB and antiapoptotic gene expression.

Yue et al[44] showed that turmeric extract in combination with bevacizumab produced comparable effects to bevacizumab plus FOLFOX with the advantage that it increased survival of tumor-bearing mice. In another study[45], the combination of curcumin with the multi-kinase inhibitor regorafenib produced an increase in cell death in KRAS-mutated CRC cells and not in wild-type KRAS CRC cells.

With regard to clinical trials[21], most involve curcumin and address the pharmacokinetic profile and toxicity of curcumin formulations. Some trials test curcumin in combination with drugs such as 5-FU or irinotecan and will provide useful information, even if enrollment is low and patients are most likely not stratified by tumor molecular subtype.

Much remains to be done with regard to the effects of phytochemicals on patients, whether isolated or in combination with chemotherapeutical drugs.

CONCLUSION
Although a diet rich in fruits and vegetables is generally beneficial with regard to CRC, variations in response among individuals to similar dietary choices strongly suggest that there may be interactions of food constituents with particular molecular targets that will benefit patients differently according to their tumor’s molecular signature.

The capacity to inhibit proliferation and induce apoptosis through effects on molecular targets of the MAPK and/or the PI3K/AKT pathways makes flavonoids potentially strong allies if used as adjuvants in combination treatment improving therapeutic efficacy and patient survival. However, in order to take full advantage of the anticancer potential of these natural compounds, a detailed and systematic characterization of the compound’s mechanisms of action is required. Although much has been published on the anticancer effects of dietary phytochemicals, such an exhaustive characterization of potential beneficial and adverse effects in the setting of cancer treatment in humans is still lacking. Results from in vitro studies cannot be extrapolated to effects in humans. Also, care should be taken that some flavonoids’ and other phytochemicals’ potential to prevent DNA damage or induce repair of the lesions produced by the therapeutic agent do not hinder treatment efficacy.

Clinical trials are needed to verify relevant effects and those remain insufficient in number and in patient enrollment. Also, patients are generally not selected on the basis of their tumor molecular profiles, which would be a requirement for a more personalized treatment approach.

Moreover, efforts should be concerted towards establishing nutritional guidelines that prescribe the ingestion of the right plant food sources or of individual flavonoids as part of nutraceutical formulations in the several phases of CRC progression and treatment. Much work remains to be done, not least the introduction of standardization and regulatory systems as well as extensive testing through clinical trials of possible toxicity of the new formulations.

Reduced bioavailability often mean that polyphenols have limited efficacy in in vivo studies. However, these issues can today be addressed by nanoencapsulation, which may increase bioavailability and increase circulating concentrations or enable delivery into the colon and cause direct exposure of colonocytes to the active compounds limiting possible systemic side effects.

FOOTNOTES

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Basic Study

Glutamine deprivation impairs function of infiltrating CD8+ T cells in hepatocellular carcinoma by inducing mitochondrial damage and apoptosis

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Abstract

BACKGROUND
The functions of infiltrating CD8+ T cells are often impaired due to tumor cells causing nutrient deprivation in the tumor microenvironment. Thus, the mechanisms of CD8+ T cell dysfunction have become a hot research topic, and there is increased interest on how changes in metabolomics correlate with CD8+ T cell dysfunction.

AIM
To investigate whether and how glutamine metabolism affects the function of infiltrating CD8+ T cells in hepatocellular carcinoma.

METHODS
Immunohistochemical staining and immunofluorescence were performed on surgically resected liver tissues from patients. Differentially expressed genes in infiltrating CD8+ T cells in hepatocellular carcinoma were detected using RNA sequencing. Activated CD8+ T cells were co-cultured with Huh-7 cells for 3 d. The function and mitochondrial status of CD8+ T cells were analyzed by flow cytometry, quantitative real-time polymerase chain reaction, and transmission electron microscopy. Next, CD8+ T cells were treated with the mitochondrial protective and damaging agents. Functional alterations in CD8+ T cells were
detected by flow cytometry. Then, complete medium without glutamine was used to culture cells and their functional changes and mitochondrial status were detected.

RESULTS
There were a large number of infiltrating PD-1+CD8+ T cells in liver cancer tissues. Next, we co-cultured CD8+ T cells and Huh-7 cells to explore the regulatory effect of hepatoma cells on CD8+ T cells. Flow cytometry results revealed increased PD-1 expression and decreased secretion of perforin (PRF1) and granzyme B (GZMB) by CD8+ T cells in the co-culture group. Meanwhile, JC-1 staining was decreased and the levels of reactive oxygen species and apoptosis were increased in CD8+ T cells of the co-culture group; additionally, the mitochondria of these cells were swollen. When CD8+ T cells were treated with the mitochondrial protective and damaging agents, their function was restored and inhibited, respectively, through the mitochondrial damage and apoptotic pathways. Subsequently, complete medium without glutamine was used to culture cells. As expected, CD8+ T cells showed functional downregulation, mitochondrial damage, and apoptosis.

CONCLUSION
Glutamine deprivation impairs the function of infiltrating CD8+ T cells in hepatocellular carcinoma through the mitochondrial damage and apoptotic pathways.

Key Words: Glutamine; Mitochondrial damage; CD8+ T cells; T cell function; Hepatocellular carcinoma

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Core Tip: This study aimed to investigate whether and how glutamine metabolism affects the function of infiltrating CD8+ T cells in hepatocellular carcinoma. Experimental validation was performed by using liver cancer tissues and cell lines. We discovered that glutamine deprivation impaired the function of infiltrating CD8+ T cells in hepatocellular carcinoma through the mitochondrial damage and apoptotic pathways.

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INTRODUCTION
CD8+ T cells are important effector immune cells in the tumor microenvironment that primarily kill tumor cells by secreting granzyme B (GZMB) and perforin (PRF1)[1]. Owing to factors that include chronic stimulation by tumor antigens, the physicochemical state of the tumor microenvironment is imbalanced, including low pH, hypoxia, and low nutrition availability[2,3], which trigger T cell dysfunction and ultimately result in cell depletion[4,5]. Exhausted T (Tex) cells are characterized by loss of effector functions, elevated and sustained expression of inhibitory receptors (IRs), and a distinct metabolic profile[6,7]. Recently, the mechanisms of CD8+ T cell exhaustion have become a hot research topic, and there is increased interest on how changes in metabolomics correlate with changes in immune cell functions.

Tumor cells consume high levels of energy sources such as glucose[8], resulting in nutrient deprivation in the tumor microenvironment. This deprives the energy sources of immune cells, altering the phenotype and function of affected immune cells[9]. Glutamine (Gln) is the most abundant free amino acid in serum[10]. Gln is not only involved in the occurrence, development, and metastasis of tumors[11,12], but also regulates the growth and function of immune cells[13]. Gln has been reported to regulate the phenotype of CD4+ cells, and increasing Gln levels can skew regulatory CD4+ T cells toward more inflammatory subtypes[14,15]. However, whether Gln also regulates CD8+ T cells and the mechanism underlying this regulation have not been reported.

Mitochondria are important intracellular organelles that provide energy and biosynthetic substrates for cell survival through oxidative phosphorylation[16]. Mitochondria are also highly nutrient-sensitive. When cells are deficient in nutrients, their mitochondria undergo depolarization and appear damaged. Damaged mitochondria can release pro-apoptotic proteins such as cytochrome c and other substances to induce the formation of apoptotic complexes that activate caspase 9, which causes apoptosis. Recent
research has demonstrated mitochondrial damage in tumor tissues that is closely related to remodeling of the tumor microenvironment. It has also been shown in the literature that after glucose deprivation, CD8+ T cells in the tumor microenvironment produce reactive oxygen species (ROS), which subsequently trigger mitochondrial damage[17]. Whether Gln similarly regulates the function of mitochondria in CD8+ T cells is still unclear.

Therefore, this study investigated whether Gln regulates CD8+ T cell function through the mitochondrial damage and apoptotic pathways to clarify the relationship between Gln metabolism and CD8+ T cell depletion, which will lay a foundation for developing new anti-tumor treatments.

**MATERIALS AND METHODS**

**Immunohistochemical staining**

Immunohistochemical staining was performed on surgically resected tissues from patients with clinicopathologically confirmed hepatocellular carcinoma (HCC) and adjacent non-cancerous tissues. The study was approved by the Medical Ethics Committee of North China University of Science and Technology (No. 2018109).

**Immunofluorescence**

Immunofluorescence staining of paraffin-embedded human liver tissue sections was performed using anti-PD-1/CD279 (66220-1-Ig, Proteintech, Rosemont, IL, United States) and anti-CD8α (PB9249, Boster Bio, Pleasanton, CA, United States) antibodies. Antigen retrieval was performed with EDTA buffer (AR0023, Boster Bio). Tissue sections were blocked with 10% goat serum (AR1009, Boster Bio), and then incubated with rabbit anti-CD8α antibody (1:200 dilution) and mouse anti-PD-1 antibody (1:4000 dilution) at 4 °C overnight. Secondary antibodies including DyLight594 fluorescein goat anti-mouse (BA1141, Boster Bio) and fluorescein DyLight488 goat anti-rabbit (BA1127, Boster Bio) IgGs were then added and incubated for 45 min at 37 °C. DAPI staining solution (AR1176, Boster Bio) was used for counterstaining at room temperature for 3 min, and then washed with PBS (pH 7.2-7.6) (AR0030, Boster Bio). Slides were mounted using anti-fluorescent quench mounting medium (AR1109, Boster Bio). Finally, whole-slide scanning (APERIOVERSA8, Leica, Wetzlar, Germany) and image acquisition (BX51, Olympus, Tokyo, Japan) were performed.

**Cell sorting**

First, 100 mL of peripheral blood was collected from healthy volunteers, from which peripheral blood mononuclear cells (PBMCs) were obtained by diluting peripheral blood with an equal volume of PBS followed by centrifugation (800 g, 20 min). The pellet was then washed with 5 × volume of PBS and centrifuged (200 g, 5 min). Cells were resuspended in x-vivo15 (SH30809.01B, Hyclone, Logan, UT, United States). PBMCs were stained with anti-human CD8 antibody (Biolegend, San Diego, CA, United States). Cell sorting was performed using a MoFloXDP sorting flow cytometer (Beckman Coulter, Brea, CA, United States). The post-classification purity of all included samples was > 85%.

**Cell culture and activation**

The hepatoma cell line HuH-7 (RRID: CVCL_0336) was confirmed via short tandem repeat markers by Procell Life Science & Technology Co., Ltd. (Hyderabad, India). The results showed that the DNA typing of this cell line matched 100% with other typing in the CRC cell bank, and no human cell cross-contamination was found. HuH-7 cells were cultured in Dulbecco’s modified eagle medium (DMEM) (Sigma-Aldrich, St. Louis, MO, United States), supplemented with 10% fetal bovine serum (FBS) (Gibco, Waltham, MA, United States) and 1 % penicillin/streptomycin (Hyclone). CD8+ T cells were cultured in vitro using Roswell Park Memorial Institute (RPMI) 1640 medium (SH30809.018; Hyclone), supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Hyclone). CD8+ T cells were stimulated with 10 μg/mL anti-human CD3 antibody (kx10-3A; Beijing Kexin Biological, Beijing, China) and 2.5 μg/mL anti-human CD28 antibody (kx10-28A; Beijing Kexin Biological) in medium containing 100 U/mL IL-2 (PeproTech, Rocky Hill, NJ, United States) for 2 d. Both cell types were maintained at 37 °C in an atmosphere containing 50 mL/L CO₂.

**Cell co-culture**

HuH-7 and CD8+ T cells were co-cultured in transwell inserts for 3 d, and the initial number of both cell types used was equal.

**RNA sequencing of CD8+ T cells**

Total RNA was extracted from CD8+ T cells, and total RNA sequencing (RNA-Seq) libraries were obtained by a three-step method: (1) RNA library construction and on-board sequencing were performed. Sequencing data were aligned to the reference genome of the project species to obtain comprehensive transcript information and to perform gene expression quantification; (2) The
transcriptome sequencing project was completed on the Illumina sequencing platform (San Diego, CA, United States), and the IlluminaPE library (approximately 300 bp) was constructed for sequencing; and (3) The obtained sequencing data were analyzed by bioinformatics after performing quality control.

**Flow cytometry analysis**

CD8⁺ T cells isolated from peripheral blood were cultured in groups and stained for intracellular markers. Analytical flow cytometry (NovoCyte, ACEA Biosciences Inc., San Diego, CA, United States) and a flow analyzer (CytoFLEXS, Beckman Coulter) were used for the analysis. CD8⁺ T cells were stained with an Annexin V-FITC Apoptosis Detection Kit (A211-02; Vazyme, Nanjing, China) to detect apoptosis. CD8⁺ T cells were stained with JC-1 fluorescent dye (J8030; Solarbio, Beijing, China) and a ROS Detection Kit (CA1410; Solarbio) to detect mitochondrial damage and ROS levels, respectively. Cells were fixed and permeabilized with Fixation/Permeabilization solution (554714; BD Biosciences, Franklin Lakes, NJ, United States), and then CD8⁺ T cells were stained with anti-GZMB (515403; Biolegend) and anti-PRF1 (154305; Biolegend) antibodies to detect their activation status. Finally, CD8⁺ T cells were stained with PE-conjugated anti-human CD279 (PD-1) antibody (329905; Biolegend) to detect their functional changes.

**Quantitative real-time polymerase chain reaction**

First, mRNA was extracted from sorted CD8⁺ T cells using the MiniBEST Universal RNA Extraction Kit (Cat.#9767; TaKaRa, Dalian, China) according to the manufacturer’s protocol. Extracted mRNA was reverse-transcribed into cDNA after mixing mRNA template with the Primescript RT Reagent Kit with gDNA Eraser (AK3920; TaKaRa) for quantitative polymerase chain reaction (qPCR). The qPCR reactions were run on a Light Cycler 480SYBRGreenI Master (04887352001; Roche, Basel, Switzerland). The primer sequences are as follows: GZMB (forward: 5′-GGTGCGGTGGCTTCCTGAT-3′ and reverse: 5′-ACTGCTGGTGCTTGCTCATG-3′); PRF1 (forward: 5′-TGCGGTCTTACAGTTTCAA-3′ and reverse: 5′-GCACCTCGTGTCCGTGAG-3′); and GAPDH (forward: 5′-TCAAGAAGGTGGTGAAGCAGG-3′ and reverse: 5′-TCAAGGTAAGGAGTGGGT-3′). The qPCR steps included initial denaturation at 95 °C for 10 min, and 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 15 s, and extension at 72 °C for 20 s. All qPCR reactions were performed on a Bio-Rad real-time quantitative fluorescence PCR instrument (CFX Connect, Bio-Rad, Hercules, CA, United States). Relative gene expression was calculated with respect to the internal standard (GAPDH). Expression levels were normalized against GAPDH using the 2^(-ΔΔCt) method.

**Transmission electron microscopy**

Cells in each group were digested to prepare cell suspensions at a density of 1 × 10⁶ cells/mL. After discarding the supernatant, the cells were fixed with 3% glutaraldehyde fixative for 2 h and 1% osmic acid for 1 h. After dehydration with a graded ethanol series and acetone, the cells were embedded in epoxy resin, ultrathin sectioned, and double stained with uranium and lead. The ultrastructural changes of mitochondria in each group of cells were observed by transmission electron microscopy (TEM).
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Figure 2 PD-1 expression by infiltrating CD8+ T cells in liver tissues. Tissues were subjected to fluorescent double staining with anti-CD8 and anti-PD-1 antibodies. A: Hepatocellular carcinoma tissues; B: Paracancerous tissues. Scale bar: 400 μm.

Statistical analysis
All analyses were performed with SPSS 21.0 software (IBM Corp., Armonk, NY, United States) and GraphPad Prism 8.0.2 (GraphPad Software, Inc., San Diego, CA, United States). Measurement data were first examined using the Kolmogorov-Smirnov test to check whether the measurement data of each group had a normal distribution. Results are expressed as the mean ± SE for measurement data with a normal distribution. Comparisons between two groups were performed using the two-tailed Student’s t test. One-way analysis of variance was used for multiple group comparisons, and the LSD-t test was used for pairwise comparisons. Significance was accepted at P < 0.05.

RESULTS

CD8+ T cell infiltration in liver tissues
To investigate T cell infiltration in the tumor microenvironment, we performed immunohistochemical staining of resected liver tissues. The results showed significantly more CD8+ T cells in HCC tissues than in paracancerous tissues (Figure 1). Next, we used immunofluorescence staining to detect PD-1 expression on CD8+ T cells. Compared with paracancerous tissues, PD-1 was more abundantly...
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Figure 3 Evaluations of CD8\(^+\) T cell function. Activated CD8\(^+\) T cells were co-cultured with Huh-7 cells for 3 d. Flow cytometry was used to detect the expression of indicated molecules in CD8\(^+\) T cells. A: PD-1; B: PRF1; C: Granzyme B (GZMB). D and E: Quantitative real-time polymerase chain reaction was used to detect the mRNA levels in CD8\(^+\) T cells (D: PRF1; E: GZMB). The data represent the mean ± SE; \( ^{a}P < 0.05; ^{b}P < 0.01; ^{c}P < 0.001; ^{d}P < 0.0001 \).

expressed on the surface of CD8\(^+\) T cells in HCC tissues (Figure 2).

**Hepatoma cells induce decreased CD8\(^+\) T cell function**

After observing a large number of infiltrating PD-1\(^+\)CD8\(^+\) T cells in HCC tissues, we next co-cultured CD8\(^+\) T cells with Huh-7 cells to evaluate the functional alterations of CD8\(^+\) T cells. Flow cytometry and
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Figure 4 Differential gene expression in CD8+ T cells. Activated CD8+ T cells were co-cultured with Huh-7 cells for 3 d. Total RNA was extracted from CD8+ T cells for transcriptome sequencing. A: GO enrichment plot of upregulated genes; B: GO enrichment plot of downregulated genes.

quantitative real-time PCR were used to examine the function of CD8+ T cells, which indicated that compared with the control group, CD8+ T cells in the co-culture group expressed higher levels of PD-1 (Figure 3A) and lower levels of GZMB and PRF1 (Figure 3B-E). Increased expression of IRs and reduced ability to secrete effector molecules suggested functional inhibition of CD8+ T cells; thus, hepatoma cells
Hepatoma cells induce differential gene expression in CD8+ T cells

To explore the potential mechanisms of the functional impairment of T cells, we performed RNA-seq of CD8+ T cells co-cultured with Huh-7 cells. We found that genes related to mitochondrial damage (such as mitochondrial disassembly) and apoptosis (such as regulation of apoptotic pathways) were upregulated (Figure 4A). The expression of genes related to cell metabolism (such as multi-organism metabolic processes) and T cell function (such as T cell proliferation) was downregulated (Figure 4B). These data illustrated that CD8+ T cells co-cultured with hepatoma cells underwent alterations associated with cellular metabolism, mitochondrial damage, and apoptosis.
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**Figure 6** Transmission electron microscopy images of mitochondria in CD8\(^+\) T cells. Activated CD8\(^+\) T cells were co-cultured with Huh-7 cells for 3 d. The morphology of mitochondria in CD8\(^+\) T cells was detected by transmission electron microscopy. A: Control; B: Co-culture. Scale bar: 2 μm.

**Figure 7** Regulation of CD8\(^+\) T cell function by Mdivi-1. A-C: Activated CD8\(^+\) T cells were co-cultured with Huh-7 cells in RPMI 1640 containing 5 μmol/L Mdivi-1 for 3 d. The levels of indicated molecules in CD8\(^+\) T cells were detected by flow cytometry. A: PD-1; B: PRF1; C: Granzyme B. The data represent the mean ± SE; \(*P < 0.001; **P < 0.0001."

**Hepatoma cells induce mitochondrial damage and apoptosis in CD8\(^+\) T cells**

Mitochondria are the primary source of ROS production, and JC-1 is a dye used to reflect mitochondrial integrity. To examine whether hepatoma cells have a regulatory effect on CD8\(^+\) T cell mitochondria,
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Figure 8 Regulation of CD8+ T cell function by carbonyl cyanide-3-chlorophenylhydrazone. A-C: Activated CD8+ T cells were cultured in RPMI 1640 supplemented with 5 μmol/L or 10 μmol/L carbonyl cyanide-3-chlorophenylhydrazone for 3 d, respectively. The levels of indicated molecules in CD8+ T cells were detected by flow cytometry. A: PD-1; B: PRF1; C: Granzyme B. The data represent the mean ± SE; *P < 0.0001. CCCP: Carbonyl cyanide-3-chlorophenylhydrazone.

CD8+ T cells were co-cultured with Huh-7 cells. Flow cytometry analysis revealed that CD8+ T cells from the co-culture group had decreased JC-1 staining (Figure 5A) and increased levels of ROS (Figure 5B) and apoptosis (Figure 5C) compared with the control group. Next, mitochondrial morphology was further studied by TEM to examine the ultrastructure of mitochondria. Mitochondria of CD8+ T cells from the control group were identified with well-defined integral two-layer membranes and regular cristae (Figure 6A). In contrast, mitochondria of CD8+ T cells in the co-culture group showed significant swelling (Figure 6B). In summary, co-culture with hepatoma cells induced damage to the mitochondria of CD8+ T cells and caused apoptosis.

Mitochondrial damage induced by hepatoma cells impairs CD8+ T cell function

Next, we explored the relationship between mitochondrial damage and T cell function in infiltrating CD8+ T cells in HCC. First, we co-cultured CD8+ T cells with Huh-7 cells, with mitochondrial division inhibitor 1 (Mdivi-1) added to the culture system, which has been shown to protect from mitochondrial damage. Flow cytometry was used to examine the function of Mdivi-1-treated CD8+ T cells in co-culture. The results showed reduced expression of PD-1 (Figure 7A) and increased expression of PRF1 (Figure 7B) and GZMB (Figure 7C) in CD8+ T cells from the Mdivi-1 group compared with CD8+ T cells from the co-culture alone group, demonstrating that the function of co-cultured CD8+ T cells was not decreased after protecting from mitochondrial damage. Next, we cultured CD8+ T cells with different concentrations of the oxidative phosphorylation uncoupler carbonyl cyanide-3-chlorophenylhydrazone (CCCP) and examined their function. As shown from flow cytometry analysis, PD-1 expression gradually increased (Figure 8A), and there was a gradual decrease in the secretion of PRF1 (Figure 8B) and GZMB (Figure 8C) by CD8+ T cells with increasing CCCP concentrations. These results illustrated that mitochondrial damage led to the inhibition of CD8+ T cell function. In summary, co-culture with hepatoma cells impaired the function of CD8+ T cells through the mitochondrial damage pathway.
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Gln deprivation induces mitochondrial damage, apoptosis, and functional impairment of CD8+ T cells

As a primary energy source, Gln affects both the development of tumor cells and the functions of immune cells. Therefore, we assessed whether Gln metabolism affects the function of CD8+ T cells. We co-cultured CD8+ T cells with Huh-7 cells and supplemented the co-culture system with RPMI 1640 with normal concentrations of Gln or without Gln. The findings demonstrated that CD8+ T cells in the co-culture system without Gln expressed lower PD-1 (Figure 9A) and higher PRF1 (Figure 9B) and GZMB (Figure 9C) levels compared with the co-culture alone group; additionally, JC-1 staining was decreased (Figure 9D), and there were increased levels of ROS (Figure 9E) and apoptosis (Figure 9F). Next, CD8+ T cells were cultured alone in RPMI 1640 with the normal Gln concentration or without Gln to further validate the effect of Gln on CD8+ T cells. The results showed that CD8+ T cells in the group lacking Gln had increased PD-1 expression (Figure 10A) and decreased secretion of PRF1 (Figure 10B) and GZMB (Figure 10C) compared with those in the control group. Similarly, JC-1 staining was decreased (Figure 10D), and the levels of ROS (Figure 10E) and apoptosis (Figure 10F) were increased. Together, these data showed that hepatoma cells competed with CD8+ T cells for Gln. Gln deficiency inhibited the function of CD8+ T cells and induced mitochondrial damage and apoptosis.

DISCUSSION

In the tumor immune microenvironment, CD8+ T cells are the main immune effector cells and play a crucial role in the host immune environment. A large number of CD8+ T cells infiltrate tumor tissues in patients. However, due to chronic stimulation by tumor antigens, most of the T cells are functionally impaired and have differentiated into CD8+ T ex cells, which mediate tumor immune escape[18-20]. Therefore, understanding the mechanism of decreased CD8+ T cell function is essential for tumor immunity. T cell effector cells are characterized by the loss of effector functions and elevated and sustained expression of IRs[6,21,22]. In this study, the expression of PD-1 was increased and the secretion of cellular effector molecules such as GZMB and PRF1 was decreased in CD8+ T cells from the co-culture group. Our observations illustrated that the function of infiltrating CD8+ T cells was suppressed by HCC cells. Therefore, we next explored the specific mechanisms via which CD8+ T cell function was inhibited.

Mitochondria are central to cellular metabolism and play key roles in the functional regulation of immune cells such as CD8+ T cells[23]. Mitochondria are slightly impaired, the damage is neutralized by fusion with healthy mitochondria. However, when mitochondrial damage exceeds the range buffered by fusion, the cell promotes mitochondrial division and damage[24]. Severely injured mitochondria usually show increased ROS production and decreased membrane potential[25], which decreases cellular function. As shown by our data, the mitochondria of CD8+ T cells co-cultured with HCC cells exhibited swelling, increased ROS production, and decreased membrane potential, and the cells underwent apoptosis, implying that HCC cells induce mitochondrial damage and apoptosis in CD8+ T cells. Next, we examined the relationship between mitochondrial damage and CD8+ T cell function by using Mdivi-1 and CCCP to regulate mitochondrial status. Mdivi-1 is a quinazolinone derivative that penetrates cell membrane and attenuates mitochondrial damage by inhibiting mitochondrial division[26]. CCCP can interrupt oxidative phosphorylation, which impairs mitochondrial function and prompts mitochondria to produce high levels of ROS[27]. Together, these data show that mitochondrial damage can impairs the function of CD8+ T cells, i.e., hepatoma cells can impairs the function of CD8+ T cells through the mitochondrial damage and apoptotic pathways.

Studies have shown that tumor cells inhibit anti-tumor immunity by competing for essential nutrients and reducing the metabolic adaptability of tumor-infiltrating immune cells, which in turn decreases the function of immune cells[28]. The nutrients required for cellular metabolism include glucose, amino
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**A**

Control

0GlIn

**B**

P (0.46%)  
P (0.65%)  

**C**

P (0.49%)  
P (0.37%)  

**D**

**E**

Control

0GlIn

**C**

PEA

**C**

FITCA

**C**

FITCA

**C**

FITCA

**b**

**b**

**b**

**b**

**b**

**b**
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Figure 10 Altered CD8+ T cell function by glutamine deficiency. Activated CD8+ T cells were cultured in RPMI 1640 containing no glutamine (0Gln) for 3 d. The levels of indicated molecules in CD8+ T cells were detected by flow cytometry. A: PD-1; B: PRF1; C: Granzyme B; D: JC-1; E: Reactive oxygen species; F: Apoptosis. The data represent the mean ± SE; bP < 0.01; cP < 0.001; dP < 0.0001. Gln: Glutamine.

CD4+ T cells lacking Gln have diminished proliferative capacity and decreased cytokine secretion. We found that CD8+ T cells lacking Gln have a reduced ability to secrete effector molecules and increased expression of inhibitory receptors such as PD-1, suggesting that Gln deprivation inhibits the function of CD8+ T cells. In states of energy deficiency, mitochondrial function is abnormal, which further activates pro-apoptotic downstream regulators that induce apoptosis[30]. A previous study suggested that upon glucose deprivation, cells largely mobilize oxidative phosphorylation to maintain energy homeostasis, causing mitochondria to produce high levels of ATP and ROS[31]. Our data showed that in the absence of Gln, the mitochondria of CD8+ T cells underwent morphological changes with reduced mitochondrial membrane potential, generated high levels of ROS, and induced apoptosis. However, the above results were obtained only from in vitro experiments, and more details need to be further studied.

CONCLUSION
We found that Gln deprivation impairs the function of CD8+ T cells through the mitochondrial damage and apoptotic pathways.

ARTICLE HIGHLIGHTS

Research background
The functions of infiltrating CD8+ T cells are often impaired due to tumor cells causing nutrient deprivation in the tumor microenvironment. Thus, the mechanisms of CD8+ T cell dysfunction have become a hot research topic, and there is increased interest on how changes in metabolomics correlate with CD8+ T cell dysfunction.

Research motivation
To explore the effect of glutamine metabolism on the function of tissue-infiltrating CD8+ T cells, so as to provide a new strategy for reversing the exhausted CD8+ T cells in hepatocellular carcinoma.

Research objectives
This study aimed to investigate whether and how glutamine metabolism affects the function of infiltrating CD8+ T cells in hepatocellular carcinoma.

Research methods
Immunohistochemical staining and immunofluorescence were performed on surgically resected liver tissues from patients. Differentially expressed genes in infiltrating CD8+ T cells in hepatocellular carcinoma were detected using RNA sequencing. Activated CD8+ T cells were co-cultured with Huh-7 cells for 3 d. The function and mitochondrial status of CD8+ T cells were analyzed by flow cytometry, quantitative polymerase chain reaction, and transmission electron microscopy. Next, CD8+ T cells were treated with the mitochondrial protective and damaging agents. Functional alterations in CD8+ T cells were detected by flow cytometry. Then, complete medium without glutamine was used to culture cells, and their functional changes and mitochondrial status were detected.
Research results
There were a large number of infiltrating PD-1+CD8+ T cells in liver cancer tissues. Next, we co-cultured CD8+ T cells and Huh-7 cells to explore the regulatory effect of hepatoma cells on CD8+ T cells. Flow cytometry results revealed increased PD-1 expression and decreased secretion of perforin (PRF1) and granzyme B (GZMB) by CD8+ T cells in the co-culture group. Meanwhile, JC-1 staining was decreased and the levels of reactive oxygen species and apoptosis were increased in CD8+ T cells of the co-culture group; additionally, the mitochondria of these cells were swollen. When CD8+ T cells were treated with the mitochondrial protective and damaging agents, their function was restored and inhibited, respectively, through the mitochondrial damage and apoptotic pathways. Subsequently, complete medium without glutamine was used to culture cells. As expected, CD8+ T cells showed functional downregulation, mitochondrial damage, and apoptosis.

Research conclusions
Glutamine deprivation impairs the function of infiltrating CD8+ T cells in hepatocellular carcinoma through the mitochondrial damage and apoptotic pathways.

Research perspectives
From this study, we confirmed the potential mechanisms of CD8+ T cell dysfunction induced by glutamine deprivation, which would provide a novel strategy for reversing the exhaustion of CD8+ T cells in hepatocellular carcinoma.

FOOTNOTES
Author contributions: Wang W participated in the writing and editing of the manuscript; Guo MN and Li N participated in the data analysis; Pang DQ participated in the clinical specimen collection; Wu JH provided the experimental idea; all the authors approved for the final version of the manuscript.

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Retrospective Cohort Study

Does the addition of Braun anastomosis to Billroth II reconstruction on laparoscopic-assisted distal gastrectomy benefit patients?

Xiong-Guang Li, Qi-Ying Song, Di Wu, Shuo Li, Ben-Long Zhang, Li-Yu Zhang, Da Guan, Xin-Xin Wang, Lu Liu

Specialty type: Gastroenterology and hepatology

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Abstract

BACKGROUND
Operation is the primary therapeutic option for patients with distal gastrectomy. Braun anastomosis is usually performed after Billroth II reconstruction, which is widely applied on distal gastrectomy because it is believed to benefit patients. However, studies are needed to confirm that.

AIM
To identify whether the addition of Braun anastomosis to Billroth II reconstruction on laparoscopy-assisted distal gastrectomy benefits patients.

METHODS
A total of 143 patients with gastric cancer underwent laparoscopy-assisted distal gastrectomy at Centre 1 of PLA general hospital between January 2015 and December 2019. Clinical data of the patients were collected, and 93 of the 143 patients were followed up. These 93 patients were divided into two groups: Group 1 (Billroth II reconstruction, 33 patients); and Group 2 (Billroth II reconstruction combined with Braun anastomosis, 60 patients). Postoperative complication follow-up data and relevant clinical data were compared between the two groups.

RESULTS
There were no significant differences between Group 1 and Group 2 in postoperative complications (6.1% vs 6.7%, P = 0.679), anal exhaust time or blood loss. The follow-up prevalence of reflux gastritis indicated no significant difference between Group 1 and Group 2 (68.2% vs 51.7%, P = 0.109). The follow-up European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 scores revealed no evident difference between Group 1
and Group 2 as well. Group 1 had a shorter operating time than Group 2 on average (234.6 min vs 262.0 min, \( P = 0.017 \)).

CONCLUSION

Combined with Billroth II reconstruction, Braun anastomosis has been applied due to its ability to reduce the prevalence of reflux gastritis. Whereas in this study, the prevalence of reflux gastritis showed no significant difference, leading to a conclusion that under the circumstance of Braun anastomosis costing more time and more money, simple Billroth II reconstruction should be widely applied.

Key Words: Gastric cancer; Billroth II reconstruction; Braun anastomosis; Bile reflux

INTRODUCTION

Billroth I, Billroth II and Roux-en-Y reconstruction are the three most wildly applied reconstructions for distal gastrectomy[1]. Among these reconstructions, Billroth II reconstruction is recognized to reduce a high proportion of patients with reflux gastritis, which decreases patient quality of life[2] and potentially leads to malignancy, gastritis and reflux esophagitis[3,4]. In accordance with recent studies, the incidence of reflux gastritis after Billroth II reconstruction varies from 40% to 90%[5-7]. The addition of Braun anastomosis has been performed after Billroth II reconstruction since 1885, aiming to reduce complications after Billroth II reconstruction.

However, based on the working experiences in the hospital, it was observed that patients who underwent Braun anastomosis could get serious reflux gastritis as well. Moreover, one recent study[8] found that the addition of a Braun anastomosis is not effective in preventing enterogastric bile reflux. Other studies suggested that Braun anastomosis has a minor impact on the incidence of reflux gastritis to pancreateoduodenectomy[9,10] and one anastomosis gastric bypass[11]. Thus, whether Braun anastomosis can truly decrease the incidence of bile reflux to distal gastrectomy remains unknown.

The current study aimed to identify whether Braun anastomosis can truly decrease the incidence of bile reflux and improve the quality of life of the patients after Billroth II reconstruction on laparoscopic distal gastrectomy.

MATERIALS AND METHODS

Study design and data source

This retrospective cohort study was approved by our ethics committee at our institution. Between January 2015 and December 2019, a total of 143 patients with distal gastric cancer converted Billroth II reconstruction were collected in the 1st center of People’s Liberation Army General Hospital (PLA general hospital), Beijing, China. Of these patients, follow-up data was available for 93. These 93 patients were divided into two groups: Group 1 (Billroth II reconstruction, 33 patients); and Group 2 (Billroth II reconstruction combined with Braun anastomosis, 60 patients).

Laparoscopic-assisted distal gastrectomy with D2 lymphadenectomy was performed on all of the patients under the conduct of the Japanese classification of gastric carcinoma and the guidelines for the treatment of gastric carcinoma[12,13]. The arteries and veins were cut in the laparoscopic vision and then a small incision (less than 10 cm) was made in the center of the abdominal wall.
Table 1 General patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Billroth 2</th>
<th>Billroth 2 + Braun</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>56.8 ± 9.9</td>
<td>57.2 ± 11.4</td>
<td>0.834</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.784</td>
</tr>
<tr>
<td>Male</td>
<td>41</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>18</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Pathological tumor stage</td>
<td></td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>234.6 ± 47.7</td>
<td>262.0 ± 64.9</td>
<td>0.017</td>
</tr>
<tr>
<td>Blood loss (mL)</td>
<td>160.6 ± 130.9</td>
<td>136.2 ± 107.9</td>
<td>0.224</td>
</tr>
<tr>
<td>Anal exhaust time (d)</td>
<td>5.0 ± 2.0</td>
<td>3.8 ± 1.1</td>
<td>0.348</td>
</tr>
</tbody>
</table>

In Group 1, a small opening was made in the jejunum 20 cm away from the Treitz ligament on the anti-mesenteric margin and the residual gastric wall. The Billroth II anastomosis was performed with a 60 mm linear stapler in the end. In Group 2, jejenum-jejunum anastomosis was made 40 cm from the afferent limb.

Clinical data of the patients was collected, and 93 of the 143 patients were followed up. The follow-up data included: (1) The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 scores of patients; and (2) The number of patients with reflux gastritis. All of the follow-up was completed between January 2021 and June 2021. Postoperative complications, relevant clinical data and follow-up data were compared as well.

**Participant selection**
The inclusion criteria included: (1) Age from 18 to 75; (2) Pathologically diagnosed as distal gastric cancer; (3) Cancer pathological stage I-III (the 8th edition of the American Joint Committee on Cancer[15]); and (4) Complete clinical details. Exclusion criteria included: patients with serious heart disease or brain disease that influenced quality of life. A total of 143 patients were selected. Clinical data of the patients were collected and 93 of the 143 patients are followed up. Among the other 50 patients, 40 patients were out of contact and 10 patients were dead.

**Outcomes definition**
The main outcomes of this study were the incidence of reflux gastritis after the operation and The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30 scores, which is wildly applied in a variety of clinical studies[16-18]. Patients were called and required to answer 30 questions from The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core-30. Scores were calculated based on five multi-item functional scales (emotional, physical, role, social and cognitive function), of which higher scores indicate better quality of life; three multi-item and six single-item symptom scores, of which higher scores indicate poorer quality of life. Reflux gastritis was diagnosed according to the gastroscope reports.

**Statistical analysis**
All statistical analyses were performed with the support of SPSS v23.0 for Windows software. Continuous variables were expressed as mean ± SD and compared by Student's t-test. Categorical variables were analyzed by Pearson χ² test. A two-tailed P value < 0.05 was considered statistically significant.

**RESULTS**
There were 33 patients in Group 1 and 60 patients in Group 2. The age, pathological tumor stage, sex, mean blood loss and mean exhaust time between the two groups was similar, while group 2 had a significantly longer mean operation time (Table 1).

In comparison of postoperative complications, 1 of the 33 patients in Group 1 suffered from bile reflux and 2 patients had anastomotic fistula. In Group 2, 1 patient had anastomosis bleeding and 3 patients had anastomotic fistula. The total incidence of postoperative complications indicated no
Table 2 Postoperative complications

<table>
<thead>
<tr>
<th>Complication</th>
<th>Billroth 2</th>
<th>Billroth 2 + Braun</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile reflux</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Anastomosis bleeding</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Anastomotic fistula</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>3</td>
<td>4</td>
<td>0.696</td>
</tr>
</tbody>
</table>

Table 3 Follow-up data

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflux gastritis</td>
<td></td>
<td></td>
<td>0.109</td>
</tr>
<tr>
<td>No</td>
<td>11</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>22</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>66.70%</td>
<td>51.70%</td>
<td></td>
</tr>
<tr>
<td>Multi-item functional scales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical function</td>
<td>97.6 ± 0.95</td>
<td>92.7 ± 1.40</td>
<td>0.107</td>
</tr>
<tr>
<td>Cognitive function</td>
<td>98.9 ± 1.01</td>
<td>95.8 ± 1.40</td>
<td>0.126</td>
</tr>
<tr>
<td>Emotional function</td>
<td>94.4 ± 1.98</td>
<td>93.6 ± 1.54</td>
<td>0.744</td>
</tr>
<tr>
<td>Role function</td>
<td>97.5 ± 1.64</td>
<td>96.4 ± 1.19</td>
<td>0.592</td>
</tr>
<tr>
<td>Social function</td>
<td>96.5 ± 1.58</td>
<td>94.2 ± 1.67</td>
<td>0.369</td>
</tr>
<tr>
<td>Total function</td>
<td>84.3 ± 3.81</td>
<td>83.1 ± 2.25</td>
<td>0.757</td>
</tr>
<tr>
<td>Symptom</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>1.85 ± 5.49</td>
<td>6.48 ± 9.21</td>
<td>0.1</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>2.78 ± 6.80</td>
<td>1.94 ± 4.70</td>
<td>0.695</td>
</tr>
<tr>
<td>Pain</td>
<td>0.01 ± 3.46</td>
<td>2.08 ± 4.76</td>
<td>0.258</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>0.51 ± 2.90</td>
<td>1.39 ± 5.57</td>
<td>0.398</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>1.52 ± 6.41</td>
<td>4.17 ± 10.00</td>
<td>0.173</td>
</tr>
<tr>
<td>Insomnia</td>
<td>3.03 ± 7.74</td>
<td>3.61 ± 9.75</td>
<td>0.769</td>
</tr>
<tr>
<td>Constipation</td>
<td>1.01 ± 5.80</td>
<td>3.06 ± 7.19</td>
<td>0.165</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4.55 ± 10.44</td>
<td>4.72 ± 10.22</td>
<td>0.937</td>
</tr>
<tr>
<td>Financial difficulty</td>
<td>2.02 ± 5.52</td>
<td>5.28 ± 11.68</td>
<td>0.135</td>
</tr>
</tbody>
</table>

Group 1: Billroth II reconstruction; Group 2: Billroth II reconstruction combined with Braun anastomosis.

During follow-up, 11 patients in Group 1 and 29 patients in Group 2 had reflux gastritis on their gastroscope report during the postoperative review. The total incidence of reflux gastritis showed no significant difference (66.7% vs 51.7%, P = 0.109). For five multi-item functional scales (physical, emotional, role, cognitive and social function), three multi-item and six single-item symptom scores, it showed no significant difference between these two groups (Figure 1 and Table 3).

DISCUSSION

Billroth II reconstruction was invented in 1885 by Billroth as a modification of Billroth I. Due to the unique structure of Billroth II reconstruction, bile will flow through the residual stomach to the afferent loop, causing reflux gastritis (Figure 2A). Billroth II reconstruction is recognized with complications including anorexia, loss of appetite, dumping syndrome, nutritional anemia and alkaline reflux esophagitis[19]. In a previous study, the addition of Braun anastomosis was regarded as a method to
reduce the incidence of reflux gastritis. That is because Braun anastomosis could relieve the afferent
loop pressure\cite{20,21}, making bile flow through the jejunum-jejunum anastomosis, rather than the
residual stomach (Figure 2B).

In this study, the incidence of reflux gastritis in Group 1 was lower than that in Group 2, but the
difference was insignificant. It is indicated that bile may flow through both the residual stomach and the
jejunum-jejunum anastomosis (Figure 2C). More experiments are needed to ensure this judgement.

In terms of postoperation complications, Group 1 and Group 2 were similar. The five multi-item
functional scales (physical, emotional, role, cognitive and social function), three multi-item and six
single-item symptom scores showed no significant difference as well.

**CONCLUSION**

In conclusion, this study indicated that the addition of Braun anastomosis to Billroth II reconstruction
made no significant difference in reducing the incidence of reflux gastritis. The addition can hardly
improve the quality of life of the patients but extends the operation time. Thus, the addition of Braun
anastomosis is not necessary, and simple Billroth II reconstruction should be wildly applied.

**ARTICLE HIGHLIGHTS**

**Research background**

Braun anastomosis is usually performed after Billroth II reconstruction on laparoscopy-assisted distal
gastrectomy because it is believed to benefit patients. But we found that patients who underwent Braun
anastomosis still had serious complications after operation. Thus, studies are needed to confirm that.

**Research motivation**

To determine whether the addition of Braun anastomosis to Billroth II reconstruction on laparoscopy-
assisted distal gastrectomy benefits patients.

**Research objectives**
To study the role of Braun anastomosis in laparoscopy-assisted distal gastrectomy.

**Research methods**
The clinical data of the addition of Braun anastomosis to Billroth II reconstruction on laparoscopy-assisted distal gastrectomy for patients with distal gastric cancer were compared. Patient follow-up data were analyzed. Operation time, blood loss, anal exhaust time and prevalence rate of reflux gastritis between the groups were examined.

**Research results**
Postoperative complications were reported in 3 of the 33 patients in the Billroth II reconstruction group and 4 out of 60 patients in the Billroth II reconstruction combined with Braun anastomosis group. The total incidence of postoperative complications indicated no significant difference between the two groups. During follow-up, 11 patients in the Billroth II reconstruction group and 29 patients in the Billroth II reconstruction combined with Braun anastomosis group had reflux gastritis. The total incidence of reflux gastritis showed no significant difference (66.7% vs 51.7%, \( P = 0.109 \)). Five multi-item functional scales (physical, emotional, role, cognitive and social function), three multi-item and six single-item symptom scores showed no significant difference between these two groups.

**Research conclusions**
The addition of Braun anastomosis to Billroth II reconstruction on laparoscopy-assisted distal gastrectomy did not show any benefit to patients with distal gastrectomy.

**Research perspectives**
A prospective study with more patients is required to verify the conclusions of this study.

**FOOTNOTES**

**Author contributions:** Li XG designed the experiment; Song QY and Wu D performed the experiment; Li S and Zhang BL collected data; Zhang LY and Guan D managed data; Liu L created the tables and figures based on data; Li XG, Song QY and Wu D wrote the initial draft; Wang XX modified the draft; Li XG, Song QY and Wu D contributed equally to this article.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of PLA General Hospital (Approval No. S2021-579).

**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

**Conflict-of-interest statement:** We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

**Data sharing statement:** No additional data are available.

**STROBE statement:** The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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**Country/Territory of origin:** China

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**S-Editor:** Yan JP
REFERENCES


Contemporary, national patterns of surgery after preoperative therapy for stage II/III rectal adenocarcinoma

Celine Soriano, Henry T Bahnson, Jennifer A Kaplan, Bruce Lin, Ravi Moonka, Huong T Pham, Hagen F Kennecke, Vlad Simianu

BACKGROUND
Contemporary treatment of stage II/III rectal cancer combines chemotherapy, chemoradiation, and surgery, though the sequence of surgery with neoadjuvant treatments and benefits of minimally-invasive surgery (MIS) is debated.

AIM
To describe patterns of surgical approach for stage II/III rectal cancer in relation to neoadjuvant therapies.

METHODS
A retrospective cohort was created using the National Cancer Database. Primary outcome was rate of sphincter-sparing surgery after neoadjuvant therapy. Secondary outcomes were surgical approach (open, laparoscopic, or robotic), surgical quality (R0 resection and 12+ lymph nodes), and overall survival.
A total of 38927 patients with clinical stage II or III rectal adenocarcinoma underwent surgical resection from 2010-2016. Clinical stage II patients had neoadjuvant chemoradiation less frequently compared to stage III (75.8% vs 84.7%, \( P < 0.001 \)), but had similar rates of total neoadjuvant therapy (TNT) (27.0% vs 27.2%, \( P = 0.697 \)). Overall rates of total mesorectal excision without sphincter preservation were similar between clinical stage II and III (30.0% vs 30.3%) and similar if preoperative treatment was chemoradiation (31.3%) or TNT (30.2%). Over the study period, proportion of cases approached laparoscopically increased from 24.9% to 32.5% and robotically 5.6% to 30.7% (\( P < 0.001 \)). This cohort showed improved survival for MIS approaches compared to open surgery (laparoscopy HR 0.85, 95%CI 0.78-0.93, and robotic HR 0.82, 95%CI 0.73-0.92).

**CONCLUSION**

Sphincter preservation rates are similar across stage II and III rectal cancer, regardless of delivery of preoperative chemotherapy, chemoradiation, or both. At a national level, there is a shift to predominantly MIS approaches for rectal cancer, regardless of whether sphincter sparing procedure is performed.

**Key Words:** Rectal cancer; Total neoadjuvant therapy; Colorectal surgery; Minimally-invasive surgery; Chemotherapy; Radiation

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**INTRODUCTION**

The management of rectal cancer has evolved, with emphasis on optimizing oncological outcomes and minimizing operative morbidity. Treatment of locally advanced rectal cancer typically involves multimodality therapies and total mesorectal excision (TME)\(^1\,^2\). Neoadjuvant therapy using chemotherapy and/or radiotherapy has several advantages, such as locoregional control and improved overall survival, compared to surgery alone\(^3\,^4\). Additionally, the administration of chemoradiation combined with induction or consolidation chemotherapy, known as total neoadjuvant therapy (TNT), has gained popularity due to increased treatment compliance without compromise of pathologic complete response or complete resection rates\(^5\,^6\).

Despite advances in multimodality treatment paradigms, the optimal sequence of surgery in relation to chemotherapy and radiation remains unknown. Recent trials have assessed pre-operative treatment regimens and improved rates of organ preservation, disease free survival, and pathological complete response rates in patients with high risk, locally advanced rectal cancer\(^7\,^8\). Several factors, including anatomic considerations, tumor features, and functional symptoms, can influence decision-making, and treatment is typically individualized. Due to the complexity of rectal cancer care, variation has been described, with differences in curative resection rates, postoperative morbidity and mortality, and long-term oncologic outcomes among both surgeons and hospitals\(^9\). Furthermore, practices of how surgery is sequenced with other modalities, especially in the era of minimally invasive surgery (MIS), is not well described.

Therefore, the aim of this study was to characterize surgical resection of locally advanced rectal adenocarcinoma in the setting of multimodal therapy at the national level, with a focus on describing patterns of surgery in sequence with neoadjuvant treatment delivery and shift in surgical approach trends over time. We hypothesized that there would be increases in the delivery of neoadjuvant chemotherapy and chemoradiation, performance of sphincter-sparing resections, and use of minimally invasive surgical approaches.
MATERIALS AND METHODS

This study was determined to be exempt from human subjects review by the Benaroya Research Institute Institutional Review Board.

Data/population

A retrospective cohort of patients with clinical stage II and stage III rectal adenocarcinoma who underwent surgical resection between 2010 and 2016 was created using the National Cancer Database (NCDB). The NCDB is a validated national cancer registry of the American College of Surgeons and American Cancer Society, collected from more than 1500 Commission on Cancer-accredited facilities. Stage was defined according to the seventh edition of the American Joint Committee on Cancer’s clinical group. The cohort was based on clinical stage, rather than pathologic stage, as treatment delivery is established once staging workup is complete. Patients with a diagnosis of multiple cancers and undergoing palliative surgery were excluded (Figure 1).

Outcomes/definitions

To describe patterns of surgical care delivery, the primary outcome was proportion of patients receiving local excision or TME with or without sphincter preservation. The frequency of sphincter preservation was characterized by surgery alone or in sequence with chemotherapy or radiation therapy. Using NCDB definitions, local excision was defined as conventional trans-anal excision or trans-anal endoscopic microsurgery. TME with sphincter preservation was defined as any rectal resection that included anastomosis [low anterior resection (LAR) and total proctocolectomy and pouch-anal anastomosis]. TME without sphincter preservation was defined as any rectal resection without anastomosis [abdominoperineal resection (APR), LAR with colostomy, and total proctocolectomy with ileostomy]. Surgical approach to TME was subcategorized into open, laparoscopic, and robotic. Conversion to open from laparoscopy and robotics was also reported, but these cases were included in their intended approach categories. Chemotherapy delivery was defined as single or multi-agent systemic administration before or after surgery. TNT was defined as delivery of both multiagent chemotherapy and radiation therapy prior to surgical date.

Secondary outcomes that were assessed include pathologic stage, quality of surgical resection, and overall survival. Quality of surgical resection included proportion of cases with negative margins, total lymph node harvest and proportion of cases with 12+ lymph nodes harvested. To explore potential variation in care delivery, patient factors (age, sex, insurance status, comorbidities) and location of care (facility information, geographic area) were described and used as covariates in the survival analysis. Comorbidities were defined using the Charlson-Deyo comorbidity index.

Statistical analysis

Categorical and continuous variables based on clinical interest were compared with chi-square and Kruskal Wallis tests, respectively. While the hypothesis did not focus on differences in treatment based on rectal cancer stage, stage-specific data are provided in supplemental text (Supplementary Table 1). Because of the expected uptake of MIS over time, we described trends in surgical approach by year. Test for trend of surgical approach were done with Chi-squared test. Univariate- and multivariate-adjusted overall survival analyses were performed using cox proportional hazards model on a subset of the analysis sample, excluding patients with multiple cancers or where treatment and diagnosis were done at different facilities, as per NCDB recommendations. The final survival model was adjusted for age, sex, race, insurance, rurality, geography, facility type, pathologic stage, cancer grade, preoperative radiation, chemotherapy type and sequence, surgery type (LE, TME with or without sphincter preservation), intent of surgical approach (open, laparoscopic, robotic), resection margin status and 12+ lymph nodes resected status. Kaplan Meier survival curves stratified by TME with and without sphincter preservation are shown, by intent of surgical approach (open, laparoscopic, robotic). Statistical significance was determined by $P < 0.05$. Survival and patient characteristics tables were run with Mayo Clinic’s SAS macros[13] on SAS version 9.4 and JMP Pro Version 15 was also used for graphics and data analysis.

RESULTS

Patient demographics and sequence of treatment

From 2010-2016, a total of 38,927 patients underwent resection of stage II/III rectal cancer (mean age 60.9 ± 12.7 years, and 61% male). Baseline patient and facility characteristics are outlined in Table 1. Sphincter was not preserved in 30.2% ($n = 11748$). Patients with clinical stage III disease represented 55% of the cohort, and stage distribution was similar whether TME with sphincter preservation (55.5%) or not (54.9%) was performed. It was rare to undergo local excision after initially presenting with clinical stage II (5.2%) or clinical stage III (2.5%) rectal cancer.
<table>
<thead>
<tr>
<th>Table 1 Patient and facility demographics of patients with clinical stage II/III rectal cancer, stratified total mesorectal excision and sphincter preservation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local excision (n = 1442)</td>
</tr>
<tr>
<td>Age at diagnosis</td>
</tr>
<tr>
<td>mean ± SD</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Charlson Comorbidity Score</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2+</td>
</tr>
<tr>
<td>Race3</td>
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<td>Black</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Insurance status3</td>
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<td>Medicare/medicaid/other government</td>
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<td>Private insurance/managed care</td>
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<tr>
<td>Urban</td>
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<tr>
<td>Facility type3</td>
</tr>
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Soriano C et al. Rectal cancer surgery after preoperative therapy

1Kruskal Wallis.
2Chi-Square.
3Race unknown for 255 patients; Insurance unknown for 521 patients; Living location unknown for 926 patients; Facility type and geographic region unknown for 1714 locations.

TME: Total mesorectal excision.

Figure 1 CONSORT diagram of inclusion and exclusion criteria for cohort creation and survival analysis.

Sequence of treatment by stage
Patients with clinical stage II disease more frequently had no radiation (16.8% vs 8.7%, P < 0.001) or no chemotherapy (14.9% vs 5.9%, P < 0.001) compared to stage III patients (Supplementary Table 1). Clinical stage II patients less frequently had neoadjuvant chemoradiation (75.2%, vs 84.1% P < 0.001), but had similar rates of TNT (27.0% vs 27.2%, respectively, P = 0.697) compared to stage III. Overall rates of TME without sphincter preservation were similar between clinical stage II and III, 30.0% vs 30.3%, respectively, and similar if preoperative treatment was neoadjuvant chemoradiation (31.3%, n = 9762 TME without sphincter preservation out of n = 31160 that received neoadjuvant chemoradiation) or TNT (30.2%, n = 1302 TME without sphincter preservation out of n = 4302 that received TNT).

Surgical approach and quality of resection
Rates of open resection in the cohort were approximately 50%, but over the period of the study decreased from 69.4% in 2010 to 36.8% in 2016. There were concomitant rises in laparoscopic resection from 24.9% to 32.5% and robotic resection 5.6% to 30.7% (P < 0.001) (Figure 2). Open approach was used for 60% of TME without sphincter preservation compared to 47% of TME with sphincter preservation (P < 0.001).

The distribution of surgical approach is described in Table 2. Conversion to an open operation was lower with robotic approach (6.9%) compared to laparoscopy (14.5%). This was maintained regardless of whether sphincter sparing procedure was performed (conversion rate 15% laparoscopic, 6.9% robotic) or not (conversion rate 16.4% laparoscopic, 7.1% robotic), or whether TNT (conversion rate 15.6% laparoscopic, 6.5% robotic) was delivered.

R0 resection was obtained 94.8% of patients who underwent TME with sphincter preservation, and 90.3% of patients who underwent TME without sphincter preservation (P < 0.001). Twelve or more lymph nodes were examined more frequently in TME with sphincter preservation (71.6%) than without sphincter preservation (68.4%). Rates of R0 resection and 12+ lymph nodes harvested were both lower
Table 2 Tumor characteristics and surgical quality by surgical approach

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<th>Total (n = 38927)</th>
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<td>323 (3.4%)</td>
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<td>3480 (36.5%)</td>
<td>2044 (36.3%)</td>
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<td>152 (1.0%)</td>
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<td>4813 (12.4%)</td>
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<td>911 (7.5%)</td>
<td>315 (4.5%)</td>
<td>2696 (6.9%)</td>
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<td>9515 (78.4%)</td>
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<td>31418 (80.7%)</td>
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<td>Total neoadjuvant therapy</td>
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<td>4302 (27.1%)</td>
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<td>TME with sphincter preservation</td>
<td>12118 (61.1%)</td>
<td>8633 (71.1%)</td>
<td>4986 (71.7%)</td>
<td>25737 (66.1%)</td>
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<tr>
<td>TME without sphincter preservation</td>
<td>7061 (35.6%)</td>
<td>2760 (22.7%)</td>
<td>1927 (27.7%)</td>
<td>11748 (30.2%)</td>
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<td>Conversion to open</td>
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<td>6568 (95.1%)</td>
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<td>R1</td>
<td>806 (4.1%)</td>
<td>413 (3.5%)</td>
<td>193 (2.8%)</td>
<td>1412 (3.7%)</td>
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<tr>
<td>R2</td>
<td>782 (4.0%)</td>
<td>352 (2.9%)</td>
<td>148 (2.1%)</td>
<td>1282 (3.3%)</td>
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<tr>
<td>Number of lymph nodes examined (mean ± SD)</td>
<td>14.7 ± 9.7</td>
<td>14.8 ± 9.8</td>
<td>15.7 ± 9.0</td>
<td>14.9 ± 9.6</td>
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<tr>
<td>12 or more lymph nodes examined</td>
<td>13198 (67.1%)</td>
<td>8148 (67.7%)</td>
<td>5088 (73.6%)</td>
<td>26434 (68.4%)</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

1Chi-Square.
TME: Total mesorectal excision.

with open, compared to minimally invasive, approaches.

Overall survival
Table 3 summarizes factors impacting overall survival in this cohort. After adjustment, TME without sphincter preservation was associated with worse survival HR 1.30 (95%CI 1.20-1.40) compared to sphincter preservation. Interestingly, this cohort showed improved survival for minimally invasive approaches compared to open surgery (laparoscopy HR 0.85, 95%CI 0.78-0.93, and robotic HR 0.82, 95%CI 0.73-0.92). This improved survival in cases approached minimally invasively was sustained after stratification into TME with and without sphincter preservation (Figure 3).
<table>
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<th>Variable</th>
<th>n</th>
<th>Events</th>
<th>5-yr survival% (95%CI)</th>
<th>Cox univariate HR (95%CI)</th>
<th>Cox univariate score P value</th>
<th>Cox multivariate HR (95%CI)</th>
<th>Cox multivariate likelihood ratio P value</th>
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<tbody>
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<td>Age at diagnosis</td>
<td>27114</td>
<td>5281 (19%)</td>
<td>73.3 (72.6, 74.0)</td>
<td>1.03 (1.03, 1.04)</td>
<td>&lt; 0.0001</td>
<td>1.02 (1.02, 1.02)</td>
<td>&lt; 0.0001</td>
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<td>Sex</td>
<td>10502</td>
<td>1869 (18%)</td>
<td>75.5 (74.4, 76.6)</td>
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<td>&lt; 0.0001</td>
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<tr>
<td>Female</td>
<td>16612</td>
<td>3412 (21%)</td>
<td>71.9 (70.9, 72.8)</td>
<td>1.19 (1.13, 1.26)</td>
<td>1.23 (1.14, 1.32)</td>
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<tr>
<td>Male</td>
<td>20949</td>
<td>3656 (17%)</td>
<td>75.7 (74.9, 76.5)</td>
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<tr>
<td>Charleson comorbidity score</td>
<td>20949</td>
<td>3656 (17%)</td>
<td>75.7 (74.9, 76.5)</td>
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<td>&lt; 0.0001</td>
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<tr>
<td>Race</td>
<td>2307</td>
<td>508 (22%)</td>
<td>69.7 (67.1, 72.2)</td>
<td>1.18 (1.08, 1.29)</td>
<td>1.04 (0.92, 1.19)</td>
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<td>Black</td>
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<td>246 (16%)</td>
<td>76.6 (73.6, 79.5)</td>
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<tr>
<td>White</td>
<td>23091</td>
<td>4498 (19%)</td>
<td>73.4 (72.7, 74.2)</td>
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<td>&lt; 0.0001</td>
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<td>Insurance status unknown</td>
<td>382</td>
<td>64 (17%)</td>
<td>71.8 (64.8, 78.8)</td>
<td>1.46 (1.14, 1.88)</td>
<td>0.93 (0.62, 1.39)</td>
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<tr>
<td>Medicare/medicaid/other government</td>
<td>11607</td>
<td>3039 (26%)</td>
<td>64.8 (63.6, 66.0)</td>
<td>2.12 (2.01, 2.25)</td>
<td>1.33 (1.22, 1.46)</td>
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<tr>
<td>Not insured</td>
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<td>1.61 (1.42, 1.82)</td>
<td>1.14 (0.97, 1.33)</td>
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<td>Private insurance/managed care</td>
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<td>80.8 (79.9, 81.7)</td>
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<td>0.98 (0.89, 1.08)</td>
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<td>Living location</td>
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<td>Chemotherapy sequence</td>
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</tr>
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<td>&lt; 0.0001</td>
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<tr>
<td>Chemotherapy before surgery</td>
<td>2387</td>
<td>14351</td>
</tr>
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<td>Chemotherapy before and after surgery</td>
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<td>2849</td>
</tr>
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<tr>
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<tr>
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<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Local excision</td>
<td>953</td>
<td>1934</td>
</tr>
<tr>
<td>TME with sphincter preservation</td>
<td>18237</td>
<td>1934</td>
</tr>
<tr>
<td>TME without sphincter preservation</td>
<td>7924</td>
<td>1934</td>
</tr>
<tr>
<td>Surgical approach</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Open</td>
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<td>3300</td>
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<tr>
<td>Robotic</td>
<td>4397</td>
<td>3300</td>
</tr>
<tr>
<td>Tumor grade</td>
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<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Other (ND/UNK/NA/high grade dysplasia)</td>
<td>3918</td>
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</tr>
<tr>
<td>Poor/undifferentiated</td>
<td>1004</td>
<td>1004</td>
</tr>
<tr>
<td>Well/moderate differentiation</td>
<td>20173</td>
<td>20173</td>
</tr>
<tr>
<td></td>
<td>7683</td>
<td>7683</td>
</tr>
<tr>
<td></td>
<td>(18%)</td>
<td>(18%)</td>
</tr>
<tr>
<td></td>
<td>74.8 (74.0, 75.6)</td>
<td>74.8 (74.0, 75.6)</td>
</tr>
</tbody>
</table>
DISCUSSION

This contemporary, nationwide cohort study identified an expected shift towards a minimally-invasive surgical approach for stage II/III rectal cancer with high quality surgical outcomes. Most of the patients are getting neoadjuvant radiation, but only a small fraction receives TNT. Neoadjuvant treatment at the population level does not seem to affect sphincter-sparing rates. Interestingly, this cohort also showed improved survival in cases approached minimally invasively - a finding that is at odds with prior, high-quality randomized control trials, but may reflect important differences between the randomized control trial population and surgeon and patient selection that occur in broader practice.

Contemporary treatment for rectal cancer is multidisciplinary. The most common neoadjuvant regimen utilizes chemoradiotherapy, which has been shown to lower the recurrence rate and is associated with less toxicity than post-operative radiation, with no difference in overall survival[14]. Additionally, neoadjuvant therapy may promote tumor shrinkage and affect sphincter-sparing rates. Still, despite recommendations in national guidelines describing neoadjuvant treatment for locally advanced rectal cancer or nodal disease[15,16], variation in radiation delivery is seen[17,18]. Midura et al [19] identified that factors such as hospital volume and facility type affected delivery of neoadjuvant therapy, including decreased use of neoadjuvant therapy for higher stage rectal cancer at lower-volume, community cancer centers. Furthermore, total neoadjuvant therapy has been increasingly promoted, in which studies have reported local disease control and decreased recurrence rates[20]. A majority of patients in our cohort underwent some type of neoadjuvant treatment, and sphincter-sparing rates were similar in patients with stage II or stage III disease. A prior meta-analysis supports the approximate rate of permanent colostomy to be approximately 30%[21]. It is important to note that certain clinical features, such as tumor distance from the anal verge or patients’ prior continence status, which might
influence the decision for a non-sphincter sparing operation, are not available in this dataset. Most decisions about sphincter preservation happen before surgery, and rates of low tumors and incontinence rates are not expected to have meaningfully changed during this time period.

The equivalence of minimally-invasive and open approaches for rectal cancer surgery continues to be debated. Laparoscopy and robotic-assisted colorectal surgery have enabled decreased length of hospital stay, better analgesia, and improved visibility and ergonomics, specifically in the pelvis[22-24]. However, adoption of MIS for rectal cancer has been controversial, as both the Z6051 and ALaCaRT trials were unable to establish non-inferiority of pathological outcomes for minimally invasive vs open resection in patients with rectal cancer[25,26]. Follow-up of these trials found no significant difference in survival between approaches, with Z6051 showing 2-year disease free survival (DFS) of 79.5% in the laparoscopic group and 83.5% in the open group and ALaCaRT showing 2-year DFS of 94% in the laparoscopic group and 93% in the open group[27,28]. Finally, the ROLARR trial found no significant difference in conversion to open laparotomy between conventional laparoscopy vs robotic-assisted surgery, and concluded no short term benefit of robotic surgery over laparoscopy[29]. Our findings of improved survival with minimally invasive approaches, even after adjustment for pathological stage, neoadjuvant treatment, and patient/center features, are at odds with these prior, high-quality studies. However, the NCDB has a wider, national representation, and the findings herein may reflect patient- and approach- selection in broader practice, including training, resources, and institutional factors that impact approach outside of randomized trial patients. For example, it is unclear if the improved resection margins and lymph node harvest in the laparoscopic and robotic subgroups are due to the approaches themselves or the cases that lent themselves to be approached minimally invasively (or the surgeons choosing a minimally-invasive approach in these cases). Additionally, our findings are limited by the absence of information regarding local recurrence rate. However, it is notable that this effect of surgical approach on survival in this national cohort was maintained even after adjustment for multiple confounders or when stratifying the analysis by the subgroups with and without sphincter preservation.

Local excision operations in the setting of stage II/III are controversial and deserve special mention in this cohort. Patients with stage II/III who underwent transanal local excision make up a minority of operations and are not the standard treatment because of the inability to evaluate mesorectal lymph nodes. Still, several studies have shown the feasibility of this approach in the setting of neoadjuvant treatment[30-33]. In select patients showing tumor response to short course radiotherapy or chemotherapy, high rates of organ preservation can be achieved. Therefore, patients and their surgeons may opt for this approach if facing a decision about permanent colostomy or if they are poor surgical candidates for the standard TME. Further randomized studies to better assess the feasibility of this approach, and long term follow up for meaningful oncologic outcomes are underway[34].

This study is further limited by the inability to address the magnitude of treatment response and the impact of treatment response on decisions for sphincter preservation and surgical approach. For instance, we were unable to assess clinical complete responders, which occurs as frequently as 20%-30%[20], and would not be included unless they underwent resection and pathology confirmed no residual tumor. Patients may avoid resection if they have a complete clinical response but would need an APR, so there is bias in this study such that APR surgery only occurred in those patients that likely did not have good response and still needed resection. This presumably also impacts overall survival estimates. Finally, there is a lack of data available regarding local staging studies that could lead to misclassification of clinical stage. For instance, it has been reported that magnetic resonance imaging, which has become the standard of care, can over-stage rectal cancer as high as 30%[35-37]. Misclassification of
stage could result in undertreatment or overtreatment, and that cannot be determined using this dataset. Despite these limitations, this study provides important information regarding treatment delivery patterns.

CONCLUSION
At a national level, minimally invasive surgery has become the predominant approach for rectal cancer. Sphincter preservation rates, when patients undergo surgical resection, do not vary with delivery of neoadjuvant treatment. In this broad national cohort, both open surgery and non-sphincter sparing operations were associated with worse overall survival for patients with stage II/III rectal adenocarcinoma.

ARTICLE HIGHLIGHTS

Research background
It is not well described whether the contemporary, multi-disciplinary approaches to stage II/III rectal cancer are resulting in meaningful changes in sphincter preservation, surgical quality, or overall survival.

Research motivation
While we push to individualize treatment decisions, it is important to recognize whether contemporary patterns to increase minimally-invasive surgery (MIS) and neoadjuvant treatment offer meaningful change the expected outcome of locally advanced rectal cancer.

Research objectives
Describe broad uptake in sphincter preservation, minimally-invasive approaches to rectal cancer, and the associated surgical outcomes of resection margins, lymph node harvest, and overall survival.

Research methods
Retrospective ‘real-world’ cohort of National Cancer Database (NCDB) sites, limited to stage II/III surgically treated rectal cancer.

Research results
Neither stage nor neoadjuvant treatment made a meaningful impact on rates of permanent colostomy, which was about 30% across all subgroups. From 2010 to 2016, there was a broad shift to MIS (laparoscopic and robotic) approaches to rectal cancer. These MIS approaches were associated with more frequent negative margins, better lymph node harvest, and improved overall survival after adjustment.

Research conclusions
There has been a shift to MIS approaches to locally advanced rectal cancer. Sphincter preservation rates remain similar in contemporary years, despite increasing neoadjuvant therapy. In recent years, more cases at NCDB sites are done MIS, which associate with better surgical quality and improved overall survival in this study.

Research perspectives
The findings of improved surgical quality and overall survival in this cohort are in contrast to randomized trial data that preceded this study. This may highlight the difference between randomized patients are ‘real-world’ practices or call into question the need for more contemporary, and pragmatic, trials for locally advanced rectal cancer surgery.

FOOTNOTES

Author contributions: All authors have made substantial contributions to conception and design of the study, acquisition of data, or analysis and interpretation of data, been actively involved in drafting the article or making critical revisions related to important intellectual content of the manuscript, and have provided final approval of the version of the article to be published.

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L-Editor: A
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REFERENCES


Retrospective Study

Clinicopathological differences, risk factors and prognostic scores for western patients with intestinal and diffuse-type gastric cancer

Cristina Díaz del Arco, Lourdes Estrada Muñoz, Luis Ortega Medina, Elena Molina Roldán, M Ángeles Cerón Nieto, Soledad García Gómez de las Heras, M Jesús Fernández Aceñero

Abstract

BACKGROUND
In the molecular era, the Laurén system is still a cost-effective and widely implemented classification for gastric cancer (GC) and it has been recently associated with clinical, histological and molecular features of these tumors. Despite recent advances in the understanding of the molecular biology of GC, there is a need to develop new prognostic tools for patient stratification in clinical practice. Thus, the identification of easily available prognostic factors in patients with intestinal and diffuse-type tumors can significantly improve risk assessment and patient stratification in GC.

AIM
To identify clinicopathological differences, risk factors, and to develop cost-effective prognostic scores for patients with intestinal and diffuse-type GC.

METHODS
Retrospective study of all patients undergoing surgery for GC at a tertiary referral center from 2001 to 2019. 286 cases met inclusion criteria (intestinal: 190, diffuse: 96). Clinical data and gross findings were collected. All specimens were reviewed by two independent pathologists and a detailed protocol for histologic evaluation
was followed. Five tissue microarrays (TMAs) were constructed and sections of the TMA block were immunostained for HERCEPTEST, MSH2, MSH6, MLH1 and PMS2. Statistical analyses were performed and prognostic scores were developed based on hazard ratios.

RESULTS

Intestinal and diffuse-type GC showed different epidemiological, clinicopathological and prognostic features. Diffuse tumors were significantly associated with younger age, less symptomatology, flat morphology, deeper invasion, perineural infiltration, advanced stage at diagnosis, administration of adjuvant therapy and poorer prognosis. Intestinal lesions were fungoid or polyoid, showed necrosis, desmoplasmia, microsatellite instability and HERCEPTEST positivity and were diagnosed at earlier stages. Tumor depth, desmoplasmia, macroscopic type and lymph node involvement were independently related to the Laurén subtype. Furthermore, intestinal and diffuse GC were associated with different risk factors for progression and death. Vascular invasion, perineural infiltration and growth pattern were important prognostic factors in intestinal-type GC. On the contrary, tumor size and necrosis were significant prognosticators in diffuse-type GC. Our recurrence and cancer-specific death scores for patients with intestinal and diffuse-type GC showed an excellent patient stratification into three (diffuse GC) or four (intestinal) prognostic groups.

CONCLUSION

Our findings support that Laurén subtypes represent different clinicopathological and biological entities. The development of specific prognostic scores is a useful and cost-effective strategy to improve risk assessment in GC.

Key Words: Gastric cancer; Clinicopathological; Score; Prognosis; Laurén; Molecular

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Core Tip: In the molecular era, the Laurén system is a cost-effective and widely implemented classification. The identification of easily available prognostic factors in intestinal and diffuse-type tumors may significantly improve patient stratification in gastric cancer (GC). In this study, the authors found that intestinal and diffuse-type GC show different epidemiological, clinical and prognostic features. Laurén subtypes were also associated with different risk factors for tumor progression and death. Finally, separate clinicopathological scores for patients with intestinal and diffuse-type GC showed an excellent prognostic stratification. The development of specific prognostic scores is a useful, cost-effective strategy to improve risk assessment in GC.

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INTRODUCTION

Gastric cancer (GC) is an aggressive tumor which is usually diagnosed at advanced stages in western countries[1,2]. It can be classified according to its location, macroscopical or microscopical features[3,4]. Although several histological-based classifications have been proposed, only the Laurén and the most recent WHO classification are currently widely used[5]. The Laurén system has been extensively adopted by clinicians and pathologists since its publication in 1965 and it can be easily evaluated in conventional paraffin-embedded hematoxylin-eosin-stained slides[6]. This classification divides GC into intestinal, diffuse and mixed types, depending on the tumor architecture, growth pattern and cell morphology. Intestinal-type GC is composed of glandular structures accompanied by papillary or solid components. On the other hand, diffuse-type GC is composed of loosely attached cells growing as small clusters or scattered cells with an infiltrative pattern. This classification has been variably associated with clinicopathological features[7,8]. Intestinal tumors occur more frequently in older men and they are related to Helicobacter pylori (H. pylori) infection and environmental factors. Furthermore, most studies have identified Laurén subtype as an independent prognosticator in GC[9-11].
Recent technological advances have allowed us to improve the understanding of the molecular biology of GC[12]. In 2014, The Cancer Genome Atlas (TCGA) Research Network defined four molecular subtypes of GC: tumors positive for Epstein-Barr virus, microsatellite unstable tumors (MSI), genomically stable tumors (GS) and tumors with chromosomal instability (CIN)[13]. Laurén classification has also been correlated with these molecular groups[14,15].

The only curative treatment for GC is surgery and localized tumors are treated by total or subtotal gastrectomy. However, patient prognosis is poor with estimated 5-year survival rates lower than 30% [16]. There is an urgent need to identify potential therapeutic targets and prognostic factors in GC in order to improve patient subclassification and response to therapy. It has been suggested that diffuse GC may benefit from broader surgical margins, extended lymphadenectomy and hyperthermic intraperitoneal chemotherapy (HIPEC) for peritoneal metastasis[17,18]. Interestingly, diffuse GC may be more resistant to standard chemotherapeutic regimens than intestinal GC, and several investigators have recommended the use of the Laurén classification in clinical trials[19].

In summary, in the era of molecular medicine, the Laurén system is a cost-effective and widely implemented classification which has been associated with clinical, pathological, prognostic and molecular features. Thus, Laurén subtypes can be considered distinct entities that differ in their histology, biology, and clinical behavior, and the identification of easily available prognostic factors in patients with intestinal and diffuse-type tumors may significantly improve risk assessment and patient stratification in GC.

In this study, our objectives were to: (1) Assess the clinicopathological differences between Laurén subtypes; (2) Identify and compare the clinicopathological risk factors for recurrence and cancer-specific death of patients with intestinal and diffuse-type GC; and (3) Develop specific cost-effective prognostic scores for overall survival (OS) and disease-free survival (DFS) for patients with intestinal and diffuse GC. As far as we know, no other study has developed specific clinicopathological prognostic scores for patients with intestinal and diffuse-type GC.

**MATERIALS AND METHODS**

**Study design**

This is a retrospective study including all patients undergoing surgery for GC at a tertiary referral hospital in Madrid (Spain) from 2001 to 2019. All tumors were treated by total or subtotal gastrectomy with D1 or D2 lymphadenectomy. Clinical records were reviewed and demographic, clinical and follow-up information was retrieved including age at diagnosis, sex, symptoms (local and systemic), smoking and drinking habits, treatment, tumor recurrence and cause of death. Gross findings (tumor size, tumor location, macroscopic type) were collected from the database of the Department of Surgical Pathology (PatWin). The study was reviewed and approved by the institutional review board of the hospital.

**Histopathological study**

All tumors were formalin-fixed and embedded in paraffin. Slides were reviewed by two independent pathologists following a detailed protocol for histologic evaluation. Discordant cases were conjointly reviewed. Main microscopic features were assessed including tumor type (Laurén classification), histologic grade, presence of signet-ring cells (independently of the percentage of signet-ring cells), tumor budding, perineural infiltration, lymphovascular invasion, growth pattern (expansive or infiltrative), desmoplasia, necrosis, surgical margins, tumor depth (T stage), number of lymph node (LN) dissected, number of metastatic LN and lymph node ratio (LNR). The LNR was defined as the ratio between the number of metastatic LN and the total number of LN retrieved from the resection specimen. LNR was treated as a quantitative variable. All cases were staged according to the 8th edition of the American Joint Committee on Cancer tumor, node, metastasis (TNM) classification of GC[20].

**Immunohistochemical study**

Five tissue microarrays (TMAs) including a subgroup of cases from the GC cohort were assembled for immunohistochemical (IHC) analysis. TMAs were constructed using the MTA-1 tissue arrayer (Beecher Instruments, Sun Prairie, United States) and contained 2 cores per case (diameter: 1 mm). Representative areas were pre-selected by a pathologist and corresponded to the center and leading edge of each tumor. Cores were punched out from the donor block and transferred into a TMA, 2 μm sections were obtained from the TMA block for IHC study. Slides were deparaffinized by incubation at 60°C for 10 min and incubated with Dako PT-Link for 20 min at 95°C in a high pH buffered solution. Holders were incubated with peroxidase blocking reagent (Dako, Denmark) to block endogenous peroxidase. Sections were incubated for 20 min with the primary antibodies followed by incubation with the appropriate anti-Ig horseradish peroxidase-conjugated polymer (EnVision, Dako, Denmark) to detect antigen-antibody reaction. Then, biopsies were visualized with 3,3′-diaminobenzidine as a chromogen for 5 min and counterstained with hematoxylin. Sections of the TMA block were immunostained for HERCEPTEST, MSH2, MSH6, MLH1 and PMS2 (all antibodies prediluted; Dako, Denmark). Positive and negative controls were included. IHC markers were evaluated by two experienced pathologists.
Staining intensity, location and percentage of cells stained were assessed for all antibodies. For the aims of this study, HERCEPTEST was evaluated according to the CAP recommendations. MSI tumors showed complete loss of MLH1, MSH2, MSH6 and/or PMS2 IHC expression.

**Inclusion criteria**

We reviewed all GC resected in our institution between 2001 and 2019. After data collection, patients receiving neoadjuvant therapy, metastatic tumors at diagnosis, patients with R1 or R2 resections and tumors of the mixed type were excluded from the study.

**Statistical analysis**

All data was stored in an anonymized Excel file and analyzed using IBM SPSS statistics (V20). Qualitative variables were described as frequencies. Quantitative variables were expressed as mean ± SD or median with range, as appropriate. For the analysis of the association between variables, we have performed χ² (chi)-squared test for qualitative data and Student’s t-test for dichotomous quantitative variables. Statistical significance was settled at a P value < 0.05.

Multivariate analyses were performed by Cox regression (backward stepwise method), and regression models were adjusted for potential confounders. Two models (OS and DFS) were calculated for each Laurén subtype.

Prognostic scores for tumor progression and death were developed based on the hazard ratios of significant independent prognostic factors, as seen in other studies[21]. Two prognostic scores (one for recurrence and one for cancer-specific death) were constructed for each Laurén subtype.

OS and DFS curves according to the prognostic scores were estimated by Kaplan Meier analysis and significance was tested with the log-rank test. Receiver operating characteristic curves for cancer-specific death and progression were plotted. The area under the curve (AUC) was calculated for each prognostic score.

**RESULTS**

A total of 377 GC were resected in our institution between 2001 and 2019. Final analyses included 286 patients with pure intestinal-type GC (n = 190) and diffuse-type GC (n = 96). Clinicopathological features of our cases are presented in **Supplementary Table 1**. Mean age at diagnosis was 72 years and most patients were symptomatic (90.2%). Mean tumor size was 43 mm and most lesions were located in the gastric antrum (56.1%) and body (34.5%). According to their macroscopic appearance, tumors were mainly fungoid (36%) or ulcerative (31.8%). Microscopically, 66.4% of GC were intestinal (n = 190) and 33.6% were diffuse (n = 96). 35.1%, 35.1% and 50.5% of cases showed vascular invasion, perineural infiltration and desmoplasia, respectively. IHC was performed in 172 GC (intestinal n = 107, diffuse n = 65): 28.5% were microsatellite unstable and most cases were HERCEPTEST 0 (91.9%). Patients were diagnosed at stages I (27.2%), II (31.8%) and III (40.5%). 18% of patients received adjuvant therapy. Mean follow-up was 46.5 mo (0-208 mo). During follow-up, 36.6% of tumors recurred and 26.8% of patients died due to tumor.

**Clinicopathological differences between Laurén subtypes**

Clinicopathological features of our cases depending on the Laurén subtype are summarized in **Supplementary Table 1**. Univariate analysis (Table 1) showed a significant association between Laurén subtypes and patient age, tumor depth, macroscopic type, local symptoms, necrosis, perineural infiltration, intratumor inflammatory infiltration, desmoplasia, MSI, HERCEPTEST, T, N, LNR, adjuvant therapy, tumor recurrence and patient death. Diffuse tumors were diagnosed at advanced stages in younger patients with less local symptoms. They infiltrated deeper into the gastric wall, had flat morphology and higher rates of perineural infiltration. MSI was infrequent and HERCEPTEST was negative (0) in all cases. Adjuvant treatment was administered more frequently to patients with diffuse GC. Intestinal lesions were more frequently fungoid or polyoid, showed necrosis and desmoplasia, and were diagnosed at earlier stages. In respect of patient prognosis, diffuse GC was significantly associated with higher rates of tumor recurrence and cancer-specific death. Multivariate analysis is presented in **Table 2**. DFS and OS curves according to the Laurén classification are shown as **Supplementary Figures 1 and 2**, respectively.

**Prognostic factors in intestinal and diffuse-type GC**

Intestinal GC: Univariate analysis is summarized in **Supplementary Table 2**. Tumor recurrence was significantly associated with perineural infiltration, vascular invasion, T, N, TNM stage and LNR. The relationship between tumor budding or MSI and recurrence tended to be significant (P = 0.057 and 0.084, respectively). Death due to GC was significantly related to infiltrative growth pattern, vascular invasion, T, N, TNM stage and LNR. Presence of necrosis and MSI approached significance (P = 0.088 and 0.096, respectively). Multivariate analysis is presented in **Table 3**: LNR, vascular invasion and T stage were independent risk factors for tumor recurrence, whereas LNR and growth pattern were
Table 1: Univariate analyses: Differences between intestinal and diffuse subtypes (Chi-squared and Student’s t tests)

<table>
<thead>
<tr>
<th>Feature</th>
<th>P value</th>
<th>OR, diffuse (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Depth</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>Macroscopic type</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Polypoid</td>
<td></td>
<td>0.86 (0.45-1.65)</td>
</tr>
<tr>
<td>Flat</td>
<td></td>
<td>3.16 (1.49-6.71)</td>
</tr>
<tr>
<td>Ulcerative</td>
<td></td>
<td>1.54 (0.9-2.65)</td>
</tr>
<tr>
<td>Fungoid</td>
<td></td>
<td>0.38 (0.21-0.69)</td>
</tr>
<tr>
<td>Local symptoms</td>
<td>0.004</td>
<td>0.4 (0.2-0.75)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.006</td>
<td>0.39 (0.2-0.77)</td>
</tr>
<tr>
<td>Perineural infiltration</td>
<td>&lt; 0.001</td>
<td>2.98 (1.78-5)</td>
</tr>
<tr>
<td>Intratumoral inflammation</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>0.63 (0.28-1.38)</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td>2.08 (1.07-4.03)</td>
</tr>
<tr>
<td>High</td>
<td></td>
<td>0.68 (0.38-1.2)</td>
</tr>
<tr>
<td>Desmoplasia</td>
<td>&lt; 0.001</td>
<td>0.29 (0.16-0.54)</td>
</tr>
<tr>
<td>Microsatellite instability</td>
<td>0.023</td>
<td>0.16 (0.02-0.28)</td>
</tr>
<tr>
<td>HERCEPTEST 2+/3+</td>
<td>0.018</td>
<td>0.07 (0.02-0.24)</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>0.41 (0.19-0.88)</td>
</tr>
<tr>
<td>T2</td>
<td></td>
<td>0.74 (0.39-1.4)</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>1.25 (0.76-2.05)</td>
</tr>
<tr>
<td>T4</td>
<td></td>
<td>2.08 (1.08-3.99)</td>
</tr>
<tr>
<td>N stage</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td></td>
<td>0.5 (0.3-0.85)</td>
</tr>
<tr>
<td>N1</td>
<td></td>
<td>0.8 (0.4-1.6)</td>
</tr>
<tr>
<td>N2</td>
<td></td>
<td>1.93 (1.04-3.55)</td>
</tr>
<tr>
<td>N3</td>
<td></td>
<td>1.62 (0.9-2.93)</td>
</tr>
<tr>
<td>Metastatic lymph nodes</td>
<td>0.005</td>
<td></td>
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<tr>
<td>Lymph node ratio</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Adjuvant therapy</td>
<td>0.022</td>
<td>2.14 (1.11-4.15)</td>
</tr>
<tr>
<td>Recurrence</td>
<td>&lt; 0.001</td>
<td>2.63 (1.55-4.47)</td>
</tr>
<tr>
<td>Death</td>
<td>&lt; 0.001</td>
<td>2.82 (1.56-5.09)</td>
</tr>
</tbody>
</table>

Odds ratios have been calculated for diffuse vs intestinal subtype.

Independently associated with tumor death.

**Diffuse GC:** Univariate analysis is summarized in Supplementary Table 2. GC recurrence was significantly related to tumor size, T, N, TNM stage and LNR; and death due to GC was significantly associated with tumor necrosis, presence of systemic symptoms, N, TNM stage and LNR. The association between cancer-specific death and vascular invasion, T stage and tumor size tended to be significant (P = 0.059, 0.058 and 0.08, respectively). Multivariate analysis (Table 3) identified tumor size and LNR as independent predictors of tumor recurrence. Necrosis and LNR were independent risk factors for death due to GC.
Table 2 Multivariate analysis: Variables independently related to Laurén subtypes

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value</th>
<th>HR</th>
<th>95%CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Lymph node ratio</td>
<td>0.012</td>
<td>9.463</td>
<td>1.655</td>
</tr>
<tr>
<td>Depth</td>
<td>0.037</td>
<td>1.108</td>
<td>1.006</td>
</tr>
<tr>
<td>Desmoplasia</td>
<td>0.014</td>
<td>0.323</td>
<td>0.131</td>
</tr>
<tr>
<td>Macroscopy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypoid</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flat</td>
<td>0.006</td>
<td>10.002</td>
<td>1.928</td>
</tr>
<tr>
<td>Ulcerative</td>
<td>0.027</td>
<td>4.536</td>
<td>1.189</td>
</tr>
<tr>
<td>Fungoid</td>
<td>0.686</td>
<td>1.318</td>
<td>0.345</td>
</tr>
</tbody>
</table>

1 Hazard ratios have been calculated using the intestinal type as a reference.

Table 3 Independent risk factors for tumor recurrence and cancer-specific death in intestinal and diffuse-type gastric cancer

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Factor</th>
<th>P value</th>
<th>HR</th>
<th>95%CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
</tr>
<tr>
<td>Intestinal type GC</td>
<td>LNR</td>
<td>0.004</td>
<td>32.424</td>
<td>3.057</td>
</tr>
<tr>
<td></td>
<td>Growth</td>
<td>0.052</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Depth</td>
<td>0.03</td>
<td>4.678</td>
<td>0.987</td>
</tr>
<tr>
<td></td>
<td>T stage</td>
<td>0.056</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T1-2</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>T3-4</td>
<td>2.193</td>
<td>0.98</td>
<td>4.909</td>
</tr>
<tr>
<td></td>
<td>Vascular invasion</td>
<td>0.005</td>
<td>2.829</td>
<td>1.379</td>
</tr>
<tr>
<td></td>
<td>Infiltrative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Necrosis</td>
<td>0.008</td>
<td>4.234</td>
<td>1.460</td>
</tr>
<tr>
<td>Tumor death (OS)</td>
<td>LNR</td>
<td>0.026</td>
<td>5.729</td>
<td>1.234</td>
</tr>
<tr>
<td>T stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence (DFS)</td>
<td>LNR</td>
<td>0.01</td>
<td>1.018</td>
<td>1.005</td>
</tr>
<tr>
<td></td>
<td>Size</td>
<td>0.001</td>
<td>11.420</td>
<td>3.895</td>
</tr>
</tbody>
</table>

DFS: disease-free survival; GC: Gastric cancer; LNR: Lymph node ratio; OS: overall survival.

Prognostic scores for patients with intestinal and diffuse-type GC

Intestinal GC: Two prognostic scores were constructed based on hazard ratios (Table 4). The recurrence score included T stage, LNR and vascular invasion; total score ranged from 0 to 9. Kaplan-Meier curves showed an excellent patient stratification into four prognostic groups (S1-S4, P < 0.001, Figure 1A). Mean DFS times were 161, 129, 83 and 61 mo for S1-S4 cases, respectively. The risk score for predicting cancer-specific death included LNR and growth pattern; total score ranged from 0 to 37. Cases were divided into four categories (S1-S4). This score showed a good prognostic performance by Kaplan-Meier analysis (P < 0.001, Figure 1B). Mean OS was 170, 132, 77 and 67 mo for S1-S4 patients. AUC values of the prognostic scores for recurrence and cancer-specific death were 0.745 and 0.763, respectively (Supplementary Table 3).

Diffuse GC: Prognostic scores for diffuse-type GC are presented in Table 5. The score for predicting tumor recurrence included tumor size and LNR; total score ranged from 0 to 120. The score for cancer-
Table 4 Prognostic scores for patients with intestinal-type gastric cancer

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Death</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth</td>
<td></td>
<td>T stage</td>
</tr>
<tr>
<td>Expansive</td>
<td>0</td>
<td>T1-2</td>
</tr>
<tr>
<td>Infiltrative</td>
<td>5</td>
<td>T3-4</td>
</tr>
<tr>
<td>LNR × 32</td>
<td></td>
<td>LNR × 4</td>
</tr>
<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Total score</strong></td>
<td>0-37</td>
<td>0-9</td>
</tr>
<tr>
<td><strong>Stages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S2</td>
<td>&gt; 0-5</td>
<td>&gt; 0-2</td>
</tr>
<tr>
<td>S3</td>
<td>&gt; 5-14</td>
<td>&gt; 2&lt; 5</td>
</tr>
<tr>
<td>S4</td>
<td>&gt; 14</td>
<td>5-9</td>
</tr>
</tbody>
</table>

LNR: Lymph node ratio.

Table 5 Prognostic scores for patients with diffuse-type gastric cancer

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Death</th>
<th>Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Features</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>4</td>
<td>Size in mm</td>
</tr>
<tr>
<td>LNR × 6</td>
<td></td>
<td>LNR × 11</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>0-10</td>
<td>0-120</td>
</tr>
<tr>
<td><strong>Stages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>0&lt; 1.5</td>
<td>0&lt; 20</td>
</tr>
<tr>
<td>S2</td>
<td>1.5-4</td>
<td>&gt; 20&lt; 60</td>
</tr>
<tr>
<td>S3</td>
<td>&gt; 4</td>
<td>&gt; 60</td>
</tr>
</tbody>
</table>

LNR: Lymph node ratio.

Specific death included tumor necrosis and LNR; total score ranged from 0 to 10. Both prognostic scores showed an excellent risk stratification of patients into three groups (S1-S3, P < 0.005, Figure 2). Mean DFS was 93, 90 and 33 mo (S1-S3 patients) and mean OS was 145, 86 and 16 mo (S1-S3 patients). AUC values of the prognostic scores for recurrence and cancer-specific death were 0.674 and 0.710, respectively (Supplementary Table 3).

DISCUSSION

The global incidence of GC has been decreasing in recent years and this fact may be due to the detection and eradication of H. pylori and improvements in food preservation[22-24]. However, the relative incidence of diffuse GC is consequently increasing[25]. As previously mentioned, the Laurén system was developed in 1965 as a “histo-clinical classification”. After this first description of intestinal and diffuse-type GC, several studies have variably associated Laurén subtypes with clinicopathological features of GC, including patient age, sex or macroscopic morphology[7,8,26]. In our series, Laurén subtypes showed significant differences in age at diagnosis, tumor depth, macroscopic type, local symptoms, necrosis, perineural infiltration, intratumoral inflammatory infiltration, desmoplasia, T, N, LNR and administration of adjuvant therapy.

In respect of GC prognosis, the relationship between Laurén subtypes and patient outcomes is still controversial. In this study, we observed that diffuse GC showed higher rates of recurrence and cancer-specific death than intestinal tumors. Furthermore, in a previous study, we identified Laurén subtype as an independent prognostic factor for both DFS and OS in a subgroup of patients with GC from our
Diaz del Arco C et al. Clinicopathological prognostic scores for Laurén subtypes

Figure 1 Intestinal-type gastric cancer. A: Recurrence score; B: Cancer-specific death score. Kaplan-Meier curves of each prognostic group (S1-S4). P value by log-rank test was P < 0.001.

Figure 2 Diffuse-type gastric cancer. A: Recurrence score, P value by log-rank test was P = 0.003; B: Cancer-specific death score, P value by log-rank test was P < 0.001. Kaplan-Meier curves of each prognostic group (S1-S3).

Most authors have found that diffuse tumors are significantly and independently related to poor prognosis[10,28,29], but other studies have not confirmed these findings[30,31]. A recent meta-analysis including 73 publications and more than 61000 patients further confirmed the prognostic value of the Laurén classification[32].

The huge impact of technological advances on GC diagnosis and pathogenesis has led to the development of new molecular-based classifications[33]. However, molecular studies are expensive and these classifications have not been implemented in practice. The most important systems have been published by TCGA in 2014 and the Asian Cancer Research Group (ACRG) in 2015[13,34]. TCGA defined four subtypes: tumors positive for Epstein-Barr virus, MSI tumors, GS tumors and tumors with CIN[13]. ACRG divided GC into p53 active, p53 inactive, mesenchymal and MSI GC[34]. Most GS and mesenchymal tumors are diffuse and most cases of MSI and CIN GC are intestinal-type tumors[35].

Intestinal tumors are associated with MSI and show higher mutation rates and more copy-number alterations than diffuse-type GC. On the other hand, diffuse GC is related to CDH1 mutation, and approximately 9% of these tumors present MSI[36]. Recent studies have also shown that HER2 positivity is more frequently seen in intestinal-type GC[37,38]. Our results support these findings: we observed that intestinal-type tumors are associated with higher rates of MSI (34.6% vs 18.5%) and HERCEPTEST positivity. 6.7%, 3.8% and 3.8% of intestinal cases were 1+, 2+ and 3+, respectively, whereas all diffuse tumors were HERCEPTEST negative (0).

Laurén classification may also play a role in patient management and response to therapy[39]. Early GC can be treated by endoscopic resection and standard criteria include well or moderately differentiated GC confined to the mucosa, size ≤ 20 mm and absence of lymphatic or venous invasion[40,41]. Current expanded criteria for endoscopic submucosal dissection include the resection of high-grade GC ≤ 2 cm in size[42]. GC in stages IB-III is treated by gastrectomy with lymphadenectomy. Diffuse tumors may benefit from more aggressive surgical options and prevention or treatment of peritoneal metastases by HIPEC[43,44]. Regarding chemotherapeutic and radiotherapeutic regimens, most treatment protocols are based on the TNM classification. However, several authors have observed that treatment
response may vary depending on the Laurén subtype and have suggested that diffuse tumors may be more resistant to standard chemotherapeutic agents than intestinal-type GC [39]. In a recent literature review, we summarized the main findings of clinical trials and comparative studies analyzing treatment regimens depending on the Laurén subtypes [11]. According to this review, studies on adjuvant therapy showed that intestinal-type GC is more chemo-sensitive than diffuse GC and treatment response may also vary depending on geographical features. The benefit of neoadjuvant therapy seems to be limited to patients with intestinal GC.

Clinicopathological prognostic features of GC patients have been previously analyzed in the literature [45]. Some authors have studied specific prognostic factors in younger patients or proximal tumors [46, 47]. In this study, our second objective was to identify clinicopathological risk factors for tumor recurrence and cancer-specific death depending on the Laurén subtype. As might be expected, the TNM system was associated with tumor recurrence and death in both GC subtypes. But several differences were observed: vascular invasion, perineural infiltration and infiltrative growth pattern were important prognostic features in intestinal-type GC. On the contrary, tumor size and necrosis were significant prognosticators in diffuse-type GC. As for molecular features, we found that the relationship between MSI and prognosis tended to be significant only in intestinal-type tumors.

Finally, we constructed prognostic scores for predicting tumor recurrence and cancer-specific survival in patients with intestinal and diffuse-type GC. Our scores included only clinicopathological variables and can be easily calculated in clinical practice. All scores showed an excellent patient stratification into three (diffuse GC) or four (intestinal GC) prognostic groups by Kaplan-Meier analyses. Previous studies have developed predictive scores for GC patients and most of them included nutritional and laboratory findings [41, 48]. Other authors have developed molecular signatures or scores including immunohistochemical parameters [49]. Recently, Bao et al. [50] developed a three-gene signature for prognostic prediction in diffuse-type GC. Clinicopathological prognostic scores, although easy to apply, have been less frequently published [51]. As far as we know, no other study has developed separate clinicopathological risk scores for patients with intestinal and diffuse-type GC.

**Strengths and limitations of our study**

The results of this study should be interpreted in the context of its strengths and limitations. Strengths: This study includes patients with pure intestinal or diffuse GC treated by curative gastrectomy. Cases with neoadjuvant therapy, mixed tumors and R1-2 resections were excluded. All patients were diagnosed and treated in a western tertiary hospital. All tumors were reviewed and pathological features were independently assessed by two pathologists following a detailed protocol. Limitations: Retrospective study. GC is not frequent in western countries so this study includes less patients than Asian studies. IHC markers were performed in TMA sections and they may not represent the full heterogeneity of the tumor. In an attempt to overcome this limitation, cores were selected from the center and the leading edge of each case. Furthermore, no significant differences were observed between the two cores of each tumor.

**CONCLUSION**

In our series, intestinal and diffuse-type GC showed different epidemiological, clinical and prognostic features and they were associated with different risk factors for progression and death. Our specific prognostic scores for predicting tumor recurrence and cancer-specific survival in patients with intestinal and diffuse-type GC showed an excellent patient stratification into three (diffuse GC) or four (intestinal GC) prognostic groups.

Laurén classification is a cost-effective and widely implemented tool in GC and it has regained importance in the last few years due to its correlation with the molecular groups of GC. Our findings support the notion that Laurén subtypes may represent different clinicopathological and biological entities and the development of specific prognostic scores could be a useful and cost-effective strategy to improve risk assessment and patient stratification in GC. Our scores include clinicopathological variables easily available in practice and patients can be stratified according to their risk without complementary tests. However, our results should be externally validated and refined in other western and eastern cohorts of patients. Thus, more studies with a larger number of patients and other ethnic groups are needed in order to confirm the prognostic validity of the proposed prognostic scores and the current role of the Laurén classification in GC.

**ARTICLE HIGHLIGHTS**

**Research background**

In the molecular era, the Laurén system is still a cost-effective and widely implemented classification for gastric cancer (GC) and it has been recently associated with clinical, histological and molecular features.
of these tumors. Laurén subtypes have also shown differences in response to systemic therapy.

**Research motivation**

Despite recent advances in the understanding of the molecular biology of GC, there is a need to develop new prognostic tools for patient stratification in clinical practice. The implementation of specific scores for patients with intestinal and diffuse-type GC may significantly improve risk assessment and management of GC.

**Research objectives**

Our aims were to: (1) evaluate the clinicopathological differences between Laurén subtypes; (2) identify specific risk factors for these subtypes; and (3) develop prognostic scores for patients with intestinal and diffuse-type GC.

**Research methods**

This is a retrospective study of all patients undergoing surgery for GC at a tertiary referral center from 2001 to 2019. Clinical data and gross findings were collected. Histological and immunohistochemical features were assessed by two independent pathologists and prognostic scores were developed based on hazard ratios.

**Research results**

In our series of western patients with GC, intestinal and diffuse-type tumors showed distinctive epidemiological, clinical and prognostic features. In addition, Laurén subtypes were associated with different risk factors for tumor progression and cancer-specific death. Our prognostic scores for predicting overall survival and disease-free survival in patients with intestinal and diffuse-type GC included clinicopathological variables that can be easily calculated in clinical practice and showed an excellent patient stratification into three (diffuse GC) or four (intestinal GC) prognostic groups.

**Research conclusions**

The stratification of GC patients depending on Laurén subtypes and the implementation of specific clinicopathological prognostic scores in intestinal and diffuse-type tumors can be useful for patient stratification, risk assessment and treatment selection.

**Research perspectives**

Our prognostic scores should be externally validated in patients from both western and eastern countries due to the geographical variation of GC. In addition, this study opens a door to the development and implementation of cost-effective and specific clinicopathological prognostic scores in patients with GC in different contexts.

**FOOTNOTES**

**Author contributions:** Díaz del Arco C participated in the data acquisition, analysis, interpretation, manuscript draft, approval and agreement; Estrada Muñoz L participated in the data acquisition, analysis, manuscript revision, approval and agreement; Ortega Medina L participated in the study design, data interpretation, manuscript revision, approval and agreement; Molina Roldán E, Cerón Nieto MA, García Gómez de las Heras S participated in the data acquisition, manuscript revision, approval and agreement; Fernández Aceñero MJ participated in the study design, data analysis and interpretation, manuscript draft, approval and agreement.

**Institutional review board statement:** This study was reviewed and approved by the Ethics Committee of the Hospital Clínico San Carlos.

**Conflict-of-interest statement:** We have no financial relationships to disclose.

**Data sharing statement:** Data will be available on request.

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Díaz del Arco C et al. Clinico-pathological prognostic scores for Laurén subtypes

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Characterizing the patient experience during neoadjuvant therapy for pancreatic ductal adenocarcinoma: A qualitative study

Lena Stevens, Zachary J Brown, Ryan Zeh, Christina Monsour, Sharla Wells-Di Gregorio, Heena Santry, Aslam M Ejaz, Timothy Michael Pawlik, Jordan M Cloyd

Abstract

BACKGROUND

Neoadjuvant therapy (NT) has increasingly been utilized for patients with localized pancreatic ductal adenocarcinoma (PDAC). It is the recommended approach for borderline resectable (BR) and locally advanced (LA) cancers and an increasingly utilized option for potentially resectable (PR) disease. Despite its increased use, little research has focused on patient-centered metrics among patients undergoing NT, including patient experiences, preferences, and recommendations. A better understanding of all aspects of the patient experience during NT may identify opportunities to design interventions aimed at improving quality of life; it may also facilitate the completion of NT and receipt of surgery, ultimately optimizing long-term outcomes.

AIM

To understand the experience of patients initiating and receiving NT to identify opportunities to improve neoadjuvant cancer care delivery.

METHODS

Semi-structured interviews of patients with localized PDAC during NT were conducted to explore their experience initiating and receiving NT. Interviews took place between August 2020 and October 2021. Due to the descriptive nature of the research, questions were open ended. Interviews were conducted over the phone, audio recorded and then transcribed. All interviews were coded by two inde-
pendent researchers using NVivo 12, iteratively identifying themes until thematic saturation was achieved. An integrative approach to qualitative analysis was used, utilizing both inductive and deductive methods.

RESULTS
A total of 12 patients with localized PDAC were interviewed. Patients with BR (n = 7), PR (n = 2), and LA (n = 3) cancers participated in the study. All patients indicated that choosing NT was the doctor’s recommendation, while most reported not being familiar with the concept of NT (n = 11) and that NT was presented as the only option (n = 8). Five themes describing the patient experience emerged: physical symptoms, emotional symptoms, coping mechanisms, access to care, and life factors. The most commonly cited recommendation for improving the experience of NT was improved education before and during NT (n = 8). Five themes describing the patient experience emerged: physical symptoms, emotional symptoms, coping mechanisms, access to care, and life factors. The most commonly cited recommendation for improving the experience of NT was improved education before and during NT (n = 7). Patients highlighted the need for more information on the rationale behind choosing NT prior to surgery, the anticipated surgery and its likelihood of surgery occurring after NT, as well as general information prior to starting NT treatment. The need for seeing different members of the healthcare team, including ancillary services was also frequently cited as a recommendation for improving the experience of NT (n = 5).

CONCLUSION
This study provides a framework to allow for a better understanding of the PDAC patient experience during NT and highlights opportunities to improve quality and quantity of life outcomes.

Key Words: Pancreatic ductal adenocarcinoma; Neoadjuvant therapy; Patient experience; Patient-centered care; Quality of life; Qualitative research

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Core Tip: This study aims to understand the experience of localized pancreatic ductal adenocarcinoma (PDAC) patients initiating and receiving neoadjuvant therapy (NT). Semi-structured interviews of patients with localized PDAC during NT were conducted; 12 patients were interviewed. All patients indicated that choosing NT was the doctor’s recommendation. Most reported not being familiar with the concept of NT. Five themes describing the patient experience emerged. This study provides a framework to allow for a better understanding of the patient experience and highlights opportunities to improve quality and quantity of life outcomes for patients with PDAC.

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DOI: https://dx.doi.org/10.4251/wjgo.v14.i6.1175

INTRODUCTION
The delivery of chemotherapy and/or radiation therapy prior to surgery, known as neoadjuvant therapy (NT), is increasingly utilized for patients with pancreatic ductal adenocarcinoma (PDAC)[1,2]. Since a significant proportion of patients are unable to receive all intended adjuvant therapies following major pancreatectomy, NT ensures the receipt of some systemic therapy and leads to improved rates of multimodality therapy. NT also improves margin-negative resection rates, enhances patient selection by ensuring the absence of rapid tumor progression prior to surgery, enables an in vivo test of the efficacy of chemotherapy, and based on emerging evidence from randomized controlled trials, may lead to improved overall survival[3-7]. Based on these advantages, NT is now the recommended approach for borderline resectable (BR) and locally advanced (LA) cancers and an increasingly utilized option for potentially resectable (PR) disease according to national guidelines[3,8,9].

Despite its increasing use in PDAC and other cancer types[2], little is known about the patient experience during NT. Indeed, the neoadjuvant time period might be particularly distressing for patients who must cope with not only the toxicity of treatment, but also side effects from the tumor itself which remains in situ. Furthermore, little is known about the psychosocial impact of NT particularly given many patients’ inherent preference for “just getting the cancer out,” as well as the uncertainty of future surgery[10]. A recent systematic review found scarce data on quality of life (QOL) during NT for
PDAC and no existing literature on other aspects of the patient experience\cite{11}. In contrast to immediate surgery, NT is also inherently multi-disciplinary in nature. As such, there may be barriers to effective care initiation and coordination that impede the completion of all scheduled therapy and the receipt of surgical resection\cite{12}.

Therefore, the purpose of this qualitative study was to characterize the patient experience during NT for PDAC. Specifically, we sought to understand patient treatment preferences, information needs, the physical and psychosocial impact of treatment, and barriers to successful initiation and delivery of NT. A better understanding of all aspects of the patient experience during NT may identify opportunities to design interventions aimed at improving QOL during NT, facilitating completion of NT and receipt of surgery, and ultimately optimizing the long-term outcomes of patients with PDAC.

\section*{MATERIALS AND METHODS}

\subsection*{Study design and population}

Patients with PDAC undergoing NT prior to planned surgery in the future were recruited to participate in this qualitative study. All treatment decisions at our institution are made at a pancreatic cancer specialty specific multidisciplinary clinic and made on an individualized basis. Participants were identified by prospectively screening ambulatory clinics at The Ohio State University Wexner Medical Center and James Comprehensive Cancer Center. Inclusion criteria included receiving at least two cycles of chemotherapy in a neoadjuvant intent, still eligible for surgical resection, and English language speaking, without restrictions on age, race, or disability. Eligible patients were contacted by phone, informed consent was obtained, and an interview was scheduled at the participant’s convenience. Due to the COVID-19 pandemic, interviews were conducted by phone between August 2020 and October 2021.

\subsection*{Interview guide and process}

The interview script was developed using evidence synthesis, stakeholder engagement, and expert opinion. The content of the interviews focused on patient treatment preferences, perspectives on the decision-making process, and all aspects of the patient experience during NT; recommendations on opportunities to improve the delivery of NT were also sought. Questions were open-ended, prompting additional questions depending on the responses of the interviewees (Supplementary material). This type of interview method was selected due to the descriptive nature of the research. Semi-structured interviews allow researchers to discuss topics of interest more in detail by elaborating on emerging themes and asking probing questions. A nominal gift card was given to participants for their participation. This study was approved by the Institutional Review Board of The Ohio State University (IRB# 2019C0155).

\subsection*{Statistical analysis}

All interviews occurred by phone, were audio recorded, and then manually transcribed verbatim by the researchers. Transcripts were then uploaded to NVivo12 (QSR International, Australia) for data extraction, synthesis, and analysis purposes. Data extraction followed an integrated approach, including both an inductive and deductive coding methodology\cite{13}. The following preliminary codes were developed before a more in-depth, inductive coding process took place: Patient Experiences; Patient Perspectives on NT; Solutions, Facilitators and Recommendations; and Sources of Information. Two researchers independently coded the transcripts for sub-themes in an iterative fashion until thematic saturation was achieved\cite{13}. Interviews were then re-reviewed and coded using the final codebook. When coding from both independent researchers was not concordant, these instances were reviewed with a third researcher at team meetings. These sections and codes were discussed until a consensus was reached. Demographic data from participants were summarized and illustrative quotes in each theme were selected.

\section*{RESULTS}

\subsection*{Participant characteristics}

A total of 12 patients participated in the interviews. On average, patients were 67 years old, ranging from 52 to 81 years. Patients with BR (n = 7, 58%), PR (n = 2, 17%), and LA (n = 3, 25%) cancers participated in the study. A majority of patients (n = 7, 58%) received chemotherapy and radiation therapy before their planned surgery while others (n = 5, 42%) received just chemotherapy. At most recent follow-up, most patients (n = 10, 83%) had completed NT with 8 patients (67%) undergoing surgical resection of their tumor. Complete participant characteristics are reported in Table 1.
# Table 1 Participant characteristics ($n = 12$)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Count</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age [mean (range), yr]</strong></td>
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</tr>
<tr>
<td>Gender</td>
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<td>5</td>
<td>42</td>
</tr>
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<td>Chemo + XRT</td>
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<tr>
<td>Length of NT</td>
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<td>10</td>
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<td>$3-6$ mo</td>
<td>6</td>
<td>60</td>
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<tr>
<td>$&gt; 6$ mo</td>
<td>3</td>
<td>30</td>
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<tr>
<td>Type of chemo</td>
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<tr>
<td>FOLFIRINOX</td>
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<tr>
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<td>17</td>
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<td>Major complications during NT</td>
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<td>3</td>
<td>25</td>
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<tr>
<td>$100+$ miles</td>
<td>2</td>
<td>17</td>
</tr>
<tr>
<td>Surgical resection</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Patient perspectives on neoadjuvant therapy

Among the 12 patients who participated in the interviews, the vast majority ($n = 11, 92\%$) were not familiar with the concept of NT at the time of initial consultation. All subjects reported that NT was the doctor’s recommendation and most ($n = 8, 67\%$) explained that NT was presented to them as the only option. All interviewees indicated that improving resectability was the main rationale for choosing NT. While some ($n = 6, 50\%$) patients indicated that before meeting with their physicians they did not have a preference for a specific treatment plan, others ($n = 4, 33\%$) expressed that they had hoped to avoid chemotherapy and undergo upfront surgery. All patients indicated that their main source of information were members of their health care team while other sources of information discussed included the internet ($n = 4, 33\%$), family and friends ($n = 3, 25\%$), and educational materials ($n = 1, 8\%$).

Patient experience during neoadjuvant therapy

Five subthemes of patient experiences during NT emerged: physical symptoms, emotional symptoms, coping mechanisms, access to care, and life factors (Figure 1 and Table 2). All participants reported elements of each of the five subthemes.

Physical symptoms: A few patients ($n = 3, 25\%$) discussed that they did not experience any major side effects and they were tolerating their therapy well (“I have never had any symptoms. No throwing up, no nothing.”). However, most patients reported experiencing major side effects from their treatment. Many patients reported feeling weak ($n = 6, 50\%$). One patient stated: “I’ll say at night, I am going to do this, that and the other and the next day comes and my body says ‘no, we’re not going to do that’.” Others mentioned challenges around weight loss, loss of appetite, and the taste of food ($n = 5, 42\%$), as well as a general feeling of sickness ($n = 4, 33\%$) (“After getting chemo for the next 5 days I’m sick as a dog.”).

Emotional symptoms: In addition to shock experienced during their diagnosis, patients reported varying rates of fear and depression (“… it scared me. It depressed me.”). A few patients ($n = 3, 25\%$) shared concerns for their family and friends’ well-being, regarding uncertainty about next steps in treatment, and about their overall prognosis. One patient stated: “I love my wife and I want to be around for her. It’s hard.” Some ($n = 3, 25\%$) also shared not wanting to think about and dwell too much on their diagnosis and treatment approach, as well as the need for not too much information, as it leads to unnecessary anxiety.

Coping and support mechanism: The main coping and support mechanism cited by most patients ($n = 10, 83\%$) was support from family members. Tangible aspects of support included family members and friends offering rides to appointments, discussing different treatment options, helping with coordinating care and reaching out to the medical team, as well as helping with chores around the house. Patients putting their trust in their religious faith was another coping and support mechanism mentioned by some ($n = 5, 42\%$). One patient stated: “I’m a religious person, so that’s enough said.” Several patients ($n = 4, 33\%$) also mentioned receiving support from different members of the medical team (“So, there is always someone here to answer my questions, which also feels good and gives you comfort.”).
Table 2 Representative quotes describing patient experience during neoadjuvant therapy

<table>
<thead>
<tr>
<th>Physical symptoms</th>
<th>Quote No. 1</th>
<th>“After getting chemo for the next 5 d I’m sick as a dog, weak, losing weight, lost about 40 lbs.”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quote No. 2</td>
<td>“Side effects of course. You’re gonna be queasy, you’re gonna be lightheaded. Definitely, I ate, but food didn’t taste good, even water didn’t taste good and I didn’t expect that.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 3</td>
<td>“I’m tired all the time. No energy. I sleep a lot. A little diarrhea. Pretty mild, never threw up yet. Only real bad thing is my appetite. Lost it about completely I have to force myself to eat until I start gagging”</td>
</tr>
<tr>
<td>Emotional symptoms</td>
<td>Quote No. 1</td>
<td>“Most times I don’t care, I really just don’t want to think about it. I just want to watch a good movie. You know what I mean. Not dwell on it all the time. I love my wife and I want to be around for her. It’s hard.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 2</td>
<td>“There are days that I get a little depressed. Because…I am used to movement. And I just don’t have the stamina, nor the willpower.”</td>
</tr>
<tr>
<td>Coping and support mechanisms</td>
<td>Quote No. 1</td>
<td>“…my wife and I, we are Christians and we know it’s up to him, the Lord.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 2</td>
<td>“I have good support from my family and friends. And prayer circles. Getting financial gifts to help us with gas money and things like that. So, they’ve all been very supportive.”</td>
</tr>
<tr>
<td>Access to care</td>
<td>Quote No. 1</td>
<td>“And I have had a lot of friends that make sure that I get to my treatments. So, I don’t have any problems there.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 2</td>
<td>“But it got to be a little bit much for [my sister-in-law] so, I had to withdraw money to get me through this [to ride the bus] that was an extra expense, as I am a senior. And I’m on a fixed income. So, I hadn’t counted on that.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 3</td>
<td>“I have no problem with transportation. My wife was always there to give me a ride.”</td>
</tr>
<tr>
<td>Life factors</td>
<td>Quote No. 1</td>
<td>“I have help with paying my bills, [grocery store], cleaning my house, things like that, doing laundry. So, I am very lucky.”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 2</td>
<td>“I worry about all the damn bills”</td>
</tr>
<tr>
<td></td>
<td>Quote No. 3</td>
<td>“I’m retired so really the work thing didn’t come into play, but I have chores around the house that I’m limited in doing”</td>
</tr>
</tbody>
</table>

Access to care: For most, access and coordination was an important but feasible aspect of NT. This included minimal obstacles associated with traveling to medical appointments (n = 8, 67%), scheduling appointments (n = 6, 50%), contacting doctors (n = 6, 50%), getting answers to questions (n = 4, 33%), getting insurance to cover treatments (n = 3, 25%), or seeing a doctors and getting referrals (n = 2, 17%). While in general patients did not experience major complications accessing and coordinating care, a minority of patients reported some barriers. A few (n = 3, 25%) highlighted that traveling to appointments was burdensome. One patient explained: “Every time we have to have something done, it’s two hours out of our day, about 2.5 h out of our day just driving to the place. But we made that choice knowing that was the case for the care, the treatment and we’ve been proceeding.”

Life factors: Finally, all patients described the need to integrate their treatment and condition with their normal life circumstances. Several patients discussed the impact of NT on other aspects of their life. NT impacting a patient’s work and financial situation were the most commonly cited sub-themes. Many patients discussed that missing work was not a major challenge they were faced with (n = 5, 42%), and that they did not experience major financial concerns (n = 6, 50%). Yet, some (n = 3, 25%) expressed concern around not being able to work and the burden it placed on them financially (n = 4, 33%). One patient stated: “I’ve been off since all this happened (…) drives me kinda nuts. I used to work all the time. But I got no energy now.” Another patient explained: “I had to withdraw money to get me through this that was an extra expense, as I am a senior. And I’m on a fixed income. So, I hadn’t counted on that.” Other life aspects mentioned were patients having to deal with other health problems (n = 1, 8%) at the same time they are on NT and needing help with daily activities (n = 2, 17%).

Recommendations for improving the experience of neoadjuvant therapy
The most commonly cited recommendation for improving the experience of NT was to provide better
education and more information on NT (n = 7, 58%). Patients highlighted the need for more information on: the rationale behind choosing NT prior to surgery, the anticipated surgery and its likelihood of occurring after NT, as well as general information prior to starting NT treatment. Patients also discussed that more discussions with physicians could potentially be helpful, but also, highlighted the need for information tailoring (not too much vs not too little). The need for seeing different members of the healthcare team, including ancillary services was also frequently cited as a recommendation for improving the experience of NT (n = 5, 42%). Patients discussed the importance of seeing psychologists, palliative care doctors, case workers, physical therapists, and nutritionists. Better coordination and communication (n = 2, 17%) and better treatments (n = 2, 17%) were also offered as potential recommendations.

**DISCUSSION**

Pancreatic ductal adenocarcinoma is a highly aggressive malignancy, often thought of as a systemic disease at the time of diagnosis, that requires multimodal therapy with a combination of surgery and chemotherapy in order to achieve meaningful long-term survival[14-17]. NT is being increasingly utilized in patients with localized PDAC[1,3,18]. Previous research on NT for PDAC has focused on its safety, efficacy, and cost-effectiveness with little data on patient-centered preferences or experiences duringNT. In this qualitative study of patients actively receiving NT for PDAC, we found several important observations. First, patients are generally unfamiliar with the concept of NT prior to meeting with an oncologist. While many have an inherent preference for upfront surgery, most understand their providers’ recommendation for NT as an attempt to improve resectability (or likelihood of achieving margin-negative resection). Second, patients have unique experiences and care needs during NT that providers should be aware of in order to optimize patient-centered outcomes. A patient-centered approach that supports physical and emotional symptoms and recognizes the importance of life integration is required. Third, specific recommendations for improving the experience of NT prior to surgery were identified.

Interestingly, although patients in our study were actively receiving NT, most were relatively unfamiliar with the concept of NT. All patients expressed that NT was the recommendation of their doctor. While a few patients expressed their desire for a surgery-first approach or to avoid chemotherapy altogether, nevertheless, all patients eventually came to understand and agree with the rationale for NT. This may highlight a disconnect between patients and providers in that systemic chemotherapy is part of the treatment of all patients with pancreatic cancer as even patients with localized cancers who have undergone resection are likely to experience disease recurrence[19,20]. Similar results were found in patients with breast cancer. A study in women with breast cancer who underwent NT found a majority of women understood that chemotherapy was given prior to surgery in order to shrink the tumor but did not grasp the concept that chemotherapy is utilized to treat systemic disease beyond simply local tumor control[21].
Missing from prior studies has been an evaluation of patient-centered preferences and outcomes regarding the use of NT for PDAC. Cancer-related treatment decisions are complex and require consideration of multiple factors; such decisions are often made in the context of shared decision making (SDM), a model in which informed and engaged patients make health-care decisions in conjunction with their providers[22]. The degree to which patients are involved in the SDM process of choosing NT or immediate surgery is unclear. Most patients with cancer desire an active role in making decisions about their care[23] and such patient-centered decision making has been shown to improve patients’ understanding of their treatment options, satisfaction with their health care, and overall quality of life (QOL)[24-26]. Previous research in breast and rectal cancer suggest patient-centered approaches to SDM regarding NT are lacking in clinical practice[27-29]. Indeed, SDM is under-utilized by surgeons in general[30]. Additionally, it is well known that strong emotions and fears may influence treatment decision making[31]. Specifically, emotions may cause behavior or decisions to diverge from more rational or practical decision making consistent with one’s values[32]. For example, patients state their desire to “just get the cancer out” even if this emotional response does not align with one’s values, priorities, or optimal treatment strategy. We found most patients believed NT was their only treatment option moving forward. This is not surprising as a majority of the patients in our study had either BR or LA disease which is currently the preferred treatment strategy based on recent randomized controlled trials[33,34]. Another study has found that most patients believed there was no other treatment option and thus accepted NT[21]. Understanding patient preferences, values, and expectations regarding NT will improve SDM which will lead to not only delivering patient-centered care but also the opportunity to overcome barriers to patient acceptance of NT.

While not previously studied in PDAC, in practice, multiple barriers to the use of NT are often expressed by patients. For example, some patients may have financial concerns secondary to missing work by “delaying” surgery. Others worry about arranging and/or affording transportation for NT due to long travel distances. Additionally, in our study, most, if not all patients, experienced physical and emotional symptoms during NT. Furthermore, the development of toxicities during the course of NT may prove to be a potential barrier that may worsen a patient’s ability to subsequently undergo an operation. A meta-analysis of 38 studies of which 1738 patients received NT found approximately 64% of patients experienced at least grade III toxicity[35]. In fact, this number may be magnified at community hospitals which may not have the same resources as tertiary referral centers to manage toxicities and progress patients through therapy[36-38].

We found that patients with PDAC receiving NT must balance their cancer treatment with other aspects of their lives such as family responsibilities and work in addition to coping with the physical and emotional symptoms that accompany their new diagnosis and treatment (Figure 1). These findings are similar to a previous qualitative study of patients with breast cancer receiving NT. Beaver et al[21] reported five themes among women receiving NT: Coping with the rapid transition from “well” to “ill”, information needs and decision making, needing support and empathy, impact on family, and creating a new “normal”. These findings suggest similar experiences among patients receiving chemotherapy prior to surgery regardless of cancer type. While patients with PDAC certainly have unique challenges such as biliary obstruction, malnutrition, gastric outlet obstruction, as well as cancer-related pain, additional research is needed on supporting the general care needs directly influenced by the neoadjuvant aspects of treatment.

The findings from our study provide a framework to allow for a better understanding of the patient experience during NT and highlight opportunities for inter-disciplinary interventions to improve patient-centered outcomes of those with PDAC. Indeed, many patients who receive NT fail to either complete NT or to undergo subsequent pancreatectomy with common reasons including disease progression or worsening performance status due to toxicity[33,39,40]. Furthermore, since failing to complete therapy or undergo surgical resection is associated with a worse prognosis, having a patient-centered approach to understand potential barriers to completion is essential. As we have demonstrated in this study, patients experience both physical and emotional symptoms during treatment and require a team approach with the help of ancillary services to help complete therapy. Involvement of patient navigators, social workers, nutritionists, and physical therapists to address patient concerns and symptoms may aid to improve the high attrition rate in patients receiving NT. Previous research has highlighted patient dissatisfaction with the lack of access to counseling services, support groups, and educational tools[41].

There are several limitations to our study. Although our study reached theme saturation, the relatively small sample size and single institution design means that the findings may not be generalizable to all patients with PDAC who are receiving NT. Additionally, our study includes patients with PR, BR and LA disease where larger sample sizes are required to investigate if the patient experience differs according to anatomic stage (e.g., patients with LA disease may have lower expectations of undergoing resection and/or greater burden of cancer-related symptoms than patients with PR disease.) Finally, it is unclear if the patient experience is temporal-dependent and since interviews were performed at a single time during NT, future research may focus on longitudinal evaluations of the patient experience.
CONCLUSION

In conclusion, this is the first qualitative study to characterize the experience of patients receiving NT for localized PDAC. Our findings clarify the lack of familiarity with the concept of NT prior to initiating treatment, the unique care needs of patients receiving NT, and recommendations to improve the delivery of cancer care in the neoadjuvant setting. These data provide a framework to allow for a better understanding of the patient experience during NT and highlight opportunities for patient-centered interventions aimed at improving quality and quantity of life outcomes of those with PDAC.

ARTICLE HIGHLIGHTS

**Research background**

Neoadjuvant therapy (NT) has increasingly been utilized for patients with localized pancreatic ductal adenocarcinoma (PDAC). It is the recommended approach for borderline resectable (BR) and locally advanced (LA) and it has also increasingly been utilized for potentially resectable (PR) disease. However, little research has focused on patient-centered metrics among patients undergoing NT, including patient experiences, preferences, and recommendations.

**Research motivation**

A better understanding of all aspects of the patient experience during NT may help identify opportunities to design interventions aimed at improving quality of life. It may also facilitate the completion of NT and receipt of surgery, ultimately optimizing long-term outcomes.

**Research objectives**

This research aims to understand the experience of patients initiating and receiving NT to identify opportunities to improve neoadjuvant cancer care delivery.

**Research methods**

Semi-structured, open-ended interviews of patients with localized PDAC during NT were conducted to explore their experience initiating and receiving NT. Interviews were conducted over the phone. All interviews were audio recorded, transcribed, and coded by two independent researchers using NVivo 12, iteratively identifying themes until thematic saturation was achieved.

**Research results**

A total of 12 patients with localized PDAC were interviewed. All patients indicated that choosing NT was the doctor’s recommendation and most reported not being familiar with the concept of NT (n = 11, 92%). Five patient experience themes emerged: physical symptoms, emotional symptoms, coping mechanisms, access to care, and life factors. Improved education before and during NT was the most commonly cited recommendation for improving the experience during NT (n = 7, 58%). Patients highlighted the need for more information on the rationale behind choosing NT prior to surgery, the anticipated surgery and its likelihood of surgery occurring after NT, as well as general information prior to starting NT treatment.

**Research conclusions**

This study provides a framework to allow for a better understanding of the PDAC patient experience during NT and highlights opportunities to improve quality and quantity of life outcomes.

**Research perspectives**

This exploratory research utilizes qualitative interviews to examine the patient experience when initiating and receiving NT.

ACKNOWLEDGEMENTS

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FOOTNOTES

Author contributions: Cloyd JM designed the research study; Stevens L, Zeh R, Monsour C and Cloyd JM performed the research; Stevens L, Brown ZJ and Cloyd JM analyzed the data and wrote the manuscript; Wells-Di Gregorio S, Santry H, Ejaz AM and Pawlik TM provided expert opinion and edits to the manuscript; and All authors have read and approve the final manuscript.

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Informed consent statement: All study participants provided informed verbal consent prior to study enrollment.

Conflict-of-interest statement: There are no conflicts of interest to report.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement – checklist of items, and the manuscript was prepared and revised according to the STROBE Statement – checklist of items.

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Jang YH, Han Y, Lee H, Kim SW, Kwon W, Lee KH, Oh DY, Chie EK, Lee JM, Heo JS, Park JO, Lim DH, Kim SH, Park
Stevens L et al. Patient experience during NT for PDAC


Randomized Controlled Trial

Biofeedback therapy combined with Baduanjin on quality of life and gastrointestinal hormone level in patients with colorectal cancer

Xiao-Ding Zhou, Hong-Gang Wei, Fu-Lu Ai

Abstract

BACKGROUND
With the change in people’s lifestyles, the incidence of colorectal cancer (CRC) is increasing. It is essential to study the efficacy of various treatment methods for CRC patients to prevent and treat CRC.

AIM
To investigate the efficacy of biofeedback therapy combined with Baduanjin in improving the quality of life and gastrointestinal hormone levels of patients with CRC.

METHODS
A total of 120 patients with CRC who were admitted to our hospital from June 2020 to June 2021 were included in the study. They were randomly divided into four groups (n = 30): the control group (group A), the biofeedback therapy intervention group (group B), the Baduanjin exercise intervention group (group C), and the combination group (group D). Patients in group A adopted the standard nursing mode and necessary health education. Patients in group B were treated with biofeedback therapy based on routine nursing care. Patients in group C were given Baduanjin intervention for 12 wk based on conventional drug treatment and care. Patients in group D were treated with biofeedback therapy and Baduanjin exercise. In this study, patients’ quality of life, gastrointestinal hormone levels, and clinical efficacy in the four groups were observed at baseline and 12 wk after intervention. Meanwhile, the correlation between gastrointestinal hormone levels and various functional areas of quality of life was analyzed.
comparing the observed indicators of patients in the four groups, the efficacy of biofeedback therapy combined with Baduanjin in improving the quality of life and gastrointestinal hormone levels of patients with CRC was explored.

RESULTS
At baseline, there were no significant differences in quality of life, gastrointestinal hormone levels, or clinical efficacy among the four groups ($P > 0.05$). Twelve weeks after the intervention, the combination group’s quality of life, gastrointestinal hormone levels, and clinical effectiveness were better than those of the three other groups.

CONCLUSION
On the basis of routine nursing care, patients with CRC combined with biofeedback therapy and Baduanjin exercise can improve the quality of life of patients with CRC and the efficacy of gastrointestinal hormone levels.

Key Words: Biofeedback therapy; Baduanjin; Colorectal cancer; Quality of life; Gastrointestinal hormone level; Clinical efficacy

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Core Tip: With the increasing incidence and recurrence rate of colorectal cancer (CRC), it is essential to study more comprehensive measures for its treatment and prevention, such as cognitive-behavioral intervention, exercise intervention, and the combination of biofeedback therapy. In this study, CRC patients were treated with 12 wk of biofeedback therapy combined with Baduanjin exercise intervention training to observe the changes in patients’ quality of life and gastrointestinal hormone levels and to explore the clinical value of biofeedback therapy combined with Baduanjin exercise from the overall perspective, which will be vital for the future research on the treatment strategies of CRC.

INTRODUCTION
Colorectal cancer (CRC), including colon and rectal cancer, is a common type of cancer. Its incidence and mortality are high[1], and it may cause hematochezia, diarrhea, constipation, local abdominal pain, weight loss, and other symptoms[2]. In China, patients with CRC are mostly the elderly, and CRC afflicts more males than females[3]. Although the specific pathogenesis of CRC is unknown, its incidence increases yearly, which may result from a combination of age, environment, dietary habits, heredity, occupation, and other factors[4,5].

At present, the treatment method for CRC is surgical treatment. As the surgical cure rate and five-year survival rate of the disease are about 50% and the local recurrence rate is high[6], researchers have been recently studying more comprehensive treatment methods[7], such as cognitive behavioral intervention, exercise intervention, and biofeedback therapy in combination, to help delay the illness and improve the quality of life of patients[8]. Exercise can promote intestinal peristalsis, help fecal discharge, and reduce the contact time of intestinal and fecal carcinogens. Fitness Qigong Baduanjin is a medium-intensity aerobic exercise with easy-to-learn and precise movements, and it is widely recognized by people[9]. Biofeedback refers to the use of instruments to process certain biological information in the body related to psychological and physiological processes, such as skin temperature, heart rhythm, and blood pressure, to be displayed in a visual and auditory manner so that people can understand and consciously control their psychological and physiological activities[10].

In this study, patients with CRC were treated with biofeedback therapy combined with Baduanjin exercise for 12 wk to observe the patients’ constipation symptoms, quality of life, and changes in gastrointestinal hormone levels and explore the clinical value of biofeedback therapy combined with Baduanjin exercise. This work provides a theoretical reference for future research on the treatment strategies of CRC.
MATERIALS AND METHODS

Patient population
A total of 120 patients with CRC who were admitted to Liaoning Provincial Tumor Hospital from June 2020 to June 2021 were selected as the research subjects, aged 45–64, and numbered according to the order of admission. Patients were divided into four groups by random number table method, including control group (group A), biofeedback treatment intervention group (group B), Baduanjin exercise intervention group (group C) and combined group (group D) with 30 patients in each group.

Inclusion and exclusion criteria
Diagnostic criteria: The diagnostic criteria of CRC were referred from Guidelines for Diagnosis and Comprehensive Treatment of Liver Metastasis from Colorectal Cancer in China[11].

Inclusion criteria: (1) Compliant with the diagnostic criteria of CRC mentioned in the Guidelines for Diagnosis and Comprehensive Treatment of Liver Metastasis from Colorectal Cancer in China; (2) Patients with clear consciousness, normal cognitive function, and being able to actively cooperate with others to complete the experiment; and (3) Surgical treatment of CRC.

Exclusion criteria: (1) Severe diseases such as severe infection and hyperpyrexia; (2) The tumor condition deteriorates rapidly and needs emergency treatment; and (3) Those who are unable to complete specific exercise intensity due to mental or physical disorders.

Off (eliminate) standard: (1) Adverse reactions occurred during the experiment; (2) Active withdrawal due to the inability or unwillingness to cooperate for various physiological or psychological reasons; and (3) Other treatment regimens were adopted during the experiment without permission.

Treatment plan
Control group (group A): We adopt a standard nursing mode and necessary health education for patients. Before the experiment, the nurse asked the patient’s medical history, evaluated the patient, and closely monitored the patient’s basic vital signs, such as blood pressure, heart rate, and gastrointestinal function recovery. Make patients keep good living habits, proper diet, and adequate sleep, and give patients regular medication.

Biofeedback therapy intervention group (group B): Based on routine nursing, the intervention program of biofeedback therapy was implemented twice a day for 12 wk, each time for 10 minutes. Before implementing this scheme, the therapist explained the experiment’s purpose, process, and precautions to the patients and put forward the areas where the patients disagreed with ensuring the patients’ active participation.

Biofeedback therapy interventions were as follows: (1) Two weeks before the start of the experiment, the therapist trains the patients to watch the monitor and sphincter contraction and relaxation twice weekly. The investigation officially began when the patients learned to manage the monitor and contract and relax the sphincter; (2) At first, the therapist uses a water-filled pressure probe connected to a colour monitor (Polygraf ID, equipped with Polygram98 instrument, Medtronics, Skovlunde, Denmark) to evaluate the contraction amplitude and duration of the patient’s autonomic anal sphincter, and monitors the contraction and electromyographic activity of the patient’s sphincter, glutaeus muscle and abdominal muscle through the probe; (3) During the experiment, the surrounding environment should be quiet, the light should be soft, and the temperature appropriate. The patient was supine, and therapists helped them untie their belts. Make their lower limbs reach out 60 degrees. During the whole experiment, patients should feel comfortable and relaxed; (4) Biofeedback training uses sensory training and strength training. Sensory training: When the patients receive balloon training, the therapist repeatedly inflates and deflates the balloon in increments of 5 mL air or normal saline for 10s each time, three times in each group, a totally of 10 groups. Therapists help patients determine the volume of bowel movement and the maximum tolerance they can maintain. Strength training: In the absence of an airbag, the patients are required to repeatedly contract the external anal sphincter and guide the patients to breathe naturally without stopping during muscle contraction. The training was repeated for 5s, six times in each group, with ten groups every day. When patients compare their sense of muscle control with the change of muscle control mode displayed on the monitor, the therapist gradually increases the intensity and frequency of their exercise. The therapist should give feedback according to the patient’s condition and constantly encourage the patients in the whole treatment process; and (5) Therapists teach patients the most basic training essentials. After discharge from the hospital, patients still need consolidation training, twice a day for 10 minutes each time, with four weeks as a course of treatment. Therapists evaluated the efficacy of patient training at 3, 6 and 12 wk after intervention.

Baduanjin exercise intervention group (Group C): The therapist gave the patients Baduanjin training based on routine medication and nursing and intervened for 12 wk. A week before the experiment, the professional coaches guided and trained the subjects in movements and postures according to the "Fitness Qigong Baduanjin" (2003) issued by the State Sports General Administration. Doctors have been
The therapist used constipation.

**Assessment of symptoms and curative effect of constipation:** The therapist used the constipation symptom evaluation table developed by the Colorectal Surgery Group of Surgery Branch of Chinese Medical Association to evaluate the constipation symptoms of patients before and after treatment. They mainly scored the patients’ difficulties, excessive excretion, fecal traits, defecation time, falling, endless, distension feeling, defecation frequency, abdominal distension, etc. The score of each item is 0-3. The higher the score, the more serious the constipation symptoms are.

**Quality of life assessment:** Therapists used CRC QLQ-CR38 developed by the European Organization for Research and Treatment of Cancer to assess patients’ quality of life before and after treatment. There are 38 entries in QLQ-CR38, including functional areas and symptom areas. The functional areas of the scale include seven aspects: physical function, future expectation, sexual function, sexual satisfaction and others. Symptom areas include eight aspects: urination problems, gastrointestinal symptoms, adverse reactions of chemotherapy, defecation problems, ostomy-related problems, male and female sexual function and weight problems. The scale has a total of 31 items, each with a score of 1-4. When the score of the functional area is higher, the patient’s function is better. The higher the domain score, the more severe the symptoms of patients.

Under the unified guidance of nurses, patients should fill out the QLQ-CR38 form by themselves. The QLQ-CR38 scale was assessed by physicians familiar with the scale at baseline and 12 wk after the intervention.

**Assessment of gastrointestinal hormone levels:** At baseline and 12 wk after the intervention, 5 mL blood samples were collected from the four groups, centrifuged using a high-speed desktop centrifuge for 5 min (3500 rpm), approximately 2 mL serum was collected, and levels of motilin (MTL) and somatostatin (SS) were measured.

**Efficacy evaluation criteria:** Record the total therapeutic effects of the four groups at 12 wk after the operation, and the real effective rate = (markedly effective + relieved + effective)/total cases × 100%. Markedly effective: the adverse symptoms and signs of the patient completely disappeared; Remission: mild hiccup, abdominal distension and constipation, and more vital bowel sounds; Effective: noticeable hiccup, abdominal distension and constipation, and weak borborygmus; No effect: Severe hiccup, abdominal distension and constipation, and weak or absent bowel sounds.

**Statistical analysis**

Data in this study were processed by SPSS25.0 software, and measurement data were expressed as mean ± SD. One-way ANOVA and two-way test analyzed primary data of patients. Quality of life scores, MTL, and SS levels was by the normal distribution, and repeated measures analysis of variance was used. α = 0.05 was considered the test level, and P < 0.05 indicated a statistically significant difference, and P < 0.01 indicated a statistically significant difference.
RESULTS

General information of four groups of patients
In this experiment, one subject in the control group (Group A) dropped out of the study, two subjects in the cognitive behaviour therapy intervention group (Group B), two subjects in the Baduanjin exercise intervention group (Group C) and three subjects in the combined group (Group D). Finally, the therapist included 29 cases in group A, 28 cases in group B, 28 cases in group C and 27 cases in group D. There was no significant difference in age, body mass index and surgical site between the two groups (P > 0.05, Table 1).

Clinical efficacy of four groups of patients
Twelve weeks after the intervention, the clinical efficacy of group d was better than that of group A, group B, and group C. The effective rates of a group A, group B, group C, and group D were 59%, 75%, 78.57%, and 92.59%, respectively. The difference was statistically significant (P < 0.05, Table 2).

Constipation symptom scores of patients in the four groups before and after treatment
Table 3 shows the constipation symptom scores in the four groups before and after the intervention. There was no significant difference in constipation symptom scores among the four groups at baseline (P > 0.05). After 12 wk of intervention, the constipation symptom scores of subjects in Group A were not significantly different from those at baseline (P > 0.05), and the constipation symptom scores of subjects in Group B, Group C, and Group D were significantly different (P < 0.01). Twelve weeks after the intervention, compared with the issues in group A, constipation symptom scores in groups B, C, and D were significantly different (P < 0.01).

QLQ-CR38 scores of four groups before and after treatment
Table 4 shows the change scores of the QLQ-CR38 scale of four groups of subjects before and after the intervention. At baseline, there was no significant difference among groups of QLQ-CR38 scale in four groups (P > 0.05). Compared with baseline, after 12 wk of intervention, the scores of body image, future expectation, sexual function, sexual satisfaction, adverse reactions of chemotherapy, and defecation problems of the subjects in Group A, Group B, Group C, and Group D all increased. Among them, there was no significant difference in the future expectation of the subjects in Group A (P > 0.05), but a significant difference in body image and sexual satisfaction (P < 0.05), and significant difference in sexual function, adverse reactions of chemotherapy and defecation problems (P < 0.01). There were substantial differences in body image, future expectation, sexual function, sexual satisfaction, adverse reactions to chemotherapy, and defecation problems between group B and group C (P < 0.01). In group D, there was no significant difference in defecation problems (P > 0.05), but a significant difference in adverse reactions of chemotherapy (P < 0.05), and a highly significant difference in body image, future expectation, sexual function, and sexual satisfaction (P < 0.01). The micturition problems, gastrointestinal symptoms, stoma-related problems, male sexual problems, female sexual problems, and body mass scores in Group A, B, C, and D decreased. Among them, there was no significant difference in micturition and gastrointestinal symptoms (P > 0.05), but a substantial difference in stoma-related problems and body mass (P < 0.05), and an extremely significant difference in male and female problems (P < 0.01). There were substantial differences in urination problems, gastrointestinal symptoms, stoma-related problems, male sexual problems, female sexual problems, and body mass among the subjects in Group B, Group C, and Group D (P < 0.01). Twelve weeks after the intervention, compared with the issues in group A, the scores of body image, future expectation, sexual function, and sexual satisfaction in groups B, C, and D all increased. Among them, there was no significant difference in body image and sexual function between group B and group C (P > 0.05), but a significant difference in future expectation and sexual satisfaction (P < 0.01). There were substantial differences in body image, future expectation, sexual function, and sexual satisfaction among subjects in group D (P < 0.01). Adverse reactions to chemotherapy, defecation problems, urination problems, gastrointestinal symptoms, stoma-related problems, male sexual problems, female sexual problems, and body mass scores in Group B, Group C, and Group D decreased. Among them, there were no significant differences in adverse reactions of chemotherapy, male problems, and female problems in group B (P > 0.05), but significant differences in urination problems, gastrointestinal symptoms, and defecation problems (P < 0.05), and highly significant differences in stoma-related problems and body mass (P < 0.01). In group C, there were no significant differences in female issues and adverse reactions of chemotherapy (P > 0.05), but significant differences in urination problems, defecation problems, male problems and body mass (P < 0.05), and highly significant differences in gastrointestinal symptoms and stoma-related problems (P < 0.01). The adverse reactions of chemotherapy, defecation problems, urination problems, gastrointestinal symptoms, stoma-related problems, male sexual problems, female sexual problems, and body mass of the subjects in group D were all significantly different (P < 0.01).
Table 1 General data of four groups of patients (n = 112, mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
<th>Statistic</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases (Male/female)</td>
<td>15/14</td>
<td>16/12</td>
<td>15/13</td>
<td>15/12</td>
<td>0.19</td>
<td>0.98</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.89 ± 6.43</td>
<td>53.93 ± 7.63</td>
<td>52.79 ± 5.85</td>
<td>54.70 ± 6.29</td>
<td>0.53</td>
<td>0.66</td>
</tr>
<tr>
<td>BMI</td>
<td>21.17 ± 1.35</td>
<td>21.49 ± 1.02</td>
<td>21.55 ± 1.14</td>
<td>20.95 ± 1.32</td>
<td>1.49</td>
<td>0.22</td>
</tr>
<tr>
<td>Operation site/case</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.93</td>
<td>0.99</td>
</tr>
<tr>
<td>Left colon</td>
<td>8</td>
<td>8</td>
<td>6</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right colon</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>15</td>
<td>13</td>
<td>14</td>
<td>13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

BMI: Body mass index.

Table 2 Clinical efficacy of four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Show effect</th>
<th>Alleviate</th>
<th>Effective</th>
<th>Be invalid</th>
<th>Total effective rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>29</td>
<td>1</td>
<td>6</td>
<td>8</td>
<td>14</td>
<td>51.72%b</td>
</tr>
<tr>
<td>Group B</td>
<td>28</td>
<td>3</td>
<td>8</td>
<td>8</td>
<td>9</td>
<td>67.86%a</td>
</tr>
<tr>
<td>Group C</td>
<td>28</td>
<td>3</td>
<td>11</td>
<td>6</td>
<td>8</td>
<td>71.43%</td>
</tr>
<tr>
<td>Group D</td>
<td>27</td>
<td>7</td>
<td>14</td>
<td>4</td>
<td>2</td>
<td>92.59%</td>
</tr>
</tbody>
</table>

bP < 0.05, between group D, group A, group B and group C 12 wk after intervention.

Table 3 Symptom scoring scale of constipation (mean ± SD, score)

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>16.52 ± 0.91</td>
<td>16.14 ± 0.99</td>
</tr>
<tr>
<td>Group B</td>
<td>16.36 ± 1.03</td>
<td>12.25 ± 1.93c,d</td>
</tr>
<tr>
<td>Group C</td>
<td>16.39 ± 0.96</td>
<td>12.11 ± 1.93c,d</td>
</tr>
<tr>
<td>Group D</td>
<td>16.22 ± 1.12</td>
<td>9.52 ± 1.72c,d</td>
</tr>
</tbody>
</table>

cP < 0.01, the intra-group comparison of each group at different time points.
dP < 0.01, the comparison of group B, group C and group D with the group A at the same time point.

Gastrointestinal hormone levels of four groups before and after treatment

Table 5 shows the changes of gastrointestinal hormone levels in peripheral blood of four groups of subjects before and after the intervention. There was no significant difference in gastrointestinal hormone levels among the four groups (P > 0.05). Compared with baseline, after 12 wk of intervention, there was no significant difference in MTL level in group A (P > 0.05). Still, there was a substantial difference in MTL level among groups B, C, and D (P < 0.01). There is no significant difference in SS level in group A (P > 0.05), but there is a substantial difference in SS level among group B, group C, and group D (P < 0.01). Twelve weeks after the intervention, compared with group A, the MTL and SS levels in group B, group C, and group D were significantly different (P < 0.01).

Correlation analysis of constipation symptom scores and gastrointestinal hormone levels with various functional areas of quality of life in CRC patients

The therapist made a Pearson correlation analysis based on the symptom score of constipation score of CRC patients, scores of gastrointestinal hormone levels and functional areas of quality of life. The results are shown in Table 6. The results showed that the constipation symptom score of CRC patients was negatively correlated with body image, future expectation, sexual function and sexual satisfaction score, negatively associated with urination problems, gastrointestinal symptoms, adverse reactions of
### Table 4 QLQ-CR38 scores of four groups before and after treatment (mean ± SD, min)

<table>
<thead>
<tr>
<th>Group dimension</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 wk</td>
<td>Baseline</td>
<td>12 wk</td>
</tr>
<tr>
<td><strong>Functional dimension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body image</td>
<td>43.17 ± 16.45</td>
<td>49.45 ± 12.25</td>
<td>41.32 ± 16.77</td>
<td>50.46 ± 13.24</td>
</tr>
<tr>
<td>Future expectation</td>
<td>42.48 ± 13.27</td>
<td>47.48 ± 11.83</td>
<td>42.14 ± 14.42</td>
<td>61.93 ± 15.58</td>
</tr>
<tr>
<td>Sexual satisfaction</td>
<td>38.52 ± 12.42</td>
<td>43.30 ± 13.72</td>
<td>39.07 ± 11.26</td>
<td>52.29 ± 11.92</td>
</tr>
<tr>
<td><strong>Symptom dimension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urination problem</td>
<td>45.28 ± 14.14</td>
<td>42.38 ± 12.05</td>
<td>44.86 ± 13.64</td>
<td>35.29 ± 10.73</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>37.34 ± 13.55</td>
<td>35.45 ± 11.06</td>
<td>38.46 ± 16.65</td>
<td>28.50 ± 12.71</td>
</tr>
<tr>
<td>Adverse reactions of</td>
<td>14.34 ± 4.53</td>
<td>29.28 ± 6.64</td>
<td>15.54 ± 7.09</td>
<td>26.18 ± 7.49</td>
</tr>
<tr>
<td>chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Defecation problem</td>
<td>23.52 ± 9.70</td>
<td>35.38 ± 8.67</td>
<td>24.18 ± 8.64</td>
<td>30.18 ± 7.83</td>
</tr>
<tr>
<td>Issue related to stoma</td>
<td>50.45 ± 11.30</td>
<td>44.52 ± 11.25</td>
<td>50.50 ± 10.90</td>
<td>36.29 ± 9.08</td>
</tr>
<tr>
<td>Male sexual problems</td>
<td>60.83 ± 14.90</td>
<td>51.07 ± 13.44</td>
<td>62.11 ± 11.25</td>
<td>45.71 ± 12.18</td>
</tr>
<tr>
<td>Female sexual problems</td>
<td>26.72 ± 8.69</td>
<td>23.38 ± 5.80</td>
<td>27.43 ± 7.71</td>
<td>21.68 ± 5.83</td>
</tr>
<tr>
<td>Body mass</td>
<td>54.48 ± 14.19</td>
<td>49.41 ± 11.97</td>
<td>55.32 ± 13.65</td>
<td>41.11 ± 9.87</td>
</tr>
</tbody>
</table>

*<i>P < 0.05</i>, the intra-group comparison of each group at different time points.

*<i>P < 0.01</i>, the intra-group comparison of each group at different time points.

*<i>P < 0.05</i>, the comparison of group B, group C and group D with the group A at the same time point.

*<i>P < 0.01</i>, the comparison of group B, group C and group D with the group A at the same time point.

### Table 5 Gastrointestinal hormone levels in peripheral blood of four groups (mean ± SD, pg·mL⁻¹)

<table>
<thead>
<tr>
<th>Group</th>
<th>MTL</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 wk</td>
</tr>
<tr>
<td></td>
<td>12 wk</td>
<td>Baseline</td>
</tr>
<tr>
<td>Group A</td>
<td>146.07 ± 28.42</td>
<td>159.28 ± 25.69</td>
</tr>
<tr>
<td>Group B</td>
<td>151.75 ± 25.54</td>
<td>192.79 ± 22.75</td>
</tr>
<tr>
<td>Group C</td>
<td>147.57 ± 20.43</td>
<td>189.46 ± 25.06</td>
</tr>
<tr>
<td>Group D</td>
<td>144.52 ± 22.41</td>
<td>269.33 ± 24.74</td>
</tr>
</tbody>
</table>

*<i>P < 0.01</i>, the intra-group comparison of each group at different time points.

*<i>P < 0.01</i>, the comparison between group d, group a, group b and group c at the same time point.

MTL: Levels of motilin; SS: Levels of somatostatin.

chemotherapy, defecation problems, stoma-related problems, female problems and bodyweight score, but not related to male issues. The MTL level of CRC patients is positively correlated with body image, future expectation, sexual function and sexual satisfaction score, but negatively associated with urination problems, gastrointestinal symptoms, adverse reactions of chemotherapy, stoma-related problems, male problems, female problems and bodyweight score, and has nothing to do with defecation problems. CRC SS level is positively correlated with urination problems, gastrointestinal symptoms, stoma-related problems, adverse reactions of chemotherapy, male problems, female problems, body weight, and negatively associated with body image, future expectation, sexual function...
Table 6 Correlation between gastrointestinal hormone levels and scores of functional areas of quality of life in colorectal cancer patients (R)

<table>
<thead>
<tr>
<th>Project</th>
<th>Constipation symptom score</th>
<th>MTL</th>
<th>SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body image</td>
<td>-0.376&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.617&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-0.532&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Future expectation</td>
<td>-0.530&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.569&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.446&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sexual function</td>
<td>-0.334&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.290&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.245&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sexual satisfaction</td>
<td>-0.375&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.369&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.250&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urination problem</td>
<td>0.393&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.448&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.334&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>0.436&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.390&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.238&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Adverse reactions of chemotherapy</td>
<td>0.329&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.431&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.305&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Defecation problem</td>
<td>0.265&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.002</td>
<td>0.166</td>
</tr>
<tr>
<td>Issue related to stoma</td>
<td>0.507&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.528&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.410&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Male sexual problems</td>
<td>0.151</td>
<td>-0.643&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.803&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Female sexual problems</td>
<td>0.305&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.244&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.190&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Body mass</td>
<td>0.314&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.284&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.195&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>P < 0.05, between gastrointestinal hormone levels and scores of functional areas of quality of life in colorectal cancer patients.

<sup>b</sup>P < 0.01, between gastrointestinal hormone levels and scores of functional areas of quality of life in colorectal cancer patients.

MTL: Levels of motilin; SS: Levels of somatostatin.

and sexual satisfaction scores, but not related to defecation problems.

DISCUSSION

Constipation symptoms, quality of life, and gastrointestinal hormone level disorder in patients with CRC

Patients with CRC may have symptoms such as abdominal pain, diarrhoea, or constipation, which may cause psychological stress and bring many adverse effects on patients’ quality of life[10]. Guérin et al[11] observed the prevalence of CRC in chronic constipation and non-constipation groups within one year; they found that most CRC in the constipation group was significantly higher than that in the non-constipation group. Moreover, with the increase in the severity of constipation, the incidence of CRC gradually increased. Watanabe et al[12] postulated that constipation increases the risk of CRC. They included 251 patients with CRC in a seven-year follow-up of subjects aged 40–64 years, which ultimately determined that constipation increases CRC risk. Akhondi-Meybodi et al[13] used the QLQ-C30 questionnaire to evaluate different aspects of the life of 120 patients with CRC. He found no significant relationship between the average quality of life score and gender and tumour stage. However, their physical, social, clinical, and economic quality of life remain inferior because CRC has serious adverse effects on people’s financial situation, social function, pain, and physical function, so the quality of life of patients with CRC is low. Faury et al[14] investigated the quality of life and fatigue of patients with CRC. He found that the quality of life and fatigue will be damaged for a long time after the cancer diagnosis and will vary with stoma status. Therefore, we should provide long-term intervention measures to improve CRC survivors’ quality of life and fatigue. Silva et al[15] reported that colostomy enhances the patients’ quality of life for 3–5 mo and improves it 6–8 mo after the operation. By contrast, late-stage radiotherapy and chemotherapy can harm the quality of life. Pate et al[16] evaluated the quality of life of 403 patients with CRC and 401 control people in nine different geographical locations. He found no significant difference in fatigue, society, emotion, function, and physical health between patients with CRC and the control group. Nevertheless, the CRC-specific quality of life index was poor. Leermakers et al[17] assessed the gastrointestinal function of 289 patients with CRC through the Health-Related Quality of Life (HRQoL) questionnaire before operation and at 3, 6, and 12 mo after the procedure. He found that the gastrointestinal function of patients with CRC improved after the process. However, the risk of postoperative gastrointestinal dysfunction in women and young patients is still high. Similarly, some studies[18] systematically evaluated and conducted a meta-analysis of patients with CRC after treatment and found that fatigue, psychological distress, and gastrointestinal symptoms of CRC survivors are the main problems that plague them and persist after cancer treatment. Therefore, specific intervention measures should be adopted to improve the quality of
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Effect of biofeedback therapy combined with Baduanjin on the quality of life of patients with CRC

Duncan et al.[19] summarized many studies related to improving the quality of life of patients with cancer. Although many interventions can improve the quality of life of patients with cancer, the efficacy of a single intervention is not very significant because the patients’ physical and psychosocial problems will vary in different periods of cancer course. The effective intervention measures in one stage may not be suitable for the other. By contrast, the combined intervention has a more significant effect on the whole course of patients with cancer than monotherapy. An increasing number of studies showed that exercise intervention could promote patients’ mental health with CRC[20]. As an aerobic exercise, Duan Jin exercise combined with biofeedback therapy can alleviate CRC symptoms and improve the quality of life of patients with CRC. Patients’ fecal incontinence is significantly related to their quality of life. Liang et al.[21] evaluated the effect of biofeedback therapy on fecal incontinence in patients with CRC through 61 patients and 48 control groups. Their results showed that patients who received biofeedback therapy for more than 15 wk had significantly improved fecal incontinence. The fecal incontinence scores, defecation frequency, and anorectal manometry were also considerably enhanced. Kuo et al.[22] treated 32 patients with electrical stimulation and biofeedback. The clinical effect of rehabilitation treatment was evaluated through the functional results, Wexner score, and anorectal manometry. The patients’ fecal incontinence and quality of life significantly improved. Similarly, Enck et al.[23] divided 109 patients with fecal incontinence into two groups. One group received biofeedback training, while the other control group did not; the efficacy of biofeedback training in improving fecal incontinence was evaluated. Biofeedback training was found to be effective in enhancing adult fecal incontinence. Kim et al.[24] assessed the quality of life, mental health, and physical activity level of 71 patients by performing aerobic exercise at home for 12 wk and finally found that aerobic exercise can improve the quality of life of patients with CRC. Pham et al.[25] searched some databases, selected various kinds of literature with high impact factors and strong credibility for analysis and evaluation, and finally found that appropriate physical activity in healthy people can prevent the occurrence of rectal cancer. Schmid et al.[26] studied CRC survivors and compared the highest and lowest levels of physical activity of CRC survivors before diagnosis and found that physical activity reduces the death risk of CRC survivors. Long or excessively strenuous exercise may have adverse physiological effects on patients. As a moderate-intensity aerobic exercise, Baduanjin significantly improves the quality of life of patients with CRC.

In this study, 38 items of the QLQ-CR38 scale were used to evaluate the efficacy of biofeedback therapy combined with Baduanjin in improving the quality of life of patients with CRC at 12 wk after the intervention. After 12 wk of intervention, male sexual problems, urination problems, gastrointestinal symptoms, stoma-related problems, and body weight of the intervention group were better than those of the control group, which indicated that biofeedback therapy combined with Baduanjin intervention had a good effect on the quality of life of patients with CRC.

Effect of biofeedback therapy combined with Baduanjin on gastrointestinal hormones in patients with CRC

Aerobic exercise can promote the transportation of intestinal gas, improve the clearance rate of intestinal gas, and relieve gastrointestinal symptoms[27]. Song et al.[28] recently analyzed the influence of diet, lifestyle, exercise, and other interventions on the prognosis of patients with CRC. He found that exercise can improve immune and metabolic homeostasis and enhance gastrointestinal function by changing intestinal microflora. Bilski et al.[29] provided a comprehensive overview of the beneficial and harmful effects of physical activity on the gastrointestinal tract in recent years. At the same time, they explored the relationship between different forms and intensities of exercise and intestinal physiological function and pathology. Studies have found that regular and moderate exercise has a beneficial effect on some gastrointestinal diseases, improving the gastrointestinal process and alleviating the symptoms. The method, duration and intensity of training can directly affect its curative effect. As a medium-intensity aerobic exercise, Baduanjin can significantly improve gastrointestinal function.

Bartlett et al.[30] evaluated the intestinal function and quality of life of 19 patients with intestinal dysfunction caused by CRC surgery by biofeedback therapy. They found that the biofeedback scheme can significantly improve the quality of life score and reduce the severity of symptoms, defecation frequency, and incontinence in patients with CRC. Kim et al.[31] studied 70 patients with CRC who received biofeedback therapy and reviewed all the data retrospectively. Finally, they found that biofeedback therapy may impact the relief of various gastrointestinal symptoms, especially on fecal incontinence. Kye et al.[32] conducted a 6-month experiment on 56 patients with rectal excision. Biofeedback therapy did not prevent anorectal dysfunction during temporary stoma intervals after reversing temporary stoma six months after rectal excision. However, biofeedback therapy is helpful to maintain the resting tension of the anal sphincter and has a particular influence on gastrointestinal hormones in patients with CRC.

In this study, MTL and somatostatin SS levels were detected to assess the efficacy of biofeedback therapy combined with Baduanjin in improving gastrointestinal hormone levels of patients with CRC at
12 wk after the intervention. After 12 wk of intervention, the MTL level of each group increased, and the SS level decreased. The curative effect of the combined intervention group was more prominent than that of the control, which indicated that biofeedback therapy combined with Baduanjin intervention played an essential role in gastrointestinal function and gastrointestinal hormone level of patients with CRC.

CONCLUSION
The limitation of this study is that it is a small sample and single-centre study. Based on the results of this study, we believe that biofeedback therapy and Baduanjin training can improve the quality of life and gastrointestinal hormone levels in patients with CRC, and the combined effect is superior to monotherapy. As an economical and effective means of clinical and family intervention, it is worthy of promotion and application in clinical and community-based family rehabilitation.

ARTICLE HIGHLIGHTS

Research background
With the change in people’s lifestyles, the incidence of colorectal cancer (CRC) is increasing. It is essential to study the efficacy of various treatment methods for CRC patients to prevent and treat CRC.

Research motivation
Exercise is becoming more and more critical in the treatment of various diseases. Baduanjin is a joint fitness exercise. It is of great significance to explore the therapeutic effect of Baduanjin combined with biofeedback therapy on CRC patients in the future.

Research objectives
This study aimed to investigate the efficacy of biofeedback therapy combined with Baduanjin in improving the quality of life and gastrointestinal hormone levels of patients with CRC.

Research methods
In this study, clinical randomized controlled trials were used to select experimental samples and SPSS 25 was used for data analysis. These research methods can make the experimental results more objective.

Research results
After a period of biofeedback therapy combined with Baduanjin intervention, the quality of life, gastrointestinal hormone levels and clinical efficacy of patients were significantly improved and better than the control group.

Research conclusions
On the basis of routine nursing care, patients with CRC combined with biofeedback therapy and Baduanjin exercise can improve the quality of life of patients with CRC and the efficacy of gastrointestinal hormone levels.

Research perspectives
In future studies, we should pay more attention to the effects of different forms of exercise on CRC. At the same time, more studies should be conducted on the therapeutic effects of the combination of several methods on CRC patients.

FOOTNOTES

Author contributions: Zhou XD performed the experiments and wrote the manuscript; Wei HG helped perform the data analysis with constructive discussions; Ai FL contributed significantly to data analysis and manuscript preparation.

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META-ANALYSIS

Does chronic kidney disease affect the complications and prognosis of patients after primary colorectal cancer surgery?

Xiao-Yu Liu, Bin Zhang, Yu-Xi Cheng, Wei Tao, Chao Yuan, Zheng-Qiang Wei, Dong Peng

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Abstract

BACKGROUND
The effect of chronic kidney disease (CKD) on the outcomes of colorectal cancer (CRC) patients after primary CRC surgery is controversial.

AIM
To analyze whether CKD had specific effect on the outcomes after CRC surgery.

METHODS
We searched the PubMed, Embase, Cochrane Library databases and CNKI, from inception to March 14, 2022. Newcastle-Ottawa Scale was used for the quality assessment in this meta-analysis, and we used RevMan 5.3 was used for data analysis.

RESULTS
A total of nine studies including 47771 patients were eligible for this meta-analysis. No significant difference was found in terms of overall postoperative complications [odds ratio (OR) = 1.78, 95%CI: 0.64-4.94, P = 0.27]. We analyzed the specific complications and found that the CKD group had higher rates of pulmonary infection (OR = 2.70, 95%CI: 1.82-4.00, P < 0.01), cardiovascular complications (OR = 3.39, 95%CI: 2.34-4.91, P < 0.01) and short-term death (OR = 3.01, 95%CI: 2.20-4.11, P < 0.01). After pooling the hazard ratio (HR), the CKD group had worse overall survival (OS) (HR = 1.51, 95%CI: 1.04-2.20, P = 0.03). We performed subgroup analyses of the dialysis and non-dialysis groups, and no significant difference was found in the non-dialysis group (HR = 1.20, 95%CI: 0.98-1.47, P = 0.08). The dialysis group had worse OS (HR = 3.36, 95%CI: 1.92-5.50, P < 0.01) than the non-dialysis group. The CKD group had worse disease-free survival (DFS) (HR = 1.41, 95%CI: 1.12-1.78, P < 0.01), and in the subgroup analysis of the dialysis and non-dialysis groups, no significant difference was found in the non-dialysis group (HR = 1.27, 95%CI: 0.97-1.66, P = 0.08). The dialysis group had worse OS (HR = 1.95, 95%CI: 1.23-3.10, P < 0.01) than the non-
CONCLUSION

Preexisting CKD was associated with higher rates of pulmonary infection, higher rates of short-term death, and worse OS and poorer DFS following CRC surgery.

Key Words: Chronic kidney disease; Colorectal cancer; Outcome; Prognosis; Meta-analysis

Core Tip: Previous studies have shown that patients with chronic kidney disease might have an increased risk of colorectal cancer, however, the impact of chronic kidney disease on complications and prognosis after colorectal cancer surgery is controversial. Furthermore, the prognosis remained unclear as well. Therefore, this study aimed to analyze whether chronic kidney disease had specific effect on the outcomes after colorectal cancer surgery. In conclusion, preexisting chronic kidney disease was associated with higher rates of pulmonary infection, higher rates of short-term death, poorer overall survival rates, and poorer disease-free survival rates following colorectal cancer surgery.

INTRODUCTION

Colorectal cancer (CRC) is the third most common malignant tumor and ranks the second leading cause of cancer-related deaths around the world. Nearly 1.8 million new CRC patients and 0.7 million cancer-related deaths occur each year[1]. Radical resection is the only curative treatment for early-stage CRC[2, 3], and comprehensive therapy (such as immune therapy, radiotherapy, chemotherapy and surgery) is recommended for advanced-stage CRC[4-8].

It is estimated that approximately 500 million adults worldwide are diagnosed with chronic kidney disease (CKD), but the prevalence varies greatly among different countries[9,10]. CKD can increase mortality and morbidity, and it involves an economic burden as well[11,12]. Some key pathophysiological causes of CKD may contribute to increased postoperative morbidity (including excessive arterial calcification, endothelial dysfunction and increased levels of inflammatory factors)[13].

As previous studies reported, patients with CKD might increase the risk of CRC[14]; however, the effect of CKD on complications and prognosis after CRC surgery remains controversial. Some studies held the view that CKD had no effect on surgical complications[15,16]. Moreover, other studies believed that CKD increased postoperative complications[17,18]. Furthermore, the prognosis remained unclear as well. Therefore, our study aimed to analyze whether CKD had an effect on the complications and prognosis of CRC patients who underwent primary CRC surgery.

MATERIALS AND METHODS

This meta-analysis conformed to the Preferred Reporting method for Systematic Reviews and Meta-Analyses (PRISMA) statement[19]. The register number of this meta-analysis was CRD42021266160.

Literature search strategy

We searched the PubMed, Embase, Cochrane Library databases and CNKI from inception to March 14, 2022. There were two key items in this search strategy: chronic kidney disease and colorectal cancer. To expand the search scope, in terms of chronic kidney disease, we used "kidney" OR "dialysis" OR "hemodialysis" OR "estimated glomerular filtration rate". Colorectal cancer was searched as follows: "rectal cancer" OR "colorectal cancer" OR "colon cancer" OR "rectal neoplasm" OR "colorectal neoplasm" OR "colon neoplasm" OR "rectal tumor" OR "colorectal tumor" OR "colon tumor". Then, two key items were combined with "AND". We restricted the search language to Chinese and English, and we limited the searching scope to titles and abstracts. In addition, we use Reference Citation Analysis (https://www.referencecitationanalysis.com) to retrieve relevant literature.
Inclusion and exclusion criteria
The inclusion criteria were as follows: (1) Studies that included patients undergoing colorectal surgery; (2) Studies that compared the CKD group and the non-CKD group of CRC patients; and (3) Study outcomes included the complications or prognosis. The exclusion criteria were as follows: (1) Studies with insufficient data on complications or prognosis; and (2) Case reports, comments, letter to editor, conferences and reviews. The procedures of inclusion and exclusion criteria were carried out by two reviewers separately, and if there was a disagreement, it was settled by discussion with another reviewer.

Study selection
Two reviewers searched the databases separately. The titles and abstracts were screened after removing duplicates, after which full texts were evaluated for eligibility. Disagreement was settled by another reviewer.

Data extraction
Two reviewers extracted and cross-checked the data. The data which were extracted were: (1) Publication year, first author, country, sample size, study design, Newcastle-Ottawa Scale (NOS) score and the definition of the CKD group and the non-CKD group; (2) The baseline information included age, sex, comorbidities, American Society of Anesthesiologists (ASA) score and tumor stage; (3) The surgery-related information included surgery type and surgery method; (4) Postoperative complications included anastomotic leakage (divided into three groups: grade A, grad B and grade C. Grade A needed no active intervention, grade B needed active intervention and grade C needed reoperation)[20], pulmonary complications, intestinal obstruction, surgical site infection, postoperative bleeding and short-term death; and (5) Overall survival (OS) and disease-free survival (DFS).

Outcomes
The primary outcome referred to the short-term outcome, which was equal to postoperative complications (including anastomotic leakage, intestinal obstruction, surgical site infection, postoperative bleeding, pulmonary complications and short-term death during the hospital stay). The second outcome was the long-term prognosis of OS and DFS.

Quality assessment
The assessment of the included studies was according to the Newcastle-Ottawa Scale (NOS)[21]. Nine points represented high-quality studies, seven to eight points represented medium-quality studies and scores less than seven points represented low-quality studies.

Statistical analysis
In the current meta-analysis, the pooled prognosis (OS and DFS) used hazard ratios (HRs) and 95% CIs, and the HRs were extracted from COX analyses. If no available data reported the HRs from COX analyses, then we extracted the HRs from Kaplan-Meier survival curves[22]. The mean ± SD was used for continuous variables, and proportions were used for categorical variables. Mean differences (MDs)/odds ratios (ORs) plus 95% CIs were calculated for continuous and dichotomous variables. Statistical heterogeneity was analyzed using the I² and the chi-square test[23,24]. When I² > 50%, we used the random effects model, in this model, P < 0.1 was considered to be statistically significant. We used the fixed effects model, in this model, when I² ≤ 50%, and P < 0.05 was considered to be statistically significant. All the statistical analysis was performed using RevMan 5.3 (The Cochrane Collaboration, London, United Kingdom).

RESULTS

Study selection
We identified 903 studies (266 studies were obtained from PubMed, 509 studies were obtained from Embase, 103 studies were obtained from the Cochrane Library and 25 studies were obtained from CNKI). A total of 903 studies were included after removed duplicate, 671 studies were left for initial evaluation. After titles and abstracts were screened, 9 studies were left for full-text screening. Finally, nine studies[15-18,25-29] were included (Figure 1).

Patient characteristics and quality assessment
A total of nine studies[15-18,25-29] including 47771 patients, were enrolled in the current meta-analysis. As for publishing countries, five of nine studies were conducted in Japan, and the other studies were in the United States, the United Kingdom, Canada and China. The publication years were from 2012 to 2022, eight studies were retrospective studies, only one study was a prospective study. The study dates were from 2001 to 2020. The NOS score and definitions of the CKD group and the non-CKD group are...
Table 1 Characteristics of the studies included in the meta-analysis

<table>
<thead>
<tr>
<th>Ref.</th>
<th>Country</th>
<th>Study design</th>
<th>Study date</th>
<th>Sample size</th>
<th>Definition of CKD and non-CKD</th>
<th>NOS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CKD group</td>
<td>Non-CKD group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>CKD group</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Non-CKD group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Currie et al[15], 2014</td>
<td>United Kingdom</td>
<td>Prospective</td>
<td>2006-2011</td>
<td>126</td>
<td>582</td>
<td>708</td>
</tr>
<tr>
<td>Nozawa et al[17], 2012</td>
<td>Japan</td>
<td>Retrospective</td>
<td>2001-2010</td>
<td>245</td>
<td>882</td>
<td>1127</td>
</tr>
<tr>
<td>Higashino et al[16], 2020</td>
<td>Japan</td>
<td>Retrospective</td>
<td>2008-2015</td>
<td>14</td>
<td>567</td>
<td>581</td>
</tr>
<tr>
<td>Hu et al[18], 2015</td>
<td>United States</td>
<td>Retrospective</td>
<td>2009-2013</td>
<td>265</td>
<td>42138</td>
<td>42401</td>
</tr>
<tr>
<td>Chen et al[26], 2016</td>
<td>China</td>
<td>Retrospective</td>
<td>2012-2015</td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>Obara et al[25], 2021</td>
<td>Japan</td>
<td>Retrospective</td>
<td>2007-2016</td>
<td>24</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Obara et al[27], 2022</td>
<td>Japan</td>
<td>Retrospective</td>
<td>2011-2015</td>
<td>59</td>
<td>204</td>
<td>263</td>
</tr>
<tr>
<td>Shiraishi et al[28], 2022</td>
<td>Japan</td>
<td>Retrospective</td>
<td>2016-2020</td>
<td>78</td>
<td>1294</td>
<td>1372</td>
</tr>
<tr>
<td>Dudani et al[29], 2021</td>
<td>Canada</td>
<td>Retrospective</td>
<td>2005-2013</td>
<td>136</td>
<td>1118</td>
<td>1254</td>
</tr>
</tbody>
</table>

CKD: Chronic kidney disease; eGFR: Estimated glomerular filtration rates (mL./min/1.73 m²); NOS: Newcastle-Ottawa Scale.

Figure 1 Flowchart of study selection.

shown in Table 1. Six studies reported that the modification of diet in renal disease equation was used to estimate the estimated glomerular filtration rates (eGFR)[16,18,25,27-29]; however, the method used in the other studies was unclear[15,17,26] (see Table 1).

Baseline information

Sex, age, ASA score, T staging and N staging were included for baseline information analysis. The CKD group had an older age (OR = 8.67, 95%CI: 5.73-11.61, P < 0.01) and a higher proportion of ASA 3-4 grade (OR = 10.27, 95%CI: 2.98-35.35, P < 0.01) after pooling the baseline information. As for other baseline information, no significant difference was found between the two groups (P > 0.05) (Table 2).
Table 2 Summary of characteristics between chronic kidney disease group and Non-chronic kidney disease group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Studies</th>
<th>Participants (CKD/non-CKD)</th>
<th>Mean difference/odds ratio (95%CI)</th>
<th>Heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline information</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>952/46819</td>
<td>0.92 (0.63, 1.35); ( P = 0.67 )</td>
<td>( I^2 = 82%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Age, yr</td>
<td>3</td>
<td>190/796</td>
<td>8.67 (5.73, 11.61); ( P &lt; 0.01 )</td>
<td>( I^2 = 60%; P = 0.08 )</td>
</tr>
<tr>
<td>ASA1-ASA2</td>
<td>3</td>
<td>218/2443</td>
<td>0.10 (0.03, 0.34); ( P &lt; 0.01 )</td>
<td>( I^2 = 88%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>ASA3-ASA4</td>
<td>3</td>
<td>218/2443</td>
<td>10.27 (2.98, 35.35); ( P &lt; 0.01 )</td>
<td>( I^2 = 88%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>T0-T2</td>
<td>4</td>
<td>508/2962</td>
<td>0.71 (0.46, 1.08); ( P = 0.11 )</td>
<td>( I^2 = 55%; P = 0.08 )</td>
</tr>
<tr>
<td>T3-T4</td>
<td>4</td>
<td>508/2962</td>
<td>1.41 (0.92, 2.16); ( P = 0.11 )</td>
<td>( I^2 = 55%; P = 0.08 )</td>
</tr>
<tr>
<td>N0</td>
<td>4</td>
<td>508/2962</td>
<td>1.09 (0.89, 1.33); ( P = 0.42 )</td>
<td>( I^2 = 0%; P = 0.40 )</td>
</tr>
<tr>
<td>N1-N3</td>
<td>4</td>
<td>508/2962</td>
<td>0.92 (0.75, 1.33); ( P = 0.42 )</td>
<td>( I^2 = 0%; P = 0.40 )</td>
</tr>
<tr>
<td>Surgery-related information</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td>4</td>
<td>409/2055</td>
<td>2.10 (0.91, 4.85); ( P = 0.08 )</td>
<td>( I^2 = 80%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Rectal cancer</td>
<td>4</td>
<td>409/2055</td>
<td>0.48 (0.21, 1.10); ( P = 0.08 )</td>
<td>( I^2 = 80%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Laparoscopy surgery</td>
<td>6</td>
<td>566/44809</td>
<td>0.88 (0.54, 1.42); ( P = 0.59 )</td>
<td>( I^2 = 72%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Open surgery</td>
<td>6</td>
<td>566/44809</td>
<td>1.14 (0.70, 1.86); ( P = 0.59 )</td>
<td>( I^2 = 72%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Emergency operation</td>
<td>2</td>
<td>324/42342</td>
<td>1.31 (0.84, 2.05); ( P = 0.23 )</td>
<td>( I^2 = 0%; P = 0.49 )</td>
</tr>
<tr>
<td>Operative time</td>
<td>3</td>
<td>309/1096</td>
<td>-3.58 (-18.95, 11.79); ( P = 0.65 )</td>
<td>( I^2 = 60%; P = 0.08 )</td>
</tr>
<tr>
<td>Intraoperative blood loss</td>
<td>3</td>
<td>309/1096</td>
<td>1.40 (-30.34, 33.15); ( P = 0.93 )</td>
<td>( I^2 = 0%; P = 0.96 )</td>
</tr>
<tr>
<td>Postoperative complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall complication</td>
<td>7</td>
<td>738/44407</td>
<td>1.78 (0.64, 4.94); ( P = 0.27 )</td>
<td>( I^2 = 95%; P &lt; 0.01 )</td>
</tr>
<tr>
<td>Specific complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>4</td>
<td>641/43612</td>
<td>3.39 (2.34, 4.91); ( P &lt; 0.01 )</td>
<td>( I^2 = 36%; P = 0.20 )</td>
</tr>
<tr>
<td>Anastomotic leakage</td>
<td>4</td>
<td>508/2962</td>
<td>1.44 (0.89, 2.32); ( P = 0.14 )</td>
<td>( I^2 = 35%; P = 0.21 )</td>
</tr>
<tr>
<td>Pulmonary infection</td>
<td>5</td>
<td>607/44891</td>
<td>2.70 (1.82, 4.00); ( P &lt; 0.01 )</td>
<td>( I^2 = 0%; P = 0.84 )</td>
</tr>
<tr>
<td>Intestinal obstruction</td>
<td>5</td>
<td>420/2971</td>
<td>0.89 (0.58, 1.37); ( P = 0.60 )</td>
<td>( I^2 = 6%; P = 0.37 )</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>6</td>
<td>685/45109</td>
<td>1.29 (1.00, 1.65); ( P = 0.05 )</td>
<td>( I^2 = 0%; P = 0.86 )</td>
</tr>
<tr>
<td>Other site infection</td>
<td>3</td>
<td>515/43030</td>
<td>1.07 (0.64, 1.80); ( P = 0.79 )</td>
<td>( I^2 = 0%; P = 0.60 )</td>
</tr>
<tr>
<td>Postoperative bleeding</td>
<td>3</td>
<td>347/2200</td>
<td>0.63 (0.17, 2.36); ( P = 0.49 )</td>
<td>( I^2 = 0%; P = 0.50 )</td>
</tr>
<tr>
<td>Short-term death</td>
<td>4</td>
<td>650/44169</td>
<td>3.01 (2.20, 4.11); ( P &lt; 0.01 )</td>
<td>( I^2 = 0%; P = 0.50 )</td>
</tr>
</tbody>
</table>

CKD: Chronic kidney disease; ASA: American Society of Anesthesiologists; T: Tumor; N: Node.

**Surgery-related information**

We compared the surgery-related information between the two groups, and it was found no significant difference in terms of laparoscopic surgery, open surgery, emergency operation, intraoperative blood loss or operation time (\( P > 0.05 \)). But, the CKD group had higher proportion of patients who underwent colon cancer surgery (\( OR = 2.10, 95\% CI: 0.91-4.85, P = 0.08 \)) (Table 2).

**Postoperative complications**

There was no significant difference in terms of overall postoperative complications (\( OR = 1.78, 95\% CI: 0.64-4.94, P = 0.27 \)). We performed subgroup analyses of the dialysis and non-dialysis groups and no significant difference was found between the non-dialysis group (\( OR = 1.21, 95\% CI: 0.97-1.50, P = 0.09 \) and the dialysis group (\( OR = 2.67, 95\% CI: 0.29-24.23, P = 0.38 \)) (Figure 2).

We conducted the analysis of specific complications, and we found that the CKD group had higher rates of pulmonary infections (\( OR = 2.70, 95\% CI: 1.82-4.00, P < 0.01 \)), cardiovascular complications (\( OR = 3.39, 95\% CI: 2.34-4.91, P < 0.01 \)) and short-term death (\( OR = 3.01, 95\% CI: 2.20-4.11, P < 0.01 \)) (Table 2).
A Overall complications

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>CKD Events Total</th>
<th>Non-CKD Events Total</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen D 2016</td>
<td>1 5</td>
<td>2 10</td>
<td>8.0%</td>
<td>1.00 [0.07, 14.64]</td>
</tr>
<tr>
<td>Currie A 2014</td>
<td>46 128</td>
<td>236 582</td>
<td>17.1%</td>
<td>0.84 [0.57, 1.26]</td>
</tr>
<tr>
<td>Higashino N 2020</td>
<td>2 14</td>
<td>142 567</td>
<td>12.7%</td>
<td>0.50 [0.11, 2.26]</td>
</tr>
<tr>
<td>Hu WH 2015</td>
<td>238 265</td>
<td>16855 42138</td>
<td>17.1%</td>
<td>13.22 [8.88, 19.70]</td>
</tr>
<tr>
<td>Nozawa H 2012</td>
<td>110 245</td>
<td>332 882</td>
<td>17.3%</td>
<td>1.35 [1.01, 1.80]</td>
</tr>
<tr>
<td>Obara N 2021</td>
<td>4 24</td>
<td>2 24</td>
<td>11.4%</td>
<td>2.20 [0.36, 13.34]</td>
</tr>
<tr>
<td>Obara S 2022</td>
<td>17 59</td>
<td>35 204</td>
<td>16.4%</td>
<td>1.95 [1.00, 3.82]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>738</strong></td>
<td><strong>44407</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>1.78 [0.64, 4.94]</strong></td>
</tr>
</tbody>
</table>

B Subgroup analysis of Non-dialysis

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Dialysis Events Total</th>
<th>Non-dialysis Events Total</th>
<th>Weight</th>
<th>Odds Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Currie A 2014</td>
<td>46 126</td>
<td>236 582</td>
<td>36.8%</td>
<td>0.84 [0.57, 1.26]</td>
</tr>
<tr>
<td>Nozawa H 2012</td>
<td>110 245</td>
<td>332 882</td>
<td>54.8%</td>
<td>1.35 [1.01, 1.80]</td>
</tr>
<tr>
<td>Obara S 2022</td>
<td>17 59</td>
<td>35 204</td>
<td>7.7%</td>
<td>1.95 [1.00, 3.82]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>435</strong></td>
<td><strong>1678</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>1.21 [0.97, 1.50]</strong></td>
</tr>
</tbody>
</table>

C Subgroup analysis of dialysis

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Dialysis Events Total</th>
<th>Non-dialysis Events Total</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Higashino N 2020</td>
<td>2 14</td>
<td>142 567</td>
<td>32.2%</td>
<td>0.50 [0.11, 2.26]</td>
</tr>
<tr>
<td>Hu WH 2015</td>
<td>238 265</td>
<td>16855 42138</td>
<td>37.5%</td>
<td>13.22 [8.88, 19.70]</td>
</tr>
<tr>
<td>Obara N 2021</td>
<td>4 24</td>
<td>2 24</td>
<td>30.3%</td>
<td>2.20 [0.36, 13.34]</td>
</tr>
<tr>
<td><strong>Total (95% CI)</strong></td>
<td><strong>303</strong></td>
<td><strong>42279</strong></td>
<td><strong>100.0%</strong></td>
<td><strong>2.67 [0.29, 24.43]</strong></td>
</tr>
</tbody>
</table>

**OS and DFS**

The CKD group had worse OS (HR = 1.51, 95% CI: 1.04-2.20, \( P = 0.03 \)) after pooling up the HRs. We performed subgroup analyses of the dialysis and non-dialysis groups and no significant difference was found in the non-dialysis group (HR = 1.20, 95% CI: 0.98-1.47, \( P = 0.08 \)). The dialysis group had worse OS (HR = 3.36, 95% CI: 1.92-5.90, \( P < 0.01 \)) than the non-dialysis group (Figure 3).

The CKD group had worse DFS (HR = 1.41, 95% CI: 1.12-1.78, \( P < 0.01 \)). We performed subgroup analyses of the dialysis and non-dialysis groups, and no significant difference was found in the non-dialysis group (HR = 1.27, 95% CI: 0.97-1.66, \( P = 0.08 \)). The dialysis group had worse OS (HR = 1.95, 95% CI: 1.23-3.10, \( P < 0.01 \)) than the non-dialysis group (Figure 4).

**Sensitivity analysis**

We performed repeated meta-analysis by omitting each study at a time, in our meta-analysis, the exclusion of any one of the included studies did not alter the results.

**DISCUSSION**

A total of nine studies were included in this meta-analysis. There was no significant difference in terms of overall postoperative complications after pooling all of the data. As for prognosis, the CKD group had worse OS and DFS. We performed subgroup analysis of the dialysis and non-dialysis groups. Dialysis was associated with a worse OS and DFS as well.

The classification of renal function is mainly based on the eGFR[30] and different renal function classifications might have an impact on surgery outcomes. As previous studies have reported, CKD could increase postoperative complications after gastric cancer surgery and hepatocellular carcinoma surgery, and the complications included anastomotic leakage and short-term postoperative death[31-
In addition, patients with concurrent CKD and cancer have a poor prognosis[32]. In terms of CRC, although an impact of renal function on postoperative complications and prognosis has been reported, it remains controversial.

In this study, there was no significant difference in terms of overall postoperative complications, and we chose the random effect model to conduct the analysis because of the high heterogeneity among studies. We conducted the analysis of specific complications, and we found that the CKD group had higher rates of pulmonary infection, cardiovascular complications and short-term death. The possible reason was that patients undergoing colorectal surgery might experience fluid overload due to nutritional support during non-oral periods. Therefore, these events might increase the risk of lung-related complications[18]. Cardiovascular complications might be associated with endothelial dysfunction in CKD patients[16,34]. Furthermore, patients with CKD are in a relatively immunosuppressive state due to nutritional deficiencies, the lymphocyte suppression and the loss of serum immune system components, which might result in an increase in infectious diseases and short-term deaths after surgery[35].

The CKD group had worse OS rates DFS rates. The probable reason was that CKD was associated with endothelial dysfunction, malnutrition, volume overload or changes in calcium and phosphorus metabolism, and the dysfunction would cause higher rates of cardiovascular events[13,36]. In addition, an association between postoperative complications and cancer-related poor prognosis has been reported in esophageal, gastric and colorectal cancer; therefore, higher rates of postoperative complications might result in a poor prognosis[37,28]. Moreover, postoperative complications and perioperative blood loss can suppress immune function, which might be a factor for promoting cancer recurrence[39,40]. We performed subgroup analyses of the dialysis and non-dialysis groups. Dialysis was associated with worse OS and DFS. Therefore, the distinction in OS and DFS between the two groups might be mainly determined by dialysis.

Some parameters that were insufficient for meta-analysis included cancer-related death, blood transfusion, reoperation rate, adjuvant chemotherapy and the R0 resection rate. However, attention should be given to these parameters. In addition to OS and DFS, cancer-specific survival (CSS) could reflect tumor-related deaths. Obara et al[25] reported that CSS was a negative parameter between the

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Figure 3 Overall survival. A: Overall survival between the chronic kidney disease (CKD) group and the non-CKD group; B: Subgroup analysis of the non-dialysis groups; C: Subgroup analysis of the dialysis groups.

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33. In addition, patients with concurrent CKD and cancer have a poor prognosis[32]. In terms of CRC, although an impact of renal function on postoperative complications and prognosis has been reported, it remains controversial.

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two groups, and the major long-term cause of death in CKD patients might be cardiovascular death. Blood transfusion, a factor that influences the prognosis, might have been more common in the CKD group. Therefore, for surgeons, careful control during the operation and perioperative management of CKD are necessary to reduce blood transfusion.

Some limitations were existing. First, only nine studies were included, and the sample size was relatively small. Second, the cutoff for CKD differed among the included study. Five studies reported that the cutoff eGFR was 60 mL/min/1.73 m², one study reported that the cutoff value was 55 mL/min/1.73 m² and the remaining three studies used hemodialysis as the cutoff. Obvious heterogeneity was seen after pooling the data in terms of complications, OS and DFS, therefore, we performed a subgroup analysis, and heterogeneity was reduced. Third, we extracted all of the data that could be analyzed in this study, however, some information was lacking, such as postoperative hospital stay, cancer-related death, blood transfusion, reoperation rate, adjuvant chemotherapy, R0 resection rate, perioperative chemo-radiotherapy and completion of the schedule of chemo-radiotherapy. Fourth, some of the survival data were extracted from the Kaplan-Meier survival curves, which might result in inaccuracies. Finally, among the specific postoperative complications, it was found that pulmonary infection, cardiovascular complications and short-term death were significantly different between the groups; however, this conclusion was based on only four studies, including two CKD studies and two dialysis studies, which would result in heterogeneity. Therefore, multicenter, high-quality and well-controlled prospective studies including comprehensive baseline information comparing the complications, OS, DFS and CSS should be conducted.

CONCLUSION

In conclusion, preexisting CKD was associated with higher rates of pulmonary infection, higher rates of short-term death, and worse OS and DFS following CRC surgery.
ARTICLE HIGHLIGHTS

Research background
Colorectal cancer (CRC) is the third most common malignant tumor and the second leading cause of cancer deaths worldwide. Several key pathophysiological causes of chronic kidney disease (CKD) may lead to increased postoperative morbidity, including excessive arterial calcification, endothelial dysfunction and increased levels of inflammatory factors. Previous studies have shown that patients with CKD might have an increased risk of CRC; however, the impact of CKD on complications and prognosis after CRC surgery is controversial.

Research motivation
The aim of this study was to conduct meta-analysis of current studies and to analyze whether CKD had specific effect on the outcomes after CRC surgery.

Research objectives
The aim of this study is to provide some recommendations for clinical work by investigating the impact of CKD on postoperative complications and prognosis in colorectal cancer.

Research methods
We searched the PubMed, Embase, Cochrane Library databases and CNKI, from inception to March 14, 2022. Newcastle-Ottawa Scale was used for the quality assessment in this meta-analysis, and we used RevMan 5.3 was used for data analysis.

Research results
A total of nine studies including 47771 patients were included in this meta-analysis. No significant difference was found in terms of overall postoperative complications. We analyzed the specific complications and found that the CKD group had higher rates of pulmonary infection, cardiovascular complications and short-term death. After pooling the hazard ratios, the CKD group had worse overall survival (OS).

Research conclusions
Preexisting CKD was associated with higher rates of pulmonary infection, higher rates of short-term death, and worse OS and poorer disease-free survival (DFS) following CRC surgery.

Research perspectives
Based on the results and limitations of this research, multicenter, high-quality and well-controlled prospective studies including comprehensive baseline information comparing the complications, OS, DFS and CSS should be performed in the future.

ACKNOWLEDGEMENTS
We thank the Department of Gastrointestinal Surgery, of The First Affiliated Hospital of Chongqing Medical University for their contributions.

FOOTNOTES

Author contributions: Liu XY and Bin Zhang are co-first authors; Liu XY and Zhang B contributed to data extraction; Peng D contributed to quality assessments and writing-origin draft; Peng D and Liu XY contributed to data analysis; Peng D, Liu XY, Zhang B, Cheng YX, Tao W, Yuan C, and Wei ZQ contributed to writing-review and editing; The final manuscript was read and approved by all of our authors.

Conflict-of-interest statement: The authors declare that they have no competing interests.

PRISMA 2009 Checklist statement: The authors have read the PRISMA 2009 Checklist, and the manuscript was prepared and revised according to the PRISMA 2009 Checklist.

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Abstract

Hepatocellular carcinoma (HCC) is one of the deadliest and most common malignancies of the liver. Considering the rich immune background of carcinogenesis in HCC, efforts have been focused on further understanding the role of the immune system in tumor suppression and promotion. The utilization of immunotherapy in HCC has led to encouraging results that has translated to longer survival and better quality of life among patients. The development of novel HCC-tailored regimens such as vaccine therapy and adoptive cellular therapy coupled with a deeper understanding of biomarkers predictive of the response to immunotherapy will lead to better treatment outcomes.

Key Words: Hepatocellular carcinoma; Immunotherapy; Biomarkers; Cancer vaccines; Adoptive cellular therapy

Core Tip: Immunotherapy has changed the treatment landscape for solid cancers. In advanced hepatocellular carcinoma (HCC), immune checkpoint inhibitors have become the standard of care due to their efficacy and safety outcomes. However, primary and acquired resistance is a major issue in the treatment paradigm, and more research is still needed to understand and identify potential predictors of the response in HCC. Other immunotherapy modalities, such as vaccine therapy and adoptive cellular therapy, could play a prominent role in certain HCC subcohorts and are currently being investigated in clinical trial settings.
TO THE EDITOR

We read with great interest the review by Mattos et al[1] on the immune landscape of hepatocellular carcinoma (HCC), which covered the immune aspects and markers of HCC as well as the immunotherapeutic modalities used in this malignancy. Considering the immunogenicity of HCC, it comes as no surprise that clinical and basic research has been directed to dive deeper into the immune-biological and therapeutic upside of HCC, especially with the rise of immunotherapy in oncology.

While the authors thoroughly discussed the therapeutic use of immune checkpoint inhibitors (ICIs), such as anti-programmed cell death protein 1 and its ligand (nivolumab, pembrolizumab, and atezolizumab) and anti-cytotoxic T-lymphocyte-associated protein 4 (ipilimumab), we would like to highlight the role of other promising immunotherapeutic modalities in HCC. The first being tumor-associated antigen vaccines, including the oncofetal antigen glypican-3 (GPC3) vaccine, which was investigated in adjuvant settings in HCC patients in a phase 2 trial and resulted in a median overall survival (mOS) of 20.1 mo[2]. Another potential vaccine antigen is the multidrug resistance-associated protein 3 (MRP3), a member of the adenosine triphosphate-binding cassette transporters highly expressed in HCC tissue[3]. MRP3-derived peptide vaccines resulted in a mOS of 19 mo in a phase 1 trial of 12 HCC patients. Oncolytic virotherapy is another immune modality that has been widely investigated in solid malignancies. Heo et al[4] conducted a phase 2 trial assessing the efficacy and safety of high- and low-dose JX-594, an oncolytic poxvirus, in HCC patients[4]. The investigators reported a significantly longer mOS with high-dose compared to low-dose JX-594 (14.1 mo vs 6.7 mo; P = 0.02). Lastly, adoptive cellular therapy, which is a promising option that is being used more in hematological and solid cancers, has been investigated in HCC, specifically through genetically modified T cells expressing chimeric antigen receptors for GPC3 in a phase 1 trial on 13 patients, which resulted in a mOS of 278 d[5]. Table 1 includes the characteristics of the clinical trials on non-ICI immunotherapeutic options for HCC patients.

We would also like to emphasize the importance of identifying biomarkers predictive of the immunotherapy response in HCC. To date, limited evidence exists on this topic, yet some preclinical and clinical data point to potential targets. For instance, emerging evidence suggests that activated Wnt/beta-catenin signaling can predict primary immunotherapy resistance in HCC[6]. There is also growing interest in the microbiome’s predictive value to ICI response in other cancers. For HCC, this is especially relevant since chronic liver disease alters the microbiome components[7]. Established ICI predictive biomarkers in other malignancies, such as microsatellite instability and high tumor mutational burden, are of limited use in HCC due to their rarity[6,8].

<table>
<thead>
<tr>
<th>Table 1 Clinical trials characteristics on vaccine therapy, oncolytic virotherapy, and adoptive cellular therapy in hepatocellular carcinoma patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ref.</strong></td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>Sawada et al[2], 2016</td>
</tr>
<tr>
<td>Mizukoshi et al[3], 2015</td>
</tr>
<tr>
<td>Palmer et al[10], 2009</td>
</tr>
<tr>
<td>Butterfield et al[11], 2014</td>
</tr>
<tr>
<td>Heo et al[4], 2013</td>
</tr>
<tr>
<td>Shi et al[3], 2020</td>
</tr>
</tbody>
</table>

AFP: Alpha fetoprotein; CAR: Chimeric antigen receptor; CI: Confidence interval; DCs: Dendritic cells; GPC3: Glypican-3; mOS: Median overall survival; MRP3: Multidrug resistance-associated protein 3; RFA: Radiofrequency ablation; RFS: Recurrence-free survival.

**FOOTNOTES**

**Author contributions:** Abushukair HA drafted the manuscript and conceptualized the concepts; Saeed A conceptualized the core concepts and critically revised the draft.
**Conflict-of-interest statement**: Anwaar Saeed reports research grants from AstraZeneca, Bristol Myers Squibb, Merck, Exelixis, KAHR Medical, and Incyte, and advisory board fees from AstraZeneca, Bristol Myers Squibb, Merck, Exelixis, and Pfizer. The other author has no conflicts of interest to declare.

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**S-Editor**: Fan JR

**L-Editor**: Filipodia

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**REFERENCES**


Insight on BRAF<sub>V600E</sub> mutated colorectal cancer immune microenvironment

Hassan Mohammed Abushukair, Sara Mu'amar Zaitoun, Anwaar Saeed

Abstract

Colorectal cancer (CRC) is the second deadliest malignancy for both sexes. The BRAF<sup>V600E</sup> mutation, one of the most common driver mutations in CRC, is known for its poor prognosis due to the increased risk of metastasis. The effect of the BRAF<sup>V600E</sup> mutation on the tumor microenvironment was the topic of the study reported in World Journal of Gastrointestinal Oncology, with special focus on immune status. The authors presented insightful findings that were exclusively based on macrophage polarity and cytokine levels, without investigating other relevant immune elements. A more comprehensive look into the dynamic immune activity of cancer environments will warrant more meaningful practical findings. In this letter, we discuss other significant immune factors and their possible implications on the tumor microenvironment of BRAF-mutated CRC.

Key Words: Colorectal cancer; BRAF<sup>V600E</sup>; Tumor microenvironment; Microsatellite instability; Macrophages; Immune checkpoint proteins

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Core Tip: The immune landscape of the tumor microenvironment is a crucial indicator of the proliferative and invasive activity of the tumor cells and serves as a predictor of response to targeted immunotherapeutic modalities. BRAF<sup>V600E</sup> is one of the most common driver mutations in colorectal cancer thought to have a unique impact on the tumor immune microenvironment. It is unknown whether this impact is of a suppressive or activating nature. Future studies on larger samples, considering a wider array of immune elements, such as the infiltration of relevant immune cells as well as immune checkpoints’ expression, are needed.

Citation: Abushukair HM, Zaitoun SM, Saeed A. Insight on BRAF<sup>V600E</sup>-mutated colorectal cancer immune microenvironment. *World J Gastrointest Oncol* 2022; 14(6): 1213-1215
DOI: https://dx.doi.org/10.4251/wjgo.v14.i6.1213

TO THE EDITOR

We read the interesting study by Zhi et al[1] on the immune status of BRAF<sup>V600E</sup>-mutated colorectal cancer (CRC), titled “BRAF<sup>V600E</sup> mutant colorectal cancer cells mediate local immunosuppressive microenvironment through exosomal long noncoding RNAs”, in which they utilized patient tissue samples, CRC cell lines as well as in silico analysis to study correlations between the BRAF<sup>V600E</sup> mutation and changes in the local immune microenvironment of CRC. The authors reported an immunosuppressive microenvironment induced by exosomal long noncoding RNAs in BRAF<sup>V600E</sup> mutant CRC as well as higher angiogenic and lymphangiogenic activity compared to wild-type CRC.

We would like to point out the complementary findings to this study from previous work that has alluded to other parts of the immune landscape of the tumor microenvironment of BRAF<sup>V600E</sup> CRC. From this study, Zhi et al[1] reported a higher level of M2 macrophages in BRAF<sup>V600E</sup>-mutated patients compared to the wild-type, with no difference in M1 macrophages levels. Yet, the sample number from which these results were obtained was relatively small (BRAF<sup>V600E</sup> mutation: 10; BRAF wild-type: 20), and this translated to high standard deviations in the M2 counts in both samples. In a recent study by Cen et al[2], which used a larger sample (mutated patients: 110, wild-type patients: 798) from the Cancer Genomic Atlas and the Gene Expression Omnibus databases, the authors reported a higher immune cell infiltration and lower tumor purity. Specifically, a higher proportion of CD8+ T cells, M1 macrophages as well as neutrophils were found in BRAF<sup>V600E</sup>-mutated CRC patients, whereas no difference was found in M2 macrophage levels. Furthermore, according to the consensus molecular subtypes’ classification, which provides the most comprehensive description of CRC heterogeneity at the gene expression level, BRAF<sup>V600E</sup> mutation is associated with consensus molecular subtype 1, which correlates with high immune infiltration and immune-response pathway activity[3].

Interestingly, subtypes of BRAF<sup>V600E</sup> based on expression patterns in CRC have been further identified. There are two subtypes regardless of microsatellite instability, PI3K mutation status, sex and sidedness: BM1 and BM2[4]. Differences between those subtypes exist, including the prognosis (BM1 was found to have a poorer prognosis than BM2) and the immune status. BM1 has an overall stronger immune profile, emphasized by the activation of pathways like IL2/STAT5, tumor necrosis factor-α signaling via nuclear factor kappa B, IL6/JAK/STAT3 and allograft rejection[4]. Taking these subtypes into consideration will reveal a deeper understanding of the tumor immune microenvironment in BRAF-mutated CRC patients.

The immune status of the tumor microenvironment is a multilayered complex subject that leads to crucial implications regarding tumor cell immune evasion, therapeutic response or distant invasion tendency. Therefore, we feel that limiting the immune landscape to the levels of tumor-associated macrophages (M1/2) and cancer-associated fibroblasts, as in the study by Zhi et al[1], would not reflect the whole story. This is particularly due to the fact that other key immune components, such as CD8+ and CD4+ T cells, neutrophils, myeloid-derived suppressor cells and regulatory T cells, were not investigated. Of note, higher levels of cytotoxic CD8+ T cells could possibly be neutralized in the tumor microenvironment by immune checkpoints, such as programmed death protein and its ligand or cytotoxic T lymphocyte-associated protein 4[5]. In addition, microsatellite status is of paramount importance in this context, since a higher abundance of CD8+ T cells, activated natural killer cells and M1 macrophages, and upregulated immune checkpoints were identified in microsatellite instability-compared to microsatellite-stable CRC[6]. Hence, future investigations including a wider array of immune components, taking into consideration significant genomic features (microsatellite instability and tumor mutational burden), will likely shed light on more reflective findings into the tumor immune status.

In conclusion, the authors presented compelling findings that provide a new perspective on BRAF<sup>V600E</sup>-mutated CRC immune microenvironment by discussing a proposed mechanism for inducing an
immunosuppressed state through the release of exosomal long noncoding RNAs. Future studies targeting this topic should take into consideration the entire spectrum of the dynamic immune activity in the tumor microenvironment, covering relevant immune cells, immune checkpoints and molecular aberrations. Such comprehensive studies will provide insight for promising therapeutic opportunities for this subset of CRC patients.

FOOTNOTES

Author contributions: Abushukair HM and Zaitoun SM drafted the manuscript and contributed to conceptualization; Saeed A contributed to conceptualization of core concepts and critically revised the draft.

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REFERENCES


Correction to "MicroRNA-320a suppresses tumor progression by targeting PBX3 in gastric cancer and is downregulated by DNA methylation"

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Abstract
We rechecked the original data of Figure 3, Part.B, and found that 0 h group in the BGC-823 cell wound scratch assay was misapplied. Therefore, we are writing to apply for the modification of Figure 3, Part.B.

Key Words: Correction; Gastric cancer; miRNA-320a; DNA methylation

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Core Tip: This is a correction to "MicroRNA-320a suppresses tumor progression by targeting PBX3 in gastric cancer and is downregulated by DNA methylation".

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Correction
Correction to: Li YS, Zou Y, Dai DQ. MicroRNA-320a suppresses tumor progression by targeting PBX3 in gastric cancer and is downregulated by DNA methylation. World J Gastrointest Oncol 2019; 11(10): 842-856 PMID: 31662823 DOI: 10.4251/wjgo.v11.i10.842.
We recently read our manuscript published in the World Journal of Gastrointestinal Oncology (Manuscript NO: 48527, DOI: 10.4251/wjgo.v11.i10.842), we have carefully rechecked the original data of Figure 3, Part.B, and found that 0 h group in the BGC-823 cell wound scratch assay was misapplied. Therefore, we are writing to apply for the modification of Figure 3, Part.B. The revised images are shown in this Correction (Figure 1). We feel deeply sorry for this mistake during the proofreading process. This correction does not alter any interpretation of the results or conclusion of this study[1].

We apologize for any inconvenience caused by this mistake.

FOOTNOTES

Author contributions: Li YS and Dai DQ submitted the final manuscript and all authors read and approved the final version.

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