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EDITORIAL

Nonalcoholic fatty liver disease in lean subjects: Prognosis, outcomes and management

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Abstract

Nonalcoholic fatty liver disease (NAFLD) accounts for most cases of chronic liver disease worldwide, with an estimated global prevalence of approximately 25% and ranges from simple steatosis to nonalcoholic steatohepatitis and cirrhosis. NAFLD is strongly connected to metabolic syndrome, and for many years, fatty liver was considered to be an exclusive feature of obese patients. However, recent studies have highlighted the presence of NAFLD in non-obese subjects, with or without increased visceral fat or even in lean subjects without increased waist circumference. "Lean NAFLD" is a relatively new concept and there is significant scientific interest in understanding the differences in pathophysiology, prognosis and management compared with NAFLD in overweight/obese patients. In the present editorial, we discuss the clinical and metabolic profiles and outcomes of lean NAFLD compared with both obese NAFLD and lean healthy individuals from Asian and Western countries. Moreover, we shed light to the challenging topic of management of NAFLD in lean subjects since there are no specific guidelines for this population. Finally, we discuss open questions and issues to be addressed in the future in order to categorize NAFLD patients into lean and non-lean cohorts.

Key Words: Lean nonalcoholic fatty liver disease; Non-obese nonalcoholic fatty liver disease; Clinical outcomes; Metabolic outcomes; Disease management; Lifestyle interventions

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Core Tip: Affecting approximately one fourth of the global population, non-alcoholic fatty liver disease (NAFLD) is the predominant cause of chronic liver disease and for many years it was considered as a disease affecting only obese people. However, a significant proportion of non-obese or even lean individuals develop NAFLD. Therefore, it is of great interest to discuss the differences in prognosis, metabolic profiles and outcomes as well as the current management of lean NAFLD patients as compared with both obese NAFLD patients and lean healthy controls.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) has been recognized as the predominant cause of chronic liver disease in the industrialized world[1]. It encompasses a wide spectrum of clinical and histological entities, ranging from simple steatosis, defined as triglyceride (TG) accumulation > 5% within the hepatic parenchyma, to nonalcoholic steatohepatitis (NASH), which is characterized by inflammation and fibrosis and can lead to cirrhosis and even hepatocellular carcinoma (HCC)[2,3]. The prevalence of NAFLD is increased in patients with type 2 diabetes mellitus (T2DM), metabolic syndrome (MetS) and obesity[4]. Although the latter is not only a risk factor for NAFLD but is also associated with more severe forms of the disease, a significant proportion of subjects develop NAFLD despite having a relatively normal body mass index (BMI), a condition referred to as non-obese or lean NAFLD[5]. Non-obese/lean NAFLD is divided into 2 major categories[5]: The first and more prevalent includes non-obese patients who may be overweight (BMI between the 85th-95th percentile for age) with or without increased waist circumference and adipose tissue, while the second category includes lean subjects with no excess visceral fat mass[5]. In the latter category, several secondary causes have been implicated, such as high fructose intake, protein malnutrition (Kwashiorkor) as well as administration of steatogenic drugs (amiodarone, tamoxifen, methotrexate, prednisolone, *etc*.) and genetic predisposition[5,6]. Regarding the latter, Romeo *et al*[7] have emphasized the involvement of the rs738409 single nucleotide polymorphism in patatin-like phospholipase domain-containing protein 3 (*PNPLA 3*) gene in NAFLD onset and progression. Yet, a plethora of other gene variants have been also associated with increased susceptibility to NAFLD/NASH and progression to liver fibrosis and even HCC, such as the transmembrane 6 superfamily member 2 (*TM6SF2*)[8-10], glucokinase regulatory gene (*GCKR*)[11,12] and membrane bound O-acyltransferase domain containing 7 (*MBOAT7*) genes[13]. In addition, a variant of interferon-λ3 (*IFN-λ3*) gene has been related with increased liver inflammation and fibrosis among NAFLD patients[14], while the rs72613567 polymorphism in hydroxysteroid 17-beta dehydrogenase 13 (*HSD17B13*) gene was recently shown to reduce the risk of liver fibrosis, NASH and HCC[15,16]. Of note, both dietary composition and socioeconomic factors have been correlated with NAFLD development. Adherence to Mediterranean diet has been demonstrated to ameliorate hepatic insulin sensitivity and reduce hepatic fat accumulation while the Western dietary pattern, which mainly consists of high fructose and saturated fats intake, has been involved in NAFLD development[17,18]. Moreover, prolonged sitting time, usually related with high calorie intake and unhealthy dietary composition, and decreased physical activity are independent risk factors for NAFLD, even in lean subjects[19].

Current data on the prevalence of non-obese/lean NAFLD worldwide is characterized by wide variability. In a recent systematic review including 84 studies with 10530308 individuals, Ye *et al*[20] demonstrated that among the general population, the prevalence of lean and non-obese NAFLD was 5.1% and 12.1%, respectively. In addition, the overall prevalence of NAFLD among the lean general population was 10.6%, while the prevalence of NAFLD in the non-obese population was 18.3%. Interestingly, the prevalence of non-obese NAFLD among the total NAFLD population

was highest in Europe (51.3%) and lowest in eastern Asia (37.8%)[20]. Of note, NAFLD patients were categorized according to the World Health Organization (WHO) and Asian Pacific recommendations as overweight and lean when their BMI was 25 to 30 kg/m^2 and < 25 kg/m^2, respectively, in non-Asian populations and 23 kg/m^2 to 27.5 kg/m^2 and < 23 kg/m^2, respectively, in Asian populations[21-23]. However, it is well-established that individuals with similar BMI may have different degrees of visceral obesity, which is closely associated with the development of NAFLD[24-26]. Waist circumference is considered a more accurate marker of visceral obesity than BMI, but is not available in the majority of the relevant studies[27]. The present editorial will discuss the metabolic profile, prognosis and related clinical outcomes, as well as the management of non-obese or lean patients suffering from NAFLD.

CLINICAL IMPACT OF NON-OBESE/LEAN NAFLD

Literature search

PubMed database was systematically searched from the date of inception of this editorial until April 2020, to identify studies focusing on non-obese/lean NAFLD. The terms used were "Lean non-alcoholic fatty liver disease" OR "Lean nonalcoholic fatty liver disease" OR "Lean NAFLD" OR "Non-obese non-alcoholic fatty liver disease" OR "Non-obese nonalcoholic fatty liver disease" OR "Non-obese NAFLD" OR "Non-overweight fatty liver disease" OR "Non-overweight NAFLD". Since we aimed to emphasize the metabolic, hepatic and cardiovascular outcomes in obese *vs* non-obese/lean NAFLD patients as well as non-obese/lean individuals with or without NAFLD, studies evaluating the histological aspects of NAFLD were excluded.

Non obese/lean NAFLD vs controls: Metabolic and clinical outcomes (Table 1)

Younossi *et al*[28] in a study performed in the United States reported that lean (BMI < 25 kg/m^2) NAFLD patients compared to lean healthy subjects had higher prevalence of insulin resistance (IR), T2DM, hypercholesterolemia and hypertension, *i.e.*, the components of MetS. In the cross-sectional NHANES III study, Golabi *et al*[29] reported that lean (BMI < 25 kg/m^2) NAFLD patients, had higher risk of all-cause [Hazard Ratio (HR): 1.54] and cardiovascular-related mortality (HR: 2.38) than lean non-NAFLD subjects after adjustment for potential confounding variables[29]. Interestingly, in another study from the United States, Zou *et al*[30] showed that in a non-obese population (BMI < 30 kg/m^2 for non-Asians and < 27 kg/m^2 for Asians), patients with NAFLD had higher blood pressure, fasting plasma glucose (FPG), insulin, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C) and TG levels and higher Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), a marker of IR, than subjects without NAFLD. In addition, the former group had increased overall, cardiovascular and cancer-related mortality during a 15-year follow-up, but these findings were not confirmed in multivariate analysis[30].

In a post hoc analysis in Japanese subjects, Yoshitaka *et al*[31] reported that lean (BMI < 23 kg/m^2) NAFLD patients had higher blood pressure, increased FPG and TG serum levels, as well as greater risk (HR: 10.4) for cardiovascular events than to lean non-NAFLD individuals, independently of potential confounders. In a retrospective cohort study of 4629 lean Japanese participants (BMI < 23 kg/m^2) who were enrolled in a regular health checkup program, Fukuda *et al*[32] showed that patients with NAFLD had more than 3 times higher incidence of T2DM than subjects without NAFLD. Regarding non-obese subjects, Nishioji *et al*[33] found that non-obese (BMI < 25 kg/m^2) Japanese NAFLD patients had a higher prevalence of MetS components compared with healthy individuals. Both retrospective and prospective studies from South Korea also showed that non-obese NAFLD patients have an increased risk for T2DM than non-NAFLD, non-obese individuals, independently of other risk factors[34,35]. Moreover, Sung *et al*[36] in a large cohort of non-obese (BMI < 27 kg/m^2) South Korean individuals, reported that non-obese NAFLD patients have higher estimated cardiovascular risk based on the Framingham risk score than healthy controls, whereas in another South Korean cross-sectional study, non-obese (BMI < 25 kg/m^2) subjects without NAFLD had better metabolic profile than non-obese patients with NAFLD[37]. Accordingly, Kwon *et al*[38], in another retrospective study from South Korea, showed that non-obese (BMI < 25 kg/m^2) NAFLD patients had higher prevalence of MetS components than non-obese controls and had.

In lean (BMI < 23 kg/m^2) Chinese individuals, the presence of NAFLD was associated with increased odds for T2DM and MetS, independently of demographic and lifestyle parameters[39]. Regarding non-obese populations, 2 independent studies in

Table 1 Main findings and outcomes of lean (or non-obese) non-alcoholic fatty liver disease patients' *vs* lean (or non-obese) healthy individuals

Ref./Year/Country	Population (lean/non-obese NAFLD population)	Metabolic profile lean/non-obese NAFLD *vs* healthy controls	Liver function tests findings, lean/non-obese NAFLD *vs* healthy controls	Histological outcomes, lean/non-obese NAFLD *vs* healthy controls	Survival-related outcomes, lean/non-obese NAFLD *vs* healthy controls
Younossi *et al*[28] /2012/United States	11613 study population; 4457 lean subjects (431)	↑ Prevalence of insulin resistance, T2DM, hypercholesterolemia and hypertension		NA	NA
Golabi *et al*[29] /2019/United States	5375 lean subjects (581)	↑ Prevalence of metabolic comorbidities	NA	NA	↑ Hazard for all-cause and cardiovascular-related mortality
Zou *et al*[30]/2020/United States	9654 controls (1528)	↑ BP, HOMA-IR, glucose, insulin, TC, LDL-C, TG, ↓ HDL-C	↑ ALT, AST, γ-GT	NA	↑ 15-yr overall, cardiovascular, cancer and other causes-related mortality (not confirmed in Cox model)
Yoshitaka *et al*[31] /2017/Japan	1647 individuals; 984 non-overweight subjects (69)	↑ BP, glucose, TG, UA, ↓ HDL-C	↑ AST, ALT, γ-GT	NA	↑ HR of CVD incident
Fukuda *et al*[32] /2016/Japan	4629 participants (2989) in the non-overweight group (139)	↑ Adjusted HR for T2DM, ↑ BP, TC, TG, ↓ HDL-C	↑ ALT, AST, γ-GT	NA	NA
Nishioji *et al*[33] /2015/Japan	3271 enrolled individuals; 2606 non-obese (511)	↑ BP, TC, TG, HbA1c, glucose	↑ ALT, AST, γ-GT	NA	NA
Kim *et al*[34]/2018/South Korea	2920 participants; 2119 in non-obese group (420)	↑ HR for T2DM, ↑ TG, TC, LDL-C, ↓ HDL-C	↑ ALT, AST, γ-GT	NA	NA
Sinn *et al*[35]/2019/South Korea	51463 total population; 21984 lean subjects (2262)	↑ HR for T2DM onset, ↑ glucose, HbA1c, TG, TC and LDL-C, ↓ HDL-C	↑ ALT and AST	NA	NA
Sung *et al*[36]/2009/South Korea	30172 all non-obese; (7101)	↑ Prevalence of hypertension, T2DM, MetS in elevated ALT, steatosis and NASH groups	NA	NA	In men: ↑ Cardiovascular risk for group with elevated ALT serum levels and for steatosis and NASH groups. In women: ↑ Cardiovascular risk for steatosis and NASH groups
Kim *et al*[37]/2013/South Korea	759 individuals (98 in NAFLD group)	↑ Glucose, TG, UA, HOMA-IR, ↓ HDL-C	↑ ALT, AST, γ-GT	NA	NA
Kwon *et al*[38]/2012/South Korea	29994 study population; 24008 non-obese (3014)	↑ BP, glucose, insulin, HOMA-IR, ↓ HDL-C	↑ AST, ALT, γ-GT	NA	NA
Feng *et al*[39]/2014/China	1779; 731 in the lean group (134)	↑ OR for hypertension, T2DM, central obesity and MetS, UA, TC, LDL-C, TG, glucose, insulin, HOMA-IR ↓ HDL-C	↑ ALT, AST, γ-GT	NA	NA
Lee *et al*[40]/2018/China	2008 enrolled subjects; 953 non-obese (208)	↑ TC, TG, glucose	↑ ALT	NA	NA
Zeng *et al*[41]/2020/China	2715 enrolled participants (1100 NAFLD patients)	↑ Prevalence of hypertension and MetS, TG, LDL-C, ↓ HDL-C	NA	NA	NA
Yu *et al*[42]/2014/China	1296 non-obese subjects of whom 246 were NAFLD	↑ Arterial stiffness, assessed by the higher brachial-ankle pulse wave velocity, TC, LDL-C,	↑ ALT, AST	NA	NA

	patients	TG, glucose, insulin, UA, HOMA-IR			
Wang *et al*[43]/2015/China	9360 women population (1194 were NAFLD patients)	↑ TG, TC, LDL-C, glucose	↑ AST, ALT	NA	NA
Kumar *et al*[44]/2013/India	205 NAFLD patients (27 lean) plus 131 lean healthy subjects	↑ Prevalence of MetS, dyslipidemia		NA	NA
Oral *et al*[45]/2019/Turkey	367 non-obese individuals (225 in NAFLD group and 142 in the control group)	↑ TG, TC, UA, creatinine, HOMA-IR	↑ AST, ALT	NA	NA
Erkan *et al*[46] /2014/Turkey	219 non-obese non diabetic individuals of whom 143 NAFLD patients	↑ Prevalence of hypertension, MetS, hyperglycemia, hypertriglyceridemia and insulin resistance, insulin, HOMA-IR	↑ AST, ALT, γ-GT	NA	NA
Feldman *et al*[47] /2017/Austria	187 subjects (116 suffering from NAFLD of whom 55 were lean)	↑ Prevalence of T2DM, glucose, ↓ adiponectin	↑ ALT, γ-GT	NA	NA
Gonzalez-Cantero *et al*[48] /2018/Spain	113 non-obese enrolled individuals (55 NAFLD patients)	↑ HOMA-IR, TG, insulin, ↓ HDL-C, adiponectin	↑ ALT, AST, γ-GT	NA	NA

NAFLD: Non-alcoholic fatty liver disease; ALT: Alanine aminotransferase; T2DM: Type 2 diabetes mellitus; NASH: Non-alcoholic steatohepatitis; NAS: NAFLD activity score; BP: Arterial blood pressure; TC: Total cholesterol; LDL-C: Low density lipoprotein-cholesterol; HDL-C: High density lipoprotein-cholesterol; TG: Triglycerides; HR: Hazard ratio; HbA1c: Glycosylated hemoglobin, type A1C; HOMA-IR: Homeostasis Model Assessment Insulin Resistance; CVD: Cardiovascular disease; HCC: Hepatocellular carcinoma; NA: Not applicable; UA: Uric acid; AST: Aspartate aminotransferase; γ-GT: γ-Glutamyl transferase; MetS: Metabolic syndrome.

China confirmed that non-obese (BMI < 25 kg/m^2) patients with NAFLD suffered more frequently from hypertension and MetS than healthy non-obese subjects[40,41], whereas a cross-sectional study in China reported that non-obese (BMI < 27.5 kg/m^2), normotensive and non-diabetic NAFLD patients had increased arterial stiffness, higher serum levels of FPG, TC, LDL-C, TG and greater HOMA-IR than non-obese, healthy subjects[42]. Similar findings were observed in Chinese women[43]. Moreover, a cohort study in India also showed that NAFLD patients are at higher risk for metabolic disorders irrespectively of the presence of obesity[44].

In accordance to the aforementioned studies, Oral *et al*[45] reported that non-obese (BMI < 30 kg/m^2) NAFLD patients from Turkey were more frequently glucose intolerant and had higher TG and TC levels than non-obese controls. Similar findings were also reported in another Turkish study, where lean (BMI < 25 kg/m^2) NAFLD patients had higher prevalence of hypertension and MetS as well as higher HOMA-IR[46]. Finally, in Europe, Feldman *et al*[47] reported that Austrian lean (BMI < 25 kg/m^2) healthy subjects were more frequently glucose tolerant and had lower prevalence of T2DM than lean NAFLD patients and these findings were confirmed by Gonzalez-Cantero *et al*[48] in a non-obese (BMI < 30 kg/m^2) Spanish cohort.

Obese vs non-obese/lean NAFLD (Table 2)

Studies with metabolic outcomes: Regarding metabolic outcomes in NAFLD obese and NAFLD non-obese/lean patients, in a retrospective study of 669 NAFLD patients in Italy, lean (BMI < 25 kg/m^2) NAFLD patients had lower prevalence of hypertension, T2DM and MetS than overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2) and obese (BMI ≥ 30 kg/m^2) NAFLD patients[49]. Notably, the former group had significantly thinner carotid intima-media, indicating less atherosclerotic burden[49]. Although this result was not confirmed in a study by Shao *et al*[50] in obese (BMI > 25 kg/m^2) and non-obese Chinese NAFLD patients, the authors showed that the latter group had lower FPG and serum TC and TG levels as well as a lower prevalence of hypertension. Moreover, Li *et al*[51] demonstrated that the proportion of Chinese patients with elevated FPG and serum TG levels was higher among obese (BMI > 25 kg/m^2) compared with non-obese NAFLD patients, while another cross-sectional study from China confirmed that obese NAFLD women (BMI > 28 kg/m^2) had higher FPG than non-obese women[43]. In addition, 2 studies from China showed a higher prevalence of MetS and hypertension in obese (BMI > 25 kg/m^2) compared to non-obese patients with NAFLD[41,52]. In the Indian population, Kumar *et al*[44] reported that among NAFLD patients, lean (BMI < 23 kg/m^2) patients had had lower serum insulin levels and HOMA-IR, as well as lower prevalence of T2DM and MetS than obese (BMI > 25 kg/m^2) patients. In a case control study from Sri Lanka, Niriella *et al*[53] reported a higher prevalence of hypertension in non-lean (BMI > 23 kg/m^2) patients with NAFLD compared with lean NAFLD patients. In studies performed in Japan, Yoshitaka *et al*[31] reported lower blood pressure and FPG and higher serum high density cholesterol (HDL-C) levels in lean (BMI < 23 kg/m^2) than in overweight NAFLD patients[44], while Honda *et al*[54] reported that FPG, insulin, TG and HOMA-IR were increased among Japanese obese (BMI > 25 kg/m^2) NAFLD patients compared with non-obese NAFLD patients. In a study form Hong-Kong, Wei *et al*[55] reported that non-obese (BMI < 25 kg/m^2) NAFLD patients had lower IR than obese NAFLD patients. Moreover, the prevalence of MetS and hypertension was increased in obese patients. A study performed in Bangladesh also showed that non-obese (BMI < 25 kg/m^2) NAFLD patients had lower TC, FPG, HOMA-IR and higher HDL-C levels than obese NAFLD patients[56].

Similar findings were observed in the cross-sectional NHANES III study, in which lean (BMI < 25 kg/m^2) NAFLD patients had less frequently hypertension, T2DM and hypercholesteremia as well as lower levels of FPG and HOMA-IR than overweight /obese NAFLD patients[28]. In a prospective study from Turkey, Akyuz *et al*[57] reported that lean (BMI < 25 kg/m^2) NAFLD patients had a less prevalence of MetS and hypertension than overweight NAFLD patients, while in a study from Spain, Gonzalez-Cantero *et al*[48] also confirmed that overweight (BMI: 25-29 kg/m^2) patients with NAFLD had higher HOMA-IR and TG and lower HDL-C serum levels than lean NAFLD patients. In a study from Austria, Feldman *et al*[47] also reported that lean (BMI < 25 kg/m^2) NAFLD patients had lower FPG, insulin and HOMA-IR and higher HDL-C levels than obese NAFLD patients.

In contrast to these findings, a retrospective study from South Korea reported a higher prevalence of MetS components in non-obese (BMI < 25 kg/m^2) NAFLD patients compared with obese NALFD patients, even after adjusting for confounders[38]. It is possible that unrecorded differences in dietary patterns, physical activity and smoking status between the 2 groups might explain this paradoxical might[38]. Lee *et al*[40] also reported that non-obese (BMI < 25 kg/m^2) NAFLD patients had higher prevalence of MetS and hypertension as well as lower serum HDL-C levels than obese NAFLD patients. However, this study was hospital- and not community-based suggesting the presence of selection bias as an explanation for these unexpected findings[40].

Studies with both metabolic and clinical outcomes: In a prospective cohort study in 307 NAFLD patients from Hong-Kong, Leung *et al*[52] reported that non-obese patients (23.5% patients of the total cohort) had lower prevalence of MetS and hypertension as well as lower NAFLD activity score, serum cytokeratin-18 fragments and decreased liver stiffness based on transient elastography than obese patients. Of note, deaths, HCC and liver failure occurred only in obese patients during a follow-up period of 49 mo[52].

In contrast, a United States study in 483 biopsy-confirmed NAFLD patients showed that lean (BMI < 25 kg/m^2) patients had higher all-cause mortality than non-lean patients during a follow-up of 133 mo, although they had lower prevalence of T2DM, MetS, hypertriglyceridemia and hypertension, and less advanced fibrosis. Notably, even after adjustment for potential confounders, lean NAFLD was an independent risk factor (HR: 11.8) for higher all-cause mortality[58]. In the NHANES study, Zou *et al*[30]

Table 2 Main findings and outcomes of lean (or non-obese) non-alcoholic fatty liver disease patients' *vs* obese ones

Ref./Year/Country	Population (lean/non-obese NAFLD patients)	Metabolic profile, lean/non-obese NAFLD *vs* non-lean/obese NAFLD	Liver function tests findings, lean/non-obese NAFLD *vs* non-lean/obese NAFLD	Histological outcomes, lean/non-obese NAFLD *vs* non-lean/obese NAFLD	Survival-related outcomes, lean/non-obese NAFLD *vs* non-lean/obese NAFLD
Younossi *et al*[28] /2012/United States	11613 study population; 2491 NAFLD patients (431 lean)	↓ Prevalence of insulin resistance, T2DM, hypocholesteremia, hypertension, HOMA score	↓ AST, ALT	NA	NA
Zou *et al*[30]/2020/ United States	4711 patients with NAFLD (1528 non-obese)	Similar prevalence of T2DM and MetS, Metabolic comorbidities: More common	NA	↑ Prevalence of advanced liver fibrosis	↑ 15-yr overall, cardiovascular, cancer and other causes related mortality (not significant in a Cox model)
Yoshitaka *et al*[31] /2017/Japan	1647 individuals; 312 NAFLD patients (69 non-overweight)	↓ BP, glucose, ↑ HDL-C	↓ AST, ALT, and γ-GT	NA	NA
Kwon *et al*[38]/2012/South Korea	29994 study population; 6039 NAFLD patients (3014 non-obese)	↑ Prevalence ratios for high BP, glucose intolerance, and ↑ TG, ↓ HDL-C especially among women population	NA	NA	NA
Feng *et al*[39]/2014/China	1779 study population; 898 NAFLD patients (134 lean)	↓ Insulin, TC, UA, HOMA-IR, ↑ HDL-C	↓ ALT and γ-GT	NA	NA
Lee *et al*[40]/2018/China	2008 enrolled subjects; 493 NAFLD patients (208 non-obese)	↑ Prevalence of MetS and hypertension, ↓HDL-C	NA	NA	NA
Zeng *et al*[41]/2020/China	2715 enrolled participants; 1100 NAFLD patients (142 lean)	↑ Prevalence of MetS	NA	Less severe hepatic steatosis, evaluated by ameliorated values of CAP and FLI	NA
Wang *et al*[43]/2015/China	9360 women population; 1194 were NAFLD patients (514 non-obese)	↑ UA, glucose	↓ ALT, AST but ↑ AST/ALT ratio	NA	NA
Kumar *et al*[44] /2013/India	205 NAFLD patients (27 lean)	↓ Hyperinsulinemia, HOMA-IR, ↓ prevalence of T2DM, MetS		↓ Mean NAS and ↓ proportion of patients with liver fibrosis	NA
Feldman *et al*[47] /2017/Austria	187 subjects; 116 NAFLD patients (55 lean)	↓ Glucose, insulin, HOMA-IR, ↑ HDL-C, adiponectin	↓ ALT	NA	NA
Fracanzani *et al*[49] /2017/Italy	669 NAFLD patients (143 lean)	↓ Prevalence of hypertension, T2DM, MetS, NASH, carotid plaques and significant thinner carotid intima-media	NA	↓ Prevalence of NAFLD and ↓ median NAS	NA
Shao *et al*[50]/2020/China	534 NAFLD patients (240 non-obese)	No ↑ risk of cardiovascular damage and ↑ TC, FFA, TG, BP, insulin resistance	↓ ALT and AST	NA	NA
Li *et al*[51]/2019/China	496 NAFLD patients (101 lean)	↑ Proportion of patients with ↑ TG, glucose	↓ Proportion of patients with ↑ ALT	NA	NA

Leung *et al*[52] /2017/Hong-Kong	307 NAFLD patients (72 non-obese)	↓ Prevalence of MetS, hypertension	NA	↓ NAS, ↓ fibrosis stage, serum cytokeratine-18 fragments and liver stiffness measurement	Severe clinical outcomes (6 deaths, 2 HCC,1 liver failure) were observed only in the obese group
Niriella *et al*[53]/2018/Sri Lanka	2985 initial cohort; 936 NAFLD patients (120 lean)	↓ Prevalence of hypertension and central obesity, no significant difference in prevalence of other metabolic comorbidities at baseline. No remarkable alterations of new onset of metabolic comorbidities at the completion of follow-up	NA	NA	NA
Honda *et al*[54] /2016/Japan	1562 enrolled subjects; 540 NAFLD patients (134 non-obese)	↑ HOMA-IR, glucose, insulin, TG, ↓ genotype prevalence of (*PNPLA3*) GG	↓ ALT and AST	↓ Lobular inflammation, steatosis grade, hepatocyte ballooning and NAS	NA
Wei *et al*[55]/2015/Hong-Kong	262 patients with NAFLD (135 non-obese)	↓ Insulin resistance, BP and cytokeratin-18 fragments and ↓ prevalence of MS, ↑ genotype (*PNPLA3*) GG prevalence	NA	Less non-obese NAFLD patients with ↑ NAFLD fibrosis score	NA
Alam *et al*[56] /2014/Bangladesh	465 NAFLD patients (119 non-obese)	Similar prevalence of T2DM and hypertension and ↓ TC, glucose, HOMA-IR, ↑ HDL-C	↓ ALT, AST, γ-GT	No significant difference in histological findings	NA
Akyuz *et al*[57] /2015/Turkey	483 NAFLD patients (37 lean)	↓ BP, ↓ prevalence of MetS, less severe hepatic steatosis, ↑ hemoglobin levels	NA	Less severe hepatic fibrosis	NA
Cruz *et al*[58]/2014/United States	1090 NAFLD patients (125 lean)	↓ Insulin resistance, ↓ prevalence of low HDL-C, hypertriglyceridemia and hypertension	↓ ALT	↓ Steatosis degree and less advanced fibrosis	↓ Cumulative survival
Hagström *et al*[59] /2018/Sweden	646 NAFLD patients (123 lean, 335 overweight, 188 obese)	↓ TG, glucose	↓ ALT, AST compared to both overweight and obese counterparts	↓ Prevalence of NASH and ↓ mean fibrosis stage compared to both overweight/obese patients	↓ Risk for overall mortality, ↑ risk for severe hepatic disease development as compared to overweight patients

NAFLD: Non-alcoholic fatty liver disease; ALT: Alanine aminotransferase; T2DM: Type 2 diabetes mellitus; BP: Blood pressure; MetS: Metabolic syndrome; TC: Total cholesterol; HDL-C: High density lipoprotein cholesterol; LDL-C: Low density lipoprotein cholesterol; TG: Triglycerides; UA: Uric acid; NASH: Non-alcoholic steatohepatitis; NAS: NAFLD activity score; HOMA-IR: Homeostasis Model Assessment Insulin Resistance; HCC: Hepatocellular carcinoma; CAP: Controlled attenuation parameter; FLI: Fatty liver index; HR: Hazard ratio; PNPLA 3: Patatin-like phospholipase domain-containing protein 3; NA: Not applicable; AST: Aspartate aminotransferase; γ-GT: γ-Glutamyl transferase; MetS: Metabolic syndrome.

also reported that non-obese NAFLD (BMI < 30 kg/m^2 for non-Asians and < 27 kg/m^2 for Asians) patients had a higher prevalence of metabolic comorbidities, more advanced fibrosis and higher mortality due to cardiovascular disease and cancer and higher all-cause mortality than obese NALFD. However, these findings were not confirmed in a multivariate analysis, where only T2DM and fibrosis stage were independent risk factors for mortality[30]. Finally, in a retrospective cohort study in 646 NAFLD patients in Sweden, Hagström *et al*[59] reported that lean (BMI < 25 kg/m^2) NAFLD patients had higher risk for cirrhosis, decompensated cirrhosis and HCC than overweight (25 kg/m^2 ≤ BMI < 30 kg/m^2) patients, independently of confounders; all-cause mortality did not differ between the 2 groups. Of note, lean patients had lower serum TG and FPG levels as well as lower prevalence of NASH and lower fibrosis stage[59].

It may seem paradoxical that most studies[30,58,59], although not all[52], reported a worse prognosis in non-obese/lean patients with NAFLD compared with obese

NAFLD patients. Zou *et al*[30] attributed the worse outcome of non-obese NAFLD patients to the advanced fibrosis stage and the higher frequency of metabolic comorbidities in this group. Hagström *et al*[59] speculated that genetic predisposition and unhealthy lifestyle were associated with the worse liver-related outcomes of lean NAFLD patients. Another explanation could be that in all studies, BMI was used as a surrogate marker to define the thresholds for leanness or obesity. However, BMI is not a specific marker of abdominal obesity; waist circumference reflects more accurately the visceral adiposity fat fraction[60]. Nonetheless, even waist circumference cannot distinguish visceral from subcutaneous fat and cannot allow quantification of adipose tissue parts. Accordingly, more accurate markers of abdominal obesity, such as magnetic resonance imaging (MRI), might be useful in distinguishing between obese and lean patients with NAFLD. Indeed, MRI is considered a more accurate and quantitative tool for evaluation of visceral adipose tissue[61]. Thus, both the definition of lean/non-obese NAFLD and the categorization of patients into lean/non-obese or obese should be based in the near future on MRI to overcome the limitation of current, BMI-based definitions.

MANAGEMENT OF NON-OBESE/LEAN NAFLD

Management of NAFLD in lean patients is particularly challenging, since the cornerstone of NAFLD treatment is weight loss, which might not apply in these patients. In addition, there are no specific guidelines for the management of NAFLD in lean subjects. However, accumulating data suggest that several interventions might be useful in this population. A summary of the key elements of management of lean NAFLD is given in Table 3.

Initial workup and assessment of disease severity

To select the most appropriate management, a thorough diagnostic workup should be performed. The initial workup of a lean patient with suspected NAFLD may include a variety of modalities. Usually, ultrasound is the screening imaging method of choice and can provide information regarding the presence and severity of steatosis and the presence of cirrhosis. The Fibrosis-4 (FIB-4) and NAFLD fibrosis score can be useful for assessing the severity of liver fibrosis in patients with NAFLD[62,63]. In patients with inconclusive findings, elastography (transient, shear wave, or magnetic resonance) is the most widely used method to assess the severity of hepatic fibrosis, otherwise liver biopsy is recommended[64,65].

Management of NAFLD in lean subjects

Weight reduction: Similar with obese patients with NAFLD, weight reduction appears effective in lean subjects with NAFLD. In a study in 333 patients with NAFLD, weight change was an independent predictor of disease progression or resolution after a mean follow-up of 28.7 mo. Interestingly, among patients who also experienced NAFLD progression, non-obese subjects had greater weight gain than obese patients whereas among patients who experienced NAFLD resolution, non-obese patients showed smaller weight loss than obese subjects[66]. Moreover, 2 studies showed that 5% of body weight reduction led to significant decrease in steatosis in lean patients with NAFLD[67,68]. In the first study (n = 120 patients with NAFLD), a 10-wk program including diet modification and exercise resulted in improvement in steatosis in the repeated liver biopsy[67], while in the second one (n = 14 Lean NAFLD patients), an 8-wk intervention consisting of intensive dietary and lifestyle measures induced a decrease in both steatosis and stiffness assessed with transient elastography[68].

Dietary modifications and physical activity: Diet appears to improve NAFLD in lean patients independently of weight loss. It has been reported that lean patients with NAFLD have comparable total caloric intake with obese patients with NAFLD[69,70]. However, the former have higher intake of cholesterol and lower intake of polyunsaturated fatty acids (PUFAs)[70]. In a study in 120 patients with NAFLD who followed a 10-wk program including diet modification and exercise, most patients achieved a reduction of steatosis without weight reduction; instead, a reduction in fat intake and in overall body fat was observed and might have contributed to the improvement in steatosis[67]. Therefore, low-fat diet appears more appropriate for lean patients with NAFLD.

There are also reports highlighting physical exercise as a contributing factor to NAFLD amelioration irrespectively of its effect on body weight. In a large

Table 3 Key elements of management of lean non-alcoholic fatty liver disease

Evaluation of severity of liver	Liver fibrosis (serological markers, elastography, biopsy)
	Presence of NASH (biopsy or serological evidence of inflammation)
Weight reduction	5% of body weight reduction can be effective in reducing steatosis
Physical activity	Positive effect regardless of weight reduction
Dietary Intervention	↓ Fat intake, ↑ protein intake
Commorbidities	Strict control of:
	Diabetes mellitus (consider pioglitazone)
	Hypertriglyceridemia (baseline triglyceride count was independently correlated with NAFLD resolution)
	Hypercholesterolemia (reduction of total cholesterol was independently correlated with steatosis reduction)
	Hypertension
Sleep patterns	Emphasize the significance of adequate sleep duration and quality
Pharmacological therapy	Pioglitazone and vitamin E as the only accepted therapies, but proposed only on an individual basis
	Possible role of probiotics
	Small number of trials for lean patients
	According to the results of trials focusing on non-lean patients

NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease.

retrospective study (n = 3718), lack of physical activity was independently associated with the presence of NAFLD, after adjusting for visceral adiposity and IR. These results might be particularly relevant for lean patients with NAFLD, who have lower prevalence of IR and visceral adiposity compared with obese patients[28,58].

Management of comorbidities: As already mentioned, lean patients with NAFLD appear to have higher incidence of T2DM compared with overweight patients without NAFLD[32]. Given that T2DM is a major risk factor for NAFLD progression[71-73], these findings highlight the importance of T2DM prevention in this population. Furthermore, it has been reported that elevated TG levels are independently associated with development or resolution of NAFLD, especially in non-obese patients[66]. In another study, a ≥ 10% reduction in TC levels was independently associated with ≥ 20% reduction of steatosis in biopsy after a 10-wk exercise and diet modification program[67]. Given the increased cardiovascular risk of lean NAFLD patients, screening for and management of cardiometabolic comorbidities are essential to reduce cardiovascular morbidity in this population.

Sleep patterns: Short duration and poor quality of sleep have been associated with increased incidence of NAFLD[74-77]. Considering that a substantial proportion of lean patients with NAFLD have disturbed sleep[69], recommendations for more rest and efforts to improve sleep quality should be considered in this population.

Pharmacological interventions

Treatment options for NAFLD include pioglitazone and vitamin E but are limited to non-diabetic patients with biopsy-proven NASH. However, in both European and American guidelines, these agents are recommended to be used with caution and in carefully selected patients[4,64]. In addition, only ezetimibe has been evaluated in lean patients with NAFLD. In a pilot study (n = 8 non-obese patients), treatment with ezetimibe for 12 mo resulted in a decrease in aminotransferase levels but had no effect on hepatic steatosis assessed with ultrasound[78]. Interestingly, BMI did not change during the study. A larger, placebo-controlled, randomized study evaluated a symbiotic supplement consisting of seven bacterial strains in 50 lean NAFLD patients who also received lifestyle recommendations[79]. The supplement resulted in a greater reduction in liver stiffness and steatosis, in serum TG and TC levels and in inflammatory markers including high-sensitivity C-reactive protein and nuclear factor-κB activity than placebo. This study supports the findings of a previous report in obese patients with NAFLD[80] and suggests a role of gut microbiota manipulation in the management of NAFLD.

CONCLUSION

Even though NAFLD is strongly associated with obesity and related comorbidities, a substantial proportion of lean subjects can also develop NAFLD. Visceral obesity as opposed to general obesity, genetic predisposition, unhealthy dietary pattern consisting of high cholesterol and fructose intake may be associated with lean NAFLD. Although lean patients appear to have a worse prognosis but a healthier metabolic profile than obese patients with NAFLD, we should bear in mind that the current categorization into lean or obese cohorts was mostly based on BMI and not on visceral fat mass evaluation. Thus, the use of MRI as a reliable and quantitative diagnostic tool for evaluating the presence and severity of abdominal obesity in NAFLD patients might be useful. Currently, lifestyle interventions including weight loss, physical activity and a healthier dietary pattern seem to have beneficial impact on lean NAFLD. Beyond that, sleep interventions and pharmacotherapy along with strict management of comorbidities should also be incorporated in the management of this disease. Without a doubt, lean NAFLD raises many challenges since the pathophysiology and the natural history of the disease has not been widely studied and physicians should have high clinical suspicion in order to identify individuals at risk of lean NAFLD who lack the common, easily recognizable phenotype of obesity.

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REVIEW

Simultaneous colorectal and parenchymal-sparing liver resection for advanced colorectal carcinoma with synchronous liver metastases: Between conventional and mini-invasive approaches

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Abstract

The optimal timing of surgery in case of synchronous presentation of colorectal cancer and liver metastases is still under debate. Staged approach, with initial colorectal resection followed by liver resection (LR), or even the reverse, liver-first approach in specific situations, is traditionally preferred. Simultaneous resections, however, represent an appealing strategy, because may have perioperative risks comparable to staged resections in appropriately selected patients, while avoiding a second surgical procedure. In patients with larger or multiple synchronous presentation of colorectal cancer and liver metastases, simultaneous major hepatectomies may determine worse perioperative outcomes, so that parenchymal-sparing LR should represent the most appropriate option whenever feasible. Mini-invasive colorectal surgery has experienced rapid spread in the last decades, while laparoscopic LR has progressed much slower, and is usually reserved for limited tumours in favourable locations. Moreover, mini-invasive parenchymal-sparing LR is more complex, especially for larger or multiple tumours in difficult locations. It remains to be established if simultaneous

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resections are presently feasible with mini-invasive approaches or if we need further technological advances and surgical expertise, at least for more complex procedures. This review aims to critically analyze the current status and future perspectives of simultaneous resections, and the present role of the available mini-invasive techniques.

Key Words: Synchronous colorectal liver metastases; Colorectal surgery; Liver surgery; Simultaneous resection; Parenchymal-sparing liver resection; Mini-invasive surgery; Intraoperative ultrasonography

Core Tip: The optimal timing of surgery in case of synchronous colorectal cancer and liver metastases is debated. Staged approaches are traditionally preferred, but simultaneous resections are increasingly performed in appropriately selected patients. Since major liver resections (LR) may determine worse perioperative outcomes, parenchymal-sparing LR should be considered whenever feasible. While mini-invasive colorectal surgery is widely diffused, mini-invasive LRs are usually reserved for limited tumours in favourable locations, and parenchymal-sparing LR is more complex. It remains to be established if simultaneous resections are presently feasible with mini-invasive approaches or further technological advances and surgical expertise are needed, at least for more complex procedures.

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INTRODUCTION

Colorectal cancer (CRC) is a very common cause of cancer-related death in developed countries, with synchronous liver metastases (SCRLM) in about 15 to 25% of patients at the time of diagnosis[1,2]. Radical liver resection (LR) of colorectal liver metastases (CRLM) may achieve 5-year overall survival (OS) rates of 37% to 58%[3-6]. However, the expanding availability of therapeutic tools, that include medical, radiological and surgical treatments, alone or in combination, has made the management of metastatic CRC increasingly complex[7,8]. Patients with CRC and synchronous metastases require specific consideration, because may have less favourable cancer biology and oncological outcomes than those with metachronous CRLM, therefore requiring appropriate multimodal treatments[1,9]. The optimal timing of surgery in these patients is still under debate. Traditionally most surgeons prefer a staged approach with initial colorectal resection (CRR) followed by LR, eventually after interval chemotherapy (CHT)[4]. Traditional staged strategies are believed to avoid increased morbidity and mortality[3,10], and may warrant better selection for LR, excluding patients who experience disease progression while awaiting hepatectomy, especially when occurred during interval CHT[9,10]. However, simultaneous procedures may be safely performed in selected patients, with perioperative results comparable to staged resections. This approach avoids a second surgical procedure and the risk of interval progression of liver disease, and permits an earlier initiation of adjuvant CHT[11-18]. At present most authors consider that simultaneous CRR and minor hepatectomy are usually safe and should be preferred in selected patients with limited liver disease[4,9,11-18], while patients requiring simultaneous colorectal and major liver resection should be accurately evaluated, since increased morbidity and mortality rates have been reported[3]. Some authors, however, suggest that simultaneous colorectal and major liver resection may have similar perioperative risks compared to major LR alone[19,20], so that even simultaneous resection of rectal tumours and major hepatectomies are considered reasonable in appropriate patients[20,21].

Major hepatectomies have been traditionally preferred to achieve curative resection of CRLM, especially in the case of large or multiple lesions; however extensive hepatectomies may determine significant perioperative complications, mostly related to posthepatectomy liver failure (PHLF)[22,23]. Several therapeutic strategies have been undertaken to minimize the risk of PHLF after LR. These include preoperative systemic and/or locoregional CHT, that may significantly reduce the neoplastic burden in the liver, thus limiting the extension of the hepatectomy[24], and specific technical innovations that increase the volume of the future remnant liver (FRL) when major LR are planned, mainly preoperative portal vein embolization (PVE) and two-stage procedures (TSH), comprising the associating liver partition and portal vein ligation for staged hepatectomy (ALPPS) strategy[25,26]. An alternative approach, termed "conservative" or "parenchymal-sparing" liver surgery (PSLR), involves the resection of liver neoplasms with the minimum sufficient resection margin (RM), to preserve as much normal liver parenchyma as possible along with the major intrahepatic vessels[27,28]. This approach is based on careful preoperative planning and expert use of intraoperative ultrasonography (IOUS). PSLR has the advantage of limiting the risk of perioperative PHLF even in patients with extensive liver disease[23], and increases the chance of repeat LR in the case of recurrence (salvageability)[27,28]. Repeat LR of CRLM has a well-demonstrated potential for cure in selected patients with recurrent disease[29,30]. In the last decade liver surgery for CRLM has progressively shifted toward more conservative procedures that result in decreased morbidity and mortality rates than major LR, with comparable oncological results[6,31-33].

Mini-invasive surgery, including laparoscopic and robotic procedures, has known a progressive diffusion in oncological colorectal surgery[34-36], even though some controversies still exist for rectal cancer surgery[36-40]. Diffusion of mini-invasive techniques of liver surgery has progressed much slower, since the acquisition of adequate experience in mini-invasive LR is difficult, requires specific complex training with a prolonged learning curve, and may be accompanied by a significant increase of costs per procedure[41-43]. Although the vast majority of laparoscopic liver resections (LLR) are minor resections and mainly involve anterior and inferior liver segments (segments S2, S3, S4b, S5 and S6)[43,44], more complex procedures, including major hepatectomies, are increasingly performed in most experienced centers[41-43,45,46]. In case of difficult procedures, some surgeons adopt hand-assisted or hybrid approaches[42,43]. Mini-invasive procedures have been recently proposed also for TSH, including the ALPPS strategy[47,48]. Mini-invasive LR has usually better perioperative results than conventional open LR, with comparable oncological outcomes[41,49-54], even though patients undergoing mini-invasive LR are in most cases highly selected, with limited liver disease in favourable locations[50,54-57].

Based on the growing consensus toward simultaneous procedures in selected patients bearing resectable CRC with SCRLM, the mini-invasive techniques have been utilized also for simultaneous colorectal and liver resections[58,59], including simultaneous major LR[60,61]. Mini-invasive simultaneous procedures usually determine better perioperative results than conventional open resections, with comparable oncological outcomes[62,63]. However, patients considered for mini-invasive simultaneous procedures are highly selected either for the site or the extension of the primary and metastatic disease, so that the perioperative and oncological outcomes cannot be generalized[64,65]. While PSLR with adequate resection margin should be considered the standard of care, there is concern that LLR may sometimes involve larger procedures resecting more nontumorous liver parenchyma, since smaller parenchymal-sparing procedures for multiple or non-favourably located tumours may be more complex with mini-invasive approaches[42-46,66,67]. Technological advances, as well as the growth of surgical experience and skills, are favouring the development of mini-invasive parenchymal-sparing approaches[45,66,68-72]. Nonetheless, simultaneous colorectal and conservative liver resections may require long operative times in complex resections[21,73,74]. Therefore, it remains to be established if the available surgical strategies for the treatment of advanced CRC with liver metastases are presently feasible with mini-invasive approach during the same procedure or if we need further technological advances and surgical expertise, at least in more complex surgical situations.

This review aims to critically examine the available data to determine whether simultaneous colorectal and conservative liver resections represent a safe and effective surgical strategy for advanced CRC with SCRLM, and which is the present role of the available mini-invasive techniques when more complex colorectal procedures along with conservative liver resections are required.

SEARCH STRATEGY AND SELECTION CRITERIA

We identified data for this review through a non-systematic literature search conducted using the Medline, Embase, and Web of Science databases, updated to December 2019. Papers in core clinical journals were included, describing studies on surgical strategies for synchronous CRLM, neoadjuvant CHT (NACHT) of resectable CRLM, conservative/parenchymal-sparing LR, anatomic *vs* nonanatomic LR, prognostic role of the resection margin, clinical and prognostic relevance of genetic mutations of CRLM, surgical strategies for multiple bilobar CRLM, mini-invasive colorectal surgery, mini-invasive liver surgery, mini-invasive *vs* open LR, mini-invasive *vs* open simultaneous colorectal and liver resection, mini-invasive *vs* open parenchymal-sparing LR. The reference lists of selected papers and prior reviews were checked manually to identify further significant papers not retrieved by the initial search.

THERAPEUTIC STRATEGIES FOR SYNCHRONOUS COLORECTAL LIVER METASTASES

Therapeutic strategies in patients with resectable CRC and upfront resectable SCRLM have been widely debated in the last decades and shared solutions are beyond to come (Table 1). The traditional "staged" or "classic" approach with initial resection of the primary CRC followed by LR is probably still preferred by most surgeons, because the risks of the colorectal and the liver procedures are not cumulated[3,10], but also because CHT can be usefully administered before the LR[9,10]. In patients with more advanced liver disease and uncomplicated primary cancer, the therapeutic strategy may be reversed to avoid the risk of liver tumour progression to unresectability. This option is termed "reverse" or "liver-first" approach[10,75,76], and is usually considered in patients with borderline resectable liver disease and uncomplicated primary tumour, or when a locally advanced rectal cancer eligible to neoadjuvant chemoradiotherapy (CHRT) is present[9,10,75,77,78]. A complete response of the rectal tumour to CHRT after initial liver surgery has been occasionally described, thus delaying or even avoiding the planned rectal resection[78]. However, simultaneous colorectal and liver resection represents the most attractive strategy, with growing consensus and a progressive expansion of resectability criteria[6,28]. Simultaneous resections improve the patient experience, by reducing the number of surgical procedures and also the duration of perioperative CHT in selected cases[4,17], and may substantially decrease the cumulative costs of hospitalization[79]. Nonetheless, the real impact on the perioperative results and the overall oncological outcome are still under debate[1,3].

Numerous experimental studies suggest that surgical manipulation of metastatic CRC can activate inflammation, immune depression, release of multiple factors and shedding of tumour cells[80]. These events can exert local tumour-promoting effects that predispose to local recurrences, but also activation of dormant tumour cells in distant organs, thus predisposing to the development of distant metastases[80]. Furthermore, LR soon activates multiple molecular changes to restore the optimal liver volume, with upregulation of multiple growth factors and cytokines, and subsequent activation and proliferation of the intrahepatic cells. These specific pro-regenerative effects result in a complex microenvironment that can promote the proliferation of residual tumour cells in the remnant hepatic parenchyma and even the spread of cancer at distant sites[80-82]. In patients with multiple CRLM, extended LR may achieve potentially curative surgery. PVE with or without TSH has been proposed in selected patients to cause hyperplasia of the FRL and augment resectability. As for liver regeneration, however, also PVE has been demonstrated to promote tumour progression, either by intrahepatic haemodynamic changes or through an upregulation of growth factors and cytokines, that may adversely affect the subsequent management of the neoplastic disease[81-83]. Taken together, this clinical and experimental evidence supports the theoretical advantages of simultaneous resection of the colorectal and the liver tumours, to avoid the disadvantages of multiple surgical procedures, and of conservative liver surgery, to contain the adverse effects of liver regeneration on tumour development and dissemination.

Preoperative evaluation

The accurate preoperative staging is of paramount importance to plan the surgical strategy and can be achieved with cross-sectional imaging by CT, MRI[1,2,8,9,84] and 18FDG-PET-CT in selected patients, mainly to detect extrahepatic disease[1,5,9,84].

Table 1 Controversial issues involving surgical strategies for colorectal cancer with synchronous resectable liver metastases

Controversial issue	Advantages	Disadvantages
Surgical strategies for synchronous CRLM:		
• Traditional "staged" or "classic" approach	Risks of CRR and LR are not cumulated; CHT can be usefully administered before the LR	May determine progression of CRLM, sometimes up to unresectability; manipulation of metastatic CRC may have adverse effects on distant metastases and oncological outcome
• "Reverse" or "liver-first" approach	Avoids progression of borderline resectable CRLM; permits appropriate NACHRT for locally advanced rectal cancer, sometimes up to complete response	Comparative results with the traditional approach are still uncertain
• Simultaneous colorectal and liver resection	Reduces the number of surgical procedures; may reduce the duration of perioperative CHT; may decrease the cumulative costs of hospitalization	Requires accurate selection of candidates; may increase perioperative morbidity and mortality; oncological outcomes are still uncertain
NACHT of resectable CRLM	May reduce the extent of LR; may increase the R0 resection rates; eradicates micrometastases; may select patients with favourable oncological prognosis after LR	May determine progression of CRLM, sometimes up to unresectability; may determine parenchymal damage and increase perioperative morbidity; its overall beneficial impact on oncological outcomes has not been confirmed
Nonanatomic/parenchymal-sparing *vs* anatomic LR	May reduce the extent of LR; may increase resectability; may achieve better perioperative results; may favour reresection in case of hepatic recurrence, with consequent improvement of oncological results	May reduce the extent of the RM; its overall impact on oncological outcomes is still controversial
The prognostic role of the RM:		
• ≥ 10 mm	May reduce the overall risk of recurrence; may achieve better oncological outcomes	May reduce resectability
• 1 to 10 mm	May reduce the extent of LR; may increase resectability	May favour tumour recurrence; may determine worse oncological outcomes
• < 1 mm (R1 resection)	May increase resectability	Determines worse oncological outcomes; perioperative CHT is mandatory
• "R1 vascular" RM (detachment of CRLM from vessels)	May reduce the extent of LR; may increase resectability	May favour tumour recurrence; may determine worse oncological outcomes
Evaluation of genetic mutations of CRLM	Predict response to CHT; may predict response to perioperative CHT; may predict oncological results of LR; may predict positive RM in candidates for LR; may suggest more extensive/anatomical LR; may predict response to local (RFTA) and loco-regional (chemo and radioembolization) treatments	Its overall role in establishing individualized therapeutic strategies is still uncertain; its overall impact on oncological outcomes is still uncertain
Treatment of multiple bilobar CRLM:		
• NACHT of multiple resectable CRLM	May favour curative LR; may reduce the extent of LR; may increase the R0 resection rates; eradicates micrometastases; may select patients with favourable oncological prognosis after LR	May determine progression of CRLM, sometimes up to unresectability; may determine parenchymal damage and increase perioperative morbidity; its overall beneficial impact on oncological outcomes is uncertain
• PSLR *vs* major LR, including PVE, TSH and ALPPS	Reduces the extent of LR; may increase resectability; reduces the risk of PHLF; may achieve better perioperative results; may favour reresection in case of hepatic recurrence	May reduce the extent of the RM; its overall impact on oncological outcomes is still controversial
• Intraoperative local ablation techniques	May reduce the extent of LR; may increase resectability; may favour curative LR	Higher risk of local recurrence, especially for larger tumours; its overall beneficial impact on oncological outcomes is still uncertain
The impact of PSLR on simultaneous resections	May reduce the extent of LR; may increase resectability of CRLM; may improve the propensity for simultaneous resection; may achieve better perioperative results	May reduce the extent of the RM of LR; its overall impact on oncological outcomes is still controversial

CRLM: Colorectal liver metastases; CRR: Colorectal resection; LR: Liver resection; CHT: Chemotherapy; CRC: Colorectal cancer; NACHRT: Neoadjuvant chemoradiotherapy; RM: Resection margin; RFTA: Radiofrequency thermal ablation; NACHT: Neoadjuvant CHT; PSLR: Parenchymal-sparing liver resection; PVE: Portal vein embolization; TSH: Two-stage hepatectomy; ALPPS: Associating liver partition and portal vein ligation for staged hepatectomy; PHLF: Posthepatectomy liver failure.

Preoperative liver imaging should define the number and the location of CRLM, the tumour-vessels relationship, the pattern of the hepatic vasculature and the FRL volumes[23,84-86].

Definition of patient performance status, coexisting morbidities and liver steatosis is mandatory to determine suitability for complex procedures, especially if major liver surgery is considered[8]. Although up to 70% of the normal liver parenchyma can be excised, prior CHT may remarkably compromise liver parenchyma. Various degrees of non-alcoholic fatty liver disease, from bland steatosis to steatohepatitis, and of sinusoidal injury, from sinusoidal dilation to hepatic sinusoidal obstruction syndrome and regenerative nodular hyperplasia, have been associated with modern CHT protocols[87]. Parenchymal damage is regimen specific: oxaliplatin-based regimens have been associated with significant sinusoidal injury, and irinotecan-based regimens with various degrees of non-alcoholic fatty liver disease[87,88]; these parenchymal alterations may prejudice the liver function and the consequent ability to tolerate extended resections[89], while the impact of targeted molecular therapies is still controversial[90]. In a meta-analysis based on 28 studies, Robinson *et al*[88] found that NACHT before resection of CRLM determines an increased risk of regimen-specific liver damage, which may impact on the functional hepatic reserve of candidates for major hepatectomy[88]. To prevent or at least limit these adverse outcomes, extended preoperative CHT should be avoided, and an appropriate interval between CHT completion and liver surgery should be planned[1,8,9,87].

Neoadjuvant chemotherapy for resectable CRLM

Evaluation of the available CHT protocols to enhance resectability of initially unresectable SCRLM is out of scope for this review. In patients with resectable CRLM, the role of NACHT is still controversial. After considerable enthusiasm toward systemic NACHT, mainly based on the fact that response to preoperative CHT may select patients with favourable oncological prognosis after LR[10], its overall beneficial role has been substantially questioned by multiple recent studies. The EORTC Intergroup randomized controlled trial (RCT) 4098386 compared perioperative oxaliplatin-based CHT plus LR to LR alone, in patients with limited resectable CRLM (≤ 4) at baseline assessment[91]; the overall results revealed an improved progression-free survival at 3 years after perioperative CHT, but significantly more frequent reversible postoperative complications. Nonetheless, this study received much criticism[16,18], and in the long-term follow-up report of the trial, the OS rates were not different between groups[92]. A systematic review evaluating the impact of systemic NACHT on resectable CRLM indicated that preoperative CHT may determine objective response with improved disease-free survival (DFS)[93], but also this review was deemed to have substantial limitations to influence the conclusions[18]. Another systematic review concluded that combination regimens increased cancer response and resectability rates in case of unresectable CRLM, while studies on NACHT failed to definitely prove a survival benefit for resectable tumours, with enhanced risks of perioperative complications[90]. In the new EPOC RCT[94], the addition of cetuximab to perioperative systemic FOLFOX CHT of *KRAS* exon 2 wild-type resectable or suboptimally resectable CRLMs resulted in unexpected shorter progression-free survival rates than systemic CHT alone; these disappointing results were related to disease progression consistent with failure of systemic micrometastatic disease control[95] and have been confirmed in the updated analysis of this study[96], where patients in the cetuximab group experienced significantly worse OS rates. Recent retrospective series do not support the use of NACHT in upfront resectable CRLM. A study based on the LiverMetSurvey International Registry could not find any survival advantage for NACHT plus LR in resectable CRLM compared to surgery alone[97]. In a multicentre series of 300 patients with upfront resectable CRLM collected between 2008 and 2015 in 2 French institutions, which favoured perioperative FOLFOX CHT, and 2 Japanese institutions, which systematically preferred upfront LR plus adjuvant CHT[98], perioperative FOLFOX CHT did not improve DFS compared to adjuvant CHT alone after LR. The potential adverse effects of NACHT on morbidity, mortality and oncological outcome of candidates for LR[90], and in determining a shift in the growth pattern of CRLM, from a pattern with a good prognosis to another with a worse prognosis[99-101], represent further controversial issues. Nevertheless, preoperative CHT is still the preferred option in case of resectable CRLMs in some Western countries[8]. In patients with CRC and resectable SCRLM, preoperative CHT is usually advocated in Western countries, while upfront simultaneous resections are usually considered, if they can be safely performed, in Asian countries, although there is no significant evidence to support either therapeutic strategy[5,9,98].

Simultaneous vs staged colorectal and liver resection

Perioperative and long-term outcomes of simultaneous *vs* staged procedures for SCRLM have been compared in many recent systematic reviews and meta-analyses[11-13]. Simultaneous procedures were usually compared to staged approaches where the SCRLM were resected at a later stage. Although these series show a somewhat shorter hospital stay and lower morbidity rates for simultaneous resections, postoperative mortality rates seem to be lower with the staged procedures in some series, while long-term survivals are similar between the strategies[11-13]. However, the studies included in these systematic reviews and meta-analyses had a general bias, since staged approaches were usually favoured in patients with left-sided CRC and more advanced liver disease. Yin *et al*[14] performed a systematic review and meta-analysis including 2880 patients and found that patients in the simultaneous group had lower perioperative complications, whereas perioperative mortality within 60 d, OS and recurrence-free survival (RFS) rates were similar. In a wider meta-analysis evaluating 3159 patients[15], the authors suggested that patients undergoing delayed LR may achieve better outcomes, since they had similar intraoperative parameters, perioperative complications and survival rates compared to patients with simultaneous resection, despite having a more extensive liver disease. However, a subsequent meta-analysis including 4494 patient[16] questioned the reliability of some previous meta-analyses as a consequence of important biases, mainly the fact that significantly more patients with mild conditions received simultaneous resections, and found comparable perioperative and long-term oncological results between simultaneous and staged resections after correction of baseline imbalance regarding primary tumour and metastases characteristics. Similar results were found in another recent meta-analysis evaluating 5300 patients[17]. However, the numerous studies comparing simultaneous and classical staged resections with CRR followed by hepatectomy should be interpreted with caution, because simultaneous resections are more likely considered for patients with better clinical conditions, right-sided CRC and more limited liver disease[11,14-18]. On the other hand, the staged groups more frequently include patients who respond to perioperative CHT[15-17], while patients who do not complete the planned LR due to disease progression during the interval CHT are excluded from evaluation: consequently, the oncological results of patients selected for staged procedures may be overestimated comprising only those with more favourable tumour biology or responsive to perioperative (neoadjuvant and/or interval) CHT. Further studies should prevent this selection bias by using "intention-to-treat" analyses, including also patients with progressive metastatic disease after CRR who missed the subsequent LR[16]. A small prospective RCT that involved 10 French tertiary referral centers specialized in colorectal and hepatobiliary surgery, has recently compared simultaneous *vs* delayed colorectal-first resection in patients with CRC and resectable SCRLMs[102]; the study was discontinued because of recruitment problems, so that only 85 patients were finally evaluable, 39 in the simultaneous and 46 in the delayed resection groups, respectively. Major perioperative complication rates were similar between groups; in the delayed resection group, 8 patients did not reach the LR stage, due to disease progression in 6 cases; 2-year OS and DFS rates tended to be improved in simultaneous resection group (P = 0.05), a tendency which persisted for OS at multivariate analysis after a median follow-up of 47 mo (P = 0.07). The authors recognized the numerous limitations of their study and cautiously suggested that simultaneous resection of the primary CRC and of the resectable SCRLMs is an acceptable strategy, even though delayed treatments still has an important role in these complex patients.

Some recent studies have compared all the available surgical strategies, simultaneous *vs* staged primary-first *vs* staged liver-first resections. In a small series of patients with rectal cancer and SCRLM, van der Pool *et al*[103] suggested an individualized approach, where both simultaneous and liver-first approaches were effective alternatives to traditional staged primary-first procedures. In another study evaluating 156 consecutive patients, Brouquet *et al*[75] found comparable 3- and 5-year OS rates for the three different strategies. Likewise, a multi-institutional study[76] with over 1000 patients found similar oncological outcomes for the three groups; male sex, a rectal primary and combined LR plus ablation were independent factors of worse long-term prognosis; thus the authors suggested that tumour biology rather than the surgical procedure is the main determinant of prognosis. More recent robust systematic reviews and meta-analyses confirmed previous results, with comparable perioperative and oncological results for the three surgical strategies[104-106]. In a population-based study referring to 1830 patients with CRC and SCRLM who underwent colorectal and liver resection with bowel-first, simultaneous or liver-first approach, and were included in the English National Bowel Cancer Audit dataset,

Vallance *et al*[107] found a progressive increase in the use of either simultaneous or liver-first approach over the study period, along with wide variations among different hospital trusts. A simultaneous approach was more frequently adopted where a local hepatobiliary unit was present. There was no difference in 4-year survival rates between the propensity score-matched groups according to surgical strategy. A very recent network meta-analysis based on 32 retrospective studies has compared the three surgical strategies and again found no significant differences in major morbidity and 5-year survival rates[108].

PARENCHYMAL-SPARING LIVER RESECTION

Resectability of liver neoplasms has considerably improved over the last decades. At present CRLMs are mostly considered resectable if complete cancer excision can be achieved with curative intent, *i.e.* when macroscopically free RMs are resulting, without unresectable extrahepatic disease, and the estimated FRL is sufficient to avoid liver failure[109]. Most surgeons still usually consider major LR, including conventional major hepatectomies and two-stage procedures, to achieve curative resection, particularly in case of large and/or multiple tumours. However, extensive LRs have been related to significant perioperative complications, mainly due to various degrees of PHLF[22,23]. The progressive expansion of IOUS as an essential tool in liver surgery has favoured the diffusion of more conservative hepatectomies to reduce the risk of PHLF[23], but also to spare major intrahepatic vessels and increase salvageability in case of recurrence[27,28] (Table 1). Conservative procedures are based on at least three factors: (1) The intrahepatic diffusion patterns of CRLMs are different from that of the hepatocellular carcinoma so that anatomic resections (AR) per se have limited or no effect on the clinical outcome; (2) The concept of "negative resection margin" without considering margin width has progressively replaced the "1-cm rule"[110]; and (3) There is increasing evidence that also multiple and/or bilobar CRLM are eligible to potentially curative hepatic surgery in the context of multimodal treatment strategies.

Anatomic vs nonanatomic liver resection

Liver tumours should be resected with enough margins to achieve potentially curative treatment and to prevent recurrence. The propensity of hepatocellular carcinoma for vascular invasion and metastatic spread through the portal venous system requires AR whenever possible as it eradicates the portal tributaries near the tumour. AR may reduce the risk of local recurrence and achieve better survival rates than nonanatomic resection (NAR)[111,123]. The expert use of IOUS has favoured the development of surgical techniques that limit the extension of hepatectomies while respecting the segmental or subsegmental distribution of intrahepatic vessels, either for primary or metastatic liver tumours[28,112-114]. Metastatic tumours can spread within and outside the liver through lymphatic vessels, portal and hepatic veins, bile ducts and perineural spaces[115,116]. Migration of tumour cells from CRLM through intrahepatic lymphatic vessels adversely affects survival[115,117], while the prognostic role of portal or hepatic vein invasion is still uncertain[115,116]. Accordingly, AR comprising portal vessels close to the cancer and the corresponding hepatic tissue should not be theoretically justified for CRLM, and NAR with adequate RM is actually regarded as a proper surgical option[23,86,118-122]. In a meta-analysis including 1662 patients with CRLM, NAR reduced the blood transfusion requirements and operation times compared to AR, while perioperative morbidity, mortality, surgical margins, OS and DFS rates were similar[118]. Another systematic review including 2505 patients compared PSLR to AR for CRLM[119] and found a similar incidence of R0 resection, postoperative length-of-stay and OS. A more recent meta-analysis based on 18 studies including 7081 CRLM patients compared the clinical outcome of PSLR (n = 3974) and non-PSLR (n = 3107)[123]; the perioperative outcomes were better in PSLR than in non-PSLR group, since non-PSLR was significantly associated with longer operative time, increased estimated blood loss, higher intraoperative transfusion rate, and more postoperative complications; OS and RFS rates were similar between groups. However, since the authors included segmental resections among PSLRs, we consider that the results of their comparison should be referred to limited *vs* major LR. The clear evidence that non-anatomical limited LR for CRLM were equivalent to major anatomic LR in patients with limited hepatic disease came from Japanese series since the early 2000s[23]. Kokudo *et al*[120] compared major AR to limited NAR in patients with unilobar single or double tumours and reported similar survival rates, concluding that major AR was unnecessary in 80.4% of the patients resectable by limited NAR. They thus

suggested to consider limited NAR as the mainstay surgical procedure for CRLM to minimize surgical stress and operative risks. In a series of 300 patients with a solitary CRLM ≤ 30 mm, Mise *et al*[121] compared PSLR to more extended hepatectomies, including right hepatectomy, left hepatectomy, or left lateral sectionectomy and found that OS, RFS, and liver-only RFS were similar between the groups, but PSLR significantly increased the opportunity of salvage repeat LR and 5-year OS in case of relapse. These results have been confirmed in a multicentric cohort of 1720 patients receiving either PSLR or right hepatectomy for a single CRLM ≤ 30 mm located in the right hemi-liver[122], where PSLR had significantly lower rates of major complications and 90-d mortality; although 5-year OS, RFS and liver recurrence rates were similar between groups, in patients with liver-only recurrence, repeat LR was more frequently performed after PSLR, with significantly higher 5-year OS rates. Taken together, these data suggest that a combination of a first parenchymal-sparing NAR followed by repeat hepatectomy in case of recurrence offers superior oncological benefits compared to major LR in most patients with limited liver disease[120-122]. Similar results have been described also in case of two or more CRLM. A recent case-matched study by Lordan *et al*[124] comparing 238 patients with PSLR to 238 patients with major LR, found fewer blood transfusions, lower incidence of severe complications, lower 90-d mortality and shorter hospital stay in PSLR patients, while OS and DFS rates were similar. The authors concluded that conservative LR should be proposed whenever technically feasible because it is safer than major LR, without compromising oncological results. Also in case of deeply placed CRLM, where major LR are traditionally preferred, PSLR resulted in similar perioperative and oncological results compared to major LR, increasing the number of patients eligible for direct LR without the need of PVE[125].

The advantages of PSLR have been confirmed also for mini-invasive LR. In a recent series of 269 patients who underwent LLR with curative intent for CRLMs, after propensity score matching 82 patients undergoing PSLR where compared to 82 who received major LR[126]; PSLR was associated with lower rates of major perioperative complications compared to major hepatectomy; RFS and liver-specific RFS rates were comparable between groups, but salvage repeat LR for hepatic recurrence was more frequently performed in the PSLR group; in case of hepatic recurrence, the OS rate was significantly higher for patients undergoing salvage repeat LR than for those who were unable to receive further curative treatment; the PSLR group also showed a trend toward higher 5-year OS rates. Thus, the authors concluded that PSLR should be the standard approach for CRLMs, even for mini-invasive procedures.

The liver resection margin

The impact of the width of the RM on the oncological outcome after LR for CRLM is controversial (Table 1). The so-called "1-cm rule", which advocates that R0 margins should be 10 mm or greater to prevent local recurrence and optimize OS, has been proposed since the 1980s and is still considered basically valid whenever technically feasible[24,110,127]. The presence of residual microscopic deposits of tumour cells on the resection margin (R1) is regarded as an important source of recurrence and a critical determinant of poor prognosis[116,127]. As for primary liver tumours, intrahepatic micrometastases (IHM) may develop in CRLM, are believed to represent re-metastasis from existing tumours, and are predominant within 4 to 10 mm of the tumour margin[28,128,129]. However, their role as a prognostic factor is controversial. One study reported that IHM is associated with higher incidence of intrahepatic recurrence and poorer survival[130]. In another study, IHM was less frequently found in patients who received NACHT than in those untreated[128]. In a study detecting tumour-specific mutant DNA in hepatic parenchyma surrounding metastases, mutant DNA was found in surrounding liver parenchyma within 4 mm of the tumour border, but not at 8, 12, and 16 mm from the tumour margin, even after tumour shrinkage due to NACHT[129]. The presence of fibrotic tissue between the CRLM and the surrounding parenchyma has also been identified as a beneficial prognostic factor and may be relevant in the assessment of the RM[115]. CRLMs showing an infiltrating growth pattern, where cancer cells spread freely through the surrounding normal liver parenchyma, have been mostly associated with worse overall oncological outcome compared to metastases with an expanding growth pattern, where cancer cells push the adjacent liver tissue, although some controversy still exists[115]. Vermeulen *et al*[131] classified metastatic growth into three different histopathological growth patterns (HGP), based on the interface between metastatic cells and the surrounding normal liver parenchyma, and the related angiogenic patterns[131]: In desmoplastic HGP, the neoplastic cells are separated from the surrounding liver parenchyma by a rim of desmoplastic tissue, there is no direct contact between cancer cells and hepatocytes, and new blood vessels

in the desmoplastic rim are formed by sprouting angiogenesis; in the pushing HGP, there is no desmoplastic rim surrounding the metastatic nodule at the interface with the liver tissue, and the surrounding liver parenchyma is pushed away and compressed, without direct contact between cancer cells and hepatocytes within the liver cell plates; in the replacement HGP, cancer cells replace hepatocytes within the liver cell plates and co-opt the sinusoidal blood vessels at the tumour–liver interface, without inducing sprouting angiogenesis, so that metastatic cells form cell plates that are in continuity with the liver cell plates, and the stromal architecture of the liver is maintained[99,101,131]. Mixed growth patterns are usually found in single patients with multiple liver metastases, but also in a single metastasis[99,101]. Desmoplastic HGP has been associated with better oncological outcomes[99,101,132], even though its prognostic role was not confirmed in patients undergoing NACHT before LR[101]. The unfavourable prognostic impact of any non-desmoplastic HGP on the incidence of R1 margin and the OS rates has been recently confirmed in a bi-institutional series of 1302 patients with surgically resected CRLM[133].

Altogether, these data demonstrate that CRLM may be well-circumscribed, with a very low incidence of satellite nodules or micrometastases, suggesting a limited effect of minimal negative RM on recurrence or survival rates in selected patients[6,24]. Pawlik *et al*[134] have reported that OS and DFS, overall recurrence risk and site of recurrence were similar after resection of CRLM with margins of 1-4 mm, 5-9 mm, and ≥ 10 mm, suggesting that a predicted RM of < 1 cm should not contraindicate liver surgery. Other studies have confirmed that sub-centimetric tumour-free RM may have limited or no negative impact on the oncological outcome after LR for CRLM[135,136]. Recent meta-analyses however still suggest that the "1-cm rule" have an independent positive prognostic effect on OS and DFS and should be pursued whenever possible, even though a predicted sub-centimetric RM should no longer be considered a contraindication to surgical resection[137-139].

Microscopically positive RM (< 1 mm) is currently believed to significantly worsen overall oncological results of LR for CRLM, due to an increased risk of recurrence at the surgical margin[117,134,140] and of intrahepatic recurrence[140,141]. An increasing number of CRLMs has been associated with greater risk of R1 resection[133,135,142]. Tranchart *et al*[143] reported that R1 LR was an independent unfavourable predictor of OS and DFS, and that only administration of postoperative CHT predicted improved DFS after R1 LR. Further studies have confirmed either the adverse effect of R1 LR on survival[133,134,136,142] or the protective effect of postoperative CHT after R1 LR[141,144,145]. A favourable impact of NACHT on the oncological outcome of R1 LR has been also observed[146], especially in tumours responsive to CHT[147,148], but this point is still controversial[133,145]. The cessation of NACHT, however, regardless of previous response, may be followed by tumour regrowth, with clusters of viable tumour cells infiltrating the normal hepatic parenchyma for several millimetres at the periphery of the metastases, a phenomenon called "dangerous halo"[100]. Similarly, NACHT may determine irregular borders of metastatic lesions, especially after significant contraction, and sometimes discrete clusters of viable cancer cells are found outside of the main lesion, but near its peripheral margin[149]. Moreover, NACHT can alter the growth pattern of CRLM favouring the emergence of more aggressive patterns[99,100]. The possible progression of the dangerous halo is particularly worrying, and LR should achieve RM wide enough to reduce the risk of local relapse[100], particularly if CHT has been suspended for a relatively long time.

Recent studies suggest that also a submillimetric clear RM can be considered adequate for CRLM in certain circumstances[142]. The detachment of CRLM from intrahepatic vessels has been proposed as part of IOUS-guided PSLR[114]. Even though this procedure formally implies a R1 RM, the reported oncological results have been similar to those of R0 LRs, suggesting that tumour detachment from intrahepatic vessels can be safely achieved to expand resectability[150]. Other studies have questioned the role of R1 margin status as an independent predictor of survival since it was not related to survival after checking for competing risk factors on multivariate analysis[134,140,141]. Tumour biology has been suggested to play a determinant role on the long-term results, where R1 resections might not have a prognostic value per se, but rather reveal more aggressive disease[24,27,127,134,141,144]. Recent changes in the prognostic value of R1 LR might be partially related to the beneficial effect of perioperative CHT[143-147]. However, a recent multicentric retrospective cohort of 1784 hepatectomies confirmed the independent adverse effect of R1 LR compared to R0 LR, affecting both OS or DFS rates in patients with CRLM[151].

Clinical and prognostic relevance of genetic mutations of CRLMs

The growing interest in genetic data and mutational status of primary and metastatic CRC is based on the increasing relevance of genetic mutation analysis of CRLM to prognosticate oncological outcomes of candidates for either systemic CHT or liver-directed therapies, including surgery[7-9,152-154]. The *RAS* oncogene (*KRAS*, *NRAS*, and *HRAS*) is involved in complex *RAS* signalling pathways that affect multiple cancer-driving processes. These include neoplastic drift of normal tissues, enhancement of tumour cell growth and suppression of cell death responses, and modulation of the tumour microenvironment by stimulating pro-angiogenic mechanisms and altering host-related immune responses, which finally promote local invasiveness and metastatic progression of tumour cells[152]. From 15% to 50% of patients receiving LR for CRLM have a *RAS* mutation[152], and the *KRAS* mutation accounts for 14% to 52% of the mutations in the *RAS* pathway in resectable CRLM[155]. *RAS* mutations have been associated with a higher prevalence of lung metastases and to specific patterns of recurrence after LR, especially at extrahepatic sites, and usually predict worse OS and DFS rates than wild-type tumours[9,152-156]. *RAS* mutations have been related to a higher incidence of positive margins after LR[157], and also the width of the RM has been suggested to have a different prognostic impact according to *RAS* mutational status[155]. Moreover, ARs determined better DFS and lower intrahepatic recurrence rates in patients with *RAS* mutations, suggesting that more extensive hepatectomies are required for more aggressive mutated CRLM[158]. *RAS* mutations determined worse oncological results also in candidates for repeat LR of recurrent resectable CRLM, in patients who received TSH for bilobar liver metastases, and in patients with synchronous liver and lung metastases undergoing liver surgery[152].

Similar to *RAS*, the *BRAF* oncogene interferes with signalling pathways involved in cell division and differentiation[152]. *BRAF* mutations occur in about 10% of patients with CRC and usually determine poor oncological outcomes[152]. *BRAF* mutations are present in a minority of patients with resectable CRLM, but have been associated with aggressive clinical behaviour and worse oncological outcome among candidates for LR, compared to both wild type *BRAF* and *KRAS* mutated tumours[8,152-156,159]. Other significant gene mutations, including *TP53*, *PIK3CA* and *SMAD4*, have been recently reported, with controversial conclusions about their prognostic impact in candidates for surgery of CRLM[8,152,153,155]. Triple mutation in *TP53*, *RAS* and *SMAD4* has recently been associated with worse OS and RFS rates after resection of CRLMs, compared to double mutations in any two of the three genes[7]. Moreover, in patients harbouring multiple CRLM, mutation heterogeneity for at least one gene across metastatic deposits determined worse prognosis after LR compared to homogeneous mutations, suggesting that worse oncological outcomes are associated with heterogeneous disease[160].

RAS mutation status may affect the oncological outcome even in candidates for percutaneous radiofrequency thermal ablation (RFTA)[152], hepatic arterial infusion, transarterial radioembolization and chemoembolization of CRLM[7,153,155]. Taken together, these data suggest that the mutational status of metastatic CRC might contribute in the future to appropriately select patients who can experience a survival benefit from LR, to define the optimal sequence of perioperative CHT, liver surgery and other effective loco-regional treatments, to identify patients at higher risk of recurrence after LR, and possibly to establish individualized therapeutic strategies[152-155].

Therapeutic strategies for multiple bilobar liver metastases

In a series of 141 patients who received LR for CRLMs published in 1984, Adson *et al*[161] found similar 5-year OS rates between patients with single and those with multiple lesions. Subsequently, Ekberg *et al*[110] suggested that poor prognostic factors contraindicating surgery included > 4 lesions, impossibility to obtain a RM ≥ 1 cm and presence of extrahepatic disease. In the following years however radical LR of multiple (≥ 4) CRLM was confirmed to be compatible with long-term survival[162,163], with a beneficial effect of NACHT in case of multiple bilobar tumours[164] (Table 1). For patients with extensive bilobar disease, surgeons from the Paul Brousse Hospital proposed complex therapeutic strategies combining ablative techniques, PVE, TSH and NACHT[165-167]. In the same period, in a series of 183 Japanese patients who underwent LR for CRLM between 1980 and 2000, Kokudo *et al*[85] reported a 5-year OS of 41.9%, with an overall outcome of 21 patients with ≥ 4 CRLM similar to that of patients with ≤ 3 CRLM. These authors actually defined the following principles of conservative surgical strategies for multiple liver metastases: Careful preoperative assessment of the number of nodules and their contiguity to the major intrahepatic

vessels; meticolous intraoperative inspection, palpation and IOUS of the liver; multiple partial resections whenever possible, rather than extended hepatectomies; resection of large intrahepatic vessels only in case of neoplastic invasion; NAR even with minimal RM; and preoperative PVE whenever the calculated volume of the FRL was less than 40% in case of major hepatectomy. The remnant liver was the most common site of relapse in the overall series, and repeated LR was achieved in about half of these patients, with a 5-year OS rate of 44.7% starting from the first LR[85]. Torzilli *et al*[168] subsequently reported a similar approach to multiple (≥ 4) bilobar CRLMs in a small series of 29 patients where the operative strategy was based on tumour-vessel relationships and findings at colour Doppler IOUS, and concluded that one-stage IOUS-guided LR is safe and effective in selected patients with multiple bilobar CRLMs, decreasing the need for TSH.

In recent years, ablative techniques that achieve local tumour destruction by heating, comprising RFTA and microwave ablation, have become increasingly widespread as an attractive option to treat primary and metastatic liver tumours, alone or in combination with LR[8]. Ablative techniques for CRLM have usually shown significantly lower complications, but also higher recurrence rates and lower OS when compared to LR[169-171]. The efficacy of RFTA is considered equivalent to liver surgery for small (≤ 2 cm) CRLM[170], and an ablation margin > 1 cm has been associated with better oncological results[7]. Therapeutic strategies combining LR with intraoperative ablation techniques proved to be effective in increasing resectability of multiple bilateral CRLM[26], with overall oncological results comparable to those of bilateral LR or TSH. They can therefore represent an effective choice for successfully pursuing parenchymal-sparing treatments for extensive disease in selected patients[7,26,67,172-174], also in case of laparoscopic procedures[126]. The choice between RFTA and microwave ablation should be based on the features of the liver tumours and their anatomical relationship with the main intrahepatic vessels[26].

Actually, a progressive shift toward more conservative procedures for bilobar CRLM, eventually including intraoperative ablations, has been recently reported by surgeons traditionally inclined to more extensive LR[32]. The beneficial results of PSLR were also documented in a retrospective multicentric series of patients with multiple (> 3) bilobar CRLM comparing PSLR to non-PSLR, defined as the resection of ≥ 3 consecutive hepatic segments[33]: PSLR was associated with lower complications and a shorter stay in the intensive care unit, while OS and DFS were similar between groups. The beneficial impact of PSLR for the treatment of multiple bilobar metastases has been confirmed by others, questioning also the consolidated role of TSH[31,67]. Also selected patients with a large number of liver metastases are potential candidates for liver surgery. In a bi-institutional series comparing 736 patients with 1-3, 4-7 and ≥ 8 CRLM, respectively, multivariate analysis revealed that decreased survival was associated with positive lymph node metastasis of the primary cancer, extrahepatic disease, tumour size > 5 cm, and tumour exposure during LR, indicating that the number of CRLM may have less impact on the prognosis than other prognostic factors[175]. In another series of 849 patients receiving LR for CRLM[176], 743 patients with 1-7 metastases were compared to 106 with ≥ 8 metastases: In the latter group, multivariate analysis recognized three preoperative adverse prognostic factors, including primary rectal cancer, no response to preoperative CHT and extrahepatic disease; patients with ≥ 2 risk factors had very poor outcomes, while those without risk factors had survival rates comparable to patients with 1-7 metastases. In a series of 529 patients with ≥ 10 CRLM derived from the LiverMetSurvey registry, a macroscopically complete (R0/R1) LR was obtained in 72.8% of patients and was correlated with a 3- and 5-year OS of 61% and 39%, respectively, being the strongest favourable factor of OS at multivariate analysis[177]; other independent favourable factors were age < 60 years, preoperative MRI, maximal tumour size < 40 mm, and adjuvant CHT. Therefore, the authors concluded that the number of CRLM per se should not contraindicate surgery, which gives the only hope of prolonged survival.

The impact of PSLR on simultaneous resection

The perioperative outcomes of simultaneous colorectal and minor liver resection, including mortality, severe morbidity, hepatic-related morbidity and blood transfusion requirements, are comparable to those observed for minor LR alone[2,4,12]. Results are much more conflicting for patients eligible for simultaneous colorectal and major LR. Some authors reported that combined procedures including major LR adversely impact on perioperative morbidity and mortality rates compared to major LR alone[3,76], while others did not observe added perioperative risks in these cases[19,20]. Currently, most authors suggest simultaneous procedures in case of uncomplicated, easily accessible CRC with liver disease requiring minor LR[13,14,178], while more

extended criteria should be reserved to units specialized in both hepatobiliary and colorectal surgery[11]. Actually, the planned extent of LR seems to represent the most important determinant of whether colorectal and hepatic procedures should be performed simultaneously[4,12,73,179] (Table 1). Since PSLR substantially decreases the need for major LR and the related perioperative risks, it may represent the most appropriate surgical strategy to associate a colorectal procedure with the resection of multiple and/or bilobar SCRLM[180]. In a small series of 39 patients who underwent simultaneous curative colorectal and liver resection for CRLM, Tanaka *et al*[73] reported that only the mean volume of the resected liver was a significant risk factor for perioperative complications (350 *vs* 150 g; $P < 0.05$); simultaneous procedures included 38.5% of low anterior resections and 5 major hepatectomies. The systematic application of PSLR criteria have been associated with higher rates of feasibility of combined resections also in case of multiple CRLM. In a series from the University of Tokyo, Minagawa *et al*[181] found that a simultaneous resection was feasible in 142 out of 148 evaluated patients (96%), regardless of the site of the primary tumour and the extent of CRLM, without perioperative mortality; half of the patients had rectal cancer, while only 11.3% of patients required a hemi-hepatectomy, based on their policy of PSLR[85]. In a more recent series of 150 patients[182] the feasibility of a simultaneous resection was 84.7%, with low postoperative major complications (18.2%), few anastomotic leaks (1.6%), and nil mortality; the 5- and 10-year OS rates were 64% and 52%, respectively. Similarly, in a small series of 45 patients who received elective resection of primary CRC and SCRLM[74], a simultaneous CRR with anastomosis and one-stage PSLR was feasible in 34 (75,6%), none of them requiring a right hepatectomy.

MINI-INVASIVE COLORECTAL AND LIVER SURGERY

Mini-invasive colorectal surgery

Laparoscopic surgery is presently considered the standard approach for surgical treatment of colon cancer[34,35], while its role for rectal cancer is still somewhat controversial (Table 2). Despite the longer operative time, laparoscopic rectal resection has shown superior short-term outcomes than open surgery, but pathological and oncological outcomes are equivocal. Vennix *et al*[37] reviewed 14 RCTs comparing laparoscopic to open rectal resection, and reported that the number of resected lymph nodes, surgical margins, long-term OS and DFS, and local recurrence rates were similar between groups. Similarly, a recent multicentric Japanese study analyzed 1500 patients operated for low rectal cancer and found significantly better perioperative results after laparoscopic than open surgery, while the 3-year OS and RFS rates were similar between groups[38]. On the contrary, a meta-analysis of 14 RCTs from Martínez-Pérez *et al*[39], comparing 1697 patients with laparoscopic rectal resection to 1292 patients with open rectal resection, found that the circumferential resection margin involvement, distal resection margin involvement, mean number of lymph nodes retrieved, mean distance to the distal and radial margins were similar between groups, but the risk of non-complete (nearly complete or incomplete) mesorectal excision was significantly higher in patients undergoing laparoscopic resection (13.2% *vs* 10.4%, $P = 0.02$). Likewise, in a subsequent meta-analysis of 14 RCTs, Nienhüser *et al*[183] found better oncological outcome for complete resection rate and the number of resected lymph nodes in favour of the open rectal surgery compared to laparoscopic surgery, but the long-term oncological outcome was similar between groups. The real impact of these histopathologic results on OS and DFS, however, is uncertain since long-term results of the ongoing RCTs are still awaited.

The role of robotic surgery in the treatment of rectal cancer is still to be established. A recent meta-analysis referred to 5 RCTs including 334 robotic and 337 laparoscopic surgery cases[36] showed that robotic surgery was associated with significantly lower conversion rate, but significantly longer operating time compared to laparoscopic surgery; perioperative mortality, rate of circumferential margin involvement, incomplete mesorectum, and mean number of harvested lymph nodes were similar between the groups. The authors noted however that, although patients were all operated by skilled surgeons, the rate of incomplete mesorectal excision was 23.5% for the robotic group and 25.6% for the laparoscopic group, comparatively higher than described in the current literature for open and conventional laparoscopic rectal resection[39]. Some recent small series suggest that robotic surgery could improve the quality of total mesorectal excision for rectal cancer compared to laparoscopic procedures[184], but these conclusions have not been confirmed by the available RCTs[36]. For all these reasons robotic surgery for rectal cancer can be selectively used,

Table 2 Controversial issues involving mini-invasive (laparoscopic and robotic) surgical strategies for colorectal cancer with synchronous resectable liver metastases

Controversial issue	Advantages	Disadvantages
Mini-invasive *vs* open colorectal surgery	Achieves better perioperative results; achieves similar oncological results	In case of rectal resection, may determine a higher risk of suboptimal oncological results at histopathology; in case of rectal resection, its overall impact on oncological outcomes is still uncertain
Mini-invasive *vs* open liver surgery	Achieves better perioperative results; achieves at least similar oncological results; rapid technological evolution; rapid growth of surgical experience and skill	Usually preferred for limited disease, in favourable locations and selected patients; may determine more complex and longer procedures; may determine more extended hepatectomies; less frequently used for major LR, including TSH and ALPPS, and for CRLM in postero-superior segments and in the caudate lobe; may determine higher costs
Mini-invasive *vs* open simultaneous colorectal and liver resection	Achieves better perioperative results; achieves similar oncological results	Usually preferred for limited liver disease, in favourable locations, and higly selected patients; may determine more complex and longer procedures; may determine higher costs
Mini-invasive *vs* open PSLR	Achieves better perioperative results; achieves similar oncological results; rapid technological evolution; rapid growth of surgical experience and skill	The principles of PSLR are time-consuming and rather difficult to apply during mini-invasive procedures; usually preferred for limited disease, in favourable locations and selected patients; may determine more complex and longer procedures; may determine higher costs
The impact of PSLR on mini-invasive simultaneous resection	May achieve better perioperative results; may achieve similar oncological results	May determine more complex and longer procedures; may have very limited indications

LR: Liver resection; TSH: Two-stage hepatectomy; ALPPS: Associating liver partition and portal vein ligation for staged hepatectomy; CRLM: Colorectal liver metastases; PSLR: Parenchymal-sparing liver resection.

giving appropriate consideration to the extra cost and time requirements[40].

Mini-invasive liver surgery

The use of minimally invasive techniques of LR, including LLR and robotic-assisted LR, has rapidly increased in the last decade[41,43] (Table 2). Nevertheless, the acquisition of adequate experience in mini-invasive LR is difficult, requires specific complex training with a prolonged learning curve, and may be accompanied by a significant increase of costs per procedure[42,43]. As for conventional open liver surgery, also mini-invasive techniques are evolving toward more complex procedures. However, at present, the vast majority of mini-invasive LR are minor and mainly involve anterior and inferior liver segments (segments S2, S3, S4b, S5 and S6)[43,44]. Major LR including 3 or more segments, and resection of the postero-superior segments (S4a, S7 and S8) and caudate lobe are still considered challenging, although increasingly performed in most experienced centers[41-43,45,46]. Mini-invasive procedures have been successfully proposed also for TSHs, including ALPPS[47,48]. Hand-assisted or hybrid approaches are selectively adopted in difficult procedures[42,43]. Multiple recent studies have underlined the advantages of mini-invasive LRs. In an extensive literature review examining the comparative benefits of laparoscopic *vs* open LR in 2473 patients[49], LLR had better perioperative results, without differences in complication rates, survival and total hospital costs. Besides, the long-term oncological results of LLR for primary or metastatic liver malignancy are believed to be similar to those of open procedures[41,50]. Likewise, a random-effects meta-analysis of 8 case-matched series by Schiffman *et al*[51] comparing LLR to open LR for CRLM, found significantly better perioperative results in the LLR group, with comparable operative times, and similar 5-year DFS and OS rates. Although a wider and more recent meta-analysis including 4591 patients confirmed previous results[52], the authors underlined that, given the selection bias in the examined series, their results might only be referred to highly selected patients with few, small, peripherally located, and unilobar CRLM. To limit the confounding effects of selection bias in nonrandomized trials comparing LLR *vs* open LR, Zhang *et al*[53] have recently conducted a meta-analysis of 10 studies with propensity score-based analysis including 2259 patients with CRLM; two studies included patients with simultaneous colorectal and liver resection, and 3 studies included > 40% of major hepatectomies in both laparoscopic and open groups. Perioperative results were better in the laparoscopic group, although with significantly longer operative time; mortality rates, R0 resection, tumour recurrence and 5-year OS were similar between groups. However, a recent meta-analysis of individual patient data from 2 RCT and 13 propensity-score matched studies have raised the question of the oncological outcome of mini-invasive compared to open liver surgery for

CRLM[185]: The authors examined 3148 patients who received LLR (n = 1,275) or open LR (n = 1,873), and found a survival benefit in favour of LLR at 3 (P = 0.0030), 5 (P = 0.0025), 10 (P = 0.0035) and 15 (P = 0.0048) years from surgery, respectively; the survival advantage was not evident for patients undergoing simultaneous colorectal and liver resections; furthermore, no survival advantage was found when the meta-analysis was limited to the 473 patients included in the 2 RCTs. The authors cautiously concluded that the unexpected long-term survival benefit in favour of LLR suggests that laparoscopy is at least not inferior to the standard open LR for CRLM. A survival advantage of LLR for CRLM at 3 years from surgery was also found in the meta-analysis reported by Parks *et al*[186], while LLR was associated with better 3-year OS but similar 5-year OS than open LR in the previously cited meta-analysis by Zhang *et al*[53]. These differences in the OS rates were not confirmed in other studies, including multicentric series[50] and meta-analyses[51,52], so that the question of the overall oncological outcome of mini-invasive techniques compared to open surgery for CRLM remains controversial. Robotic LR is currently considered an effective alternative to LLR[155,188]. Compared to laparoscopic procedures, robotic-assisted LR has been associated with longer operative times, higher rates of Pringle manoeuvre, higher intraoperative blood loss and higher costs, while the other perioperative outcomes are comparable[54,189]. Oncological outcomes, including margin status, DFS and OS rates, were similar in a recent multicentric study comparing the two mini-invasive techniques[54].

It should be underlined however that in these series, patients undergoing mini-invasive LR were in most cases highly selected with regards to tumour size, number of liver lesions and tumour location, so it seems inappropriate to generalize their perioperative and oncological results to the current population of patients with resectable CRLM, who frequently have more severe liver disease. In recent multicentric series where case-matched analyses were adopted to obtain well-balanced cohorts and appropriately compare outcomes, the unmatched initial cohorts of patients with open LR had significantly more advanced metastatic disease than those with LLR[50,55,56], as reflected by more frequent preoperative CHT, higher incidence of concomitant extrahepatic disease, bilobar distribution, and a higher number of tumours and larger tumour size. Besides, the surgical procedures were substantially different, since patients with open LR underwent more limited resections, multiple resections, with more use of preoperative PVE, hepatic pedicle clamping, or combined treatments with RFTA. Also in case of CRLM located in the postero-superior liver segments, still considered challenging locations for mini-invasive procedures, LLR has been selectively adopted for superficial, solitary, and small CRLM (up to 30 mm), not proximal to major vessels[57]. Taken together, these data demonstrate that most surgeons still consider mini-invasive procedures for highly selected patients with limited liver disease in favourable locations, which in fact represent a minority of potential candidates for curative resection of CRLM.

Mini-invasive vs open simultaneous colorectal and liver resection

Based on the growing consensus toward simultaneous resection of CRC and SCRLM, mini-invasive techniques have been applied also for simultaneous procedures (Table 2), even including major LR[58,60,61]. In a recent meta-analysis, the authors compared 164 mini-invasive to 213 open simultaneous resections of CRC and SCRLM[62]: The mini-invasive approach resulted in lesser surgical blood loss and shorter length of postoperative stay, while operating time, operative blood transfusion, intestinal function recovery time, postoperative complications, OS and DFS rates were similar between the groups. In another meta-analysis involving 502 patients with CRC and SCRLM[63], 216 receiving a mini-invasive procedure and 286 an open procedure, mini-invasive surgery was associated with less intraoperative blood loss and blood transfusion, faster recovery of intestinal function and diet, and shorter postoperative hospital stay; operation time and overall postoperative complication rates were similar between groups, as were the OS and DFS rates, respectively. However, also these series mainly included patients with limited liver disease, since mean/median tumour size of CRLM was 19 to 55 mm, and mean/median number of nodules was 1.0 to 2.0. Therefore, as previously discussed for mini-invasive LR, also for simultaneous resections the perioperative and oncological outcomes of mini-invasive procedures cannot be extended to the current population of candidates for simultaneous colorectal and liver resection, which frequently includes patients with more advanced neoplasms or requiring more complex procedures. The attitude to select patient with limited liver disease and favourable location of CRC for mini-invasive simultaneous procedures is confirmed by a recent multicenter study[64] of 142 patients treated by combined laparoscopic resection of CRC and SCRLM: patients with solitary lesions of < 50 mm,

located in segments S2 to S6 were considered as more suitable to LLR; even though 40.8% of patients had rectal cancer, only 3.5% had preoperative CHRT, suggesting that patients with low rectal cancer and SCRLM were not usually considered for simultaneous resection; simultaneous rectal and major liver resection was performed in 4.2% of patients. Moreover, the authors pointed out that the average contribution of each institution to the overall series reached approximately one patient per year and per institution, that is the evident consequence of the strict selection criteria for simultaneous mini-invasive procedures. The same authors subsequently compared this series of 142 patients with laparoscopic simultaneous procedures to 241 patients who received open simultaneous resections in the same period and concluded that appropriate candidates for simultaneous laparoscopic procedures were patients without severe comorbidities, presenting with one, small (up to 30 mm) CRLM resectable by a wedge resection or a left lateral sectionectomy[65]. Mini-invasive simultaneous resections have similar oncological outcomes than open procedures[62,63,65]. In a very recent unicentric series from South Korea, 109 patients out of 126 undergoing simultaneous laparoscopic resection were compared, by propensity score matching, with 109 out of 318 undergoing an open approach between 2008 and 2016[61]: The 3-year OS and DFS rates were similar between groups, despite some perioperative advantages for the laparoscopic group. The authors however suggested among the limitations of their retrospective study, a natural selection bias for more simple cases to undergo LLR.

Mini-invasive vs open PSLR

Although PSLR with negative resection margins is now accepted as the standard of care for CRLM[126], there is some concern that mini-invasive LR may sometimes involve larger procedures resecting more liver parenchyma, since smaller PSLR may be more complex with laparoscopic approaches[42,43,66,67,126]. This might be the case especially for multiple and/or bilobar tumours and for tumours located in the postero-superior liver segments. In a small series of 35 patients undergoing LLR for CRLM, 54% of patients underwent major LR, even though the median number of nodules was one, with mean tumour size of 40 mm[190]. Likewise, in a multicentric series of 176 patients with LLR[55], 45.5% of patients underwent a major LR even though patients had a mean tumour number of 2.2 nodules, with bilobar distribution in 18.2% and maximum tumour size > 50 mm in 6.8% of the cases. In another series of 133 patients undergoing LLR for CRLM[191], the authors reported 65 (48.9%) major hepatectomies in a patient population where the size of the biggest lesion was > 5 cm in 15.8% of the cases, and the tumours were solitary in 40.6%, bilobar in 26.3% and with a postero-superior location in 37.6% of the cases, respectively. Altogether, these data suggest that candidates for mini-invasive LR of CRLM frequently receive major hepatectomies despite limited liver disease. This situation is not really surprising when we consider that all the principles of parenchymal-sparing LR[85] are time-consuming and rather difficult to apply during mini-invasive procedures: The careful intraoperative inspection and palpation of the liver is possible only for hand-assisted or for hybrid laparoscopic procedures[192]; the assiduous use of IOUS is more time-consuming during laparoscopy[192,193]; multiple partial resections instead of extended hepatectomies, and NAR even with a minimum surgical margin, are complex procedures also for expert laparoscopic surgeons, especially when tumours are located centrally or in postero-superior segments[126]; and detachment of tumours in contact with large intrahepatic vessels is hazardous because of the problematic control of major intraoperative bleeding during mini-invasive procedures[126]. Actually, patients with relatively limited liver disease are being more frequently addressed with mini-invasive major LR or staged hepatectomies[43,66], while in recent years open procedures are evolving toward more complex parenchymal-sparing resections[31,114,120-122].

However, even though the preservation of functional hepatic volume may be more difficult during LLR, and mini-invasive LR is less frequently performed for tumours in difficult locations[44,45], an increasing number of reports demonstrate that technological advances and growth of surgical experience and skill are favouring the development of mini-invasive parenchymal-sparing approaches[126,193], although the transection planes require expert use of IOUS to delimit segments, define the anatomy of intrahepatic vessels, and prevent bleeding[126], and the transection areas are larger and more difficult to manipulate than those of hemi-hepatectomies[43]. In a series of 62 IOUS-guided laparoscopic segmentectomies reported by Ishizawa *et al*[68], laparoscopic resection of the postero-superior segments (S1, S4a, S7 and S8) was performed in 26 patients with satisfactory results, but determined longer operation time and increased blood loss than the other laparoscopic segmentectomies. Other series have reported limited anatomic LLR in case of liver tumours deeply located in the postero-superior

segments[45,66,68,193], in the central segments[69], in the caudate lobe[45,70,71], and for centrally located tumours proximal to major intrahepatic vessels[72]. These reports, however, mainly come from skilled laparoscopic surgeons and usually refer to patients with single lesions, smaller than 30 to 40 mm[45], so that the reported perioperative and oncological results cannot be generalized to patients with more severe liver disease. Two RCT have recently compared the outcome of patients undergoing mini-invasive and open PSLR, respectively. In the OSLO-COMET RCT[193], 280 patients with resectable CRLM were recruited between 2012 and 2016, to compare mini-invasive (n = 133) and open (n = 147) LR; patients were included if the CRLMs could be radically resected by a PSLR, including repeat LR; exclusion criteria included, among others, the need of concomitant RFTA, vascular or biliary reconstruction, simultaneous colorectal and liver resection; patients selection resulted in a mean (SD) number of CRLMs of 1.5 (1.1) and 1.6 (1.1) in the laparoscopic and open group, respectively, while the median (interquartile range) pathology weight of resected specimen was 83 g (38-185) and 64 g (31-204) in the laparoscopic and open group, respectively. There were no differences in blood loss, operation time, and RMs; postoperative complications were lower and the postoperative hospital stay was shorter for LLR, respectively; mortality was similar between groups; although the cost of the procedure was significantly higher for LLR, in a 4-mo perspective the costs were equal. In the LapOpHuva RCT[192], 193 patients with resectable CRLMs were enrolled between 2005 and 2016, to compare mini-invasive (96 patients) and open (97 patients) PSLR, among 540 patients operated for CRLMs in the same period; exclusion criteria included, among others, high tumour load with multiple and bilobar metastases, huge liver metastases > 10 cm, metastases close to major vessels, metastases requiring non-standardized surgical techniques, including repeated LR, simultaneous colorectal and liver resection, right/extended right/extended left hepatectomy, TSH. There were no differences regarding surgical time, blood loss and transfusion requirement between groups; LLR group required more frequently a Pringle manoeuvre; LLR group showed lower global morbidity, but similar severe complications and mortality; OS and DFS rates were similar between groups. In both studies however the patient selection was quite stringent, and the laparoscopic procedures were performed by very experienced laparoscopic surgeons. In the LapOpHuva trial, 195 patients among 540 (36.1%) were finally considered resectable by laparoscopy, while 179 (33.1%) were excluded because required complex resection of single or multiple metastases, including repeat LR and simultaneous colorectal and liver resection, and the others because of more complex LR. These figures represent the real-life experience of a reference Liver Unit, and probably depict the actual limits of mini-invasive liver surgery.

The impact of PSLR on mini-invasive simultaneous resection

PSLR may have a positive impact also in simultaneous laparoscopic procedures (Table 2), since major hepatectomies have been associated with worse perioperative results. However, simultaneous colorectal and conservative liver resection may require very long operative times with sometimes complex liver procedures already with conventional open surgery. Tanaka *et al*[73] reported a series of 39 simultaneous procedures including 38.5% of patients with rectal cancer requiring low anterior resection; the CRLM were bilobar in 35.9% of patients; LR included 23 partial resections, 3 segmentectomies, 8 sectionectomies, 4 left hepatectomies and 1 right hepatectomy; the median (SD) duration of operation was 510 (154) min. In another recent series of 38 patients who received simultaneous PSLR and restorative CRR[74], low anterior resection was performed in 44.7% of patients, after preoperative neoadjuvant CHRT in 21.1%; 47.7% of patients had bilobar CRLM and 28.9% had multiple (≥ 4) bilobar CRLM; a simultaneous major LR (≥ 3 segments) was performed in 13.2% of patients; the mean (SD) duration of the surgical procedure was 382 (139) min in patients without hepatic pedicle clamping and 564 (122) min in patients requiring intermittent hepatic pedicle clamping because of more extended liver disease and more complex LR. In a recent series of 145 patients with rectal cancer and SCRLM, who received a simultaneous resection[21], LR included 41% wedge resections, 39% segmentectomies and 21% major resections (≥ 3 segments), while a pump for adjuvant chemotherapy was placed in 20% of patients; the mean (SD) duration of operation was 354 (96) min. We should consider if these complex procedures, eventually including low or ultra-low rectal resection, major hepatic resections, atypical or anatomic segmental LRs, intraoperative ablations during the same procedure, are presently feasible with mini-invasive approaches, or if we need further technological advances and surgical expertise to pursue PSLR for complex surgical situations.

CONCLUSION

In conclusion, simultaneous resections in selected patients with resectable CRC and SCRLM have postoperative risks comparable to staged resections, may reduce the length of perioperative CHT and usually decrease the overall costs of cure. A staged approach is still advisable in patients requiring urgent CRR because of complicated CRC. All the other patients can be theoretically considered for simultaneous resection. In the case of rectal cancer, preoperative CHRT should be considered according to the tumour stage and its potential benefits. However, simultaneous resections should be reserved for surgical teams experienced in both fields. Concerning the LR, a systematic approach using IOUS to pursue oncological radicality while reducing the extent of hepatectomy may represent the best choice to reduce the perioperative risks of simultaneous procedures. Mini-invasive approaches have a standardized role in oncological colorectal surgery, while LLR is still usually reserved for limited tumours in favourable locations. Conservative LRs, that may be considered standard of care for CRLM, especially in case of simultaneous procedures, are more complex with mini-invasive approaches, notably for larger or multiple tumours in difficult locations. It remains to be established if the available surgical strategies of simultaneous colorectal and liver resection are presently feasible with mini-invasive procedures, or if conventional open procedures are still safer and more effective, at least for more complex tumours, while awaiting for further technological advances and surgical expertise in mini-invasive surgery.

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REVIEW

What could microRNA expression tell us more about colorectal serrated pathway carcinogenesis?

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Abstract

In the last two decades, the vision of a unique carcinogenesis model for colorectal carcinoma (CRC) has completely changed. In addition to the adenoma to carcinoma transition, colorectal carcinogenesis can also occur *via* the serrated pathway. Small non-coding RNA, known as microRNAs (miRNAs), were also shown to be involved in progression towards malignancy. Furthermore, increased expression of certain miRNAs in premalignant sessile serrated lesions (SSLs) was found, emphasizing their role in the serrated pathway progression towards colon cancer. Since miRNAs function as post-transcriptional gene regulators, they have enormous potential to be used as useful biomarkers for CRC and screening in patients with SSLs particularly. In this review, we have summarized the most relevant information about the specific role of miRNAs and their relevant signaling pathways among different serrated lesions and polyps as well as in serrated adenocarcinoma. Additional focus is put on the correlation between gut immunity and miRNA expression in the serrated pathway, which remains unstudied.

Key Words: MicroRNA; Serrated pathway; Carcinogenesis; Colorectal carcinoma; Sessile

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Core Tip: In addition to the adenoma to carcinoma transition, colorectal carcinogenesis can also occur *via* the serrated pathway. In most serrated polyps, the pathway is believed to include the acquisition of a mutation in a gene that regulates mitogen-activated protein kinase (MAPK) pathway, disruptions to the Wnt signaling pathway and widespread methylation of CpG islands. Moreover, there are less data about different microRNAs (miRNAs) expression profiling in serrated adenomas with different grades of dysplasia. In contrast to the conventional colorectal carcinogenesis, the pivotal role of miRNAs and their relevant signaling pathways in the serrated pathway of carcinogenesis is still to be elucidated because of an insufficient number of studies conducted to clarify separate steps in the process.

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INTRODUCTION

Colorectal cancer (CRC) is the most prevalent cancer in Western countries and the second cause of cancer-related death[1]. Obesity, sedentary lifestyle, tobacco and alcohol consumption are considered the driving factor behind the growth of CRC[2]. In the last two decades the vision of a unique carcinogenesis model for CRC has completely changed. The most prevalent genetic events accompanying CRC development are mutations that de-regulate the Wnt signaling cascade. In particular, inactivating mutations in the tumor suppressor adenomatous polyposis coli (APC) are considered the earliest genetic lesions sufficient to initiate tumorigenesis[3].

In addition to the adenoma to carcinoma sequence, colorectal carcinogenesis can also occur *via* the serrated pathway. After the identification of serrated carcinomas by Jass *et al*[4] in 1992, the underlying genetic and epigenetic alterations have been described. In most serrated polyps, the pathway is believed to be the acquisition of a mutation in a gene that regulates mitogen-activated protein kinase (MAPK) pathway, disruptions to the Wnt signaling pathway and widespread methylation of CpG islands[5,6].

A class of small non-coding RNAs, designated as microRNAs (miRNAs), are involved in progression towards malignancy. miRNAs act as tumor suppressors or oncogenes depending on the characteristics of their downstream targets[7]. They function as post-transcriptional gene regulators and have been increasingly recognized as useful biomarkers for CRC[8].

A plethora of studies have documented aberrant miRNA levels in CRC, but only a few of them relate to serrated pathway carcinogenesis[9]. There is even less data about different miRNA expression profiling in serrated adenomas with different grades of dysplasia[10]. In contrast to the conventional colorectal carcinogenesis, the pivotal role of miRNAs in the serrated pathway is still to be elucidated because of the insufficient number of studies conducted to clarify separate steps in serrated carcinogenesis[11].

Many of the published reviews in the English literature about the serrated pathway have been focused on histological, endoscopic, and molecular features[12,13]. However, there are a few data about post-transcriptional gene regulation, in particular, the expression of miRNAs in the serrated pathway in CRC. We aimed to interrogate the role of miRNAs in relevant signaling pathways in serrated carcinogenesis.

Emerging new approaches revealed increased expression of certain miRNAs in premalignant sessile serrated lesions (SSLs), emphasizing their role in the serrated pathway progression towards colon cancer[14]. This could make miRNAs potential biomarkers for screening in patients with SSLs[15,16].

In this review, we summarized the most relevant information about the specific role of miRNAs among different serrated lesions and polyps as well as in serrated adenocarcinoma (SAC). Additionally, the review is the first that looks at the correlation between gut immunity and miRNA expression in the serrated pathway.

MORPHOLOGICAL ASPECTS OF SERRATED POLYPS AND SAC

Based on the literature, the percentage prevalence of serrated pathway is highly variable, ranging from 15% up to 30% of all CRCs[17-20].

According to the 5th edition of WHO classification of colorectal serrated lesions and polyps, they are classified into three histopathological subtypes: Hyperplastic polyps (HPs), SSLs, and traditional serrated adenomas (TSAs)[21] (Figure 1). TSAs are extremely rare < 1% of all colorectal polyps, while HPs are the most common, comprising approximately 75% of all serrated polyps. SSLs (previously known as sessile serrated adenomas or sessile serrated polyps) cause nearly 25% of serrated polyps[22].

HPs are usually small, rarely cause symptoms, and have minimal malignant potential. However, it was established that HPs could progress to SSLs or TSAs for a period of 7.5 years[23]. In this context, HPs may predispose to cancer because of their ability to transform into serrated lesions[24]. These lesions could be found anywhere in the colon, but they are mostly placed in the distal colon (70%-80%)[25]. It was established that HPs, with right-side localization, are more likely to have malignant potential[26-28].

Clinical characteristics, such as size, location, and endoscopic appearance, can support the identification of SSLs but are not sufficient for their identification. Approximately 10% of SSLs could lead to sporadic CRCs *via* the serrated polyp-carcinoma sequence[29].

In most series, TSAs account for < 1% of all colorectal polyps, represent about 1%-2% of the serrated lesions and are located predominantly in the left colon[30-32].

SAC is characterized by mainly right-sided location of the colon, specific molecular features and female predominance. Percentage prevalence of SAC is about 7.5%-8.7% of all CRCs and according to the literature it has worse prognosis than conventional CRC[6,33].

EPIGENETIC AND GENETIC ASPECTS IN SERRATED PATHWAY

CpG methylator phenotype

Toyota *et al*[34] introduced the CpG island methylator phenotype (CIMP) in 1999. Methylation is an epigenetic process where a methyl group (CH3) is added to the cytosine nucleotide at a CpG dinucleotide group. The process of methylation of gene promoters is a physiological mechanism by which gene expression is regulated without altering the DNA sequence[35,36].

Transcriptional silencing of essential tumor suppressor genes, caused by aberrant DNA methylation, could promote neoplastic growth. This aberrant methylator has been called the CIMP and is thought to be important in the serrated pathway in CRC[37].

Using eight markers, Ogino *et al*[38] classified CIMP in CRC into three subgroups, CIMP-low (CIMP-L), CIMP-high (CIMP-H), and CIMP-negative, according to the numbers of methylated promoters.

With the growing impact of translational research and molecular pathology, the CRC pathogenesis became more elucidated based on the association of CIMP and key mutations in *KRAS*, *BRAF*, *PIK3CA*, *TP53*, and *APC*. Furthermore, microsatellite instability (MSI), caused by dysfunction of DNA mismatch repair (MMR) genes, is considered another critical pathway in carcinogenesis[39].

MSI mechanism in CRC

The MSI mechanism in CRC was first described in relation to Lynch syndrome, where germline mutations take place in specific MMR genes such as *MLH1*, *MSH2*, *MSH6*, and *PMS2*[40]. Germline deletions at 3′ end of the *EPCAM* gene which lead to decreased MSH2 expression were also demonstrated as a recurrent cause of Lynch syndrome[41]. Furthermore, functional relevance of *MSH3* mutations for the development and inheritance of CRC were reported, but their role in the serrated pathway needs further

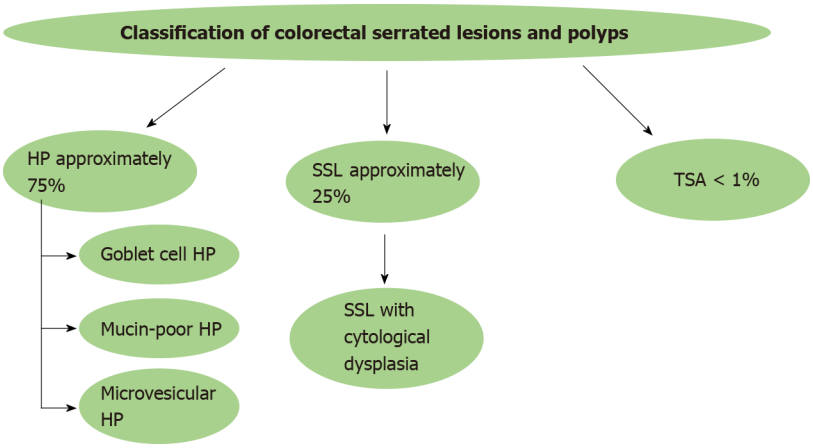

Figure 1 Schematic presentation of classification of colorectal serrated lesions and polyps. HP: Hyperplastic polyp; SSL: Sessile serrated lesion; TSA: Traditional serrated adenoma.

analysis and more cohort studies[42,43]. Evidence has shown that mutations in MSI are vital points in the developing malignancy in 3%-15% of all CRC[42,43]. About 80% of MSI CRCs are characterized by the hypermethylation of *MLH1*, while 20% of MSI CRCs by mutations in MMR genes[44]. MSI status could be subclassified into MSI-high (MSI-H), MSI-low (MSI-L) and microsatellite stable (MSS) according to the number of mutations in microsatellite sequences[45].

Alteration of MMR genes due to epigenetic silencing by sporadic, acquired hypermethylation of the *MLH1* gene promoter leads to the serrated pathway in CRC[44].

Serrated colorectal malignancies are characterized by CIMP-H, *MLH1* promoter hypermethylation, and MSI and *BRAF* mutations[46].

BRAF / *KRAS* gene mutations in serrated CRC

Serrated colorectal lesions rarely bear truncating *APC* mutations, but the most frequent genetic alterations involve *BRAF* mutations, whereas *KRAS* mutations are less common[47]. Both *KRAS* and *BRAF* belong to the MAPK signaling pathway, mediating cell proliferation, apoptosis and differentiation[48].

BRAF gene encodes a protein called B-Raf, which plays a pivotal role in regulating the MAPK/ERKs signaling pathway[49]. Recent findings in molecular biology demonstrated that mutations in *BRAF* are found in about 10% of CRC patients[50]. *BRAF*-mutated CRCs are associated with the female gender, often right-sided, mucinous histology, and advanced stage[51]. *BRAF* mutations are considered as early events in CIMP cancers by inhibition of normal apoptosis in colonic mucosa[52]. Many recent studies classified two different molecular phenotypes of CRC based on *BRAF* mutation status: *BRAF* V600E- and non-V600-mutated CRC[53]. A correlation between serrated carcinogenesis and *BRAF* V600E mutation was established, which induce CIMP-H status and methylation of *MLH1* promoter[54]. In contrast to the conventional adenomas, the earliest event in serrated precursor lesions are *BRAF* mutations and hypermethylation, which leads to transformation of aberrant crypt foci (ACF) to microvesicular HP and then to SSLs. Methylation and loss of key tumor suppressor genes such as *p16* and *MLH1* are the key points in SSLs' progression to SAC[55]. Interesting information about the *BRAF* mutated/MSS SACs was reported by Bond *et al*[55]. They found out that hypermethylation events occurred in *BRAF* mutated SACs more often than in conventional pathway (respectively 60% and 3%)[55]. *BRAF* V600E-mutated CRCs are with dismal prognosis and resistance to standard systemic chemotherapy[56,57].

Another significant driver in the serrated pathway is *KRAS* mutations[58]. Opposite to the traditional model of Vogelstein, where aberrant activation of Wnt pathway has been observed, high frequency of *KRAS* mutations was established in TSAs. In contrast to SSLs, TSA lesions showed *MGMT* hypermethylation, but not *MLH1* promoter hypermethylation. Based on this evidence, a non-*MLH1* mutating SSL could progress to a TSA and ultimately develop into a *BRAF*-mutated MSS tumor (Figure 2)[59,60].

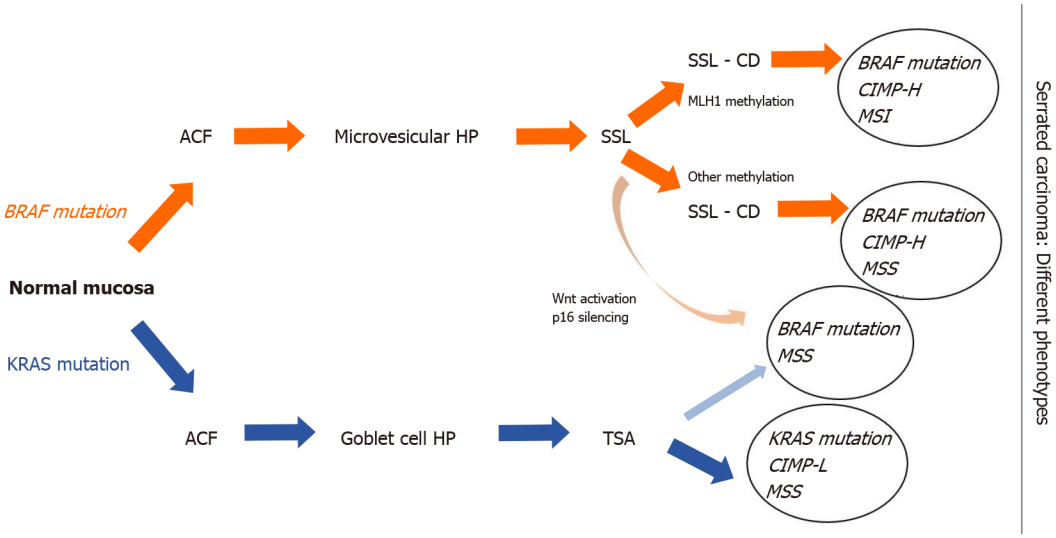

Figure 2 Outline of the schematic serrated pathway progression. In red color we indicate the steps of transformation of *BRAF*-mutated serrated lesions. *BRAF* mutations and hypermethylation lead to transformation of aberrant crypt foci to microvesicular hyperplastic polyp then to sessile serrated lesions (SSLs). Methylation and loss of key tumor suppressor genes such as *p16* and *MLH1* are the key points in SSLs' progression to serrated adenocarcinoma. In blue color we indicate *KRAS* mutations in traditional serrated adenomas (TSAs), which showed *MGMT* hypermethylation, but not *MLH1* promoter hypermethylation. In light red shading we indicate a non-*MLH1* mutating SSL, which could progress to a TSA and ultimately develop into a *BRAF*-mutated microsatellite stability tumor. ACF: Aberrant crypt foci; HP: Hyperplastic polyp; SSL: Sessile serrated lesion; SSL-CD: Sessile serrated lesion with cytological dysplasia; TSA: Traditional serrated adenoma; CIMP: CpG island hypermethylator phenotype; CIMP-H: CIMP-high; CIMP-L: CIMP-low; MSI: Microsatellite instability; MSS: Microsatellite stability.

MIRNA PROFILE FROM PREMALIGNANT SERRATED LESIONS TO CRC

miRNAs were discovered in Caenorhabditis elegans by Lee *et al*[61] in 1993 while studying the gene *lin-14*. However, the scientific community became aware of the importance of miRNAs seven years later when they were recognized as a specific class of biological regulators. miRNAs are small, single-stranded, non-coding RNAs (18-24 nucleotides) that can post-transcriptionally regulate the expression of various oncogenes and tumor suppressor genes[62]. Also, they play an essential role in cancer development, proliferation, regression, and metastasis. Even though their role in cancer progression is yet to be elucidated, several studies reported the influence of specific miRNA alterations in premalignant and malignant lesions[63-66]. miRNA expression profiling gives us the opportunity to understand and identify differences between benign and malignant lesions of the colon mucosa, as well as to stratify benign lesions according to their malignant potential[67].

The role of miRNA-125b, miRNA-222, miRNA-214, miRNA-335 in CRC carcinogenesis

In this scenario, several studies showed a unique miRNA signature in different types of colonic polyps, as well as in the progression of serrated lesions.

Tsikitis *et al*[68] profiled miRNA patterns in screen-detected polyps in relation to histologic features and cancer-related risk. miRNA expression analysis was carried out on biopsy specimens from 109 patients. The specimens were obtained from normal mucosa (NM), HPs, tubular adenomas (TAs), tubulovillous adenomas, or high-grade dysplasia (TVHGs), SSLs, and TSAs. They have not found a significant difference in the expression of miRNA between TSAs and SSLs. miRNAs expression pattern was similar in TSAs and HGTVs, whilst there were several differentially expressed miRNAs between HPNMs and TSAs. Additionally, they performed pairwise comparisons of non-serrated tissues and serrated lesions. miRNAs-222 and miRNA-214 were significantly downregulated by 2.35- and 1.51-fold respectively in serrated polyps, whereas miRNA-335 was significantly overexpressed by two-fold in non-serrated tissues. Tsikitis *et al*[68] drew the conclusion that the downregulation of miRNA-125b and miRNA-320a in the serrated pathway may be used as independent predictors of progression with a concordance index of 84.7%.

Opposite to the serrated pathway, in the conventional adenoma-carcinoma sequence, many studies showed a high expression of miRNA-125b in advanced tumor size. Another correlation was found between the overexpression of miRNA-125b, which leads to repression of the endogenous level of p53 protein in human CRC cells. Cancer progression and poor outcomes were associated with overexpression of miRNA-125b in the conventional colorectal pathway[69].

The role of miRNA-31 in carcinogenesis of serrated pathway of the colorectum

However, many studies showed that miRNA-31 plays a pivotal role in serrated carcinogenesis. In this scenario, miRNA-31 is located at 9p21.3 and is frequently overexpressed in sessile serrated adenomas. Aoki *et al*[70] analyzed in their case report miRNA-31 expression using quantitative reverse transcription-PCR in patients with early invasive CRC with HP component. Their results showed higher miRNA-31 expression in the carcinoma component compared to HP component. They revealed that progression of HP (or SSLs) to SAC is likely to be associated with overexpression of miRNA-31.

To shed light on the role of miRNA31 on the serrated pathway, Kanth *et al*[11] conducted a study of 108 colon biopsies with distinct histology types. Different expression was established in 23 miRNAs between NM and serrated lesions. Additionally, six miRNAs showed a different expression pattern between SSLs and HPs, as miRNA-31-5p has been the most significantly modulated.

Nosho *et al*[71] based on miRNA array analysis, identified that miRNA-31 was the most upregulated in *BRAF* (V600E) mutation, compared to *BRAF*-wild type CRCs. Moreover, they performed transfection of the miRNA-31 inhibitor and consequently showed that miRNA-31 might regulate *BRAF* activation in CRCs. Therefore, miRNA-31 could be used as a diagnostic biomarker as well as a feasible therapeutic target in the future. Finally, they proved that high miRNA-31 expression was associated with shorter prognosis in patients with CRC.

Higher miRNA-31 expression was associated with cell proliferation and survival in development in CRC, as well as tumor invasion and poor prognosis[72-75]. Kubota *et al*[76] pointed out that miRNA-31 could be a potential prognostic biomarker in their study of patients with stage IV of CRC. They also found out a correlation between miRNA-31 overexpression and poor tumor differentiation, as well as advanced disease stages.

Recent studies showed the presence of miRNA-31 in the serum of patients with metastatic CRC, who were treated with anti-EGFR therapy. Igarashi *et al*[77] found out a correlation between high miRNA-31-5p expression and shorter PFS in CRC patients treated with anti-EGFR therapeutics. Their theory suggested that miRNA-31-5p could be a useful prognostic biomarker for anti-EGFR therapy.

Even though the underlying mechanisms of the role of miRNA-31-5p in CRC remain unknown. It has been postulated that miRNA-31 can directly bind to the 3' untranslated region (3' UTR) of SATB2, which takes part in regulation of transcription and chromatin remodeling. Overexpression of miRNA-31-5p could induce epithelial-mesenchymal transition, tumorigenesis, and progression in CRC[78].

Furthermore, another correlation between the expression of miRNA-31 and CRC-associated fibroblast (CAFs) was established, but not *in vivo* experimental models. Yang *et al*[79] elucidated that miRNA-31 inhibits autophagy in CAFs and alters colorectal proliferation and invasion of CRC cells. Thus, more studies must be conducted in this direction because of the lack of *in vivo* experimental models.

Relevance of miRNA-135-B in CRC

In many studies, it has been reported that overexpression of miRNA-135-B has been associated with *APC* dysfunction in CRC, leading to the promotion of tumor-proliferation, progression, and invasion[63,80]. It was established that miRNA-135-B had been associated with the serrated pathway and colorectal carcinogenesis.

Only few studies indicate that specific miRNA profiles can be used to distinguish neoplastic from benign lesions in colon mucosa[6]**.** A study by Kanth *et al*[11] was the first that showed the overexpression of specific miRNAs in serrated polyps or serrated carcinoma. In summary, they provided a comprehensive analysis of miRNA gene expression in SSLs, by identifying miRNA-135B, miRNA-378A, miRNA-548, miRNA-9, and miRNA-196B. miRNA-378A-3p was significantly downregulated in SSLs compared to normal colon mucosa. They suggested that these miRNAs are good predictors in SSLs to carcinoma transformation. Additionally, they discovered that miRNA-9 and miRNA-196b were also de-regulated in SSL compared to HP. These miRNAs showed different expression patterns in *BRAF* mutated-MSI tumors. Interestingly, reduced expression of miRNA-196B has been detected in the plasma of patients with CIMP-positive SSLs or MSI colon cancers[11].

The involvement of miRNA-21 in CRC

MiRNA-21 is one of the most eminent miRNAs involved in the genesis and progression of CRC. Evidence implied that miRNA-21 negatively regulates tumor suppressor phosphatase and tensin homolog (*PTEN*) gene, which played an essential role in cell proliferation and invasion in CRC[81-84]. An interesting study by Ghareib

et al[85] established that miRNA-21 in serum could be feasible, non-invasive biomarker with high sensitivity and specificity (95.8% and 91.7%) for early detection and prognosis in patients with CRC.

In addition, Chen *et al*[86] report a correlation between tissue and serum miRNA-21 overexpression and poor prognosis in patients with CRC. It is more significant in colon cancers, compared to rectal.

Another interesting study by Yau *et al*[87] presents the potential role of fecal-based miRNA-21 and miRNA-92a as non-invasive biomarkers for CRC screening. They reported higher expression of miRNA-21 and miRNA-92a in patients with advanced distal CRC compared to the proximal localization, without significant value in the detection of early CRC.

miRNA-21 down-regulates tumor suppressor PDCD4, thus stimulating cancer cell invasion and intravasation. Moreover, the high level of miRNA-21 was associated with metastasis and resistance to chemotherapy of 5-FU in CRC. Thus, it makes miRNA-21 a potential non-invasive biomarker for diagnostic and prognosis for CRC[88].

Recently, several studies have reported the correlation between expression of miRNA-21 and serrated pathway in CRC. A study by Schmitz *et al*[89] demonstrated different expression of miRNA-21 among NM, HPs, and SSLs. They found overexpression of miRNA-21 in SSLs, whereas normal colon mucosa and HPs exhibited no differences. Opposite to them, Kanth *et al*[11] proved that there was no statistically significant expression of miRNA-21 in SSLs.

Future investigations are necessary to find out the correlation between expression levels of miRNA-21 and genetic and epigenetic alterations of SSLs.

The role of miRNA-181a-2 in the development of serrated pathway in CRC

miRNA-181 plays a pivotal role in regulation at the post-transcriptional level in many different types of cancer. More specifically, the expression of miRNA-181a and miRNA-181b are strongly associated with the mutation status of the tumor suppressor gene *p53* in colorectal carcinogenesis[90]. The underlying mechanism of how miRNA-181a influences conventional colorectal carcinogenesis could be based on up-regulation miRNA-181a through the activation of the Wnt/β-catenin pathway[91].

Little is known about the expression of miRNA-181a in the serrated pathway. A comprehensive analysis of miRNA profile in SACs and MSI-H CRC has been carried out by Kondelova *et al*[10] Interesting information about the molecular features of miRNA expression in SACs and MSI-H CRC has been elucidated. Microarray assay showed that 223 miRNAs were differently expressed, as 75 of them were downregulated in SACs compared to MSI-H CRC. On the other hand, 148 miRNAs were upregulated in the same comparison group. Notably, only miRNA-181a-2 showed significant overexpression in MSI-H CRC compared to SACs. It has been established that miRNA-181a-2 has an inverse correlation with nicotinamide phosphoribosyl transferase, which is a transcription factor playing a significant role in organogenesis and stem cell development[92].

In conclusion, their analysis showed that miRNA-181a-2 plays a role in development in different subtypes of CRC from the serrated pathological pathway. Additionally, the up-regulation of miRNA181a-2 was associated with MSI-H status. This study may be a foundation for further researches aiming to elucidate the function of miRNA-181a-2 in CRC[10].

Other significant miRNAs in serrated pathway

Slattery *et al*[15] have carried out promising research about different miRNA expression between NM and different types of polyps. They made a comprehensive analysis of miRNA expression among adenomatous polyp (AD), SSLs, and HPs. This study identified 19 differently expressed miRNAs between AD and HP such as let-7i-5p, miRNA-1229-5p, miRNA-1234-5p, miRNA-1249, miRNA-1268B, miRNA-1275, miRNA-194-5p, miRNA-215, miRNA-2392, miRNA-30b-5p, miRNA-331-3p, miRNA-3653, miRNA-3960, miRNA-4281, miRNA-4689, mRNA-4739, miRNA-518a-5p, miRNA-6510-5p and miRNA-939-5p. They concluded that the expression of the above-mentioned miRNAs in HP and SSLs are down-regulated and are related to MSI and CIMP. On the other hand, ADs have upregulated miRNA expression and are associated with TP53 and *KRAS*-mutations. Additionally, their study aimed to identify different miRNA expression and molecular pathways in colorectal carcinogenesis through genomic landscaping of colon polyps[15]. An overview of putative miRNA profile expression in the serrated colorectal pathway is presented in Figure 3.

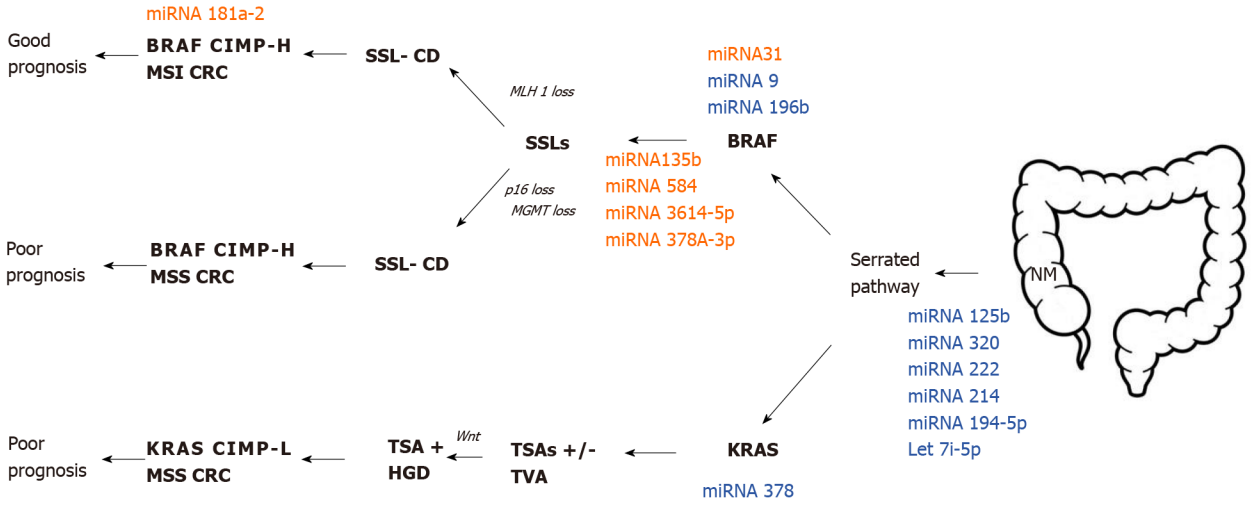

Figure 3 Putative microRNA profile expression in the serrated colorectal pathway. microRNAs in red color showed up-regulation, while the ones in blue color showed down-regulation. miRNA: microRNA; SSL: Sessile serrated lesion; SSL-CD: Sessile serrated lesion with cytological dysplasia; TSA: Traditional serrated adenoma; HGD-H: High-grade dysplasia; TVA: Tubulovillous adenoma; CIMP: CpG island methylator phenotype; CIMP-H: CIMP-high; CIMP-L: CIMP-low; MSI: Microsatellite instability; MSS: Microsatellite stability; NM: Normal mucosa.

HUMAN GUT MICROBIOTA, MUCOSAL IMMUNITY, AND MIRNA IN SERRATED PATHWAY

Human gut microbiota comprises approximately 39 trillion microorganisms that colonize the adult gut system[93]. It plays a significant role in maintaining homeostasis of the intestinal immune system, which represents a natural barrier to pathogen infection[94] but also maintain oral tolerance in the gut. Gut homeostasis can be disturbed by environmental factors such as lifestyle, diets, infections, and antibiotics, leading to dysbiosis. Many recent studies have demonstrated the association between gut dysbiosis and colorectal carcinogenesis[95]. Evidence suggest that *Fusobacterium nucleatum* (*F. nucleatum*) has overabundance in gut microbiota in dysbiosis[96]. This finding is in agreement with the fact that *F. nucleatum* is involved in mucosal inflammation and contributes to the progression of CRC[97,98]. There are plenty of studies that investigate interactions between *F. nucleatum* and conventional adenoma to carcinoma sequences[99-101]. Ito *et al*[102] focused on *F. nucleatum* and serrated carcinoma pathway. In particular, they investigated the putative correlation between *F. nucleatum* and miRNA-31 expression. However, the results of the study did not indicate a significant association between miRNA-31 and *F. nucleatum*. Nevertheless, Yu *et al*[103] showed that invasive *F. nucleatum* might play a role in developing proximal colon carcinogenesis through the serrated neoplasia process, which may play a less significant role in the traditional adenomas-carcinoma sequence. Bacterial biofilms may not support *F. nucleatum* infiltrate tumor tissues.

Longitudinal studies of immune infiltrate in resected CRC tumors have shown the role of the immune response in the pathophysiology of CRC. miRNAs, as non-coding RNAs, are capable of controlling several post-transcription target genes and performing essential roles in cell proliferation, differentiation, and apoptosis, including the immune cells[104]. In other words, miRNAs are necessary for maintaining the functioning of the immune system. However, abnormal expression of miRNAs is often found in various forms of tumors that contributes to immune deficiencies or immune evasion. Li *et al*[104] focused on the possible functions of miRNAs in CRC immune response control and the use of specific miRNA targets for CRC therapy. It is assumed that miRNAs possess an immunomodulatory role and can potentially be a part of the anti-cancer target pipeline. However, there may be some drawbacks and threats of using miRNAs as immunotherapeutics.

As discussed above, different miRNA profile variations from the transition of NM to adenoma and CRC identified some miRNA as contributors to those transformations. Moreover, serum miRNAs may be used as markers to track certain changes accompanying carcinogenesis[105]. miRNA profiles obtained in standard colorectal mucosa differ from those in adenomas and CRC. Oncogenes such as *c-Met* and *KRAS*, together with the miRNAs could also have pro- or anti-CRC effects, including influencing the immune system. More interestingly, some miRNAs increased their

expression in developing CRC, whereas others reduced their expression, such as miRNA-30b[106]. Furthermore, evidence indicates that miRNAs not only participate in colorectal carcinogenesis, but can be used as biomarkers for diagnosing, managing, and follow up the patients.

It is well-known that one of the mechanisms for cancer invasion is to establish complex pathways for disarming the immune system and evading immune surveillance. Nakanishi *et al*[106] demonstrated that in human serrated tumors, the expression of atypical protein kinases C (PKC) is decreased. Simultaneous inactivation of the encoding genes in the intestinal epithelium of the mouse culminated in random serrated tumorigenesis with a highly reactive and immunosuppressive stroma leading to advanced cancer development. Whereas epithelial PKC deficiency resulted in the death of immunogenic cells and the infiltration of CD8+ T cells that repressed tumor initiation, IFN, and CD8+ T cell responses were impaired by PKC loss, resulting in tumorigenesis[106].

Some tumors may stimulate the immune cells in the tumor stroma to produce a variety of inhibiting cytokines such as transforming growth factor (TGF-β) and IL-10, which suppress the recruitment and activation of antitumor T lymphocytes[107]. Furthermore, IL-6 suppresses the ability of dendritic cells to present antigens by activating the signal transducer and transcription activator 3 (STAT3) and lessens CD4+ T cell-mediated immune response[108]. Thus, an immunotherapy that utilizes monoclonal antibodies that antagonize immunosuppressive cytokines or inactivate immunosuppressive cells may enhance tolerance to cancer and prevent tumor growth[16]. Our team also documented that IL-6 upregulation is crucial for developing both IBD and CRC well before the upregulation of other Th17/Treg associated genes (TGFb1, IL-10, IL-23, and FoxP3 transcription factor) that are critical primarily for the development of CRC[109]. An additional study revealed that intratumoral IL-17-mediated signaling might inhibit immunotherapy responses[110].

In line with this, synergistic therapeutic efficacy was demonstrated by combined therapy with TGF-β receptor inhibitor and anti-PD-L1 checkpoint blockade. A study of human samples confirmed the importance of atypical PKCs during the immunosurveillance defects in human serrated CRC. These results give insight into how this poor-prognosis subtype of CRC to be diagnosed and treated[106].

Since miRNAs modify the differentiation, activation, and distribution of the various immune cells and the intricate cytokine network, miRNAs play an essential role in both innate and adaptive immune responses. miRNAs are closely involved in processes such as control of innate and adaptive immunity activation, regulation of inflammation and cytokine network, trafficking and cytokine crosstalk between the tumor and its microenvironment, miRNAs are promising targets for immunotherapy of different gastroenterological cancers[111]. Thus, miRNAs exert regulatory and protective functions in the digestive system and antitumor defense against gastroenterological cancers development.

In line with this, KRAS-IRF2 (interferon regulatory factor 2) axis also impacts the immune system towards immune suppression[112]. The clinical significance of this observation is the immunotherapy resistance in CRC. However, the biological functions and mechanisms of oncogenic *KRAS* in resistance to immune checkpoint blockade therapy are not fully understood.

Additionally, although various studies have examined the immune environment of CRCs with MSI, only one analysis assessed the immune microenvironment of serrated precursor lesions, including sessile serrated adenoma with dysplasia (SSA-D)[113]. Rau *et al*[113] studied the density of intraepithelial lymphocytes (IELs) in various serrated polyps and SSAs-D. The investigators observed that the amount of IELs was substantially higher in SSA-D than in SSAs, which displayed significantly higher numbers of IELs relative to HPs and typical adenomas. In their research, Acosta-Gonzalez *et al*[114] examined the immune properties of the serrated carcinogenesis system and its association with morphological stepwise dysplasia-carcinoma development and MSI status. They confirmed the higher density of IELs in lesions of MSI-H tumors. Additionally, other studies have shown that the total number of frameshift mutations in MSI CRCs correlates with lymphocyte infiltrating tumor density, specifically CD8+ lymphocyte density[115].

Nevertheless, the serrated pathway has two outcomes that differ in their clinical and prognostic characteristics as well as in their methylome profile and histological and molecular characteristics: (1) SSLs; or (2) Sporadic CRC showing MSI-H[42]. The latter subtype of CRC is correlated with deep immune invasion and has a better prognosis than the former[116].

The latest approaches in transcriptomics used to classify human CRC have shown that mesenchymal and/or desmoplastic involvement, together with an

immunosuppressive microenvironment, are essential determinants of the worst prognosis of CRC. Importantly, these aggressive CRCs harbor the traits of serrated tumors, suggesting that how aggressive the CRC becomes is determined by initiation by this alternate mechanism. Moreover, molecular markers and profiles of gene expression have indicated that at least two CRC subgroups exist within the serrated pathway: (1) An inflammatory subtype with features of stromal/mesenchymal high immune infiltration (referred to "mesenchymal serrated" CRCs); and (2) MSI ("classical serrated"). *BRAF* mutation characterized with immune suppression in the tumor environment[117].

However, the tumor stroma's possible activation and the type of immune response associated with the CRC tumor stroma are not yet well understood. SAC may be infiltrated by CD45+ cells that express PD-L1 and decrease CD8+ T cells, which determines that there are multiple immune mechanisms to avoid the immune response[106]. Nevertheless, to create more efficient therapies, understanding the pathogenesis, including the tumor environment on the immunological settings, for both forms of serrated CRC is essential. Although emerging data show that immunotherapy is a promising choice for patients with multiple cancer forms still, there is a substantial clinical gap between the identification of serrated precursor lesions and the effective therapies for treating them.

CONCLUSION

With the growing influence of translational research and molecular pathology, the serrated pathway carcinogenesis became more elucidated based on the association of CIMP and key mutations in *BRAF*, *KRAS*, *PIK3CA*, *TP53*, and *APC*. Furthermore, MSI caused by dysfunction of DNA MMR genes, is considered as another critical pathway in carcinogenesis.

In this review we summarized the most relevant information that have been published in the literature so far about miRNA expression in serrated pathway. Furthermore, we intended to answer the question could miRNA expression tell us more about colorectal serrated pathway carcinogenesis. The answer may come from several studies that have been published related to this issue. The data showed a unique miRNA signature in different types of colonic polyps, as well as in the progression of serrated lesions. Besides, those miRNAs play an important role in serrated carcinogenesis, proliferation, regression, and metastasis. Existing evidence support that miRNAs expression profiling, including miRNA-125b, miRNA-222, miRNA-214, miRNA-335 miRNA-31 miRNA-135-B miRNA-21 miRNA-181a-2, *etc.*, allows us to understand and identify differences between benign and malignant lesions of the colon mucosa, as well as to stratify benign lesions according to their malignant potential.

Moreover, serum miRNAs may be used as markers to track specific changes accompanying serrated carcinogenesis. This assertion is based on the fact that there is a significant difference of miRNA expression between serrated and conventional pathway in colorectal carcinogenesis.

The immunopathology of CRC attracted growing attention since an association between gut dysbiosis and colorectal carcinogenesis was suggested by recent authors. miRNAs are putative regulators of several post-transcription target genes and are thought to play essential role in differentiation and proliferation of immune cells. It is assumed that, different miRNA profile pattern may contribute to alterations in gut immunity and dysbiosis, leading to transition events of NM to adenoma.

The specific miRNA expression in serrated pathway, could be useful tool to find appropriate diagnostic, prognostic and treatment response markers in clinical practice. Thus, in order to understand the real significance of miRNAs in this clinical setting, further studies must be conducted.

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MINIREVIEWS

Modern surgical strategies for perianal Crohn's disease

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Abstract

One of the most challenging phenotypes of Crohn's disease is perianal fistulizing disease (PFCD). It occurs in up to 50% of the patients who also have symptoms in other parts of the gastrointestinal tract, and in 5% of the cases it occurs as the first manifestation. It is associated with severe symptoms, such as pain, fecal incontinence, and a significant reduction in quality of life. The presence of perianal disease in conjunction with Crohn's disease portends a significantly worse disease course. These patients require close monitoring to identify those at risk of worsening disease, suboptimal biological drug levels, and signs of developing neoplasm. The last 2 decades have seen significant advancements in the management of PFCD. More recently, newer biologics, cell-based therapies, and novel surgical techniques have been introduced in the hope of improved outcomes. However, in refractory cases, many patients face the decision of having a stoma made and/or a proctectomy performed. In this review, we describe modern surgical management and the most recent advances in the management of complex PFCD, which will likely impact clinical practice.

Key Words: Crohn's disease; Inflammatory bowel disease; Surgical treatment; Perianal fistulas; Anorectal fistula

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Core Tip: Perianal Crohn's disease (CD) occurs in up to 50% of patients who also have symptoms in other parts of the gastrointestinal tract. One of the most challenging phenotypes of CD is perianal fistulizing disease. Treatment is difficult, often requiring more aggressive medical and surgical interventions than luminal disease. Seton placement is the most common technique. However, with the advent of biological therapy, especially anti-TNF agents (infliximab and adalimumab), the approach to these fistulas has changed. Thus, this article aims to review the methods currently available for the management of perianal fistulizing disease.

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INTRODUCTION

In Crohn's disease (CD), perianal symptoms occur in up to 50% of patients with concurrent symptoms involving other portions of the gastrointestinal tract; in 5% of cases, perianal symptoms occur as the first manifestation of CD[1]. A challenging phenotype of CD is perianal fistulizing CD (PFCD), an aggressive, debilitating condition associated with significant morbidity that can negatively affect quality of life[2]. Treatment is difficult, often requiring more aggressive medical and surgical interventions than luminal disease. In addition, it predicts a worse disease course, requiring rigorous monitoring to identify those who are at risk of worsening, sub-optimal levels of biological drugs, and signs of neoplasia[3].

PATHOPHYSIOLOGY

Although the pathophysiology of cryptoglandular fistulas is well understood, that of CD-related fistulas has not yet been defined. Some theories have been proposed, but none have been confirmed[4].

CLASSIFICATION

Historically, perianal fistulas have been classified according to Parks' anatomical model[5]. However, the American Gastroenterology Association has proposed that PFCDs should be classified into 2 categories: Simple and complex (*i.e.*, those with a high internal orifice and multiple or rectovaginal fistulas associated with abscesses or stenosis)[6]. The Van Assche score assesses the severity of CD throughout the anal canal based on magnetic resonance imaging findings[7].

The treatment of PFCD has traditionally been surgical, and seton placement is the most common technique. However, with the advent of biological therapy, especially anti-TNF agents (infliximab and adalimumab), the approach to these fistulas has changed. Thus, this article aims to review the currently available methods for managing PFCD.

TREATMENT OF PERIANAL FISTULAS IN CD

The initial approach is to control sepsis and take measures to prevent recurrent abscesses and the appearance of additional tracts by seton placement. Cutting setons should be avoided due to the risk of fecal incontinence[8].

SIMPLE FISTULAS

Fistulotomy is appropriate for superficial or low transsphincteric fistulas without associated proctitis, in addition to subanodermal, submucosal and subcutaneous fistulas. The recurrence rate is low (< 10%)[8]. However, incontinence rates vary from 0% to 50%, which leads to conservative techniques, such as seton placement[4]. Fistulotomy should not be performed anteriorly due to the risk of keyhole defects at the site where the sphincter is shortest, particularly in women. In the presence of proctitis, the fistulotomy wounds might not heal.

COMPLEX FISTULAS

Complex fistulas require an average of 6 procedures, while simple fistulas require 3 procedures[4]. At 10 years of follow-up, one-third require diversion and 13% require a proctectomy[9].

TREATMENT OPTIONS FOR COMPLEX FISTULAS

Long-term setons

Non-cutting setons can be maintained long term, *i.e.*, months or years. Two issues remain controversial during combination therapy (anti-TNF): Timing of withdrawal and number of procedures. The absence of secretion and proctitis are important factors. According to GETECCU recommendations, it is the option of choice in the presence of proctitis[10]. Kotze *et al*[11] found that the average time until seton removal was 7.3 mo, ranging from 1 to 36 mo. The advantages of this technique are the low cost, the prevention of new abscesses or recurring tracts, and a decreased need for temporary or permanent stoma, in addition to the low rate of reintervention (10% to 20%)[8]. On the other hand, the fistula does not close with the seton *in situ*, and the rate of clinical closure of the fistula after removal is 42% when used alone and 64% when in combination therapy with anti-TNF[12]. Another issue to be addressed is patient quality of life. The seton should be removed if the treatment goal is to close the fistula, usually prior to the end of the induction phase of TNF-inhibitors[13,14] (Table 1)[15-18].

Endorectal advancement flap

For endorectal advancement flap procedures, a tissue flap is mobilized from the mucosa, submucosa, or circular muscle layer of the rectum and advanced to cover the fistula's internal opening, resulting in an intact sphincter apparatus. Healing of the excluded fistula pathway is expected over time. In the absence of proctitis or stenosis, this is a good therapeutic option with the advantage of avoiding extensive or difficult-to-heal wounds and a success rate of approximately 50%[10,19] (Table 2)[20-25].

The ligation of the intersphincteric fistula tract

The procedure for ligation of the intersphincteric fistula tract (LIFT) involves the ligation and removal of the fistula pathway *via* the intersphincteric space, followed by removal of the remaining fistulous tract by curettage and closure of the defect by suture in the external sphincter[26], so that the sphincter is not affected[27]. Kamiński *et al*[28] followed 23 patients with transsphincteric fistulas due to CD who were treated with LIFT. After 23 mo the healing rate was 48%. However, most reports of LIFT procedures describe patients without CD, and only a few studies have been published exclusively on PFCD treatment.

In CD, patients without proctitis who have lateral fistulas with long tracks, previous seton treatment, and small intestine disease would be the best candidates for the LIFT procedure. However, prospective randomized studies comparing LIFT to other techniques are needed to define the role of this method in the treatment algorithm for PFCD[29] (Table 3)[24,25,27,28].

Fibrin glue and plugs

Two anal fistula plugs are frequently used in the management of perianal fistulas: The Surgisis (Cook Surgical, Bloomington, IN, United States), a bioabsorbable xenograft made of lyophilized porcine intestinal submucosa; and the GORE (Bio-A; WL Gore and Associates, Flagstaff, AZ, United States), a synthetic plug made of polyglycolic acid and trimethylene carbonate, which contains 2 absorbable synthetic materials in

Table 1 The results of long-term seton procedures

Ref.	Year	*n*	Follow-up, mo (range)	Recurrence (%)
William *et al*[15]	1991	55	54 (6-120)	0
Thornton *et al*[16]	2005	28	13 (2-81)	21
Takesue *et al*[17]	2002	32	62 (25-133)	3 (33)
Galis-Rozen *et al*[18]	2010	17	8 (6-9)	40

Table 2 The results of flap procedures

Ref.	Year	*n*	Healing (%)	Recurrence (R) or incontinence (I) (%)
Van Koperen *et al*[20]	2009	9	45	55 (R)
Soltani *et al*[21]	2010	91	64	9.4 (I)
Church *et al*[22]	2011	19	87	NR
Roper *et al*[23]	2019	39	92,6	19.5 (R)
Stellingwerf *et al*[24]	2019	64	61	7.8 (I)
Praag *et al*[25]	2019	21	60	19 (R) 15.8 (I)

NR: Not reported.

Table 3 The results of ligation of the intersphincteric fistula tract procedures

Ref.	Year	*n*	Healing (%)	Recurrence (R) or incontinence (I) (%)
Gingold *et al*[27]	2014	15	60	40 (R)
Kaminski *et al*[28]	2017	23	48	52 (R)
Praag *et al*[25]	2019	19	89.5	21.1 (R), 21.4 (I)
Stellingwerf *et al*[24]	2019	64	53	1.6 (I)

the fistula path that allow fixation to the fistula's internal opening[13]. The basic principle of the plug's action is to occlude the fistula path and promote healing. A controlled, randomized, multicenter study by the GETAID group compared the removal of the seton alone (control group) with plug insertion and found a healing rate of 31.5% in the plug group and 23.1% in the group control[30].

Heterologous fibrin glue is a 2-component material whose first component consists of fibrinogen, factor XIII, plasminogen, and aprotinin, whereas the second component consists purely of human thrombin. Simultaneous injection of the 2 components creates a fibrin clot that will mechanically seal the fistula path. Grimaud *et al*[31] conducted the first randomized, controlled clinical trial using fibrin glue to treat PFCD. They found healing rates of 38% in the glue group and 16% in the control group[31]. With unfavorable results for PFCD healing, both techniques were abandoned[4] (Table 4)[32-36].

Video-assisted anal fistula treatment

The main steps in Video-assisted anal fistula treatment (VAAFT) include excision of the fistula's external orifice, insertion of a fistuloscope to visualize the main and secondary pathways, correction of the location of the internal orifice under direct vision and irrigation, followed by electrocauterization of the paths. Schwander, the first author to demonstrate the results of VAAFT through a prospective, randomized study, compared the results of the VAAFT with the endorectal advancement flap technique. After a 9-mo follow-up, the success rate was 82% (9/11)[37]. Since this is a high-cost method with a long learning curve, the results of long-term studies are necessary[10,28] (Table 5)[37-40].

Table 4 The results of fibrin glue and plug procedures

Ref.	Year	*n*	Healing (%)	Recurrence (%)
Champagne *et al*[32]	2006	20	80	20
Schwandner *et al*[33]	2009	9	77	23
Ellis *et al*[34]	2010	12	66	34
Cintron *et al*[35]	2013	8	50	50
Herold *et al*[36]	2016	4	25	75

Table 5 The results of video-assisted anal fistula treatment procedures

Ref.	Year	*n*	Healing (%)	Recurrence (R) Incontinence (I) (%)
Schwandner *et al*[37]	2013	13	82	0 (I)
Garg *et al*[38]	2017	786	76	0 (I)
Adegbola *et al*[39]	2018	25	84	NR
Emili *et al*[40]	2018	788	54,3	17.7 (R)

NR: Not reported.

Fistula-tract Laser Closure

Lasers were first described in perianal fistula treatment in 2006. A carbon dioxide laser was used in 27 patients with CD, and most improved[41]. In 2011, Wilhelm described a new surgical technique using a radial laser probe [Fistula-tract Laser Closure (FiLaC™), Biolitec AG, Jena, Germany] to treat PFCD[42]. The basic principle of this technique is to destroy the epithelium of the fistulous path with the laser, although without direct visualization. In the initial study with this technique the internal orifice was closed with advancement of the endorectal flap. Wilhelm recently performed a for 2-year follow-up of 13 patients who underwent FiLaC combined with endorectal advancement flap and observed a 69% primary healing rate, which rose to 92% after the second surgery (secondary healing)[43]. The main advantages of this procedure are a shorter learning curve compared to VAAFT, faster recovery, and preservation of the sphincter. The disadvantages are the cost of the equipment and the absence of direct visualization of the paths. Thus, secondary paths may not be visualized and the healing rate could be reduced[29].

A recently published systematic review and meta-analysis concluded that FiLaC can be considered an effective and safe sphincter preservation technique with low complication rates. However, the review emphasized that studies comparing the laser to other techniques will be necessary to substantiate these promising results[44] (Table 6)[41,43,45,46].

Fistulectomy with primary sphincter reconstruction

Recent retrospective studies have assessed fistulectomy with primary sphincter reconstruction, finding excellent results. After an average follow-up of 11 mo (7 to 200 mo), the primary healing rate was 88.2%, with low recurrence rates[47]. However, no prospective studies have been published yet[28] (Table 7)[47,48].

Stem cell injection

The use of mesenchymal stem cells (MSC) is the most recent and promising strategy in PFCD treatment. MSC are a cell population similar to fibroblasts that can differentiate into several mesodermal cell lines[5]. They have potent anti-inflammatory and immunomodulatory activity[49]. The use of MSC in PFCD treatment is supported by the hypothesis that epithelial defects give rise to fistulas, which are maintained open by continuous inflammation occurring along the path. Injection of MSC into the fistula pathway is believed to reduce inflammation, thus promoting its healing[3]. MSC may be derived from adipose system (adipose stem cells – ASC) or from bone marrow. Despite the lack of clinical trials comparing bone marrow MSC to ASC, there are some reports of potential advantages of using ASC. Liposuction or excisional fat biopsy can

Table 6 The results of fistula-tract laser closure procedures

Ref.	Year	*n*	Healing (%)	Recurrence (%)
Moy *et al*[41]	2006	27	NR	NR
Wilhelm *et al*[43]	2017	13	69.2	0 (I)
Stijins *et al*[45]	2019	20	20	NR
Alam *et al*[46]	2020	20	54	NR

NR: Not reported.

Table 7 The results of fistulectomy with primary sphincter reconstruction

Ref.	Year	*n*	Healing (%)	Recurrence (%)
Herold *et al*[48]	2009	10	86	14
Seyfried *et al*[47]	2018	24	> 85	?

be used to ensure the harvest of a large number of stable raw cells that are readily available for clinical use. ASC also have a greater proliferative and angiogenic capacity, in addition to being more genetically and morphologically stable[5]. However, to date, no study has directly compared the use of autologous *vs* allogeneic MSC. It may take several weeks to expand autologous MSC *in vitro*. In addition, the patient's age and disease status can also affect cell quality. Nevertheless, allogeneic therapy with MSC has gained increasing popularity because of the immediate availability of high-quality cells for treatment. Thus, allogeneic products are likely to be used in the future[50].

Evidence about the effectiveness of ASC for complex PFCD treatment comes mainly from the ADMIRE-CD, a multicenter, randomized, double-blind, placebo-controlled study of 212 patients who did not respond to conventional medical treatment and were randomly assigned to receive an injection of 120 million ASC into the fistula pathways or placebo. Patients were allowed concomitant treatment with immunosuppressants and/or anti-TNFs at stable doses throughout the study. Combined remission at week 24 was the primary endpoint, defined as the clinical closure of all treated fistulas (absence of draining), as assessed by gentle finger compression, and absence of collections > 2 cm on magnetic resonance imaging. Significantly better results were obtained for combined remission in the ASC group than in the control group (50% *vs* 34%, $P = 0.024$)[51].

The STOMP study, conducted by the Mayo Clinic, was the first study to report the use of autologous ASC in a bioabsorbable matrix for the treatment of patients with a single fistula and no associated proctitis who did not respond to anti-TNF therapy. At 3 mo, 9 of the 12 patients (75%) had complete clinical healing, while at 6 mo 10 patients (83.3%) did, with similar rates of remission found in magnetic resonance imaging[52].

Injecting stem cells may be a valid alternative for complex PFCD that cannot be treated by conventional surgical methods. More evidence is required from adequately powered randomized clinical trials.

PISA trial

The PISA trial was a multicenter, prospective, randomized, controlled study comparing 3 groups: One that received a long-term seton (1 year), one that received anti-TNFs for 1 year, and a third that underwent surgical closure of the PFCD with either an endorectal advancement flap or LIFT, after 2 mo of anti-TNF. Before randomization, all patients underwent seton placement under general anesthesia, received antibiotics (metronidazole) for 2 wk and 6-mercaptopurine. The results showed a higher rate of reintervention for the long-term group seton group (10/15 *vs* 6/15 anti-TNF *vs* 3/14 surgical closure). The results suggest that chronic treatment with a long-term seton cannot be recommended as the only treatment for PFCD[12].

Endoscopic therapy for perianal disease

Partial endoscopic fistulotomy can be performed on intersphincteric fistulas through incision and endoscopic drainage. Although incision and endoscopic drainage can also

be performed with PFCD-associated perianal abscesses, it would be a temporary measure since more definitive therapy is needed, such as seton placement or fistulotomy. Abscesses associated with a perianal fistula can also be treated with endoscopy-guided seton placement[29,49].

Intralesional anti-TNF

In 7 case series, all with a small sample size (from 9 to 33 patients), infliximab (15 and 25 mg every 4 wk) or adalimumab (20 or 40 mg every 2 wk) was injected locally around the fistula, and closure was reported in 31%-75% of cases. The advantage is that injections can be easily repeated[10]. In a recent review evaluating 6 case series (including 2 studies with adalimumab injection), for a total of 92 patients enrolled, short-term efficacy (defined as complete or partial response) ranged from 40% to 100% without any significant adverse events[53]. Although local injection of infliximab appears to be safe and possibly effective, these studies involved few patients, had a short follow-up and no control group, in addition to a lack of standardization of the evaluated criteria and results.

Hyperbaric oxygen therapy

It has been proposed that hypoxia contributes to the onset and maintenance of inflammation, either as a causative or modifying factor, and its role as a trigger of inflammation has been demonstrated both *in vitro* and *in vivo*. Hyperbaric oxygen therapy (*i.e.*, inhaling pure oxygen in chambers at pressure > 1 atm) provides an option to optimize fibroblast proliferation and leukocyte activity[49], as well as to reduce hypoxia duration by altering the secretion of interleukin 1 (IL-1), IL-6, IL-2, and TNF and promoting angiogenesis. This technique has been effectively used to treat perianal disease, pyoderma gangrenosum, steroid-refractory ulcerative colitis, and persistent perineal sinus following proctectomy in inflammatory bowel disease. Regarding response to hyperbaric oxygen therapy among patients with perineal or fistulizing CD, rates range from 50% to 70% for complete response, from 9% to 41% for partial response, and from 12% to 20% for no response; a response rate of 88% has been reported in a systematic review of 40 patients with perianal disease refractory to conventional therapy[49,54,55].

Mild adverse effects have been associated with hyperbaric oxygen therapy, and they appear to be related to alterations in oxygen toxicity and barometric pressure. Trauma to the middle ear or sinus is reported as the most common complication, whereas rare complications have been observed in patients with underlying pulmonary disease and include pneumothorax, air embolism, and transient vision loss. Cataract maturation has been reported in more than 150 treated patients[49,56].

Hyperbaric oxygen therapy may be suggested as a last-line option in the treatment of chronic perianal CD refractory to other therapies or as an adjuvant to surgery, but controlled trials are still needed before it can be recommended for the management of PFCD[10]. This treatment is very time consuming and the effect might not continue if treatment is stopped.

Deviation

Deviation is a therapeutic option for patients with refractory perianal CD. However, due to its temporary character, it is not always feasible. In a systematic review including 15 studies, for a total of 556 patients enrolled, a low rate (33%) of intestinal transit reconstruction was observed after deviation[57].

Proctectomy

Proctectomy is the final treatment option for severe perianal CD refractory to aggressive medical treatment and to surgery. Proctocolectomy is preferred to rectal preservation in patients with concurrent Crohn's colitis and perineal disease because of the high rate of persistent rectal stump disease in cases in which the stump is left in place[4]. A feared complication after these techniques is inadequate healing of the perineal wound or the emergence of a perineal sinus of persistent drainage[58]. Proctectomy must include the mesorectum, since proinflammatory cells in the Crohn's mesorectum might fuel persistent inflammation in the pelvis. The cavity produced after a TME-type proctectomy can be filled with omentum[59].

A preoperative diagnosis of CD is generally considered a contraindication to ileal pouch-anal anastomosis (IPAA), although restorative proctocolectomy with the IPAA technique is a possibility in some Crohn's colitis patients. No significant difference was found in pouch failure between CD and ulcerative colitis[60]. Li *et al*[61] suggested a very select group of patients in whom surgery may be an appropriate treatment: Those

without perianal, small bowel, or mesenteric disease. Shen *et al*[62], on the other hand, reported that patients with a preoperative diagnosis of CD who undergo IPAA often develop CD in the pouch after surgery. Multicenter studies with a large number of patients will be necessary to better define indications for IPAA in CD.

CONCLUSION

Although medical treatment is the basic approach to perianal CD, surgical treatment is also essential. Before treating the fistula medically or surgically, a seton must be placed. However, there is still no consensus about the best approach. There is no doubt that, in the presence of serious or recurrent disease, aggressive surgical treatment should be considered. In addition, some patients will require a stoma or even a proctectomy. In cases of deviation, always consider closure after controlling for proctitis. It should also be noted that perianal CD should be managed by a multidisciplinary team.

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MINIREVIEWS

Vascular anomalies associated with hepatic shunting

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Abstract

Congenital vascular anomalies affecting the liver have been described in the scientific literature for decades. Understanding these malformations begins with knowledge of hepatic vascular embryology. Surgeons have applied numerous classification systems to describe both intrahepatic and extrahepatic shunts, which can confuse the reader and clinician. In our experience, focusing on one classification system for extrahepatic shunts and one for intrahepatic shunts is better. Today many patients with these shunts carry good long-term prognosis thanks to advances in imaging to better detect shunts earlier and classify them. Timely intervention by skilled radiologists and surgeons have also limited complications arising from dynamic shunts and can avoid a liver transplant. Congenital hepatic shunts are not the only vascular condition affecting the liver. Hereditary hemorrhagic telangiectasia, also known as Osler Weber Rendu syndrome, particularly type 2, may have varying severity of hepatic involvement which warrants longitudinal care from an experienced hepatologist. Lastly, congenital hemangiomas, often first identified on the skin and oral mucosa, also can affect the liver. While most will resolve in infancy and childhood, the pediatric hepatologist must understand how and when to treat persistent lesions and their complications. This article serves as a concise reference to help clinicians better care for patients with these rare conditions.

Key Words: Hepatic; Shunt; Pediatric; Hemangioma; Congenital; Vascular

Core Tip: Hepatic shunts present from birth, hepatic hemangiomas, and hereditary hemorrhagic telangiectasia have all been described in the scientific literature over the

quality classification
Grade A (Excellent): 0
Grade B (Very good): B, B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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decades. Most reviews were written by radiologists or surgeons, but none have adequately covered all these topics from the gastroenterologist's perspective. Our review serves as a reference for most congenital vascular anomalies that present in the liver. Our goal is to provide knowledge to help clinicians understand the burden of disease of these conditions and guide management decisions.

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INTRODUCTION

Congenital hepatoportal shunts (CHS) are rare but represent a unique entity and prognosis for pediatric patients. This review is an effort to succinctly describe various vascular anomalies and conditions associated with hepatic shunts including congenital hepatic shunts, hereditary hemorrhagic telangiectasia (HHT), and hepatic hemangiomas. We will also discuss various treatment considerations for such diseases and long-term prognosis.

EMBRYOLOGY

When discussing vascular abnormalities, it is important to first understand the origins of the normal hepatic vasculature. Embryological development of the liver occurs between the fourth and tenth weeks of life. Hepatic tissue originates from endoderm foregut tissue. This tissue undergoes specification after exposure to fibroblast growth factor and bone morphogenic protein, followed by morphogenesis into a liver bud. This process is orchestrated by multiple complex signaling molecules including Hex and GATA6[1].

The afferent and efferent hepatic venous vasculature develops from a complex evolution of the cardinal veins, vitelline veins, and the umbilical veins. The right and left cardinal veins run vertically through the developing fetus. Both veins have cranial and caudal segments that interface at a confluence beneath the developing right atrium and on top of the primordial liver called the sinus venosus (Figure 1A). The sinus venosus also receives blood from the terminal ends of the umbilical veins and incorporates an anastomosis between the two vessels. This confluence of vessels along with drainage from developing hepatic veins will eventually develop into the inferior vena cava by the eighth week of gestation[1].

The right and left vitelline veins and their bridging anastomoses originate on the anterior surface of the yolk sac and surround the primitive foregut at four weeks gestation. The vasculature is symmetric at this stage and structured like rungs on a ladder. By the tenth week of gestation, the inferior segments of the right vitelline vein and superior portions of the left vitelline vein regress leaving an S-shaped dominant vessel that carries blood from the maturing intestines into the liver: The main portal vein (Figure 1B). Within the liver, this venous web eventually organizes to form the right and left hepatic veins. The left portal vein typically arises from one of the vitelline anastomoses.

The umbilical veins also transform from the symmetric right and left vessels which flow into the sinus venosus and directly into the liver to a single left vessel which ends in the liver parenchyma. In utero, this vessel supplies oxygenated, nutrient-rich blood to the body and forms a main intrahepatic bypass vessel through to the systemic venous drainage called the ductus venosus (DV). Approximately 40%-50% of the blood from the umbilical veins flows through the DV and onto systemic circulation. The remaining blood flow is distributed through the liver sinusoids. After birth, umbilical venous blood flow is disrupted when the umbilical cord is cut. The umbilical vein normally becomes the ligamentum teres and the DV regresses into the ligamentum venosum. This process occurs within minutes after birth but can take up to three weeks to complete.

The fully developed portal vein supplies 75% of the blood flow into the liver. It

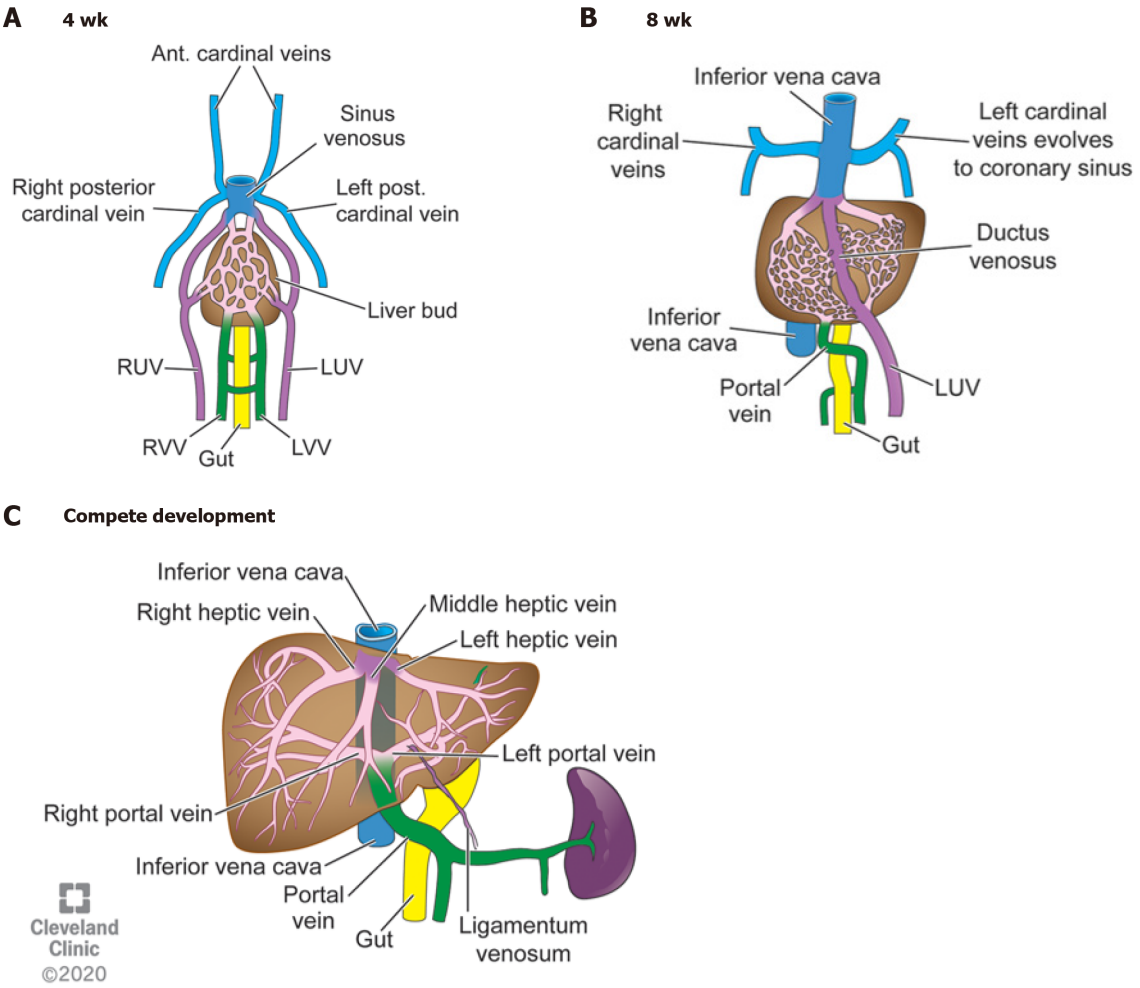

Figure 1 Four weeks gestation, eight weeks gestation, and the mature liver vasculature after birth. A: Right and left umbilical, cardinal, and vitelline veins making up the primitive vasculature to the developing liver bud. Cardinal and umbilical veins converge on top of the liver to form the sinus venosus. The vitelline veins return blood from the developing gut; B: Posterior cardinal veins coalesce to form the upper part of the inferior vena cava. The right umbilical vein involutes and left umbilical vein makes up the ductus venosus (DV). The intrahepatic vessels start to form mature hepatic veins. The vitelline veins start to mature into the portal venous system; C: The DV collapses at birth after the umbilical cord is cut and becomes the ligamentum venosum. The portal veins and hepatic veins are mature. RUV: Right umbilical vein; LUV: Left umbilical vein; RRV: Right vitelline vein; LVV: Left vitelline vein; IVC: Inferior vena cava. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2020. All rights reserved.

receives blood directly from the spleen, gallbladder, pancreas, and entire gastrointestinal tract, aside from the rectum, *via* the connecting superior and inferior mesenteric veins. The splenic vein typically flows into the superior mesenteric vein (SMV). Once in the liver, the portal vein normally divides into a left and right portal vein. The right portal vein further divides into an anterior and posterior branch (Figure 1C).

The complexity of hepatic vasculature development leads to several opportunities for abnormalities, namely from the failed closure of embryologic vessels. Other abnormalities arise from the proliferation of end vessels. This article will further review the formation, presentation, and therapeutic considerations of each of these congenital abnormalities.

CONGENITAL HEPATIC SHUNTS

A vascular shunt is any connection or orientation of blood vessels that bypass its intended organ. Congenital hepatic shunts usually present early in life either through incidental findings on imaging, on workup for liver injury secondary to the shunt itself, or work up for other causes of systemic disease either secondary to the shunt or associated with shunts. They are thought to arise from the persistence of the vitelline venous system in relation to the developing hepatic sinusoids. The incidence is

roughly 1:30000-1:50000 live births. Several classification systems exist; however, the practicality of such systems has led to much debate because: (1) Anatomical characteristics can be complex; and (2) It may not make a difference clinically or with regards to management. There are two broad categories of congenital shunts: Extrahepatic where portal blood flows bypass the liver and connect to the systemic circulation, or intrahepatic where blood flow through the liver connects to the systemic circulation before it is filtered by the hepatocytes. Intrahepatic and extrahepatic shunts may overlap within the same patient. For simplification, we will only discuss two classification systems that cover most extrahepatic and intrahepatic shunts.

Congenital extrahepatic porto-systemic shunts

Congenital extrahepatic porto-systemic shunts (CEPSS), are best classified using Morgan and Superina's[2] system developed in 1994 which divides these shunts into complete or partial hepatic diversion (Figure 2 and Table 1). CEPSS Type I is eponymously named Abernethy's malformation, in honor of Dr. John Abernethy who first described the malformation in 1793[3]. In Abernethy's malformation blood flow from the portal system bypasses the liver entirely and empties directly into the systemic venous circulation *via* an end-to-side anastomosis with the inferior vena cava. The portal system within the liver is, as best as can be identified, non-existent. While the pathophysiology is not completely understood, early involution of the peri duodenal vitelline plexus is the proposed mechanism for Abernethy's malformation[4]. Diagnosis can be difficult as some cases of CEPSS Type I on initial imaging later may show hypoplastic intrahepatic portal venous flow with more invasive investigation such as cardiac catheterization and angiography. CEPSS Type I malformations can further be subcategorized depending on whether the splenic and SMV enter the systemic venous drainage separately (Type 1a) or if they converge to a common vessel before entering the systemic venous system (Type 1b) (Figure 3). CEPSS Type II malformations describe extrahepatic shunts where some of the portal flow is still intact in the presence of a smaller side-to-side anastomosis between the portal and systemic venous systems (Figure 4A-D). Persistence of the left vitelline vein leads to Type II shunts draining above the hepatic confluence or connect to the right atrium (Figure 4D).

Congenital cavernous malformations are a separate extrahepatic portal malformation worth mentioning. Cavernous malformations are typically secondary to portal venous thrombus and as a response to portal hypertension, but congenital idiopathic malformations have been described. Liver vascular ultrasound may be read erroneously as antegrade flow if the collateral vessels are densely packed. It can be best identified on computed tomography (CT) angiography (Figure 5).

Congenital intrahepatic porto-systemic shunts

Congenital intrahepatic porto-systemic shunts (CIPSS) occur inside the liver where some normal portal venous blood flow through the liver is preserved. The embryological origins of these rare anomalies can arise from the failed fusion of the right vitellin and umbilical venous plexus which create communications between intrahepatic portal and hepatic or perihepatic veins[3,5]. CIPSS are sub-typed by location and extent of the shunt. The most widely used classification system for intrahepatic shunts was outlined by Park *et al*[6] in a case series from 1990. There he described 14 cases of various intrahepatic shunts (Figure 6 and Table 2). Type 1 is the most common (Figure 7). They are associated with cirrhosis if they persist but often close on their own. A patent DV is technically an intrahepatic shunt as it arises from a persistent connection between the left portal vein and a left hepatic vein *via* the partially involuted left umbilical vein which fails to close after birth to create the ligamentum venosum. This may be secondary to congenital heart disease causing altered hemodynamics and delayed ductal closure[7]. Patency may induce hypoplasia of the portal venous system[3].

Presentations and complications

CHS may be found in isolation, but associations with other congenital abnormalities have been described. Several cardiac congenital anomalies are associated with CHS including atrial and ventricular septal defects, patent foramen ovale, tetralogy of Fallot, and occasionally meso and dextrocardia[4]. Situs ambiguous with malrotation and polysplenia has also been described. Other GI manifestations include malrotation, annular pancreas, and biliary atresia. About eight percent of extrahepatic shunt patients can also have polysplenia or continuation of the azygos or hemiazygos systems into the inferior vena cava[3]. There is also an increased incidence of CHS in

Table 1 Morgan and Superina extrahepatic shunts

Morgan and Superina extrahepatic shunts
Type I: "Abernethy malformation" portal blood flow bypasses the liver entirely and empties into the IVC
The splenic vein and SMV enter the IVC separately
The splenic vein and SMV form common vessel before entering IVC
Type II: Partial shunt where an "H type" connection between the portal system and the IVC. Some portal flow to the liver is still intact

IVC: Inferior vena cava; SMV: Superior mesenteric veins.

Table 2 Park's classification of congenital intrahepatic portosystemic shunts

Park's classification of congenital intrahepatic portosystemic shunts[5]
Large connection of constant diameter from the right portal vein to the intrahepatic IVC
Localized peripheral shunt from multiple or single communications between the peripheral branches of the portal and hepatic veins within one hepatic segment
An aneurism between connecting peripheral portal and hepatic veins
Multiple communications between peripheral and hepatic veins peripherally throughout the liver
Patent ductus venosus

IVC: Inferior vena cava.

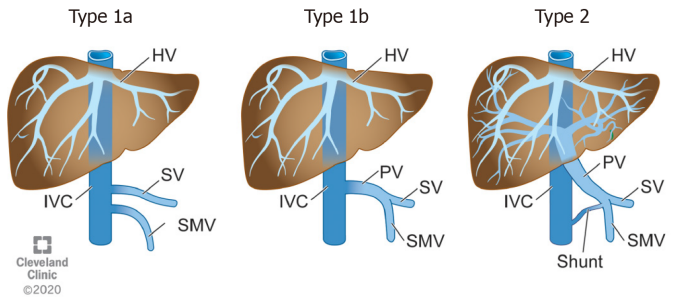

Figure 2 Morgan and Superina congenital extrahepatic portosystemic shunts. Refer to Table 1 for descriptions. HV: Hepatic vein; SV: Splenic vein; SMV: Superior mesenteric vein; IVC: Inferior vena cava; PV: Portal vein. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2020. All rights reserved.

patients with various genetic conditions such as Down's syndrome[7]. Clinically, hepatic shunts manifest a variety of symptoms or can be indolent depending on the degree of shunting. While no longer believed to be exclusively female, CEPSS Type I are often reported with a female predominance[4]. CEPSS Type II is more male predominant. CHS reduce vital nutrition to the developing liver in utero. As 75% of the hepatic blood flow arrives *via* the portal vein, CHS can significantly affect liver growth and function. Liver atrophy occurs due to loss of nutrient flow to the liver as well as stimulating growth factors such as insulin and glucagon. In CHS, liver volumes can be 50%-60% of normal[8]. The body compensates for decreased portal blood flow by increasing hepatic arterial flow; however, this blood is low in nutrients, insulin, and glucagon. As a result, the cellular regenerative capacity of the liver is compromised leading to liver nodules in 20%-50% of patients[4,9]. These nodules are typically regenerative, but malignant lesions including hepatoblastoma and hepatocellular carcinoma have been described. There are currently eight reported cases of hepatoblastoma secondary to CHS in the literature as young as 17-months-old[9]. Correction of the shunt is associated with resolution of benign nodules[10].

De Vito *et al*[11] described histological characteristics of hepatic tissue in CHS based on a review of autopsied livers, wedge and core needle biopsies. These biopsies showed small portal venules, prominent thin walled channels, and otherwise observed

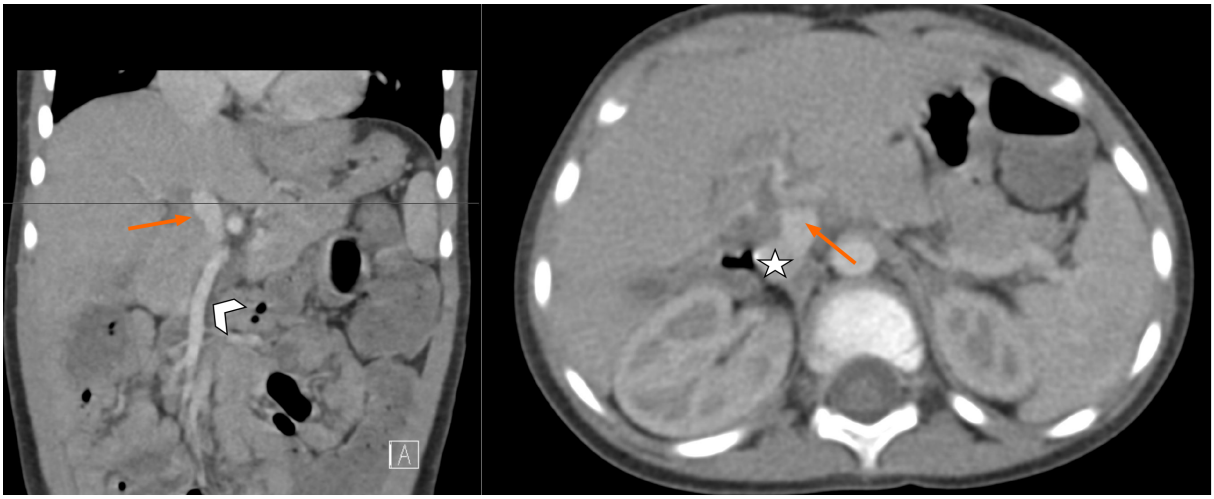

Figure 3 Two-year-old with Abernethy Malformation Type 1b (arrow). A connection from superior mesenteric vein (chevron) to the inferior vena cava (star).

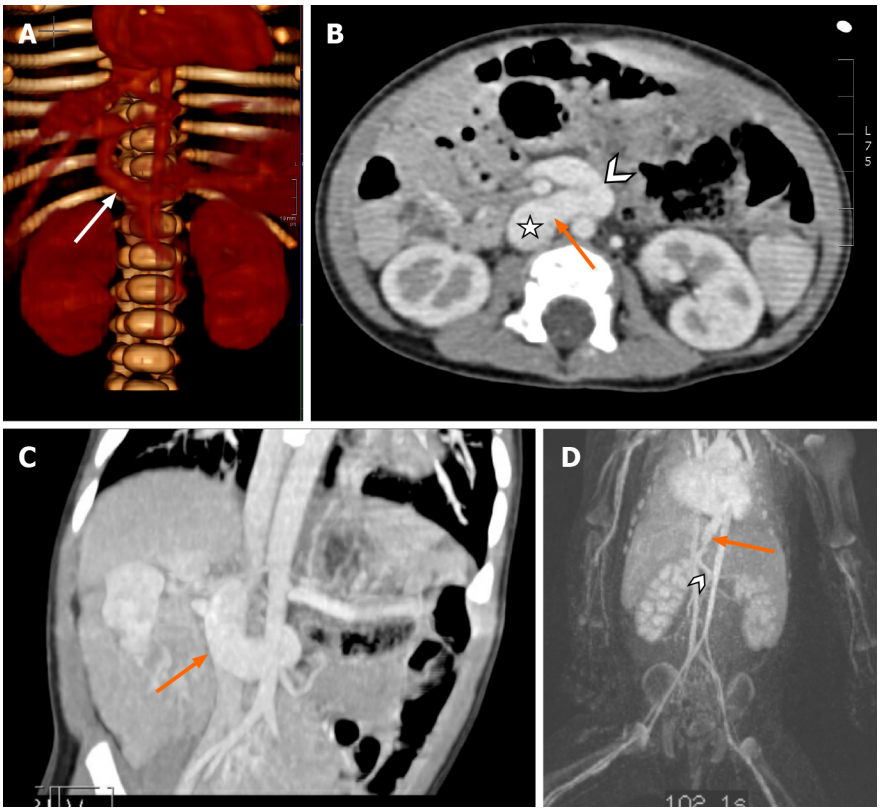

Figure 4 Examples of congenital extrahepatic portosystemic shunts. A: Nineteen-month-old with congenital extrahepatic porto-systemic shunts (CEPSS) Type II. Connection (arrow) of portal vein to inferior vena cava (IVC). Reconstruction 3D rendering of a computed tomography scan; B and C: One year old with a history of heterotaxia, intestinal malrotation, pulmonary arteriovenous malformations and CEPSS Type II (orange arrow), portal vein (chevron) to IVC (star); D: Type II extrahepatic shunt superior mesenteric vein (chevron) and shunt (arrow) draining directly into the right atrium.

portal arterio-biliary dyads suggesting atrophied venules. Increased arterial profiles were also evident, in keeping with the known compensatory mechanism of blood flow to the hepatocytes as described above. Vacuolization of hepatic nuclei, a sign of hepatocyte aging, was not evident. While the cause is not fully understood, this appears to be a result of decreased exposure to anabolic and catabolic hormones such as insulin and glucagon[12]. In the developing pediatric liver, the exposure of these hormones is likely essential to normal liver remodeling which is interrupted in patients with CHS[11].

Presentations in the neonatal period include hyperammonemia or hyper-

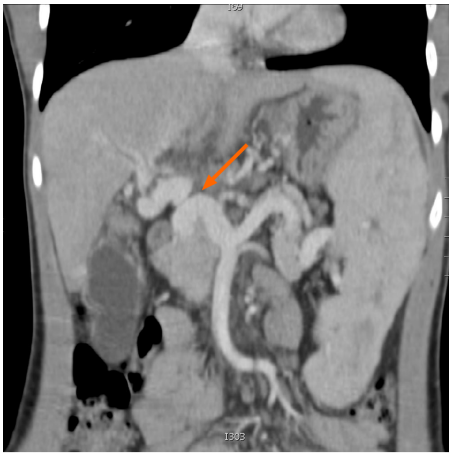

Figure 5 Computed tomography abdomen showing congenital cavernous malformation of the portal vein (arrow).

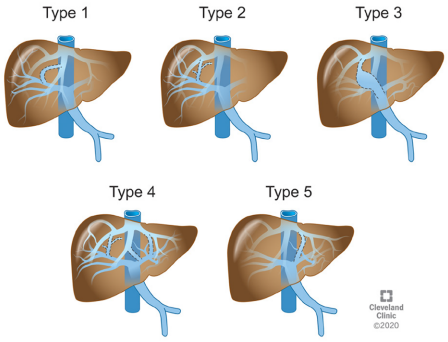

Figure 6 Park's classifications of congenital intrahepatic portosystemic shunts. Refer to Table 2 for description. Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography ©2020. All rights reserved.

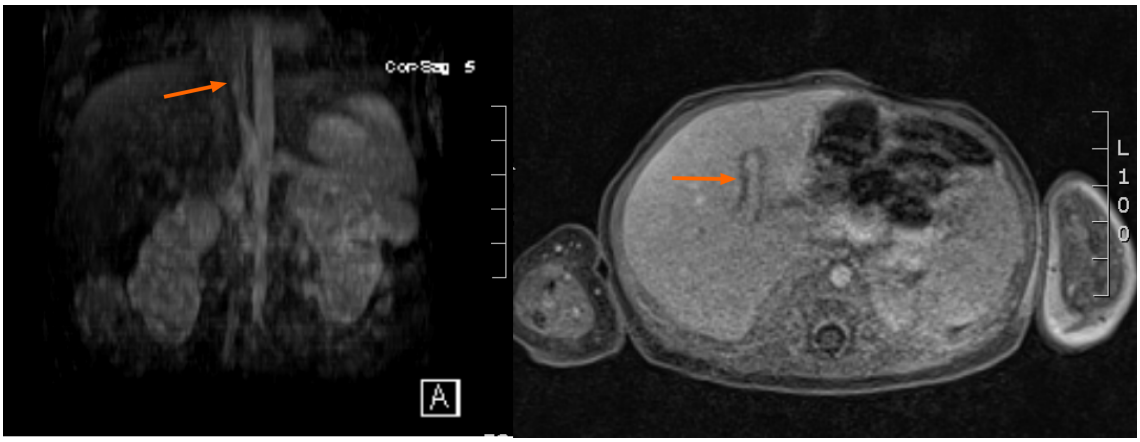

Figure 7 Intrahepatic shunt, main portal vein to the left hepatic vein (arrow).

galactosemia along with elevated bile acids. Galactose is typically processed in the liver by uridine diphosphate (UDP) enzymes to be converted into glucose and stored as glycogen. Similarly, bile acids are excreted into the intestines and reabsorbed and reprocessed in the liver. In CHS, galactose rich blood partially or totally bypasses the liver leading to galactosemia without UDP enzyme deficiency. As such, CHS should remain on a differential diagnosis for galactosemia in newborns with bile acidemia[13]. Neonates may present with cholestasis. It is unclear if the cholestasis is a result of the hepatic shunting or an inciting factor which increases the resistance of blood flow through the liver favoring extrahepatic diversion.

Stigmata of chronic liver disease can be present overtime including spider nevi,

digital clubbing, generalized fatigue, ascites, and growth failure. As the child ages, ongoing stress on the liver and chronic systemic exposure or toxic metabolites that have bypassed the liver will predispose the patient to hepatic encephalopathy, hepatopulmonary syndrome (HPS), or pulmonary hypertension[3,4]. Hepatic encephalopathy occurs when unprocessed ammonia produced by gut flora and absorbed by the intestines is processed into glutamine in the brain, which is neurotoxic[10]. While testing for hepatic encephalopathy in pediatrics is not well defined, monitoring ammonia levels is commonly done, especially if acute neurologic changes occur. Baseline elevated ammonia levels are seen in 66%-100% of patients with CHS[10]. While this was initially described in adults more than children, neurologic disease in children appears to be more indolent and the cause of seizures, irritability, and cognitive deficits. Hepatic encephalopathy is correlated well with shunt size[3]. Shunt ratios between 30%-60% are at increased risk of developing hepatic encephalopathy following systemic stress such as illness. If a shunt is > 60% then patients are at risk of spontaneous encephalopathy and warrants treatment[4].

HPS occurs in about 10% of patients with CHS[10]. While the cause is not completely understood, poor hepatic clearance of vasodilator substances acting on pulmonary endothelium is believed to be involved. Neonatal presentation may be subtle hypoxia. Older children will present with dyspnea on exertion and increased cardiac output. Orthodeoxia and platypnea, shown clinically as paradoxical improvement in dyspnea when transitioned to a supine position from an upright position, may also be seen in older children. Diagnosis can be made with either a "bubble" echocardiogram or technetium 99m-labeled macroaggregated albumin study. Both can measure the severity of HPS. Liver transplant remains a cornerstone of treatment for HPS for chronic liver disease[10].

Portopulmonary hypertension can occur in 13%-66% of CHS patients. It is often asymptomatic but can cause altered consciousness or syncope. It is thought to be caused by micro emboli and vasoconstrictive substances which typically are filtered by the liver. It is defined on cardiac catheterization as pulmonary artery wedge pressure of > 25 mmHg. The degree of severity does not correlate with shunt size. Early diagnosis and monitoring of portopulmonary hypertension is necessary as it is irreversible, even after shunt closure, and caries up to a 50% mortality rate[10].

Diagnosis: Images and biopsies

Imaging is essential for the diagnosis of CHS. Non-radioactive modalities are preferred for initial investigation. Ultrasonography (US) is widely utilized in the neonatal period, which is highly specific if a portal vein is absent. A small sized liver is further evidence of compromised portal venous blood flow. The ability to apply Doppler can help provide qualitative data such as direction of blood flow within the shunt. Intrahepatic shunts will have antegrade blood flow on color Doppler. US is generally well tolerated and exposes the patient to no ionizing radiation[3,4]. Unfortunately, there is considerable operator variability and the acoustic window remains relatively small. As such, it may not highlight intrahepatic shunts and more detailed follow-up imaging with magnetic resonance angiography (MRA) or computed tomography angiography (CTA) is required.

Multidetector CTA is the gold standard for diagnosis and characterization of hepatic shunts given easy availability, rapid processing time, and detailed imaging with three-dimensional reconstruction of the hepatic vasculature. This provides the clearest picture for treatment either by a surgical team or interventional radiologist. The benefits of CT must be weighed against the increased risks of cancer from radiation exposure and nephrotoxicity secondary to contrast medium. CTA is also advantageous for patients who will not tolerate the lengthy scan time or in those with metal implants which prohibit the use of MR[4].

MRA provides excellent imaging of hepatic vasculature as well as characterize focal hepatic lesions without the associated ionizing radiation found in CT scans. Liver nodules are best defined on MR. Hyperplastic lesions are usually multiple and have increased signal intensity on T1-weighted imaging during the arterial phase and remain bright in the venous phase[4]. They vary in size from 0.5 cm to 4 cm in diameter.

For CEPSS Type II, it is helpful to determine the degree of partial shunting. Nuclear medicine may be used to measure the degree of shunting by calculating a portosystemic shunt index. In transrectal portal sintigraphy, radiolabeled ^{123}I Iodoamphetamine is introduced into the distal colon *via* enema and absorbed through the inferior mesenteric vein. In patients without CHS, the isotope is taken up only by the liver. In patients with CHS, some or all of the isotopes travel through the shunt into the systemic circulation and accumulate in the lungs. By taking images of the liver and lungs, a shunt ratio is calculated thus determining the severity of the shunt. Shunt

ratios > 5% are considered abnormal[4].

Diagnosis of CHS can be mostly made with the imaging modalities described above; however, there is a role for liver biopsy for patients who appear to have Abernathy's malformation. If liver venules are present within portal triads, this may be evidence for the diagnosis of Type II CEPSS which would help management planning. Biopsies of suspicious hepatic nodules to rule out malignancy is also recommended.

Treatments and prognosis

CHS are rare and there are no large studies to drive treatment guidelines; however, some general principles are described here. Management of CHS first involves work up to rule out other causes and for assessment of other congenital anomalies. Shunt anatomy and severity of diverted portal venous blood flow is paramount. Before committing to intravascular or surgical management, one must first decide if or when intervention is necessary. The timing of intervention has also been greatly debated. Small type II CEPSS and many intrahepatic shunts may close spontaneously by 12 mo of age and only require close monitoring. Shunts that persist beyond 24 mo are unlikely to close spontaneously. Persistent DV and large Type II CEPSS with shunt ratio > 60% are at increased risk of developing hepatic encephalopathy and are unlikely to close spontaneously. Prophylactic closure prior to 24 mo is advised[3,14]. Symptomatic sequelae such as cardiovascular involvement, hepatic encephalopathy, or the existence of liver nodules warrant immediate treatment regardless of the shunt type. Regularly monitoring liver enzymes, alpha-fetoprotein, PT/INR, and ammonia levels as well as hepatic blood flow *via* ultrasound with doppler every 3-6 mo is essential. MR of liver annually is also often utilized in monitoring.

Hyperammonemia is treated by lowering the nitrogen load through diet modification and disruption of ammonia production and gut absorption. A low protein diet will limit the nitrogen load; however, this must be balanced with meeting the body's needs for growth. We recommend a daily limit of 0.8-1.0 g/kg of dietary protein to meet nutritional needs. Co-management with a dietician is recommended. Interrupting ammonia reabsorption in the intestines can be achieved by adding lactulose, a non-absorbable sugar which acidifies the stool and promotes ammonia (NH_3) conversion to non-absorbable ammonium (NH_4^+). Alternatively, using non-absorbable antibiotics such as rifaximin or neomycin reduces bacterial load in the intestines and stops NH_3 production[4].

Intravascular closure: A definitive diagnosis of the shunt using angiography can map the vascular anatomy and assess how blood flow dynamics change with temporary shunt occlusion with the angiocatheter balloon. Sometimes, presumed CEPSS Type I are found to have open collateral vessels during temporary shunt occlusion, thus redefining them as CEPSS Type II (Figure 8A and B). Closure of Type II CEPSS as well as persistent isolated intrahepatic shunts serves to redirect portal blood flow back through the functioning liver. Portal pressure readings within the occluded vessel will determine further treatment. If portal pressure exceeds 30 mmHg, then total occlusion could cause sudden portal hypertension leading to hepatic stress and dysfunction. Therefore, a two-stage closure is recommended to allow the liver to gradually accommodate the re-routed blood flow[3] (Figure 8A-D). A variety of occlusion devices can be deployed including coils and intravascular plugs. Large shunts may be amenable to vascular plugs which can be adjusted prior to deployment for two-stage occlusion (Figure 9). Utilization in small peripheral extrahepatic shunts such as splenorenal shunts has also been described. Careful planning by skilled interventionalists is paramount. Intravascular plugs placed in short shunts with large diameter are at risk of plug migration into the systemic circulation.

Surgical management: Alternatively, laparoscopic surgical ligation may be safer for large diameter and/or short extrahepatic shunts which would make intravascular coils or plugs difficult to place. Intraoperative temporary occlusion can also be performed to assess for large hemodynamic shifts that necessitate two-stage closure. For large intrahepatic shunts which do not close spontaneously, a partial liver resection is also an option if device closure is not possible, failed, or if there is a concurrent hepatocellular mass. Shunt occlusion with N-butyl cyanoacrylate lipiodol has also been used to sclerose shunts[3,10].

Liver transplant: CEPSS Type I often requires liver transplant as there is an absence of portal vasculature through the liver. Symptomatic CEPSS Type I is a clear indication for an expedient liver transplant. Liver transplant is also reserved for severe complications such as hepatic encephalopathy, hepatoblastoma or hepatocellular carcinoma, and HPS[3,10]. Prophylactic liver transplantation in asymptomatic CHS

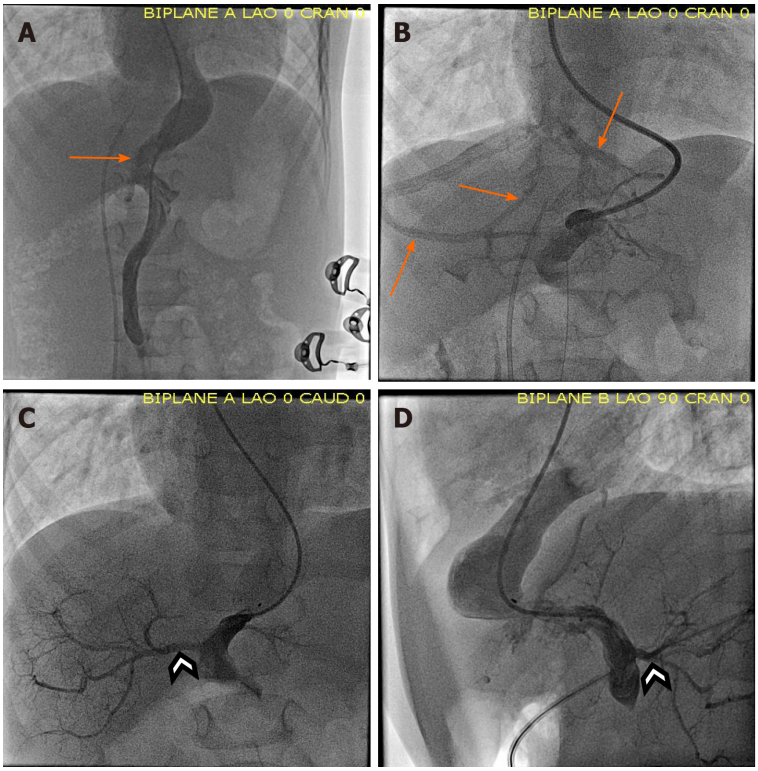

Figure 8 Intravascular closure. A: Angiography showing a large congenital extrahepatic porto-systemic shunts (CEPSS) Type II (arrow); B: Balloon occlusion of the shunt revealing multiple large intrahepatic collateral vessels (arrows) (CIPSS Type IV); C: Anteroposterior; D: Lateral views of repeat angiography several months post partial occlusion of shunt showing improvement in portal venous flow after partial occlusion with better visualization of the right portal vein (chevron). Occlusion device not seen.

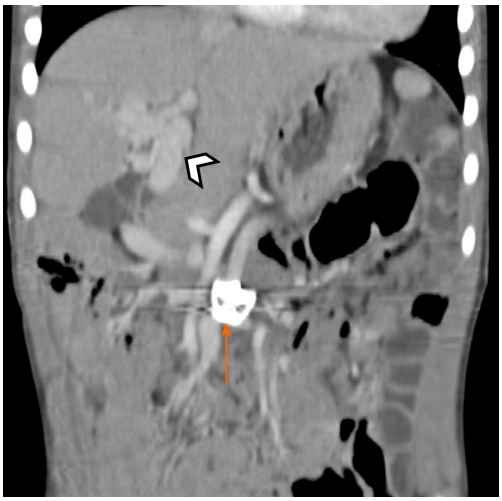

Figure 9 Four-year-old boy with heterotaxia and congenital extrahepatic porto-systemic shunts Type II two years post device closure (arrow). He subsequently developed a cavernous transformation of the portal vein (chevron).

patients has been debated and warrants careful consideration. Holding off until the inevitable pulmonary complications (PH and HPS) arise make perioperative care difficult; however, early transplantation increases the lifetime exposure to immunosuppressive medications (Figure 10). Techniques for connecting the transplanted PV to the recipient PV have different risks and benefits. End-to-end anastomosis is complicated by small bowel venous congestion. End-to-side anastomosis has less risk of small bowel congestion but may require an additional interposition venous graph to make the connection.

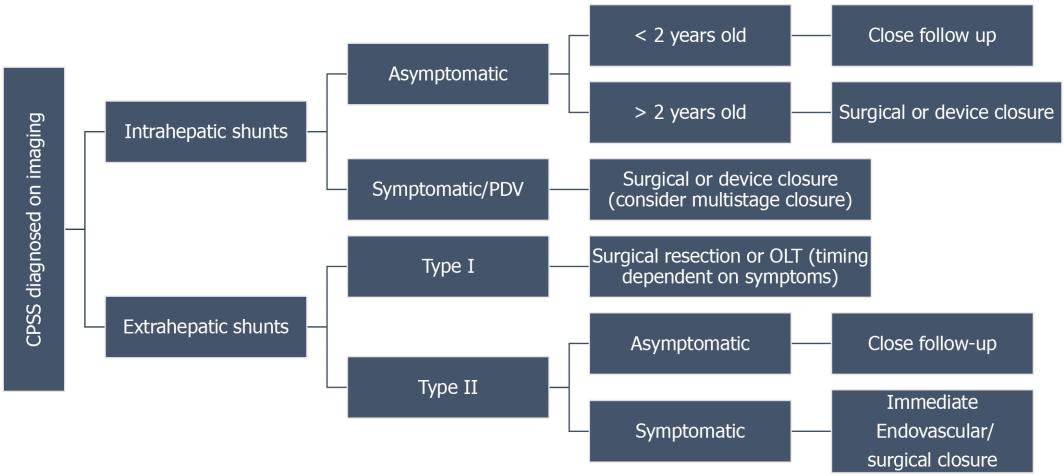

Figure 10 Congenital portosystemic shunts treatment algorithm. OLT: Orthotopic liver transplant; CPSS: Congenital portosystemic shunts.

Prognosis and complications

Patients still require regular follow-up after shunt closure. Reversal of hepatic encephalopathy and HPS is typical; however, pulmonary hypertension is unlikely to resolve following shunt occlusion but may respond to pulmonary vasodilator medication. Liver nodule regression is common. Transient portal hypertension often follows shunt occlusion. This can resolve as the liver gradually accommodates more portal blood flow, but persistent portal hypertension may spawn secondary shunts such as splenorenal shunts. Altered blood flow after shunt closure also increases the risk of portal vein thrombosis leading to portal hypertension[14]. As previously stated, migration of closure devices may theoretically occur, and patients must be made aware of this risk.

HHT (OSLER WEBER RENDU SYNDROME)

HHT is a rare autosomal dominant condition occurring in 1-2 cases per 10000, characterized by multiple angiodysplasia lesions which classically present in the skin and mucous membranes. In mucosa, they occur at the capillary level where postcapillary venules dilate and fuse with arterioles creating an arteriovenous shunt[13]. Clinical diagnostic criteria are listed[14] (Table 3). Frequent epistaxis is the most common clinical manifestation. Visceral organ involvement can occur in the liver (most common), pulmonary system, intestines, or brain and spinal cord[15]. Earlier diagnosis is on the rise following improvements in multidetector CT which can produce a clearer definition of vascular abnormalities within the organs. HHT has been categorized into two distinct types associated with distinct gene mutations with a third type currently undergoing investigation[15]. Types 1 and 2 both involved genes which control the transforming growth factor beta (TGF-beta) pathway[15]. TGF-beta signaling pathway will go on to stimulate vascular endothelial growth factor which induces vascular proliferation. The genetic mutation for type 1 is in a gene called *ENG*, found on chromosome 9, which codes for Endoglin, a TGF-beta receptor. Type 2 is caused by a mutation on chromosome 12 which codes for activin receptor-like kinase type 1 (ALK-1). Hepatic involvement in HHT is almost always associated with ALK-1 mutation and type 2 HHT[16,17]. There have also been cases of patients with juvenile polyposis syndrome (SMAD4 mutation) with HHT overlap, presenting with anemia, epistaxis, and pulmonary and liver telangiectasia[18]. Studies have suggested that between 15%-22% of patients with SMAD4 mutation can develop JPS-HHT overlap[18].

Liver involvement with HHT was first proposed in the late 19th century. By the mid-20th century medicine had described three categories of HHT based on if hepatic telangiectasia were present and if the patients developed fibrosis or cirrhosis[19]. Hepatic involvement can occur in 74%-79% of patients and can be identified at an early age; however, symptoms typically do not manifest before the third decade of life[16]. Liver biopsy will show fibrosis and cord atrophy, capillary hyperplasia, and hyperplastic vascular ectasia[16]. The type and extent of the shunt can determine the

Table 3 Hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (must have at least three of the following)
Recurrent spontaneous epistaxis
Mucocutaneous telangiectasia
Family history of HHT
Presence of visceral involvement

HHT: Hereditary hemorrhagic telangiectasia.

involvement. Only eight percent of patients with HHT and liver involvement will become symptomatic[16].

Liver vascular malformations are unique to other telangiectasias given the three vascular pathways which interact with the liver: Hepatic arteries, hepatic veins, and portal veins. Three types of intrahepatic shunts can develop: Arteriovenous, arterioportal, and portovenous. More than one type of shunt can develop in the same patient, but one may dominate functionally[15]. Arteriovenous shunts are the most common (50%). They can classically induce hepatomegaly following congestive heart failure and pulmonary hypertension. Arterioportal shunts are less common and patients usually have arteriovenous shunting as well. Arterioportal shunts often induce portal hypertension from increased blood flow and back pressure on the portal tree. Portal hypertension, classically defined as a hepatoportal venous gradient > 10 mmHg, develops in the fifth or sixth decade of life. It presents with classic transudative ascites and varices are prone to hemorrhage. Lastly, portovenous shunts are typically only seen on microscopy in childhood but may become more prominent shunts by the fifth or sixth decade of life[15].

Presentation and diagnosis

Complications from liver involvement typically occur in middle age. High output cardiac failure is the most common symptom and is associated with vascular malformations large enough to produce a bruit or palpable thrill in the epigastrium on exam[15]. The presentation of cardiac failure with orthopnea, dyspnea on exertion, and edema is classic. Pregnancy may be a precipitating or exacerbating event in women. Abdominal angina secondary to mesenteric artery "steal" phenomenon has also been described[15]. Portal hypertension is the second most common complication and is associated with arterioportal malformations. They can eventually cause ascites and gastric and esophageal variceal bleeding. Altered blood flow through the hepatocytes can create perfusion abnormalities. This can lead to focal nodular hyperplasia (2.9% of cases) and periportal fibrosis. Focal nodular hyperplasia and concomitant portal hypertension may be misdiagnosed as liver cirrhosis. Unlike in cirrhosis, these livers typically maintain synthetic function[15] (Table 4).

Biliary disease is also well described in HHT with liver involvement. It is thought to be related to shunt induced biliary ischemia and manifests as strictures of the gallbladder neck or intra or extrahepatic bile ducts. This typically affects women in their late 30 s. Often serum alkaline phosphatase and gamma-glutamyl transferase are elevated, thus patients may be erroneously diagnosed with cholecystitis and undergo cholecystectomy. In another case series of HHT patients, three of 12 patients developed bilomas[16]. Surprisingly, each of these patients had intrahepatic arteriovenous shunting and elevated alkaline phosphatase, but with normal bilirubin. Lastly, large portovenous malformations leading to hepatic encephalopathy have been rarely reported.

Diagnosis of liver involvement typically begins with a high index of suspicion following history and exam and is confirmed with imaging. Patients with known HHT without pulmonary arterio-venous malformations (AVMs) who present with dyspnea and ascites may be in cardiac failure. Liver ultrasound with Doppler and contrast spiral CT are recommended as initial, non-invasive investigations. These tests will show evidence of intrahepatic telangiectasias and an enlarged common hepatic artery in symptomatic individuals[15]. Biliary abnormalities seen on magnetic resonance cholangiopancreatography (MRCP) or endoscopic retrograde cholangiopancreatography (ERCP) are seen even in patients without biliary symptoms suggesting a progressive course of the disease. Cardiac catheterization and angiography are invasive but considered the gold standard for determining the shunt severity as well

Table 4 Vascular shunts associated with hereditary hemorrhagic telangiectasia

Vascular shunts associated with HHT	Associated systemic/Hepatic manifestations
Arteriovenous	Hepatomegaly
	Pulmonary hypertension
	High output cardiac failure
	Biliary ischemia/biloma
	Abdominal angina
Arterioportal	Focal nodular hyperplasia
	Non-cirrhotic portal hypertension
	Hepatic encephalopathy
Portovenous	Hepatomegaly
	Hepatic encephalopathy
	High output cardiac failure
	Non-cirrhotic portal hypertension
	Focal nodular hyperplasia

HHT: Hereditary hemorrhagic telangiectasia.

as the degree of heart failure if present. Portal wedge pressures can also be measured to confirm portal hypertension if present. Mesenteric steal syndrome can also be confirmed in patients presenting with abdominal pain.

Treatment

Treatment of HHT is largely symptomatic control. Patients may require blood transfusions for ongoing blood loss from cutaneous bleeding. Iron deficiency is common, and supplementation is frequently required. Laser therapy may be needed for treatment of skin telangiectasias. Endoscopy typically utilizes argon plasma coagulation for gastrointestinal AVMs. Esophageal varices should be treated as they are for any other cases of portal hypertension. Liver AVMs can predispose to high output cardiac failure and may be treated with diuretics. Previously used as a compassionate care drug to treat childhood cancers, bevacizumab is an anti-VEGF antibody that has been shown to treat bleeds from cutaneous and gastrointestinal telangiectasia leading to a significantly decreased need for transfusions. Interferon has been utilized for control of cutaneous telangiectasia. In adults, hepatic arterial embolization has been described as a more of a temporizing, palliative care option in patients with arteriovenous and arterioportal shunts who failed medical management. For extensive, medically refractory disease or portovenous disease, liver transplant remains an option. This is often used for extensive hepato-biliary necrosis and or heart failure (Table 5).

MULTIFOCAL VASCULAR HEMANGIOMAS WITH EXTRACUTANEOUS DISEASE

Infantile hemangiomas remain the most common tumor in neonates with a prevalence estimated at 4%-5% of all infants[20]. They are benign endothelial tumors but can lead to comorbidities based on size, location, and the number of lesions. Isolated cutaneous lesions are the most common, but visceral involvement, most commonly in the liver, is also seen with and without cutaneous lesions.

While isolated hemangiomas are common, multiple lesions are more likely to have a genetic cause and carry higher morbidity and mortality if untreated. Diffuse neonatal hemangiomas were first described in the early 1970s and 1980s, but the term suffered from ambiguity over the decades. It has meant to cover several conditions that have now been isolated through immunohistochemistry studies and better clinical characterization[21]. The term multifocal vascular hemangiomas with or without

Table 5 Hereditary hemorrhagic telangiectasia hepatic involvement treatment considerations

HHT hepatic involvement treatment considerations
Symptom control: Iron deficiency, heart failure, esophageal varices
Anti-VEGF antibodies *(i.e.*, bevacizumab)
Hepatic arterial embolization: Typically, an adult palliative option
Liver transplant: In setting of extensive hepato-biliary necrosis or heart failure

HHT: Hereditary hemorrhagic telangiectasia.

extracutaneous disease are now the preferred terminology; however, most of the literature still uses a variety of terms.

A recent publication in the Journal of Pediatrics[22] classified hemangiomas in the first year of life as either congenital or infantile as they follow different courses and have different treatment and management guidelines. Congenital hemangiomas grow in-utero and are present at birth. They are often identified on antenatal ultrasound. Lesions typically stain negative for glucose transporter 1 (GLUT-1) if biopsied. Large lesions have high vascular flow and are associated with hemodynamic instability and heart failure which may be the presenting symptom at birth. Other complications from large lesions include mild anemia, thrombocytopenia, and hypofibrinogenemia; however, these are typically transient and not as dramatic as what is seen in the Kasabach-Merritt phenomenon. Congenital hemangiomas are subcategorized into one of three patterns: Rapidly involuting congenital hemangiomas (RICH) where there is a complete self-resolution of the lesion within two years, partially involuting congenital hemangiomas where size reduces but never fully resolves, and non-involuting congenital hemangiomas where lesion size remains the same. Monitoring is the mainstay of treatment for these lesions with regular complete blood counts in the neonatal periods to assess for cytopenias and echocardiogram to monitor heart function. Hepatic lesions should be monitored with ultrasound every two weeks initially and extending the image interval by two weeks when lesion(s) are stable or start to involute. Patients should be followed for at least one year. RICH will have 80% total remission by 12 mo of age. Overall, 50% of congenital hemangiomas resolve by age five and 90% by age nine.

In contrast, infantile hemangiomas that develop in the neonatal period follow a different pattern. They typically grow over the first 6-12 mo of life. They often stain positive for GLUT-1 and are multifocal. Their progression in size through infancy means that their risk of complications increases during the first year of life compared to congenital hemangiomas. Heart failure and compartment syndrome are the most severe risk and carry a 16% mortality if not treated. Lastly, cytopenias may develop over time.

Hepatic hemangiomas

Hemangiomas presenting in the liver require thorough workup and close observation. They may be present in 0.4% to 20% of the population at any time, and between 0.4% and 7.3% based on autopsy studies[23,24]. Most are incidental findings on imaging for abdominal pain which are often unrelated to the hemangioma, particularly if it is small. Outcomes are dependent on the level of hepatic involvement. Hepatic hemangiomas can be categorized by size: Small (0-3 cm), medium, (3-10 cm), and large (greater than 10 cm)[24]. Solitary small and medium hepatic hemangiomas are more likely to behave like solitary cutaneous hemangiomas. These lesions may self-involute, while others can have high flow and persist. As such, they may be amenable to embolization *via* coiling or enucleation. If lesions are multifocal or diffuse, they are more reflective of infantile hepatic hemangiomatosis (IHH). IHH can be associated with high output cardiac failure, and coagulopathy depending on the level of involvement. Hepatic hemangiomatosis can either be present in the nodular or the non-nodular patterns which can be identified on CT or magnetic resonance imaging (Figure 11). The non-nodular pattern is more common overall. The latter will show coalescing ovoid low attenuation nodules measuring between 5-10 mm[25]. Contrast images may find vascular pooling within the lesion and centripetal enhancement[26]. Biopsy of these lesions will show endothelial-lined sinusoidal proliferation with erythrocyte content. Often, they are GLUT-1 positive. Of note, there has been one incidental case of diffuse hereditary hemangiomatosis in a 68-year-old adult with only

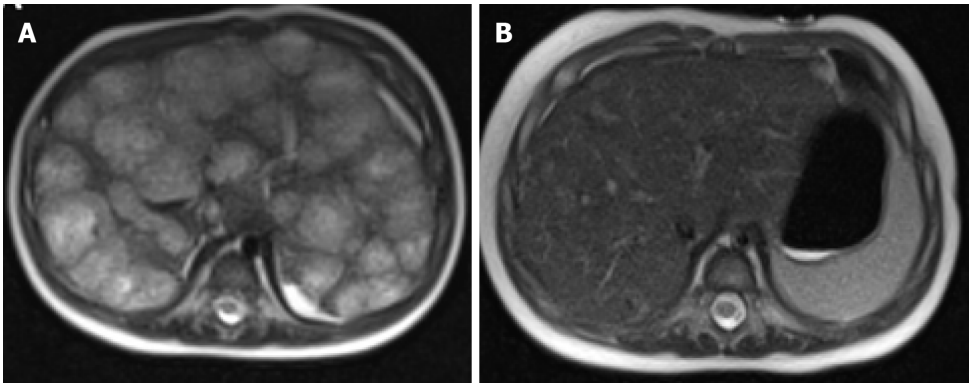

Figure 11 Magnetic resonance imaging results. A: Magnetic resonance imaging (MRI) of liver in a 4-month-old at the time of the diagnosis, axial T2-weighted (HASTE) image showing multiple nodular hyperintense lesions with centripetal fill-in on the delayed phase; B: MRI of the liver 3 mo after starting atenolol treatment, subsequent axial T2-weighted image showing interval decrease in size of enhancing lesions and improving hepatomegaly.

liver involvement[27]. Large hemangiomas, up to 20 cm in some reports, can cause compressive symptoms causing pain and cholestasis in some cases[23].

Multifocal lymphangioendotheliomatosis with thrombocytopenia (MLT) should be on the differential for any child who presents with multiple hemangiomas. This diagnosis is distinct with notable thrombocytopenia, caused by a consumptive process, as well as the presence of lymphatic vessel endothelial receptor 1 (LYVE-1) on skin biopsies. GLUT-1 is negative in MLT. Hemangiomas are smaller 1-2 mm in diameter by comparison and grow at a slower rate than hemangiomatosis. GI bleeding is common, but liver involvement is rare. Platelet consumption is also seen in kaposiform hemangioendothelioma or tufted hemangiomas. This carries a high risk of developing Kasabach-Merritt syndrome[21].

Treatment and prognosis

As stated above, most isolated cutaneous hemangiomas will self-resolve without the need for medical or surgical management. Treatment considerations for the gastroenterologist are outlined here (Table 6). Large hepatic hemangiomas are associated with hypothyroidism secondary to increased type III thyronine deiodinase activity which binds and eliminates circulating T3 thyroid hormone[28]. Thyroid hormone screening on all infants with IHH is recommended and replacement is advised to prevent complications of hypothyroidism[28]. Of note, hypothyroidism on newborn screen is not typically detected in these patients.

The prognosis of infantile hemangiomas is favorable and needs only conservative treatment; however, multiple lesions and visceral organ involvement warrant medical therapy, as high output heart failure and coagulopathy carry a high mortality if untreated[21,29]. Propranolol has been proven to be effective in the treatment of hepatic as well as cutaneous hemangiomas[28,30-32]. Meta-analysis has found it to be superior to placebo and oral steroids[33]. Commonly reported adverse events with oral propranolol include diarrhea, constipation, and bronchial hyperreactivity. Propranolol's mechanism of action is thought to be related to regression of hemangioma cells and peripheral vasoconstriction leading to permanent involution within a couple of months (Figure 11). Daily dosing on 2 mg/kg/d is commonly utilized, but up to 3 mg/kg/d has been effective in high-risk airway and facial/orbital hemangiomas[34]. Corticosteroids and weekly IV vincristine have also been studied as a treatment, but the results are inferior to propranolol[35]. Co-involvement of a dermatologist is crucial to diagnosis and management.

Lastly, surgical, ligation, enucleation, or resection of large and or symptomatic lesions not amenable to medical therapy[23]. Enucleation is technically easier with peripherally located hepatic hemangiomas and is associated with lower morbidity when compared with resection[23]. Resection is typically reserved for centrally located lesions. Laparoscopic resection has decreased morbidity over open surgery. Artery embolization, or radiofrequency ablation have been used for management of acute bleeding or to shrink large lesions prior to surgery. Liver transplant is reserved for very large lesions with severe complications such as heart failure, or Kasabach-Merritt syndrome[23].

Table 6 Treatment considerations for hepatic hemangiomas

Treatment considerations for hepatic hemangiomas
Monitoring for self-involution
Propranolol (2-3 mg/kg/d) superior to corticosteroids or IV vincristine
Surgical ligation or resection of internal or complex hemangiomas
Enucleation for peripherally located hemangiomas
Artery embolization or radiofrequency ablation for emergency bleeding or in preparation for surgical intervention
Liver transplant for exceptionally large lesions or diffuse lesions, with severe complications such as heart failure and Kasabach-Merritt syndrome not amenable to medical management

IV: Intravenous.

CONCLUSION

We have discussed various conditions that can cause congenital hepatic shunts. Many review articles have been written on these conditions separately and through the lens of various specialties such as radiological or surgical perspectives. Our goal was to create a concise review of all congenital shunts from the stance of the pediatric hepatologist. As imaging techniques and interventional therapeutics evolve, we are better able to diagnose and study these conditions. Early detection and monitoring best serve patients and clinicians in making medical management decisions.

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ORIGINAL ARTICLE

Basic Study

Reactive oxygen species-induced activation of Yes-associated protein-1 through the c-Myc pathway is a therapeutic target in hepatocellular carcinoma

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Abstract

BACKGROUND

The Hippo signaling pathway regulates organ size by regulating cell proliferation and apoptosis with terminal effectors including Yes-associated protein-1 (YAP-1). Dysregulation in Hippo pathway has been proposed as one of the therapeutic targets in hepatocarcinogenesis. The levels of reactive oxygen species (ROS) increase during the progression from early to advanced hepatocellular carcinoma (HCC).

AIM

To study the activation of YAP-1 by ROS-induced damage in HCC and the involved signaling pathway.

METHODS

The expression of YAP-1 in HCC cells (Huh-7, HepG2, and SNU-761) was quantified using real-time polymerase chain reaction and immunoblotting. Human HCC cells were treated with H_2O_2, which is a major component of ROS in living organisms, and with either YAP-1 small interfering RNA (siRNA) or control siRNA. To investigate the role of YAP-1 in HCC cells under oxidative stress, MTS assays were performed. Immunoblotting was performed to evaluate the signaling pathway responsible for the activation of YAP-1. Eighty-eight surgically resected frozen HCC tissue samples and 88 nontumor liver tissue samples were used for

gene expression analyses.

RESULTS

H_2O_2 treatment increased the mRNA and protein expression of YAP-1 in HCC cells (Huh-7, HepG2, and SNU-761). Suppression of YAP-1 using siRNA transfection resulted in a significant decrease in tumor proliferation during H_2O_2 treatment both *in vitro* and *in vivo* (both $P < 0.05$). The oncogenic action of YAP-1 occurred *via* the activation of the c-Myc pathway, leading to the upregulation of components of the unfolded protein response (UPR), including 78-kDa glucose-regulated protein and activating transcription factor-6 (ATF-6). The YAP-1 mRNA levels in human HCC tissues were upregulated by 2.6-fold compared with those in nontumor tissues ($P < 0.05$) and were positively correlated with the ATF-6 Levels (Pearson's coefficient = 0.299; $P < 0.05$).

CONCLUSION

This study shows a novel connection between YAP-1 and the UPR through the c-Myc pathway during oxidative stress in HCC. The ROS-induced activation of YAP-1 *via* the c-Myc pathway, which leads to the activation of the UPR pathway, might be a therapeutic target in HCC.

Key Words: Hepatocellular carcinoma; Yes-associated protein-1; C-Myc; Reactive oxygen species; Unfolded protein response; Activating transcription factor-6

Core Tip: We found a novel connection between Yes-associated protein-1 (YAP-1) and the unfolded protein response (UPR) through the c-Myc pathway during oxidative stress in hepatocellular carcinoma (HCC). As the Hippo pathway and c-Myc pathway share many important functions, including the regulation of growth, death and survival in cells and the regulation of stress resistance and life spans in organisms, we speculate that the interaction between YAP-1 and c-Myc is a point of convergence that allows HCC proliferation. The reactive oxygen species-induced activation of YAP-1 *via* the c-Myc pathway, which leads to the activation of the UPR pathway, might be a therapeutic target in HCC.

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INTRODUCTION

Reactive oxygen species (ROS), such as H_2O_2, superoxide radicals, and hydroxyl radicals, contribute to tumor progression by enhancing DNA damage and altering cell signaling pathways[1,2]. It has been recently suggested that ROS are involved in tumor metastasis, which is a complex process that includes angiogenesis, epithelial-to-mesenchymal transition, invasion, and migration within the tumor micro-environment[3]. ROS also control the expression of matrix metalloproteinases and mitogen-activated protein kinases (MAPKs), the activation of the Ras pathway, and the downregulation of E-cadherin expression[4].

Hepatocellular carcinoma (HCC) is one of the common fatal malignancies which results in approximately one million worldwide deaths every year[5]. Oxidative stress is known to be the most important factor of HCC development[6,7]. The major etiologies of HCC, including chronic hepatitis B or C, alcohol-related liver disease, and nonalcoholic fatty liver disease, increase ROS levels[8,9]. ROS levels are also positively correlated with HCC progression[10,11].

The Hippo signaling pathway regulates organ size by regulating both cell proliferation and apoptosis with terminal effectors such as yes-associated protein

Review Board (CHAMC 2018-02-037). All the human tissues were provided by the Bundang CHA Biobank of Bundang CHA Medical Center. For the gene expression analyses, 88 surgically resected frozen HCC tissue samples and 88 nontumor liver tissue samples were analyzed. Cases were prospectively and consecutively identified at Bundang CHA Medical Center between 2012 and 2018.

Institutional animal care and use committee statement: The *in vivo* study protocol was approved by the Institutional Animal Care and Use Committee (IACUC-180027) of CHA University. All the *in vivo* surgical procedures were performed under anesthesia with 2,2,2-tribromoethanol, and all efforts were made to minimize suffering.

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(YAP)[12,13]. The key components of the Hippo pathway include sterile 20-like kinases (Mst1 and Mst2; homologues of D. hippo), large tumor suppressors (Lats1 and Lats2; homologues of warts), YAP, its paralog protein transcriptional coactivator with PDZ-binding motif (TAZ), transcriptional coactivators, and homologues of yorkie. Inactivation of the Hippo pathway leads to uncontrolled cell proliferation in epithelial cells and stem cells[14,15] and oncogenic transformation[16], both of which are mediated by the upregulation of YAP. Dysregulation of the Hippo pathway has been proposed as one of the therapeutic targets in hepatocarcinogenesis[17-19]. A previous study showed that YAP is an independent predictive marker for the overall survival and disease-free survival of HCC patients and that it is associated with tumor differentiation[20]. The Hippo pathway, which regulates tumorigenesis, also has an important role in mediating oxidative stress[21]. Shao *et al*[13] suggested the involvement of YAP in causing cardiomyocyte survival during oxidative stress[13].

Thus, the activation of YAP-1 by ROS-induced damage has been hypothesized to exacerbate the progression of HCC, but it remains unclear which signaling pathway is involved. Here, we investigated ROS-induced YAP-1 activation in HCC and the associated signaling pathway.

MATERIALS AND METHODS

Cell lines and coculture

Human HCC cell lines including Huh-7 and HepG2, which are well-differentiated HCC cell lines, and SNU-761, which is a poorly differentiated HCC cell line were used in this study. We used Dulbecco's modified Eagle medium (DMEM; Huh-7 and HepG2) or in RPMI 1640 (SNU-761) supplemented with 10% fetal bovine serum (FBS), 100000 U/L penicillin, and 100 mg/L streptomycin, with or without 100 nmol/L insulin for cell culture.

Cell proliferation analysis (MTS assay)

HCC cell proliferation was measured with the Cell Titer 96 Aqueous One Solution cell proliferation assay (Promega, Madison, WI, United States), on the basis of the cellular conversion of the colorimetric reagent3, 4-(5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazoliumsalt (MTS) into soluble formazan by the dehydrogenase enzyme found in metabolically proliferating cells. Following each treatment, 20 μL of the dye solution was added to each well of a 96-wellplate and incubated for 2 h. Then, the absorbance was recorded at a wavelength of 490 nm using an enzyme-linked immunosorbent assay plate reader (Molecular Devices, Sunnyvale, CA, United States).

Small interfering RNA transfection

Cells were seeded in a 6-well culture plate (2×10^5 cells per well) in 2 mL antibiotic-free medium supplemented with 10% FBS. Once the cells reached 60%-80% confluence, they were transfected with small interfering RNA (siRNA) using the siRNA Transfection Reagent (Santa Cruz Biotechnology Inc., Santa Cruz, CA, United States) according to the manufacturer's instructions. The cells were treated with siRNA for 6 h at 37 °C, and then, growth medium containing 20% FBS and antibiotics was added. After 18 h, the medium was replaced with fresh medium containing 10% FBS and antibiotics. Twenty-four hours after transfection, the cells were used in further experiments.

In vivo subcutaneous xenograft model

Briefly, H_2O_2 (100 μmol/L)-treated MH134 cells (5×10^7 cells per mouse) were subcutaneously transplanted into the flanks of C3H mice in the control group (n = 10). The tumor volume was measured using a Vernier caliper and calculated as [length × (width)^2]/2. YAP-1 siRNA transfected MH134 cells were subcutaneously implanted on the flank of mice in YAP siRNA group, and control siRNA transfected MH134 cells were implanted in control siRNA group. The maximal diameter of each nodule was measured every day for 13 d.

Immunoblot analysis

The cells were lysed for 20 min on ice with lysis buffer and centrifuged at 14000 g for 10 min at 4 °C. The samples were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, blotted with the

appropriate primary antibodies at a dilution of 1:1000, and treated with peroxidase-conjugated secondary antibodies (Biosource International, Camarillo, CA, United States). The bound antibodies were visualized using a chemiluminescent substrate (ECL; Amersham, Arlington Heights, IL, United States) and exposed to Kodak X-OMAT film (Kodak, New Haven, CT, United States). The primary antibodies, including rabbit anti-phospho-p42/44 MAPK, anti-phosphorylated-Akt, and rabbit anti-c-Myc, were purchased from Cell Signaling Technology (Danvers, MA, United States). The goat anti-β-actin antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, United States). The densitometric analyses were performed with Image J software (National Institutes of Health, Bethesda, MD, United States).

Real time-polymerase chain reaction analysis

The total ribonucleic acids (RNAs) were extracted from Huh-7, HepG2, and SNU-761 cells using TRIzol Reagent (Invitrogen, Carlsbad, CA, United States). The complementary deoxyribonucleic acid (cDNA) templates were prepared using oligo (dT) random primers and Moloney Murine Leukemia Virus (MoMLV) reverse transcriptase. After the reverse transcription reaction, the cDNA template was amplified by polymerase chain reaction (PCR) using Taq polymerase (Invitrogen). YAP-1 mRNA expression was quantified by real-time PCR (Light Cycler; Roche Molecular Biochemicals, Mannheim, Germany) using SYBR green as the fluorophore (Molecular Probes, Eugene, OR, United States). The primers for YAP-1 were as follows: Forward: 5′-TGAACAAACGTCCAGCAAGATAC-3′; and reverse: 5′-CAGCCCCCAAAATGAACAGTAG-3′. The primers for c-Myc were as follows: Forward: 5′- CCCGCTTCTCTGAAAGGCTCTC-3′; and reverse: 5′-CTCTGCTGCTGCTGCTGCTGGTAG-3′. For the unfolded protein response (UPR) markers, the following primers were used: Glucose-regulated protein 78 (GRP78), forward: 5′-GACGGGCAAAGATGTCAGGAA-3′ and reverse: 5′-TCATAGTAGACCGGAACAGATCCA-3′; XBP1, forward: 5′-TTGTCACCCCTCCAGAACATC-3′ and reverse: 5′-TCCAGAATGCCCAACAGGAT-3′; activating transcription factor-6 (ATF-6), forward: 5′-TTGGCATTT ATAATACTGAACTATGGA-3′ and reverse: 5′-TTTGATTTGCAGGGCTCAC-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression was used as a control. The level of YAP-1 mRNA expression was calculated as the relative intensity of the PCR product band compared with that of the GAPDH gene using the $2^{-\Delta\Delta Ct}$ method. All the PCR experiments were performed in triplicate.

Statistical analysis

The statistical analyses were performed using PASW version 21.0 (SPSS Inc., Chicago, IL, United States). All the experimental results were obtained from three independent experiments using cells from three separate isolations and are presented as the mean ± standard deviation (SD). For comparisons between groups, the data were analyzed by the Mann–Whitney *U* test or one-way ANOVA. For all the tests, $P < 0.05$ was regarded as statistically significant.

Ethics statement

Ethical approval was obtained from the ethics committee at CHA University. We carried out this study in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The *in vivo* study protocol was approved by the Institutional Animal Care and Use Committee (IACUC-180027) of CHA University. All the *in vivo* surgical procedures were performed under anesthesia with 2, 2, 2-tribromoethanol, and all efforts were made to minimize suffering.

All the experiments using human tissues were approved by the Bundang CHA Medical Center Institutional Review Board (CHAMC 2018-02-037). All the human tissues were provided by the Bundang CHA Biobank of Bundang CHA Medical Center. For the gene expression analyses, 88 surgically resected frozen HCC tissue samples and 88 nontumor liver tissue samples were analyzed. Cases were prospectively and consecutively identified at Bundang CHA Medical Center between 2012 and 2018.

RESULTS

ROS enhanced the mRNA and protein expression of YAP-1 in HCC cells

To analyze the potential ROS-induced changes in YAP-1 expression in HCC cells, we treated human HCC cells (Huh-7, HepG2, and SNU-761 cells) with 150 μmol/L H_2O_2. Real-time PCR and immunoblot analyses indicated that H_2O_2 treatment increased the mRNA (Figure 1A) and protein (Figure 1B) expression of YAP-1 in the HCC cells. These effects were inhibited following treatment of the cells with the antioxidant N-acetylcysteine (NAC) (Figure 1C). The antioxidant treatment significantly suppressed the protein expressions of YAP-1 in HCC cells.

Modulation of YAP-1 expression in ROS-exposed HCC cells showed antitumor effects in vitro

Next, to investigate whether exposure to H_2O_2 impacts HCC cell survival, HCC cells were treated with H_2O_2 (0-350 μmol/L), and the ROS levels were increased by intervals of 50 μmol/L. As shown in Figure 2A, exposure to H_2O_2 (0-350 μmol/L) did not reduce HCC cell survival. Then, we examined the efficacy YAP-1 siRNA transfection with real-time PCR. YAP-1 siRNA transfection significantly suppressed YAP-1 mRNA expression compared to control siRNA transfection in HCC cells (Figure 2B; $P < 0.05$). Next, we performed an MTS assay to evaluate whether YAP-1 modulates HCC cell proliferation. Suppression of YAP-1 using siRNA transfection or verteporfin treatment (YAP-1 inhibitor) resulted in a significant decrease in tumor proliferation during exposure 150 μmol/L H_2O_2 *in vitro* (Figure 2C and D; both $P < 0.05$).

Modulation of YAP-1 expression in ROS-exposed HCC cells showed antitumor effects in an in vivo xenograft tumor mouse model

The antitumor effects of YAP-1 siRNA were examined using an *in vivo* xenograft model. First, we evaluated whether exposure to ROS changes the expression of YAP-1 in the murine HCC cell line MH134. H_2O_2 treatment significantly increased the proliferation of the MH134 cells (Figure 3A; $P < 0.05$). We also confirmed that suppression of YAP-1 using siRNA transfection resulted in significantly decreased mRNA expression of YAP-1 in the MH134 cells treated with 150 μmol/L H_2O_2 (Figure 3B). In the xenograft tumor model, the YAP-1 siRNA group showed significantly suppressed tumor growth compared to the control siRNA group at days 11, 12, and 13 after tumor budding (Figure 3C; all $P < 0.05$).

The oncogenic action of YAP-1 was reciprocally activated by the c-Myc pathway in ROS-exposed HCC cells

The immunoblot assay results showed that the downregulation of YAP-1 caused by siRNA transfection or verteporfin treatment decreased the protein expression of c-Myc in the ROS-exposed HCC cell lines (Figure 4A and B). When the ROS-exposed HCC cells were treated with a c-Myc inhibitor (10058-F4, 60 μmol/L), the protein expression of YAP-1 was significantly decreased compared with that in the control-treated cells (Figure 5A). Moreover, treatment with the antioxidant NAC downregulated the expression of c-Myc in the ROS-exposed HCC cell lines (Figure 5B). We also performed real-time PCR and immunoblot analyses to evaluate whether up-regulation of the c-Myc pathway was dependent on YAP-1 expressions. YAP-1 siRNA transfection significantly suppressed c-Myc mRNA expression compared to control siRNA transfection in ROS-exposed HCC cells (Figure 5C; all $P < 0.05$). Immunoblot analyses of c-Myc also revealed that ROS-exposed HCC cells transfected with YAP-1 siRNA showed suppressed protein expression of c-Myc as compared to those transfected with control siRNA (Figure 5D).

The ROS-induced oncogenic action of YAP-1 in HCC cells led to an enhanced UPR

To determine whether the oncogenic action of YAP-1, which occurs *via* the activation of the c-Myc pathway, leads to the upregulation of components of the UPR, we performed real-time PCR on cells treated with or without H_2O_2 for 78-kDa GRP78/BiP, ATF-6, and XBP1 (Figure 6A). ROS exposure significantly enhanced the mRNA expression of GRP78, ATF-6, and XBP1 in the HCC cell lines. The downregulation of YAP-1 by siRNA transfection also significantly suppressed the expression of the UPR markers compared to control siRNA transfection. We also performed immunoblot analysis to evaluate the endoplasmic reticulum (ER) stress marker phosphorylated eIF-2α (Figure 6B); the results revealed that the transfection of YAP-1 siRNA attenuated the protein expression of phosphorylated eIF-2α compared to control siRNA

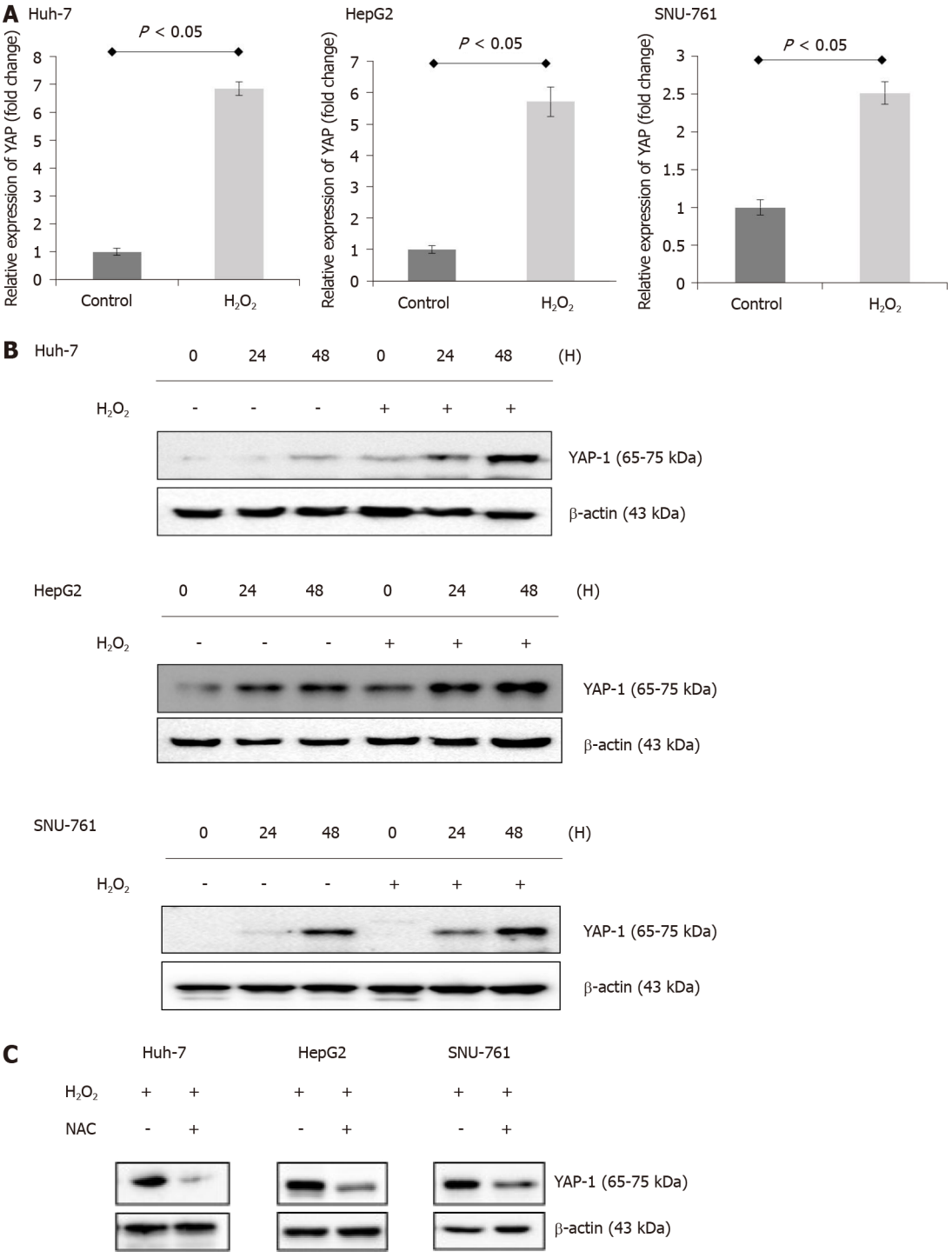

Figure 1 Reactive oxygen species enhanced the mRNA and protein expression of yes-associated protein-1 in hepatocellular carcinoma cells. A: Yes-associated protein-1 (YAP-1) mRNA was significantly enhanced in hepatocellular carcinoma (HCC) cells treated with 150 μmol/L H_2O_2. YAP-1 mRNA expression was quantified using quantitative polymerase chain reaction and normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA expression. The experiment was repeated three times. The data are expressed as the mean ± SD. The error bars represent the SD; B: The protein expression of YAP-1 in HCC cells was significantly enhanced when the HCC cells were exposed to 150 μmol/L H_2O_2, especially at 48 h. The experiment was repeated three times; C: Treatment with the antioxidant N-acetylcysteine inhibited the protein expression of YAP-1 in HCC cells. The experiment was repeated three times. YAP-1: Yes-associated protein-1; HCC: Hepatocellular carcinoma; SD: Standard deviation; NAC: N-acetylcysteine.

transfection.

Upregulated mRNA expression of YAP-1was correlated with the expression of ATF-6 in human HCC tissues

For the gene expression analyses, 88 surgically resected frozen HCC tumor tissue samples and 88 paired nontumor liver tissue samples were evaluated. The majority of the patients (n = 71, 80.7%) had stage I HCC according to the American Joint

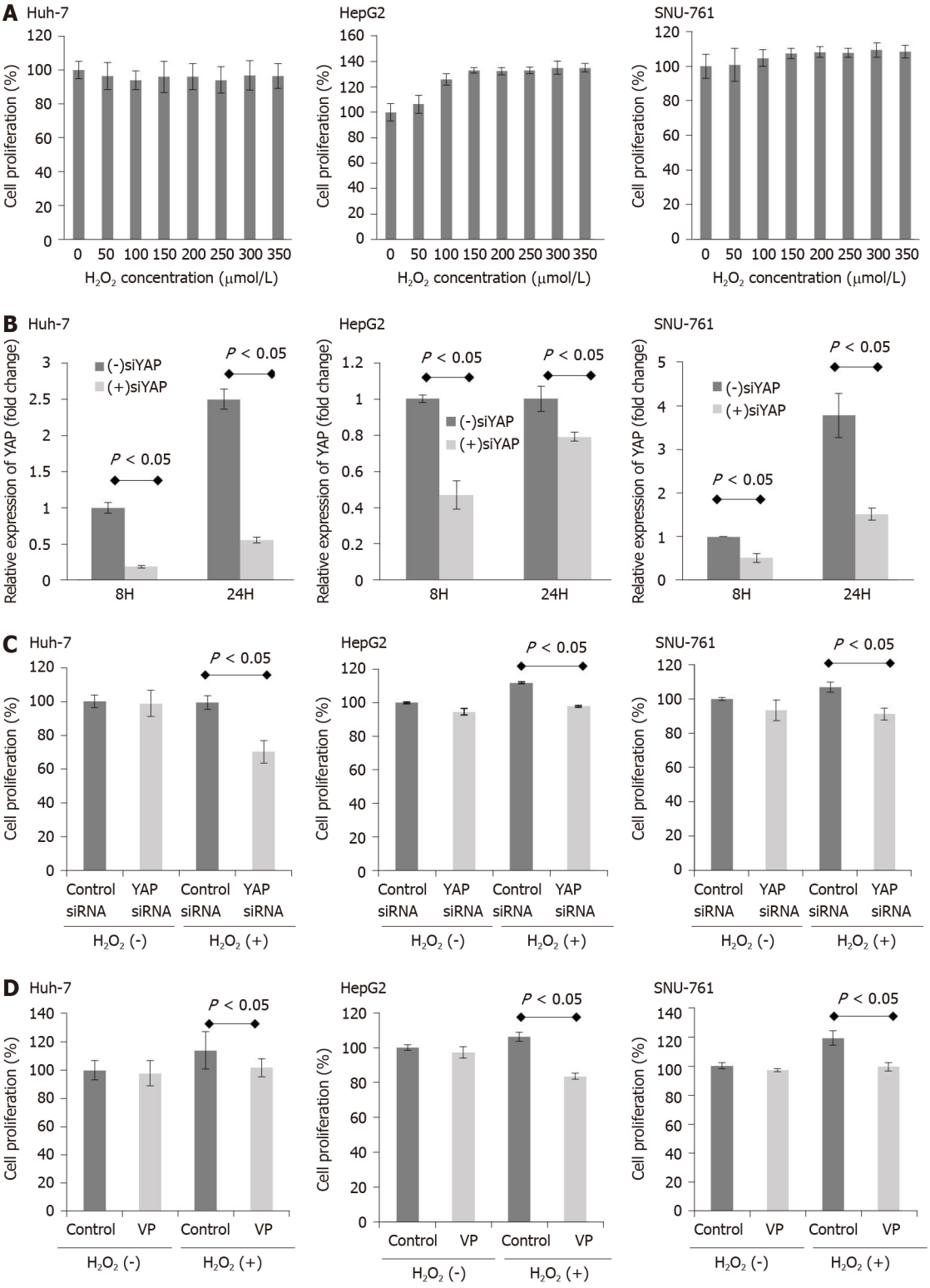

Figure 2 The effects of Yes-associated protein-1 on the proliferation of reactive oxygen species reactive oxygen species -exposed hepatocellular carcinoma cells. A: An MTS assay was performed on hepatocellular carcinoma (HCC) cells that were treated with H_2O_2 (0-350 μmol/L), and the reactive oxygen species levels were increased by intervals of 50 μmol/L. The data are expressed as the mean ± SD of percent changes of optical densities. The experiment was repeated three times; B: Yes-associated protein-1 (YAP-1) small interfering RNA (siRNA) transfection significantly suppressed YAP-1 mRNA expression compared to control siRNA transfection in HCC cells ($P < 0.05$). The data are expressed as the mean ± SD. The experiment was repeated three times; C: When HCC cells were transfected with YAP-1 siRNA, the proliferation of HCC cells was significantly decreased compared with control siRNA transfection based on the MTS assay results ($P < 0.05$). The data are expressed as the mean ± SD of percent changes of optical densities. The experiment was repeated three times; D: When HCC cells were treated with verteporfin (1000 nmol/L), the proliferation of HCC cells was significantly decreased compared with the control treatment based on the MTS assay results ($P < 0.05$). The data are expressed as the mean ± SD of percent changes of optical densities. The experiment was repeated three times. YAP: Yes-associated protein; siRNA: Small interfering RNA; VP: Verteporfin.

Commission on Cancer 8th edition HCC staging system. 11 patients (12.5%) and 6 patients (6.8%) had stage II and stage III HCC, respectively. No patient had major vascular invasion or lymph node/distant metastasis. The expression of YAP-1 was further determined in the resected HCC tissues and adjacent nontumor tissues using real-time PCR. The mean mRNA expression of YAP-1 was upregulated by 2.6-fold in the HCC tissues compared with the nontumor tissues (Figure 7A; $P < 0.05$). Among the 88 HCC tumor tissues, YAP-1 RNA expression was upregulated in 42 samples (47.7%) compared to the nontumor tissues, and YAP-1 expression was positively correlated with ATF-6 expression (Figure 7B; Pearson's coefficient = 0.299; $P < 0.05$). For one patient whose YAP-1 expression in HCC tissue was 15.5-fold higher than that in nontumor tissue, we performed immunohistochemical staining for YAP-1 with HCC tissue, which is shown in Figure 7C.

DISCUSSION

This study revealed that the ROS-induced activation of YAP-1 *via* the c-Myc pathway, which leads to the activation of the UPR, might be a therapeutic target in HCC. We have elucidated the molecular mechanism by which YAP-1 mediates the survival of HCC cells under oxidative stress.

Carcinogenesis leads to the accumulation of misfolded proteins in the ER[22]. Then, the UPR is activated to restore normal cellular function by degrading the misfolded proteins and activating the production of chaperones, such as GRP78. However, under pathological conditions, prolonged UPR activation can promote apoptosis, leading to cell death. Overall, if ER stress is too severe, the UPR leads to translational arrest and induces specific factors for cell survival or cell death. In several cancers, the expression of UPR components is enhanced, indicating the dependency of these cancers on the UPR[23]. Thus, there is a possibility that modification of the UPR might have anticancer effects.

Hypoxia is one of the major mediators of UPR-inducing pathways. Human fibrosarcoma and lung carcinoma cells upregulated GRP78 expression and XBP1 splicing under hypoxic conditions *in vitro*[24]. Tumor formation with aberrant microcirculation might lead to hypoxic conditions, which induce the UPR. Gradually, the UPR increases cell survival and tumor proliferation, which thereby increases hypoxia in the core of the tumor. After the sequestration of GRP78 by misfolded proteins, ATF-6, inositol requiring protein 1, and protein kinase RNA-like endoplasmic reticulum kinase (PERK) act as transducers to transmit the ER stress signal to the cytosol and nucleus. Activated ATF-6 translocates to the Golgi, where proteases cleave it and release its fragments into the cytosol[25]. Indeed, enhanced nuclear translocation of the ATF-6 fragment is observed in various cancers, including HCC. In this study, we identified the potential of ATF-6 to act as an effector of HCC under oxidative stress.

The c-Myc pathway undergoes chromosomal translocation and gene amplification in many cancers, including HCC. Activated c-Myc pathway upregulates oncogenes which are involved in ribosome biogenesis. Previous studies reported that elevated protein synthesis due to increased c-Myc expression in cancer cells lead to UPR activation[26,27]. Activation of UPR signaling promotes autophagy in tumor cells under conditions of hypoxia, oxidative stress, and nutrient limitation. Our findings suggest a key link between YAP-1-mediated oncogenic transformation and HCC cell survival *via* the c-Myc-mediated UPR under oxidative stress.

There are increasing lines of evidence suggesting that the loss-of-function mutations in components of the Hippo pathway and hyperactivation of YAP-1 have been observed in many cancers. Thus, we speculate that the regulating the YAP-1-c-Myc pathway might be a crucial mechanism through which the Hippo pathway regulates hepatocarcinogenesis.

Several multikinase inhibitors that have been approved for advanced HCC, including sorafenib, regorafenib, and lenvatinib, have shown modest survival advantages[28,29]. Recent evidence suggests that long-term treatment of HCC leads to hypoxia-mediated sorafenib resistance in patients with HCC because tumor-driving pathways, including YAP-1, become activated[30-32]. However, the molecular mechanism of sorafenib resistance is unclear. Here, we found that ROS are the primary triggers of YAP-1-c-Myc-UPR signaling hyperactivation during oxidative stress, and this phenomenon is also observed in human HCC tissues.

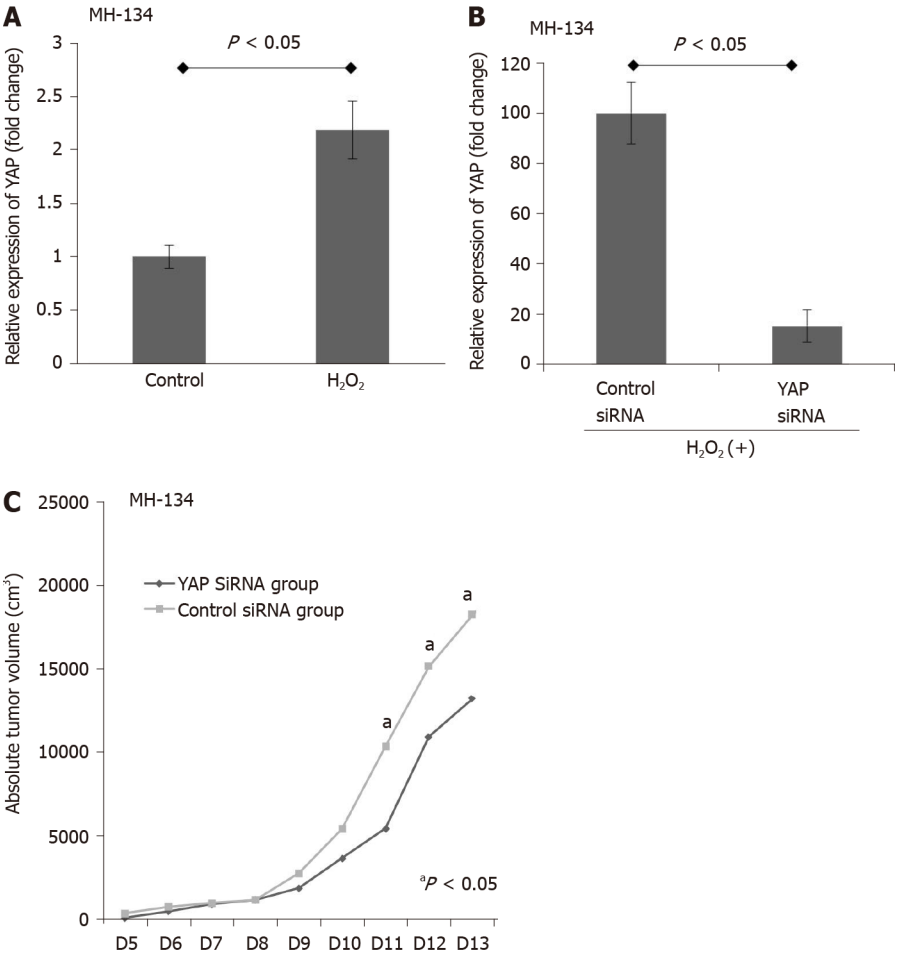

Figure 3 Modulation of yes-associated protein-1 in reactive oxygen species-exposed hepatocellular carcinoma cells showed antitumor effects in an *in vivo* xenograft tumor mouse model. A: H_2O_2 treatment significantly increased the proliferation of MH134 cells based on the MTS assay results ($P < 0.05$). The data are expressed as the mean ± SD. The experiment was repeated three times; B: Yes-associated protein-1 (YAP-1) small interfering RNA (siRNA) transfection significantly suppressed YAP-1 mRNA expression compared to control siRNA transfection in reactive oxygen species-exposed MH134 cells ($P < 0.05$). The data are expressed as the mean ± SD. The experiment was repeated three times; C: In the xenograft tumor model, the YAP-1 siRNA group showed significantly suppressed tumor growth compared to the control siRNA group at days 11, 12, and 13 after tumor budding (all $^aP < 0.05$). The data are expressed as the mean ± SD. YAP: Yes-associated protein; siRNA: Small interfering RNA.

CONCLUSION

In conclusion, our study shows a novel connection between YAP-1 and the UPR through the c-Myc pathway during oxidative stress in HCC. As the Hippo pathway and c-Myc pathway share many important functions, including the regulation of growth, death and survival in cells and the regulation of stress resistance and life spans in organisms, we speculate that the interaction between YAP-1 and c-Myc is a point of convergence that allows HCC proliferation. The ROS-induced activation of YAP-1 *via* the c-Myc pathway, which leads to the activation of the UPR pathway, might be a therapeutic target in HCC.

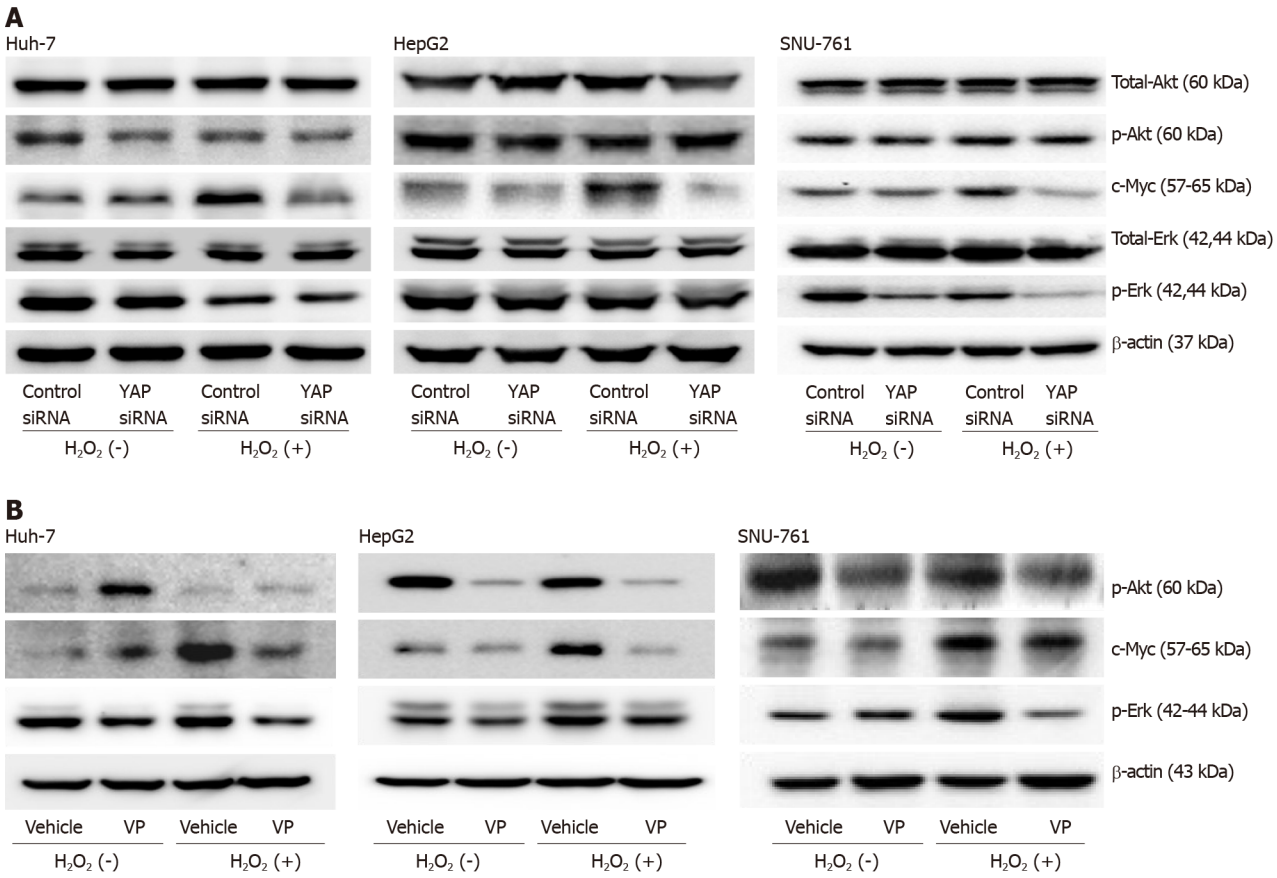

Figure 4 The oncogenic action of yes-associated protein-1 was activated by the c-Myc pathway in reactive oxygen species-exposed hepatocellular carcinoma cells. A: Immunoblot analyses of phosphorylated-Akt, total-Akt, c-Myc, phosphorylated-p42/44 (Erk), total-p42/44 (Erk), and yes-associated protein-1 (YAP-1) were performed in reactive oxygen species (ROS)-exposed hepatocellular carcinoma (HCC) cells transfected with YAP-1 small interfering RNA (siRNA) or control siRNA. The experiment was repeated three times; B: Immunoblot analyses of phosphorylated-Akt, total-Akt, c-Myc, phosphorylated-p42/44 (p-Erk), total-p42/44 (Erk), and YAP-1 were performed in ROS-exposed HCC cells treated with verteporfin or control. The experiment was repeated three times. YAP: Yes-associated protein; siRNA: Small interfering RNA; VP: Verteporfin.

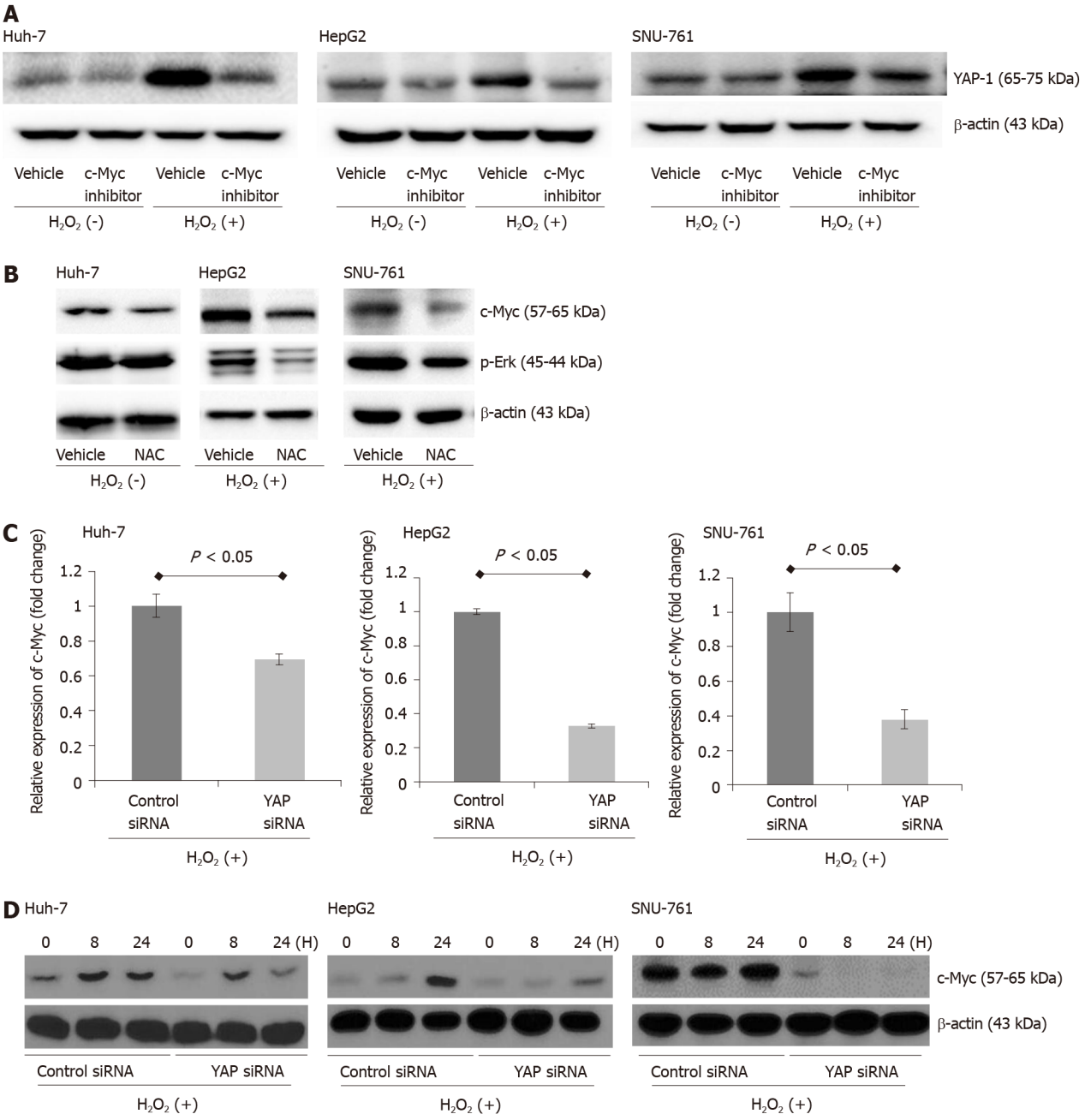

Figure 5 Up-regulation of the c-Myc pathway was dependent on yes-associated protein-1 expressions in reactive oxygen species-exposed hepatocellular carcinoma cells. A: A c-Myc inhibitor (10058-F4, 60 μmol/L) significantly decreased the protein expression of yes-associated protein-1 (YAP-1) in reactive oxygen species (ROS)-exposed hepatocellular carcinoma (HCC) cells. The experiment was repeated three times; B: N-acetylcysteine treatment downregulated c-Myc protein expression in ROS-exposed HCC cell lines. The experiment was repeated three times; C: YAP-1 small interfering RNA (siRNA) transfection significantly suppressed c-Myc mRNA expression compared to control siRNA transfection in ROS-exposed HCC cells (all $P < 0.05$). The c-Myc mRNA expression was quantified using quantitative PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase mRNA expression. The data are expressed as the mean ± SD. The experiment was repeated three times; D: Immunoblot analyses of c-Myc were performed in ROS-exposed HCC cells transfected with YAP-1 siRNA or control siRNA. The experiment was repeated three times. YAP: Yes-associated protein; siRNA: Small interfering RNA; NAC: N-acetylcysteine.

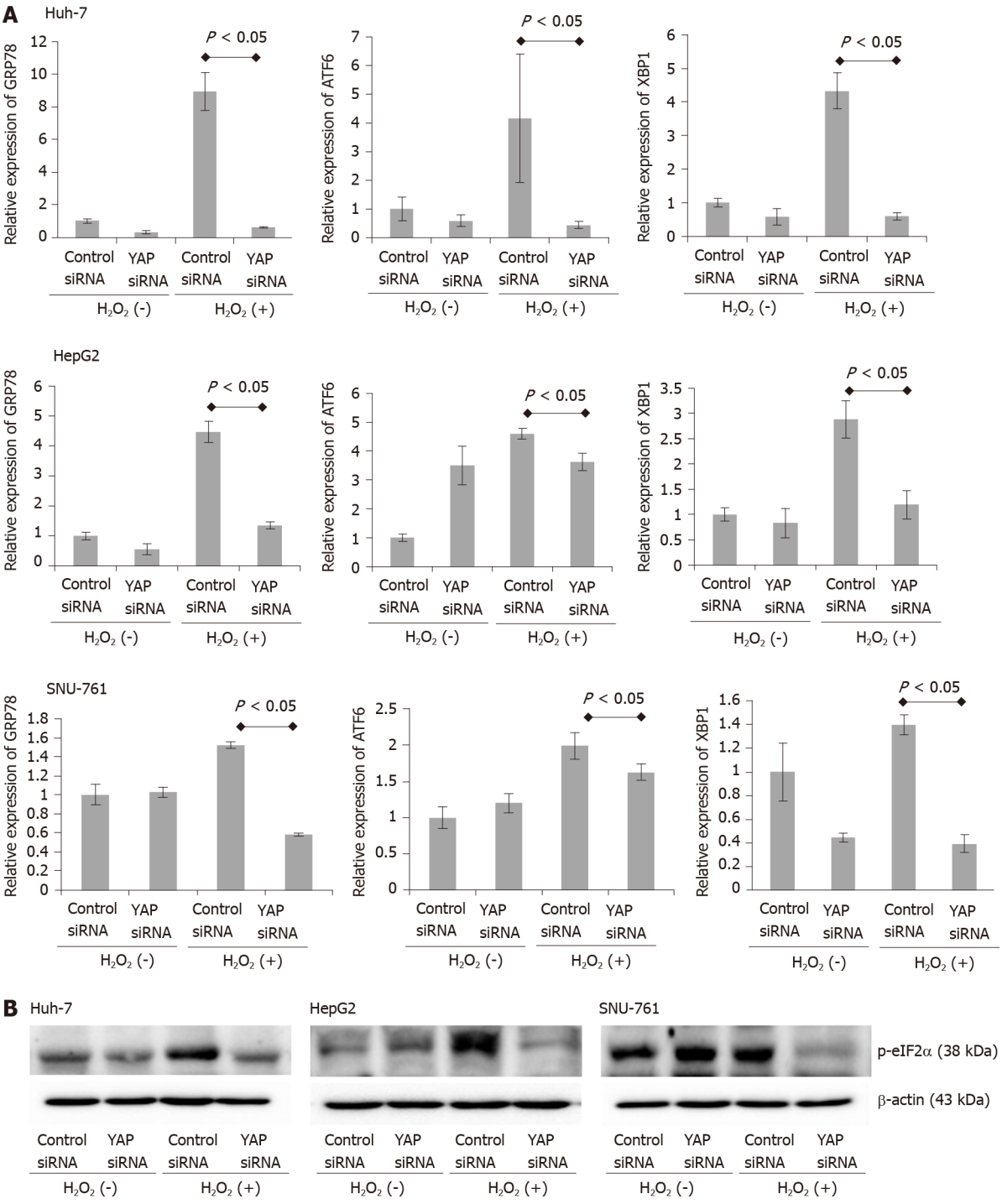

Figure 6 The reactive oxygen species-induced oncogenic action of yes-associated protein-1 in hepatocellular carcinoma cells led to an enhanced unfolded protein response. A: Yes-associated protein-1 (YAP-1) small interfering RNA (siRNA) significantly decreased the mRNA expression of unfolded protein response markers, including 78-kDa (glucose-regulated protein 78/BiP), activating transcription factor-6, and XBP1, in reactive oxygen species (ROS)-exposed hepatocellular carcinoma (HCC) cells ($P < 0.05$). The experiment was repeated three times. The data are expressed as the mean ± SD; B: Immunoblot analyses of YAP-1 and phosphorylated-eIF-2α were performed in ROS-exposed HCC cells transfected with YAP-1 siRNA or control siRNA. The experiment was repeated three times. GRP78: Glucose-regulated protein 78; ATF-6: Activating transcription factor-6; YAP: Yes-associated protein; siRNA: Small interfering RNA.

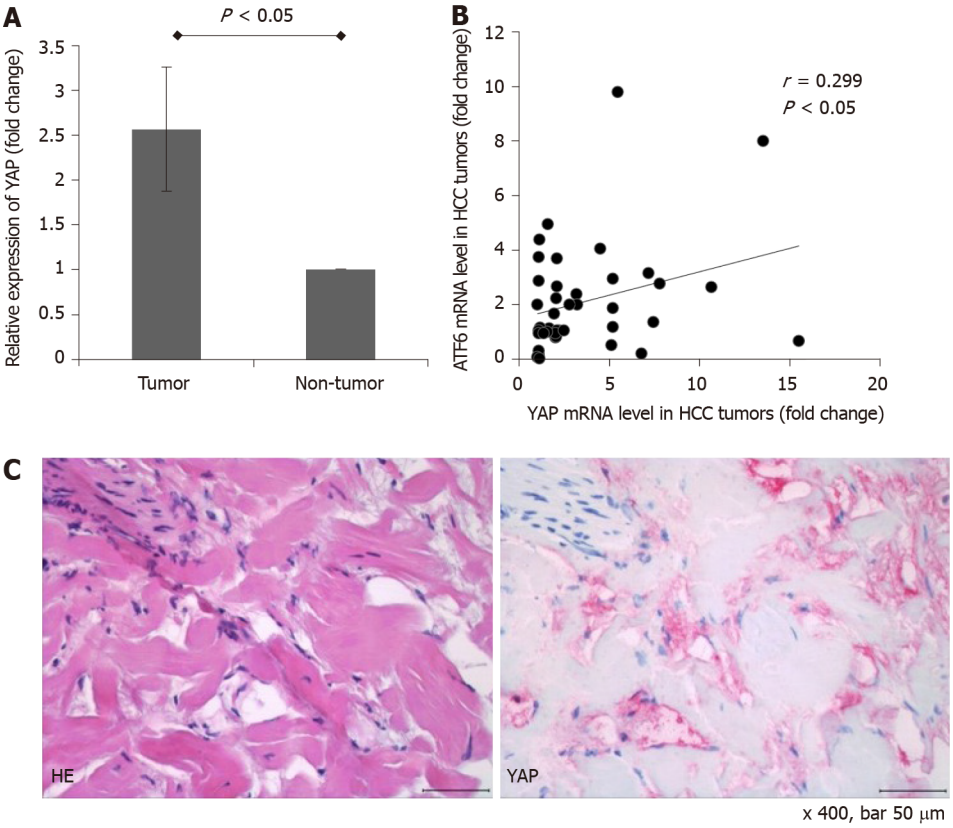

Figure 7 Upregulated mRNA expression of yes-associated protein-1 was correlated with the expression of activating transcription factor-6 in human hepatocellular carcinoma tissues. A: The mean mRNA expression of yes-associated protein-1 (YAP-1) was upregulated by 2.6-fold in hepatocellular carcinoma (HCC) tissues compared with nontumor tissues (n = 88). The data are expressed as the mean ± SD; B: The mRNA expression of YAP-1 was positively correlated with the mRNA expression of ATF6 (Pearson's coefficient = 0.299; P < 0.05); C: The expression of YAP-1 in human HCC tissue was detected by immunohistochemistry (400 × magnification). Scale bars, 50 μm. YAP: Yes-associated protein; HCC: Hepatocellular carcinoma; SD: Standard deviation; ATF-6: Activating transcription factor-6; HE: Hematoxylin-eosin.

ARTICLE HIGHLIGHTS

Research background

Reactive oxygen species (ROS) contribute to tumor progression by promoting DNA damage and altering cell signaling pathways. It has been recently suggested that ROS are involved in tumor metastasis, which is a complex process that includes epithelial-to-mesenchymal transition, migration, invasion, and angiogenesis within the tumor microenvironment.

Research motivation

Oxidative stress is the most important causative factor of hepatocellular carcinoma (HCC). The major etiologies of HCC, including chronic hepatitis B or C, alcohol-related liver disease, and nonalcoholic fatty liver disease, increase ROS levels. Thus, the activation of yes-associated protein-1 (YAP-1) by ROS-induced damage has been hypothesized to exacerbate the progression of HCC.

Research objectives

We investigated the activation of YAP-1 by ROS-induced damage in HCC and the involved signaling pathway.

Research methods

The expression of YAP-1 was quantified using real-time PCR and immunoblotting. Human HCC cells were treated with H_2O_2, and with either YAP-1 small interfering RNA (siRNA) or control siRNA. MTS assays were performed to evaluate HCC cell proliferation. To investigate the signaling pathway, immunoblotting was performed. Eighty-eight surgically resected frozen HCC tissues and 88 nontumor paired liver tissues were used for gene expression analyses.

Research results

H_2O_2 treatment increased the mRNA and protein expression of YAP-1 in HCC cells. Suppression of YAP-1 resulted in a significant decrease in tumor proliferation during H_2O_2 treatment both *in vitro* and *in vivo*. The oncogenic action of YAP-1 occurred *via* the activation of the c-Myc pathway, leading to the upregulation of components of the unfolded protein response, including 78-kDa glucose-regulated protein and activating transcription factor-6 (ATF-6). The YAP-1 mRNA levels in human HCC tissues were upregulated by 2.6-fold compared with those in nontumor tissues and were positively correlated with the ATF-6 Levels.

Research conclusions

This study shows a novel connection between YAP-1 and the unfolded protein response (UPR) through the c-Myc pathway during oxidative stress in HCC. We speculate that the interaction between YAP-1 and c-Myc is a point of convergence that allows HCC proliferation.

Research perspectives

The ROS-induced activation of YAP-1 *via* the c-Myc pathway, which leads to the activation of the UPR pathway, might be a therapeutic target in HCC.

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ORIGINAL ARTICLE

Basic Study

Fedora-type magnetic compression anastomosis device for intestinal anastomosis

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Abstract

BACKGROUND

Although previous studies have confirmed the feasibility of magnetic compression anastomosis (MCA), there is still a risk of long-term anastomotic stenosis. For traditional MCA devices, a large device is associated with great pressure, and eventually increased leakage.

AIM

To develop a novel MCA device to simultaneously meet the requirements of pressure and size.

METHODS

Traditional nummular MCA devices of all possible sizes were used to conduct ileac anastomosis in rats. The mean (± SD) circumference of the ileum was 13.34 ± 0.12 mm. Based on short- and long-term follow-up results, we determined the appropriate pressure range and minimum size. Thereafter, we introduced a novel "fedora-type" MCA device, which entailed the use of a nummular magnet with a larger sheet metal.

RESULTS

With traditional MCA devices, the anastomoses experienced stenosis and even closure during the long-term follow-up when the anastomat was smaller than Φ5 mm. However, the risk of leakage increased when it was larger than Φ4 mm. On comparison of the different designs, it was found that the "fedora-type" MCA

device should be composed of a Φ4-mm nummular magnet with a Φ6-mm sheet metal.

CONCLUSION

The diameter of the MCA device should be greater than 120% of the enteric diameter. The novel "fedora-type" MCA device controls the pressure and optimizes the size.

Key Words: Magnetic compression anastomosis; Anastomotic stenosis; Size of anastomat; Compression pressure; Fedora-type magnetic compression anastomosis device

Core Tip: To address some of the deficiencies in the current magnetic compression anastomosis (MCA) model, we explored the optimal size and pressure of the MCA device for intestinal anastomosis in rats. We found that the suggested diameter of the MCA device should be larger than 120% of the enteric diameter to avoid stenosis. Further, we developed a novel "fedora-type" MCA device for the current model, using a Φ4-mm nummular magnet with a Φ6-mm sheet metal. This model safely formed anastomosis and ensured long-term anastomosis. This novel anastomat controlled pressure and optimized the size, thus meeting our stipulated requirements.

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INTRODUCTION

Since Obora *et al*[1] used magnetic compression anastomosis (MCA) to successfully reconstruct vessels for the first time in 1978, MCA has been proven to be capable of compressing and penetrating various tissues[2]. Thus, MCA has been applied in many scenarios, especially for conditions in the digestive tract, such as esophageal[3-5], intestinal[6-8], gastrointestinal[9-11], biliary-intestinal[12-14], and pancreas-intestinal anastomoses[15]. However, research has shown that there is a risk of long-term anastomotic stenosis and even closure after MCA[15-20]; this eventually restricted further clinical application of MCA.

Therefore, effective and reliable MCA must satisfy all of the following criteria: Appropriate pressure, safe formation of anastomosis without leakage in the short-term follow-up, adequate size, and avoidance of anastomotic stenosis or closure in the long-term follow-up. Unfortunately, previous studies mostly focused on the formation of anastomosis[10,21,22], and thus long-term outcomes were neglected. Conversely, for traditional MCA devices, the compression force was positively correlated with the size. Thus, larger anastomosis was associated with a higher risk of leakage[22].

Thus, for MCA, there are three uncertainties that require clarification. First, the minimum initial size of anastomosis needs to be determined for reconstruction of the digestive tract of a certain size. Second, the suitable compression pressure range to form anastomosis without leakage needs to be determined for the particular tissue to be anastomosed. Third, clarity is required to determine the most effective design of a novel MCA device to simultaneously meet the compression pressure and size requirements.

To address these gaps, we designed the following two experiments. First, based on the anatomical characteristics of the rat intestine, we used traditional nummular MCA devices of all possible sizes to conduct ileac side-to-side anastomosis. Based on the short-term follow-up results, we determined the appropriate pressure range required for MCA. According to the long-term follow-up results, we confirmed the minimum size required to avoid anastomotic stenosis or closure. Second, based on the results of the former experiment, we introduced a novel design concept, known as the "fedora-

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type," to the MCA device to simultaneously meet the requirements of both pressure and size, so that stable anastomosis could be formed.

MATERIALS AND METHODS

Study design and ethical considerations

All experimental protocols were approved by the Committee on the Ethics of Animal Experiments of Xi'an Jiaotong University (No. XJTULAC2020-1281). This research was conducted based on the guidelines for the Care and Use of Laboratory Animals from Xi'an Jiaotong University Health Science Center. A total of 105 male Sprague-Dawley rats weighing 240-260 g were obtained from the Experimental Animal Center, Xi'an Jiaotong University, Xi'an, China. The circumference of the intestine was measured for each rat during the operation, and the mean (± standard deviation, SD) was 13.34 ± 0.12 mm. All rats were anesthetized by isoflurane inhalation and were commonly treated pre- and post-operation. Postoperative complications and survival rates were observed.

Experiment 1: Comparison of traditional nummular MCA devices

Sixty rats were divided into four groups (groups 1.1-1.4), with 15 rats in each group. Traditional nummular MCA devices with different sizes were used in each group. As shown in Figure 1A, the MCA device involved a pair of nummular magnets (parent and daughter parts, NdFeB and N45). The diameters of the MCA devices in groups 1.1-1.4 were 3, 4, 5, and 6 mm, respectively, and the corresponding mean (± SD) compression pressures were 54.56 ± 1.40, 126.07 ± 1.38, 147.56 ± 3.42, and 152.60 ± 2.67 kPa, respectively.

After anesthesia, a 3-cm midline incision was made, and the small intestine was removed and covered with sterile gauze in normal warm saline. Then, a 6-mm incision was made 12 cm distal to the cecum. Afterwards, the parent and daughter parts of the MCA device were inserted into the intestine from the incision, reaching 6 cm proximal and distal to the incision, respectively. After adjusting the locations of the magnets, they were gently coupled to compress the ileum wall. The incisions made in the intestine and abdominal wall were sutured (Figure 2).

Experiment 2: Development of a fedora-type MCA device

Forty-five rats were randomly divided into three groups (groups 2.1-2.3) with 15 rats in each group. Based on experiment 1, a self-made "fedora-type" MCA device with different designs was adopted in each group. This device also consisted of parent and daughter parts. Each part involved a nummular magnet (NdFeB, N45) and a larger sheet metal (Ti_6Al_4V), just like a fedora cap, as shown in Figure 1B and C. The nummular magnets for all the groups were Φ4 mm, and the sheet metals for groups 2.1-2.3 were Φ4, Φ5, and Φ6 mm, respectively. Additionally, the mean (± SD) compression pressures for the different groups were 126.07 ± 1.38, 80.69 ± 0.88, and 56.03 ± 0.61 kPa, respectively.

The surgical procedure used was the same as that described in experiment 1.

X-ray examination

X-ray fluoroscopy was conducted to confirm the accurate coupling of daughter and parent parts immediately after the operation (Figure 2E2 and F2). Routine X-rays were performed every day to verify the device's movement and stable coupling in the digestive tract until the devices were discharged.

Tissue harvest and analysis

On postoperative days 30, 90, and 180, five rats in each group were euthanized to collect the anastomotic tissue specimens. The gross appearance of specimens was assessed based on a widely accepted scale, as shown in supplementary Table 1[23]. The sizes of the anastomosis were measured and analyzed using ImageJ_v1.8.0. The mechanical properties were evaluated based on bursting pressure using a self-made manometer. The histological morphology of ileac stomas was evaluated using Masson's trichrome staining and hematoxylin and eosin (HE) staining.

Statistical analysis

SPSS Statistics Software version 23.0 (IBM Corporation, Armonk, NY, United States) was used for all analyses. Categorical variables are reported as numbers and

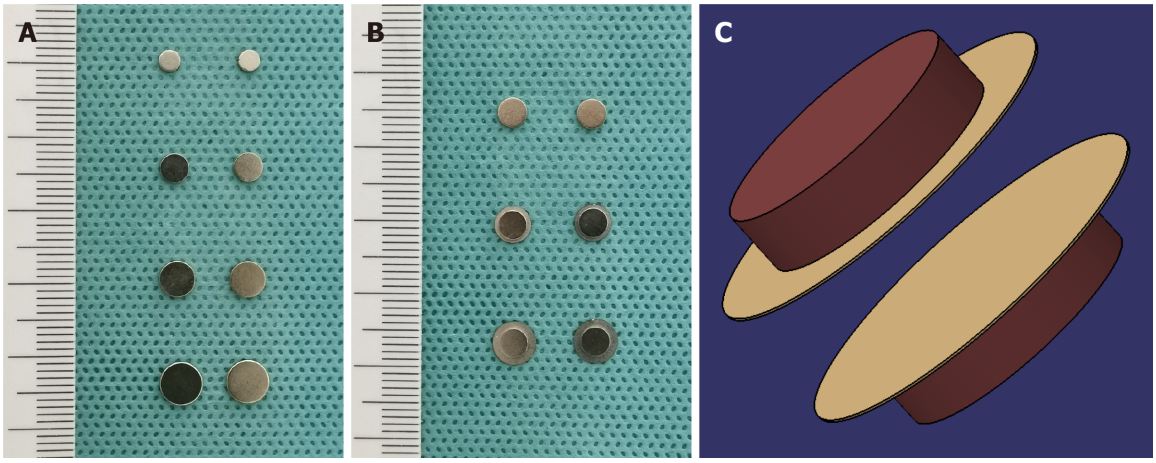

Figure 1 Magnetic compression anastomosis devices. A: Traditional nummular magnetic compression anastomosis (MCA) devices of different sizes used in experiment 1; B: Fedora-type MCA devices with different design used in experiment 2; C: Schematic diagram of the fedora-type MCA device.

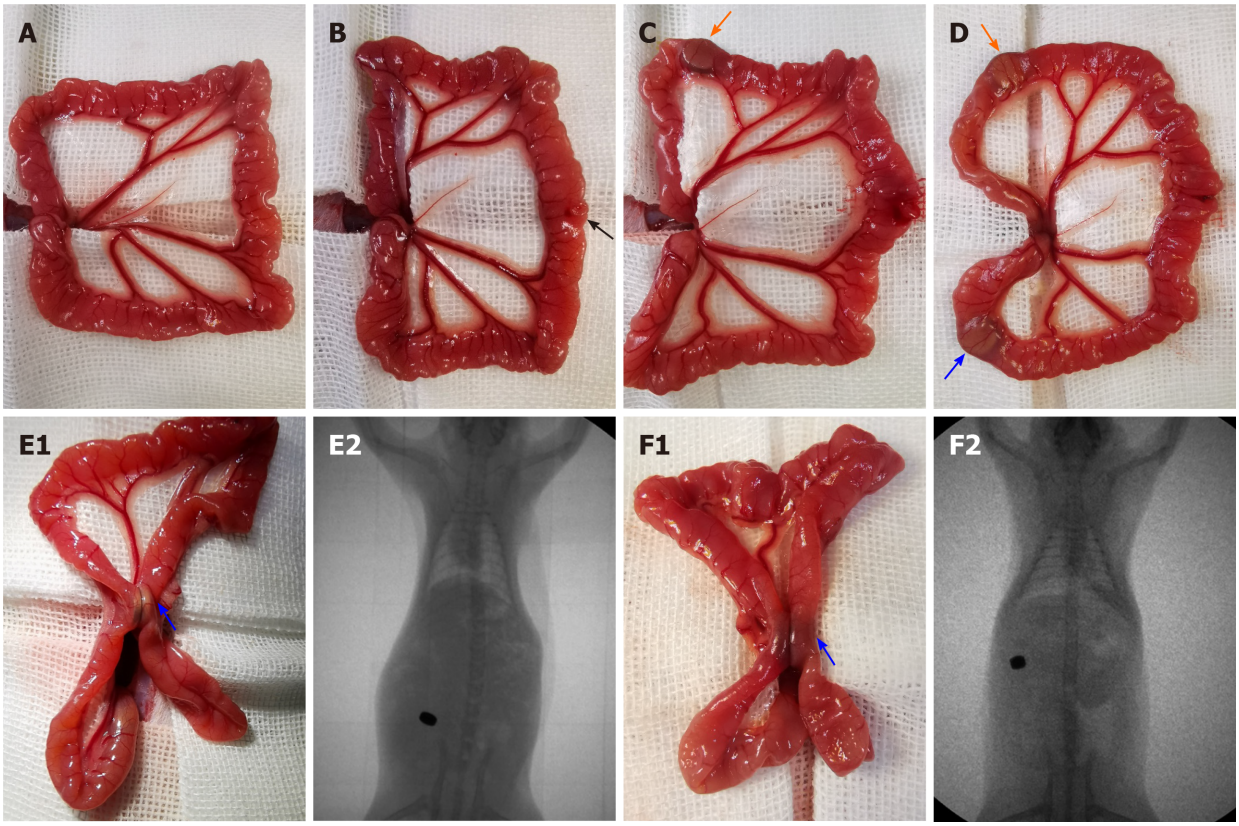

Figure 2 Surgical procedure and X-ray fluoroscopy. A: The small intestine was removed; B: A 6 mm incision was made 12 cm distal to the cecum (black arrow); C: The daughter part (orange arrow) was inserted; D: The parent part (blue arrow) was inserted; E1: Two magnets of the traditional nummular magnetic compression anastomosis (MCA) device were coupled (blue arrow) to compress the ileac wall; E2: Accurate coupling of the daughter and parent magnets in experiment 1 was confirmed using X-ray; F1: Two parts of the fedora-type MCA device were coupled (blue arrow); F2: Accurate coupling of the daughter and parent parts in experiment 2 was confirmed using X-ray.

proportions, and were compared using Chi-squared or nonparametric tests as appropriate. Normal continuous variables are reported as the mean ± SD and were compared using analysis of variance tests. Abnormal variables are reported as medians [interquartile range (IQR)] and were compared using nonparametric tests. All hypothesis tests were two-sided, and P values < 0.05 were considered statistically significant. The significance levels (α) for *post hoc* tests were adjusted accordingly.

RESULTS

Experiment 1: Comparison of traditional nummular MCA devices

Survival rate, expulsion time, and bursting pressure: No notable difficulties were encountered, and blood loss during the surgical procedure was minimal. There were no significant differences in the survival rates between the groups (groups 1.1-1.4, 93.3%, 100%, 73.3%, and 73.3%; $P = 0.083$) (Table 1). However, the combined survival rate for groups 1.1 and 1.2 was significantly higher than that of groups 1.3 and 1.4 (96.7% *vs* 73.3%, $P = 0.026$).

Routine X-ray fluoroscopy showed that all traditional nummular MCA devices coupled tightly after operation. The larger devices appeared to require shorter expulsion time. The median expulsion times were 3 (IQR 3-4), 3 (IQR 3-4), 2 (IQR 1-3), and 2.5 (IQR 2-3) d for groups 1.1-1.4, respectively ($P = 0.002$) (Table 1).

The bursting pressure for group 1.4 was lower than that in the other groups on the 30th postoperative day ($P = 0.032$) (Table 1). There was no significant difference in the bursting pressure between any of the groups on postoperative days 90 and 180 (Table 1).

Size of anastomosis: On postoperative days 30 and 90, it was observed that as the size of the MCA device increased, the circumference of the anastomosis increased ($P < 0.008$, adjusted $\alpha = 0.008$). On the 180th postoperative day, the circumference also increased with size, with the exception of that in group 1.1 when compared to group 1.2 (group 1.1 *vs* group 1.2, $P = 0.044$; $P < 0.008$ for other comparisons; adjusted $\alpha = 0.008$) (Table 1).

For the smaller groups (groups 1.1 and 1.2), the anastomosis circumferences decreased as time progressed (group 1.1: 2.47 ± 0.18, 1.20 ± 0.18, and 0.35 ± 0.19 mm for postoperative days 30, 90, and 180, respectively, $P < 0.001$; group 1.2: 8.84 ± 0.31, 5.90 ± 0.27, and 2.07 ± 0.37 mm for postoperative days 30, 90, and 180, respectively, $P < 0.017$, adjusted $\alpha = 0.017$) (Figure 3A1-A3 and B1-B3) In group 1.1, the anastomoses were nearly closed by the 90th postoperative day. In group 1.2, closure of anastomoses occurred by the 180th postoperative day. As for the larger groups (groups 1.3 and 1.4), no significant differences in the circumference were found between the different time points (group 1.3, $P = 0.811$; group 1.4, $P = 0.830$) (Figure 3C1-C3 and D1-D3).

Morphological analysis: On the 30th postoperative day, the gross appearance of the anastomoses in the smaller groups was better than that in the larger groups. In groups 1.1 and 1.2, the anastomoses were clean and intact, and the mucosa was smooth and flat without any ulcers or erosions (Figure 3A4, A5, B4, and B5). However, the adhesion around the anastomoses was severe in groups 1.3 and 1.4, and the mucosa was not smooth and flat (Figure 3C4, C5, D4, and D5). As shown in Table 1, the adhesion scores for groups 1.3 and 1.4 were significantly higher than those in groups 1.1 and 1.2, respectively ($P < 0.008$ for both, adjusted $\alpha = 0.008$).

The histological morphology showed that the serosal, submucosal, and mucosal layers were interrupted by scar tissue in the larger groups (Figure 4A1 and A2). However, it was continuous in the smaller groups (Figure 4B1 and B2).

Experiment 2: Development of a fedora-type MCA device

Survival rate, expulsion time, and bursting pressure: The surgical procedures went well for all of the different fedora-type MCA devices used. After the operation, X-ray fluoroscopy showed that the daughter and parent parts for all the fedora-type MCA devices were tightly coupled. There was no significant difference in the survival rates (groups 2.1-2.3: 93.33%, 100%, and 93.33%, respectively, $P = 0.434$) or expulsion time (groups 2.1-2.3: 3 (IQR 3-3.25), 4 (IQR 2-5), and 4 (IQR 3-5) d, respectively, $P = 0.175$) between different fedora-type MCA devices. Additionally, there was no significant difference in the bursting pressure based on the different fedora-type MCA devices used (Table 2).

Size of anastomosis: On the 30th, 90th, and 180th postoperative days, the larger fedora-type MCA devices had a larger anastomosis circumference ($P < 0.017$ for all, adjusted $\alpha = 0.017$) (Table 2). Based on the findings from the former experiment, the circumferences of the anastomoses in the smaller fedora-type MCA device (group 2.1) decreased as time progressed (8.04 ± 0.62 mm, 5.36 ± 0.32 mm, and 2.45 ± 0.67 mm for postoperative days 30, 90, and 180, respectively; $P < 0.017$ for all, adjusted $\alpha = 0.017$), and the stomas were nearly closed by the 180th postoperative day (Figure 5A1-A3). There were no significant differences in the circumference at the different postoperative time points for the large fedora-type MCA devices (group 2.2: $P = 0.749$;

Table 1 Results of traditional nummular magnetic compression anastomosis devices with different sizes

	Group 1.1	Group 1.2	Group 1.3	Group 1.4	*P* value
Survival rate	93.3% (14/15)	100% (15/15)	73.3% (11/15)	73.3 (11/15)	0.083
Discharge time (d)	3 (IQR 3-4)	3 (IQR 3-4)	2 (IQR 1-4)	2.5 (IQR 2-3)	0.002
Adhesion score					
0	92.9% (13/14)	86.7% (13/15)	27.3% (3/11)	27.3% (3/11)	< 0.001
1	7.1% (1/14)	13.3% (2/15)	27.3% (3/11)	9.1 (1/11)	
2	0 (0/14)	0 (0/15)	9.1% (1/11)	27.3% (3/11)	
3	0 (0/14)	0 (0/15)	18.2% (2/11)	18.2% (2/11)	
4	0 (0/14)	0 (0/15)	18.2% (2/11)	18.2% (2/11)	
Circumference of anastomotic stomas (mm)					
30 d	2.47 ± 0.18	8.84 ± 0.31	13.54 ± 0.31	15.98 ± 0.73	< 0.001
90 d	1.20 ± 0.18	5.90 ± 0.27	13.73 ± 0.49	16.43 ± 0.30	< 0.001
180 d	0.35 ± 0.19	2.07 ± 0.37	13.24 ± 0.68	16.33 ± 0.37	< 0.001
Bursting pressure (mmHg)					
30 d	247.64 ± 10.78	245.18 ± 7.77	242.90 ± 11.56	205.725 ± 8.06	0.032
90 d	264.55 ± 7.87	269.46 ± 9.30	261.47 ± 9.72	256.03 ± 15.63	0.830
180 d	263.32 ± 10.85	258.62 ± 10.19	261.08 ± 12.06	265.05 ± 11.26	0.978

Table 2 Results of fedora-type magnetic compression anastomosis devices with different designs

	Group 2.1	Group 2.2	Group 2.3	*P* value
Survival rate	93.3% (14/15)	100% (15/15)	93.3% (14/15)	0.434
Discharge time (d)	3 (IQR 3-3.25)	4 (IQR 2-5)	4 (IQR 3-5)	0.175
Adhesion score				
0	85.7% (12/14)	86.7% (13/15)	85.7% (12/14)	0.985
1	7.1% (1/14)	13.3% (2/15)	7.1% (1/14)	
2	7.1% (1/14)	0 (0/15)	0 (0/14)	
3	0 (0/14)	0 (0/15)	7.1% (1/14)	
4	0 (0/14)	0 (0/15)	0 (0/14)	
Circumference of anastomotic stomas (mm)				
30 d	8.04 ± 0.62	13.10 ± 0.43	15.85 ± 0.47	< 0.001
90 d	5.36 ± 0.32	13.56 ± 0.58	16.20 ± 0.52	< 0.001
180 d	2.45 ± 0.67	13.57 ± 0.47	16.42 ± 0.31	< 0.001
Bursting pressure (mmHg)				
30 d	242.80 ± 8.90	239.32 ± 9.18	250.88 ± 7.71	0.634
90 d	259.14 ± 7.42	267.00 ± 9.38	261.14 ± 12.01	0.842
180 d	258.35 ± 14.46	260.82 ± 11.78	265.85 ± 14.07	0.972

group 2.3: $P = 0.712$) (Figure 5B1-B3 and C1-C3).

Morphological analysis: On the 30th postoperative day, the gross appearance of anastomoses in all groups did not significantly differ. The anastomoses were clean and intact for all designs of the fedora-type MCA devices on the 30th postoperative day, and all the mucosae were smooth and flat, without any ulcers or erosions (Figure 5A4, A5, B4, B5, C4, and C5). As shown in Table 2, the difference in the adhesion score

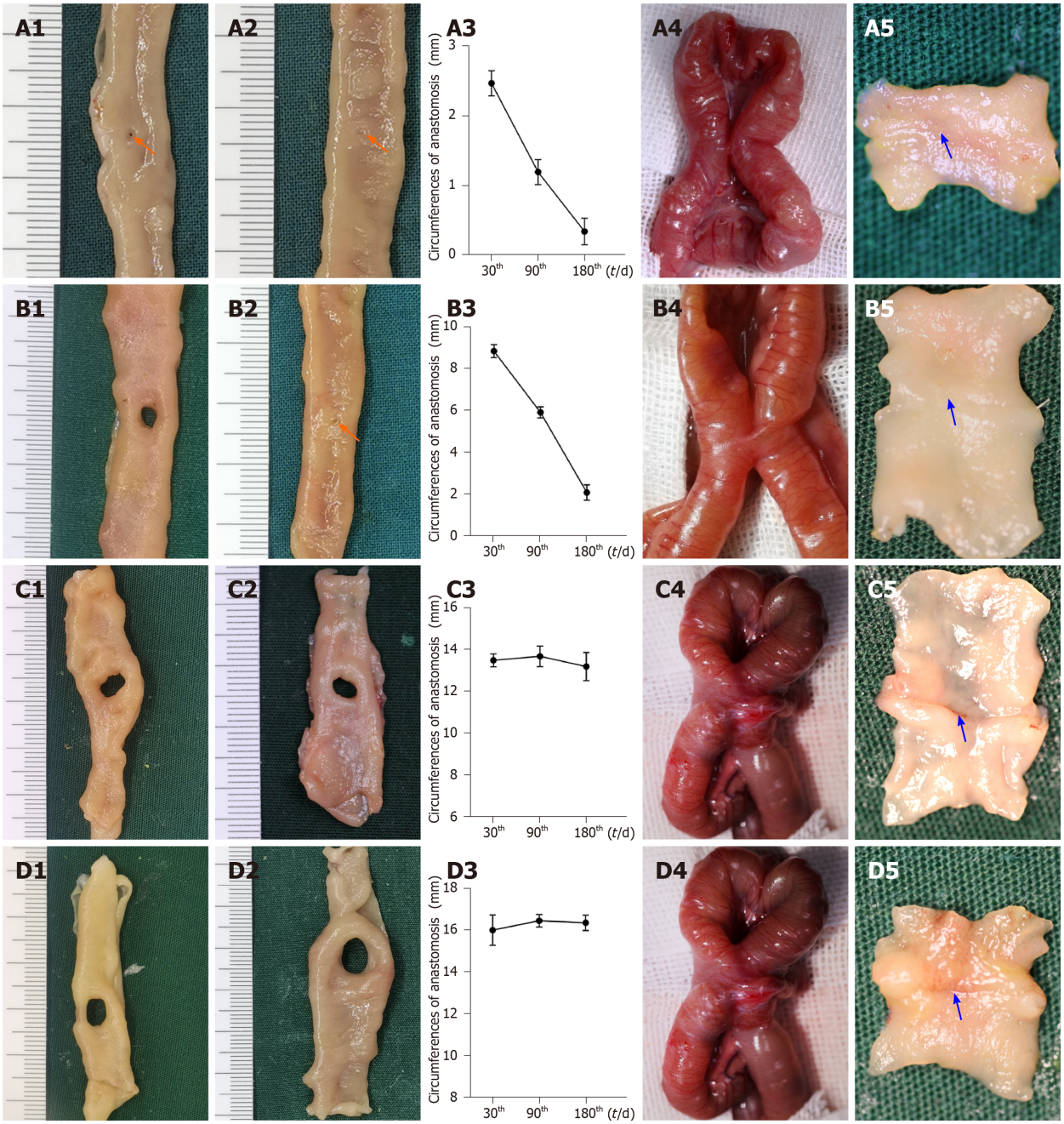

Figure 3 Gross appearance of anastomoses using traditional nummular magnetic compression anastomosis devices. A: Group 1.1 (Φ3 mm): The size of anastomosis 30 d after magnetic compression anastomosis (MCA) (A1), the size of anastomosis 180 d after MCA (A2), the change in anastomosis circumferences after MCA (A3), serosa side of anastomosis (A4), and mucosa side of anastomosis (A5); B: Group 1.2 (Φ4 mm): The size of anastomosis 30 d after MCA (B1), the size of anastomosis 180 d after MCA (B2), the change in anastomosis circumferences after MCA (B3), serosa side of anastomosis (B4), and mucosa side of anastomosis (B5); C: Group 1.3 (Φ5 mm): The size of anastomosis 30 d after MCA (C1), the size of anastomosis 180 d after MCA (C2), the change in anastomosis circumferences after MCA (C3), serosa side of anastomosis (C4), and mucosa side of anastomosis (C5); D: Group 1.4 (Φ6 mm): The size of anastomosis 30 d after MCA (D1), the size of anastomosis 180 d after MCA (D2), the change in anastomosis circumferences after MCA (D3), serosa side of anastomosis (D4), and mucosa side of anastomosis (D5). Orange arrows: Anastomosis; blue arrows: Anastomotic line.

between the groups was not significant (P = 0.985). The HE and Masson's trichrome staining in all groups showed that the serosal, submucosal, and mucosal layers were continuous (Figure 4C1 and C2).

DISCUSSION

Although previous studies have confirmed the feasibility of MCA in animal experiments[24-26] and clinical practice[27-29], there is still a risk of anastomotic stenosis or

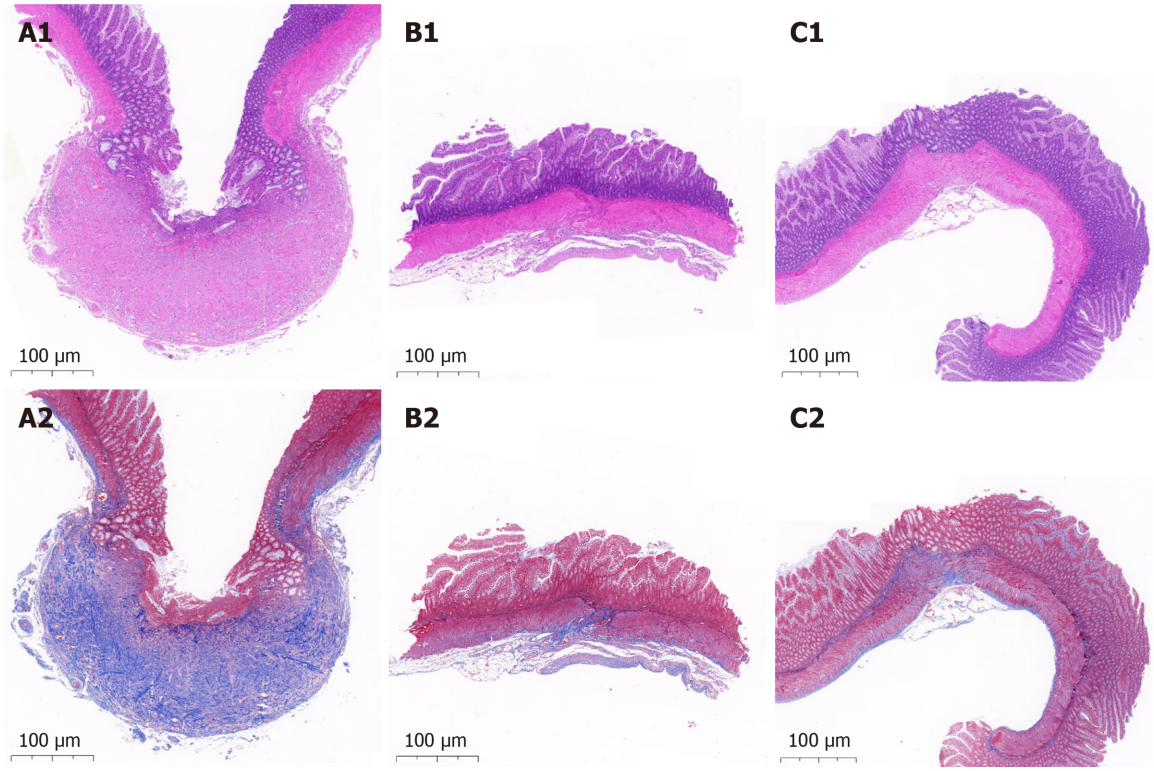

Figure 4 Microscopic appearance of anastomosis. A: Larger size groups of traditional nummular magnetic compression anastomosis (MCA) devices (Group 1.3 and 1.4): Hematoxylin and eosin staining (A1) and Masson's trichrome staining (A2); B: Smaller size groups of traditional nummular MCA devices (Group 1.1 and 1.2): Hematoxylin and eosin staining (B1) and Masson's trichrome staining (B2); C: Fedora-type MCA device: Hematoxylin and eosin staining (C1) and Masson's trichrome staining (C2).

even closure in the long run after MCA[15-17]. One interesting finding regarding MCA is the correlation between the size of anastomosis and the MCA device. Therefore, in the current study, traditional nummular MCA devices with different sizes were used to explore the suitable size and pressure for MCA. However, for traditional MCA devices, as the pressure increased, the size also increased[22]. Larger MCA devices increased the risk of leakage; therefore, we developed a novel "fedora-type" MCA device to allow for a large size but low pressure. Each part of the fedora-type MCA device had a nummular magnet with a larger sheet metal. After comparison, the optimal design for the fedora-type MCA device was that with a Φ4-mm nummular magnet and a Φ6- mm sheet metal.

The anastomat influenced the outcome of MCA in terms of pressure and size. The pressure affects the ischemic necrosis speed of the compressed tissue. If this speed surpasses the healing of anastomotic tissue, leakage could occur[21,22]. However, if the pressure is too low, dissociation of the MCA device might occur[10]. Furthermore, the importance of size is embodied in the following two aspects. First, if the size is too small, the anastomosis would narrow or even close with time; this is perhaps due to the insufficient shunt. Conversely, if the size is too large, placement and discharge of the anastomat will be difficult[30]. Thus, pressure influenced the short-term outcome of anastomotic formation for MCA, while size influenced the long-term outcome of anastomotic stenosis or closure for MCA. The existing limited basic work regarding MCA devices has mostly been focused on the effect of pressure, with a relatively short-term follow-up period (no more than 3 mo)[10,21,22]. These previous studies have ignored the importance of the size, which required subgroups and long-term follow-up. However, anastomotic stenosis or closure was identified as the real challenge for MCA devices in the gut[15,16,20].

To our knowledge, this is the first study to simultaneously explore the optimal size and pressure of traditional nummular MCA devices for intestinal anastomosis in the rat model, with a 6-mo follow-up period. The rat model simplified the subgroups. Thus, all sizes of traditional MCA devices were explored; this was crucial to investigate the relationship between anastomat, gut sizes, and anastomotic stenosis. This study showed that 5-6 mm was the optimal size range for ileac side-to-side anastomosis in the rat model. When the size was smaller than 5 mm, the anastomosis formed was small, and anastomotic stenosis or closure occurred in the long-term

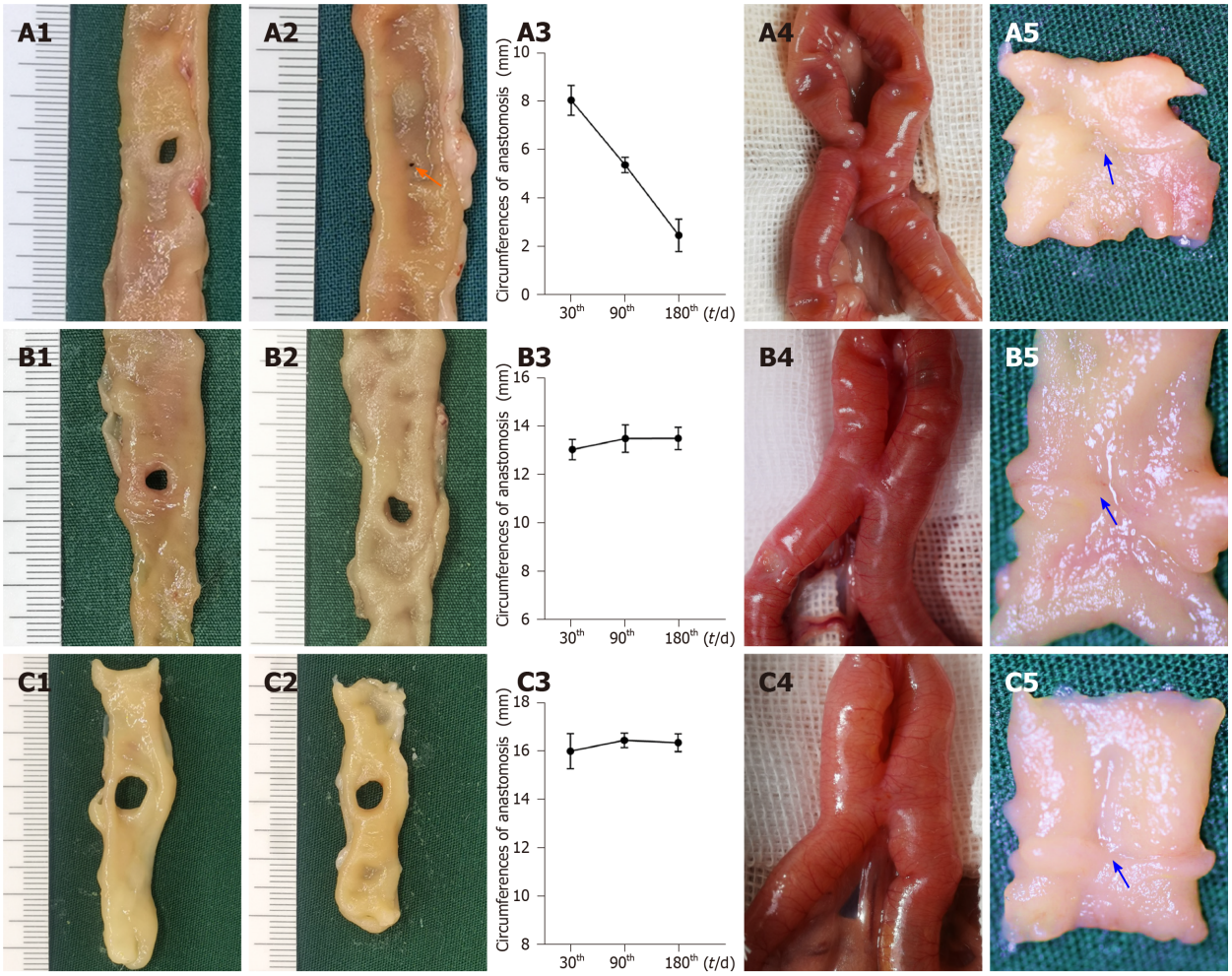

Figure 5 Gross appearance of anastomoses using the fedora-type magnetic compression anastomosis devices. A: Group 2.1 (with a Φ4 mm sheet metal): The size of anastomosis 30 d after magnetic compression anastomosis (MCA) (A1), the size of anastomosis 180 d after MCA (A2), the change in anastomosis circumferences after MCA (A3), serosa side of anastomosis (A4), and mucosa side of anastomosis (A5); B: Group 2.2 (with a Φ5 mm sheet metal): The size of anastomosis 30 d after MCA (B1), the size of anastomosis 180 d after MCA (B2), the change in anastomosis circumferences after MCA (B3), serosa side of anastomosis (B4), and mucosa side of anastomosis (B5); C: Group 2.3 (with a Φ6 mm sheet metal): The size of anastomosis 30 d after MCA (C1), the size of anastomosis 180 d after MCA (C2), the change in anastomosis circumferences after MCA (C3), serosa side of anastomosis (C4), and mucosa side of anastomosis (C5). Orange arrows: Anastomosis; blue arrows: Anastomotic line.

follow-up. While the size reached up to 7 mm, it was difficult to insert it into the intestine. In the current model, the mean (± SD) circumference of the intestine was 13.34 ± 0.12 mm, meaning that the diameter was approximately 4.2 mm. Thus, we speculated that the size of the MCA device should be larger than 120% of the enteric diameter, otherwise the anastomosis stoma would not receive sufficient shunt. This would result in stenosis or closure in the long-term follow-up. Unfortunately, the size was only approximately 58%-66% of the enteric diameter in a previously published study[6-8]. This study also demonstrated that 54.56 ± 1.40 kPa to 126.07 ± 1.38 kPa was the optimal compression pressure range, in accordance with previously published studies.

Although we determined the optimal size and pressure, they were almost impossibly achieved by traditional MCA devices, which were either of large or small size and achieved high or low pressure, respectively. The high pressure increased the risk of leakage, while the small size caused anastomotic stenosis or even closure. Devices that were large in size and led to a low amount of pressure were the ideal design for MCA devices in the gut. Therefore, we developed a novel MCA device to meet these parameters, which we called a "fedora-type" MCA device. Both parts of the novel anastomat consisted of a nummular magnet and a larger sheet metal. This allowed for control of the compression pressure by adjustment of the magnet, and for optimal size by allowing for the sheet metal to be changed. The novel design broke the internal connection between size and compression pressure in MCA devices and allowed for a large size and low pressure. Of all the different designs for the fedora-

type MCA device used, the Φ4-mm nummular magnet with a Φ6-mm sheet metal could safely form anastomosis after operation and ensure long-term stability. It should be noted that the pressure produced by this design was almost the same as that of the Φ3-mm traditional nummular MCA device, which was the smallest one used in the first experiment in this study (54.56 ± 1.40 kPa *vs* 56.03 ± 0.61 kPa). However, the circumference of anastomosis at 6 mo was comparable to that of the Φ6- mm traditional MCA device (16.33 ± 0.37 mm *vs* 16.42 ± 0.31 mm, P = 0.893). This confirmed that the anastomotic stenosis was associated with the size of the MCA device, instead of the pressure.

This study was subject to several limitations that merit consideration. These results are only applicable to rats; models in larger animals and further clinical trials are needed to test this hypothesis and guide clinical application. Although some results of the current work cannot be directly translated into clinical practice, such as the size of MCA device, other results would provide important guidance for further clinical application. For example, with an adequate number of animals, we demonstrated that the diameter of MCA device should be greater than 120% of the enteric diameter to ensure the stability of intestinal anastomosis. In this study, the anastomotic specimens at postoperative days 30, 90, and 180 were analyzed. The anastomotic specimens from a longer follow-up duration might be more convincing. However, we suspect that if the anastomosis remained stable for 6 mo, stenosis would rarely occur.

CONCLUSION

To address some of the deficiencies in the current MCA model, we explored the optimal size and pressure of the MCA device for intestinal anastomosis in rats. We found that the suggested diameter of the MCA device should be larger than 120% of the enteric diameter to avoid stenosis. Then, we developed a novel "fedora-type" MCA device for the current model, using a Φ4-mm nummular magnet with a Φ6-mm sheet metal. This model safely formed anastomosis and ensured long-term anastomosis. This novel anastomat controlled pressure and optimized the size, thus meeting our stipulated requirements for a large size and small force device.

ARTICLE HIGHLIGHTS

Research background

The feasibility of magnetic compression anastomosis (MCA) has been confirmed by previous studies; however, there is still a risk of long-term anastomotic stenosis. In fact, anastomat influences the outcome of MCA in terms of pressure and size. High pressure increases the risk of leakage, while small size causes anastomotic stenosis or even closure. One defect of traditional MCA lies in the correlation between the size of anastomosis and the MCA device. For traditional MCA devices, a large size has represented large pressure, eventually leading to increased leakage, meaning "large size & large force".

Research motivation

Studies have shown that there is a risk of long-term anastomotic stenosis and even closure after MCA; this has restricted further clinical application of MCA.

Research objectives

This study aimed to explore the optimal size and pressure of the MCA device for intestinal anastomosis in rats. Thereafter, a novel MCA device ("fedora-type" MCA device) was developed to simultaneously meet the requirements of pressure and size.

Research methods

We designed the following two experiments. First, based on the anatomical characteristics of rat intestines, we used traditional nummular MCA devices with all possible sizes to conduct ileac side-to-side anastomosis. Based on the short-term results, we determined the appropriate pressure range required for MCA. According to the long-term results, we confirmed the minimum size required to avoid anastomotic stenosis or closure. Second, based on the results of the former experiment, we introduced a novel design concept, referred to as the "fedora-type" MCA device, to

simultaneously meet the requirements of both pressure and size, so that stable anastomosis could be formed.

Research results

The optimal size range was 5-6 mm for ileac side-to-side anastomosis in the rat model (the diameter of the MCA device should be within 120%-140% of the enteric diameter). When the size was smaller than 5 mm, anastomotic stenosis or closure occurred. This study also demonstrated that 54.56 ± 1.40 kPa to 126.07 ± 1.38 kPa was the optimal compression pressure range. Traditional MCA cannot meet both of these requirements. This newly developed "fedora-type" MCA device consisted of a nummular magnet and a larger sheet metal. This allowed for control of the compression pressure by adjustment of the magnet, and for optimal size by allowing for the sheet metal to be changed. The novel design broke the internal connection between size and compression pressure in MCA devices and allowed for a large size and low pressure. Of all the different designs for the fedora-type MCA device used, the Φ4 mm nummular magnet with a Φ6 mm sheet metal could safely form anastomosis after operation and ensure long-term stability.

Research conclusions

The diameter of the MCA device should be larger than 120% of the enteric diameter to avoid stenosis. This novel anastomat controlled pressure and optimized the size respectively, thus meeting our stipulated requirements for a large size and small force device. The "fedora-type" MCA device for this model, using a Φ4 mm nummular magnet with a Φ6 mm sheet metal, safely formed anastomosis and ensured long-term anastomosis.

Research perspectives

Models in larger animals and further clinical trials are needed to test this hypothesis and guide clinical application.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Attention deficit hyperactivity disorder and gastrointestinal morbidity in a large cohort of young adults

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Abstract

BACKGROUND

Although the association of attention deficit hyperactivity disorder (ADHD) with psychiatric disorders is well known, its association with somatic diseases is unclear. Only few studies have investigated the gastrointestinal (GI) morbidity in adult patients with ADHD.

AIM

To measure gastrointestinal comorbidity and its burden on healthcare in young adults with ADHD.

METHODS

The cohort included subjects aged 17-35 years recruited to the Israel Defense Forces in 2007-2013, 33380 with ADHD and 355652 without (controls). The groups were compared for functional and inflammatory conditions of the gastrointestinal

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tract and clinic and specialist visits for gastrointestinal symptoms/disease during service (to 2016). Findings were analyzed by generalized linear models adjusted for background variables.

RESULTS

Compared to controls, the ADHD group had more diagnoses of functional gastrointestinal disorders (referred to as FGID), namely, dyspepsia [odds ratio (OR): 1.48, 95% confidence interval (CI): 1.40-1.57, $P < 0.001$], chronic constipation (OR: 1.64, 95%CI: 1.48-1.81, $P < 0.001$), and irritable bowel syndrome (OR: 1.67, 95%CI: 1.56-1.80, $P < 0.001$) but not of organic disorders (inflammatory bowel disease, celiac disease). They had more frequent primary care visits for gastrointestinal symptoms [rate ratio (RR): 1.25, 95%CI: 1.24-1.26, $P < 0.001$] and referrals to gastrointestinal specialists (RR: 1.96, 95%CI: 1.88-2.03, $P < 0.001$) and more episodes of recurrent gastrointestinal symptoms (RR: 1.29, 95%CI: 1.21-1.38, $P < 0.001$). Methylphenidate use increased the risk of dyspepsia (OR: 1.49, 95%CI: 1.28-1.73, $P < 0.001$) and constipation (OR: 1.42, 95%CI: 1.09-1.84, $P = 0.009$).

CONCLUSION

ADHD in young adults is associated with an excess of FGID and increased use of related health services. Research is needed to determine if an integrative approach treating both conditions will benefit these patients and cut costs.

Key Words: Functional gastrointestinal disorders; Irritable bowel syndrome; Dyspepsia; Constipation; Adolescents

Core Tip: The association of attention deficit hyperactivity disorder (ADHD) with gastrointestinal morbidity and gastrointestinal-associated healthcare burden is unclear. We measured it on a large cohort of young adults, containing 33380 subjects with ADHD and 355652 without. We showed for the first time that ADHD is associated with dyspepsia, chronic constipation, and irritable bowel syndrome but not with inflammatory bowel disease and celiac disease. Furthermore, young adults with ADHD have more frequent primary care visits for gastrointestinal symptoms and referrals to gastrointestinal specialists. ADHD in young adults is associated with an excess of functional gastrointestinal disorders and increased use of related health services.

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INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a chronic condition of inappropriate levels of inattention and/or hyperactivity-impulsiveness that interferes with the quality of social, academic, or occupational functioning. ADHD is one of the most common neuropsychiatric disorders of childhood, with the majority of cases persisting through adulthood[1,2]. The estimated prevalence of ADHD in the 18-44-year age group is 3.4% worldwide[3].

The association of ADHD to psychiatric comorbidity has been well described[4-9], but its association to somatic diseases is less established. According to current literature, ADHD is related to obesity, sleep disorders, and asthma, and may also be associated with otitis media, allergic rhinitis, motor disturbances, urinary symptoms, migraine and celiac disease[10-13].

The literature on gastrointestinal (GI) morbidity in ADHD is scarce in adults. There are more data for children but the findings are inconsistent[14]. A few studies reported an increased prevalence of ADHD in children with GI symptoms, such as encopresis,

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constipation, chronic diarrhea, and irritable bowel syndrome (IBS)[15,16], and others noted higher rates of abdominal distention, abdominal pain, overweight, and food allergy in children with ADHD[17-20]. Some studies, however, found no association between ADHD and GI symptoms or body mass index (BMI)[21,22].

The aim of this study was to investigate the prevalence and types of gastrointestinal comorbidities in young adults with ADHD and their burden on the healthcare system.

MATERIALS AND METHODS

Study population

In Israel, one year prior to mandatory recruitment to the Israel Defense Forces (IDF), all eligible men of Jewish, Druze, and Circassian origin, and the majority of women of Jewish origin, undergo a medical assessment which includes review of their primary care medical files, medical history taking, physical examination, and if necessary, referral for further evaluation. The findings are recorded and coded as medical profile. If a major medical problem develops, the profile is adjusted accordingly based on type, duration, and severity[23].

The population of the present study consisted of all young adults of both sexes who were recruited to the IDF between January 2007 and February 2013 and assigned to active duty. This population accounts for about 50% of all Israeli young adult population. The three main groups that are underrepresented in the database are ultra-orthodox men and women, orthodox women and Arabs that are not recruited to active military service. Data were collected retrospectively from the central Medical Corp database for each participant, from recruitment until discharge from military service (mandatory or career) or the end of the study (February 29, 2016).

The study was approved on June 29th 2015 by the institutional review board (IRB) of the IDF Medical Corps in accordance with the Helsinki Declaration. Since it was a database study and participants could not be identified, exemption from informed consent was given by the IRB.

Anthropometric and sociodemographic data

Height and weight were measured by trained personnel during the obligatory medical board examination using a stadiometer and a beam balance scale. BMI was calculated as weight in kilograms divided by height in meters squared. The following sociodemographic data were collected: Year of birth; age at the time of examination; country of birth, categorized as western countries (Europe, America, Australia, South Africa), former Soviet Union, Asia (other than the former Soviet Union; predominantly Western Asia), Africa (other than South Africa; predominantly Maghreb), Ethiopia or Israel; education, categorized as less than 12 years, 12 years, or more than 12 years; and socioeconomic status, ranked on a 10-point Central Bureau of Statistics scale according to place of residence as low (1-4), middle (5-7) or high (8-10).

Identification of recruits with ADHD

In Israel, the diagnosis of ADHD in children and adolescents is based on formal questionnaires given to parents and teachers and objective computer-based tests and psychologic tests as needed. All diagnoses are based on DSM and made by MDs or psychologists, expert in this field. The study population was divided by the absence or presence of ADHD using four sources: (1) The medical files of the primary care physicians, reviewed as part of the medical assessment at recruitment to the IDF; (2) The IDF medical profile; (3) Medical records during active duty documenting ICD-9 codes 314.0, 314.00 or 314.01; and (4) The IDF pharmacy database documenting dispensation of methylphenidate. To account for the possibility that ADHD was under-reported before and during IDF service, for the purpose of this study, any patient who met at least one of the four criteria was considered to have ADHD. In some of the analysis, patients who received methylphenidate were analyzed separately from those who did not, because methylphenidate may adversely affect the GI tract. We further divided the ADHD participants who did not receive methylphenidate into two more groups: those who were diagnosed before recruitment to the IDF, did not receive specific medical profile and did not seek for medical help regarding ADHD per-se during their active medical service (mild ADHD); and those who got a specific profile or approached their physician because of their ADHD.

Identification of recruits with GI symptoms/disease

Data on GI symptoms/diseases were collected from the central medical records database of the IDF Medical Corps. Diagnoses of inflammatory bowel diseases (IBDs) and celiac disease were based on the medical profile alone because these are major diseases affecting medical service and therefore would need to be determined very precisely at recruitment. For IBD and celiac disease, the diagnosis was based on endoscopy and histologic findings, and for celiac disease also on serology. Diagnoses of IBS, dyspepsia, and constipation were based on several sources to ensure inclusion of only well-established cases: (1) The IDF medical profile; (2) Medical records during active duty documenting ICD-9 codes 564, 564.1, 564.4, 564.10, 536.9 for IBS, ICD-9 code 536.8 for dyspepsia, or ICD-9 codes 564.0, 564.01, 564.02 for constipation, as assigned by a gastroenterologist expert; or (3) Medical records during active duty documenting these ICD-9 codes assigned by a physician other than a gastroenterologist if the two recordings were separated by an interval of at least 6 mo. Constipation was diagnosed for this study only after hypothyroidism, diabetes and hypercalcemia were ruled out. Functional gastrointestinal disorder (FGID) was defined as the presence of either IBS, dyspepsia, and/or constipation.

GI symptoms besides dyspepsia and constipation were categorized into 12 groups based on ICD-9 codes (Supplementary Table 1).

Recurrent symptoms were defined as any of the GI symptoms recorded more than twice during a period of 3-12 mo.

Outcome measures

Outcome measures for the present study were as follows: Diagnosis of IBS, dyspepsia, constipation, IBD, and celiac disease; GI symptoms as the reason for a primary care clinic visit, referral to a GI specialist, and recurrent GI complaints. Independent variables included ADHD and other medical, demographic, and anthropometric data.

Statistical analysis

The characteristics of the participants are presented as arithmetic mean and standard deviation (± SD) for continuous variables or as number and percentage for categorical variables. The association between ADHD and continuous variables was measured by Student's *t*-test and validated by Mann-Whitney test when the distribution of the continuous variables was abnormal. The association of ADHD with categorical variables was measured with chi-square test (χ^2) or Fisher's exact test as appropriate. For regression analysis, we used generalized linear models with ADHD as the independent binary logistic variable. The recruits without ADHD served as the reference group, and the confounders were the sociodemographic and anthropometric variables. Gender and suspected confounders that showed a significant association on univariate analysis at a P level of < 0.10 were entered into the multivariate model. All data were generated with IBM-SPSS software, version 23 (IBM Corp., Armonk, NY, United States).

RESULTS

Patients' characteristics

The cohort included 389032 recruits, 41.3% female, aged 17-35 years, of whom 33380 (8.6%) had ADHD. Table 1 describes the sociodemographic characteristics of the cohort. Data were missing on country of birth for 0.87% of subjects, socioeconomic status for 1.63%, and education for 1.21%. Most ADHD patients (n = 23,138, 69.3%) had mild ADHD, and only 3980 subjects (11.9%) received anti-ADHD drugs during the study period. The ADHD group had a higher percentage of females than the control group (43.3% *vs* 41.1%, P < 0.001), but this higher proportion occurred only in the mild ADHD group. The ADHD group also had a higher mean socioeconomic class and a higher BMI (P < 0.001 for all).

GI-related diseases and syndromes

Compared to controls, the ADHD group had a higher rate of dyspepsia [$399/10^4$ *vs* $273/10^4$, odds ratio (OR): 1.48, 95% confidence interval (CI): 1.40-1.57, P < 0.001], constipation ($129/10^4$ *vs* $79/10^4$, OR: 1.64, 95%CI: 1.48-1.81, P < 0.001), IBS ($263/10^4$ *vs* $156/10^4$, OR: 1.67, 95%CI: 1.56-1.80, P < 0.001) and FGID ($672/10^4$ *vs* $449/10^4$, OR: 1.53, 95%CI: 1.47-1.61, P < 0.001). There was no between-group difference in the rate of diagnosis of IBD [$30/10^4$ *vs* $31/10^4$, OR: 0.97, 95%CI: 0.79-1.19, P = not significant (NS)]

Table 1 Sociodemographic characteristics of young adults with and without attention deficit hyperactivity disorder

		ADHD	
		No	Yes
	Number of participants	355652	33380
Gender	Female	41.10%	43.30%
	Male	58.90%	56.70%
Socioeconomic status	Low	26.30%	14.70%
	Medium	53.30%	50.30%
	High	20.40%	35.00%
Education	12	94.20%	93.20%
	< 12	0.30%	0.10%
	> 12	5.50%	6.70%
Comorbidities	None or mild	68.10%	63.20%
	Mild to moderate	9.20%	9.80%
	Moderate to severe	22.60%	27.00%
Country of origin	Western countries	21.80%	25.30%
	Africa	23.00%	21.70%
	Asia	20.40%	25.50%
	Former Soviet Union	20.00%	13.00%
	Ethiopia	3.50%	1.10%
	Other	0.50%	0.60%
	Israel	10.80%	12.70%
BMI, mean ± SD	Males	21.93 ± 0.02	22.42 ± 0.06
	Females	21.52 ± 0.02	21.88 ± 0.07
Height in cm, mean ± SD	Males	174.2 ± 0.03	174.4 ± 0.10
	Females	162.2 ± 0.03	162.2 ± 0.10

ADHD: Attention deficit hyperactivity disorder; BMI: Body mass index.

and celiac disease ($16/10^4$ *vs* $15/10^4$, OR: 1.03, 95%CI: 0.78-1.37, P = NS) (Figure 1). The effect of ADHD on the rate of dyspepsia, constipation, IBS and FGID was larger in females, although still significant in males as well (OR for dyspepsia 1.51 in females and 1.39 in males; for constipation, OR of 1.60 for females and 1.58 for males; for IBS, OR of 1.83 for females and 1.47 for males; and for FGID, the OR was 1.57 for females and 1.43 for males. $P < 0.001$ for all associations in both genders). Among participants with ADHD, methylphenidate prescription was associated with an increased risk of dyspepsia and constipation, but not of IBS, IBD and celiac disease (Figure 2). These effects were unrelated to the severity of ADHD or the cumulative dose of the drug. On multivariate analysis adjusted for male sex, country of origin, country of birth, socioeconomic status, education and BMI, ADHD was significantly associated with higher rates of dyspepsia, constipation and IBS (Table 2). The association of ADHD with dyspepsia and constipation was more prominent in the subjects taking methylphenidate during the study period. The association of ADHD with IBS remained only in those not taking methylphenidate.

In order to assess the risk factors for FGID among participants with ADHD, we compared the characteristics between ADHD with and without FGID and found that in the ADHD group, FGID was associated with female gender, other comorbidities and use of methylphenidate, and was negatively associated with low SES (Supplementary Table 2).

Table 2 Results of logistic regression of the association of attention deficit hyperactivity disorder with gastrointestinal pathology and impact of gastrointestinal morbidity on use of healthcare resources

		Univariate model			Multivariate model		
	Methylphenidate	OR	95%CI	*P* value	RR	95%CI	*P* value
Celiac	+	0.66	0.25-1.77	0.539			
	-	1.08	0.81-1.45	0.592			
IBD	+	0.65	0.32-1.30	0.252			
	-	1.01	0.82-1.25	0.919			
IBS	+	1.63	1.35-1.98	C0.001	1.02	0.81-1.30	0.842
	-	1.67	1.54-1.80	< 0.001	1.29	1.18-1.41	< 0.001
Constipation	+	2.12	1.66-2.69	< 0.001	1.6	1.21-2.13	0.001
	-	1.56	1.40-1.74	< 0.001	1.43	1.26-1.62	< 0.001
Dyspepsia	+	2.03	1.77-2.32	< 0.001	1.75	1.49-2.06	< 0.001
	-	1.4	1.31-1.49	< 0.001	1.2	1.11-1.29	< 0.001
Total complaints	+	1.82	1.55-2.12	< 0.001	1.85	1.56-2.18	< 0.001
	-	1.26	1.17-1.35	< 0.001	1.26	1.17-1.36	< 0.001
Gastroenterologist referrals	+	2.34	2.14-2.57	< 0.001	2.29	2.07-2.53	< 0.001
	-	1.98	1.90-2.06	< 0.001	1.99	1.90-2.07	< 0.001
Visits in a primary care clinic	+	1.53	1.50-1.57	< 0.001	1.56	1.52-1.59	< 0.001
	-	1.23	1.22-1.24	< 0.001	1.23	1.22-1.24	< 0.001

CI: Confidence interval; IBD: Inflammatory bowel disease; IBS: Irritable bowel syndrome; OR: Odds ratio; RR: Rate ratio.

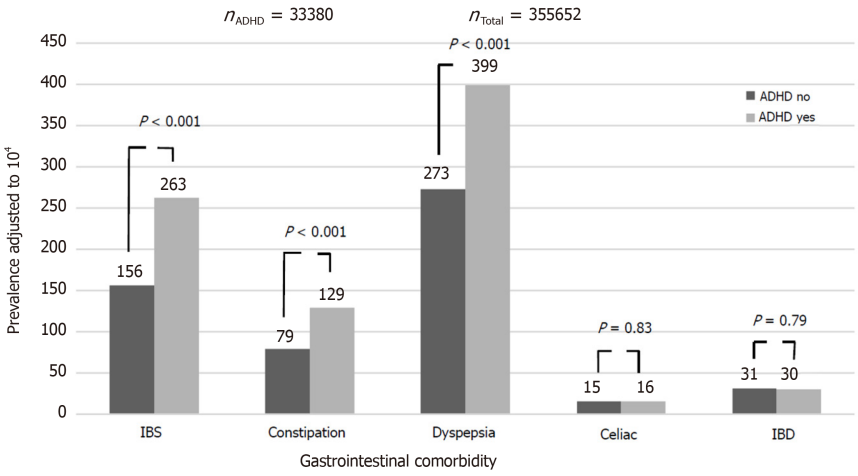

Figure 1 Gastrointestinal comorbidity among young adults with or without attention deficit hyperactivity disorder. ADHD: Attention deficit hyperactivity disorder; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome.

GI-related primary care physician visits, referrals to a GI specialist and recurrent GI symptoms

Table 3 summarizes the association between ADHD and referral to a GI specialist according to each GI symptom. Supplementary Tables 3 and 4 summarize the same association for GI-related primary care physician visits and recurrent GI symptoms, respectively. All three tables show a positive association of heartburn and gastroesophageal reflux disease, nausea and vomiting, abdominal pain, and diarrhea with ADHD. On univariate analysis (Table 2), compared to controls, the subjects with ADHD were referred more often to a GI specialist [rate ratio (RR): 1.96, 95%CI: 1.88-2.03, P < 0.001], examined more frequently by a primary care physician for GI

Table 3 Association of attention deficit hyperactivity disorder and reason for referrals to a gastrointestinal specialist by specific symptom

	Gastrointestinal symptom	RR	95%CI	*P* value
Gastroenterologist referrals	Perianal symptoms	1.69	1.32-2.15	< 0.001
	Heartburn and GERD	2.15	1.88-2.45	< 0.001
	Bowel habit changes	2.04	1.36-3.06	0.001
	Nausea and vomiting	2.53	2.21-2.89	< 0.001
	Weight loss	1.88	1.34-2.64	< 0.001
	Abdominal pain	1.94	1.84-2.04	< 0.001
	Rectal bleeding and melena	1.62	1.39-1.89	< 0.001
	Abdominal gas and bloating	1.58	1.13-2.22	0.008
	Diarrhea	2.05	1.88-2.23	< 0.001
	Abdominal mass	0.98	0.30-3.24	0.973
	Others	2.72	1.56-4.77	< 0.001
	Overall	1.96	1.88-2.03	< 0.001

CI: Confidence intervals; GERD: Gastroesophageal reflux disease; OR: Odds ratio; RR: Rate ratio.

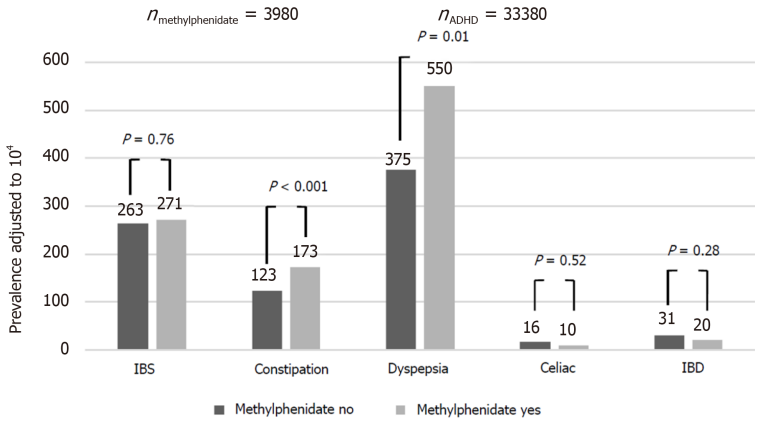

Figure 2 Association between gastrointestinal comorbidity and methylphenidate prescription to young adults with attention deficit hyperactivity disorder. ADHD: Attention deficit hyperactivity disorder; IBD: Inflammatory bowel diseases; IBS: Irritable bowel syndrome.

symptoms (RR: 1.25, 95%CI: 1.24-1.26, $P < 0.001$) and had more episodes of recurrent GI symptoms (RR: 1.29, 95%CI: 1.21-1.38, $P < 0.001$). The association of ADHD with increased use of health resources was independent of methylphenidate prescription, although its magnitude was higher in the subjects taking the drug (Table 2). Among ADHD patients, medical visits due to weight loss were higher only in those who had not received medications. On multivariate analysis adjusted for male sex, country of origin, country of birth, socioeconomic status, education, and BMI, ADHD (with or without medication) was significantly associated with primary care visits for GI symptoms, referrals to a GI specialist, and recurrent GI symptoms (Table 2).

DISCUSSION

The present study of a large cohort of young adults with ADHD showed that ADHD is associated with an increased rate of comorbid FGID (IBS, constipation, and dyspepsia) but not with somatic immune-mediated GI conditions, such as IBD and celiac disease. In addition, the ADHD group had a significantly increased rate of primary care visits for GI symptoms, referrals to GI specialists, and recurrent GI symptoms than the

control group, pointing to the high burden of GI morbidity in individuals with ADHD on healthcare resources. These associations were not related to the use of methylphenidate, although those who received methylphenidate had a higher relative risk of all the measured outcomes except IBS.

The largest study to date on physical comorbidities of ADHD was a symptom-based survey of a nationally representative sample in the United States[20]. The results showed a significant association between ADHD and "serious stomach or bowel problems" which were not specified or categorized by type (inflammatory or functional). Another population-based survey revealed an association between ADHD and recurrent complaints of vomiting and diarrhea within the previous 2 wk or frequent diarrhea and colitis[23].

Psychiatric comorbidities are known to be more common in patients with ADHD, particularly depression, anxiety, and bipolar disorder[4-9,24]. Unfortunately, since young adults with major psychiatric illnesses are not eligible for recruitment to the IDF, we were not able to study the association between FGID and major psychiatric comorbidities in our cohort. The increased utilization of healthcare services by the ADHD population, as shown in our study and in others[25], can be partially explained by the mental stress associated with serving in the army and by these psychiatric comorbidities, respectively. Therefore, patients with ADHD who have GI symptoms might best be treated with an integrative approach by a multidisciplinary team of primary care physician, GI specialist, and psychiatrist.

The association between ADHD and IBS or dyspepsia has not been intensely investigated. There are studies of ADHD and constipation but the results are controversial[7,16,22,23,26]. The present study yielded a positive association between ADHD and constipation that was more prominent in the patients receiving methylphenidate (RR: 1.60 *vs* 1.43, $P < 0.01$).

The relatively high prevalence of constipation and FGID in patients with ADHD has several possible explanations. First, it may be attributable to a miscommunication or impaired cross-talk between the central and enteric nervous systems, resulting in altered perceptions of intestinal distension and disordered GI motility[16]. Second, a single neurobiological mechanism may underlie both disorders. This possibility is supported by the known association of ADHD with urinary voiding dysfunction[26]. Third, the behavioral disorders and the high rate of comorbid psychiatric disorders in individuals with ADHD may be related to the pathogenesis of FGID[27], and fourth and most interesting, an evolving hypothesis suggests an important role of the gut-brain axis and intestinal microbiota in modulating ADHD, therefore explaining the overlap between ADHD and FGID[28,29].

In contrast to FGID, immune-mediated conditions such as IBD and celiac disease were not associated with ADHD. A previous small study of 50 children reported a higher prevalence of ADHD among those with IBD[30] but, unlike our study, it did not examine the rate of IBD in patients with ADHD. Likewise, several studies found a higher rate of ADHD among patients with celiac disease[31-33], but whether celiac disease is more prevalent among patients with ADHD is less clear[21,34]. In a recent systematic review of eight studies of ADHD and celiac disease, Ertürk *et al*[35] concluded that the results were inconsistent, as only three reported a positive correlation. It is worth mentioning that a recently published study from Germany, showed an association between childhood ADHD and immune-mediated diseases, such as type I diabetes, juvenile rheumatoid arthritis and asthma; however, no association was recorded with IBD and celiac disease[36].

Methylphenidate prescriptions were given to 3980 participants (11.92%) during the study period. We considered the receipt of medical treatment a marker of severe disease. However, methylphenidate itself has been associated with adverse GI effects, mainly abdominal pain, decreased or loss of appetite, weight loss, nausea, and vomiting. Indeed, the methylphenidate-treated subjects had a higher relative risk for most of the ADHD-associated outcome measures than the untreated subjects. Moreover, the association of methylphenidate with symptoms of nausea, vomiting, and abdominal pain was high in the assessment of medical visits to either primary care physicians or GI specialists. This finding may have been due either to side effects of the drug or the effects of a more severe form of ADHD.

Since we used a broad definition of ADHD, the rate of ADHD in our population (8.5%) was higher than previously published[3]; the majority of ADHD cases in the study (69.3%) had mild ADHD, and did not consume anti-ADHD drugs or seek help for ADHD symptoms during military service. Nevertheless, ADHD remained associated with FGID (IBS, dyspepsia and constipation) regardless its severity.

The association between ADHD and GI-related functional morbidity may affect clinical decisions and treatment. Attention should be addressed to GI problems in

patients with known ADHD, including a careful medical history focused on GI-related morbidity, so as not to miss some of the common GI problems. The presence of ADHD in a patient with GI symptoms, normal laboratory results and no red flags may by itself support the diagnosis of a functional GI disorder. Since FGIDs are now considered disorders of gut-brain interaction and centrally acting neuromodulators are amongst the mainstays of refractory FGIDs, these drugs may be considered in treating patients suffering from both FGID and ADHD.

Previous studies of GI-related comorbidity in ADHD were performed in children; this is the first study to focus on young adults. The main strength of this population-based study is its large size: 389032 participants of whom 33380 had ADHD. Moreover, our control group was well defined and based on a representative sample of the general population. We based the diagnosis of ADHD on medical documentation and not parental or patient reports, which also eliminated the risk of recall bias. Since methylphenidate is associated with substantial GI morbidity, we stratified our data regarding to medication consumption.

The present study has some limitations. We used a broad definition of ADHD, so some of the participants in the ADHD group may have had a mild form of the disease or inactive disease based on childhood medical reports. Our dependence on ICD-9 coding may have allowed for the inclusion of misdiagnoses, and diagnoses that were not strictly based on the ROME criteria; although, our strict criteria for the diagnosis of FGID in terms of duration of symptoms may have helped to overcome this limitation. Also, dyspepsia in this study is mainly uninvestigated dyspepsia, since upper GI endoscopy and *Helicobacter pylori* testing were not requested. Since the study design was cross-sectional, our results can show only an association between ADHD and GI-related morbidity but not causality. The medication data should be interpreted with caution because it is based on prescriptions and not on confirmed consumption.

CONCLUSION

In conclusion, ADHD is associated with FGID and a high need for GI-related health services. This study emphasizes the complex interaction between mind and body. Further research is needed to explore the possible combination of treatment of FGID with the neuropsychological therapeutic modalities for ADHD, and to determine if the presence of ADHD can assist in the diagnosis of FGID.

ARTICLE HIGHLIGHTS

Research background

Attention deficit hyperactivity disorder (ADHD) is a very common chronic condition of inappropriate levels of inattention and/or hyperactivity that interferes with the quality of social, academic, or occupational functioning. Although ADHD is associated with some gastrointestinal (GI) symptoms in children, the association of ADHD to GI disorders in adults is not well characterized.

Research motivation

The motivation for the research came from the clinical observation that many young adults attending the GI clinic with functional gastrointestinal disorders (FGID) mention ADHD as a chronic condition they suffer from. Therefore, we decided to conduct a study to confirm this association. Finding an association between ADHD and GI-related functional morbidity might affect clinical decisions and treatment; in such patients who have both ADHD and FGID, treatment should be taken by an integrative approach combined of a multidisciplinary team of primary care physician, GI specialist, and psychiatrist, and centrally acting neuromodulators should be considered in the treatment plan.

Research objectives

The aim of this study was to investigate the prevalence and types of GI comorbidities in young adults with ADHD and their burden on the healthcare system. Indeed, we found an association between ADHD and FGID, such as irritable bowel syndrome (IBS), dyspepsia, and chronic constipation. ADHD was not associated with IBD or celiac disease.

Research methods

This was a retrospective cohort study, consisting of all young adults of both sexes recruited to the Israeli Defense Forces (IDF) between January 2007 and February 2013 and assigned to active duty. This population accounts for about 50% of the entire Israeli young adult population. Several sources were used to accurately identify ADHD patients as well as to use only well-established diagnoses of IBS, dyspepsia, and constipation. The following sociodemographic data were collected: year of birth; age at the time of examination; country of birth; education; and socioeconomic status. Outcome measures were diagnosis of IBS, dyspepsia, constipation, IBD, and celiac disease, as well as GI symptoms as the reason for a primary care clinic visit, referral to a GI specialist, and recurrent GI complaints.

Research results

The cohort included 389032 recruits, 41.3% female, aged 17-35 years, of whom 33380 (8.6%) had ADHD. Most ADHD patients (n = 23138, 69.3%) had mild ADHD, and only 3980 subjects (11.9%) received anti-ADHD drugs during the study period. Compared to controls, the ADHD group had a higher rate of dyspepsia, constipation, IBS and FGID. There was no between-group difference in the rate of diagnosis of IBD and celiac disease. The effect of ADHD on the rate of dyspepsia, constipation, IBS and FGID was larger in females, although still significant in males as well. Among participants with ADHD, methylphenidate prescription was associated with an increased risk of dyspepsia and constipation, but not of IBS, IBD, and celiac disease. Compared to controls, the subjects with ADHD were referred more often to a GI specialist, examined more frequently by a primary care physician for GI symptoms, and had more episodes of recurrent GI symptoms. Participants with ADHD suffered more from recurrent heartburn and gastroesophageal reflux disease, nausea and vomiting, abdominal pain, and diarrhea.

The study contributes to the research in the field since this is the first study to focus on young adults and it is a large size population-based study.

Research conclusions

The present study of a large cohort of young adults with ADHD showed that ADHD is associated with an increased rate of comorbid FGID (IBS, constipation, and dyspepsia) but not with somatic immune-mediated GI conditions, such as IBD and celiac disease. In addition, the ADHD group had a significantly increased rate of primary care visits for GI symptoms, referrals to GI specialists, and recurrent GI symptoms than the control group, pointing to the high burden of GI morbidity in individuals with ADHD on healthcare resources. These associations were not related to the use of methylphenidate; although, those who received methylphenidate had a higher relative risk of all the measured outcomes, except IBS. The association between ADHD and GI-related functional morbidity may affect clinical decisions and treatment. Attention should be addressed to GI problems in patients with known ADHD, including a careful medical history focused on GI-related morbidity, so as not to miss some of the common GI problems. The presence of ADHD in a patient with GI symptoms, normal laboratory results and no red flags may by itself support the diagnosis of a functional GI disorder. Since FGIDs are now considered disorders of gut-brain interaction and centrally acting neuromodulators are amongst the mainstays of refractory FGIDs, these drugs may be considered in treating patients suffering from both FGID and ADHD.

Research perspectives

ADHD is associated with FGID and a high need for GI-related health services. This study emphasizes the complex interaction between mind and body. Further research is needed to explore the possible combination of treatment of FGID with the neuropsychological therapeutic modalities for ADHD, and to determine if the presence of ADHD can assist in the diagnosis of FGID.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Nomograms and risk score models for predicting survival in rectal cancer patients with neoadjuvant therapy

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Abstract

BACKGROUND

Colorectal cancer is a common digestive cancer worldwide. As a comprehensive treatment for locally advanced rectal cancer (LARC), neoadjuvant therapy (NT) has been increasingly used as the standard treatment for clinical stage II/III rectal cancer. However, few patients achieve a complete pathological response, and most patients require surgical resection and adjuvant therapy. Therefore, identifying risk factors and developing accurate models to predict the prognosis of LARC patients are of great clinical significance.

AIM

To establish effective prognostic nomograms and risk score prediction models to predict overall survival (OS) and disease-free survival (DFS) for LARC treated with NT.

METHODS

Nomograms and risk factor score prediction models were based on patients who received NT at the Cancer Hospital from 2015 to 2017. The least absolute shrinkage and selection operator regression model were utilized to screen for prognostic risk factors, which were validated by the Cox regression method. Assessment of the performance of the two prediction models was conducted using receiver operating characteristic curves, and that of the two nomograms was conducted by calculating the concordance index (C-index) and calibration curves. The results were validated in a cohort of 65 patients from 2015 to 2017.

RESULTS

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Seven features were significantly associated with OS and were included in the OS prediction nomogram and prediction model: Vascular_tumors_bolt, cancer nodules, yN, body mass index, matchmouth distance from the edge, nerve aggression and postoperative carcinoembryonic antigen. The nomogram showed good predictive value for OS, with a C-index of 0.91 (95%CI: 0.85, 0.97) and good calibration. In the validation cohort, the C-index was 0.69 (95%CI: 0.53, 0.84). The risk factor prediction model showed good predictive value. The areas under the curve for 3- and 5-year survival were 0.811 and 0.782. The nomogram for predicting DFS included ypTNM and nerve aggression and showed good calibration and a C-index of 0.77 (95%CI: 0.69, 0.85). In the validation cohort, the C-index was 0.71 (95%CI: 0.61, 0.81). The prediction model for DFS also had good predictive value, with an AUC for 3-year survival of 0.784 and an AUC for 5-year survival of 0.754.

CONCLUSION

We established accurate nomograms and prediction models for predicting OS and DFS in patients with LARC after undergoing NT.

Key Words: Neoadjuvant therapy; Rectal cancer; Nomogram; Overall survival; Disease-free survival; Risk factor score prediction model

Core Tip: The manuscript focuses on the risk factors after administration of neoadjuvant therapy for locally advanced rectal cancer. We utilized the least absolute shrinkage and selection operator and Cox regression to identify risk factors for overall survival and disease-free survival and explore their prognostic value. Based on the factors, we built two nomograms and two risk factor score prediction models to predict survival time. The nomograms were validated by calibration and the concordance index, and the prediction model was validated with receiver operating characteristic curves. The risk factors included in the model and nomograms are associated with survival and recurrence and can aid physicians to improve patient survival.

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INTRODUCTION

In recent years, neoadjuvant therapy (NT) has been increasingly implemented because it can reduce the risk of local recurrence and toxicity[1,2]. Numerous international guidelines recommend NT as the standard treatment for locally advanced rectal cancer (LARC)[3]. Because of the different sensitivities to adjuvant therapy, approximately 15%-27% of patients achieve a pathological complete response (pCR), and the majority of patients with stage II/III rectal cancer require surgery or adjuvant therapy[4]. Therefore, achieving a pCR is closely related to the need for subsequent treatment. Unlike patients who directly undergo surgical resection, those who first receive NT have more vulnerable immune systems, which can affect surgical outcomes[5] and influence overall survival (OS) and disease-free survival (DFS).

Global studies have reported that colorectal cancer accounts for approximately 1 of 10 newly diagnosed cancer cases and cancer-related deaths, and approximately one-third of colorectal cancer cases are rectal cancer[6,7]. Identifying prognostic factors and accurately predicting OS and DFS can provide individualized treatments for patients and improve their quality of life.

Previous studies have revealed that the number of lymph nodes, response to NT, neoadjuvant rectal score (NAR score), ypTNM stage, and family history[3,8-10] are related to OS and DFS. However, few modules or nomograms use clinical features to

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predict OS and DFS for LARC after NT. Therefore, identifying clinical features that can serve as prognostic factors and developing accurate models to predict OS and DFS could easily determine clinical treatments and improve the prognosis of patients who have received NT.

In this study, we screened preoperative and postoperative clinical features and constructed a nomogram and risk factor prediction model to predict OS and DFS. To the best of our knowledge, this study is the first attempt to build a nomogram to predict OS and DFS by screening risk factors using least absolute shrinkage and selection operator (LASSO) regression.

MATERIALS AND METHODS

Patients

We analyzed 220 patients who were clinically diagnosed with LARC and divided them into two groups: 165 patients in the primary cohort and 65 patients in the validation cohort. All patients were admitted to the Colorectal Surgery Department of the National Cancer Hospital from 2015 to 2017 and were administered preoperative NT followed by laparoscopic total mesorectal excision (TME).

We collected available demographic and clinical characteristics before NT and after TME surgery as follows: Age, sex, body mass index (BMI), clinical T (cT) and N stages (cN), pathological T (yT) and N stages (yN), ypTNM, total number of lymph nodes, positive lymph node status, preoperative chemotherapy cycle, radiotherapy cycle, distance of the tumor from the anal verge before NT and after NT, pathological response, preoperative chemotherapy regimen, radiotherapy dose, operating time, matchmouth distance from the edge, surgical procedure, preoperative carcinoembryonic antigen (CEA), postoperative CEA, and follow-up data.

This study was approved by the ethics committee at our institution. The clinical information and characteristics were recorded and analyzed after consent was obtained from the patients and their families.

Therapy

Regarding preoperative radiotherapy, the long-course regimen radiation dose ranged from 45.0-50.5 Gy; for patients who received the short-course regimen, the total dose was 25 Gy. Radiation was delivered to the pelvic cavity and tumor bed at 10 MV. All patients received TME approximately 2-60 weeks after NT based on their physical conditions. For patients who had received adjuvant therapy, three chemotherapeutic regimens were completed following radiotherapy: XELOX, capecitabine or 5-fluorouracil (5-FU) alone and capecitabin or 5-FU combined with other medicine.

Follow-up

Clinical data were obtained from follow-up visits conducted by the outpatient clinic and by telephone or email. For patients who visited the outpatient clinic, the medical history was collected, and a complete physical examination was carried out. Serum tumor marker CEA measurements and enhanced CT examinations of the pelvis were performed to detect and monitor recurrence and physical condition[3,8-10]. A colonoscopy was performed every 6 months for the first two years and once a year after two years. All patients were followed up every three months after surgery, and the last follow-up month was March 2020. DFS was defined as the time from the date of surgery to the time of recurrence or death, whereas OS was defined as the time from the date of surgery to the time of death or the last date of follow-up.

Statistical analysis

LASSO regression and nomogram construction were conducted with R software (version 3.6.1). The prognostic factors were initially screened *via* LASSO regression through the R packages "survival" and "glmnet". We utilized Cox regression to validate the prognostic factors. Then, the Kaplan-Meier (K-M) prognosis curves were drawn using the online tool Sanger box. Continuous variables were analyzed by Cox regression, and the R package "survival" was utilized to analyze variables. Each sample was categorized, and the differences in the K-M prognosis curves between the two groups were analyzed. Then, the cycle was repeated, and the *P* value of each sample was calculated and assessed using the log-rank test. The nomograms were established based on the key factors screened by the LASSO regression R package "rms". The C-index and calibration curves of the nomograms for OS and DFS reflect

the accuracy between the predicted and observed results. Risk factor prediction models were built using the R package "survival", and ROC curves were constructed with the R package "survivalROC". LASSO regression, Cox regression, K-M curves and prediction models were based on 220 patients, and nomograms were built according to the primary cohort and validated using the validation cohort.

RESULTS

Characteristics of patients

Figure 1 shows the workflow of our study. All patients underwent TME surgery. In the primary cohort, 99 (63.9%) patients were men, and 56 (36.1%) were women; 30 patients experienced recurrence, while 18 died. In the validation cohort, 53 (81.5%) patients were men, and 12 (18.5%) were women;, and 17 patients experienced recurrence, and 15 died (Tables 1-4). The median follow-up time was 41 months, and the median OS was 40.73 months (range, 2 to 62 mo). The 1-year, 3-year, and 5-year OS rates were 99.35%, 67.74%, and 4.52%, respectively. The median DFS was 38.54 (range, 2 to 62 mo), and the 1-year, 3-year, and 5-year DFS rates were 92.26%, 61.29%, and 3.23%, respectively.

Prognostic factor selection

Based on the clinical data, there were 10 potential prognostic factors in the LASSO regression model for OS selected out of 50 clinical features: Vascular_tumors_bolt, cancer nodules, yN, cT, ypTNM, BMI, matchmouth distance from the edge, nerve aggression, postoperative CEA and operation time (Figure 2A and B). We utilized Cox regression to validate the prognostic value. Among the factors, there were three factors with a value of $P > 0.05$: Operation time, cT and ypTNM (Table 5).

There were two potential prognostic factors for DFS in the LASSO regression model based on 50 clinical features: ypTNM and nerve aggression (Figure 3A and B). We utilized Cox regression to validate the two factors, which were shown to have a good prognostic value for DFS (Table 6).

As shown in Figure 4A-C, all continuous variables were grouped into high expression and low expression groups. The K-M curve of the prognosis difference between the two groups for each variable was analyzed to determine which prognostic factors were associated with a good prognosis of LARC patients treated with NT. K-M curves of classified variables are also shown to highlight the prognostic value (Figure 4D-I). The result of Kaplan-Meier curves for the prognostic factors of OS and DFS are shown in Tables 7 and 8.

Prognostic nomogram for OS and DFS

The nomogram integrated all of the prognostic factors for OS and DFS as shown in Figure 5A and B; these factors were screened by LASSO regression. The C-index for prediction of OS was 0.91 (95%CI: 0.85-0.97), and that for DFS prediction was 0.77 (95%CI: 0.69-0.85).

Validation of the nomograms

The effectiveness of the nomograms was tested in the validation cohort, and the C-index and calibration plot revealed the prognostic value of these models for OS and DFS. The C-index for prediction of OS was 0.69 (95%CI: 0.53-0.84), and that for prediction of DFS was 0.71 (95%CI: 0.61-0.81). Therefore, the established nomograms were well calibrated and showed good predictive value for OS and DFS (Figure 6).

Risk factor score prediction models for OS and DFS

We utilized Cox proportional hazards regression analysis of the clinical characteristics to develop the prognostic models (Figure 7A-F). According to the prognostic risk score, all patients were divided into a low-risk and a high-risk group. The risk scores reflected the 3-year and 5-year survival rates of the patients. K-M curves were used to show the relationship of the risk score with OS and DFS in the low-risk and high-risk groups, and these curves verified that a low risk score had a stronger positive association with OS and DFS (OS: $P = 3.576e-05$; DFS: $P = 2.91e-06$; Figure 7A and D). The AUCs of ROC curves for 3-year and 5-year OS were 0.811 and 0.782 (Figure 7B and C). The AUC for 3-year DFS was 0.784, and that for 5-year DFS was 0.754, as shown in Figure 7D and F.

Table 1 Patient demographics

Variable	Primary cohort (*n* = 155)		Validation cohort (*n* = 65)	
	No. of patients	%	No. of patients	%
Age				
Median	60.00		61.00	
Range	52.00-66.00		51.00-65.50	
BMI				
Median	24.13		23.44	
Range	21.78-26.50		21.80-25.27	
Death				
Yes	18	11.6	15	23.1
No	137	88.4	50	76.9
Her-2				
1	34	21.9	18	27.7
2	17	11	6	9.2
3	3	1.9	2	3.1
4	1	0.6		
5	100	64.5	39	60
BRAF-V600E				
1	105	67.7	45	69.2
2	6	3.9		
3	1	0.6		
4	1	0.6	1	1.5
5	42	27.1	19	29.2
P53				
1	11	7.1	2	3.1
2	7	4.5	1	1.5
3	1	0.6	2	3.1
4	15	9.7	7	10.8
5	121	78.1	53	81.5
ASA				
1	3	1.9	3	4.6
2	122	78.7	48	73.8
3	30	19.4	14	21.5
Sex				
Male	99	63.9	53	81.5
Female	56	36.1	12	18.5

BMI: Body mass index; ASA: American Society of Anesthesiologists; Her-2: 1-, 2+, 3++, 4+++, 5no; BRAF-V600E: 1-, 2+, 3++, 4no; P53: 1-, 2+, 3++, 4++, 5no.

DISCUSSION

Recently, NT has emerged as the standard treatment for LARC patients[11-14]. Patients who cannot achieve a pCR usually undergo surgery and receive adjuvant therapy. Compared to patients who undergo traditional surgery and adjuvant therapy without NT, patients who receive NT have a more complex physical condition because of the

Table 2 Clinical data before surgery

Variable	Primary cohort (*n* = 155)		Validation cohort (*n* = 65)	
	No. of patients	%	No. of patients	%
Preoperative chemotherapy cycle				
Median	2.00		3.00	
Range	2.00-3.00		2.00-3.00	
Surgery a few weeks after radiotherapy				
Median	8.00		9.00	
Range	7.00-11.00		7.00-15.00	
Distance from margin before NT				
Median	5.00		5.00	
Range	3.00-7.00		3.00-7.00	
Distance from margin after NT				
Median	5.00		5.00	
Range	3.00-7.00		3.00-7.00	
Preoperative CEA				
Median	2.85		3.35	
Range	1.60-4.73		1.52-6.21	
cT				
2			1	1.5
3	120	77.4	52	80
4	35	22.6	12	18.5
cN				
0	54	34.8	21	32.3
1	73	47.1	33	50.8
2	28	18.1	11	16.9
cM				
0	146	94.2	62	95.4
1	9	5.8	3	4.6
cTNM				
2	52	33.5	21	32.3
3	94	60.6	41	63.1
4	9	5.8	3	4.6
yT				
0	22	14.2	8	12.3
1	4	26	1	1.5
2	34	21.9	15	23.1
3	84	54.2	35	53.8
4	11	7.1	6	9.2
yN				
0	88	56.8	33	50.8
1	47	30.3	23	35.4
2	19	12.3	9	13.8

3	1	0.6		
yM				
0	146	94.2	62	95.4
1	9	5.8	3	4.6
ypTNM				
0	21	13.5	8	12.3
1	28	18.1	12	18.5
2	39	25.2	15	23.1
3	58	37.4	27	41.5
4	9	5.8	3	4.6
Pathological changes after treatment				
1	85	54.8	38	58.5
2	48	31	19	29.2
3	22	14.2	8	12.3
TRG				
0	3	1.9	2	3.1
1	27	17.4	14	21.5
2	62	40	26	40.0
3	41	26.5	15	23.1
4	22	14.2	8	12.3
Preoperative simultaneous chemotherapy				
Yes	126	81.3	51	78.5
No	29	18.7	14	21.5
Preoperative radiotherapy				
Yes	3	1.9	4	6.2
No	152	98.1	61	93.8
Preoperative chemotherapy				
Yes	26	16.8	10	15.4
No	129	83.2	55	84.6

CEA: Carcinoembryonic antigen; TRG: Tumor regression grade. Pathological changes after treatment, 1: no-downstaging; 2: downstaging; 3: Polymerase chain reaction.

influence of NT[15,16]. Additionally, the prognostic factors for OS and DFS also change. Thus, exploring the prognostic factors that can predict OS and DFS has become necessary.

Many studies have revealed that lymph node metastasis, low BRCA2 expression and other variables can be prognostic factors for patients administered NT. In our study, we developed and validated risk score prediction models and nomograms for OS and DFS based on clinical characteristics. Preliminary screening of potential factors by LASSO regression can reduce the number of features included and screen only critical factors[17,18]. Cox regression and K-M curves can further verify the prognostic value of key factors. The followings were included in the nomogram for OS: Vascular_tumors_bolt, cancer nodules, yN, BMI, matchmouth distance from the edge, nerve aggression and postoperative CEA. The nomogram of DFS included the following variables: ypTNM and nerve aggression. The risk factor score prediction models included the same risk factors as the nomograms. The AUCs for the prediction models for both OS and DFS were high and showed that a low risk score had a strong positive association with the years of survival, indicating that the risk factor and prognostic models had good prognostic value for LARC.

Table 3 Surgical and pathological data

Variable	Primary cohort (*n* = 155)		Validation cohort (*n* = 65)	
	No. of patients	%	No. of patients	%
Total number of lymph nodes				
Median	16.00		17.00	
Range	12.00-22.00		11.00-22.00	
Positive lymph node status				
Median	0.00		0.00	
Range	0.00-1.00		0.00-2.00	
Operating time				
Median	193.00		209.00	
Range	158.00-237.00		148.00-257.00	
Matchmouth distance from the edge				
Median	3.00		2.00	
Range	0.00-4.00		1.00-4.00	
Amount of bleeding during surgery				
Median	50.00		50.00	
Range	20.00-100.00		20.00-60.00	
Joint organ cut				
Yes	8	5.2	3	4.6
No	147	94.8	62	95.4
Side-side lymph node sweep				
Yes	5	3.2	3	4.6
No	150	96.8	62	95.4
Preventive mouth-building				
Yes	35	22.6	15	23.1
No	120	77.4	50	76.9
Retention of the left colon artery				
Yes	9	5.8	6	9.2
No	146	94.2	59	90.8
Postoperative pathology				
1	3	1.9	1	1.5
2	128	82.6	52	80
3	19	12.3	11	16.9
4	1	0.6	1	1.5
5	4	2.6		
Cancer nodules				
Yes	17	11	11	16.9
No	138	89	54	83.1
Nerve aggression				
Yes	30	19.4	23	35.4
No	125	80.6	42	64.6
Vascular_tumors_bolt				

Yes	17	11	9	13.8
No	138	89	56	86.2

Postoperative pathology, 1: Highly differentiated adenocarcinoma; 2: Moderately and Second differentiated adenocarcinoma; 3: Poorly and medium differentiated adenocarcinoma; 4: Signet-ring cell carcinoma; 5: mucinous adenocarcinoma.

Regarding the prognostic factors of OS, 50 candidate clinical features were reduced to 10 potential predictors, and through Cox regression analysis, three factors could be eliminated: Operation time, cT and ypTNM. The *P* values of operation time, cT and ypTNM were higher than 0.05. The distance of the tumor from the anal margin is closely related to operation time and other important factors[19-21] because if the tumor is close to the anus, anal preservation will be prioritized. However, removing the anus or preserving the lower anus can be a lengthy procedure; therefore, the operation time may be related to the tumor location after NT. In addition to the distance from the margin after NT, the matchmouth distance from the edge can more comprehensively reflect the tumor type. Changes in the size of the tumor can influence the type of surgery, which will also affect the distance of the matchmouth from the edge. Changes in tumor size before and after NT were related to the tumor response to treatment. Therefore, although the operation time and ypTNM can reflect the different statuses, they also have a close relationship with the matchmouth distance from the edge, thus we excluded the two variables. Regarding the distance from the margin to the anus, a shorter distance from the matchmouth to the anus corresponds to shorter survival time.

Laparoscopic surgery for colorectal cancer has a shorter postoperative exhaust time than conventional left hemicolectomy[22]. Postoperative exhaust time is an important postoperative indicator that is closely related to obstructive colorectal cancer[23,24]. In our cohort, only one patient presented with obstruction; therefore, the prognostic value of postoperative exhaust time was not screened out by the LASSO regression analysis.

The appearance of cancer nodules is an important factor associated with primary tumor metastasis and has been suggested to reflect the effects of adjuvant therapy. With the development of UICC/AJCC staging standard, the definition and staging of cancerous nodules have gradually improved, and the prognostic value of nodules in colon cancer is also increasing. In previous studies, cancer nodules were thought to significantly increase the rates of local recurrence and metastasis in colorectal cancer[25]. Cancer nodules had the lowest contribution to our nomogram for OS; if patients have cancer nodules, the nomogram score will increase, and OS will decrease.

yN was evaluated after surgery. For tumors located in or near the rectum, the N stage significantly more frequently either remained stable or progressed, but treatment with surgery and adjuvant therapy could also have an effect. yN is a good prognostic factor for DFS and cancer-specific survival[26-28]. Pathological examination is very important for patients who receive NT because it can ensure the appropriate staging and treatment. In our study, both LASSO regression and the K-M curves revealed that yN had good prognostic value; thus, we included this variable to ensure that our nomogram fully reflects the condition after adjuvant therapy. Regarding yN, in the nomogram, as the N stage progresses, the nomogram score increases and survival decreases. Of note, yN3, which is to the left of yN0 and yN1, may be due to lymph node changes after NT, which was found at a high rate by the surgeon performing the resection.

BMI reflects the patients' weight and height. As a risk factor for colorectal cancer[29,30], the BMI value is an important prognostic indicator. Patients with a higher BMI tend to be more obese and have shorter survival based on our nomogram. We also explored the level of the serum tumor marker CEA because it is an important and strong diagnostic biomarker both before therapy and after surgery[31]. In our nomogram, a higher CEA level indicates shorter survival.

LARC poses several challenges, including recurrence[32]. Tumor recurrence is an important factor affecting the prognosis and survival of tumor patients[33]. A lower probability of recurrence leads to a higher survival rate. In previous studies, recurrence has been linked with biomarkers such as BRAF-6000E, RAS and CD8-positive T-cells[11,34,35], and an early diagnosis[25] can take advantage of the patients' clinical information. In identifying predictive factors of DFS, 50 clinical features were reduced to 2 potential predictors of DFS. The DFS nomogram included ypTNM and nerve aggression. Pathologic TNM (ypTNM) has been considered a good prognostic

Table 4 Clinical data after surgery

Variable	Primary cohort (*n* = 155)		Validation cohort (*n* = 65)	
	No. of patients	%	No. of patients	%
Number of cycles of postoperative chemotherapy regimens				
Median	4.00		4.00	
Range	0.00-6.00		0.00-6.00	
Postoperative exhaust				
Median	3.00		3.00	
Range	3.00-5.00		3.00-5.00	
Postoperative defecation				
Median	5.00		5.00	
Range	3.00-6.00		4.00-6.00	
Postoperative ureter removal time				
Median	4.00		4.00	
Range	4.00-5.00		3.00-5.00	
Postoperative CEA				
Median	2.41		2.55	
Range	1.59-3.705		1.70-3.41	
Postoperative adjuvant therapy				
Yes	101	65.2	45	69.2
No	54	34.8	20	30.8
Postoperative bleeding				
Yes	2	1.3		
No	153	98.7	65	100
Postoperative intestinal fistula				
Yes	5	3.2		
No	150	96.8	65	100
Intestinal obstruction after surgery				
Yes			1	1.5
No	155	100	64	98.5
Unplanned postoperative surgery				
Yes	3	1.9		
No	152	98.1	65	100
Cardiovascular accidents				
Yes	1	0.6		
No	154	99.4	65	100
Postoperative complications				
Yes	7	4.5	1	1.5
No	148	95.5	64	98.5
Recurrence				
Yes	30	19.4	17	26.2
No	125	80.6	48	73.8

CEA: Carcinoembryonic antigen.

Table 5 Cox regression analysis for the prognostic factors of overall survival

Variable	*P* value	OR	95%CI
yN	0.003		
1 *vs* 0	0.947	576.353	0.000-4.138E+84
2 *vs* 0	0.935	2450.459	0.000-1.758E+85
3 *vs* 0	0.934	2902.876	0.000-2.084E+85
Cancer nodules	0.003	3.278	1.506-7.134
Nerve aggression	< 0.0001	3.446	1.726-6.882
Vascular_tumors_bolt	0.009	2.924	1.309-6.531
ypTNM	0.112		
1 *vs* 0	0.110	0.267	0.053-1.346
2 *vs* 0	0.962	0.000	0.000-2.397E+244
3 *vs* 0	0.102	0.299	0.071-1.268
4 *vs* 0	0.801	0.856	0.254-2.886
cT	0.057		
3 *vs* 2	0.018	14.337	1.585-129.724
4 *vs* 2	0.192	2.011	0.705-5.735
Matchmouth distance from the edge	0.012	0.805	0.679-0.953
Postoperative CEA	0.037	1.017	1.001-1.034
BMI	0.031	1.113	1.010-1.226
Operation time	0.068	1.004	1.000-1.008

CEA: Carcinoembryonic antigen; BMI: Body mass index.

Table 6 Cox regression analysis for the prognostic factors of disease-free survival

Variable	*P* value	OR	95%CI
ypTNM	0.001		
1 *vs* 0	0.003	0.089	0.018-0.445
2 *vs* 0	0.001	0.032	0.004-0.266
3 *vs* 0	0.017	0.291	0.105-0.805
4 *vs* 0	0.198	0.558	0.230-1.355
Nerve aggression	< 0.0001	3.01	1.681-5.388

factor in many studies. Utilizing ypTNM, our study also confirmed that ypTNM is a strong predictor for DFS[36-38]. Nerve aggression was also an important predictive factor in our study. A higher ypTNM or presence of nerve aggression corresponds to a shorter survival time.

There are limitations to our study. The data included here were all from a single network of tumor hospitals, thus lacking representation of the general population. Additionally, our research in the field of molecular target design is poorly established.

Table 7 Kaplan-Meier curves for the prognostic factors of overall survival

Variable	*P* value	HR	95%CI
yN	0.00083	0.51	0.35-0.75
Cancer nodules	0.0015	3.29	1.51-7.15
Nerve aggression	0.00018	3.45	1.73-6.89
Vascular tumors bolt	0.0059	2.93	1.31-6.55
Matchmouth distance from edge	0.0035	0.80	0.67-0.95
Postoperative CEA	0.55	1.02	1.00-1.03
BMI	0.036	1.12	1.02-1.23

CEA: Carcinoembryonic antigen; BMI: Body mass index.

Table 8 Kaplan-Meier curves for the prognostic factors of disease-free survival

Variable	*P* value	HR	95%CI
ypTNM	< 0.0001	0.73	0.56-0.96
Nerve aggression	< 0.0001	3.02	1.69-5.4

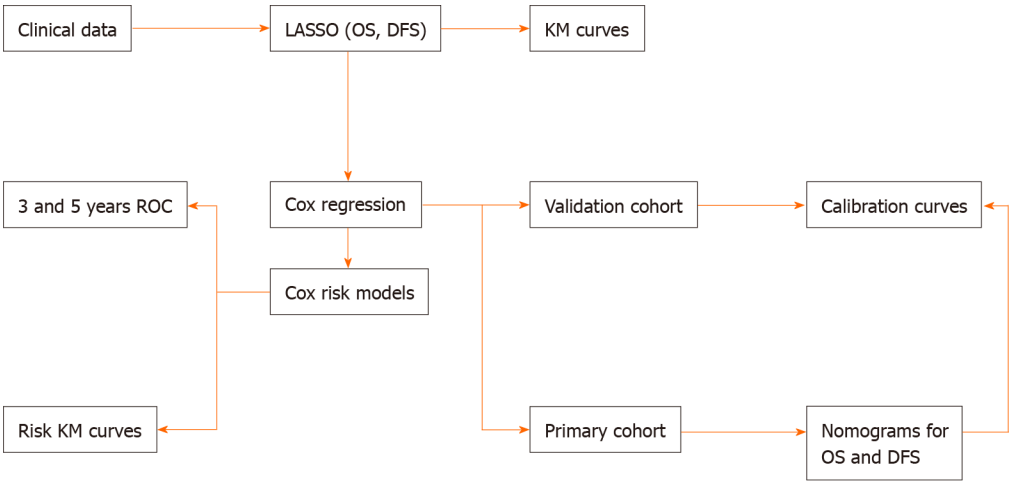

Figure 1 Analysis workflow in this study. ROC: Receiver operating characteristic; OS: Overall survival; DFS: Disease-free survival; KM: Kaplan-Meier.

CONCLUSION

Recurrence, cancer nodules, yN, positive lymph node status, BMI, matchmouth distance from the edge, distance from the margin after NT and postoperative CEA were prognostic factors for OS, and ypTNM and nerve aggression were prognostic value for DFS. We created and validated nomograms and prediction models that can objectively and accurately predict OS and DFS in LARC patients.

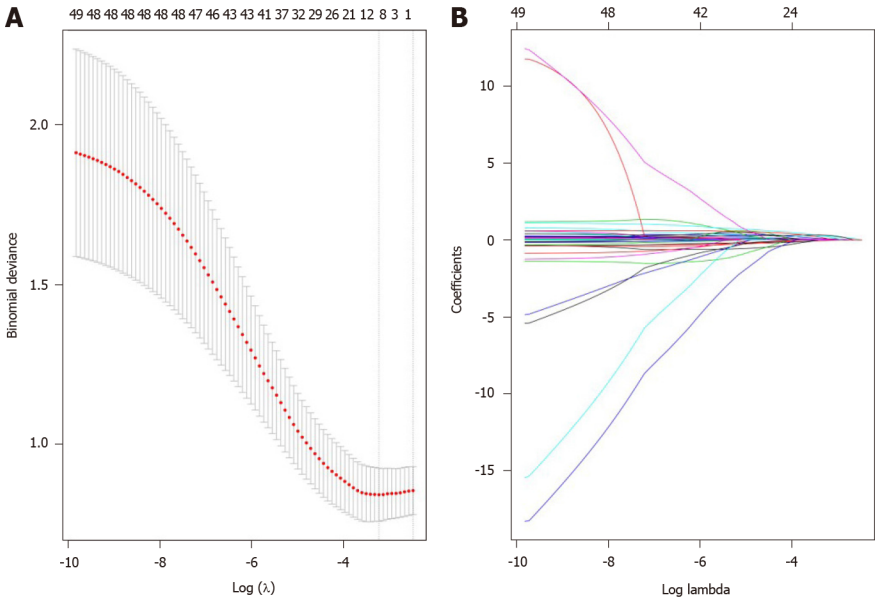

Figure 2 Selection of prognostic factors using the least absolute shrinkage and selection operator regression model. A: A graph of the error rate of cross-validation; B: least absolute shrinkage and selection operator coefficient profiles of the 151 texture features.

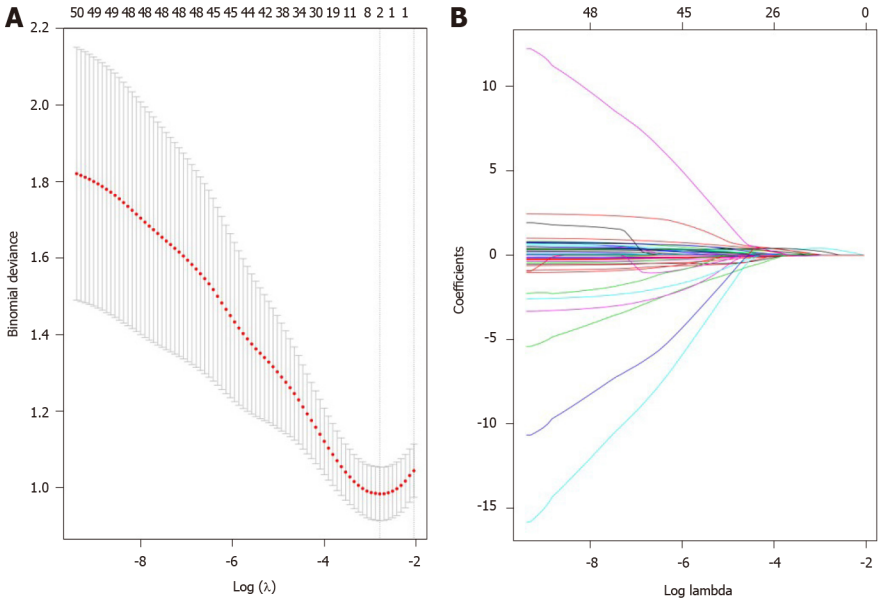

Figure 3 Prognostic factor selection using the least absolute shrinkage and selection operator. A: A graph of the error rate of cross-validation; B: Least absolute shrinkage and selection operator coefficient profiles of the 150 texture features.

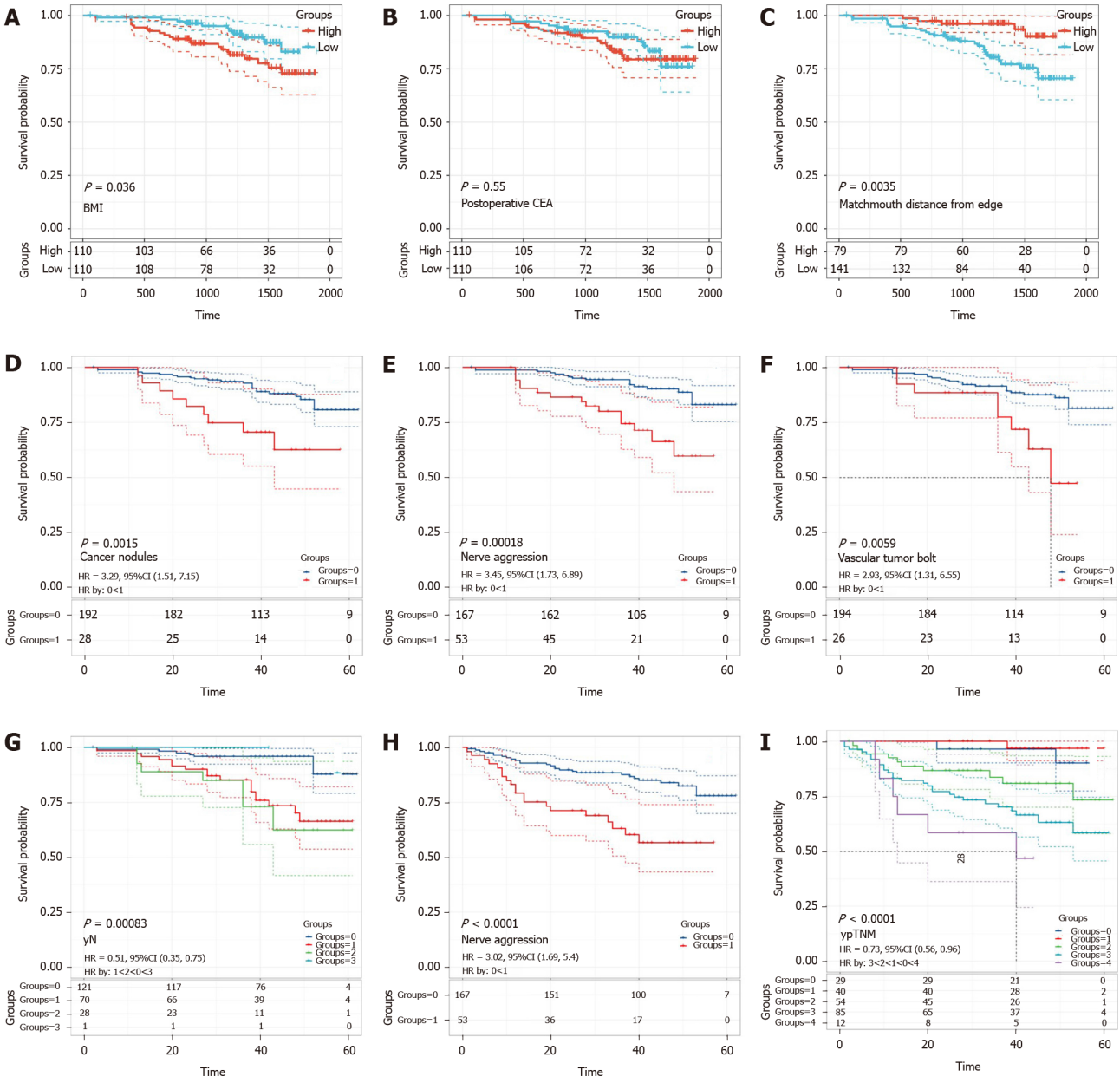

Figure 4 Kaplan-Meier survival curves for the prognostic factors of overall survival and disease-free survival. A-G: The prognostic factors for overall survival; H, I: The prognostic factors for disease-free survival. BMI: Body mass index; CEA: Carcinoembryonic antigen.

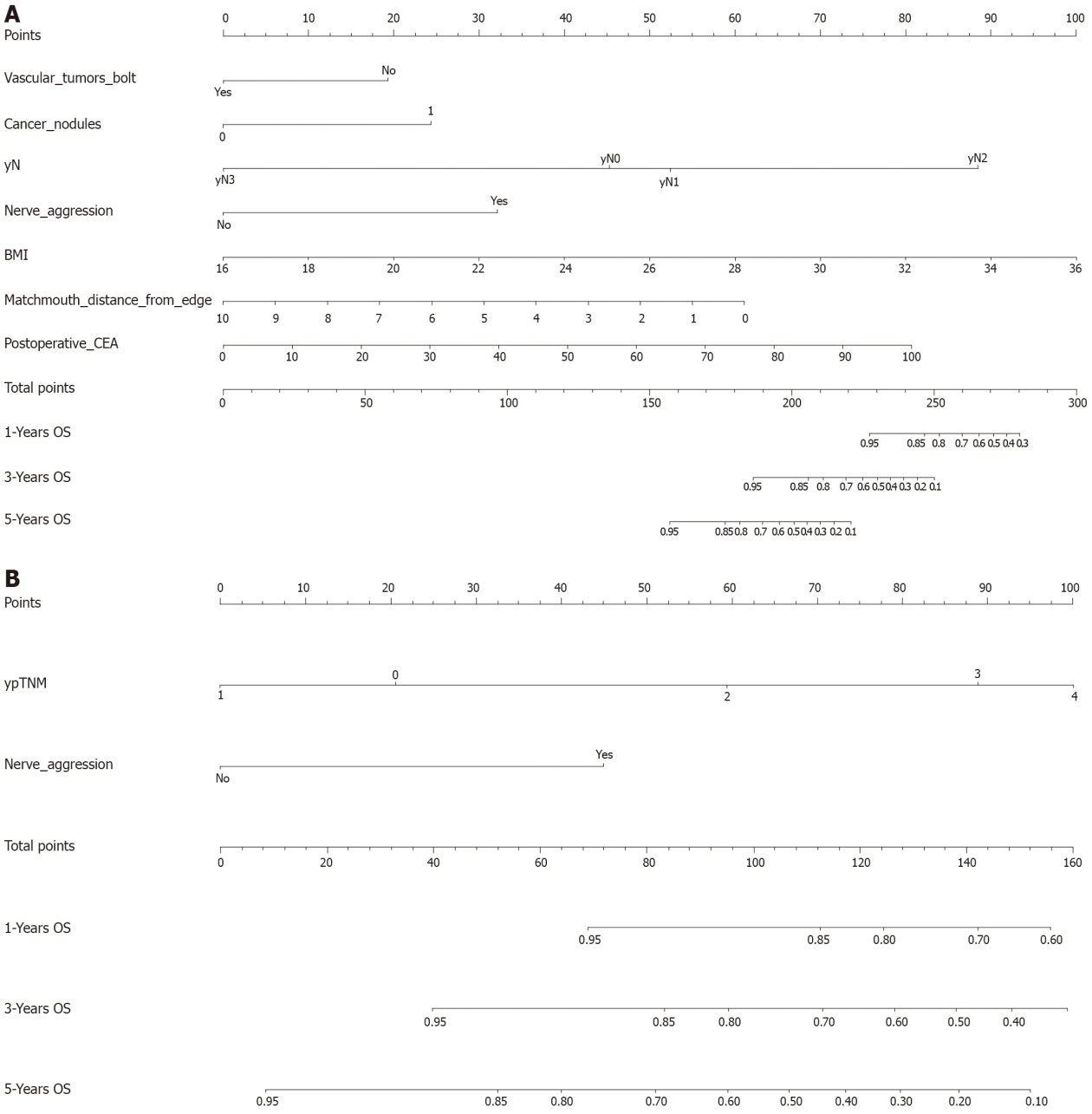

Figure 5 Survival nomogram. A: The nomogram for overall survival was developed in the primary cohort with eight prognostic factors: recurrence, cancer nodules, yN, positive lymph node status, body mass index, matchmouth distance from the edge, distance from the margin after neoadjuvant therapy and postoperative carcinoembryonic antigen; B: The nomogram for disease-free survival was developed in the primary cohort with two prognostic factors: ypTNM and nerve aggression.

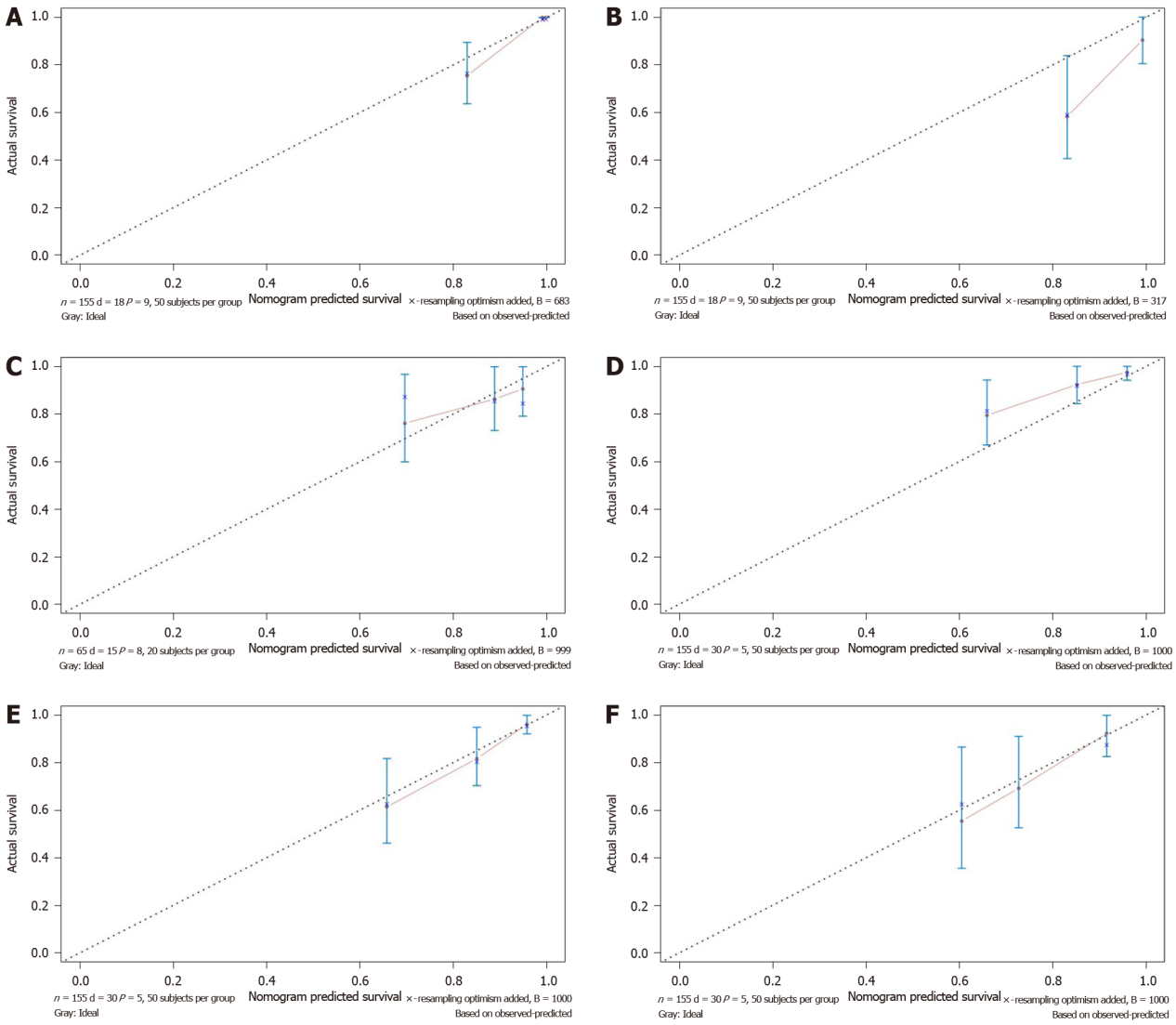

Figure 6 Calibration curve for predicting patient survival. A: 3-year and B: 5-year overall survival (OS) rates in the primary cohort; C: 3-year OS rate in the validation cohort; D: 1-year and E: 3-year disease-free survival (DFS) rates in the primary cohort; F: 3-year DFS rate in the validation cohort.

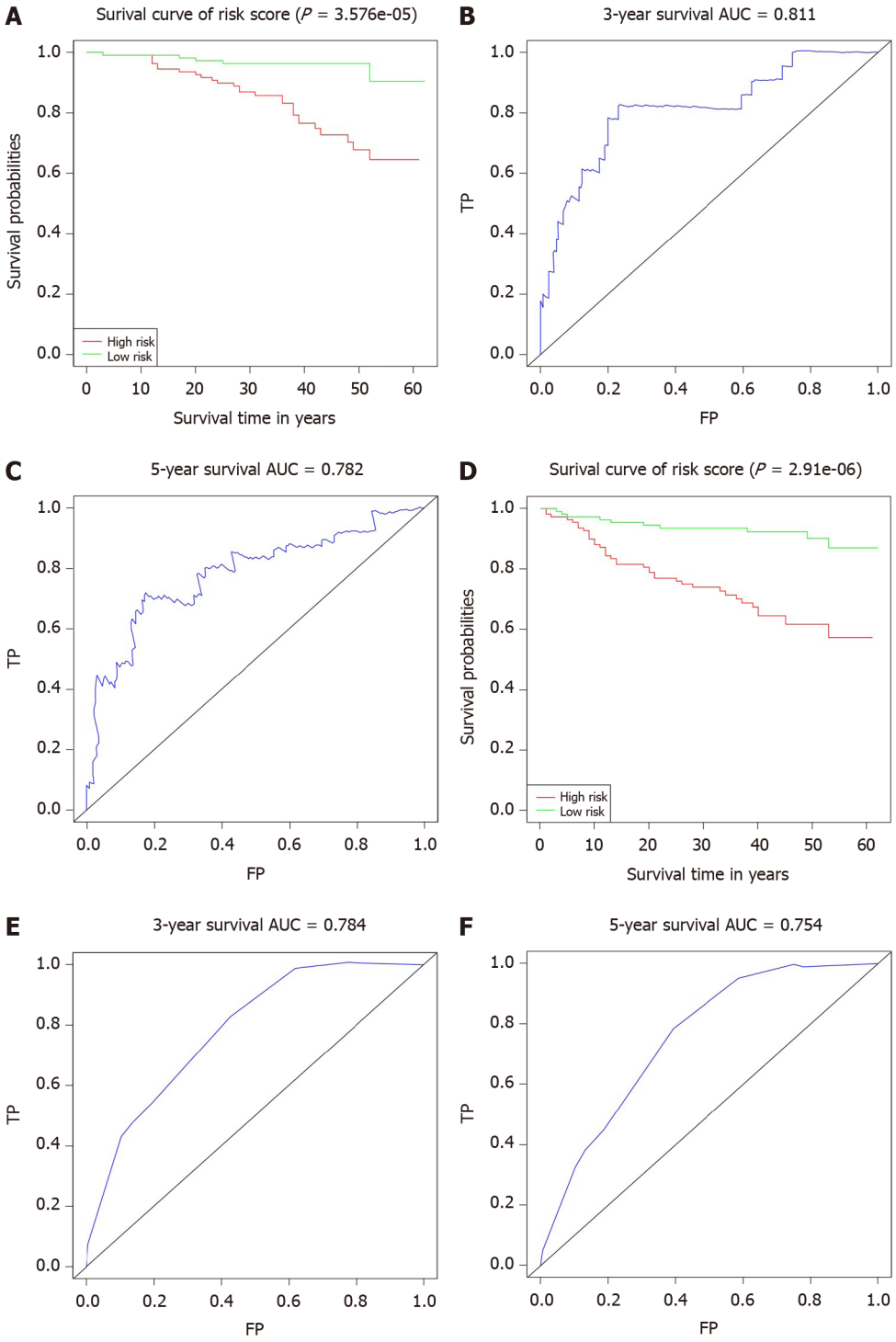

Figure 7 Kaplan-Meier and receiver operating characteristic curve for the risk factor score prediction model. A: Kaplan-Meier (K-M) overall survival (OS) curves for the low-risk and high-risk groups; B: Receiver operating characteristic (ROC) curves for the 3-year and C: 5-year OS rates of locally advanced rectal cancer (LARC); D: K-M disease-free survival (DFS) curves for the low-risk and high-risk groups; E: ROC curves for the 3-year and F: 5-year DFS rates of LARC.

ARTICLE HIGHLIGHTS

Research background

Neoadjuvant therapy (NT) has been increasingly used as the standard treatment for clinical stage II/III rectal cancer. Risk factors after administration of neoadjuvant therapy for locally advanced rectal cancer (LARC) are still under debate.

Research motivation

There is a lack of consensus concerning the risk factors after administration of neoadjuvant therapy for LARC. Nomograms and risk prediction models for survival can help clinicians to choose therapy according to patient's individual risk.

Research objectives

The main aim of this study was to explore the prognostic factors and establish effective prognostic nomograms and risk score prediction models to predict overall survival (OS) and disease-free survival (DFS) for LARC treated with NT.

Research methods

Nomograms and risk factor score prediction models were based on patients who received NT. LASSO regression was utilized to screen for prognostic risk factors, which were validated by the Cox regression. ROC curves, C-index and calibration curves were performed to evaluate the prediction models and nomograms.

Research results

Seven features, including vascular_tumors_bolt, cancer nodules, yN, body mass index (BMI), matchmouth distance from the edge, nerve aggression and postoperative carcinoembryonic antigen (CEA), were significantly associated with OS. The nomogram for predicting DFS included ypTNM and nerve aggression. The primary and validate cohort showed good predictive value. The prediction model for OS and DFS had good predictive value.

Research conclusions

We established accurate nomograms and prediction models for predicting OS and DFS in patients with LARC after undergoing NT.

Research perspectives

Larger prospective multicenter clinical studies need to be performed to validate the nomograms and risk score prediction models of OS and DFS.

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ORIGINAL ARTICLE

Observational Study

Estimation of visceral fat is useful for the diagnosis of significant fibrosis in patients with non-alcoholic fatty liver disease

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Abstract

BACKGROUND

Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD), although obese patients with NAFLD do not always develop significant fibrosis. The distribution of body fat could predict the risk of NAFLD progression.

AIM

To investigate the role of bioelectrical impedance-estimated visceral fat (VF) in assessing NAFLD severity.

METHODS

In this cross-sectional study, patients with biopsy-proven NAFLD were prospectively included. All patients underwent anthropometric evaluation, blood tests and bioelectrical impedance analysis.

RESULTS

Between 2017 and 2020, 119 patients were included [66.4% male, 56 years (SD 10.7), 62.2% obese, 61.3% with metabolic syndrome]. Sixty of them (50.4%) showed significant fibrosis (≥ F2) in liver biopsy. Age, VF and metabolic syndrome were associated with significant fibrosis (61 years *vs* 52 years, 16.4 *vs*

13.1, 73.3% *vs* 49.2%, respectively; $P < 0.001$ for all). In the multivariate analysis, VF and age were independently associated with significant fibrosis (VF, OR: 1.11, 95%CI: 1.02-1.22, $P = 0.02$; age, OR: 1.08, 95%CI: 1.03-1.12, $P < 0.01$). A model including these variables showed and area under the receiver operating characteristic curve (AUROC) of 0.75, which was not inferior to transient elastography or NAFLD fibrosis score AUROCs. We developed a nomogram including age and VF for assessing significant fibrosis in routine practice.

CONCLUSION

VF is a surrogate marker of liver fibrosis in patients with NAFLD. Bioelectrical impedance analysis is an inexpensive and simple method that can be combined with age to guide patient referral when other resources may be unavailable.

Key Words: Non-alcoholic fatty liver disease; Visceral fat; Liver fibrosis; Bioimpedanciometry; Metabolic syndrome; Obesity

Core Tip: Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD), although obese patients with NAFLD do not always develop significant fibrosis. The distribution of body fat could predict the risk of NAFLD progression. Our study demonstrates that bioimpedanciometry-estimated visceral fat is useful for detecting advanced NAFLD. Our proposed simple method would allow referral to specialized care in a wide variety of resource-limited settings. Future studies will aim at validating this tool in larger prospective cohorts.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a major cause of chronic liver disease in the world, ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), which can lead to significant fibrosis, liver cirrhosis and hepatocellular carcinoma[1,2]. As the hepatic manifestation of metabolic syndrome (MetS), NAFLD is more prevalent within patients with obesity, type 2 diabetes mellitus, dyslipidemia and/or hypertension[3-5]. Particularly, metabolic unhealthy status may have a greater impact on NASH and significant fibrosis than obesity itself[6]. Obese subjects do not always develop NAFLD and NAFLD can occur in non-obese subjects[7]. In this regard, abdominal fat deposition is closely related with MetS[8]. Waist circumference (WC), waist-to-height ratio and waist-to-hip ratio are surrogate markers of abdominal fat, which can rule in MetS[9]. However, the visceral component of abdominal fat is most intimately associated with MetS and adverse outcomes, probably through pro-inflammatory adipokines[8,10,11]. Visceral fat (VF) is a key element in the pathogenesis of NAFLD, independently of insulin resistance and liver steatosis[12-17]. However, VF cannot be captured by the aforementioned indices. Several works have proposed measuring VF as an indirect marker of NAFLD by using different techniques and thresholds[13,15,16,18-23]. None of these studies assessed a possible correlation of VF with liver fibrosis while the prognosis of NAFLD patients is strongly conditioned by fibrosis[24].

Methods for assessing VF and liver fibrosis in NAFLD patients include computed tomography (CT) scan, magnetic resonance imaging and histological analysis, which are impractical in real clinics. Even though transient elastography is simple, non-invasive and reliable for estimating fibrosis in NAFLD, it is not always available[25]. On

and design, analysis and interpretation of data, manuscript preparation, final drafting of the manuscript and study supervision.

Institutional review board statement: The protocol was approved by the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda (PI 05-18, 12/03/2018) and it was conducted according to the 1975 Declaration of Helsinki and the Good Clinical Practice guidelines.

Informed consent statement: Written informed consent was obtained from all patients prior to inclusion.

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the contrary, bioelectrical impedance analysis (BIA) is innocuous and easy to use. In addition, it is operator-independent and less expensive than CT scan and magnetic resonance imaging[3].

Currently, it is unknown if VF may be a reliable measure of NAFLD severity. On the other hand, BIA may have all the features to become a preferred method for VF estimation. Therefore, we aimed at assessing the role of BIA as a non-invasive tool for assessing NAFLD severity. To this end, we compared BIA with liver biopsy, transient elastography and other indirect methods.

MATERIALS AND METHODS

Study design

This is a cross-sectional study prospectively including consecutive biopsy-proven NAFLD adult outpatients in a third-level hospital. Exclusion criteria encompassed any other liver comorbidity, history of bariatric or ileal surgery, liver or kidney transplantation, malignancy or treatment with any drug known to induce liver steatosis or insulin sensitization, such as estrogens, amiodarone, methotrexate and tamoxifen. The protocol was approved by the Ethics Committee of the Hospital Universitario Puerta de Hierro-Majadahonda (PI 05-18, 12/03/2018) and it was conducted according to the 1975 Declaration of Helsinki and the Good Clinical Practice guidelines. Written informed consent was obtained from all patients prior to inclusion.

Data collection

Prior to liver biopsy, all the patients underwent abdominal ultrasound, liver transient elastography (FibroScan® 502 Touch, Echosens, Paris, France) and controlled attenuation parameter (CAP, Echosens, Paris, France), as clinically indicated. M or XL probes were used as needed[26]. CAP (dB/m) was considered only when the associated elastography measurement was valid [median measurement/interquartile range ≥ 0.3 (kPa)]. Liver biopsy was performed as part of the clinical work-up for NAFLD diagnosis. For our study, all the slides were reviewed by an experienced liver pathologist (C.S.) using the NAFLD activity score (NAS)[27]. Significant fibrosis was defined as fibrosis stage ≥ 2.

All the patients underwent a complete anthropometric evaluation, blood tests and BIA after overnight fasting by the same investigator, mostly the same day of the liver biopsy. Height, weight and WC were measured with patients in light clothing, after removing their shoes and emptying their bladders. Total and visceral adipose tissue were measured by BIA (DC430PMA, Tanita, Amsterdam, The Netherlands). A rating between 1 and 12 indicates a healthy level of VF and a rating between 13 and 59 indicates an excessive accumulation of VF. Obesity was defined as a body mass index (BMI) (weight/height^2) of ≥ 30 kg/m^2 and overweight as 25-30 kg/m^2. An increased WC was defined as ≥ 102 cm for men and ≥ 88 cm for women[28]. Insulin resistance was calculated by the homeostatic model assessment[29]. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III definitions when three or more criteria were met[30].

Statistical analysis

Quantitative variables were described as mean ± SD or median and range where appropriate. Categorical variables were described in percentages. For bivariate analysis, quantitative variables were compared using Student´s *t*-test. When normality or equality of variances was not observed, non-parametric tests were used. Categorical variables were compared using Chi-squared and Chi-squared for trend tests, or Fisher's exact test. Correlations between quantitative variables were assessed using Pearson or Spearman rank correlations, as appropriate. To compare variables in more than two groups, Kruskal-Wallis test was used. Multivariable logistic-regression standardized models were constructed by introducing explanatory variables other than transient elastography measurements, with a $P < 0.2$, using a backward elimination method. Diagnostic accuracy was determined by the area under the receiver operating characteristic curve (AUROC) and 95%CI. Sensitivity, specificity, positive predictive value and negative predictive value were calculated for the models. Youden index was used to determine the optimal cut-off value for these. Statistical analyses were performed with STATA software 14 (Stata Corporation, College Station, TX, United States) and $P < 0.05$ was considered statistically significant.

RESULTS

Between September 2017 and February 2020, 390 NAFLD patients were screened for the study, 119 of who were included (Supplementary Figure 1). Patient characteristics are shown in Table 1. The mean age was 56 ± 10.7 years, 66.4% of the patients were male and 95% were overweight or obese. Type 2 diabetes mellitus and MetS were predominant (55.5% and 61.3%, respectively). Accordingly, mean WC and VF were elevated (109.3 ± 14 cm, 14.8 ± 5.3, respectively). Significant fibrosis was present in 60 patients (50.4%) and cirrhosis was found in 18 patients (15.1%).

VF measurements positively correlated with WC, BMI and liver fat measurement by CAP (r = 0.67; r = 0.64 and r = 0.32, respectively; P < 0.001) (Table 2). We assessed possible associations for all these parameters with the several components of NAS in liver histology. None of these parameters was associated with the presence of NASH, excepting CAP (343 dB/m *vs* 319 dB/m; P = 0.018), which positively correlated with the degree of steatosis and overall activity score (Supplementary Table 1). However, VF was the only parameter associated with histological fibrosis stage (r^2 = 0.112; P < 0.01). VF measurements were lowest for those patients with F0-1 in liver biopsy and highest for those patients showing F4, with intermediate levels for those with F2-3 (P < 0.01) (Figure 1A), therefore displaying a linear increase (r^2 = 0.11, P < 0.01). Even though WC and BMI correlated with transient elastography measurements (r = 0.23 and r = 0.25, respectively; P < 0.05), they did not correlate with the gold standard. When focusing on patients with significant fibrosis, VF was the only parameter that was statistically significantly associated (16.4 *vs* 13.1, P < 0.001) (Table 3 and Figure 1B). In addition, these patients were older and showed a higher frequency of MetS than those without significant fibrosis (61 years *vs* 52 years, 73.3% *vs* 49.2%; P < 0.01 for both). In multivariable regression analysis excluding transient elastography, age and VF were the only variables independently associated with histological significant fibrosis (VF, OR: 1.11, 95%CI: 1.02-1.22, P = 0.021; age, OR: 1.08, 95%CI: 1.03-1.12, P = 0.001). A model including these variables showed an AUROC of 0.75 (95%CI: 0.66-0.84), with a sensitivity of 70%, a specificity of 67.8%, as well as positive and negative predictive values of 68.9% and 69%, respectively (Figure 2A). When comparing our model AUROC with the AUROCs for transient elastography and NAFLD fibrosis score, we found no significant differences among them (0.82 and 0.78 *vs* 0.75, P = 0.099 and 0.345, respectively, Figure 2B). Based on our results, we built a simple nomogram including age and VF for the prediction of significant fibrosis in routine practice (Figure 3). A nomogram probability of 50% was the cut-off that best identified patients with significant fibrosis, showing an AUROC of 0.7 (sensitivity, 67%; specificity, 73%).

DISCUSSION

NAFLD is one of the most prevalent chronic liver diseases worldwide, which can progress to steatohepatitis, fibrosis, cirrhosis and rarely hepatocellular carcinoma without cirrhosis[1,2]. NAFLD is associated with diet, MetS, obesity and adverse cardiovascular events[3,31-33]. Even though fat deposition is a key pathophysiologic element, the distribution of fat deposits must be underscored. Large population studies have shown markers of increased VF to be independent predictors of cardiovascular and overall mortality[10,34]. In addition, central body fat distribution has been associated with the development of NAFLD[22]. CT scan is the most effective method to differentiate subcutaneous from visceral obesity. However, it has many limitations such as price, radiation and availability[35]. Therefore, identifying simple anthropometric markers of VF in clinical practice may be extremely useful to assess metabolic status. In our study including 119 patients with biopsy-proven NAFLD, we investigate the value of VF estimated by BIA as a non-invasive marker of NAFLD severity

A number of studies show that simple anthropometric indices related with abdominal obesity, such as BMI and WC, are able to predict the presence of NAFLD[19,36,37]. In our study including patients already diagnosed with NAFLD, all WC, BMI and CAP showed increased values, and VF measurements positively correlated with them. Yet, when assessing liver histology, which is the gold standard, associations with NAS features were overall poor. Here, VF was the only parameter associated with fibrosis stage, even though VF was not associated with the degree of steatosis. Liver fibrosis is the strongest histological feature influencing outcomes in the long term and late stages of NAFLD may have waning degrees of steatosis[24]. All these

Table 1 Patient characteristics

	***n* = 119**
Age (yr), mean ± SD	56 ± 10.7
18-30, *n* (%)	2 (1.7)
31-50, *n* (%)	30 (25.2)
51-70, *n* (%)	79 (66.4)
> 70, *n* (%)	8 (6.7)
Sex (male), *n* (%)	79 (66.4)
Metabolic syndrome, *n* (%)	73 (61.3)
Increased waist circumference, *n* (%)	91 (76.5)
Hypertension, *n* (%)	63 (52.9)
Type 2 diabetes mellitus, *n* (%)	66 (55.5)
Increased Triglyceride levels, *n* (%)	61 (51.3)
Low HDL-cholesterol levels, *n* (%)	53 (44.5)
HOMA-IR, mean ± SD	7.5 ± 13.1
BMI (kg/m^2), mean ± SD	32.5 ± 5.2
Obese, *n* (%)	74 (62.2)
Normal BMI, *n* (%)	6 (5)
Waist circumference (cm), mean ± SD	109.3 ± 14
Visceral fat, mean ± SD[1]	14.8 ± 5.3
Visceral fat ≥ 13, *n* (%)[2]	77 (63.6)
CAP (dB/m), mean ± SD	330.9 ± 50.4
Liver elastography (Kpa), mean ± SD	11.7 ± 8
Histological fibrosis stage, *n* (%)	
F0-1	59 (49.6)
F2	18 (15.1)
F3	24 (20.2)
F4	18 (15.1)

[1]Measured by bioimpendanciometry analysis.
[2]Upper threshold of normality provided by the manufacturer. HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; HDL-cholesterol: High-density lipoprotein cholesterol; CAP: Controlled attenuation parameter.

findings concur with previous studies suggesting that body composition is capital to assess NAFLD and metabolic risk factors as a whole. Although BMI is a robust marker for obesity, it does not provide any information about the anatomic distribution of fat[21,23,38]. Similarly, WC is a well-known and simple parameter included in the definition of MetS, but it may fail to distinguish visceral from subcutaneous fat and is influenced by patient height[19,39]. In addition to depending on weight gain, visceral adipose tissue also accumulates more rapidly with increasing age, which allows time for disease progression as well[40]. Thus, an increased prevalence and severity of NAFLD is expected for older ages[41]. In our study, those patients with significant fibrosis were older than F0-1 patients.

Our hypothesis was supported by the multivariable model, which confirmed VF and age as the only independent risk factors for significant liver fibrosis measured by liver biopsy. The fact that MetS and its components lost their significance in the multivariable analysis, points again to VF as an active mediator, rather than just a marker of MetS. Although obesity is a risk factor for NAFLD, insulin resistance and cardiovascular diseases, not every obese patient is insulin resistant or at high risk for liver and cardiovascular diseases. In fact, VF seems to influence NAFLD genesis

Table 2 Correlations of visceral fat with anthropometric parameters, liver fat and liver fibrosis

	HOMA-IR	BMI (kg/m²)	WC (cm)	Hepatic fat (CAP) (dB/m)	Liver elastography (kPa)	Histological fibrosis stage
Visceral fat	0.16	0.64[b]	0.67[b]	0.32[b]	0.33[b]	0.112[b]
Hepatic fat (CAP) (dB/m)	0.001	0.45[b]	0.38[b]		0.20	0.002
WC (cm)	0.24[a]	0.81[b]		0.38[b]	0.23[a]	0.009
BMI (kg/m^2)	0.21[a]		0.81[b]	0.45[b]	0.25[b]	0.003

The values correspond with *r* correlation coefficient or r^2 coefficient for histological fibrosis stage.
[a]$P < 0.05$.
[b]$P < 0.01$. HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; CAP: Controlled attenuation parameter; WC: Waist circumference; BMI: Body mass index.

Table 3 Patient characteristics according to significant liver fibrosis (F ≥ 2)

	F0-1 (*n* = 59)	F ≥ 2 (*n* = 60)	*P*
Age (yr), mean ± SD	52 ± 10.5	61 ± 9.4	< 0.001
Sex (male), *n* (%)	34 (57.6)	45 (75)	0.054
Metabolic syndrome, *n* (%)	29 (49.2)	44 (73.3)	0.007
Number metabolic risk factors, *n* (%)			0.002[1]
0	4 (6.8)	2 (3.3)	
1	11 (18.6)	5 (8.3)	
2	15 (25.4)	9 (15)	
3	17 (28.8)	17 (28.3)	
4	9 (15.3)	18 (30)	
5	3 (5.1)	9 (15)	
Type 2 diabetes mellitus, *n* (%)	24 (40.7)	42 (67.7)	0.003
BMI (kg/m^2), mean ± SD	32.5 ± 5.6	32.6 ± 4.8	0.966
Obese, *n* (%)	36 (61)	38 (63.3)	0.794
Normal BMI, *n* (%)	5 (8.5)	1 (1.7)	0.090
Waist circumference (cm), mean ± SD	108.6 ± 14.9	109.8 ± 13.3	0.663
Visceral fat, mean ± SD	13.1 ± 5	16.4 ± 5.1	< 0.001
Visceral fat ≥ 13, *n* (%)	29 (49.2)	48 (77.4)	0.001
CAP (dB/m), mean ± SD	330.5 ± 58	331.2 ± 44	0.946
Liver elastography (kPa), mean ± SD	8.8 ± 5.6	14.5 ± 8.8	< 0.001

Significant *P* values are shown in bold font.
[1]Chi-squared for trend test.CAP: Controlled attenuation parameter; BMI: Body mass index.

independently of insulin resistance[12,42,43]. The precise mechanisms by which VF exerts its damaging consequences remain controversial, but it has been suggested that visceral adipose tissue may be infiltrated with inflammatory cells and release inflammatory cytokines which travel through the portal vein to the liver, in addition to free fatty acids[10,31,37,43-46]. Visceral obesity is probably the most important target for future interventions in MetS and NAFLD.

Because NAFLD has become a major Public Health concern, it is essential to find screening tools to identify patients at risk of NASH or significant fibrosis for specialist referral, before they present with important complications[47]. Accurate assessment of liver fibrosis in primary care and other settings is limited by a reliance on blood tests,

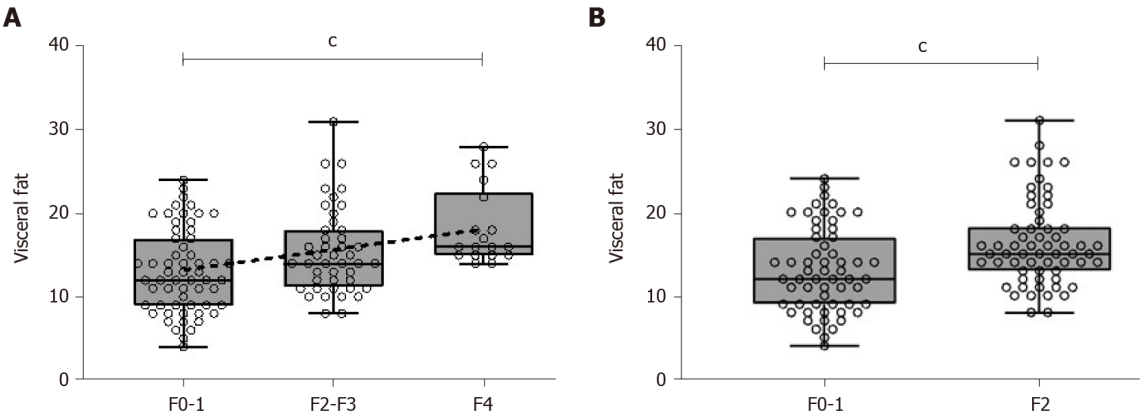

Figure 1 Visceral fat measurement by bioimpedanciometry, according to histological fibrosis stage. A: Visceral fat measurements increased along with fibrosis stage assessed by histological analysis (F0-1, 12; F2-3, 14; F4, 16; Kruskal-Wallis $^{c}P < 0.001$). A line can be fit by linear regression, showing linear association ($r^2 = 0.11$, $^{c}P < 0.001$); B: Visceral fat measurements were greater for those patients with significant fibrosis (16.3 *vs* 13.1, $^{c}P < 0.001$).

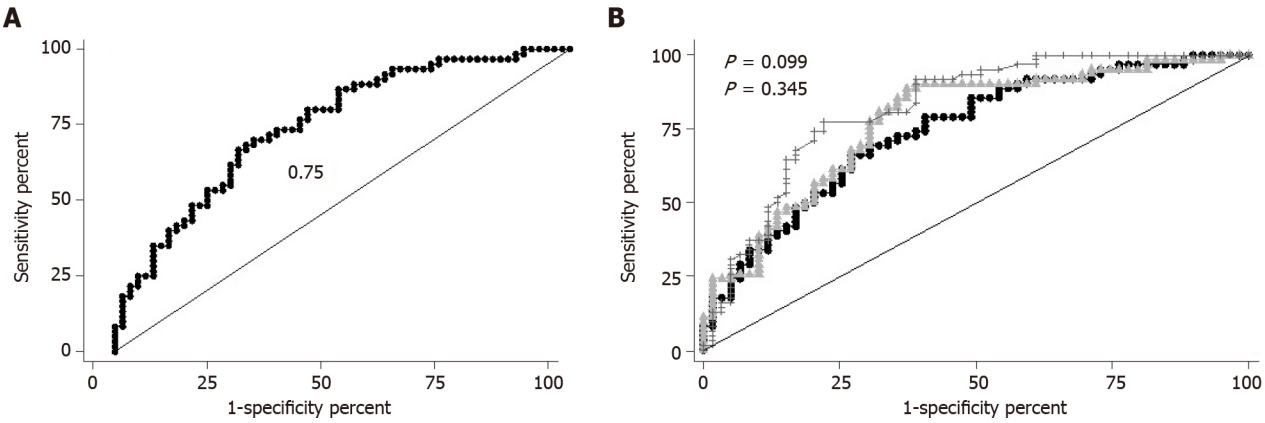

Figure 2 Area under the receiver operating characteristic curve. A: Receiver operating characteristic (ROC) curve for non-invasive diagnosis of significant liver fibrosis by a model including age and visceral fat; B: Comparison of the areas under ROC curves for a model using age and visceral fat versus liver elastography measurement, to predict significant liver fibrosis. Circles denote our model, triangles indicate non-alcoholic fatty liver disease fibrosis score and crosses denote liver elastography.

which correlate poorly with liver fibrosis, as well as a restricted access to more discriminatory tests such as transient elastography[48]. Our model was built excluding transient elastography and is able to identify advanced liver fibrosis with an AUROC of 0.75 by using BIA measurement and age. Of note, this AUROC was not significantly different from that of transient elastography or NAFLD fibrosis score. To simplify the model and enhance its utility, we built a nomogram, which provides visual means of calculating the probability for a given patient to have significant fibrosis. Potentially, this would allow initial assessment in a wide variety of clinical and resource-availability settings, since no blood draw would be needed and bioimpedanciometry devices are less costly than other equipment, with no or minimal training.

Certainly, our study has a number of limitations. The cross-sectional design does not allow for causation and prognosis assessment. On the other hand, sample size is relatively limited, although biopsies were available. The population studied was Caucasian while other populations may be more or less prone to abdominal obesity and VF accumulation, thus needing specific calibration. The absence of a control group may be controversial as a limitation since liver biopsy is indicated only for those NAFLD patients with suspicion of significant fibrosis. Finally, VF was not evaluated by CT scan but BIA has been shown to have a high correlation with CT scan[49]. Additionally, BIA is easy to operate, inexpensive, highly reproducible, and radiation free.

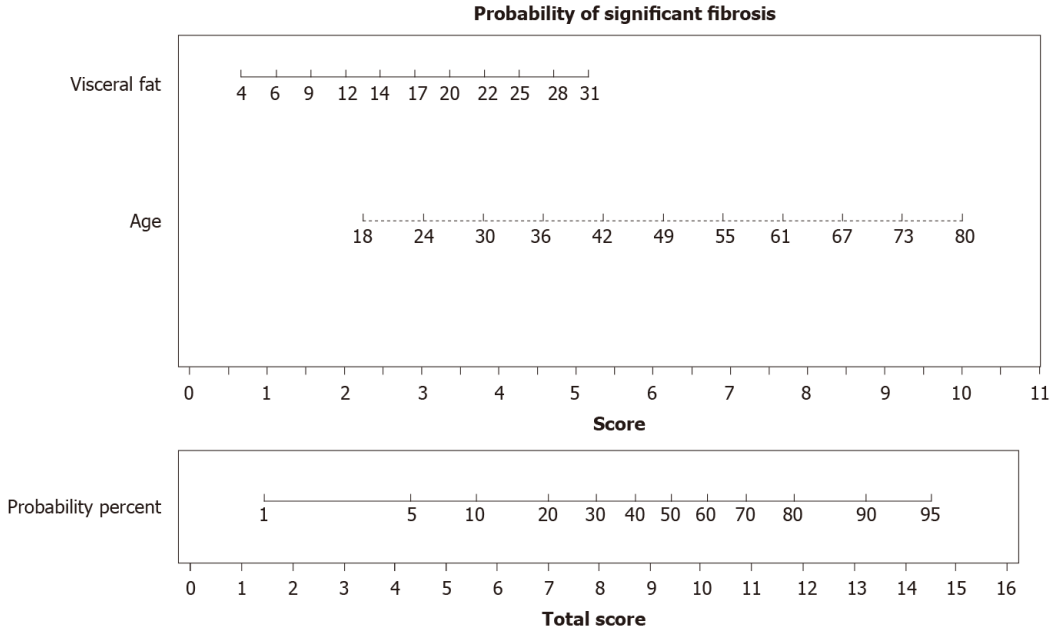

Figure 3 Nomogram for assessing the probability of significant liver fibrosis in a clinically useful manner. With the variables resulting from the multivariate regression model, we built an easy-to-use visual tool. In an individual patient, visceral fat levels and age correspond to a score. Combining these scores gives a total score that can be converted to a probability of that patient having significant fibrosis in liver biopsy. For example, a patient with a visceral fat level of 12 (score 2) and with 55 years old (score 7) would have a total score of 9 and a corresponding probability of histological significant fibrosis of 43%.

CONCLUSION

In conclusion, our study demonstrates that BIA-estimated visceral adipose tissue is useful for detecting advanced NAFLD, independently of MetS. Our proposed simple method would allow referral to specialized care in a wide variety of resource-limited settings. Future studies will aim at validating this tool in larger prospective cohorts.

ARTICLE HIGHLIGHTS

Research background

Obesity is a risk factor for non-alcoholic fatty liver disease (NAFLD), although obese patients with NAFLD do not always develop significant fibrosis.

Research motivation

The distribution of body fat could predict the risk of NAFLD progression.

Research objectives

Our aim was to investigate the role of bioelectrical impedance-estimated visceral fat (VF) in assessing NAFLD severity.

Research methods

It is a cross-sectional study. In which patients with biopsy-proven NAFLD were prospectively included.

Research results

In the multivariate analysis, VF and age were independently associated with significant fibrosis (VF, OR: 1.11, 95%CI: 1.02-1.22, $P = 0.02$; age, OR: 1.08, 95%CI: 1.03-1.12, $P < 0.01$). A model including these variables showed and area under the receiver operating characteristic curve (AUROC) of 0.75, which was not inferior to transient elastography or NAFLD fibrosis score AUROCs. We developed a nomogram including age and VF for assessing significant fibrosis in routine practice.

Research conclusions

Bioelectrical impedance analysis is an inexpensive and simple method that can be

combined with age to guide patient referral when other resources may be unavailable.

Research perspectives

Future studies will aim at validating this tool in larger prospective cohorts.

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ORIGINAL ARTICLE

Prospective Study

Accuracy of carbon dioxide insufflation for endoscopic retrograde cholangiopancreatography using double-balloon endoscopy

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Abstract

BACKGROUND

Retrograde cholangiopancreatography using double-balloon endoscopic retrograde cholangiography (DBERC) is a valuable technique to treat biliary stone and jejunobiliary anastomotic stenosis in patients with altered gastrointestinal anatomy. The accurate selection of the route at the anastomosis branch is one of the most important factors in reaching the target in a timely manner.

AIM

To determine the accuracy of carbon dioxide insufflation enterography (CDE) at the branch for selecting the correct route during DBERC.

METHODS

We enrolled 52 consecutive patients scheduled for DBERC at our institution from June 2015 to November 2017. Route selection *via* two methods (visual observation and CDE) was performed in each patient. We determined the correct rate of route selection using CDE.

RESULTS

Thirty-three patients had a jejunojejunal anastomosis and 19 patients had a gastrojejunal anastomosis. The therapeutic target region was reached in 50 patients. The mean procedure times from the teeth to the target (total insertion time), from the teeth to the branch, and from the branch to the target, and the

mean total examination time were 15.2, 5.0, 8.2, and 60.3 min, respectively. The rate of correct route selection using visual observation and CDE were 36/52 (69.2%) and 48/52 (92.3%), respectively (P = 0.002). The rate of correct route selection using CDE in patients with a jejunojejunal anastomosis was 29/33 (87.8%), and the rate in patients with a gastrojejunal anastomosis was 19/19 (100%).

CONCLUSION

CDE is helpful in selecting the route at the branch in the anastomosis for more timely access to the target in patients with altered gastrointestinal anatomy undergoing DBERC.

Key Words: Retrograde cholangiopancreatography; Double-balloon endoscopy; Carbon dioxide insufflation; Anastomosis; Accuracy; Prospective study

Core Tip: Carbon dioxide insufflation enterography (CDE) may be useful for selecting route at branch in patients with altered gastrointestinal anatomy in double-balloon endoscopy. The endoscopist inserts the tip of the endoscope into one of the two tracts at the branch and insufflate carbon dioxide with an obstruction created by the inflation of an endoscopic balloon. Fluoroscopy is used to determine the direction of carbon dioxide flow. This prospective study evaluated the usefulness of CDE during double-balloon endoscopic retrograde cholangiography in patients with altered gastrointestinal anatomy by prospectively investigating the accuracy of route selection using CDE at the branch of the anastomosis. The mean procedure times from the teeth to the target (total insertion time), from the teeth to the branch, and from the branch to the target, and the mean total examination time were 15.2, 5.0, 8.2, and 60.3 min, respectively. The rate of correct route selection using CDE in patients with a jejunojejunal anastomosis was 29/33 (87.8%), and the rate in patients with a gastrojejunal anastomosis was 19/19 (100%).

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INTRODUCTION

Previously, biliary stones in patients with altered gastrointestinal (GI) anatomy were treated *via* a percutaneous trans-hepatic approach, however, this approach is sometimes challenging and may require a long therapeutic period in order to reach the stones[1]. In 2008, retrograde cholangiopancreatography (ERCP) using a short type of double-balloon endoscopy (DBE) called double-balloon endoscopic retrograde cholangiography (DBERC) was reported by Matsushita *et al*[2] and biliary stones were able to be treated during a single endoscopic procedure. Since then, improvement in the endoscopic equipment was made and access to the blind end and subsequent treatment became easier[3-5]. A multicenter prospective study demonstrated that the mean time required to reach the blind end was 22.4 min and the therapeutic success rate was 97.9%[6].

However, in patients with a longer blind loop, severe adhesions, or a past history of hepatectomy, reaching the blind end for biliary drainage is still challenging[7]. The proper route at the bifurcation of the jejunojejunal anastomosis, as in Roux-en-Y reconstructions, or the gastrojejunal anastomosis, as in Billroth II reconstructions, is sometime difficult to be identified. When the incorrect route is initially selected, the examination and treatment time becomes much longer, as the endoscopist must return

the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethics Committee of Nagoya University Hospital.

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to the anastomosis in order to choose the correct path. It has been reported that the type of reconstruction may also affect the time required to reach the blind end as well as the ERCP success rate[7]. The correct selection of the route at the anastomosis can lead to a decreased insertion time. Yano *et al*[8] reported that the direction in which sprayed indigo carmine solution flowed due to peristalsis indicates the afferent loop of a Roux-en-Y anastomosis, and that the alternate route should be selected. The correct route was selected in 80% of the patients in their study Fukuba *et al*[9] used carbon dioxide insufflation enterography (CDE) to confirm the correct route. In this method, the endoscopist inserts the tip of the endoscope into one of the two tracts at the branch and insufflate carbon dioxide (CO_2) with an obstruction created by the inflation of an endoscopic balloon. Fluoroscopy is used to determine the direction of CO_2 flow. However, their study had retrospective fashion and included small number of cases. The aim of this prospective study was to evaluate the usefulness of CDE during DBERC in patients with altered GI anatomy by prospectively investigating the accuracy of route selection using CDE at the branch of the anastomosis.

MATERIALS AND METHODS

Inclusion criteria was the consecutive patients who were scheduled to undergo DBERC from June 2015 to November 2017 at our institution. Exclusion criteria were the Patients with a poor general condition and emergent cases. Informed consent was obtained from each patient prior to his or her involvement in this study. A short-type double-balloon endoscope consisting of an EI-530B endoscope (effective length: 1.550 mm, working channel: 2.8 mm, FUJIFILM, Tokyo, Japan) and a TS13101 overtube (FUJIFILM, Tokyo, Japan) were used for each examination. CO_2 insufflation was performed in all procedures[7]. DBE insertion was performed by experienced endoscopists (Nakamura M and Yamamura T) and their assistants who held the overtube. Patients were placed under conscious sedation with diazepam (0.02 mg/kg) and pentazocine (7.5 mg) with left lateral decubitus position. Analgesics were additionally and repeatedly used for 7.5 mg as necessary, based on the consciousness and pain of the patient during the procedure. Dexmedetomidine (loaded at 6 μg/kg/h for 10 min and maintained at 0.4 μg/kg/h) was administered concomitantly in patients in whom sufficient sedation was not achieved using diazepam and pentazocine[10]. General anesthesia was used in child and adolescent patients. The pancreatobiliary team (Kawashima H, Ohno E, and Ishikawa T) performed the ERCPs. After reaching the target site, the body position was changed to dorsal or abdominal to perform ERCP. We performed both visual observation and CDE route selection in each patient from the jejunojejunal or gastrojejunal anastomosis to the target of the jejunobiliary anastomosis or the original Vater papilla and compared the accuracies of both route selection methods. When the endoscope reached the anastomosis during DBERC, the main endoscopist selected one of two lumens as the visual observation (Figure 1, Evaluation 1). The lumen on the left was initially selected, and the lumen that made a sharp angle if side selection was not available (Figure 1). The endoscopist then advanced the endoscope by one stroke and inflated the balloon on the tip of the endoscope to avoid a backflow of carbon dioxide, as previously reported[9]. Carbon dioxide was added up to ten seconds under fluoroscopy until the endoscopist could estimate whether the selected route lead to the target (Figure 1, Evaluation 2). When carbon dioxide could be seen in the patient's upper, right abdomen (Video 1), the selected route was considered to be correct. Then the endoscope was advanced and ERCP was performed. When CDE enhanced the pelvis (Video 2), the selected route was considered incorrect, and the endoscopist pulled back to the anastomosis and continued the procedure using the other route. In patients with the Billroth II reconstruction and a Braun anastomosis leading to the original Vater papilla, we initially selected the left route at Braun anastomosis. If the left route did not lead to the target, the center route was chosen. The definitions of correct and incorrect routes are shown in Figure 1. The primary endpoint was the correct rate of CDE for selection of the route to the target. Secondary endpoints were the comparison of correct rate between visual observation and CDE around the anastomosis and examination times. Regarding the relation between patient's burden, the factors associated with the dose of sedation and analgesic were analyzed using logistic regression analysis. The study was registered in the University Hospital Medical Information Network and in a clinical trial registry (UMIN000018357), and was approved by ethic committee at Nagoya University Hospital (registration No. 2015-0228).

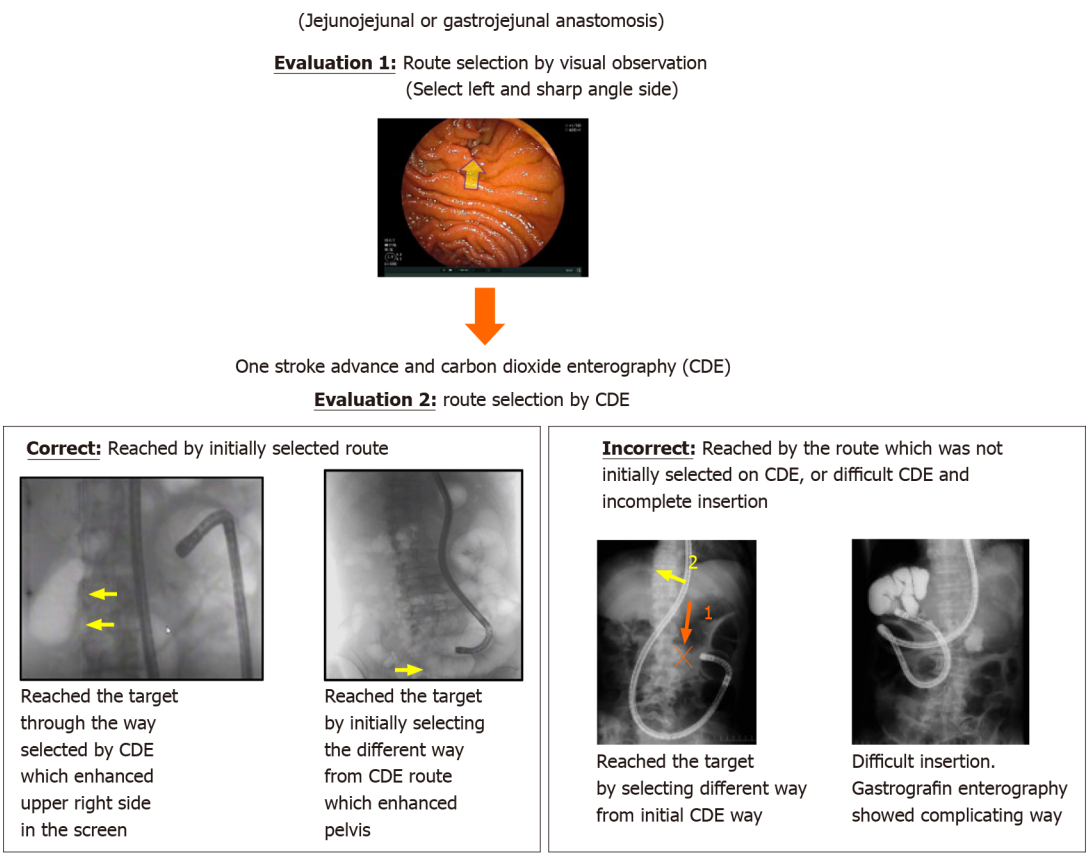

Figure 1 Jejunojejunal or gastrojejunal anastomosis. Evaluation 1 for route selection by visual observation and Evaluation 2 for route selection by carbon dioxide insufflation enterography. CDE: Carbon dioxide insufflation enterography.

Statistical analysis

SPSS version 26 for Windows (SPSS Inc., Chicago, IL, United States) was used to analyze the data in this study. The McNemar test was used to compare the rates of correct route selection between the two methods. The patients' clinical results were compared using the Kruskal-Wallis test and the Mann-Whitney *U* test. Multiple logistic regression using the stepwise selection method was used to determine the effects of the dosages of sedation and analgesics in each patient. Statistical significance was set at $P < 0.05$.

RESULTS

We were able to reach the target in 50/52 patients (Table 1). The remaining two patients had severe adhesions that prevented the endoscopist from reaching the target. Thirty-three patients were included in the jejunojejunal anastomosis group (due to Roux-en-Y reconstruction and liver transplantation) and the gastrojejunal anastomosis group (due to Billroth II reconstruction and pancreatoduodenectomy) included 19 patients, six of whom had Braun anastomoses. The most frequent indication for ERCP was the treatment of biliary stones. Time from the branch to the target was likely to be longer than that from the incisor tooth to the branch.

CDE was more accurate than visual observation in both groups (Table 2). The rate of correct route selection using CDE was higher in the gastrojejunal anastomosis group than in the jejunojejunal anastomosis group. Incorrect CDE in the patients with Braun anastomoses was occurred in 2/6 (33.3%) and higher than those without Braun anastomosis. Table 3 shows the patients' clinical results for each group. Time from the branch to the target and total examination time were longer in patients with incorrect selection by CDE ($n = 4$). Of these four patients, the target was reached in two patients, one of who had too sharp angle at the branch to occlude the lumen and the other in whom the balloon attached on tip of endoscope was prolapsed to the anastomosis during CDE. Pancreatobiliary interventions were performed in 38 patients. To evaluate the relation between patient's burden and DBERC, the factors associated with the dose

Table 1 Clinical results of ouble-balloon endoscopic retrograde cholangiography

Clinical results	*n* = 52
Male:female	32:20
Age (years old, mean ± SD)	62.5 ± 17.6
Types of branch	
Jejuno-jejunal anastomosis	33
(Roux-en Y reconstruction, liver transplantation)	
Gastro-jejunal anastomosis	19
(Billroth II, panceatoduodenectomy)	
Indications	
Cholangitis	20
Biliary stone	13
Jaundice	7
Suspected tumor	5
Hyperamilasemia	3
Stenosis at anastomosis	2
Foreign body in the bile duct	1
Abdominal pain	1
Reached target, *n* (%)	50/52 (96.1)
Exmination time	
Insertion time, minutes (range)	15.2 (5.0-90.7)
Teeth-branch, minutes (range)	5.0 (1.3-25.5)
Branch-target, minutes (range)	8.2 (3.3-72.4)
Total examination, minutes (range)	60.3 (20.6-165.6)
Sedations	
Midazolam, *n* [median (range)]	49 [10 mg (2.5-40))
Pentazocine, *n* [median (range)]	49 [15 mg (7.5-45)]
Dexmedetomidine, *n* (dose)	3 (137, 103, 80 μg)
General anethtesia, *n*	3
Interventions	
EPBD with biliary stone extraction	12
Biliary stone extraction	10
Balloon dilation of the anastomosis stricture	7
ENBD	4
Metallic stent placement	3
Endoscopic sphincterotomy	1
Extraction of foreign body	1

EPBD: Endoscopic papillary balloon dilatation; ENBD: Endoscopic nasobiliary drainage.

of sedation and analgesic were analyzed using logistic regression analysis. We found no significant relationships between patient factors and the required dose of midazolam, though a higher analgesic dose was significantly associated with an age < 65 years (Tables 4 and 5). There were not any adverse events related to DBE insertion in this study.

Table 2 Correct rate for route selection

Total	
Correct on visual (%)	36/52 (69.2)
Correct on CDE (%)	48/52 (92.3)[1]
Jejuno-jejunal anastomosis	
Correct on visual (%)	20/33 (60.6)
Correct on CDE (%)	29/33 (87.8)[2]
Billroth II, Pancreatoduodenectomy	
Correct on visual (%)	16/19 (82.3)
Correct on CDE (%)	19/19 (100)[3]

Visual *vs* carbon dioxide insufflation enterography,
[1]*P* = 0.002,
[2]*P* = 0.012,
[3]*P* = 0.250. CDE: Carbon dioxide insufflation enterography.

Table 3 Comparison of clinical results according to evaluation groups

Group	A	B	C	D	*P* value[1]
Evaluation 1: Visual observation	Correct	Correct	Incorrect	Incorrect	
Evaluation 2: CDE	Correct	Incorrect	Correct	Incorrect	
n	35	1	13	3	
Age	59.4 (21.4)	76	56.3 (23.3)	67.3 (6.0)	0.568
Male	20	0	9	3	
Insertion time [minutes, mean (SD)]	16.9 (14.9)[2]	90	25.5 (22.3)	68.3 (45.0)	0.008
Incisor tooth to branch [minutes, mean (SD)]	4.8 (4.1)	20	8.5 (7.7)	16.6 (12.3)	0.042
Branch- target [minutes, mean (SD)]	12.0 (13.2)[3]	70	17.0 (18.1)	52.6 (32.5)	0.014
Total examination time [minutes, mean (SD)]	62.9 (26.6)	165	73.0 (33.9)	82.0 (33)	0.229
Treatment, *n*	26	0	7	0	
Baseline CRP (mg/dL, mean (SD))	2.2 (4.0)	3.5	2.6 (2.7)	0.10 (0.11)	
Baseline serum amylase [IU/L, mean (SD)]	175 (220)	793	138 (100)	118 (58)	

[1]Krustal-Wallis test.
[2]*P* = 0.042 (*vs* Group D).
[3]*P* = 0.047 (*vs* Group D), Mann-Whitney *U* test, Bonferroni correction. CRP: C-reactive protein; CDE: Carbon dioxide insufflation enterography.

DISCUSSION

This was the first prospective study to evaluate the results of CDE for selecting the route to the target during DBERC. These results indicated that CDE accurately selected the correct route at the anastomosis in patients with GI reconstruction who underwent DBERC. The mean total insertion time in this study was 15 min, which was shorter than that in the previous report[6]. When CDE accurately selected the route, the total insertion time was shorter. When visual observation is used to select a route, its accuracy cannot be determined until the target is reached. The use of CDE allows endoscopists to estimate the direction and distance of the target prior to reaching it, which results in a decrease in the total insertion time. The CDE method takes approximately 30 s to complete, including 10 s of CO_2 insufflation. However, when CDE leads the endoscopist to choose the incorrect route, a longer total insertion time results. This emphasizes the importance of the accuracy of CDE.

When a balloon is used to occlude the lumen, insufflated CO_2 can only go forward.

Table 4 Univariate analysis influencing factors for dose of sedation

Factors	Univariate analysis			
	P value	Odds ratio	95%CI	
			Lower limit	Upper limit
Age (less than 65 yr)	0.241	0.500	0.157	1.594
Gender	0.556	0.708	0.224	2.240
Correct visual selection	0.700	1.286	0.358	4.617
Correct CDE	0.770	0.655	0.039	11.119
Intervention	0.466	0.643	0.196	2.108
Insertion time (more than 22 min.)	0.895	0.917	0.251	3.350
Total examination time (more than 80 min.)	0.797	1.179	0.377	4.125
CRP level normal	0.805	1.167	0.344	3.956
Serum amylase level normal	0.432	0.583	0.152	2.240
Billroth II and PD	0.721	0.808	0.250	2.612
Previous surgery more than 2 times	0.270	0.467	0.120	1.810

CDE: Carbon dioxide insufflation enterography; CRP: C-reactive protein.

Table 5 Univariate and multivariate analyses influencing factors for dose of pentazocine

Factors	Univariate analysis				Multivariate analysis			
	P value	Odds ratio	95%CI		*P* value	Odds ratio	95%CI	
			Lower limit	Upper limit			Lower limit	Upper limit
Age (less than 65 yr)	0.025	12.429	1.362	113.410	0.033	11.338	1.232	105.219
Gender	0.868	0.872	0.173	4.392				
Correct visual selection	0.999	-	-	-				
Correct CDE	0.999	-	-	-				
Intervention	0.744	1.339	0.231	7.751				
Insertion time (more than 22 min)	0.283	2.475	0.473	12.961				
Total examination time (more than 80 min)	0.353	2.182	0.421	11.318				
CRP level normal	0.834	1.207	0.208	7.012				
Serum amylase level normal	0.867	1.164	0.197	6.891				
Billroth II and PD	0.582	0.612	0.106	3.521				
Previous surgery more than 2 times	0.166	0.305	0.057	1.639	0.313	0.389	0.062	2.431

CDE: Carbon dioxide insufflation enterography; CRP: C-reactive protein.

However, CO_2 can sometimes flow back to the main route to the cecum *via* the small space between the lumen and balloon, in which situation, it is difficult to assess the routes as CO_2 is observed in all areas of the abdomen. CDE should be performed as soon as the balloon is inflated, when there is no space between the lumen and the balloon. In contrast, visual observation of the jejunojejunal branch was accurate in 60% of patients, which is comparable to the 50% that would be predicted based on having two, equal choices. The left side lumen often had a sharp angle at the branch and the endoscopist chose that way; however, it was not always correct. We believe that it was easy to rotate the anastomosis and the position was changeable by several factors, namely air insufflation volume, insertion technique, and bowel movement. The accuracy of the visual observation method was slightly higher in patients with

gastrojejunal anastomoses, which are unlikely to be influenced by these factors.

Yane *et al*[11] reported that a pancreatic indication, the first ERCP attempt, and no transparent hood were statistically significant factors affecting procedural failure for short-type single-balloon enteroscope-assisted ERCP. Other insertion-related items besides transparent hood were not investigated. However, the procedural failure is also related to the procedure time, which can affect adverse events such as aspiration pneumonia and acute pancreatitis[12,13]. DBERC is a sequential procedure involving an insertion technique and biliary intervention. Adhesions and other factors can result in a challenging insertion of the endoscope into the GI tract. When insertion requires more than 60 min, a delicate technique should be used for subsequent biliary interventions. Based on our study, incorrect CDE may lead to an insertion requiring more than 60 min (Table 3). In patients < 65 years old, longer insertion times may lead to abdominal pain (Table 5). Therefore, accurate CDE is important to reduce the patient's burden and improve safety.

In patients with reconstructed GI tracts, success of DBERC is highly dependent on the exact anatomy. The DBERC endoscope insertion and procedural success rates in patients with stenosis of the anastomosis site after liver transplantation have been reported as 68%-85% and 78%-88.2%, respectively, and are lower than the success rates in patients who underwent other GI reconstruction procedures[14-17]. This may be due to the fact that endoscope insertion and therapeutic procedures are more difficult due to changes in hepatic volume and afferent loop length after such surgery[7]. In patients who have undergone a hepatectomy, the selection of the correct route at the hepaticojejunostomy anastomosis is important to access the target site in a timely manner.

DBERC has a learning curve. The time required to complete the DBERC procedure in this study, especially the time required to reach the blind end, is less than that in previous reports[18,19]. This indicates that endoscopists who have experience maneuvering the DBERC may have shorter examination times. However, the procedure duration time still had a wide range. Some difficult cases inevitably require a long duration to complete the procedures. It is challenging to perform procedures within the expected duration, and this problem may be overcome by the improvement of endoscopes and devices[20].

This study had several limitations. First, it includes a small sample size in which both methods were used in the same patient. The result and performance of the second evaluation method depended on the first evaluation. A randomized, comparative study between CDE and visual observation for the proper route selection is necessary.

CONCLUSION

In conclusion, CDE is able to accurately select the route at the anastomosis in patients with GI reconstruction who are undergoing DBERC.

ARTICLE HIGHLIGHTS

Research background

Double-balloon endoscopic retrograde cholangiography (DBERC) has been widely used for pancreatobiliary diseases after reconstruction in gastrointestinal surgery, but sometimes it is complicating.

Research motivation

The accurate selection of the route at the anastomosis branch is one of the most important factors for the success of DBERC. We used carbon dioxide insufflation enterography (CDE) for selecting the route.

Research objectives

The aim of this study was to determine the accuracy of CDE at the branch for selecting the correct route during DBERC.

Research methods

Route selection *via* two methods (visual observation and CDE) was performed in each patient in DBERC. We determined the correct rate of route selection using CDE. The

primary endpoint was the correct rate of CDE for selection of the route to the target. Secondary endpoints were the comparison of correct rate between visual observation and CDE around the anastomosis and examination times.

Research results

We enrolled 52 consecutive patients scheduled for DBERC at our institution from June 2015 to November 2017. We were able to reach the target in 50/52 patients. The rate of correct route selection using visual observation and CDE were 36/52 (69.2%) and 48/52 (92.3%), respectively (P = 0.002). The rate of correct route selection using CDE in patients with a jejunojejunal anastomosis was 29/33 (87.8%), and the rate in patients with a gastrojejunal anastomosis was 19/19 (100%).

Research conclusions

CDE was able to accurately select the route at the anastomosis in patients with gastrointestinal reconstruction who are undergoing DBERC.

Research perspectives

Using CDE, DBERC will be performed safely and easily for patients who underwent any gastrointestinal reconstruction. A randomized, comparative study between CDE and visual observation for the proper route selection is necessary.

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SYSTEMATIC REVIEWS

Prognostic role of artificial intelligence among patients with hepatocellular cancer: A systematic review

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Abstract

BACKGROUND

Prediction of survival after the treatment of hepatocellular carcinoma (HCC) has been widely investigated, yet remains inadequate. The application of artificial intelligence (AI) is emerging as a valid adjunct to traditional statistics due to the ability to process vast amounts of data and find hidden interconnections between variables. AI and deep learning are increasingly employed in several topics of liver cancer research, including diagnosis, pathology, and prognosis.

AIM

To assess the role of AI in the prediction of survival following HCC treatment.

METHODS

A web-based literature search was performed according to the Preferred Reporting Items for Systemic Reviews and Meta-Analysis guidelines using the keywords "artificial intelligence", "deep learning" and "hepatocellular carcinoma" (and synonyms). The specific research question was formulated following the patient (patients with HCC), intervention (evaluation of HCC treatment using AI), comparison (evaluation without using AI), and outcome (patient death and/or tumor recurrence) structure. English language articles were retrieved, screened, and reviewed by the authors. The quality of the papers was assessed using the Risk of Bias In Non-randomized Studies of Interventions tool. Data were extracted and collected in a database.

RESULTS

Manuscript source: Invited manuscript

Specialty type: Gastroenterology and hepatology

Country/Territory of origin: Italy

Peer-review report's scientific quality classification
Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): 0
Grade D (Fair): 0
Grade E (Poor): 0

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Among the 598 articles screened, nine papers met the inclusion criteria, six of which had low-risk rates of bias. Eight articles were published in the last decade; all came from eastern countries. Patient sample size was extremely heterogenous (n = 11-22926). AI methodologies employed included artificial neural networks (ANN) in six studies, as well as support vector machine, artificial plant optimization, and peritumoral radiomics in the remaining three studies. All the studies testing the role of ANN compared the performance of ANN with traditional statistics. Training cohorts were used to train the neural networks that were then applied to validation cohorts. In all cases, the AI models demonstrated superior predictive performance compared with traditional statistics with significantly improved areas under the curve.

CONCLUSION
AI applied to survival prediction after HCC treatment provided enhanced accuracy compared with conventional linear systems of analysis. Improved transferability and reproducibility will facilitate the widespread use of AI methodologies.

Key Words: Deep learning; Artificial neuronal network; Recurrence; Liver transplantation; Resection; Hepatocellular cancer

Core Tip: Prediction of survival after the treatment of hepatocellular carcinoma (HCC) has been widely investigated yet remains inadequate. The application of artificial intelligence (AI) is an emerging adjunct to traditional statistics due to its ability to process vast amounts of data and find hidden interconnections between variables. The current study aimed to assess the role of various methodologies of AI in the prediction of survival after treatment of HCC by performing a systematic review of the literature.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and the third most common cause of cancer-related death worldwide. Surgery, in the form of liver transplantation and resection, is the mainstay of treatment as the only potentially curative treatment option. Ablation has emerged as an alternative treatment to resection for small tumors. In contrast, intra-arterial treatments and chemotherapy can offer disease control and be used as part of a multimodal therapeutic strategy[1].

Many factors affect survival following the treatment of HCC. Among them, we can consider background liver condition, radiologic and histologic characteristics of the tumor, biologic markers, and comorbidities.

Traditionally, conventional linear models, such as the survival analysis and the Cox proportional hazard models, have been used to evaluate the prognosis of HCC[2-4]. Nevertheless, linear systems can have considerable limitations and often fail to capture the complexity of the interactions among clinicopathological characteristics[5]. With the intent to overcome such constraints, artificial intelligence (AI) has been employed with growing interest in healthcare research during the last decade, in particular applying deep learning (DL) techniques in artificial neural networks (ANN)[6]. ANN is a mathematical model that resembles the structure and function of a biological neural system using computer technology. It consists of a highly interconnected set of units, beginning with an input layer (the data to be analyzed), one or more hidden layers that process the data, and an output layer that provides the outcomes. The peculiarity

of ANN is that it can be trained by exposing the network to examples of input/output pairs, thus improving its reliability[7]. During DL, the model reassigns a different weight to the connections within each hidden layer. ANN can learn from errors by comparing any generated output with desired outputs. The error is backpropagated, and the existing weights between connections are modified accordingly. Once learning is complete, ANN can create connections and make predictions on datasets that have not been observed before.

AI has been used to build models to predict a variety of outcomes related to HCC, such as tumor diagnosis, pathology characteristics, response to treatment, and survival[7,8]. With the growing availability of big data from fields such as genomics, AI can unravel otherwise hidden connections between tumor elements because of the increasing computational power of modern technology[9].

The objective of the current study was to systematically review the application of AI and DL in the prediction of survival among patients who were treated for HCC, as well as compare the performance of AI methods relative to linear prediction models.

MATERIALS AND METHODS

Search sources and study design

A systematic review of the published literature focused on the prognostic impact of AI in the management of HCC was undertaken. The search strategy was performed following the Preferred Reporting Items for Systemic Reviews and Meta-Analysis (PRISMA) guidelines[10].

The specific research question formulated in the present study includes the following PICO components: (1) Patient: Patient with a confirmed HCC; (2) Intervention: Evaluation of HCC treatment using AI; (3) Comparison: Evaluation of HCC treatment without using AI; and (4) Outcome: Patient death and/or tumor recurrence. A search of the PubMed and Cochrane Central Register of Controlled Trials Databases was conducted using the following terms: (Artificial intelligence OR deep learning) AND (HCC OR hepatocellular carcinoma OR hepatocellular cancer). The search period was from "1985/01/01" to "2020/02/29".

The systematic qualitative review included only English studies that included human patients. Published reports were excluded based on several criteria: (1) Data on animal models; (2) Lacked enough clinical details; and (3) Had non-primary source data (*e.g.*, review articles, non-clinical studies, letters to the editor, expert opinions, and conference summaries). In the case of studies originating from the same center, possible overlapping of clinical cases was examined, and the most informative study was considered eligible.

Data extraction and definitions

Following a full-text review of the eligible studies, two independent authors (Lai Q and Larghi Laureiro Z) performed the data extraction and crosschecked all outcomes. During the selection of articles and extraction of the data, potential discrepancies were resolved following a consensus with a third reviewer (Mennini G). Collected data included the first author of the publication, year of publication, country, number of reported cases, research question/purpose, the method used, and key findings.

Quality assessment

Selected studies were systematically reviewed with the intent to identify potential sources of bias. The quality of the papers was assessed using the Risk of Bias In Non-randomized Studies of Interventions tool[11].

RESULTS

Search results and study characteristics

The PRISMA flow diagram schematically depicts the article selection process (Figure 1). Among the 598 articles screened, a total of 127 studies reported on the use of AI in HCC. Among these articles, only 9 (7.1%) studies referred to the use of AI in the prediction of survival among patients with HCC and were included in this review[12-20]. Other studies using AI in HCC were excluded; specifically, these studies reported on the use of AI for the diagnosis of the tumor (n = 76, 59.8%), identification of specific genes or pathways (n = 17, 13.4%), prediction of tumor response after

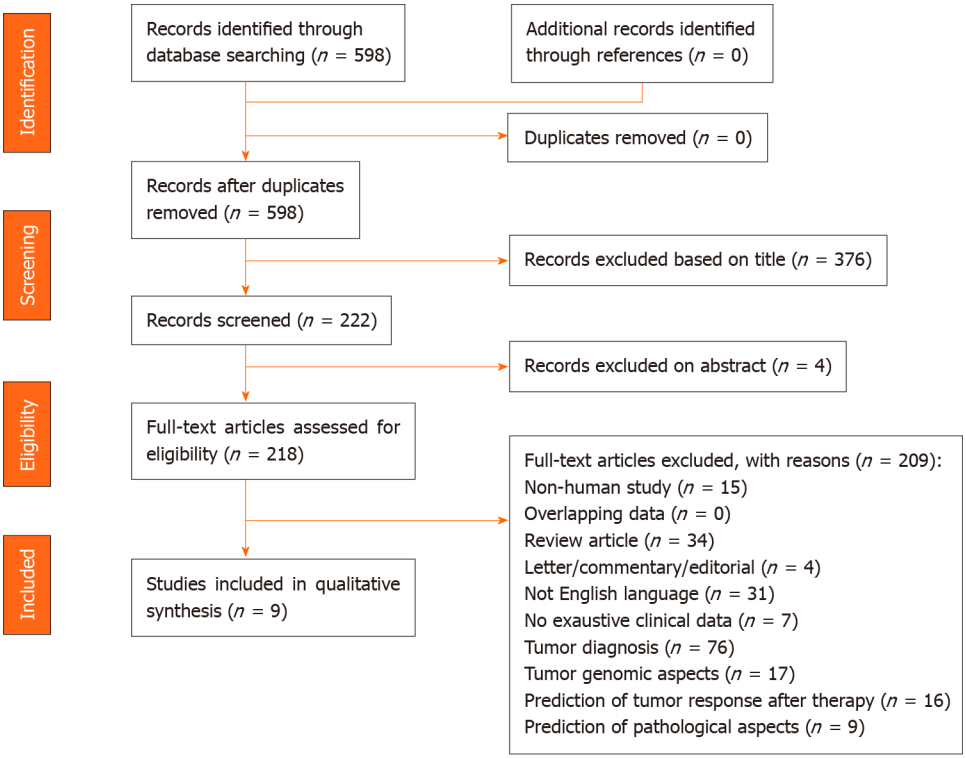

Figure 1 Preferred Reporting Items for Systemic Reviews and Meta-Analysis flowchart of the literature search and study selection.

therapy (n = 16, 12.6%), and the prediction of pathological aspects (n = 9, 7.1%) (Figure 2). All studies included in the analytic cohort were published in the last decade except for one that was published in 1995[12]. All articles were from Asia; five studies were based on a population from Taiwan[13-17], two from China[18,20], one from Japan[12], and one from India[19].

Qualitative assessment of the included studies

Results from the qualitative assessment of the included studies are depicted in Figure 3. Six studies had a low risk of bias, while two studies were at high risk for bias, mainly due to the presence of potential confounders. In one study, due to the absence of clear data explaining the characteristics of the comparison groups, the risk of bias was unclear.

Review of the eligible studies

Data extracted from the nine eligible articles are reported in detail in Table 1. The largest studies were based on the same population of patients coming from the Taiwan Bureau of National Health Insurance. All patients had a diagnosis of a malignant neoplasm of the liver and underwent a hepatectomy between 1998-2009 (n = 22926)[14,15]. In all other studies, the sample size was smaller than 1000 cases, and in two cases, the sample size was smaller than 100[12,17].

The use of ANN in populations of patients who underwent surgery was reported in six articles[12-16,18]. The outcomes investigated included in-hospital postoperative mortality[14], long-term overall survival[12,15,16,18], and disease-free survival after hepatic resection[13]. Several other studies used different AI systems rather than ANN. Specifically, a support vector machine was used for the development of predictive models relative to the recurrence of HCC following radiofrequency ablation[17]. Besides, an Artificial Plant Optimization algorithm was used to assess the effectiveness and efficiency to predict HCC recurrence[19]. Peritumoral radiomics was used to predict early recurrence after HCC curative-intent resection or ablation[20].

A cohort was used in the majority of studies to train the AI network[12-16,18,20]; in one study, a double five-fold cross-validation loop method was adopted[17]. In all studies, AI demonstrated superior predictive performance compared with other traditional models. In several studies, the ANN outperformed logistic regression or Cox regression models[13-16,18]. In all cases, the prediction accuracy of the AI models expressed as the areas under the curve was significantly improved compared with traditional statistical techniques[13-16,18].

Table 1 Articles focused on the role of artificial intelligence in the prediction of survival

Ref.	Country/region	*n*	Research question/purpose	Method used	Key findings
Hamamoto *et al*[12], 1995	Japan	11	ANN for the prediction of survival after HCC resection.	ANN was trained with the data of 54 resected patients and then prospectively used.	The outcomes in the prospective cohort were successfully predicted in all the cases (10 successful, 1 died).
Ho *et al*[13], 2012	Taiwan	482	To validate the use of ANN model for predicting 1-, 3-, and 5-yr disease-free survival after hepatic resection, and to compare it with LR and decision tree model.	Training set: 80% of the cases; validation set: Remaining 20% of the cases.	The ANN model outperformed the other models in terms of prediction accuracy (AUC for 5-yr disease-free survival: 0.864 *vs* 0.627-0.736).
Shi *et al*[14], 2012	Taiwan	22926	ANN model for predicting in-hospital mortality in HCC surgery patients and to compare it with LR models.	This study analyzed administrative claims data obtained from the Taiwan Bureau of National Health Insurance.	Compared to the LR models, the ANN models had a better accuracy rate in 97.28% of cases, and a better ROC curve in 84.67% of cases.
Shi *et al*[15], 2012	Taiwan	22926	To validate the ANN models for predicting 5-yr mortality in HCC resected patients, and to compare them with LR models.	This study analyzed administrative claims data obtained from the Taiwan Bureau of National Health Insurance.	Compared to the LR models, the ANN models had a better accuracy rate in 96.57% of cases, and a better receiver operating characteristic curves in 88.51% of cases.
Chiu *et al*[16], 2013	Taiwan	434	To compare significant predictors of mortality for HCC resected patients between ANN and LR models, and to evaluate the predictive accuracy of ANN and LR in different survival year estimation models.	Training set: 80% of the cases; validation set: Remaining 20% of the cases.	The results indicated that ANN had double to triple numbers of significant predictors at 1-, 3-, and 5-yr survival models as compared with LR models. Scores of accuracy, sensitivity, specificity, and AUC using ANN were superior to those of LR.
Qiao *et al*[17], 2014	China	543; 182; 104	ANN for the prediction of survival in early HCC cases following partial hepatectomy.	Training set: 75% of the cases; internal validation set: Remaining 25% of the cases; external validation set.	In the training cohort, the AUC of the ANN was larger than that of the Cox model (0.855 *vs* 0.826, $P = 0.0115$). These findings were confirmed with the internal and external validation cohorts.
Liang *et al*[18], 2014	Taiwan	83	Use of support vector machine for the development of recurrence predictive models for HCC patients receiving RFA treatment.	Five feature selection methods including genetic algorithm, simulated annealing algorithm, random forests and hybrid methods were utilized.	The developed support vector machine-based predictive models using hybrid methods had averages of the sensitivity, specificity, and AUC as 67%, 86%, and 0.69.
R *et al*[19], 2019	India	152	To use artificial plant optimization algorithm to select optimal features and parameters of classifiers to improve the effectiveness and efficiency of prediction of HCC recurrence.	Different methods tested.	The sampling based multiple measurement artificial plant optimized random forest classifier with statistical measure showed the best results (balanced accuracy: 0.955).
Shan *et al*[20], 2019	China	156	Peritumoral radiomics for the prediction of early recurrence after HCC curative resection or ablation.	Training cohort ($n = 109$) and validation cohort ($n = 47$). Using CT images, two regions of interest were delineated around the lesion for feature extraction o tumoral radiomics and peritumoral radiomics.	In the validation cohort, the ROC curves, calibration curves and decision curves indicated that the CT-based peritumoral radiomics model had better calibration efficiency and provided greater clinical benefits.

ANN: Artificial neural network; HCC: Hepatocellular carcinoma; AUC: Area under the curve; LR: Logistic regression; RFA: Radiofrequency ablation; CT: Computed tomography; ROC: Receiver operating characteristic.

DISCUSSION

The use of AI in healthcare began in the early 1970s and has gained increased acceptance over the last decades. In particular, the development of AI in medical research and its clinical applications have gained popularity, in part because of the widespread use of AI in almost all fields of human life[21]. The current literature search revealed that many AI studies focused on diagnosis, and the application of AI to distinguish the radiological features of HCC. The identification and diagnostic

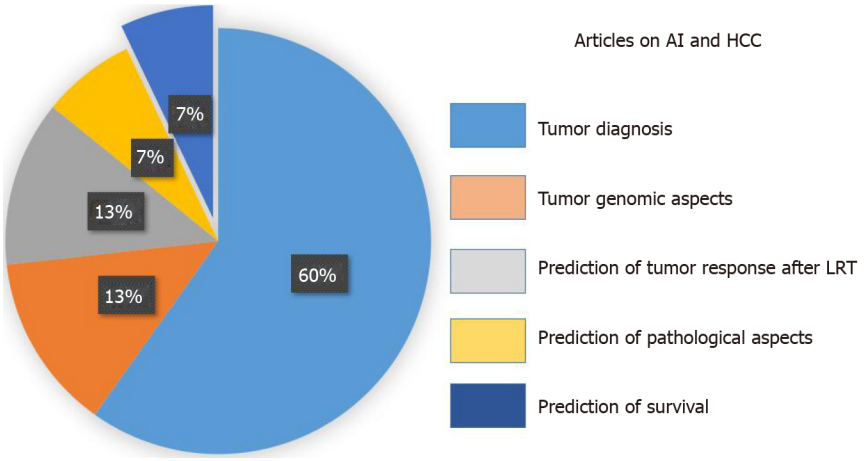

Figure 2 Different articles exploring the impact of artificial intelligence as diagnostic or prognostic tool in the setting of hepatocellular carcinoma management. AI: Artificial intelligence; HCC: Hepatocellular carcinoma; LRT: Locoregional therapy.

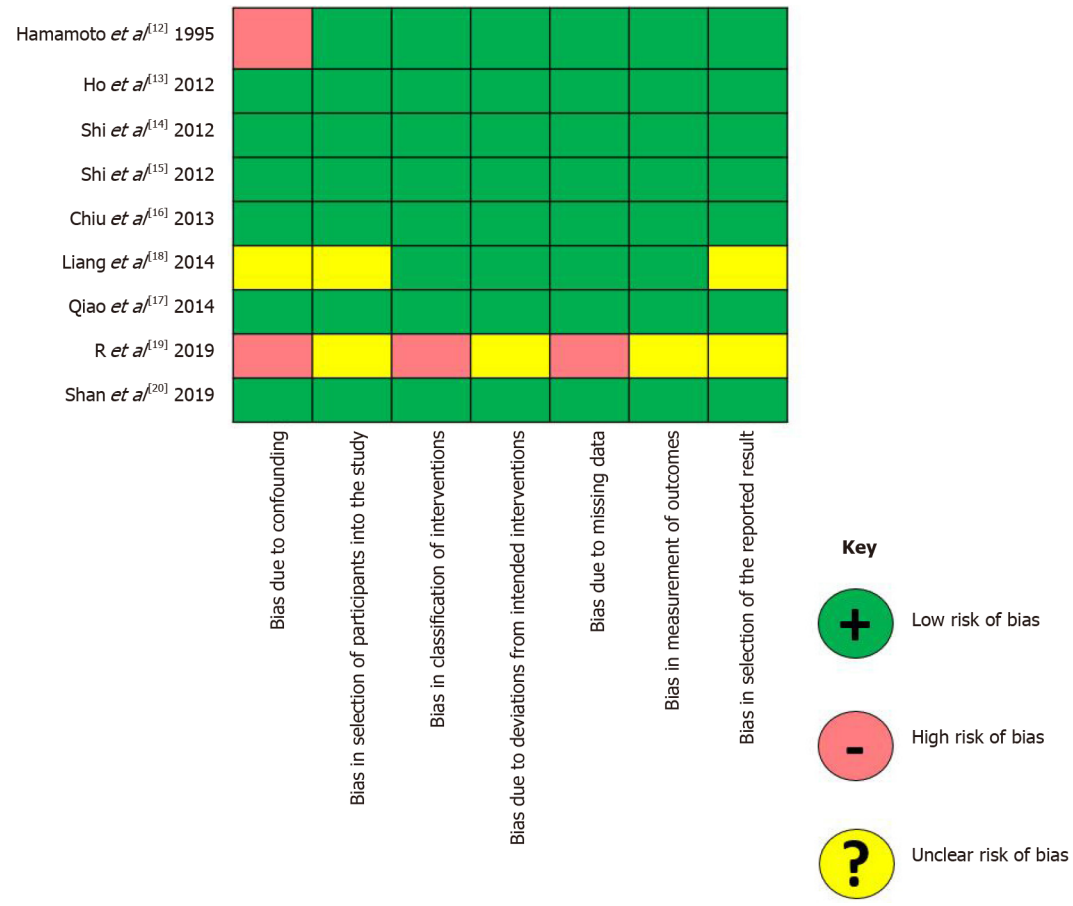

Figure 3 Results of the Risk of Bias In Non-randomized Studies of Interventions tool for the extracted articles.

discrimination of benign *vs* malignant liver masses has been the objective of a previous systematic review that noted AI could differentiate liver cancer and, in particular, HCC from other lesions better compared with other methods such as Bayesian models and expert radiologists image inspection[8]. The present systematic review is important because it is the first to summarize the ability of AI systems to predict patient survival following treatment of HCC. Our results revealed that different types of AI methods have been employed in the existing studies with heterogeneous patient sample sizes. The majority of the included studies (n = 6/9) utilized ANN for the analysis of predictors of post-treatment survival, which is in line with the results of other systematic reviews on the prediction of outcomes[22,23]. Considering the need for more

accurate prediction, investigators have compared AI techniques with traditional linear models to optimize treatment decision-making. Although several prediction models have utilized both pre- and postoperative variables, these models have not proved useful in clinical decision-making since they require information that can only be available after resection or other treatment. In contrast, models with only preoperative variables can help guide treatment strategies in the preoperative setting[24,25].

Importantly, our systematic review revealed that the prediction of survival using AI methodology was highly accurate and remained robust in studies with limited sample sizes, although current knowledge in prediction modeling using AI has noted that AI performs better when applied to larger sample sizes[26]. Although the reason for the consistent high predictive accuracy of AI models is multifactorial, the complexity of AI models (*e.g.*, a higher number of events per variable) further reinforces the superiority of their performance, which might explain the outstanding results even when used in smaller size studies[27].

Reproducibility and applicability of AI models in clinical practice and across different centers might be questioned due to the difficulties in acquiring and utilizing a dedicated software to process the data. In addition, as ANN learns from examples, one may argue that ANN needs to be trained before it can be applied to varying datasets that are different from the one it was initially built on. Nevertheless, what emerged from this systematic review was that AI could be an outstanding adjunct to conventional linear systems of analysis to predict post-treatment survival. Cucchetti *et al*[7] made their ANN available online so that other centers can test and possibly enrich their model aiming to predict HCC tumor grade and micro-vascular invasion preoperatively. Besides, when applied to other aspects of HCC, AI is particularly useful for exploring interconnections of big data such as in genomics. ANN combined with genotyping for microsatellite mutations/deletions was able to predict HCC recurrence after liver transplantation with an 85% accuracy in the center where the model was developed, and with 89.5% accuracy when examined in data from another center[28]. AI applied to radiomics is increasingly investigated: Machine learning has been used to provide a quantitative interpretation of computed tomography scans to reclassify indeterminate nodules and potentially avoid biopsy and improve patients safety[29]. Similarly, neural network algorithms have been built with the intent to objectively and reproducibly provide liver imaging reporting and data system categories concordant with the expert radiologists classifcation[30].

One of the downsides associated with the application of ANN in clinical practice might be the disproportionate number of input factors per patient (too many, *e.g.*, thousands of proteins for gene expression) relative to the number of patients (too little). The risk of overfitting the dataset can be mitigated by strictly filtering out potentially irrelevant variables[31]. In particular, selecting the variables to use as input factors in ANN using traditional statistics has been employed as a strategy to improve efficiency and reduce redundancy of the AI model, as confirmed by all of the studies using ANNs included in this systematic review. When analyzing cancer patient data (*i.e.*, too many dimensions for a relatively small number of samples), combining DL with other techniques of machine learning have been used to identify prognostic gene signatures and differentiate between better and worse prognosis in patients with various types of tumors including HCC[32].

CONCLUSION

Artificial intelligence can provide an enhanced prediction of survival following treatment of HCC compared with conventional linear models. The use of AI can be particularly helpful to process large amounts of data, as well as help identify patterns and associations that are not evident with traditional techniques given the complexity of the biological systems. AI has a promising role in health-care research and its application to HCC. While an increasing amount of data becomes available per patient, it is important to identify to what extent AI can help guide clinical decision-making and optimize the prediction of long-term outcomes based on the unique characteristics of each patient.

ARTICLE HIGHLIGHTS

Research background

Prediction of survival after the treatment of hepatocellular carcinoma (HCC) has been widely investigated, yet remains inadequate. The application of artificial intelligence (AI) is emerging as a valid adjunct to traditional statistics due to the ability to process vast amounts of data and find hidden interconnections between variables. AI and deep learning are increasingly employed in several topics of liver cancer research, including diagnosis, pathology, and prognosis.

Research motivation

AI applied to survival prediction after HCC treatment should provide enhanced accuracy compared with conventional linear systems of analysis.

Research objectives

Improved transferability and reproducibility will facilitate the widespread use of AI methodologies.

Research methods

A web-based literature search was performed according to the Preferred Reporting Items for Systemic Reviews and Meta-Analysis guidelines using the keywords "artificial intelligence", "deep learning" and "hepatocellular carcinoma" (and synonyms).

Research results

Among the 598 articles screened, nine papers met the inclusion criteria, six of which had low-risk rates of bias. Eight articles were published in the last decade; all came from eastern countries. Patient sample size was extremely heterogenous (n = 11-22926). AI methodologies employed included artificial neural networks (ANN) in six studies, as well as support vector machine, artificial plant optimization, and peritumoral radiomics in the remaining three studies. All the studies testing the role of ANN compared the performance of ANN with traditional statistics. Training cohorts were used to train the neural networks that were then applied to validation cohorts. In all cases, the AI models demonstrated superior predictive performance compared with traditional statistics with significantly improved areas under the curve.

Research conclusions

AI applied to survival prediction after HCC treatment provided enhanced accuracy compared with conventional linear systems of analysis.

Research perspectives

Improved transferability and reproducibility will facilitate the widespread use of AI methodologies.

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CASE REPORT

Case series of three patients with hereditary diffuse gastric cancer in a single family: Three case reports and review of literature

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Abstract

BACKGROUND

Hereditary diffuse gastric cancer (HDGC) is a familial cancer syndrome often associated with germline mutations in the *CDH1* gene. However, the frequency of *CDH1* mutations is low in patients with HDGC in East Asian countries. Herein, we report three cases of HDGC harboring a missense *CDH1* variant, c.1679C>G, from a single Japanese family.

CASE SUMMARY

A 26-year-old female (Case 1) and a 51-year-old male (father of Case 1), who had a strong family history of gastric cancer, were diagnosed with advanced diffuse gastric cancer. After genetic counselling, a 25-year-old younger brother of Case 1 underwent surveillance esophagogastroduodenoscopy that detected small signet ring cell carcinoma foci as multiple pale lesions in the gastric mucosa. Genetic

genetic testing and contributed to manuscript drafting; all authors issued final approval for the version to be submitted.

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analysis revealed a *CDH1* c.1679C>G variant in all three patients.

CONCLUSION

It is important for individuals suspected of having HDGC to be actively offered genetics evaluation. This report will contribute to an increased awareness of HDGC.

Key Words: Hereditary diffuse gastric cancer; Signet ring cell carcinoma; *CDH1*; E-cadherin; Endoscopic findings; Case report

Core Tip: Hereditary diffuse gastric cancer (HDGC) has rarely been reported in East Asian countries. We report a Japanese HDGC family with a missense CDH1 variant, c.1679C>G (p.T560R). We clearly detected early signet ring cell carcinoma foci by esophagogastroduodenoscopy with white light imaging, non-magnifying narrow band imaging (NBI) and magnifying NBI. In this family, active genetics evaluation and intensive endoscopic surveillance resulted in early diagnosis and treatment of HDGC.

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INTRODUCTION

Gastric cancer (GC) is the fifth most common neoplasm and the third most deadly cancer worldwide, with an estimated 783000 deaths per year[1]. Although most instances of GC are sporadic, approximately 1%-3% of cases arise as a result of inherited cancer syndromes[2]. Hereditary diffuse gastric cancer (HDGC) is an autosomal dominant cancer syndrome. The relationship between HDGC and germline mutation of *CDH1*, encoding the tumor-suppressor protein E-cadherin, was first identified in New Zealand families[3]. To date, over 155 germline *CDH1* mutations, of which the majority are pathogenic and a number of variants are unclassified, have been described[2]. However, the detection rate of *CDH1* germline mutations in patients with HDGC is low and few cases have been reported in East Asian countries[4-10]. In the current report, we present the clinical courses of three cases with HDGC harboring a germline pathogenic variant of *CDH1*, c.1679C>G, from a single family.

CASE PRESENTATION

Chief complaints

Cases 1-3: Unremarkable.

History of present illness

Case 1: The proband is a 26-year-old female. She was referred to our hospital for screening esophagogastroduodenoscopy (EGD) because her older brother died of GC 3 years ago at another hospital.

Case 2: A 51-year-old male (father of Case 1) visited our hospital for screening EGD because he had a strong family history of gastric cancer.

Case 3: As a result of taking the detailed family history, we noted that Cases 1 and 2 had several family members with GC. We suspected HDGC and performed genetic counselling for a 25-year-old younger brother of Case 1.

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History of past illness
Cases 1-3: The patients had a free previous medical history.

Personal and family history
Cases 1-3 had several family members with GC. Pedigree of this family is shown in Figure 1.

Physical examination
Cases 1-3: Unremarkable.

Laboratory examinations
Cases 1-3: The serum levels of CEA and CA 19-9 were within normal limits.

Imaging examinations
Case 1: EGD revealed advanced GC at the lower and middle body of the stomach on a background of non-atrophic gastric mucosa (Figure 2A and B). The biopsy specimens demonstrated diffuse type adenocarcinoma without *Helicobacter pylori* co-infection. Computed tomography (CT) revealed lymph node metastases along the lesser curvature of the stomach (Figure 2C).

Case 2: The patient had surveillance EGD that showed a Borrmann type 3 tumor at the fundus on a background of non-atrophic gastric mucosa (Figure 3A). A histopathological examination of the biopsy specimens revealed diffuse type adenocarcinoma without *Helicobacter pylori* co-infection. Furthermore, advanced colon cancer at the ascending colon was also detected by screening colonoscopy, although histopathological analysis indicated this was an intestinal adenocarcinoma (Figure 3B). No distant metastases were identified by CT (Figure 3C).

Case 3: He received surveillance EGD that detected multiple small pale lesions, mainly in the greater curvature of the stomach (Figure 4A). Narrow band imaging (NBI) without magnification showed clearly isolated whitish areas, and NBI with magnification detected "wavy" microvessels, indicating diffuse type GC, in these lesions (Figure 4B and C). We took 6 targeted biopsies from these lesions, which revealed signet ring cell carcinoma (SRCC) in all the specimens.

Further diagnostic work-up
The presence of germline *CDH1* c.1679C>G (p.T560R) variant: As the three patients (Cases 1, 2 and 3) fulfilled the International Gastric Cancer Linkage Consortium (IGCLC) criteria for HDGC[2], we tested all of them for germline *CDH1* mutation. This genetic testing revealed a *CDH1* c.1679C>G (p.T560R) variant in all three patients.

FINAL DIAGNOSIS

Case 1
The final diagnosis of Case 1 is HDGC.

Case 2
The final diagnosis of Case 2 is HDGC and colon cancer.

Case 3
The final diagnosis of Case 3 is HDGC.

TREATMENT

Case 1
The patient underwent total gastrectomy with D2 Lymphadenectomy (pT4aN1M0, Stage IIIA).

Case 2
The patient underwent total gastrectomy with D2 Lymphadenectomy (pT4aN3aM0, Stage IIIB) and right hemicolectomy with D3 Lymphadenectomy (pT2N0M0, Stage I).

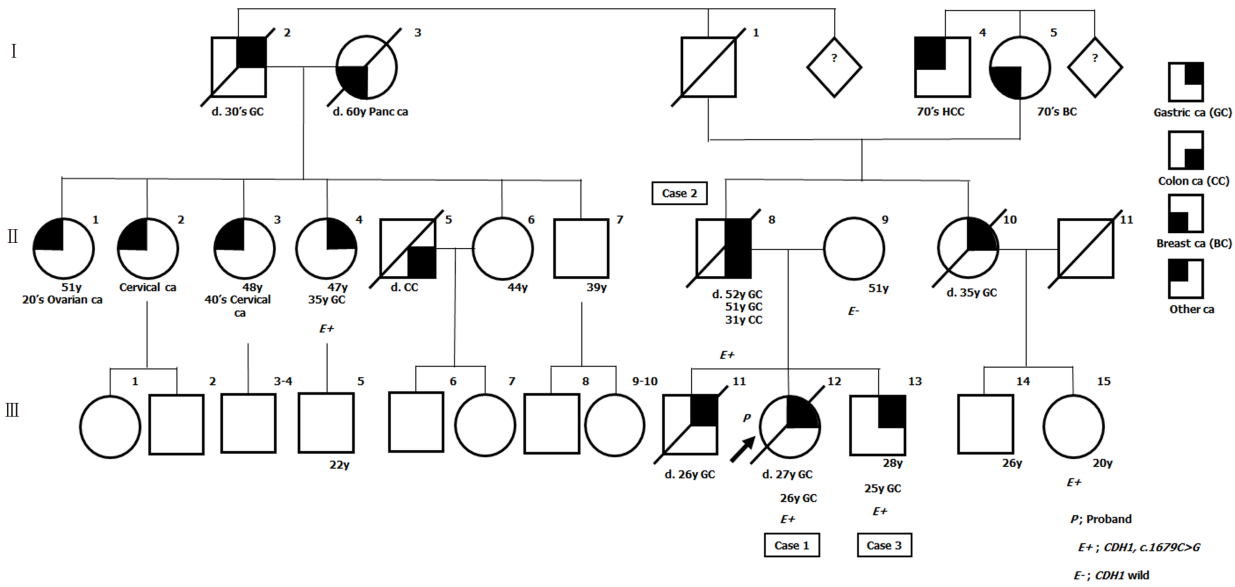

Figure 1 Pedigree of this family. Several individuals with gastric cancer were confirmed in this family. In addition to Cases 1, 2 and 3, the *CDH1* c.1679C>G variant was detected in II-4 and III-15 by further genetic analysis. GC: Gastric cancer; BC: Breast cancer; HCC: Hepatocellular carcinoma.

Case 3

Total gastrectomy with D1 Lymphadenectomy was performed (pT1N0M0, Stage IA). A total of 36 SRCC foci were observed by histological examination of the entire gastric mucosa (Figure 4D). Immunohistochemistry revealed loss of E-cadherin expression in areas corresponding to SRCC foci, which was compatible with the findings in HGDC (Figure 4E)[3].

OUTCOME AND FOLLOW-UP

Case 1

Ovarian metastasis was detected by CT during the adjuvant chemotherapy (Figure 2D). Although systemic chemotherapy was continued, the patient died two years after the diagnosis.

Case 2

The GC was treated with adjuvant chemotherapy. Despite treatment, the disease progressed due to peritoneal carcinomatosis during the adjuvant chemotherapy (Figure 3D), and the patient died one year after the diagnosis.

Case 3

No evidence of GC recurrence has been observed in the 3 years after diagnosis.

Relatives of cases 1, 2 and 3

Based on the result of genetic analysis, we further performed genetic counselling and genetic testing for their relatives to the extent that this was possible, and detected this variant in two of them (Figure 1). As the two p.T560R variant carriers refused prophylactic gastrectomy, we are currently continuing endoscopic surveillance for them.

DISCUSSION

Here we present an HDGC family with a missense *CDH1* substitution variant, c.1679C>G (p.T560R). The p.T560R variant had been reported three times in patients with HDGC[11-13]. Yelskaya *et al*[12] reported that the p.T560R mutation created a novel 5¢ splice donor site that led to truncation of E-cadherin. Furthermore, Pena-Couso *et al*[13]

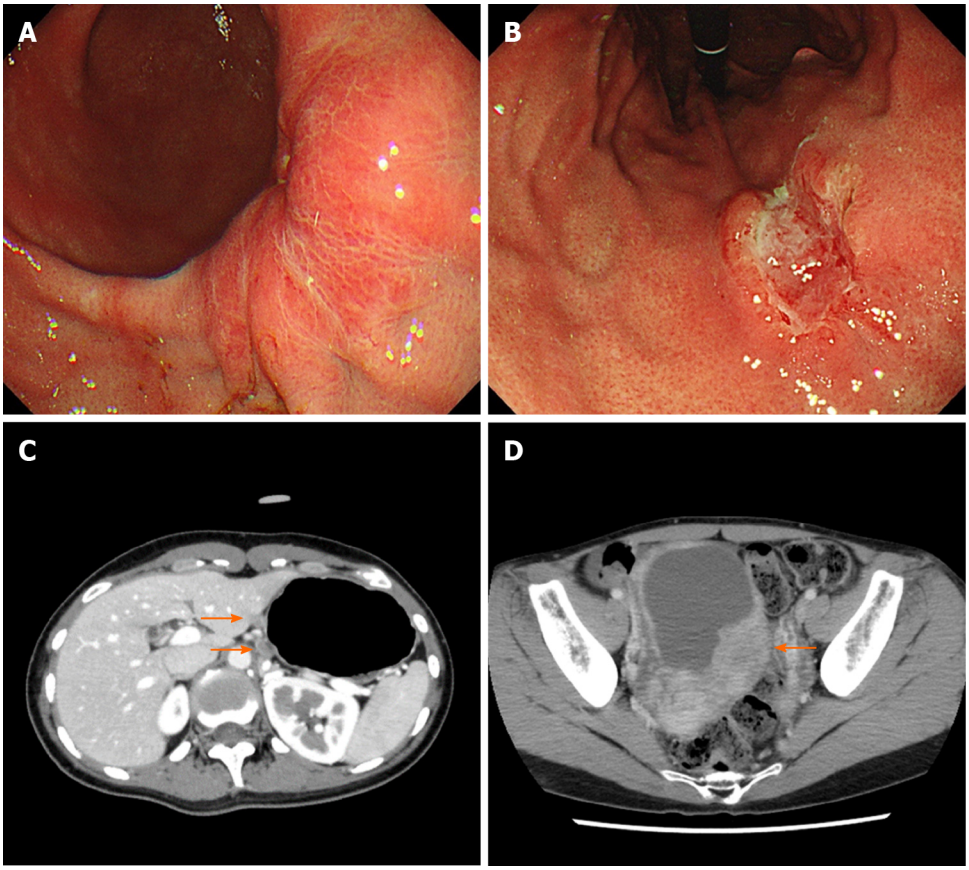

Figure 2 Representative images obtained from esophagogastroduodenoscopy and computed tomography in Case 1. A and B: Advanced gastric cancer was observed at the posterior wall of the lower gastric body (A) and at the lesser curvature of the middle body (B) in esophagogastroduodenoscopy; C: Metastatic lymph nodes were detected at the lesser curvature of the proximal stomach by abdominal computed tomography (CT) (orange arrows); D: Abdominal CT showed ovarian metastasis during adjuvant chemotherapy (orange arrow).

performed functional analyses, which revealed that the p.T560R mutation causes an abnormal pattern of E-cadherin expression in the cytoplasm, disrupts cell-cell adhesion and promotes cellular invasion. Consistent with these reports, loss of E-cadherin expression at SRCC foci was observed in Case 3. Furthermore, we observed early recurrence and rapid progression of GC after radical resection in Cases 1 and 2. E-cadherin is a member of the cadherin family and mediates calcium-dependent cell-cell adhesion[14]. Reduction of E-cadherin expression promotes invasion and metastasis in various cancer types through initiation of the epithelial-mesenchymal transition[15]. Indeed, HDGC patients with germline *CDH1* mutations have shorter survival times compared to those without germline *CDH1* mutations[16]. On the other hand, the loss of E-cadherin may not be sufficient for the development of invasive gastric adenocarcinoma, because signet ring-like cells are observed in gastric mucosa of E-cadherin-deficient mice, but this does not lead to development of carcinomas that invade the submucosa[17]. In addition to the loss of E-cadherin, other genes, such as *Smad4* and *p53*, may play important roles in tumorigenesis and metastasis in HDGC[18].

With respect to gastric endoscopic findings, multiple small pale lesions were observed with white light imaging in Case 3 and all biopsy specimens from the pale lesions revealed SRCC. Pale lesions in HDGC patients possibly reflect microscopic foci of early SRCC, although their presence is not diagnostic for this disease[2,7,10,19]. On the other hand, Hüneburg and colleagues[20] reported that combining targeted biopsies from abnormal findings (including pale lesions) with random biopsies did not improve detection of SRCC foci in *CDH1* mutation-positive HDGC patients. Currently, the IGCLC guidelines for endoscopic surveillance of HDGC recommend that all endoscopically visible lesions (including pale areas) are biopsied, and after sampling of all visible lesions, five random biopsies should be taken from each of the following anatomical zones: prepyloric, antrum, transitional zone, body, fundus and cardia[18]. Nevertheless, the rate at which SRCC foci are detected in *CDH1* mutation carriers following endoscopy is 45%-60%, which is relatively low[19,21-23]. Further studies are needed to improve the accuracy of endoscopic diagnosis of HDGC. Additionally, we

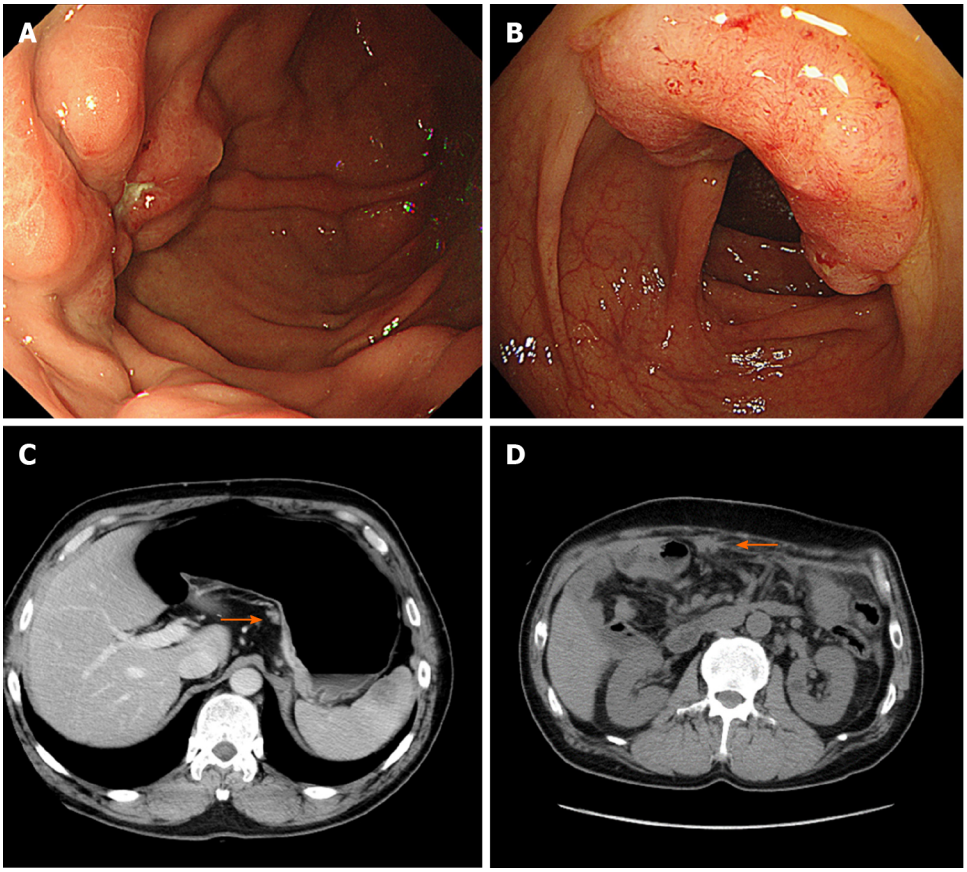

Figure 3 Representative images obtained from esophagogastroduodenoscopy, colonoscopy and computed tomography in Case 2. A: Advanced gastric cancer was observed at the fundus in esophagogastroduodenoscopy; B: Colonoscopy showed advanced colon cancer at the ascending colon; C: Metastatic lymph nodes at the lesser curvature of the proximal stomach without distant metastasis were identified by abdominal computed tomography (CT) (orange arrow); D: Peritoneal dissemination were observed by abdominal CT during the adjuvant chemotherapy (orange arrow).

recognized the SRCC foci as clearly isolated whitish areas by NBI and observed wavy microvessels inside the lesions by magnifying NBI. NBI has not previously been validated as a method for diagnosis of patients with HDGC[19,23]. Interestingly, the NBI findings that we observed in Case 3 are similar to those previously reported in studies of early SRCC patients[24-27]. Although the detection of small intramucosal SRCC foci is not easy because most of them are covered by a normal foveolar epithelium, the endoscopic findings that we observed in Case 3 are informative for the detection of early SRCC foci in *CDH1* mutation-positive HDGC patients.

Lastly, it is well known that germline *CDH1* mutations increase the lifetime risk of developing lobular breast cancer. Although we performed breast cancer screening for Case 1, no breast cancer was detected. In contrast, coexistence of colon cancer was revealed in Case 2. Currently, it is unclear whether *CDH1* germline mutations also increase the risk of colorectal cancer. There are several case reports of colorectal SRCCs in germline *CDH1* mutation carriers[28-31]. However, as the histopathology of colon cancer in Case 2 indicated intestinal adenocarcinoma, the relationship between *CDH1* mutation and development of colon cancer in Case 2 is not certain. Interestingly, Salahshor *et al*[32] reported that the colorectal cancer subtype associated with HDGC can be intestinal adenocarcinoma. Further studies are needed to clarify whether germline *CDH1* mutations cause colorectal carcinogenesis.

CONCLUSION

We report an HDGC family with a missense *CDH1* variant, c.1679C>G (p.T560R), where active genetics evaluation and intensive endoscopic surveillance in Case 3 resulted in early diagnosis and treatment of HDGC. HDGC has rarely been reported in East Asian countries. However, the rarity of HDGC in East Asian Countries may be related to insufficient surveillance or overlooked cases and may not reflect the actual prevalence. We therefore recommend that individuals suspected of having HDGC (

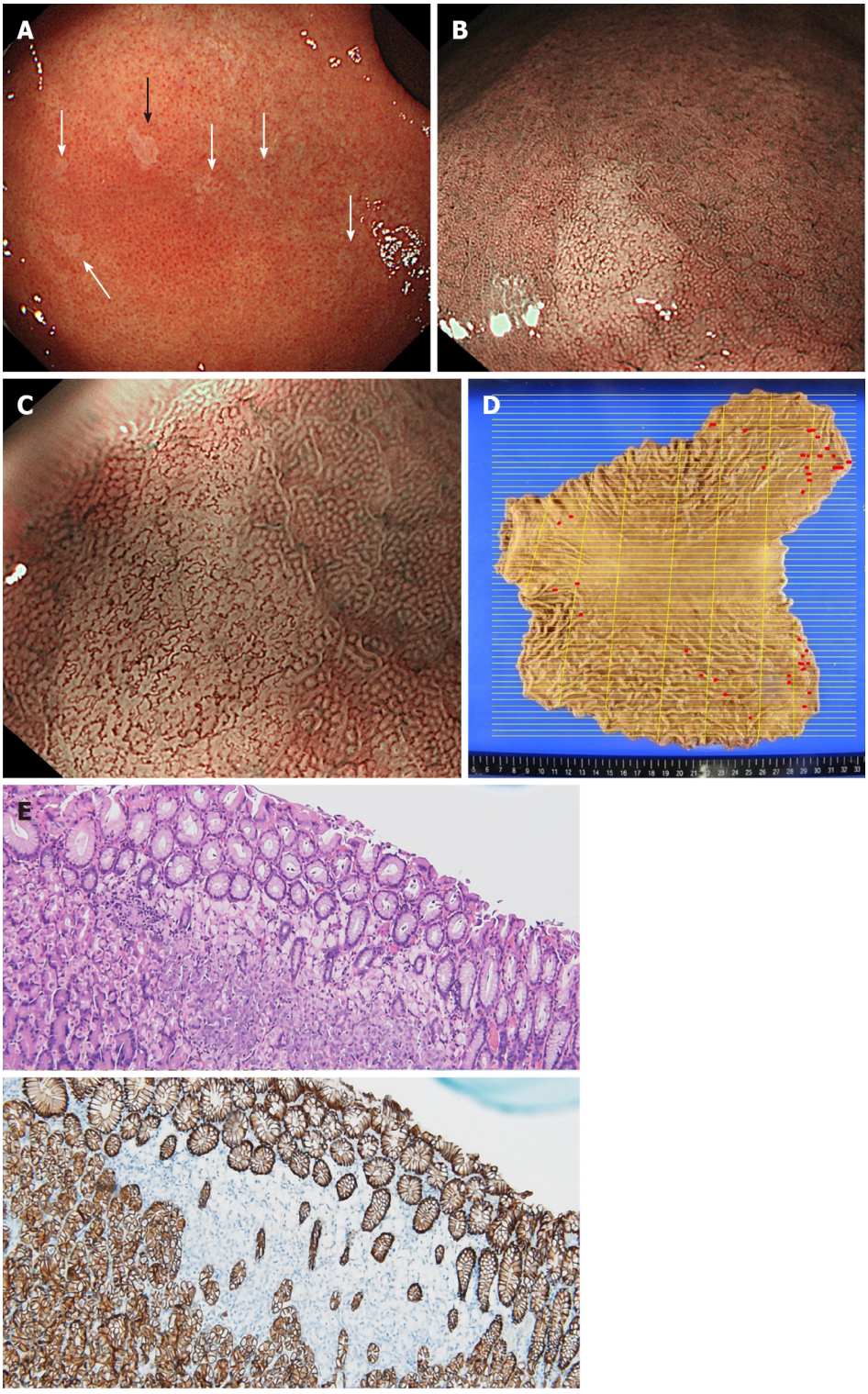

Figure 4 Representative images obtained from esophagogastroduodenoscopy and pathological findings in Case 3. A: Multiple small pale lesions were observed mainly at the greater curvature of the gastric body in esophagogastroduodenoscopy (white and black arrows); B: Clearly isolated whitish areas were detected by non-magnifying narrow band imaging (NBI). The image is the lesion indicated by the black arrow in (A); C: Magnifying NBI detected wavy microvessels inside the lesions; D: A gastrectomy mapping study revealed 36 signet ring cell carcinoma (SRCC) foci in the entire gastric mucosa. Red lines indicate SRCC foci; E: Hematoxylin and eosin staining (upper panel) and immunohistochemistry for E-cadherin (lower panel) of the lesion. Loss of immunoreactivity at SRCC foci was confirmed.

e.g., fulfilling the IGCLC criteria for HDGC, existence of multiple SRCC foci) should be offered genetic counselling and mutation analysis in cooperation with cancer genetics professionals. The present report will contribute to an increased awareness of HDGC and will improve the performance of endoscopic diagnosis for early SRCC foci in HDGC patients harboring a *CDH1* mutation.

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CASE REPORT

Intussusception due to hematogenous metastasis of hepatocellular carcinoma to the small intestine: A case report

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Abstract

BACKGROUND

The commonest sites of extrahepatic metastases from hepatocellular carcinoma (HCC) are the lungs, bones, adrenal glands, and regional lymph nodes. Hematogenous metastasis to the gastrointestinal (GI) tract is a rare condition in patients with HCC, and the prognosis is usually poor. We report, herein, an extremely rare case of a patient with intussusception due to hematogenous metastasis of HCC to the ileum and his long-term survival with multidisciplinary therapy.

CASE SUMMARY

The patient was a 71-year-old man with a history of chronic hepatitis B, who had undergone three surgeries for HCC. He was treated with sorafenib for peritoneal metastases of HCC. He was admitted to our hospital with chief complaints of abdominal pain and vomiting. Abdominal contrast-enhanced computed tomography imaging revealed a small intestinal tumor, presenting with intussusception and small bowel obstruction. Conservative treatment was started, but due to repeated exacerbation of symptoms, surgery was planned on the 28th d of hospitalization. Partial ileal resection without reducing the intussusception and end-to-end anastomosis was performed. On histological examination, tumor cells were not observed on the serosal surface, but intravascular invasion of tumor cells was seen. Immunohistochemistry was positive for immunohistochemical markers, and a diagnosis of hematogenous metastasis of HCC to the ileum was made. He

remains alive 82 mo after the first surgery.

CONCLUSION
Prognosis of HCC patients with GI tract metastasis is usually poor, but in some cases, multidisciplinary therapy may prolong survival.

Key Words: Hepatocellular carcinoma; Hematogenous metastases; Extrahepatic metastasis; Small intestinal metastasis; Intussusception; Case report

Core Tip: Intussusception due to hematogenous metastasis of hepatocellular carcinoma (HCC) to the gastrointestinal (GI) tract is an extremely rare condition in patients with HCC. Patients with GI tract metastasis of HCC usually have a poor prognosis because of the advanced tumor stage. Surgical treatment of extrahepatic metastasis of HCC has still not been established. However, this case report suggests that selected patients with extrahepatic metastasis of HCC may achieve prolonged survival with multidisciplinary therapy including surgical resection.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a highly prevalent disease and accounts for 800000 deaths per year globally[1]. Despite the development of novel treatment modalities and newer surgical instruments, the long-term outcomes of HCC are not satisfactory because of high rates of recurrence and metastasis. Intrahepatic metastasis is the most common recurrence pattern of HCC, accounting for approximately 85%-90% of cases[2,3]. Extrahepatic metastases have been reported in 13%-64% of HCC patients, with the lungs, bones, adrenal glands, and regional lymph nodes as the commonest sites of metastases[4-6]. Metastasis of HCC to the gastrointestinal (GI) tract is infrequent, and the distant hematogenous metastasis of HCC to the small intestine is extremely unusual. We report, herein, a case of intussusception due to hematogenous metastasis of HCC to the ileum.

CASE PRESENTATION

Chief complaints

A 71-year-old man was admitted to our hospital with chief complaints of abdominal pain and vomiting.

History of present illness

A 71-year-old man was on treatment for chronic hepatitis B for 22 years when he was diagnosed with HCC. He was noted to have tumor nodules of size 20 mm in diameter, located in segment 8, on a follow-up abdominal computed tomography (CT) (Figure 1) and had undergone partial liver resection 7 years previously. Based on the 8th Union for International Cancer Control classification of HCC, the tumor was classified as pT1N0M0 stage 1. Seven months after the first surgery, abdominal CT revealed recurrent HCC with nodules 10 mm in diameter, in segment 6 of the liver (Figure 2A). When laparotomy was performed, a peritoneal mass was found that was not apparent preoperatively; therefore, partial liver resection and peritoneal tumor resection were performed (Figure 2B and C). The peritoneal tumor with peritoneal metastasis of HCC was diagnosed based on histopathological findings. Fourteen months after the first

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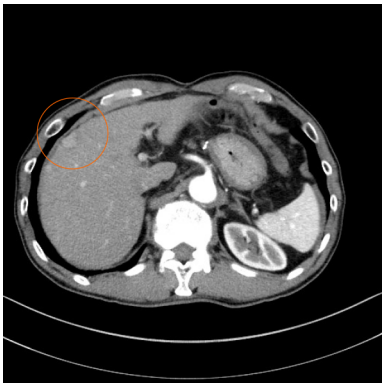

Figure 1 Abdominal contrast-enhanced computed tomography before the first surgery. Arterial phase of abdominal contrast-enhanced computed tomography before the first surgery showed a tumor nodule 20 mm in diameter with early staining located in segment 8 of the liver (orange circle).

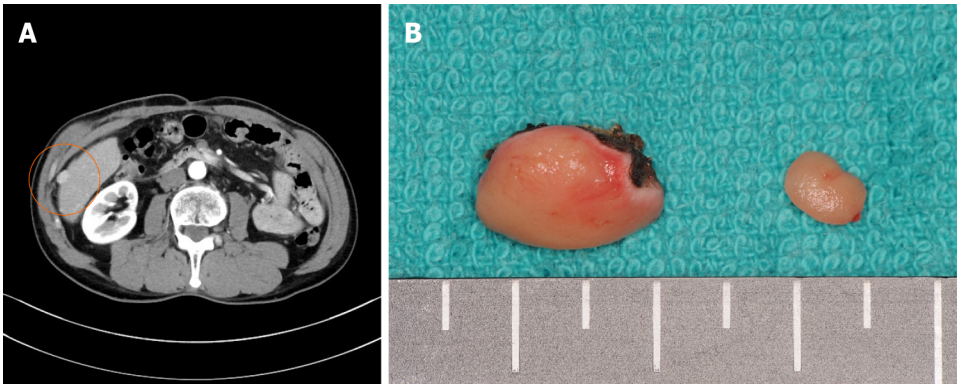

Figure 2 Abdominal contrast-enhanced computed tomography and the surgical specimen from the second surgery. A: Arterial phase of abdominal contrast-enhanced computed tomography before the second surgery showed a tumor 10 mm in diameter, located in segment 6 (orange circle), and protruding to the surface of the liver with early staining; B: Surgical specimen of the liver tumor and peritoneal tumor at the second surgery.

surgery, abdominal CT revealed a tumor nodule 32 mm in diameter in the pelvis, which was diagnosed as a peritoneal recurrence of HCC (Figure 3A). We determined that the recurrent tumor was solitary and decided to perform tumor resection. However, many small peritoneal nodules were found at the time of laparotomy, and radical resection was impossible (Figure 3B). Subsequently, the patient was followed up by the department of gastroenterology of our hospital, and 16 mo after the initial resection of HCC, administration of sorafenib of 400 mg/d was started. Since he developed a grade 2 hand-foot syndrome, the dosage was reduced to 200 mg/d. The administration was continued for 54 mo without any other major adverse events, and the disease was well controlled. Seventy months after the first surgery, he was admitted to our hospital with chief complaints of abdominal pain and vomiting.

History of past illness

The patient's history was significant for extensive gastrectomy for duodenal ulcer at the age of 22 years. In addition, he had a history of hypertension since the age of 65, for which he was on treatment with amlodipine besilate (10 mg/d) and azilsartan (20 mg/d).

Personal and family history

The patient's social history consisted of a 40-pack year history and an alcohol intake of 350 mL beer per day. He had discontinued smoking and drinking alcohol 10 years previously. There was no history of cancer or liver disease in his family.

Physical examination

The height and weight of the patient at admission were 172 cm and 52 kg, respectively. There were no abnormalities in the vital signs. The abdomen was soft and slightly swollen. Tenderness was noted in the right lower abdomen.

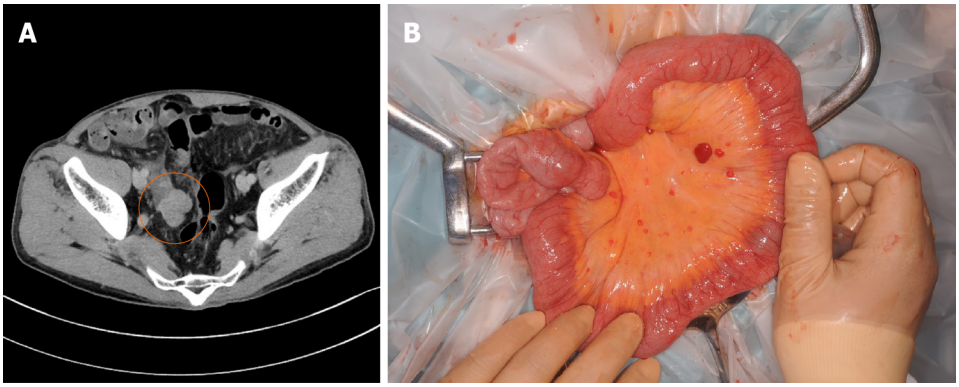

Figure 3 Abdominal contrast-enhanced computed tomography before the third surgery and the intraoperative findings. A: Abdominal contrast-enhanced computed tomography showed a tumor 32 mm in diameter in the pelvis (orange circle); B: Many small peritoneal nodules were found at the time of laparotomy.

Laboratory examinations

No abnormal findings were found other than a high C-reactive protein level (4.95 mg/dL) in blood biochemical tests. Liver function tests revealed a class A Child-Pugh score.

Imaging examinations

Abdominal contrast-enhanced CT revealed a well-defined, rounded, enhancing endoluminal tumor in the small intestine, leading to intussusception and small bowel obstruction (Figure 4). An ileus tube was inserted to decompress the small intestine.

FINAL DIAGNOSIS

On the basis of these findings, the diagnosis was a small intestinal tumor (primary or metastasis), which had caused the intussusception and small bowel obstruction.

TREATMENT

Initially, conservative treatment was initiated because of the peritoneal dissemination of HCC. X-ray examination after contrast infusion through the ileus tube showed no tumor or stenosis in the small intestine other than that at the intussusception site. The patient had fluctuating symptoms, and surgery was planned on the 28th d of hospitalization. During surgery, the intussusception site was found 130 cm distal to the ligament of Treitz. We performed partial ileal resection without reduction of the intussusception, followed by end-to-end anastomosis (Figure 5A). The resected specimen showed a polypoid tumor of size 50 mm protruding into the lumen (Figure 5B).

OUTCOME AND FOLLOW-UP

The postoperative period was uneventful, and the patient was discharged on the 18th postoperative day. The histological examination revealed tumor cells with a cytoplasm rich in eosinophilic granules, enlarged nuclei, and distinct nucleoli that showed dense proliferation in the lesion (Figure 6A). No tumor cells were observed on the serosal surface, but intravascular invasion of tumor cells was observed (Figure 6B). Immunohistochemistry was positive for alpha-fetoprotein (AFP), Hep Par1, and Glypican3[7], and a diagnosis of hematogenous metastasis of HCC to the ileum was made (Figure 6C-E). Since right adrenal metastasis was found on a follow-up abdominal CT 78 mo after the first surgery, administration of Lenvatinib of 8 mg/d was started. The patient continues to survive 82 mo after the initial surgery without any major adverse events of Lenvatinib.

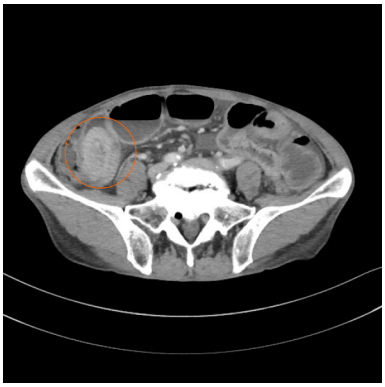

Figure 4 Abdominal contrast-enhanced computed tomography demonstrated an intussusception of the small intestine due to a well-defined, rounded, enhancing endoluminal mass (orange circle).

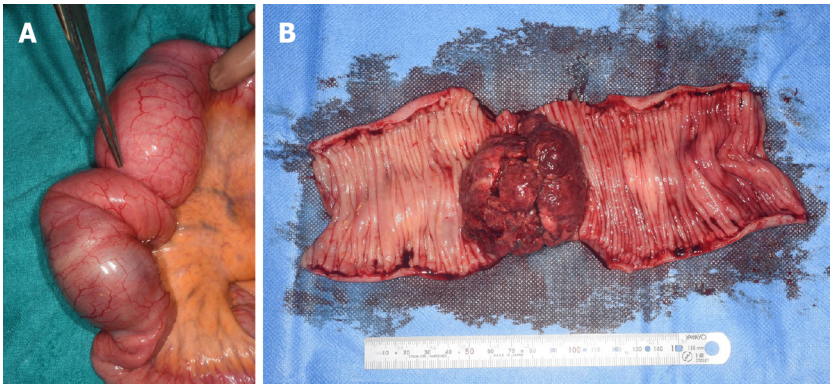

Figure 5 Intraoperative findings and the resected specimen. A: The intussusception site was found 130 cm distal to the ligament of Treitz; B: The resected specimen showed a polypoid tumor 50 mm in diameter protruding into the lumen.

DISCUSSION

HCC is one of the most common malignancies globally, and its incidence has been increasing in the recent years. The long-term outcomes of HCC are disappointing because of the high rates of recurrence and metastasis. In an autopsy series, GI involvement of HCC was found in only 4%-12% of cases[8,9]. The metastasis of HCC to the GI tract is mostly through direct invasion to the adjacent GI tract *via* adhesion to the serosal side. The most frequently involved sites are the duodenum, stomach, hepatic flexure of the colon, and jejunum.

Park *et al*[10] reported that the modes of metastases were direct invasion of contiguous HCC (66.7%), hematogenous metastasis (16.7%), and peritoneal dissemination (5.6%). Thus, another mode of metastasis of HCC to the GI tract comprises the hematogenous spread. This is caused by tumor thrombosis and invasion *via* the portal system, and is disseminated by the hepatofugal portal blood flow to the GI tract. According to the literature, the interval between diagnosis of HCC and detection of the GI tract involvement ranged from 3 mo to 8 years[11,12]. Metastatic lesions in the small intestine are usually asymptomatic and are not easily discovered. GI metastasis is mostly found in HCC patients with an advanced stage, and it has a poor prognosis, with a median survival period of 7 mo[5].

In our patient, the serosal side of the ileum was free from tumor cells, and intravascular invasion of tumor cells was observed. Hence, we diagnosed that hematogenous metastasis to the ileum had occurred and it had spread in the lumen. Unlike previous reports, the tumor size of HCC was not large, and portal vein thrombosis was not detected at both the primary HCC and recurrent HCC stage. However, peritoneal dissemination was observed during the second surgery, and recurrence occurred relatively early after the first surgery. On the contrary, metastasis to the ileum occurred 70 mo after the first surgery. It was determined that disease control was good with sorafenib, a multikinase inhibitor with antiproliferative, antiangiogenic, and proapoptotic properties.

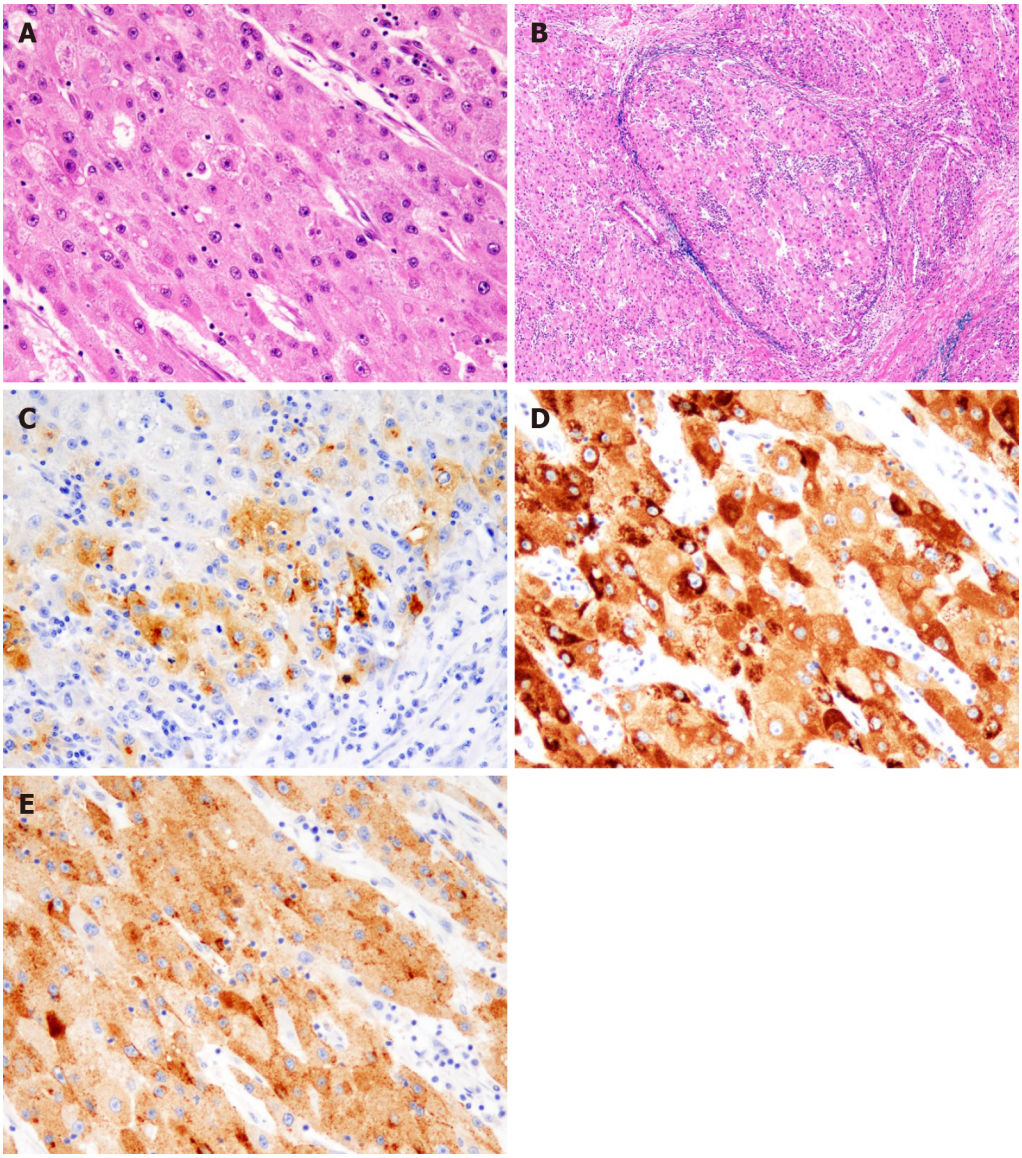

Figure 6 Histopathological findings and immunohistochemistry. A: Histological findings showed tumor cells with cytoplasm rich in eosinophilic granules, enlarged nuclei, and clear nucleoli that showed dense proliferation on hematoxylin and eosin staining (× 400); B: Intravascular invasion of tumor cells were observed on Victoria blue staining (× 40); C: Alpha-fetoprotein (AFP) positive cells were observed on immunostaining (× 400); D: Hep Par1 positive cells were observed on immunostaining (× 400); E: Glypican3 positive cells were observed on immunostaining (× 400).

Intussusception is common in children, whereas it is a rare condition in adults, who account for only 5% of the cases of intussusceptions. It is a rare cause of intestinal obstruction in adults (< 1% cases)[13,14]. According to the etiology of adult intussusception, the rates of malignant tumor, benign tumor, and idiopathic causes were 32.9%, 37.4%, and 15.1%, respectively[15]. Breast cancer, lung cancer, and malignant melanoma are reported to be the major causes of small bowel obstruction due to metastatic tumors[16]. Reports of intussusception and small bowel obstruction due to small intestinal metastasis of HCC are extremely rare. Based on the review of previously published studies, there are only two cases reported so far, including our own case[17].

Surgical treatment of extrahepatic metastasis of HCC has still not been established. The prognosis of patients at this stage continues to be poor due to limited effective treatment options. However, despite the limited number of cases, it has been reported that the prognosis improved after surgical resection of isolated extrahepatic metastases of HCC. Resection of isolated lung metastasis of HCC has been reported to improve prognosis in selective patients. Takahashi *et al*[18] reported that disease-free interval of more than 12 mo was significantly associated with favorable outcomes in both overall survival (5-year rate, 59.3% *vs* 28.7%; P = 0.026) and disease-specific survival (5-year rate, 62.5% *vs* 36.2%; P = 0.038) in patients who underwent pulmonary resection. Chan *et al*[19] reported that surgical resection of extrahepatic metastasis from HCC should be

considered in patients with one or two isolated extrahepatic metastases if they had a good performance status, good liver function, and well-controlled intrahepatic HCC. Uka *et al*[20] also reported that in the treatment of patients with extrahepatic metastases of HCC, relieving portal venous invasion may improve survival. Chua *et al*[21] suggested that when resection of extrahepatic metastasis of HCC is performed, it should be combined with the most effective systemic therapy that is currently available.

In general, GI metastasis of HCC has a poor prognosis. However, as in this case, extrahepatic metastasis can occur even in patients with an early tumor stage and negative portal vein invasion or occlusion. Since good disease control of intrahepatic lesions and metastatic lesions was accomplished by systemic chemotherapy, and because of the long interval before the patient developed small intestinal metastasis, it is considered that the patient achieved long-term survival due to multidisciplinary therapy.

CONCLUSION

We herein report an extremely rare case of intussusception due to hematogenous metastasis of HCC to the ileum. Even if the prognosis of patients with GI tract metastasis of HCC is poor, selected patients may have prolonged survival because of multidisciplinary therapy including surgical resection.

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