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Contents

Weekly Volume 28 Number 34 September 14, 2022

REVIEW

MINIREVIEWS

ORIGINAL ARTICLE

Basic Study

Retrospective Cohort Study

Retrospective Study

World Journal of Gastroenterology

Weekly Volume 28 Number 34 September 14, 2022

Contents

World Journal of Gastroenterology

Weekly Volume 28 Number 34 September 14, 2022

Contents

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REVIEW

Therapeutic strategies for post-transplant recurrence of hepatocellular carcinoma

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Abstract

Despite stringent selection criteria, hepatocellular carcinoma recurrence after liver transplantation (LT) still occurs in up to 20% of cases, mostly within the first 2–3 years. No adjuvant treatments to prevent such an occurrence have been developed so far. However, a balanced use of immunosuppression with minimal dose of calcineurin inhibitors and possible addition of mammalian target of rapamycin inhibitors is strongly advisable. Moreover, several pre- and post-transplant predictors of recurrence have been identified and may help determine the frequency and duration of post-transplant follow-up. When recurrence occurs, the outcomes are poor with a median survival of 12 mo according to most retrospective studies. The factor that most impacts survival after recurrence is timing (within 1–2 years from LT according to different authors). Several therapeutic options may be chosen in case of recurrence, according to timing and disease presentation. Surgical treatment seems to provide a survival benefit, especially in case of late recurrence, while the benefit of locoregional treatments has been suggested only in small retrospective studies. When systemic treatment is indicated, sorafenib has been proved safe and effective, while only few data are available for lenvatinib and regorafenib in second line. The use of immune checkpoint inhibitors is controversial in this setting, given the safety warnings for the risk of acute rejection.

Key Words: Liver transplantation; Hepatocellular carcinoma; Immunosuppression; Recurrence; Surgical treatment; Locoregional treatment; Systemic treatment

Core tip: Hepatocellular carcinoma (HCC) is becoming the most common indication for liver transplantation (LT). The problem of tumor recurrence after LT, that occurs in up to 20% of cases, is becoming of increasing interest. We reviewed of the available literature on HCC recurrence after LT. The best preventive measures still rely on pretransplant selection criteria, since no dedicated follow-up guidelines exist and no post-LT adjuvant treatments are available. When recurrence occurs, the prognosis is poor. However, aggressive surgical treatment, particularly in the case of late recurrence, may provide a significant survival benefit.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common liver cancer and the fourth leading cause of cancer-related mortality[1,2]. From the time of its initial developments in the early 1960s, liver transplantation (LT) appeared as the ideal cure for HCC on liver cirrhosis because of the perspective to cure at the same time both the tumor and the underlying liver disease. However, the first experiences were disappointing with many authors reporting 5-year survival < 40%, mainly because of recurrences of the primary tumor[3-6]. A retrospective review of these discouraging results progressively led to the observation that survival of patients was directly related to the stage of HCC at the time of LT. This was the basis on which a prospective study was conducted in Milan applying *a priori* restrictive criteria for the selection of HCC candidates for LT (namely a single nodule ≤ 5 cm or two or three nodules ≤ 3 cm, each with no macrovascular invasion at pretransplant imaging). The seminal paper published in 1996 demonstrated that LT under such criteria achieved better long-term results than any other therapy, with outcomes similar to LT for nononcological indications[7]. The so called Milan criteria (MC) were subsequently validated by many other groups reporting 5-year survival rates of ≥ 70%, and became the benchmark for selecting patients with HCC for LT. Pooled recurrence rates have been reported to be around 8% for patients within MC *versus* 28% for patients beyond these criteria, according to a recent meta-analysis[8]. Thus, HCC recurs in a proportion of recipients who are within MC, while LT may provide cure for some patients who are beyond these criteria. When recurrence occurs, survival is poor and post-LT HCC recurrence is the factor that most affects long-term outcomes in this setting. Considering that HCC represents the most common indication for LT in the USA, and that since the introduction of direct antiviral agents against hepatitis C virus the proportion of patients undergoing LT for HCC is increasing worldwide[9,10], the problem of tumor recurrence will probably affect a growing number of patients. However, to date, treatment of HCC recurrence following LT is largely understudied and dedicated guidelines are lacking. The aim of this paper is to provide a review of the current evidence on therapeutic strategies for patients with HCC recurrence after LT.

PREVENTION OF POST-LT HCC RECURRENCE

Tumor recurrence may be linked to remaining (previously undetected) extrahepatic HCC at the time of LT, or result from the post-LT engraftment of circulating HCC clones[11]. It is extrahepatic in 50%–60% of cases, with lung, bones and adrenal glands being the most frequently affected sites[12]. Timing of HCC recurrence is variable, but in most cases it occurs within 3 years after LT. Early recurrence (< 1 year after LT) is associated with a significantly worse prognosis, while later recurrence might result in better outcomes and even in cure in selected cases[13].

Prevention of recurrence through selection criteria

Considering that the risk of post-LT recurrence is strictly related to pretransplant HCC stage and treatment, recurrence is firstly prevented by the application of pre-LT selection criteria able to identify patients at higher risk. Proposals for expansion of MC have been initially developed using tumor morphology, namely size and number of nodules. In fact, these factors have been demonstrated as surrogate markers of microvascular invasion (MVI) and/or poor tumor differentiation, which are the principal determinants of biological aggressiveness and therefore of the risk of post-LT recurrence[14]. Expanded criteria increased the acceptable size and number of HCC nodules with respect to MC, but the considerable heterogeneity coupled with differences in accuracy of liver imaging techniques

probably represent the greatest limitation of criteria based only on morphology.

To overcome these limits, criteria incorporating serum markers as surrogates of biological tumor features such as α-fetoprotein (AFP) have been proposed. In particular, by combining the morphological characteristics of the tumor and AFP values it was possible to develop selection criteria for LT definitively exceeding MC, while even decreasing the risk of post-LT recurrence[15,16]. A strategy combining tumor burden with the assessment of response to pre-LT locoregional treatment (LRT) as a marker of favorable tumor biology has gained broader acceptance[17]. For patients beyond MC, a common strategy is to downstage patients by means of LRT or surgical therapy. In fact, patients successfully downstaged within accepted criteria share the same prognosis as patients within the criteria *ab initio* and so far[18], response to therapies appears as one of the best surrogates of favorable tumor biology and thus an optimal selection tool for candidates for LT[17,19,20]. Patients progressing in the pre-LT period despite LRT have significantly worse post-LT outcomes with respect to patients with stable or responding disease. Finally, tumor differentiation, MVI, presence of circulating cancer cells and genomic markers have also been suggested as selection criteria for LT, but these assessments require biopsy, which might induce tumor seeding. Furthermore, it is well known that tumors are heterogeneous and show areas of varying degrees of differentiation and genomic features.

Post-LT surveillance

Considering that post-LT recurrence is mostly asymptomatic, and that early detection of recurrence may have a positive impact on long-term outcomes, post-LT surveillance has an important role in this setting. However, no guidelines from the major Hepato-bilio-pancreatic societies are available and surveillance protocols are mostly center-specific, often with a high heterogeneity between centers as recently reported[21]. Few retrospective studies on post-LT surveillance have been published to date, and several questions regarding frequency, duration, and imaging modality for a cost-effective surveillance remain open.

For imaging modality, cross-sectional imaging of the abdomen [with either multiphase computed tomography (CT) or magnetic resonance imaging] and noncontrast lung CT scan allow detection of the most frequent sites of recurrent HCC[21,22]. Cross-sectional imaging of the brain, bone scintigraphy or positron emission tomography–CT are indicated only in case of clinical suspicion and not on a regular basis, while it seems reasonable to check for AFP levels at each surveillance visit even if no data is available to support this indication.

Given that the majority of recurrences occur within the first 3 years after LT, there is general agreement to indicate surveillance imaging and visits more frequently in this time frame (*i.e.*, every 4–6 mo) and yearly thereafter[22]. Some authors suggest interrupting surveillance after 5 years. However, recurrences (either *de novo* tumors or true recurrences) have been repeatedly reported up to 10–15 years after LT[23]; considering that late HCC relapse is associated with a better prognosis with respect to earlier events and that it is sometimes curable, it seems reasonable to prolong yearly surveillance for at least ten years.

Ideally, frequency and duration of surveillance would be based on the assessed risk of post-LT HCC recurrence. Several proposals have been made in this sense, and the RETREAT score[24] (that includes AFP at LT, presence of MVI and sum of maximum size + number of vital nodules) is the most recent and promising predictor in terms of discriminative power and validation on a large scale registry. However, no prospective validation is available to date, and the cost-effectiveness of a surveillance program based on the risk of recurrence has yet to be demonstrated.

The impact of surveillance programs on post-recurrence survival has been scarcely studied. A recent multicenter study on 232 patients who experienced HCC recurrence found that increasing number of post-LT surveillance scans (with cut-off at three surveillance scans within the first 2 years) was associated with improved survival and possibility of undergoing potentially curative treatments[25].

Role of immunosuppression

Improvements in the management of immunosuppression reduced rejection episodes favoring long-term graft survival; calcineurin inhibitors (CNIs) tacrolimus and cyclosporine played a fundamental role in this improvement. However, several studies demonstrated that CNI exposure is associated with an increased risk of tumor recurrence with a dose-dependent effect[26]. It is likely that the immunosuppression induced by CNIs prevents the immune system from detecting and destroying circulating or dormant HCC cells and therefore, dosage of CNIs should be maintained with the aim of balancing this risk without increasing the risk of rejection episodes.

Mammalian target of rapamycin inhibitors (mTORis) sirolimus and everolimus are another class of immunosuppressants targeting some HCC pathways, which showed antiangiogenic and antiproliferative effects in experimental models[27]. Data from retrospective studies and meta-analyses suggest that, compared to CNIs, the use of mTORis reduces the risk of post-LT HCC recurrence and increases long-term survival. In the most recent meta-analysis including 23 comparative studies [17 observational and 6 randomized controlled trials (RCTs)] with 6495 patients, recurrence-free survival (RFS) was significantly increased with mTORi-based therapy at 1 and 3 years with a nonsignificant increase at 5 years[28]. Overall survival (OS) was also significantly improved, as well as recurrence rate being lower in the mTORi arm without differences based on the type of mTORi. However, only one RCT that

compared post-LT immunosuppression containing mTORi (sirolimus) *versus* not containing mTORi [29]. In this international RCT of 525 patients there appeared to be an advantage in the sirolimus group regarding RFS in the first 3–5 years. However, this benefit was subsequently lost with further follow-up and the trial failed to meet the primary endpoint of demonstrating a significant reduction of recurrences in the mTORi-containing immunosuppression group.

Adjuvant treatments after LT

Several attempts had been performed with chemotherapy as an adjuvant treatment to prevent HCC recurrence after LT[30,31]. HCC is a chemoresistant tumor; therefore, cytotoxic systemic therapies have failed to provide any consistent benefit in this setting and have been abandoned in the last decade[32]. Sorafenib, an oral multikinase inhibitor that shows significant improvement in survival of patients with advanced HCC, has been tested in small studies in the setting of adjuvant treatment for HCC after LT. Despite some initial signs of efficacy, with one phase I study showing a significant reduction in the risk of HCC recurrence with a maximum tolerable dose of sorafenib 200 mg twice daily[33], other single-center case series failed to confirm these data and to date no RCTs are available. Lenvatinib, a more recent targeted therapy for advanced HCC, has not been prospectively tested in the adjuvant setting; a small retrospective case series confirmed an acceptable drug safety and patient tolerance but did not show any significant reduction in terms of HCC recurrence[34]. Immune checkpoints inhibitors (ICIs) have emerged as a treatment option for advanced-stage HCC. No studies are available on ICIs as post-LT adjuvant treatment. A recent systematic review and pooled analysis reviewed 14 patients receiving ICIs for recurrent disease after LT for HCC: 11 of them (78.6%) died, and graft rejection was the cause of death in five cases (45.4%). The high rejection rate raises the question of safety of ICIs in transplanted patients, even in the setting of overt recurrence[35]. Thus, to date, no anticancer treatments can be recommended to prevent HCC recurrence after LT, and it is unlikely that they will become available in the near future.

TREATMENT OF POST-LT HCC RECURRENCE

Literature concerning the efficacy of each treatment modality is scarce, with the many limitations related to the small number of patients included, the frequent use of combined treatment and the different patterns of recurrence, all acting as confounding factors. In the majority of cases (50%–60%) recurrence is extrahepatic and affects the following sites: lungs (40%–60%), bones (25%–30%), adrenal glands (10%), lymph nodes (10%) and peritoneum (10%)[12]. Liver-only recurrence occurs in 15%–40% of patients, while combined liver and extrahepatic recurrence accounts for 30%–40% of cases. The therapeutic options clearly depend on location, multifocality and clinical presentation of recurrence (Figure 1).

Surgery

Liver resection is safe and provides a survival benefit in case of intrahepatic oligorecurrence[36,37], with a median survival of 28–65 mo observed for patients receiving surgery, compared to 5–15 mo in those receiving systemic treatment only[38-41]. Surgical treatment is feasible in 25%–50% of cases with higher morbidity rate (60%–80%) with respect to primary liver resections[42-44], mainly because of the risk of infections in the context of immunosuppression.

Sapisochin *et al*[39] retrospectively analyzed 121 patients with HCC recurrence after LT, finding that not being amenable to resection or ablation was an independent predictor of poor prognosis [hazard ratio (HR) = 4.7, 95% confidence interval (CI): 2.7–8.3). An Italian multicenter study analyzed 21 patients with recurrence and reported a significantly better 4-year survival rate in patients treated with surgical resection for intra- and extrahepatic recurrence compared to those with unresectable disease (57% *vs* 14%, P = 0.02)[45]. In another series of 106 patients, treatment for recurrent HCC most commonly included chemotherapy (73.5%), surgical resection (23.3%), external beam radiation (13.6%), and ablation (3.9%), with the majority of patients receiving nonsurgical therapies (59.2%). The highest survival rates at 3 years were observed in patients receiving surgical therapy alone (60%), followed by patients receiving both surgical and nonsurgical therapy (37%), patients receiving only nonsurgical therapy (11%), and patients receiving no treatment (0%)[40]. Time from LT to recurrence is one of the most important prognostic factors, and patients with late recurrence show more favorable 5-year outcomes survival with resection compared to those of patients who recur earlier[46].

Surgery may enhance long-term survival also in patients with pulmonary recurrences amenable to resection, with 5-year survival rates ranging from 34% to 44% in those undergoing metastasectomy[47-52]. A benefit from surgical treatment is also reported for other sites of recurrence in smaller case series, including vertebrae[53], adrenal glands[54,55], lymph nodes[56], peritoneum[57] and pharynx[58]. In patients with multiple recurrences, some benefits have also been gained from repeated resections, probably reflecting less aggressive tumor biology[41].

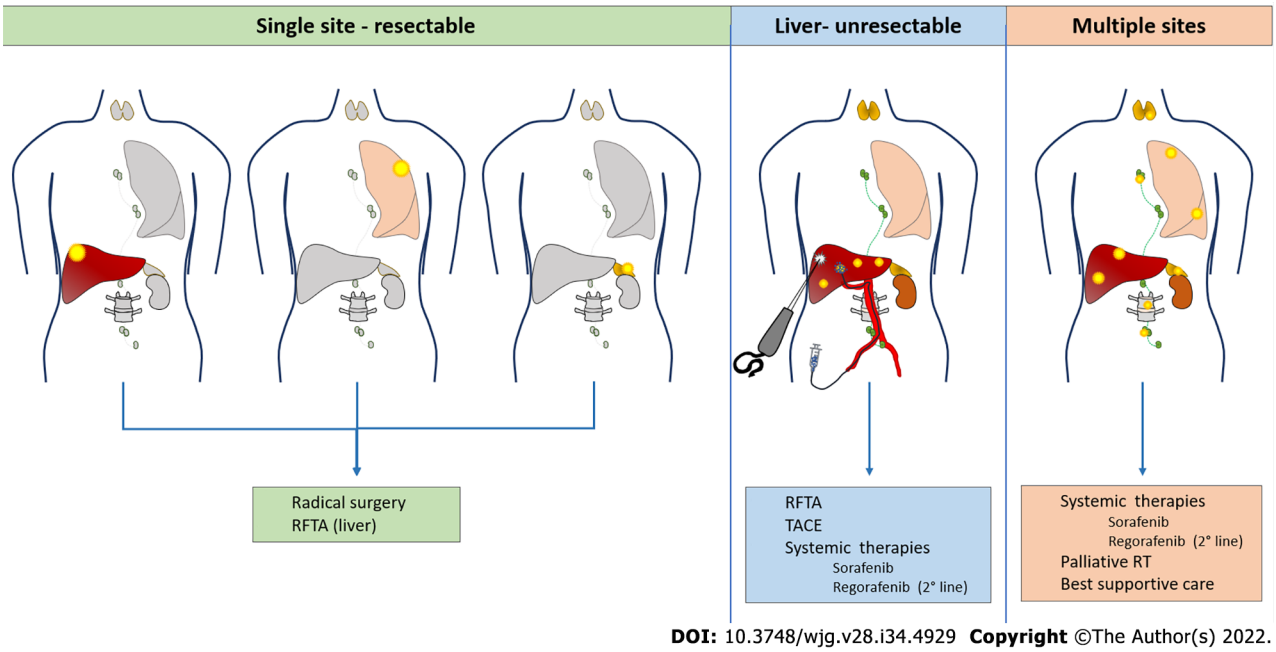

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Figure 1 Hepatocellular carcinoma recurrence after liver transplantation: Treatment possibilities according to disease presentation. RT: Radiotherapy; RFTA: Radiofrequency thermal ablation; TACE: Transarterial chemoembolization.

Locoregional therapies

Radiofrequency ablation (RFA) for liver recurrences may be proposed with a curative intent for small lesions, with the advantages of a percutaneous approach. In a retrospective single-center series, Huang *et al*[44] compared 15 patients with post-LT HCC recurrence treated surgically with 11 patients treated with RFA. This study demonstrated similar 5-year OS (35% for surgery *vs* 28% for RFA) but a tendency to a worse 5-year disease-free survival in the RFA group (16% *vs* 0%). Another study evaluating safety and efficacy of microwave ablation on a series of 11 patients found this technique safe and tolerable, with a 15.8% rate of local tumor progression after treatment and 15.3% survival at 2 years[59].

Multifocal intrahepatic recurrences may be amenable to transarterial chemoembolization (TACE). The largest series collected 28 patients treated by conventional TACE[60]. There were no significant post-treatment complications and the targeted tumor reduced in size by ≥ 25% in 19 patients (67.9%). However, intrahepatic recurrence or extrahepatic metastases occurred in 26 patients (92.9%) within 6 mo. The 3- and 5-year survival rates following TACE were 6% and 0%, respectively, with a mean survival time of 9 mo. A single-center retrospective investigation compared 14 patients treated with TACE with 14 matched controls who did not receive TACE but chemotherapy, radiotherapy or supportive care. Eight of the 14 patients treated with TACE (57%) showed partial tumor response and had a significantly longer survival compared to those who did not[61].

Systemic therapies

The effectiveness of systemic therapies for HCC recurrence following LT is largely unstudied because these patients have been routinely excluded from clinical trials. Sorafenib has been increasingly administered for treatment of post LT recurrences, and some data have been collected in the literature regarding its safety and efficacy in this setting. In a case–control study from Sposito *et al*[62], sorafenib provided improved median survival after HCC recurrence untreatable by surgery or LRT with respect to best supportive care (10.6 *vs* 2.2 mo). A meta-analysis published 2 years later reported a pooled 1-year survival of 36% (range 18%–90%)[63]. The main limitation of sorafenib in transplanted patients is toxicity, often leading to dose reduction, as reported by several studies[62,64]. Close monitoring is warranted for these patients, particularly in case of immunosuppression with mTORis, since this association may lead to severe adverse events[65-70]. Regorafenib may be proposed as a second-line treatment in case of progression under sorafenib[71]. In a recent multicenter study, second line treatment with regorafenib after sorafenib discontinuation provided a median survival of 13.1 mo compared with 5.5 mo with best supportive care in post-LT HCC relapse[72]. Recently approved tyrosine kinase inhibitors (lenvatinib[73] and cabozantinib[74]) and monoclonal antibodies (ramucirumab[75]) will soon be introduced in clinical practice also for the treatment of post-LT recurrence, giving us the opportunity to collect data about their efficacy and toxicity in this setting[76]. Currently, immunotherapy is changing the landscape of systemic therapies for HCC[77-79], but its safety after LT represents a point of concern, since ICIs may cause allograft rejection and other serious adverse events[80-85].

FACTORS IMPACTING SURVIVAL AFTER HCC RECURRENCE

Studies evaluating the outcome of patients with post-LT HCC recurrence mostly consist of small and heterogeneous series burdened by significant biases in terms of transplant criteria, availability of different treatments and patients' selection to curative and palliative options[86]. Survival of post-LT HCC recurrence is dismal and significantly worse than relapse after resection (median OS around 12 mo *vs* nearly 2 years in transplanted and resected patients, respectively), and immunosuppression is a potential driver of such a difference[87,88]. A number of factors have an impact on survival, and there is a small subset of patients with more favorable prognosis in whom curative treatments may be undertaken. Table 1 summarizes the results of studies evaluating the prognostic factors and outcome of treatment for HCC relapse after LT.

Time to recurrence and primary tumor features

Several studies showed that time from LT to recurrence has a primary role on outcomes, with early relapse being associated with poor prognosis either when defined as occurring within 6 mo[89-93], 1 year[39,94,95] or 2 years[96]. Many factors related to the primary tumor biology and aggressiveness affect time to recurrence and/or post-relapse survival: size (with cutoffs > 30 or > 50 mm)[45,97], staging outside MC[45], bilobar spread[97], absence of peritumoral capsule[45], poorly differentiated tumors[91,94,95], total tumor volume[92], presence of micro- or macrovascular invasion and pre-LT lymphocyte to neutrophil ratio[39,40,91,92]. The use of mTORi in the post-LT setting seems to be related to better post-recurrence outcomes, as shown both in eastern and western series[93,95]. Moreover, a history of graft rejection has also been associated with improved outcomes, possibly due to more active anticancer immunity[92].

It has been suggested that the observed difference in outcomes between early and late recurrences lies in different underlying biological mechanisms. However, this does not turn into a difference in the site of recurrence. In fact, occurrence of extrahepatic, combined intra- and extrahepatic or intrahepatic relapses do not seem to be different in early *versus* late recurrences[97]. While early relapses may be due to undetected extrahepatic metastases or circulating HCC clones implanting in a target organ during or soon after LT, late recurrences are possibly related to a second hit leading to late engrafting of HCC cells remaining latent during the initial post-LT period. In the latter, immunosuppression may also play a role[23]. As for intrahepatic late relapses, a further mechanism to be considered is *de novo* occurrence of HCC, usually arising in the context of chronic liver disease or cirrhosis due to recurrence of primary hepatitis, ischemic biliary injury or chronic rejection, several years after LT[98]. In such instances, results are expected to parallel those of nontransplant recipients with localized HCC, in which surgery or LRTs are effective in controlling the disease.

Pattern, features and resectability of recurrence

Aside from primary disease features, other studies have focused on the pattern of recurrence as a relevant prognostic factor for post-recurrence survival. As expected, limited disease spread with localized nodules (oligorecurrence), either hepatic or extrahepatic, has been associated to better outcomes than disseminated multifocal recurrence in several series[36-38,41,45,90]. In addition, a different prognostic impact of hepatic *versus* extrahepatic localization has been repeatedly reported. Hong *et al* showed that liver involvement as the first recurrence site was associated with worse survival, with fewer patients amenable to resection among intrahepatic rather than extrahepatic localizations[93]. A monocentric French series on 70 HCC recurrences also identified intrahepatic location as an unfavorable prognostic factor, which was confirmed in a Latin American series on 105 post-LT recurrences showing a lower probability of treatment in patients with hepatic relapses[41,94]. It may be speculated that this is related to the biological mechanism underlying tumor relapse, with recurrences due to undetected metastases at the time of LT more likely to occur at extrahepatic sites and associated with decreased burden as compared to circulating HCC clones, biologically more aggressive tumors, and being more likely to implant in the new liver. Of note, peritoneal and bone localizations were also reported as a poor prognostic factors[37,91,95]. Nevertheless, evidence deriving from several studies shows that the best outcomes are observed in patients with unifocal, often extrahepatic disease, easily amenable to surgical resection[36-38,41,45]. In the large series by Sapisochin *et al*[39] cited above, not being amenable to curative-intent treatment (resection or ablation) was an independent indicator of poor survival, together with AFP ≥ 100 ng/mL at the time of relapse. In another single-center study from the USA by Bodzin *et al*[40] on 106 recurrences, a prominent prognostic role of recurrence-related factors (AFP at relapse, > 3 nodules, maximum size of recurrence and bone spread) rather than primary disease features was shown. By combining such factors, a risk score model was built, with accurate stratification of recurrent patients into low-risk (median survival of 70.6 mo), medium-risk (12.2 mo) and high-risk (3.4 mo) subgroups.

Due to selection bias of surgical patients towards later recurrences, more favorable localizations, less aggressive disease and better performance status, the independent prognostic role of either recurrence pattern or resectability is questionable. The limited disease spread may make patients more likely to undergo surgical excision on the one hand, or simply reflect a different tumor biology, etiology, and

Table 1 Studies evaluating the prognostic factors and outcome of treatment for hepatocellular carcinoma relapse after liver transplantation

Ref.	No. of patients	Type of study	Site of recurrence	Treatment	mTTR	mOS	Negative outcome predictors
Sapisochin *et al* [39], 2015	121 (15.5%)	Retrospective multicenter	18.4% liver, 47.4% extrahepatic, 34.2% liver + extra	31.4% surgery/ablation, 42.1% palliative, 26.4% BSC	14 mo	12.2 mo (1 yr 54%; 3 yr 19%; 5 yr 14%)	No curative treatment. RFS < 1 yr. AFP > 100 ng/mL
Ho *et al*[96], 2020	349 (16.4%)	National registry	≥ 38.1% liver, ≥ 20.3% extrahepatic/(liver + extra)	4.6% surgery, 6.3% ablation, 32.1% RT, 27.2% TACE, 20.3% Sorafenib, 20.3% BSC	17.8 mo	11.2 mo (1 yr 57%; 3 yr 24.7%; 4 yr 19%)	LT era > 2008 (due to listing of downstaged patients). No curative treatment. Sorafenib/RT
Hong *et al*[93], 2019	92 (17.3%) (LDLT)	Retrospective multicenter	37% liver, 34.8% lung, 28.3% bone, 16.3% lymph nodes	38% surgery, 51.1% TACE, 38% RT, 45.7% Sorafenib	11.3 mo	11.7 mo (1 yr 59.5%; 3 yr 23%; 5 yr 11.9%)	TTR < 6 mo. No curative treatment. Multiorgan involvement. Explant tumor size > 5 cm. mTORi (late)
Toso *et al*[92], 2013	30 (12.8%)	Retrospective multicenter	46.6% liver, 43.3% lung, 23.3% bone, 13.3% other	20% surgery, 10% TACE/RF/PEI, 70% CT/BSC	14.2 mo	33 mo	Graft rejection 0-6 mo. TTR
Bodzin *et al* [40], 2017	106 (12.4%)	Retrospective multicenter	37.8% liver, 55.7% lung, 25.5% bone, 3.8% brain	23.3% surgery, 3.9% RFA, 13.6% RT, 73.5% CT, 17% BSC	15.8 mo	10.6 mo	MELD at LT > 23. TTR. > 3 recurrent nodules. Size of recurrence. Bone recurrence. AFP at recurrence. Donor Na. Pre-LT NLR
Fernandez-Sevilla *et al*[41], 2017	70 (14.2%)	Retrospective single center	2.8% liver, 72.9% extrahepatic, 24.3% liver + extra	31.4% surgery, 8.6% TACE, 28.6% Sorafenib	17 mo	19 mo (1 yr 65%; 3 yr 26%; 5 yr 5%)	AFP > 100 ng/mL. Intrahepatic. Multifocal. No surgical treatment
Maccali *et al* [94], 2021	105 (16.6%)	Retrospective multicenter	23.8% liver, 21% liver + extra, 55.2% extrahepatic	9.5% surgery, 2.9% TACE, 4.8% RT, 44.8% CT/Sorafenib	13 mo	6.2 mo	RFS < 1 yr. No surgical, loco-regional or systemic treatment
Ekpanyapong *et al*[95], 2020	96 (13.5%)	Retrospective single center	21.9% liver, 78.1% extrahepatic/(liver + extra)	27.1% surgery. 5.2% RFA. 1% TACE. 10.4% RT. 39.6% Sorafenib. 16.7% BSC	17.1 mo	10.1 mo (1 yr 48%; 3 yr 16%)	AFP > 1000 ng/mL. Poorly differentiated HCC. Bilirubin ≥ 1.2 mg/dL and albumin < 3.5 mg/dL at recurrence. Peritoneal disease
Regalia *et al* [45], 1998	21 (15.9%)	Retrospective multicenter	19% liver, 19% lung, 14% bone, 38% multiple sites	33.3% surgery, 19% CT, 14.3% RT/CT-RT, 23.8% BSC	7.8 mo	1 yr 62%; 3 yr 29%; 4 yr 23%	Related to early recurrence: Explant tumor size > 3 cm; outside Milan Criteria; absence of capsule
Kornberg *et al* [38], 2010	16 (26.7%)	Retrospective single center	25% liver, 25% bone, 31.2% lung,6.2% brain, 6.2% peritoneum, 6.2% adrenal gland	43.7% surgery, 18.7% RT, 6.2% TACE, 6.2 Sorafenib, 31.2 %BSC	23 mo	10.5 mo	No surgical treatment. Early recurrence (< 24 mo)
Alshahrani *et al* [57], 2018	232 (15.6%)	Retrospective single center	31% liver, 57.8% extrahepatic, 13.4% multiple sites	-	-	1 yr 60.2%; 3 yr 28.3%; 5 yr 20.5%; 10 yr 7%	Early recurrence
Taketomi *et al* [100], 2010	17 (16.8%) (LDLT)	Retrospective single center	-	53% surgery, 47% other	12.9 mo	1 yr 76.5%; 3 yr 51.3%; 5 yr 34.2%	No surgical treatment. Early recurrence
Roh *et al*[90], 2014	63 (13.8%)	Retrospective single center	22% liver, 16% lung, 52% multiple sites, 10% other	6% surgery, 38% local treatment, 16% systemic treatment, 33% combined treatment, 13% BSC	12.9 mo	12.2 mo	Bone involvement. Early recurrence (< 6 mo). Multi-organ
Valdivieso *et al* [36], 2010	23 (12.6%)	Retrospective single center	8.7% liver, 21.7% liver + extra, 69.5% extrahepatic	47.8% surgery, 17.4 systemic treatment, 34.8% BSC	23.4 mo	R0 33.2 mo, other 11.9 mo	R0 surgical treatment
Mehta *et al* [101], 2020	84 (11.6%)	Retrospective multicenter	26.2% liver, 48.8% extrahepatic, 25% multiple sites	-	13 mo	-	-
Sharma *et al*	17 (18%)	Retrospective	35.3% liver, 64.7%	-	25.2	-	-

[102], 2012		single center	multiple sites		mo		
Shin *et al*[91], 2010	28 (20.3%) (LDLT)	Retrospective single center	50% liver, 25% extrahepatic, 25% multiple sites	Liver: TACE. Extrahepatic: Systemic therapy/RT	7.9 mo	11.7 mo (1 yr 52.8%; 3 yr 15.8%)	Major vascular invasion. Poorly differentiated HCC. No surgical treatment. Bone metastases
Schlitt *et al* [103], 1999	39 (56.5%)	Retrospective single center	23.1% liver, 38.5 liver + extra, 38.5% extrahepatic	38.4% surgery, 41% BSC, 12.8% systemic treatment, 2.5% TACE, 5.1% RT	14.5 mo	8 mo (non-surgical treatment)	-
Escartin *et al* [104], 2007	28 (15.2%)	Retrospective single center	14.3% liver, 46.4% extrahepatic, 39.3% multiple sites	-	-	7 mo	-
Cescon *et al* [105], 2010	34 (12%)	Retrospective single center	8.8% liver, 20.6% extrahepatic, 70.6% multiple sites	100% systemic treatment (in combination with: 5.9% surgery, 3% RT, 3% RFA, 3% IA CT)	12 mo	-	-
Roayaie *et al* [37], 2004	57 (18.3%)	Retrospective single center	15.8 % liver, 52.6% extrahepatic, 31.6% multiple sites	31.6% surgery, 5.2% TACE, 26.3% systemic treatment, 7% RT, 29.8% BSC	12.2 mo	8.7 mo	Bone metastases. No surgical treatment. Early recurrence

RFS: Relapse free survival; OS: Overall survival; RT: Radiotherapy; BSC: Best supportive care; IA CT: Intra-arterial chemotherapy. RFA: Radiofrequency ablation; LT: Liver transplant; mTTR: Median time to recurrence; mOS: Median overall survival; TACE: Transarterial chemoembolization; HCC: Hepatocellular carcinoma; PEI: Percutaneous ethanol injection; LDLT: Living donor liver transplantation.

stage of recurrence on the other hand, as compared to more advanced cases of multifocal recurrence. Even when radical resection cannot be undertaken, it is widely accepted that any kind of treatment of recurrence has a positive prognostic impact. In the multicenter Latin American study, propensity score matching was used to evaluate the adjusted treatment effect considering selection bias. Patients treated with both sorafenib and surgery/TACE had better survival compared to the best supportive care regardless of time to recurrence[95]. Although randomized data are unlikely to be available in this context and retrospective comparisons are impaired by intrinsic differences between single site/oligometastatic and disseminated recurrence, surgical treatment remains an independent predictor of improved outcome following post-LT recurrence[37-39,41].

Serum markers

As for primary disease and post-LT outcome, AFP at recurrence as an indicator of disease spread, MVI and biological aggressiveness was frequently reported as a strong predictor of prognosis, with cutoffs varying from 100 to 1000 ng/mL[39-41,95]. The difference in survival for patients with high AFP was evident regardless of curative-intent treatment, confirming its value as a marker of unfavorable biological features, and its ability to guide the clinical management of patients affected by HCC recurrence. Finally, other biochemical markers at recurrence were associated with shorter survival: High bilirubin, possibly as a reflection of graft dysfunction, and low albumin, related to poor nutritional status as a general prognostic factor outlined in several series[96,99].

CONCLUSION

HCC recurrence after LT is still a dreadful event, occurring in up to 20% of cases. It might be prevented by stringent pretransplant selection criteria incorporating biological markers of aggressiveness (such as response to therapy, serum markers, histological factors) in addition to size and number of tumors. Several advances in this sense have been made in the last decade, allowing patients with HCC broader access to LT with more precise prediction of outcomes. In the post-LT period, surveillance should be driven by post-LT risk stratification, and the RETREAT score seems to be the best cost-effective approach. No adjuvant treatments after LT have been validated to prevent HCC recurrence; however, a balanced use of immunosuppression with minimal dose of CNIs and possibly the addition of mTORi is strongly advisable. Median post-recurrence survival is 12 mo: the interplay between time to recurrence (with a negative impact of earlier events) and the possibility of a radical treatment is the strongest determinant of survival.

FOOTNOTES

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REFERENCES

1 **Villanueva A**. Hepatocellular Carcinoma. *N Engl J Med* 2019; **380**: 1450-1462 [PMID: 30970190 DOI: 10.1056/NEJMra1713263]
2 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
3 **Iwatsuki S**, Starzl TE, Sheahan DG, Yokoyama I, Demetris AJ, Todo S, Tzakis AG, Van Thiel DH, Carr B, Selby R, Madariaga J. Hepatic resection vs transplantation for hepatocellular carcinoma. *Ann Surg* 1991; **214**: 221-229 [DOI: 10.1097/00000658-199109000-00005]
4 **Yokoyama I**, Carr B, Saitsu H, Iwatsuki S, Starzl TE. Accelerated growth rates of recurrent hepatocellular carcinoma after liver transplantation. *Cancer* 1991; **68**: 2095-2100 [PMID: 1655200 DOI: 10.1002/1097-0142(19911115)68:10<2095::aid-cncr2820681002>3.0.co;2-y]
5 **Bismuth H**, Chiche L, Adam R, Castaing D, Diamond T, Dennison A. Liver resection versus transplantation for hepatocellular carcinoma in cirrhotic patients. *Ann Surg* 1993; **218**: 145-151 [PMID: 8393649 DOI: 10.1097/00000658-199308000-00005]
6 **Ringe B**, Pichlmayr R, Wittekind C, Tusch G. Surgical treatment of hepatocellular carcinoma: experience with liver resection and transplantation in 198 patients. *World J Surg* 1991; **15**: 270-285 [PMID: 1851588 DOI: 10.1007/BF01659064]
7 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]
8 **Tan DJH**, Wong C, Ng CH, Poh CW, Jain SR, Huang DQ, Muthiah MD. A Meta-Analysis on the Rate of Hepatocellular Carcinoma Recurrence after Liver Transplant and Associations to Etiology, Alpha-Fetoprotein, Income and Ethnicity. *J Clin Med* 2021; **10** [PMID: 33440759 DOI: 10.3390/jcm10020238]
9 **Yang JD**, Larson JJ, Watt KD, Allen AM, Wiesner RH, Gores GJ, Roberts LR, Heimbach JA, Leise MD. Hepatocellular Carcinoma Is the Most Common Indication for Liver Transplantation and Placement on the Waitlist in the United States. *Clin Gastroenterol Hepatol* 2017; **15**: 767-775.e3 [PMID: 28013117 DOI: 10.1016/j.cgh.2016.11.034]
10 **Kwong AJ**, Kim WR, Flemming JA. De Novo Hepatocellular Carcinoma Among Liver Transplant Registrants in the Direct Acting Antiviral Era. *Hepatology* 2018; **68**: 1288-1297 [PMID: 29672886 DOI: 10.1002/hep.30045]
11 **Toso C**, Mentha G, Majno P. Liver transplantation for hepatocellular carcinoma: five steps to prevent recurrence. *Am J Transplant* 2011; **11**: 2031-2035 [PMID: 21831154 DOI: 10.1111/j.1600-6143.2011.03689.x]
12 **Sapisochin G**, Bruix J. Liver transplantation for hepatocellular carcinoma: outcomes and novel surgical approaches. *Nat Rev Gastroenterol Hepatol* 2017; **14**: 203-217 [PMID: 28053342 DOI: 10.1038/nrgastro.2016.193]
13 **Goldaracena N**, Mehta N, Scalera I, Sposito C, Atenafu EG, Yao FY, Muiesan P, Mazzaferro V, Sapisochin G. Multicenter validation of a score to predict prognosis after the development of HCC recurrence following liver transplantation. *HPB (Oxford)* 2019; **21**: 731-738 [PMID: 30391218 DOI: 10.1016/j.hpb.2018.10.005]
14 **Mazzaferro V**, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, Camerini T, Roayaie S, Schwartz ME, Grazi GL, Adam R, Neuhaus P, Salizzoni M, Bruix J, Forner A, De Carlis L, Cillo U, Burroughs AK, Troisi R, Rossi M, Gerunda GE, Lerut J, Belghiti J, Boin I, Gugenheim J, Rochling F, Van Hoek B, Majno P; Metroticket Investigator Study Group. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. *Lancet Oncol* 2009; **10**: 35-43 [PMID: 19058754 DOI: 10.1016/S1470-2045(08)70284-5]

15 **Duvoux C**, Roudot-Thoraval F, Decaens T, Pessione F, Badran H, Piardi T, Francoz C, Compagnon P, Vanlemmens C, Dumortier J, Dharancy S, Gugenheim J, Bernard PH, Adam R, Radenne S, Muscari F, Conti F, Hardwigsen J, Pageaux GP, Chazouillères O, Salame E, Hilleret MN, Lebray P, Abergel A, Debette-Gratien M, Kluger MD, Mallat A, Azoulay D, Cherqui D; Liver Transplantation French Study Group. Liver transplantation for hepatocellular carcinoma: a model including α-fetoprotein improves the performance of Milan criteria. *Gastroenterology* 2012; **143**: 986-94.e3; quiz e14 [PMID: 22750200 DOI: 10.1053/j.gastro.2012.05.052]

16 **Mazzaferro V**, Sposito C, Zhou J, Pinna AD, De Carlis L, Fan J, Cescon M, Di Sandro S, Yi-Feng H, Lauterio A, Bongini M, Cucchetti A. Metroticket 2.0 Model for Analysis of Competing Risks of Death After Liver Transplantation for Hepatocellular Carcinoma. *Gastroenterology* 2018; **154**: 128-139 [PMID: 28989060 DOI: 10.1053/j.gastro.2017.09.025]

17 **Lai Q**, Avolio AW, Graziadei I, Otto G, Rossi M, Tisone G, Goffette P, Vogel W, Pitton MB, Lerut J; European Hepatocellular Cancer Liver Transplant Study Group. Alpha-fetoprotein and modified response evaluation criteria in solid tumors progression after locoregional therapy as predictors of hepatocellular cancer recurrence and death after transplantation. *Liver Transpl* 2013; **19**: 1108-1118 [PMID: 23873764 DOI: 10.1002/lt.23706]

18 **Yao FY**, Mehta N, Flemming J, Dodge J, Hameed B, Fix O, Hirose R, Fidelman N, Kerlan RK Jr, Roberts JP. Downstaging of hepatocellular cancer before liver transplant: long-term outcome compared to tumors within Milan criteria. *Hepatology* 2015; **61**: 1968-1977 [PMID: 25689978 DOI: 10.1002/hep.27752]

19 **Otto G**, Herber S, Heise M, Lohse AW, Mönch C, Bittinger F, Hoppe-Lotichius M, Schuchmann M, Victor A, Pitton M. Response to transarterial chemoembolization as a biological selection criterion for liver transplantation in hepatocellular carcinoma. *Liver Transpl* 2006; **12**: 1260-1267 [PMID: 16826556 DOI: 10.1002/Lt.20837]

20 **Millonig G**, Graziadei IW, Freund MC, Jaschke W, Stadlmann S, Ladurner R, Margreiter R, Vogel W. Response to preoperative chemoembolization correlates with outcome after liver transplantation in patients with hepatocellular carcinoma. *Liver Transpl* 2007; **13**: 272-279 [PMID: 17256758 DOI: 10.1002/Lt.21033]

21 **Aggarwal A**, Te HS, Verna EC, Desai AP. A National Survey of Hepatocellular Carcinoma Surveillance Practices Following Liver Transplantation. *Transplant Direct* 2021; **7**: e638 [PMID: 33324743 DOI: 10.1097/TXD.0000000000001086]

22 **Berenguer M**, Burra P, Ghobrial M, Hibi T, Metselaar H, Sapisochin G, Bhoori S, Kwan Man N, Mas V, Ohira M, Sangro B, van der Laan LJW. Posttransplant Management of Recipients Undergoing Liver Transplantation for Hepatocellular Carcinoma. Working Group Report From the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020; **104**: 1143-1149 [PMID: 32217940 DOI: 10.1097/TP.0000000000003196]

23 **Verna EC**, Patel YA, Aggarwal A, Desai AP, Frenette C, Pillai AA, Salgia R, Seetharam A, Sharma P, Sherman C, Tsoulfas G, Yao FY. Liver transplantation for hepatocellular carcinoma: Management after the transplant. *Am J Transplant* 2020; **20**: 333-347 [PMID: 31710773 DOI: 10.1111/ajt.15697]

24 **Mehta N**, Heimbach J, Harnois DM, Sapisochin G, Dodge JL, Lee D, Burns JM, Sanchez W, Greig PD, Grant DR, Roberts JP, Yao FY. Validation of a Risk Estimation of Tumor Recurrence After Transplant (RETREAT) Score for Hepatocellular Carcinoma Recurrence After Liver Transplant. *JAMA Oncol* 2017; **3**: 493-500 [PMID: 27838698 DOI: 10.1001/jamaoncol.2016.5116]

25 **Lee DD**, Sapisochin G, Mehta N, Gorgen A, Musto KR, Hajda H, Yao FY, Hodge DO, Carter RE, Harnois DM. Surveillance for HCC After Liver Transplantation: Increased Monitoring May Yield Aggressive Treatment Options and Improved Postrecurrence Survival. *Transplantation* 2020; **104**: 2105-2112 [PMID: 31972705 DOI: 10.1097/TP.0000000000003117]

26 **Rodríguez-Perálvarez M**, Tsochatzis E, Naveas MC, Pieri G, García-Caparrós C, O'Beirne J, Poyato-González A, Ferrín-Sánchez G, Montero-Álvarez JL, Patch D, Thorburn D, Briceño J, De la Mata M, Burroughs AK. Reduced exposure to calcineurin inhibitors early after liver transplantation prevents recurrence of hepatocellular carcinoma. *J Hepatol* 2013; **59**: 1193-1199 [PMID: 23867318 DOI: 10.1016/j.jhep.2013.07.012]

27 **Semela D**, Piguet AC, Kolev M, Schmitter K, Hlushchuk R, Djonov V, Stoupis C, Dufour JF. Vascular remodeling and antitumoral effects of mTOR inhibition in a rat model of hepatocellular carcinoma. *J Hepatol* 2007; **46**: 840-848 [PMID: 17321636 DOI: 10.1016/j.jhep.2006.11.021]

28 **Grigg SE**, Sarri GL, Gow PJ, Yeomans ND. Systematic review with meta-analysis: sirolimus- or everolimus-based immunosuppression following liver transplantation for hepatocellular carcinoma. *Aliment Pharmacol Ther* 2019; **49**: 1260-1273 [PMID: 30989721 DOI: 10.1111/apt.15253]

29 **Geissler EK**, Schnitzbauer AA, Zülke C, Lamby PE, Proneth A, Duvoux C, Burra P, Jauch KW, Rentsch M, Ganten TM, Schmidt J, Settmacher U, Heise M, Rossi G, Cillo U, Kneteman N, Adam R, van Hoek B, Bachellier P, Wolf P, Rostaing L, Bechstein WO, Rizell M, Powell J, Hidalgo E, Gugenheim J, Wolters H, Brockmann J, Roy A, Mutzbauer I, Schlitt A, Beckebaum S, Graeb C, Nadalin S, Valente U, Turrión VS, Jamieson N, Scholz T, Colledan M, Fändrich F, Becker T, Söderdahl G, Chazouillères O, Mäkisalo H, Pageaux GP, Steininger R, Soliman T, de Jong KP, Pirenne J, Margreiter R, Pratschke J, Pinna AD, Hauss J, Schreiber S, Strasser S, Klempnauer J, Troisi RI, Bhoori S, Lerut J, Bilbao I, Klein CG, Königsrainer A, Mirza DF, Otto G, Mazzaferro V, Neuhaus P, Schlitt HJ. Sirolimus Use in Liver Transplant Recipients With Hepatocellular Carcinoma: A Randomized, Multicenter, Open-Label Phase 3 Trial. *Transplantation* 2016; **100**: 116-125 [PMID: 26555945 DOI: 10.1097/TP.0000000000000965]

30 **Olthoff KM**, Rosove MH, Shackleton CR, Imagawa DK, Farmer DG, Northcross P, Pakrasi AL, Martin P, Goldstein LI, Shaked A. Adjuvant chemotherapy improves survival after liver transplantation for hepatocellular carcinoma. *Ann Surg* 1995; **221**: 734-41; discussion 731 [PMID: 7794077 DOI: 10.1097/00000658-199506000-00012]

31 **Hsieh CB**, Chou SJ, Shih ML, Chu HC, Chu CH, Yu JC, Yao NS. Preliminary experience with gemcitabine and cisplatin adjuvant chemotherapy after liver transplantation for hepatocellular carcinoma. *Eur J Surg Oncol* 2008; **34**: 906-910 [PMID: 18166289 DOI: 10.1016/j.ejso.2007.11.014]

32 **Fujiki M**, Aucejo F, Kim R. Adjuvant treatment of hepatocellular carcinoma after orthotopic liver transplantation: do we really need this? *Clin Transplant* 2013; **27**: 169-177 [PMID: 23216662 DOI: 10.1111/ctr.12042]

33 **Siegel AB**, El-Khoueiry AB, Finn RS, Guthrie KA, Goyal A, Venook AP, Blanke CD, Verna EC, Dove L, Emond J, Kato T, Samstein B, Busuttil R, Remotti H, Coffey A, Brown RS Jr. Phase I trial of sorafenib following liver transplantation in

patients with high-risk hepatocellular carcinoma. *Liver Cancer* 2015; **4**: 115-125 [PMID: 26020033 DOI: 10.1159/000367734]

34 **Han B**, Ding H, Zhao S, Zhang Y, Wang J, Gu J. Potential Role of Adjuvant Lenvatinib in Improving Disease-Free Survival for Patients With High-Risk Hepatitis B Virus-Related Hepatocellular Carcinoma Following Liver Transplantation: A Retrospective, Case Control Study. *Front Oncol* 2020; **10**: 562103 [PMID: 33365268 DOI: 10.3389/fonc.2020.562103]

35 **Ziogas IA**, Evangeliou AP, Giannis D, Hayat MH, Mylonas KS, Tohme S, Geller DA, Elias N, Goyal L, Tsoulfas G. The Role of Immunotherapy in Hepatocellular Carcinoma: A Systematic Review and Pooled Analysis of 2,402 Patients. *Oncologist* 2021; **26**: e1036-e1049 [PMID: 33314549 DOI: 10.1002/onco.13638]

36 **Valdivieso A**, Bustamante J, Gastaca M, Uriarte JG, Ventoso A, Ruiz P, Fernandez JR, Pijoan I, Testillano M, Suarez MJ, Montejo M, Ortiz de Urbina J. Management of hepatocellular carcinoma recurrence after liver transplantation. *Transplant Proc* 2010; **42**: 660-662 [PMID: 20304217 DOI: 10.1016/j.transproceed.2010.02.014]

37 **Roayaie S**, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, Krieger NR, Schwartz ME. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl* 2004; **10**: 534-540 [PMID: 15048797 DOI: 10.1002/Lt.20128]

38 **Kornberg A**, Küpper B, Tannapfel A, Katenkamp K, Thrum K, Habrecht O, Wilberg J. Long-term survival after recurrent hepatocellular carcinoma in liver transplant patients: clinical patterns and outcome variables. *Eur J Surg Oncol* 2010; **36**: 275-280 [PMID: 19857941 DOI: 10.1016/j.ejso.2009.10.001]

39 **Sapisochin G**, Goldaracena N, Astete S, Laurence JM, Davidson D, Rafael E, Castells L, Sandroussi C, Bilbao I, Dopazo C, Grant DR, Lázaro JL, Caralt M, Ghanekar A, McGilvray ID, Lilly L, Cattral MS, Selzner M, Charco R, Greig PD. Benefit of Treating Hepatocellular Carcinoma Recurrence after Liver Transplantation and Analysis of Prognostic Factors for Survival in a Large Euro-American Series. *Ann Surg Oncol* 2015; **22**: 2286-2294 [PMID: 25472651 DOI: 10.1245/s10434-014-4273-6]

40 **Bodzin AS**, Lunsford KE, Markovic D, Harlander-Locke MP, Busuttil RW, Agopian VG. Predicting Mortality in Patients Developing Recurrent Hepatocellular Carcinoma After Liver Transplantation: Impact of Treatment Modality and Recurrence Characteristics. *Ann Surg* 2017; **266**: 118-125 [PMID: 27433914 DOI: 10.1097/SLA.0000000000001894]

41 **Fernandez-Sevilla E**, Allard MA, Selten J, Golse N, Vibert E, Sa Cunha A, Cherqui D, Castaing D, Adam R. Recurrence of hepatocellular carcinoma after liver transplantation: Is there a place for resection? *Liver Transpl* 2017; **23**: 440-447 [PMID: 28187493 DOI: 10.1002/lt.24742]

42 **Sommacale D**, Dondero F, Sauvanet A, Francoz C, Durand F, Farges O, Kianmanesh R, Belghiti J. Liver resection in transplanted patients: a single-center Western experience. *Transplant Proc* 2013; **45**: 2726-2728 [PMID: 24034033 DOI: 10.1016/j.transproceed.2013.07.032]

43 **Chok KSh**. Management of recurrent hepatocellular carcinoma after liver transplant. *World J Hepatol* 2015; **7**: 1142-1148 [PMID: 26052403 DOI: 10.4254/wjh.v7.i8.1142]

44 **Huang J**, Yan L, Wu H, Yang J, Liao M, Zeng Y. Is radiofrequency ablation applicable for recurrent hepatocellular carcinoma after liver transplantation? *J Surg Res* 2016; **200**: 122-130 [PMID: 26277218 DOI: 10.1016/j.jss.2015.07.033]

45 **Regalia E**, Fassati LR, Valente U, Pulvirenti A, Damilano I, Dardano G, Montalto F, Coppa J, Mazzaferro V. Pattern and management of recurrent hepatocellular carcinoma after liver transplantation. *J Hepatobiliary Pancreat Surg* 1998; **5**: 29-34 [PMID: 9683751 DOI: 10.1007/pl00009947]

46 **Chok KS**, Chan SC, Cheung TT, Chan AC, Fan ST, Lo CM. Late recurrence of hepatocellular carcinoma after liver transplantation. *World J Surg* 2011; **35**: 2058-2062 [PMID: 21597889 DOI: 10.1007/s00268-011-1146-z]

47 **Tomimaru Y**, Sasaki Y, Yamada T, Eguchi H, Takami K, Ohigashi H, Higashiyama M, Ishikawa O, Kodama K, Imaoka S. The significance of surgical resection for pulmonary metastasis from hepatocellular carcinoma. *Am J Surg* 2006; **192**: 46-51 [PMID: 16769274 DOI: 10.1016/j.amjsurg.2005.12.006]

48 **Jeong YH**, Hwang S, Lee GD, Choi SH, Kim HR, Kim YH, Park SI, Kim DK. Surgical Outcome of Pulmonary Metastasectomy for Hepatocellular Carcinoma Recurrence in Liver Transplant Patients. *Ann Transplant* 2021; **26**: e930383 [PMID: 33972494 DOI: 10.12659/AOT.930383]

49 **Bates MJ**, Farkas E, Taylor D, McFadden PM. Pulmonary resection of metastatic hepatocellular carcinoma after liver transplantation. *Ann Thorac Surg* 2008; **85**: 412-415 [PMID: 18222234 DOI: 10.1016/j.athoracsur.2007.10.065]

50 **Han KN**, Kim YT, Yoon JH, Suh KS, Song JY, Kang CH, Sung SW, Kim JH. Role of surgical resection for pulmonary metastasis of hepatocellular carcinoma. *Lung Cancer* 2010; **70**: 295-300 [PMID: 20353879 DOI: 10.1016/j.lungcan.2010.02.014]

51 **Finn RS**, Qin S, Ikeda M, Galle PR, Ducreux M, Kim TY, Kudo M, Breder V, Merle P, Kaseb AO, Li D, Verret W, Xu DZ, Hernandez S, Liu J, Huang C, Mulla S, Wang Y, Lim HY, Zhu AX, Cheng AL; IMbrave150 Investigators. Atezolizumab plus Bevacizumab in Unresectable Hepatocellular Carcinoma. *N Engl J Med* 2020; **382**: 1894-1905 [PMID: 32402160 DOI: 10.1056/NEJMoa1915745]

52 **Hwang S**, Kim YH, Kim DK, Ahn CS, Moon DB, Kim KH, Ha TY, Song GW, Jung DH, Kim HR, Park GC, Namgoong JM, Yoon SY, Jung SW, Park SI, Lee SG. Resection of pulmonary metastases from hepatocellular carcinoma following liver transplantation. *World J Surg* 2012; **36**: 1592-1602 [PMID: 22411088 DOI: 10.1007/s00268-012-1533-0]

53 **Hu JG**, Lu Y, Lin XJ. En Bloc lumpectomy of T12 vertebra for progressive hepatocellular carcinoma metastases following liver transplantation: A case report. *Medicine (Baltimore)* 2020; **99**: e18756 [PMID: 31914098 DOI: 10.1097/MD.0000000000018756]

54 **Abdel Wahab M**, Shehta A, Ibrahim EM, Eldesoky RT, Sultan AA, Zalata KR, Fathy O, Elshoubary M, Salah T, Yassen AM, Elmorshedi M, Monier A, Farouk A, Shiha U. Adrenalectomy for solitary recurrent hepatocellular carcinoma five years after living donor liver transplantation: A case report. *Int J Surg Case Rep* 2019; **54**: 23-27 [PMID: 30513494 DOI: 10.1016/j.ijscr.2018.11.062]

55 **Jalbani IK**, Nazim SM, Tariq MU, Abbas F. Adrenalectomy for solitary metastasis of Hepatocellular carcinoma post liver transplantation: Case report and literature review. *Pak J Med Sci* 2016; **32**: 1044-1046 [PMID: 27648064 DOI: 10.12669/pjms.324.10339]

56 **Ikegami T**, Yoshizumi T, Kawasaki J, Nagatsu A, Uchiyama H, Harada N, Harimoto N, Itoh S, Motomura T, Soejima Y, Maehara Y. Surgical Resection for Lymph Node Metastasis After Liver Transplantation for Hepatocellular Carcinoma. *Anticancer Res* 2017; **37**: 891-895 [PMID: 28179348 DOI: 10.21873/anticanres.11395]

57 **Alshahrani AA**, Ha SM, Hwang S, Ahn CS, Kim KH, Moon DB, Ha TY, Song GW, Jung DH, Park GC, Cho HD, Kwon JH, Kang SH, Lee SG. Clinical Features and Surveillance of Very Late Hepatocellular Carcinoma Recurrence After Liver Transplantation. *Ann Transplant* 2018; **23**: 659-665 [PMID: 30237389 DOI: 10.12659/AOT.910598]

58 **Tohyama T**, Sakamoto K, Tamura K, Nakamura T, Watanabe J, Wakisaka H, Takada Y. Pharyngeal metastasis following living-donor liver transplantation for hepatocellular carcinoma: a case report and literature review. *World J Surg Oncol* 2020; **18**: 109 [PMID: 32466780 DOI: 10.1186/s12957-020-01873-0]

59 **Zhai H**, Liang P, Yu XL, Cheng Z, Han ZY, Liu F, Yu J. Microwave ablation in treating intrahepatic recurrence of hepatocellular carcinoma after liver transplantation: An analysis of 11 cases. *Int J Hyperthermia* 2015; **31**: 863-868 [PMID: 26608701 DOI: 10.3109/02656736.2015.1091953]

60 **Ko HK**, Ko GY, Yoon HK, Sung KB. Tumor response to transcatheter arterial chemoembolization in recurrent hepatocellular carcinoma after living donor liver transplantation. *Korean J Radiol* 2007; **8**: 320-327 [PMID: 17673843 DOI: 10.3348/kjr.2007.8.4.320]

61 **Zhou B**, Shan H, Zhu KS, Jiang ZB, Guan SH, Meng XC, Zeng XC. Chemoembolization with lobaplatin mixed with iodized oil for unresectable recurrent hepatocellular carcinoma after orthotopic liver transplantation. *J Vasc Interv Radiol* 2010; **21**: 333-338 [PMID: 20116286 DOI: 10.1016/j.jvir.2009.11.006]

62 **Sposito C**, Mariani L, Germini A, Flores Reyes M, Bongini M, Grossi G, Bhoori S, Mazzaferro V. Comparative efficacy of sorafenib versus best supportive care in recurrent hepatocellular carcinoma after liver transplantation: a case-control study. *J Hepatol* 2013; **59**: 59-66 [PMID: 23500153 DOI: 10.1016/j.jhep.2013.02.026]

63 **Mancuso A**, Mazzola A, Cabibbo G, Perricone G, Enea M, Galvano A, Zavaglia C, Belli L, Cammà C. Survival of patients treated with sorafenib for hepatocellular carcinoma recurrence after liver transplantation: a systematic review and meta-analysis. *Dig Liver Dis* 2015; **47**: 324-330 [PMID: 25641331 DOI: 10.1016/j.dld.2015.01.001]

64 **Toso C**, Mentha G, Majno P. Integrating sorafenib into an algorithm for the management of post-transplant hepatocellular carcinoma recurrence. *J Hepatol* 2013; **59**: 3-5 [PMID: 23567081 DOI: 10.1016/j.jhep.2013.03.029]

65 **Invernizzi F**, Iavarone M, Zavaglia C, Mazza S, Maggi U, Cesarini L, Antonelli B, Airoldi A, Manini MA, Sangiovanni A, Rossi G, Donato MF, Saverio Belli L, Lampertico P. Experience With Early Sorafenib Treatment With mTOR Inhibitors in Hepatocellular Carcinoma Recurring After Liver Transplantation. *Transplantation* 2020; **104**: 568-574 [PMID: 31517781 DOI: 10.1097/TP.0000000000002955]

66 **Piguet AC**, Saar B, Hlushchuk R, St-Pierre MV, McSheehy PM, Radojevic V, Afthinos M, Terracciano L, Djonov V, Dufour JF. Everolimus augments the effects of sorafenib in a syngeneic orthotopic model of hepatocellular carcinoma. *Mol Cancer Ther* 2011; **10**: 1007-1017 [PMID: 21487053 DOI: 10.1158/1535-7163.MCT-10-0666]

67 **Herden U**, Fischer L, Schäfer H, Nashan B, von Baehr V, Sterneck M. Sorafenib-induced severe acute hepatitis in a stable liver transplant recipient. *Transplantation* 2010; **90**: 98-99 [PMID: 20606568 DOI: 10.1097/TP.0b013e3181daac69]

68 **Waidmann O**, Hofmann WP, Zeuzem S, Trojan J. mTOR inhibitors and sorafenib for recurrent heptocellular carcinoma after orthotopic liver transplantation. *J Hepatol* 2011; **54**: 396-398 [PMID: 21111506 DOI: 10.1016/j.jhep.2010.08.038]

69 **De Simone P**, Crocetti L, Pezzati D, Bargellini I, Ghinolfi D, Carrai P, Leonardi G, Della Pina C, Cioni D, Pollina L, Campani D, Bartolozzi C, Lencioni R, Filipponi F. Efficacy and safety of combination therapy with everolimus and sorafenib for recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc* 2014; **46**: 241-244 [PMID: 24507059 DOI: 10.1016/j.transproceed.2013.10.035]

70 **Bhoori S**, Toffanin S, Sposito C, Germini A, Pellegrinelli A, Lampis A, Mazzaferro V. Personalized molecular targeted therapy in advanced, recurrent hepatocellular carcinoma after liver transplantation: a proof of principle. *J Hepatol* 2010; **52**: 771-775 [PMID: 20347502 DOI: 10.1016/j.jhep.2010.01.025]

71 **Iavarone M**, Invernizzi F, Czauderna C, Sanduzzi-Zamparelli M, Bhoori S, Amaddeo G, Manini MA, López MF, Anders M, Pinter M, Rodríguez MJB, Cristóbal MR, Soteras GA, Piñero F, Villadsen GE, Weinmann A, Crespo G, Mazzaferro V, Regnault H, Giorgio M, González-Diéguez ML, Donato MF, Varela M, Wörns MA, Bruix J, Lampertico P, Reig M. Preliminary experience on safety of regorafenib after sorafenib failure in recurrent hepatocellular carcinoma after liver transplantation. *Am J Transplant* 2019; **19**: 3176-3184 [PMID: 31365177 DOI: 10.1111/ajt.15551]

72 **Iavarone M**, Invernizzi F, Ivanics T, Mazza S, Zavaglia C, Sanduzzi-Zamparelli M, Fraile-López M, Czauderna C, Di Costanzo G, Bhoori S, Pinter M, Manini MA, Amaddeo G, Yunquera AF, Piñero F, Blanco Rodríguez MJ, Anders M, Aballay Soteras G, Villadsen GE, Yoon PD, Cesarini L, Díaz-González Á, González-Diéguez ML, Tortora R, Weinmann A, Mazzaferro V, Romero Cristóbal M, Crespo G, Regnault H, De Giorgio M, Varela M, Prince R, Scudeller L, Donato MF, Wörns MA, Bruix J, Sapisochin G, Lampertico P, Reig M. Regorafenib Efficacy After Sorafenib in Patients With Recurrent Hepatocellular Carcinoma After Liver Transplantation: A Retrospective Study. *Liver Transpl* 2021; **27**: 1767-1778 [PMID: 34388851 DOI: 10.1002/lt.26264]

73 **Kudo M**, Finn RS, Qin S, Han KH, Ikeda K, Piscaglia F, Baron A, Park JW, Han G, Jassem J, Blanc JF, Vogel A, Komov D, Evans TRJ, Lopez C, Dutcus C, Guo M, Saito K, Kraljevic S, Tamai T, Ren M, Cheng AL. Lenvatinib versus sorafenib in first-line treatment of patients with unresectable hepatocellular carcinoma: a randomised phase 3 non-inferiority trial. *Lancet* 2018; **391**: 1163-1173 [PMID: 29433850 DOI: 10.1016/S0140-6736(18)30207-1]

74 **Abou-Alfa GK**, Meyer T, Cheng AL, El-Khoueiry AB, Rimassa L, Ryoo BY, Cicin I, Merle P, Chen Y, Park JW, Blanc JF, Bolondi L, Klümpen HJ, Chan SL, Zagonel V, Pressiani T, Ryu MH, Venook AP, Hessel C, Borgman-Hagey AE, Schwab G, Kelley RK. Cabozantinib in Patients with Advanced and Progressing Hepatocellular Carcinoma. *N Engl J Med* 2018; **379**: 54-63 [PMID: 29972759 DOI: 10.1056/NEJMoa1717002]

75 **Zhu AX**, Kang YK, Yen CJ, Finn RS, Galle PR, Llovet JM, Assenat E, Brandi G, Pracht M, Lim HY, Rau KM, Motomura K, Ohno I, Merle P, Daniele B, Shin DB, Gerken G, Borg C, Hiriart JB, Okusaka T, Morimoto M, Hsu Y, Abada PB, Kudo M; REACH-2 study investigators. Ramucirumab after sorafenib in patients with advanced hepatocellular carcinoma and increased α-fetoprotein concentrations (REACH-2): a randomised, double-blind, placebo-controlled, phase

3 trial. *Lancet Oncol* 2019; **20**: 282-296 [PMID: 30665869 DOI: 10.1016/S1470-2045(18)30937-9]

76 **Rimassa L**, Personeni N, Czauderna C, Foerster F, Galle P. Systemic treatment of HCC in special populations. *J Hepatol* 2021; **74**: 931-943 [PMID: 33248171 DOI: 10.1016/j.jhep.2020.11.026]

77 **El-Khoueiry AB**, Sangro B, Yau T, Crocenzi TS, Kudo M, Hsu C, Kim TY, Choo SP, Trojan J, Welling TH Rd, Meyer T, Kang YK, Yeo W, Chopra A, Anderson J, Dela Cruz C, Lang L, Neely J, Tang H, Dastani HB, Melero I. Nivolumab in patients with advanced hepatocellular carcinoma (CheckMate 040): an open-label, non-comparative, phase 1/2 dose escalation and expansion trial. *Lancet* 2017; **389**: 2492-2502 [PMID: 28434648 DOI: 10.1016/S0140-6736(17)31046-2]

78 **Finn RS**, Ryoo BY, Merle P, Kudo M, Bouattour M, Lim HY, Breder V, Edeline J, Chao Y, Ogasawara S, Yau T, Garrido M, Chan SL, Knox J, Daniele B, Ebbinghaus SW, Chen E, Siegel AB, Zhu AX, Cheng AL; KEYNOTE-240 investigators. Pembrolizumab As Second-Line Therapy in Patients With Advanced Hepatocellular Carcinoma in KEYNOTE-240: A Randomized, Double-Blind, Phase III Trial. *J Clin Oncol* 2020; **38**: 193-202 [PMID: 31790344 DOI: 10.1200/JCO.19.01307]

79 **Zhu AX**, Finn RS, Edeline J, Cattan S, Ogasawara S, Palmer D, Verslype C, Zagonel V, Fartoux L, Vogel A, Sarker D, Verset G, Chan SL, Knox J, Daniele B, Webber AL, Ebbinghaus SW, Ma J, Siegel AB, Cheng AL, Kudo M; KEYNOTE-224 investigators. Pembrolizumab in patients with advanced hepatocellular carcinoma previously treated with sorafenib (KEYNOTE-224): a non-randomised, open-label phase 2 trial. *Lancet Oncol* 2018; **19**: 940-952 [PMID: 29875066 DOI: 10.1016/S1470-2045(18)30351-6]

80 **Kittai AS**, Oldham H, Cetnar J, Taylor M. Immune Checkpoint Inhibitors in Organ Transplant Patients. *J Immunother* 2017; **40**: 277-281 [PMID: 28719552 DOI: 10.1097/CJI.0000000000000180]

81 **Friend BD**, Venick RS, McDiarmid SV, Zhou X, Naini B, Wang H, Farmer DG, Busuttil RW, Federman N. Fatal orthotopic liver transplant organ rejection induced by a checkpoint inhibitor in two patients with refractory, metastatic hepatocellular carcinoma. *Pediatr Blood Cancer* 2017; **64** [PMID: 28643391 DOI: 10.1002/pbc.26682]

82 **Biondani P**, De Martin E, Samuel D. Safety of an anti-PD-1 immune checkpoint inhibitor in a liver transplant recipient. *Ann Oncol* 2018; **29**: 286-287 [PMID: 29293878 DOI: 10.1093/annonc/mdx548]

83 **DeLeon TT**, Salomao MA, Aqel BA, Sonbol MB, Yokoda RT, Ali AH, Moss AA, Mathur AK, Chascsa DM, Rakela J, Bryce AH, Borad MJ. Pilot evaluation of PD-1 inhibition in metastatic cancer patients with a history of liver transplantation: the Mayo Clinic experience. *J Gastrointest Oncol* 2018; **9**: 1054-1062 [PMID: 30603124 DOI: 10.21037/jgo.2018.07.05]

84 **Gassmann D**, Weiler S, Mertens JC, Reiner CS, Vrugt B, Nägeli M, Mangana J, Müllhaupt B, Jenni F, Misselwitz B. Liver Allograft Failure After Nivolumab Treatment-A Case Report With Systematic Literature Research. *Transplant Direct* 2018; **4**: e376 [PMID: 30255136 DOI: 10.1097/TXD.0000000000000814]

85 **Lipson EJ**, Bodell MA, Kraus ES, Sharfman WH. Successful administration of ipilimumab to two kidney transplantation patients with metastatic melanoma. *J Clin Oncol* 2014; **32**: e69-e71 [PMID: 24493726 DOI: 10.1200/JCO.2013.49.2314]

86 **de'Angelis N**, Landi F, Carra MC, Azoulay D. Managements of recurrent hepatocellular carcinoma after liver transplantation: A systematic review. *World J Gastroenterol* 2015; **21**: 11185-11198 [PMID: 26494973 DOI: 10.3748/wjg.v21.i39.11185]

87 **Pelizzaro F**, Gambato M, Gringeri E, Vitale A, Cillo U, Farinati F, Burra P, Russo FP. Management of Hepatocellular Carcinoma Recurrence after Liver Transplantation. *Cancers (Basel)* 2021; **13** [PMID: 34638365 DOI: 10.3390/cancers13194882]

88 **Shalaby S**, Burra P. De novo and recurrent malignancy. *Best Pract Res Clin Gastroenterol* 2020; **46-47**: 101680 [PMID: 33158464 DOI: 10.1016/j.bpg.2020.101680]

89 **Jeyarajah DR**, Doyle MBM, Espat NJ, Hansen PD, Iannitti DA, Kim J, Thambi-Pillai T, Visser BC. Role of yttrium-90 selective internal radiation therapy in the treatment of liver-dominant metastatic colorectal cancer: an evidence-based expert consensus algorithm. *J Gastrointest Oncol* 2020; **11**: 443-460 [PMID: 32399284 DOI: 10.21037/jgo.2020.01.09]

90 **Roh YN**, David Kwon CH, Song S, Shin M, Man Kim J, Kim S, Joh JW, Lee SK. The prognosis and treatment outcomes of patients with recurrent hepatocellular carcinoma after liver transplantation. *Clin Transplant* 2014; **28**: 141-148 [PMID: 24372624 DOI: 10.1111/ctr.12286]

91 **Shin WY**, Suh KS, Lee HW, Kim J, Kim T, Yi NJ, Lee KU. Prognostic factors affecting survival after recurrence in adult living donor liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2010; **16**: 678-684 [PMID: 20440777 DOI: 10.1002/lt.22047]

92 **Toso C**, Cader S, Mentha-Dugerdil A, Meeberg G, Majno P, Morard I, Giostra E, Berney T, Morel P, Mentha G, Kneteman NM. Factors predicting survival after post-transplant hepatocellular carcinoma recurrence. *J Hepatobiliary Pancreat Sci* 2013; **20**: 342-347 [PMID: 22710887 DOI: 10.1007/s00534-012-0528-4]

93 **Hong SK**, Lee KW, Yoon KC, Kim HS, Ahn SW, Kim H, Lee JM, Cho JH, Yi NJ, Suh KS. Different prognostic factors and strategies for early and late recurrence after adult living donor liver transplantation for hepatocellular carcinoma. *Clin Transplant* 2019; **33**: e13703 [PMID: 31464006 DOI: 10.1111/ctr.13703]

94 **Maccali C**, Chagas AL, Boin I, Quiñonez E, Marciano S, Vilatobá M, Varón A, Anders M, Hoyos Duque S, Lima AS, Menendez J, Padilla-Machaca M, Poniachik J, Zapata R, Maraschio M, Chong Menéndez R, Muñoz L, Arufe D, Figueroa R, Soza A, Fauda M, Perales SR, Vergara Sandoval R, Bermudez C, Beltran O, Arenas Hoyos I, McCormack L, Mattera FJ, Gadano A, Parente García JH, Tani CM, Augusto Carneiro D'Albuquerque L, Carrilho FJ, Silva M, Piñero F. Recurrence of hepatocellular carcinoma after liver transplantation: Prognostic and predictive factors of survival in a Latin American cohort. *Liver Int* 2021; **41**: 851-862 [PMID: 33217193 DOI: 10.1111/liv.14736]

95 **Ekpanyapong S**, Philips N, Loza BL, Abt P, Furth EE, Tondon R, Khungar V, Olthoff K, Shaked A, Hoteit MA, Reddy KR. Predictors, Presentation, and Treatment Outcomes of Recurrent Hepatocellular Carcinoma After Liver Transplantation: A Large Single Center Experience. *J Clin Exp Hepatol* 2020; **10**: 304-315 [PMID: 32655233 DOI: 10.1016/j.jceh.2019.11.003]

96 **Ho CM**, Lee CH, Lee MC, Zhang JF, Chen CH, Wang JY, Hu RH, Lee PH. Survival After Treatable Hepatocellular Carcinoma Recurrence in Liver Recipients: A Nationwide Cohort Analysis. *Front Oncol* 2020; **10**: 616094 [PMID: 33598433 DOI: 10.3389/fonc.2020.616094]

97 **El-Domiaty N**, Saliba F, Vibert E, Karam V, Sobesky R, Ibrahim W, Pittau G, Ciacio O, Salloum C, Amer K, Saeed MA, Shawky JA, Sa Cunha A, Rosmorduc O, Cherqui D, Adam R, Samuel D. Early Versus Late Hepatocellular Carcinoma Recurrence After Transplantation: Predictive Factors, Patterns, and Long-term Outcome. *Transplantation* 2021; **105**: 1778-1790 [PMID: 32890134 DOI: 10.1097/TP.0000000000003434]

98 **Au KP**, Chok KSH. Multidisciplinary approach for post-liver transplant recurrence of hepatocellular carcinoma: A proposed management algorithm. *World J Gastroenterol* 2018; **24**: 5081-5094 [PMID: 30568386 DOI: 10.3748/wjg.v24.i45.5081]

99 **Nagai S**, Mangus RS, Kubal CA, Ekser B, Fridell JA, Klingler KR, Maluccio MA, Tector AJ. Prognosis after recurrence of hepatocellular carcinoma in liver transplantation: predictors for successful treatment and survival. *Clin Transplant* 2015; **29**: 1156-1163 [PMID: 26458066 DOI: 10.1111/ctr.12644]

100 **Taketomi A**, Fukuhara T, Morita K, Kayashima H, Ninomiya M, Yamashita Y, Ikegami T, Uchiyama H, Yoshizumi T, Soejima Y, Shirabe K, Maehara Y. Improved results of a surgical resection for the recurrence of hepatocellular carcinoma after living donor liver transplantation. *Ann Surg Oncol* 2010; **17**: 2283-2289 [PMID: 20204531 DOI: 10.1245/s10434-010-0999-y]

101 **Mehta N**, Bhangui P, Yao FY, Mazzaferro V, Toso C, Akamatsu N, Durand F, Ijzermans J, Polak W, Zheng S, Roberts JP, Sapisochin G, Hibi T, Kwan NM, Ghobrial M, Soin A. Liver Transplantation for Hepatocellular Carcinoma. Working Group Report from the ILTS Transplant Oncology Consensus Conference. *Transplantation* 2020; **104**: 1136-1142 [PMID: 32217938 DOI: 10.1097/TP.0000000000003174]

102 **Sharma P**, Welch K, Hussain H, Pelletier SJ, Fontana RJ, Marrero J, Merion RM. Incidence and risk factors of hepatocellular carcinoma recurrence after liver transplantation in the MELD era. *Dig Dis Sci* 2012; **57**: 806-812 [PMID: 21953139 DOI: 10.1007/s10620-011-1910-9]

103 **Schlitt HJ**, Neipp M, Weimann A, Oldhafer KJ, Schmoll E, Boeker K, Nashan B, Kubicka S, Maschek H, Tusch G, Raab R, Ringe B, Manns MP, Pichlmayr R. Recurrence patterns of hepatocellular and fibrolamellar carcinoma after liver transplantation. *J Clin Oncol* 1999; **17**: 324-331 [PMID: 10458250 DOI: 10.1200/JCO.1999.17.1.324]

104 **Escartin A**, Sapisochin G, Bilbao I, Vilallonga R, Bueno J, Castells L, Dopazo C, Castro E, Caralt M, Balsells J. Recurrence of hepatocellular carcinoma after liver transplantation. *Transplant Proc* 2007; **39**: 2308-2310 [PMID: 17889173 DOI: 10.1016/j.transproceed.2007.06.042]

105 **Cescon M**, Ravaioli M, Grazi GL, Ercolani G, Cucchetti A, Bertuzzo V, Vetrone G, Del Gaudio M, Vivarelli M, D'Errico-Grigioni A, Dazzi A, Di Gioia P, Lauro A, Pinna AD. Prognostic factors for tumor recurrence after a 12-year, single-center experience of liver transplantations in patients with hepatocellular carcinoma. *J Transplant* 2010; **2010** [PMID: 20862199 DOI: 10.1155/2010/904152]

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MINIREVIEWS

Diagnosis, treatment, and current concepts in the endoscopic management of gastroenteropancreatic neuroendocrine neoplasms

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Abstract

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are rare tumors derived from the neuroendocrine cell system, which that have increased in incidence and prevalence in recent years. Despite improvements in radiological and metabolic imaging, endoscopy still plays a pivotal role in the number of GEP-NENs. Tumor detection, characterization, and staging are essential in management and treatment planning. Upper and lower gastrointestinal (GI) endoscopy is essential for correct localization of the primary tumor site of GI NENs. Endoscopic ultrasonography (EUS) has an important role in the imaging and tissue acquisition of pancreatic NENs and locoregional staging of GI neuroendocrine tumors. Correct staging and histological diagnosis have important prognostic implications. Endoscopic operating techniques allow the removal of small GI NENs in the early stage of mucosal or submucosal invasion of the intestinal wall. Preoperative EUS-guided techniques may help the surgeon locate small and deep tumors, thus avoiding formal pancreatic resections in favor of parenchymal-sparing surgery. Finally, locoregional ablative treatments have been proposed in recent studies with promising results in selected patients.

Key Words: Neuroendocrine neoplasms; Gastrointestinal endoscopy; Endoscopic resection; Endoscopic ultrasound; Ablative technique; Tissue acquisition

Core Tip: Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are complex neoplasms that present many clinical challenges. This review reports endoscopic management of patients with GEP-NENs. Endoscopic procedures allow diagnosis, local staging, and tissue acquisition. Early NENs of the stomach, duodenum, or rectum are generally removed by endoscopic operating techniques. New endoscopic ultrasonography-guided operative techniques may help the surgeon locate small and deep tumors or treat small pancreatic NENs.

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INTRODUCTION

Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are rare neoplasms arising from neuroendocrine cells distributed in the GEP tract. Most commonly, the primary lesion is located in the gastric mucosa, small and large intestine, rectum, and pancreas[1].

The incidence of GEP-NENs has substantially increased over the last decades. Data from the Surveillance, Epidemiology, and End Results registry show that the incidence of GEP-NENs has increased 6.4 fold since the program's inception in 1973. This phenomenon may be related to the increasing number of radiological imaging and endoscopic examinations performed[1,2].

Approximately 15%-30% of GEP-NENs are functioning tumors with hormone-related symptoms; the remaining 70%-85% are nonfunctioning and detected incidentally or because the patient has symptoms of mass effects or distant metastases[3]. The management of GEP-NENs requires a multidisciplinary approach. Since GEP-NENs are less frequent than other malignancies, endoscopic management of these tumors may not be fully understood.

This review focuses on the endoscopic diagnosis and treatment of GEP-NENs.

Site

The GEP tract represents the most common localization of NENs. Gastrointestinal NENs (GI-NENs) develop from the stomach (23%), appendix (21%), small bowel (15%), and rectum (14%). Esophageal and colonic NENs are rare tumors that account for a small percentage of GI-NENs. Pancreatic NENs (Pan-NENs) represent about 1% of all pancreatic neoplasms but their prevalence is around 10%[1].

Grade

The World Health Organization subclassifies GEP-NENs based on the mitotic count and Ki-67 index (Table 1). This classification defines both well-differentiated neuroendocrine tumors (NETs) and poorly differentiated neuroendocrine carcinomas (NECs). NETs are divided into grade 1 (Ki-67 index < 3, mitotic rate < 2), grade 2 (Ki-67 index 3-20, mitotic rate 2-20), and grade 3 (Ki-67 index > 20, mitotic rate > 20). NECs exhibit poorly differentiated morphology with significant atypia and frequently have geographic necrosis. Tumors involving both neuroendocrine and non-neuroendocrine cells are classified as mixed NENs and non-NENs[4].

Staging

There are two major classifications in current clinical use: the European Neuroendocrine Tumor Society (ENETS) tumor, node and metastasis (TNM) system and the American Joint Committee on Cancer TNM system[5,6].

Functional status

Clinically, GEP-NENs can be classified into nonfunctioning and functioning tumors. The functioning tumors secrete substances that cause appreciable clinical symptoms, whereas the nonfunctioning neoplasms do not secrete any substance or the substance produced is inactive. The hormones produced and clinical symptoms vary by site of the primary GEP-NENs. Clinical symptoms include hypoglycemic syndrome, carcinoid syndrome, Zollinger-Ellison syndrome, watery diarrhea-hypokalemia-achlorhydria syndrome, and glucagonoma (Table 2)[7].

Biomarker levels

Chromogranin A is currently the most commonly used biomarker for GEP-NENs. It has a 10%-35% specificity, and its sensitivity ranges from 32% to 92%. Serotonin and its metabolite 5-hydroxyindole

Table 1 2019 World Health Organization classification of neuroendocrine neoplasms of the gastrointestinal tract and hepatopancreatobiliary organs

NENs	Differentiation	Grade	Mitotic rate	Ki-67 index %
NET, G1	Well differentiated	Low	< 2	< 3
NET, G2	Well differentiated	Intermediate	2-20	3-20
NET, G3	Well differentiated	High	> 20	> 20
NEC, small cell type	Poorly differentiated	High	> 20	> 20
NEC, large cell type	Poorly differentiated	High	> 20	> 20
MiNEN	Well or poorly differentiated	Variable	Variable	Variable

NEN: Neuroendocrine neoplasm; NET: Neuroendocrine tumor; NEC: Neuroendocrine carcinoma; MiNEN: Mixed neuroendocrine-non-neuroendocrine neoplasm.

Table 2 The hormones produced by the primary gastroenteropancreatic neuroendocrine neoplasms

Tissue	Hormones	Symptoms/Syndrome
Gastric	Histamine, CGA	Atypical flush, wheeze, angioedema
Duodenal	CGA, somatostatin, gastrin	Cholelithiasis, steatorrhea, diabetes, ZE syndrome (gastrinoma)
Jejuno-ileal, appendiceal, cecal	Serotonin, CGA, pancreastatin	Carcinoid syndrome
Colorectal	Pancreatic polypeptide	No hormonal symptoms
Pancreatic	Insulin	Recurrent hypoglycemia
	Glucagon	Diarrhea, glossitis, necrolytic migratory erythema, weight loss, hyperglycemia, blood clots
	VIP	Diarrhea, hypokalemia, achlorhydria
	ACTH	Cushingoid facies, weight gain, diabetes, hypertension
	GHRH	Acromegalic features, diabetes
	PTHRP	Hypercalcemia
	Gastrin	Pain, diarrhea (ZE syndrome)
	Somatostatin	Diabetes, cholelithiasis, steatorrhea, weight loss
	Serotonin	Flushing, diarrhea (carcinoid syndrome)

5HIAA: 5-hydroxyindoleacetic acid; ACTH: Adrenocorticotrophic hormone; CGA: Chromogranin A; F-PNET: Functional pancreatic neuroendocrine tumor; GHRH: Growth hormone releasing hormone; MEN1: Multiple endocrine neoplasia type 1; NF1: Neurofibromatosis type 1; PTHRP: Parathyroid hormone-related peptide; VIP: Vasoactive intestinal polypeptide; ZE: Zollinger Ellison.

acetic acid have been measured in blood and urine samples, respectively, as markers of carcinoid syndrome. However, the sensitivity of this biomarker is as low as 35% in the absence of carcinoid syndrome[8].

Several other potential biomarkers include neuron-specific enolase, human chorionic gonadotropin, alpha-fetoprotein, and pancreatic polypeptide. These circulating biomarkers are useful to aid diagnosis, but are of insufficient value to accurately identify the primary tumor site, correlate with tumor grade, and differentiate low-level malignancy from high-grade disease[9].

In recent years, new biomarkers have been evaluated that may correlate with clinical outcomes and be useful as better prognostic indicators. These include microRNAs, long noncoding RNAs, circulating tumor cells, and DNA methylation patterns[10].

Any associated syndromes

Most GEP-NENs are sporadic, but they also can arise as part of inherited familial syndromes. About 5% of patients with GEP-NENs harbor genomic mutations with well-characterized familial syndromes such as multiple endocrine neoplasia type 1 (MEN1), von Hippel-Lindau (VHL) disease, tuberous sclerosis (TSC), and neurofibromatosis type 1 (NF1)[11].

MEN1 is an autosomal-dominant syndrome, characterized by NENs of the anterior pituitary, parathyroid glands, and pancreas. VHL syndrome is an autosomal-dominant syndrome characterized by a variety of benign and malignant neoplasms including clear renal cell carcinomas, pheochromocytomas, hemangioblastomas, retinal angiomas, paragangliomas, and pNENs. TSC is an autosomal-dominant syndrome characterized by widespread, low-grade tumors, and hamartomas in multiple organs including the brain, heart, skin, eyes, kidney, lung, and liver. pNENs are described in only 1% to 5% of cases. NF1 is an autosomal-dominant syndrome characterized by ubiquitous neurofibromas; multiple cafe-au-lait skin spots; and susceptibility to gliomas, myeloid leukemia, pheochromocytomas, and occasionally pNENs[12].

DIAGNOSIS

Gastric NENs

Gastric NENs (G-NENs) are neoplasms derived from the enterochromaffin-like cells of the gastric mucosa. They are classified into types I, II and III according to their clinical and pathophysiological characteristics (Table 3)[13].

Type I G-NENs correspond to the majority of G-NENs found in the stomach (70%-80%) and are associated with autoimmune chronic atrophic gastritis and hypergastrinemia, type II G-NENs lesions are caused by gastrinomas and commonly found in patients with Zollinger-Ellison syndrome (ZES) and MEN1, and type III G-NENs lesions consist of a sporadic lesion and are unrelated to gastrin hypersecretion.

Patients with G-NENs typically present with nonspecific symptoms and the diagnosis is made by upper gastrointestinal (GI) endoscopy for symptoms such as abdominal pain, nausea, bleeding, and anemia. Despite the low incidence of G-NET, recent data have demonstrated an increase due to an expanding use of upper endoscopy, improvement of endoscopes, and more attention to identification and characterization of gastric lesion[14]. Type I G-NENs occur in the corpus and/or fundus and are multiple small reddish polyps, usually subcentimetrics. They are associated with a chronic atrophic gastritis with an excellent prognosis. Tumor extension is limited to the mucosa or submucosa.

Type II G-NENs are similar to type I lesions but the adjacent gastric folds have a hypertrophic gastric mucosa and occasionally have multiple areas of ulceration of gastric and duodenal mucosa. Type II G-NENs are involved in MEN1 and ZES and have a good prognosis, similar to type I. Type III G-NENs are single, large (> 2 cm), sometimes ulcerated, more aggressive and associated with local and distant spread, and involve the muscular layer (Figure 1)[15]. The prognosis of this subgroup is poor.

At the time of diagnosis, biopsy samples should be taken from the lesions and multiple gastric biopsies (antrum, body, and fundus) should be performed for etiologic orientation.

Endoscopic ultrasonography (EUS) should be performed if the lesion is greater than 1 cm in order to assess the layer of origin, tumor size, echogenicity, margins, wall invasion, and regional lymph nodes. At EUS, G-NENs present as rounded isoechoic or hypoechoic tumors, localized in the second (deeper mucosal) or third (submucosal) echo layers. EUS diagnostic accuracy for evaluation of subepithelial lesion including G-NET is suboptimal (43%-67%)[16]. EUS is a very accurate technique for assessing tumor size and muscularis propria integrity, factors that seem to condition the potential for distant metastasis[17].

In type I and II G-NENs, staging EUS is frequently performed to evaluate indication to endoscopic treatment. For patients suspected of having type II G-NENs, EUS evaluation must be performed for the assessment of any duodenal or Pan-NENs. In Type III G-NENs, EUS is indicated to stage the disease by assessing the presence of regional lymph node involvement[18].

Duodenal NENs

Duodenal NENs (D-NENs) are solitary, small lesions generally discovered incidentally on imaging studies or endoscopy. They are generally classified into five different tumor types: duodenal gastrinomas, somatostatinomas, nonfunctional D-NENs, duodenal gangliocytic paragangliomas, and high-grade poorly differentiated NEC[19].

Most D-NENs are localized predominantly in the first and second duodenal portion and are nonfunctioning with a low risk of local or distant metastases. Functioning D-NENs are typically associated with higher metastatic power. The presence of multiple D-NENs should raise suspicion of MEN1-ZES[19].

D-NENs located in the ampullary/periampullary region have more aggressive behavior and poorer overall survival than D-NENs located elsewhere in the duodenum[20,21].

On upper GI endoscopy, D-NENs have the appearance of submucosal tumors with a surface color often identical to the surrounding mucosa (Figure 2). As the tumor enlarges, a depression may form in the center, which is eventually replaced by an ulcer crater[22].

EUS evaluation is useful for duodenal subepithelial lesions characterization; the accuracy of combined endoscopic/EUS imaging for all duodenal lesions is 84.9%[23].

Table 3 Characteristics of the subtypes of neuroendocrine neoplasms of the stomach

Characteristics	Type I	Type II	Type III
Prevalence	70%-80%	5%-10%	10%-20%
Background	Autoimmune chronic atrophic gastritis	Gastrinomas (Zollinger-Ellison syndrome)	Normal mucosa
Number of lesions	Multiple	Multiple	Single
Size of tumors	1-2 cm	1 cm	> 2 cm
Site of tumor	Corpus and/or fundus	Corpus and/or fundus	Anywhere
Serum gastrin levels	Elevated	Elevated	Normal
Gastric pH	High	Low	Normal
Invasion	Rare	More common	Common
Prognosis (5-yr survival)	Excellent (90%-95%)	Good (70%-90%)	Worse (less than 35%)

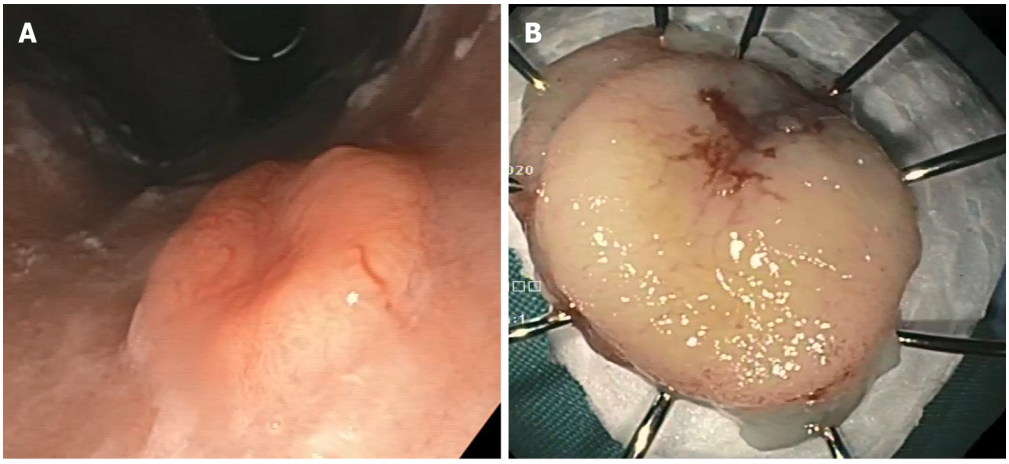

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Figure 1 Gastric neuroendocrine neoplasm. A: Endoscopic image demonstrates a flat lesion in the stomach fundus with depressed center; B: Endoscopic en bloc resection was achieved.

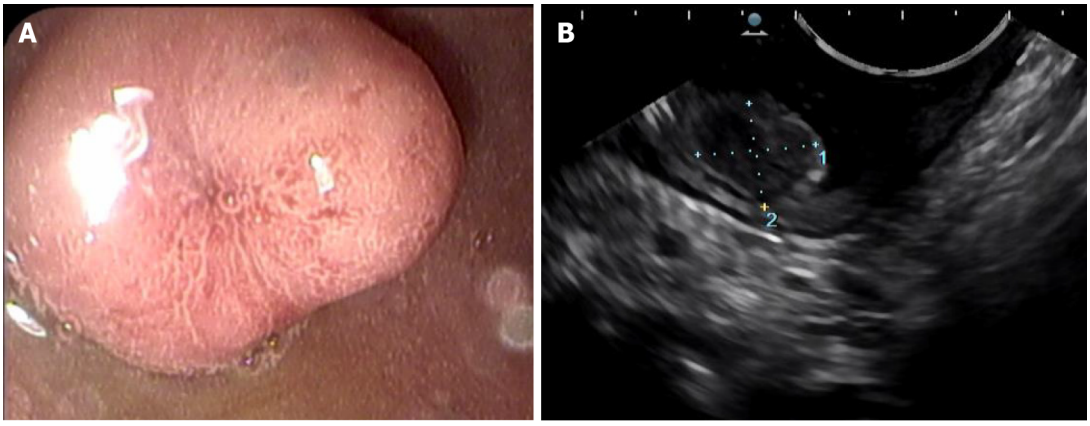

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Figure 2 Duodenal neuroendocrine neoplasm. A: Endoscopic image demonstrates a sessile polyp with central depression; B: Endoscopic ultrasound demonstrates a hypoechoic intramural structure in the submucosal layer of the duodenal wall.

EUS is also useful to exclude locoregional lymph node metastases and thus the indication for endoscopic mucosal resection in case of lesions > 1 cm. At EUS evaluation, D-NENs are usually located in the submucosal layer and are rounded, hypoechoic, well-demarcated small lesions. Fine needle aspiration (FNA) should also be performed in cases of nondiagnostic histopathology[18]. Duoden-

oscopy and EUS are indicated to identify the primary tumor in the case of ZES and in patients with MEN1[24].

Small bowel NENs

Small bowel NENs (SB-NENs) are the most common NENs developing distant metastases; however, their prognosis remains favorable[25]. They tend to present in later stages because clinical symptoms are often insidious and the diagnosis often occurs in the course of an intervention for intestinal obstruction or bleeding[26].

Accurate localization and staging of SB-NENs often involve a combination of several imaging modalities. Endoscopic examinations such as capsule endoscopy and double-balloon enteroscopy are useful in identifying occult SB-NENs when no primary tumor was found on conventional imaging. Macroscopically, most SB-NENs are sessile nodules or ulcerated lesions usually measuring between 1 and 2 cm or as multiple tumors[27,28].

Appendix NENs

Appendiceal NENs (A-NENs) are usually discovered incidentally at final pathological examination after an appendectomy is performed for acute appendicitis. Colonoscopic examination of the entire large bowel is mandatory given the frequency of synchronous colorectal neoplasia[27]. A-NENs usually have a good prognosis. In most cases, appendicectomy alone is considered curative; however, in selected cases with high malignant potential, the right hemicolectomy could be considered[29].

Colorectal NENs

Colonic NENs (C-NENs) and rectal NENs (R-NENs) are two different clinical entities. C-NENs have more aggressive features and much worse prognosis than R-NENs[30]. Nearly 70% of C-NENs are located in the ascending colon, particularly in the cecum with a mean size of 5 cm at presentation. The patients generally experience late symptoms such as abdominal pain, GI bleeding, and weight loss, and most of them have local or distant metastasis[15].

Most R-NENs are diagnosed through screening sigmoidoscopy and/or colonoscopy while only a small fraction have symptoms such as diarrhea, abdominal pain, or rectal bleeding.

The majority of R-NENs are small size lesions less than 1 cm and only 5% present are larger than 2 cm [31].

Endoscopically, R-NENs appear as sessile or semipedunculated polypoid lesions with normal or a yellow-discolored mucosa, frequently located in the midrectum. Larger lesions can have different endoscopic characteristics such as ulcerations, depressions or hyperemic color, which can be suggestive of aggressive disease[30].

At EUS, R-NENs have the aspect of nodular, hypoechoic, or isoechoic submucosal tumor clearly demarcated from the surrounding tissue[18].

EUS-FNA can be helpful to make a differential diagnosis with other subephitelial lesion[32] with a diagnostic accuracy of 85.1%[33].

According to the current ENETS consensus, an EUS should also be performed in order to assess the depth of rectal wall invasion and regional lymphadenopathy prior to endoscopic resection[34].

A complete colonoscopy is indicated after a diagnosis of a R-NENs to exclude concomitant colon cancer and other NENs[35].

Pan-NENs

Pan-NENs comprise 1% to 2% of pancreatic neoplasms[36]. Pan-NENs are classified as functioning or nonfunctioning depending on whether they cause hormonal overproduction syndrome. Functioning Pan-NENs include insulinoma, gastrinoma, VIPoma, and glucagonoma. Nonfunctioning Pan-NENs comprise the largest group of Pan-NENs and do not produce syndromes of hormonal excess. Nonfunctioning Pan-NENs often manifest later in the course of the disease or are discovered incidentally during abdominal imaging examinations performed for other diseases[37].

EUS is a very useful tool in the management of Pan-NENs and has been considered the imaging study of choice to be performed after other negative noninvasive imaging studies are negative[38].

Puli *et al*[39] assessed the diagnostic accuracy of EUS for detection of Pan-NENs. This meta-analysis demonstrated an excellent accuracy of EUS in this setting with a sensitivity of 87.2% and a specificity of 98.0%.

A meta-analysis of 2015 assessed incremental benefit of preoperative EUS for the detection of suspected Pan-NENs after other investigative modalities have been attempted. EUS increased the overall Pan-NENs detection by over 25% and was particularly useful in functioning Pan-NENs, typically smaller in size (*i.e.* insulinomas or gastrinoma)[40].

EUS is also very useful in assessing the presence of multiple lesions, size lesion and especially the distance between the lesion and the main pancreatic duct, a factor that can drive the decision on which surgical approach (*i.e.* enucleation *vs* resection)[18]. Pancreatic enucleation is commonly performed for Pan-NENs with a low risk of malignant progression but post-operative pancreatic fistula risk is higher for neoplasms located close to the duct[41].

EUS is of course useful for differential diagnosis of Pan-NENs with other solid pancreatic lesions. Pan-NENs are solid lesions and on EUS examination, appear as well-demarcated, hypoechoic lesions with a homogeneous pattern. However, because Pan-NENs grow expansively, they may also appear as cystic or indistinguishable from pancreatic adenocarcinomas.

In Pan-NENs EUS guided tissue acquisition is considered the procedure of choice to reach cytologic diagnosis, but also ascertainment of tumor grade by determining the Ki-67 proliferation index and mitotic count (Figure 3).

EUS-FNA provides a cytological specimen with a sensitivity ranging between 80% and 90%, specificity at 96%[18]. The adequacy and concordance of Ki-67 evaluation of EUS-FNA compared with histology remains unclear especially for tumor > 20 mm[42,43]. To overcome these limitations of cytological Ki-67 determination, sampling with needles for EUS-guided fine-needle biopsy (EUS-FNB) have been introduced with good results[44,45]. In a recent retrospective study EUS-FNB outperformed EUS-FNA for Ki-67 proliferation index determination[46].

EUS elastography (EUS-E) is a newer tool of diagnostic EUS for differential diagnosis of solid pancreatic lesions. It is a technique that analyzes pancreatic stiffness being a useful tool for the differential diagnosis of pancreatic masses with a qualitative or quantitative elastographic assessment. A meta-analysis by Zhang *et al*[47] showed a sensitivity and specificity of quantitative EUS-E for the differentiation of benign and malignant pancreatic masses of 0.95 and 0.61, respectively. Iglesias-García *et al*[48] evaluated the accuracy of quantitative EUS-E for the differential diagnosis of solid pancreatic masses. EUS-E was helpful in differentiating pancreatic cancer from Pan-NENs with a sensitivity of 100% and a specificity of 88%. In another study, elastographic analysis was accurate in discriminating between benign and malignant pancreatic lesions, without difference between NENs and other nonmalignant lesions[49].

Recently, shear wave elastography (SWE) has been introduced as a quantitative absolute measurement of tissue hardness. Ohno *et al*[50] compared the diagnostic performances of EUS-SWE and conventional strain elastography for solid pancreatic lesions without significant differences.

To date EUS-E does not provide sufficient diagnostic accuracy to replace tissue diagnosis but it plays a significant role in those cases of suspected pancreatic cancer where biopsy sampling was inconclusive and can help to select the area of the pancreatic lesion to be sampled[51].

Contrast-enhanced EUS (CE-EUS) consists of an intravenous injection of contrast media during the EUS examination so parenchymal perfusion and the microvasculature of the pancreas can be visualized. Pancreatic cancer is observed as a hypoenhanced heterogeneous lesion whereas Pan-NENs are observed as well-demarcated lesions with hyperenhancement in the arterial phase.

CE-EUS increased the accuracy of EUS for both the detection and characterization of solid pancreatic lesions[52].

Kitano *et al*[53] prospectively evaluated how accurately CE-EUS characterizes pancreatic lesions and hyperenhanced lesions were diagnosed as Pan-NENs with a sensitivity of 79% and specificity of 99%. Several studies have examined CE-EUS in the differentiation between malignant and benign Pan-NENs [39,40]. Heterogeneous enhancement at an early arterial phase with fewer vessels and more fibrosis is associated with an aggressive tumor[54]. Palazzo *et al*[55] reported a sensitivity of 86% and a specificity of 96% in prediction tumor aggressiveness in Pan-NENs with the use of CE-EUS.

NET could be found also in the bile ducts[56]; however, the incidence is very low. In the literature, only 100 cases of biliary tree NETs were described and they have an excellent prognosis[57]. In these cases, complete surgical excision offers optimal treatment with no evidence of chemotherapy or radiotherapy's role in the management.

ENDOSCOPIC THERAPY

In the last 10 years, the approach of GEP-NENs progressively included endoscopic resection technique. The choice of the treatment modality needs of a careful assessment and depends by multiple factors. The indication of endoscopic respective therapy does not include NET of appendix, colon and biliary tree while for pancreatic NET the suitable endoscopic approaches are ablative techniques.

G-NENs

Most type I G-NENs are limited to the mucosa or submucosa and they rarely invade the muscularis propria or metastasize to local lymph nodes if < 10 mm. Current guidelines suggested removing all tumors ≥ 10 mm[16]. EUS is recommended to identify possible involvement of regional lymph nodes and invasion beyond the submucosa prior to resection. Endoscopic resection (ER) either by endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) is the treatment of choice when EUS demonstrates the lesion to be localized to the mucosa or submucosa[58].

Surgical treatment is the option for lesions which are predicted to be T2 (lesion larger than 20 mm or grading G3) or lesions with positive margins[58].

Sato *et al*[59] showed in a low number of cases that about 66% of type I G-NENs resected by EMR had a positive vertical margin, whereas no case with ESD had positive vertical or horizontal margins. Kim *et*

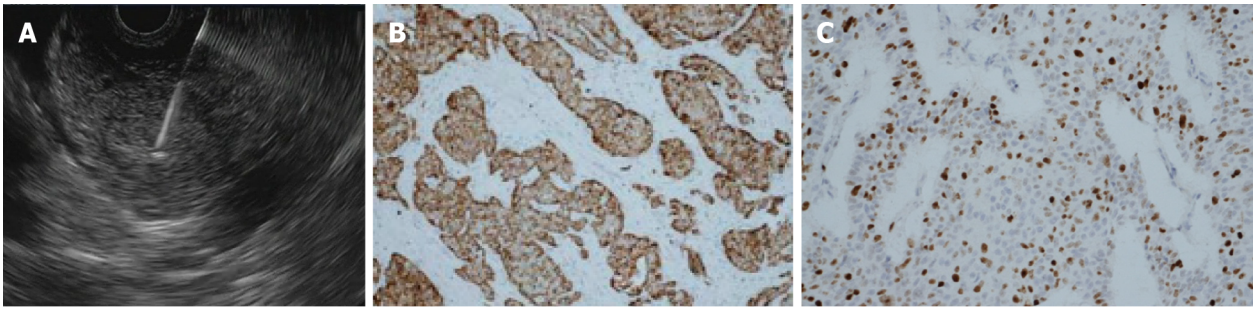

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Figure 3 Pancreatic neuroendocrine neoplasm. A: Endoscopic ultrasound revealed a 15 mm hypoechoic lesion of the pancreas; B: Stained immunohistochemically for chromogranin showing diffuse and strong positivity (original magnification 400 ×), consistent with a poorly differentiated neuroendocrine tumor infiltrating the entire thickness of the muscle tissue to the head of the pancreas; C: The proliferation index (Ki-67) < 20%.

al[60] evaluated the clinical usefulness of ESD with that of EMR for resection of type I G-NENs with an estimated size of ≤ 10 mm. ESD yielded a significantly higher histologic complete resection rate than EMR, particularly in the vertical resection margin.

Noh *et al*[61] evaluated outcomes of endoscopic treatment for type 1 G-NENs below 20 mm in diameter. The complete resection rate was significantly higher in the ESD group than in the EMR group with similar procedure-related adverse events. For type II G-NENs local or limited excision can be recommended and indication for treatment type is similar to type 1 G-NENs[24,62]. Type III G-NENs are more associated with deeper invasion of the gastric wall, higher risk of nodal metastasis than type I and II G-NENs and surgery is considered the initial therapeutic approach[58]. ER has been proposed for small lesions. Kwon *et al*[63] investigated the clinical outcomes of type 3 G-NENs (mean tumor size of 10.2 ± 6.3 mm) after endoscopic treatment with a median follow-up of 46 mo. Of the 45 included in the follow-up, no evidence of tumor recurrence was found. Authors concluded that endoscopic treatment could be applied for type 3 G-NENs smaller than 2 cm, confined in the submucosal layer and without lymphovascular invasion. In Figure 4, we summarize the current recommendations for G-NENs.

D-NENs

Most D-NENs are located in the first or second part of the duodenum, with 20% of them occurring in the periampullary region[21]. Current guidelines indicate ER for small (≤ 10 mm) nonperiampullary D-NENs confined to the submucosal layer, without lymph node or distant metastasis. Either endoscopic or surgical resection is allowed, for nonperiampullary D-NENs measuring 10-20 mm without metastatic risk (G1, no muscolaris invasion, no lymph-vascular invasion and no lymph-node metastasis)[24,58].

There are different ER techniques, such as cap technique, EMR, EMR with ligation device and ESD [58].

Gincul *et al*[64] evaluated the feasibility and outcome of endoscopic treatment of D-NENs (including 7 ampullary G1/G2 NETs with ≤ 20 mm) with EMR. The resection rate was R0 only in 51.6% (16/31) of patients. During a median follow-up period of 56 mo, 2 patients (8.3%) presented a tumor recurrence. Morbidity was 38 % (11/29) and mortality was 3% (one severe bleeding).

Nishio *et al*[65] assessed the efficacy, safety and the long-term outcomes of ESD for nonperiampullary D-NENs ≤ 10 mm in diameter. En bloc, R0 and curative resection were achieved in 100% (8/8), 88% (7/8), and 88% (7/8) of tumors, respectively. During a median follow-up of 34.0 mo none of the patients showed evidence of local recurrence or distant metastasis. Perforation occurred in 2 patients (16%) without need for surgery.

In a recent review, Brito *et al*[66] evaluated the effectiveness and complications of ER techniques in patients with D-NETs ≤ 20 mm. Polypectomy was associated with a high occurrence of incomplete resections. Among the mucosectomies, EMR with cap or EMR with injection was associated with lower frequencies of compromised margin and recurrent surgery. Endoscopic submucosal dissection was not associated with recurrence but it was associated with a higher occurrence of bleeding and perforation.

ER of D-NENs is complex and associated with significant morbidity. EMR is associated with a lower R0 resection rate than ESD. ESD is associated with better pathologically confirmed resection but with a higher morbidity rate and should be restricted to expert centers[67]. ESGE guidelines suggests choosing between EMR, ESD, and endoscopic full thickness resection (EFTR) to resect nonampullary, nonfunctional duodenal NENs of < 15 mm, depending on size, location, depth of invasion, and local expertise [16].

Ampullary D-NENs have a less favorable prognosis than nonampullary D-NENs and pancreaticoduodenectomy is recommended regardless of size[24].

Local resection might be sufficient for small highly differentiated ampullary D-NENs without node metastases especially in patients with comorbidities[24].

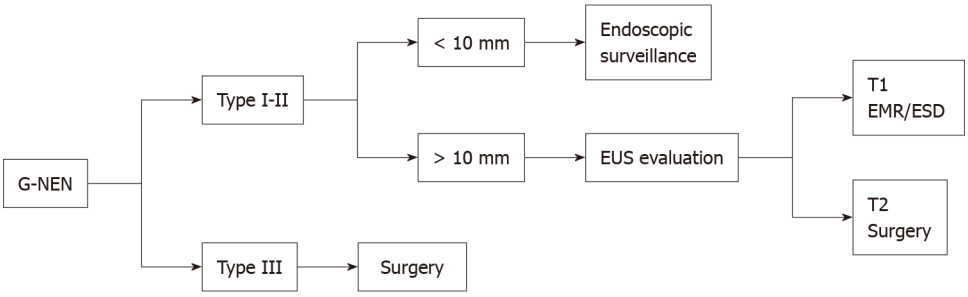

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Figure 4 Algorithm for gastric neuroendocrine neoplasm management. EMR: Endoscopic mucosal resection; ESD: Endoscopic submucosal dissection; EUS: Endoscopic ultrasonography; G-NEN: Gastric neuroendocrine neoplasm.

In one of the largest studies of EMR of D-NENs above reported, Gincul *et al*[64] included 7 ampullary G1/G2 D-NENs with ≤ 20 mm. In this subgroup of patients, R0 resection was achieved in 5 patients (71%) without recurrence with a median follow-up period of 56 mo.

Shimai *et al*[68] reported 3 patients with an ampullary D-NENS, who underwent endoscopic papillectomy without tumor recurrence or metastasis after a follow-up of 2 years. In Figure 5, we summarize the current recommendations for D-NENs.

R-NENs

The current guidelines recommend ER for R-NENs < 10 mm in size with no risk factors for metastasis. R-NENs larger than 20 mm are candidates for surgical resection. There is controversy over R-NENs of intermediate size 10-19 mm (Figure 6). R-NENs with these sizes have a poorer prognosis compared with those < 10 mm[34]. The main factors associated with the risk of lymph-node metastases are atypical endoscopic aspect (presence of mucosal depression or ulceration), suspicious pararectal lymph node at EUS, invasion of the muscularis propria, G2, and lymphovascular invasion. Rectal NET with any risk factor for metastasis should be considered for surgical resection with lymphadenoctomy. For lesions measuring 10-19 mm without factors associated with metastatic risk the most appropriate resection techniques may be ESD or transanal endoscopic microsurgery (TEM)[24-34].

The appropriate endoscopic technique resection should allow for en bloc oncological excision. Optimal endoscopic treatment modality for R-NENs has not yet been achieved.

Conventional polypectomy is associated with a low rate of complete resection as most of the R-NENs are submucosal. Son *et al*[69] reported a complete resection rate by conventional polypectomy of 30.9%.

EMR is simple and has low complication rates but can sometimes cause incomplete resection and difficulty in pathologic evaluation because even small R-NENs can invade the submucosa. Several studies, assessing efficacy of EMR, have reported complete resection rates ranging from 30%-70%[70-74].

Therefore, several m-EMR methods have been reported and included cap-assisted EMR (EMR-C), EMR with a ligating device (EMR-L), and EMR after circumferential precutting (EMR-P).

EMR-L is performed suctioning the lesion into the ligating device and cutting by using a round snare after placing bands around the base of tissue suctioned. EMR-C is performed with a transparent cap fitted to the scope, followed by snare cautery resection. These techniques allow the cutting of the submucosal layer from the muscularis propria[75].

Lee *et al*[75] performed a retrospective study comparing EMR-L and EMR-C, concluding that EMR-L may achieve both a higher endoscopic rate and a histologic complete resection rate. An analysis of 17 studies reported that the complete resection rate of R-NENs using EMR-L was 94.8% compared with 72.4% for EMR-C[76].

EMR-P is performed by lifting the mucosa with a saline injection, making a circumferential incision using the tip of the snare or special endoknives and resecting the tumor with a snare. This technique has the advantages, unlike the other m-EMR procedures, of not being affected by lesion size. Several studies have investigated the usefulness of EMR-P for resection of R-NENs showing a complete resection rate from 81.2% to 96.7% with a short procedure time, and an acceptable safety profile[77-79].

Recently Park *et al*[80] evaluated the safety and efficacy of underwater endoscopic mucosal resection in the treatment of small R-NENs (< 10 mm) with high R0 resection rates similar to ESD.

ESD is an advanced endoscopic technique employing a submucosal injection to lift the lesion away from the muscularis propria layer. Dedicated devices are then used to dissect around the entire lesion in the submucosal plane. This technique results in a high en bloc resection rate although more complicated, time consuming and with higher risk of complications than EMR and m-EMR[81].

Zhou *et al*[82] performed a meta-analysis comparing ESD with EMR and m-EMR in the treatment of R-NENs smaller than 15 mm in diameter. Complete resection rate was significantly higher in the ESD group than in the EMR group and comparable between the ESD group and the m-EMR group. A recent meta-analysis compared efficacy of ESD and EMR in curing R-NENs. This study showed that ESD is

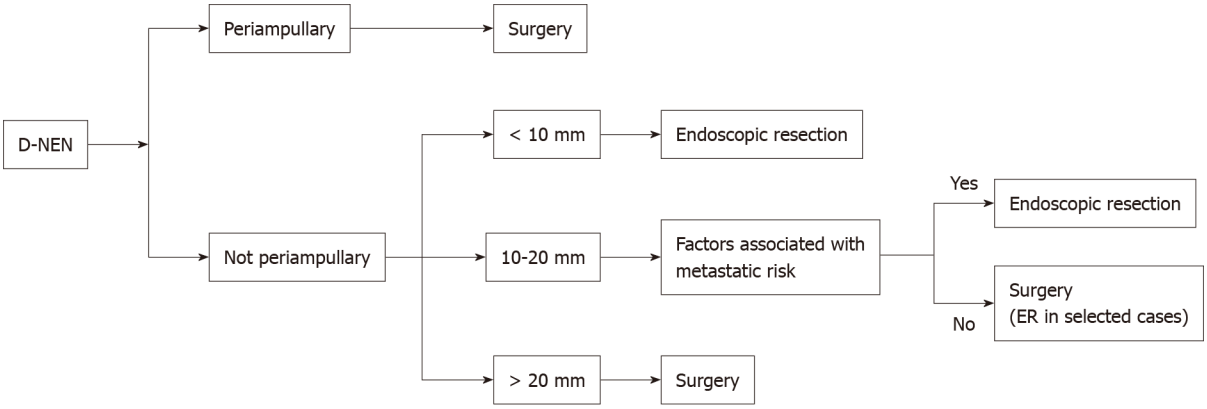

Figure 5 Algorithm for duodenal neuroendocrine neoplasms management. D-NEN: Duodenal neuroendocrine neoplasm; ER: Endoscopic resection.

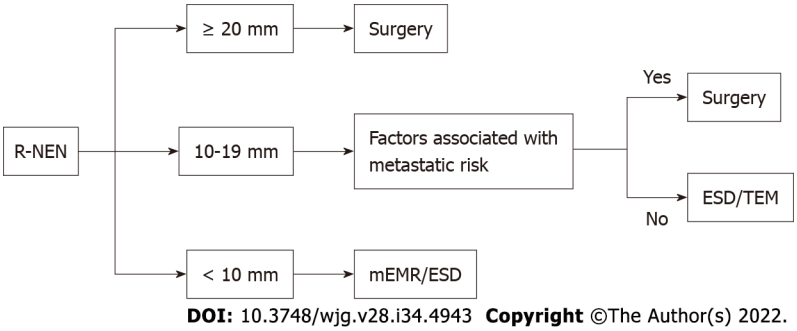

Figure 6 Algorithm for rectal neuroendocrine neoplasms management. ESD: Endoscopic submucosal dissection; mEMR: Modified endoscopic mucosal resection; R-NEN: Rectal neuroendocrine neoplasm; TEM: Transanal endoscopic microsurgery.

more effective in curing R-NENs of 10-20 mm in size than EMR without significant differences for R-NENs smaller than 10 mm[83].

In patients with an incomplete resection from EMR techniques, ESD may be indicated as salvage therapy[24,84].

A recent new endoscopic technique is EFTR, which is performed using a full thickness resection device (FTRD). The FTRD uses a transparent cap with a modified over-the-scope clip (OTSC) mounted over a standard colonoscope. The lesion is pulled into the cap and the pseudopolyp created by the OTSC closure is then resected with the snare preloaded in the tip of the cap. Meier *et al*[85] conducted a study evaluating EFTR in 40 cases of R-NENs showing effectiveness of this method (R0 resection rate in 95%) in addition to feasibility (median time, 18.5 min) and safety.

Pan-NENs

Patients with small Pan-NENs are candidates for pancreatic-sparing procedures such as central pancreatectomy or enucleation. Small Pan-NENs can be difficult to detect intra-operatively by palpation only. EUS-guided techniques to facilitate small Pan-NENs localization especially during laparoscopy surgery are tattooing or fiducial markers implantation. These techniques allow a precise localization of lesions ensuring adequate margins of resection and preserving normal pancreatic parenchyma[86].

Endoscopic ultrasound-guided fine needle tattooing (EUS-FNT) is a safe, easy to perform and useful new method to mark preoperatively small Pan-NENs. Generally, a 22-gauge standard needle allows easy injection of the tattooing solution. The needle is inserted inside the target lesion or immediately near the lesion borders into the normal parenchyma. The most frequently solution utilized is a sterile carbon-based ink which is nondegradable and remains in the tissues indefinitely[87].

Recently Rosa *et al*[88] evaluated EUS-FNT in facilitating intra-operative detection of Pan-NENs (8 insulinoma and 8 nonfunctional Pan-NENs.) The tattoo mark was detected in all but one patient. Only a small hematoma secondary to the EUS-FNT was observed.

The placement of fiducial markers implantation under EUS guidance is another technique to facilitate Pan-NENs localization during surgery. Fiducials are implantable radiographic markers that have been used for many years to mark soft tissue in radiology. The fiducial is easily visible during an intraoperative ultrasound[86].

Law *et al*[89] reported on two consecutive patients with small Pan-NENs who underwent fiducial placement. In both of the reported cases, the fiducials were visualized by intraoperative ultrasound, and the surgical resection was successful without procedure-related complications.

In recent years endoscopic ultrasound-guided ablation therapy has emerged as a new therapeutic option for solid pancreatic tumors, especially for Pan-NENs in elderly patients and candidates unfit for surgery. There are two main techniques used, EUS-guided radiofrequency ablation (EUS-RFA) and EUS-guided ethanol ablation (EUS-EA)[90]. Functioning and multiple Pan-NENs seem to be the ideal target for endoscopic ultrasound -guided ablation therapy resolving symptomatic hormonal syndromes. In case of nonfunctioning Pan-NENs, these techniques could be a therapeutic option in the case of patients unfit to surgery[91].

EUS-EA can be safe and useful for the control of symptoms in patients with small insulinomas[91-93].

Jürgensen *et al*[92] reported the first case of EUS–EA of a 13 mm pancreatic insulinoma in a 78-year-old woman. The patient exhibited no further hypoglycemic episodes with no recurrence of the tumor on follow-up.

Levy *et al*[93] reported a small series of 5 patients for EUS-EA of insulinomas; they observed that symptomatic relief was relieved almost immediately after the procedure and maintained during the follow-up.

Qin *et al*[94] reported a series of 4 patients for EUS-EA of insulinomas with no recurrence of hypoglycemia and complications during follow-up.

Choi *et al*[95] reported the largest cohort of 32 patients with nonfunctioning Pan-NENs treated with EUS–EA. In 24 out of 40 tumors (60%) complete ablation was achieved.

The other less invasive locoregional therapy for Pan-NENs is EUS-RFA. Multiple case reports of EUS-RFA of insulinomas showed complete regression of the clinical syndrome[96-98].

Regarding nonfunctioning Pan-NENs, Barthet *et al*[99] conducted a prospective multicenter study including 12 patients with 14 Pan-NENs (mean size 13.1 mm) treated with EUS-RFA. Among the 14 Pan-NENs, at 1-year follow-up, 12 had completely disappeared (86% tumor resolution). Two adverse events occurred (one pancreatitis, one pancreatic ductal stenosis). Another case series included 11 patients with nonfunctioning Pan-NENs. A complete radiological response was achieved in 8 of 11 patients treated with EUS-RFA. Two cases of mild pancreatitis occurred[100].

CONCLUSION

GEP-NENs are on the rise. The reasons for this phenomenon are a better awareness of an increased and more widespread use of GI endoscopy and advanced radiological imaging[1]. The overall survival rate for patients with GEP-NENs has improved in the last years[2]. This achievement is due to both early detection and better therapeutic strategies of GEP-NENs.

Endoscopy is the only method of choice to detect asymptomatic GI-NENs at an early stage. Most patients with early, GI-NENs can be treated with ER or surveillance.

Pan-NENs are relatively rare tumors but their number is increasing, mainly because of the advances in various diagnostic imaging modalities as EUS. Newer advancement in the field of EUS such as the evolution of needles, EUS-E and CE-EUS can provide useful information with an improvement in the management of Pan-NENs. EUS-guided tumor ablation therapies can be a therapeutic option in selected patients unfit for surgery.

The literature evidence on this field is growing every day. For the nature of our study (mini-review) and the very wide issue, we collected and summarized the most important evidence regarding the endoscopic treatment underlying current guideline. We believe that a systematic review the literature with meta-analysis (when applicable) for every specific GI-NENs is needed to prove and confirm the role of endoscopy in diagnosis and therapy of many types of GI-NENs.

FOOTNOTES

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REFERENCES

1 **Niederle MB**, Hackl M, Kaserer K, Niederle B. Gastroenteropancreatic neuroendocrine tumours: the current incidence and staging based on the WHO and European Neuroendocrine Tumour Society classification: an analysis based on prospectively collected parameters. *Endocr Relat Cancer* 2010; **17**: 909-918 [PMID: 20702725 DOI: 10.1677/ERC-10-0152]
2 **Dasari A**, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, Shih T, Yao JC. Trends in the Incidence, Prevalence, and Survival Outcomes in Patients With Neuroendocrine Tumors in the United States. *JAMA Oncol* 2017; **3**: 1335-1342 [PMID: 28448665 DOI: 10.1001/jamaoncol.2017.0589]
3 **Milan SA**, Yeo CJ. Neuroendocrine tumors of the pancreas. *Curr Opin Oncol* 2012; **24**: 46-55 [PMID: 22080942 DOI: 10.1097/CCO.0b013e32834c554d]
4 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
5 **Luo G**, Javed A, Strosberg JR, Jin K, Zhang Y, Liu C, Xu J, Soares K, Weiss MJ, Zheng L, Wolfgang CL, Cives M, Wong J, Wang W, Sun J, Shao C, Tan H, Li J, Ni Q, Shen L, Chen M, He J, Chen J, Yu X. Modified Staging Classification for Pancreatic Neuroendocrine Tumors on the Basis of the American Joint Committee on Cancer and European Neuroendocrine Tumor Society Systems. *J Clin Oncol* 2017; **35**: 274-280 [PMID: 27646952 DOI: 10.1200/JCO.2016.67.8193]
6 **Amin MB**, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR, Winchester DP. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA Cancer J Clin* 2017; **67**: 93-99 [PMID: 28094848 DOI: 10.3322/caac.21388]
7 **Dillon JS**. Workup of Gastroenteropancreatic Neuroendocrine Tumors. *Surg Oncol Clin N Am* 2020; **29**: 165-183 [PMID: 32151354 DOI: 10.1016/j.soc.2019.10.002]
8 **Fang JM**, Li J, Shi J. An update on the diagnosis of gastroenteropancreatic neuroendocrine neoplasms. *World J Gastroenterol* 2022; **28**: 1009-1023 [PMID: 35431496 DOI: 10.3748/wjg.v28.i10.1009]
9 **Oberg K**, Modlin IM, De Herder W, Pavel M, Klimstra D, Frilling A, Metz DC, Heaney A, Kwekkeboom D, Strosberg J, Meyer T, Moss SF, Washington K, Wolin E, Liu E, Goldenring J. Consensus on biomarkers for neuroendocrine tumour disease. *Lancet Oncol* 2015; **16**: e435-e446 [PMID: 26370353 DOI: 10.1016/S1470-2045(15)00186-2]
10 **Sansone A**, Lauretta R, Vottari S, Chiefari A, Barnabei A, Romanelli F, Appetecchia M. Specific and Non-Specific Biomarkers in Neuroendocrine Gastroenteropancreatic Tumors. *Cancers (Basel)* 2019; **11** [PMID: 31382663 DOI: 10.3390/cancers11081113]
11 **Fernandez CJ**, Agarwal M, Pottakkat B, Haroon NN, George AS, Pappachan JM. Gastroenteropancreatic neuroendocrine neoplasms: A clinical snapshot. *World J Gastrointest Surg* 2021; **13**: 231-255 [PMID: 33796213 DOI: 10.4240/wjgs.v13.i3.231]
12 **Cives M**, Strosberg JR. Gastroenteropancreatic Neuroendocrine Tumors. *CA Cancer J Clin* 2018; **68**: 471-487 [PMID: 30295930 DOI: 10.3322/caac.21493]
13 **Rindi G**, Luinetti O, Cornaggia M, Capella C, Solcia E. Three subtypes of gastric argyrophil carcinoid and the gastric neuroendocrine carcinoma: a clinicopathologic study. *Gastroenterology* 1993; **104**: 994-1006 [PMID: 7681798 DOI: 10.1016/0016-5085(93)90266-f]
14 **Basuroy R**, Srirajaskanthan R, Prachalias A, Quaglia A, Ramage JK. Review article: the investigation and management of gastric neuroendocrine tumours. *Aliment Pharmacol Ther* 2014; **39**: 1071-1084 [PMID: 24628514 DOI: 10.1111/apt.12698]
15 **Ahmed M**. Gastrointestinal neuroendocrine tumors in 2020. *World J Gastrointest Oncol* 2020; **12**: 791-807 [PMID: 32879660 DOI: 10.4251/wjgo.v12.i8.791]
16 **Deprez PH**, Moons LMG, O'Toole D, Gincul R, Seicean A, Pimentel-Nunes P, Fernández-Esparrach G, Polkowski M, Vieth M, Borbath I, Moreels TG, Nieveen van Dijkum E, Blay JY, van Hooft JE. Endoscopic management of subepithelial lesions including neuroendocrine neoplasms: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2022; **54**: 412-429 [PMID: 35180797 DOI: 10.1055/a-1751-5742]
17 **Varas MJ**, Gornals JB, Pons C, Espinós JC, Abad R, Lorente FJ, Bargalló D. Usefulness of endoscopic ultrasonography (EUS) for selecting carcinoid tumors as candidates to endoscopic resection. *Rev Esp Enferm Dig* 2010; **102**: 577-582 [PMID: 21039065 DOI: 10.4321/s1130-01082010001000002]
18 **Zilli A**, Arcidiacono PG, Conte D, Massironi S. Clinical impact of endoscopic ultrasonography on the management of neuroendocrine tumors: lights and shadows. *Dig Liver Dis* 2018; **50**: 6-14 [PMID: 29102525 DOI:

10.1016/j.dld.2017.10.007]
19 **Hoffmann KM**, Furukawa M, Jensen RT. Duodenal neuroendocrine tumors: Classification, functional syndromes, diagnosis and medical treatment. *Best Pract Res Clin Gastroenterol* 2005; **19**: 675-697 [PMID: 16253893 DOI: 10.1016/j.bpg.2005.05.009]
20 **Yao JC**, Hassan M, Phan A, Dagohoy C, Leary C, Mares JE, Abdalla EK, Fleming JB, Vauthey JN, Rashid A, Evans DB. One hundred years after "carcinoid": epidemiology of and prognostic factors for neuroendocrine tumors in 35,825 cases in the United States. *J Clin Oncol* 2008; **26**: 3063-3072 [PMID: 18565894 DOI: 10.1200/JCO.2007.15.4377]
21 **Randle RW**, Ahmed S, Newman NA, Clark CJ. Clinical outcomes for neuroendocrine tumors of the duodenum and ampulla of Vater: a population-based study. *J Gastrointest Surg* 2014; **18**: 354-362 [PMID: 24114680 DOI: 10.1007/s11605-013-2365-4]
22 **Sato Y**, Hashimoto S, Mizuno K, Takeuchi M, Terai S. Management of gastric and duodenal neuroendocrine tumors. *World J Gastroenterol* 2016; **22**: 6817-6828 [PMID: 27570419 DOI: 10.3748/wjg.v22.i30.6817]
23 **Pavlovic Markovic A**, Rösch T, Alempijevic T, Krstic M, Tomic D, Dugalic P, Sokic Milutinovic A, Bulajic M. Endoscopic ultrasound for differential diagnosis of duodenal lesions. *Ultraschall Med* 2012; **33**: E210-E217 [PMID: 23129520 DOI: 10.1055/s-0032-1313135]
24 **de Mestier L**, Lepage C, Baudin E, Coriat R, Courbon F, Couvelard A, Do Cao C, Frampas E, Gaujoux S, Gincul R, Goudet P, Lombard-Bohas C, Poncet G, Smith D, Ruszniewski P, Lecomte T, Bouché O, Walter T, Cadiot G; Thésaurus National de Cancérologie Digestive (TNCD). Digestive Neuroendocrine Neoplasms (NEN): French Intergroup clinical practice guidelines for diagnosis, treatment and follow-up (SNFGE, GTE, RENATEN, TENPATH, FFCD, GERCOR, UNICANCER, SFCD, SFED, SFRO, SFR). *Dig Liver Dis* 2020; **52**: 473-492 [PMID: 32234416 DOI: 10.1016/j.dld.2020.02.011]
25 **Scott AT**, Howe JR. Management of Small Bowel Neuroendocrine Tumors. *Surg Oncol Clin N Am* 2020; **29**: 223-241 [PMID: 32151357 DOI: 10.1016/j.soc.2019.11.006]
26 **Scott AT**, Howe JR. Management of Small Bowel Neuroendocrine Tumors. *J Oncol Pract* 2018; **14**: 471-482 [PMID: 30096273 DOI: 10.1200/JOP.18.00135]
27 **Hirabayashi K**, Zamboni G, Nishi T, Tanaka A, Kajiwara H, Nakamura N. Histopathology of gastrointestinal neuroendocrine neoplasms. *Front Oncol* 2013; **3**: 2 [PMID: 23346552 DOI: 10.3389/fonc.2013.00002]
28 **Sivero L**, Telesca DA, Ruggiero S, Russo T, Amato M, Bianco T, Amato B, Formisano C, Avellino M, Napolitano V. Endoscopic diagnosis and treatment of neuroendocrine tumors of the digestive system. *Open Med (Wars)* 2016; **11**: 369-373 [PMID: 28352822 DOI: 10.1515/med-2016-0067]
29 **Toumpanakis C**, Fazio N, Tiensuu Janson E, Hörsch D, Pascher A, Reed N, O Apos Toole D, Nieveen van Dijkum E, Partelli S, Rinke A, Kos-Kudla B, Costa F, Pape UF, Grozinsky-Glasberg S, Scoazec JY; The ENETS 2016 Munich Advisory Board Participants; ENETS 2016 Munich Advisory Board Participants. Unmet Needs in Appendiceal Neuroendocrine Neoplasms. *Neuroendocrinology* 2019; **108**: 37-44 [PMID: 30235454 DOI: 10.1159/000493894]
30 **Broecker JS**, Ethun CG, Postlewait LM, Le N, McInnis M, Russell MC, Sullivan P, Kooby DA, Staley CA, Maithel SK, Cardona K. Colon and Rectal Neuroendocrine Tumors: Are They Really One Disease? *Am Surg* 2018; **84**: 717-726 [PMID: 29966574 DOI: 10.1177/000313481808400525]
31 **McDermott FD**, Heeney A, Courtney D, Mohan H, Winter D. Rectal carcinoids: a systematic review. *Surg Endosc* 2014; **28**: 2020-2026 [PMID: 24584484 DOI: 10.1007/s00464-014-3430-0]
32 **Cazacu IM**, Singh BS, Luzuriaga Chavez AA, Koduru P, Ejaz S, Weston BR, Ross WA, Lee JH, Roy-Chowdhuri S, Bhutani MS. EUS and EUS-guided FNA/core biopsies in the evaluation of subepithelial lesions of the lower gastrointestinal tract: 10-year experience. *Endosc Ultrasound* 2020; **9**: 329-336 [PMID: 32913150 DOI: 10.4103/eus.eus_51_20]
33 **Chen HT**, Xu GQ, Teng XD, Chen YP, Chen LH, Li YM. Diagnostic accuracy of endoscopic ultrasonography for rectal neuroendocrine neoplasms. *World J Gastroenterol* 2014; **20**: 10470-10477 [PMID: 25132764 DOI: 10.3748/wjg.v20.i30.10470]
34 **Caplin M**, Sundin A, Nillson O, Baum RP, Klose KJ, Kelestimur F, Plöckinger U, Papotti M, Salazar R, Pascher A; Barcelona Consensus Conference participants. ENETS Consensus Guidelines for the management of patients with digestive neuroendocrine neoplasms: colorectal neuroendocrine neoplasms. *Neuroendocrinology* 2012; **95**: 88-97 [PMID: 22261972 DOI: 10.1159/000335594]
35 **Kamp K**, Damhuis RA, Feelders RA, de Herder WW. Occurrence of second primary malignancies in patients with neuroendocrine tumors of the digestive tract and pancreas. *Endocr Relat Cancer* 2012; **19**: 95-99 [PMID: 22194442 DOI: 10.1530/ERC-11-0315]
36 **Ito T**, Sasano H, Tanaka M, Osamura RY, Sasaki I, Kimura W, Takano K, Obara T, Ishibashi M, Nakao K, Doi R, Shimatsu A, Nishida T, Komoto I, Hirata Y, Nakamura K, Igarashi H, Jensen RT, Wiedenmann B, Imamura M. Epidemiological study of gastroenteropancreatic neuroendocrine tumors in Japan. *J Gastroenterol* 2010; **45**: 234-243 [PMID: 20058030 DOI: 10.1007/s00535-009-0194-8]
37 **Massironi S**, Sciola V, Peracchi M, Ciafardini C, Spampatti MP, Conte D. Neuroendocrine tumors of the gastro-entero-pancreatic system. *World J Gastroenterol* 2008; **14**: 5377-5384 [PMID: 18803349 DOI: 10.3748/wjg.14.5377]
38 **Falconi M**, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Klöppel G, Reed N, Kianmanesh R, Jensen RT; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for the Management of Patients with Functional Pancreatic Neuroendocrine Tumors and Non-Functional Pancreatic Neuroendocrine Tumors. *Neuroendocrinology* 2016; **103**: 153-171 [PMID: 26742109 DOI: 10.1159/000443171]
39 **Puli SR**, Kalva N, Bechtold ML, Pamulaparthy SR, Cashman MD, Estes NC, Pearl RH, Volmar FH, Dillon S, Shekleton MF, Forcione D. Diagnostic accuracy of endoscopic ultrasound in pancreatic neuroendocrine tumors: a systematic review and meta analysis. *World J Gastroenterol* 2013; **19**: 3678-3684 [PMID: 23801872 DOI: 10.3748/wjg.v19.i23.3678]
40 **James PD**, Tsolakis AV, Zhang M, Belletrutti PJ, Mohamed R, Roberts DJ, Heitman SJ. Incremental benefit of preoperative EUS for the detection of pancreatic neuroendocrine tumors: a meta-analysis. *Gastrointest Endosc* 2015; **81**: 848-56.e1 [PMID: 25805462 DOI: 10.1016/j.gie.2014.12.031]

41 **Giuliani T**, Marchegiani G, Girgis MD, Crinò SF, Muthusamy VR, Bernardoni L, Pea A, Ramera M, Paiella S, Landoni L, Gabbrielli A, Salvia R, Donahue TR, Bassi C. Endoscopic placement of pancreatic stent for "Deep" pancreatic enucleations operative technique and preliminary experience at two high-volume centers. *Surg Endosc* 2020; **34**: 2796-2802 [PMID: 32180000 DOI: 10.1007/s00464-020-07501-y]

42 **Paiella S**, Landoni L, Rota R, Valenti M, Elio G, Crinò SF, Manfrin E, Parisi A, Cingarlini S, D'Onofrio M, Scarpa A, Lawlor RT, Bernardoni L, Capelli P, Nessi C, Miotto M, Gabbrielli A, Bassi C, Salvia R. Endoscopic ultrasound-guided fine-needle aspiration for the diagnosis and grading of pancreatic neuroendocrine tumors: a retrospective analysis of 110 cases. *Endoscopy* 2020; **52**: 988-994 [PMID: 32498099 DOI: 10.1055/a-1180-8614]

43 **Boutsen L**, Jouret-Mourin A, Borbath I, van Maanen A, Weynand B. Accuracy of Pancreatic Neuroendocrine Tumour Grading by Endoscopic Ultrasound-Guided Fine Needle Aspiration: Analysis of a Large Cohort and Perspectives for Improvement. *Neuroendocrinology* 2018; **106**: 158-166 [PMID: 28494461 DOI: 10.1159/000477213]

44 **Leeds JS**, Nayar MK, Bekkali NLH, Wilson CH, Johnson SJ, Haugk B, Darne A, Oppong KW. Endoscopic ultrasound-guided fine-needle biopsy is superior to fine-needle aspiration in assessing pancreatic neuroendocrine tumors. *Endosc Int Open* 2019; **7**: E1281-E1287 [PMID: 31579710 DOI: 10.1055/a-0990-9611]

45 **Kamata K**, Ashida R, Yasukawa S, Chiba Y, Fukutake N, Nebiki H, Kurita A, Takaoka M, Ogura T, Shiomi H, Asada M, Yasuda H, Shigekawa M, Yanagisawa A, Kudo M, Kitano M. Histological diagnosis and grading of pancreatic neuroendocrine tumor by endoscopic ultrasound-guided fine needle biopsy using a 25-gauge needle with a core trap: A multicenter prospective trial. *Pancreatology* 2020; **20**: 1428-1433 [PMID: 32952043 DOI: 10.1016/j.pan.2020.08.023]

46 **Crinò SF**, Ammendola S, Meneghetti A, Bernardoni L, Conti Bellocchi MC, Gabbrielli A, Landoni L, Paiella S, Pin F, Parisi A, Mastrosimini MG, Amodio A, Frulloni L, Facciorusso A, Larghi A, Manfrin E. Comparison between EUS-guided fine-needle aspiration cytology and EUS-guided fine-needle biopsy histology for the evaluation of pancreatic neuroendocrine tumors. *Pancreatology* 2021; **21**: 443-450 [PMID: 33390343 DOI: 10.1016/j.pan.2020.12.015]

47 **Zhang B**, Zhu F, Li P, Yu S, Zhao Y, Li M. Endoscopic ultrasound elastography in the diagnosis of pancreatic masses: A meta-analysis. *Pancreatology* 2018; **18**: 833-840 [PMID: 30093353 DOI: 10.1016/j.pan.2018.07.008]

48 **Iglesias-Garcia J**, Larino-Noia J, Abdulkader I, Forteza J, Dominguez-Munoz JE. Quantitative endoscopic ultrasound elastography: an accurate method for the differentiation of solid pancreatic masses. *Gastroenterology* 2010; **139**: 1172-1180 [PMID: 20600020 DOI: 10.1053/j.gastro.2010.06.059]

49 **Carrara S**, Di Leo M, Grizzi F, Correale L, Rahal D, Anderloni A, Auriemma F, Fugazza A, Preatoni P, Maselli R, Hassan C, Finati E, Mangiavillano B, Repici A. EUS elastography (strain ratio) and fractal-based quantitative analysis for the diagnosis of solid pancreatic lesions. *Gastrointest Endosc* 2018; **87**: 1464-1473 [PMID: 29329992 DOI: 10.1016/j.gie.2017.12.031]

50 **Ohno E**, Kawashima H, Ishikawa T, Iida T, Suzuki H, Uetsuki K, Yashika J, Yamada K, Yoshikawa M, Gibo N, Aoki T, Kataoka K, Mori H, Yamamura T, Furukawa K, Nakamura M, Hirooka Y, Fujishiro M. Diagnostic performance of endoscopic ultrasonography-guided elastography for solid pancreatic lesions: Shear-wave measurements versus strain elastography with histogram analysis. *Dig Endosc* 2021; **33**: 629-638 [PMID: 32662150 DOI: 10.1111/den.13791]

51 **Cosgrove D**, Piscaglia F, Bamber J, Bojunga J, Correas JM, Gilja OH, Klauser AS, Sporea I, Calliada F, Cantisani V, D'Onofrio M, Drakonaki EE, Fink M, Friedrich-Rust M, Fromageau J, Havre RF, Jenssen C, Ohlinger R, Săftoiu A, Schaefer F, Dietrich CF; EFSUMB. EFSUMB guidelines and recommendations on the clinical use of ultrasound elastography. Part 2: Clinical applications. *Ultraschall Med* 2013; **34**: 238-253 [PMID: 23605169 DOI: 10.1055/s-0033-1335375]

52 **He XK**, Ding Y, Sun LM. Contrast-enhanced endoscopic ultrasound for differential diagnosis of pancreatic cancer: an updated meta-analysis. *Oncotarget* 2017; **8**: 66392-66401 [PMID: 29029521 DOI: 10.18632/oncotarget.18915]

53 **Kitano M**, Kudo M, Yamao K, Takagi T, Sakamoto H, Komaki T, Kamata K, Imai H, Chiba Y, Okada M, Murakami T, Takeyama Y. Characterization of small solid tumors in the pancreas: the value of contrast-enhanced harmonic endoscopic ultrasonography. *Am J Gastroenterol* 2012; **107**: 303-310 [PMID: 22008892 DOI: 10.1038/ajg.2011.354]

54 **Takada S**, Kato H, Saragai Y, Muro S, Uchida D, Tomoda T, Matsumoto K, Horiguchi S, Tanaka N, Okada H. Contrast-enhanced harmonic endoscopic ultrasound using time-intensity curve analysis predicts pathological grade of pancreatic neuroendocrine neoplasm. *J Med Ultrason (2001)* 2019; **46**: 449-458 [PMID: 31377939 DOI: 10.1007/s10396-019-00967-x]

55 **Palazzo M**, Napoléon B, Gincul R, Pioche M, Pujol B, Lefort C, Fumex F, Hautefeuille V, Fabre M, Cros J, Felce M, Couvelard A, Sauvanet A, Lévy P, Ruszniewski P, Palazzo L. Contrast harmonic EUS for the prediction of pancreatic neuroendocrine tumor aggressiveness (with videos). *Gastrointest Endosc* 2018; **87**: 1481-1488 [PMID: 29325706 DOI: 10.1016/j.gie.2017.12.033]

56 **De Luca L**, Tommasoni S, de Leone A, Bianchi ML, de Nictolis M, Baroncini D. Neuroendocrine tumor of the extrahepatic bile duct: a tumor in an unusual site visualized by cholangioscopy. *Endoscopy* 2013; **45** Suppl 2: E338-E339 [PMID: 24150737 DOI: 10.1055/s-0032-1326453]

57 **Altiti M**, Al-Sa'afin AJ, Al-Tawarah T, Suleihat A, Abulhaj S, Mahseeri M. Biliary tree neuroendocrine tumor, an incidental finding. *Int J Surg Case Rep* 2021; **82**: 105940 [PMID: 33964717 DOI: 10.1016/j.ijscr.2021.105940]

58 **Delle Fave G**, O'Toole D, Sundin A, Taal B, Ferolla P, Ramage JK, Ferone D, Ito T, Weber W, Zheng-Pei Z, De Herder WW, Pascher A, Ruszniewski P; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for Gastroduodenal Neuroendocrine Neoplasms. *Neuroendocrinology* 2016; **103**: 119-124 [PMID: 26784901 DOI: 10.1159/000443168]

59 **Sato Y**, Takeuchi M, Hashimoto S, Mizuno K, Kobayashi M, Iwafuchi M, Narisawa R, Aoyagi Y. Usefulness of endoscopic submucosal dissection for type I gastric carcinoid tumors compared with endoscopic mucosal resection. *Hepatogastroenterology* 2013; **60**: 1524-1529 [PMID: 23933946 DOI: 10.5754/hge121185]

60 **Kim HH**, Kim GH, Kim JH, Choi MG, Song GA, Kim SE. The efficacy of endoscopic submucosal dissection of type I gastric carcinoid tumors compared with conventional endoscopic mucosal resection. *Gastroenterol Res Pract* 2014; **2014**: 253860 [PMID: 24693280 DOI: 10.1155/2014/253860]

61 **Noh JH**, Kim DH, Yoon H, Hsing LC, Na HK, Ahn JY, Lee JH, Jung KW, Choi KD, Song HJ, Lee GH, Jung HY.

Clinical Outcomes of Endoscopic Treatment for Type 1 Gastric Neuroendocrine Tumor. *J Gastrointest Surg* 2021; **25**: 2495-2502 [PMID: 33825119 DOI: 10.1007/s11605-021-04997-0]

62 **De Luca L**, Di Berardino M, Mangiavillano B, Repici A. Gastric endoscopic submucosal dissection in Western countries: Indications, applications, efficacy and training perspective. *World J Gastrointest Surg* 2021; **13**: 1180-1189 [PMID: 34754386 DOI: 10.4240/wjgs.v13.i10.1180]

63 **Kwon YH**, Jeon SW, Kim GH, Kim JI, Chung IK, Jee SR, Kim HU, Seo GS, Baik GH, Choi KD, Moon JS. Long-term follow up of endoscopic resection for type 3 gastric NET. *World J Gastroenterol* 2013; **19**: 8703-8708 [PMID: 24379589 DOI: 10.3748/wjg.v19.i46.8703]

64 **Gincul R**, Ponchon T, Napoleon B, Scoazec JY, Guillaud O, Saurin JC, Ciocirlan M, Lepilliez V, Pioche M, Lefort C, Adham M, Pialat J, Chayvialle JA, Walter T. Endoscopic treatment of sporadic small duodenal and ampullary neuroendocrine tumors. *Endoscopy* 2016; **48**: 979-986 [PMID: 27494453 DOI: 10.1055/s-0042-112570]

65 **Nishio M**, Hirasawa K, Ozeki Y, Sawada A, Ikeda R, Fukuchi T, Kobayashi R, Makazu M, Sato C, Maeda S. Short- and long-term outcomes of endoscopic submucosal dissection for non-ampullary duodenal neuroendocrine tumors. *Ann Gastroenterol* 2020; **33**: 265-271 [PMID: 32382229 DOI: 10.20524/aog.2020.0477]

66 **Brito HP**, Torres IT, Turke KC, Parada AA, Waisberg J, Botelho RV. Comparison of endoscopic resection techniques for duodenal neuroendocrine tumors: systematic review. *Endosc Int Open* 2021; **9**: E1214-E1221 [PMID: 34447867 DOI: 10.1055/a-1487-5594]

67 **Amoyel M**, Belle A, Dhooge M, Ali EA, Hallit R, Prat F, Dohan A, Terris B, Chaussade S, Coriat R, Barret M. Endoscopic management of non-ampullary duodenal adenomas. *Endosc Int Open* 2022; **10**: E96-E108 [PMID: 35047339 DOI: 10.1055/a-1723-2847]

68 **Shimai S**, Yamamoto K, Sofuni A, Tsuchiya T, Ishii K, Tanaka R, Tonozuka R, Honjo M, Mukai S, Fujita M, Nagai K, Asai Y, Matsunami Y, Kurosawa T, Kojima H, Honma H, Minami H, Yamaguchi H, Itoi T. Three Cases of Ampullary Neuroendocrine Tumor Treated by Endoscopic Papillectomy: A Case Report and Literature Review. *Intern Med* 2020; **59**: 2369-2374 [PMID: 32611953 DOI: 10.2169/internalmedicine.4568-20]

69 **Son HJ**, Sohn DK, Hong CW, Han KS, Kim BC, Park JW, Choi HS, Chang HJ, Oh JH. Factors associated with complete local excision of small rectal carcinoid tumor. *Int J Colorectal Dis* 2013; **28**: 57-61 [PMID: 22821140 DOI: 10.1007/s00384-012-1538-z]

70 **Kim KM**, Eo SJ, Shim SG, Choi JH, Min BH, Lee JH, Chang DK, Kim YH, Rhee PL, Kim JJ, Rhee JC, Kim JY. Treatment outcomes according to endoscopic treatment modalities for rectal carcinoid tumors. *Clin Res Hepatol Gastroenterol* 2013; **37**: 275-282 [PMID: 22959100 DOI: 10.1016/j.clinre.2012.07.007]

71 **Yang DH**, Park Y, Park SH, Kim KJ, Ye BD, Byeon JS, Myung SJ, Yang SK. Cap-assisted EMR for rectal neuroendocrine tumors: comparisons with conventional EMR and endoscopic submucosal dissection (with videos). *Gastrointest Endosc* 2016; **83**: 1015-22; quiz 1023 [PMID: 26460225 DOI: 10.1016/j.gie.2015.09.046]

72 **Nakamura K**, Osada M, Goto A, Iwasa T, Takahashi S, Takizawa N, Akahoshi K, Ochiai T, Nakamura N, Akiho H, Itaba S, Harada N, Iju M, Tanaka M, Kubo H, Somada S, Ihara E, Oda Y, Ito T, Takayanagi R. Short- and long-term outcomes of endoscopic resection of rectal neuroendocrine tumours: analyses according to the WHO 2010 classification. *Scand J Gastroenterol* 2016; **51**: 448-455 [PMID: 26540372 DOI: 10.3109/00365521.2015.1107752]

73 **Jeon JH**, Cheung DY, Lee SJ, Kim HJ, Kim HK, Cho HJ, Lee IK, Kim JI, Park SH, Kim JK. Endoscopic resection yields reliable outcomes for small rectal neuroendocrine tumors. *Dig Endosc* 2014; **26**: 556-563 [PMID: 24447261 DOI: 10.1111/den.12232]

74 **Kim HH**, Park SJ, Lee SH, Park HU, Song CS, Park MI, Moon W. Efficacy of endoscopic submucosal resection with a ligation device for removing small rectal carcinoid tumor compared with endoscopic mucosal resection: analysis of 100 cases. *Dig Endosc* 2012; **24**: 159-163 [PMID: 22507089 DOI: 10.1111/j.1443-1661.2011.01190.x]

75 **Lee J**, Park YE, Choi JH, Heo NY, Park J, Park SH, Moon YS, Nam KH, Kim TO. Comparison between cap-assisted and ligation-assisted endoscopic mucosal resection for rectal neuroendocrine tumors. *Ann Gastroenterol* 2020; **33**: 385-390 [PMID: 32624659 DOI: 10.20524/aog.2020.0485]

76 **de Mestier L**, Brixi H, Gincul R, Ponchon T, Cadiot G. Updating the management of patients with rectal neuroendocrine tumors. *Endoscopy* 2013; **45**: 1039-1046 [PMID: 24163193 DOI: 10.1055/s-0033-1344794]

77 **Cheung DY**, Choi SK, Kim HK, Kim SS, Chae HS, Seo KJ, Cho YS. Circumferential submucosal incision prior to endoscopic mucosal resection provides comparable clinical outcomes to submucosal dissection for well-differentiated neuroendocrine tumors of the rectum. *Surg Endosc* 2015; **29**: 1500-1505 [PMID: 25277474 DOI: 10.1007/s00464-014-3831-0]

78 **So H**, Yoo SH, Han S, Kim GU, Seo M, Hwang SW, Yang DH, Byeon JS. Efficacy of Precut Endoscopic Mucosal Resection for Treatment of Rectal Neuroendocrine Tumors. *Clin Endosc* 2017; **50**: 585-591 [PMID: 29020763 DOI: 10.5946/ce.2017.039]

79 **Huang J**, Lu ZS, Yang YS, Yuan J, Wang XD, Meng JY, Du H, Wang HB. Endoscopic mucosal resection with circumferential incision for treatment of rectal carcinoid tumours. *World J Surg Oncol* 2014; **12**: 23 [PMID: 24472342 DOI: 10.1186/1477-7819-12-23]

80 **Park SS**, Han KS, Kim B, Chang Kim B, Hong CW, Sohn DK, Chang HJ. Comparison of underwater endoscopic mucosal resection and endoscopic submucosal dissection of rectal neuroendocrine tumors (with videos). *Gastrointest Endosc* 2020; **91**: 1164-1171.e2 [PMID: 31904380 DOI: 10.1016/j.gie.2019.12.039]

81 **Maione F**, Chini A, Milone M, Gennarelli N, Manigrasso M, Maione R, Cassese G, Pagano G, Tropeano FP, Luglio G, De Palma GD. Diagnosis and Management of Rectal Neuroendocrine Tumors (NETs). *Diagnostics (Basel)* 2021; **11** [PMID: 33923121 DOI: 10.3390/diagnostics11050771]

82 **Zhou X**, Xie H, Xie L, Li J, Cao W, Fu W. Endoscopic resection therapies for rectal neuroendocrine tumors: a systematic review and meta-analysis. *J Gastroenterol Hepatol* 2014; **29**: 259-268 [PMID: 24118068 DOI: 10.1111/jgh.12395]

83 **Yong JN**, Lim XC, Nistala KRY, Lim LKE, Lim GEH, Quek J, Tham HY, Wong NW, Tan KK, Chong CS. Endoscopic submucosal dissection versus endoscopic mucosal resection for rectal carcinoid tumor. A meta-analysis and meta-regression with single-arm analysis. *J Dig Dis* 2021; **22**: 562-571 [PMID: 34472210 DOI: 10.1111/1751-2980.13048]

84 **Ramage JK**, De Herder WW, Delle Fave G, Ferolla P, Ferone D, Ito T, Ruszniewski P, Sundin A, Weber W, Zheng-Pei Z, Taal B, Pascher A; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for Colorectal Neuroendocrine Neoplasms. *Neuroendocrinology* 2016; **103**: 139-143 [PMID: 26730835 DOI: 10.1159/000443166]

85 **Meier B**, Albrecht H, Wiedbrauck T, Schmidt A, Caca K. Full-thickness resection of neuroendocrine tumors in the rectum. *Endoscopy* 2020; **52**: 68-72 [PMID: 31614372 DOI: 10.1055/a-1008-9077]

86 **Melita G**, Pallio S, Tortora A, Crinò SF, Macrì A, Dionigi G. Diagnostic and Interventional Role of Endoscopic Ultrasonography for the Management of Pancreatic Neuroendocrine Neoplasms. *J Clin Med* 2021; **10** [PMID: 34203922 DOI: 10.3390/jcm10122638]

87 **Rimbas M**, Larghi A, Fusaroli P, Dong Y, Hollerbach S, Jenssen C, Săftoiu A, Sahai AV, Napoleon B, Arcidiacono PG, Braden B, Burmeister S, Carrara S, Cui XW, Hocke M, Iglesias-Garcia J, Kitano M, Oppong KW, Sun S, Di Leo M, Petrone MC, B Teoh AY, Dietrich CF. How to perform EUS-guided tattooing? *Endosc Ultrasound* 2020; **9**: 291-297 [PMID: 32883923 DOI: 10.4103/eus.eus_44_20]

88 **Rosa F**, Rimbaș M, Rizzatti G, Quero G, Fiorillo C, Impagnatiello M, D'Aversa F, Costamagna G, Alfieri S, Larghi A. EUS-guided fine needle tattooing (EUS-FNT) for preoperative localization of small pancreatic neuroendocrine tumors (p-NETs): a single-center experience. *Surg Endosc* 2021; **35**: 486-492 [PMID: 32959183 DOI: 10.1007/s00464-020-07996-5]

89 **Law JK**, Singh VK, Khashab MA, Hruban RH, Canto MI, Shin EJ, Saxena P, Weiss MJ, Pawlik TM, Wolfgang CL, Lennon AM. Endoscopic ultrasound (EUS)-guided fiducial placement allows localization of small neuroendocrine tumors during parenchymal-sparing pancreatic surgery. *Surg Endosc* 2013; **27**: 3921-3926 [PMID: 23636530 DOI: 10.1007/s00464-013-2975-7]

90 **Zhang L**, Tan S, Huang S, Zhong C, Lü M, Peng Y, Tang X. The safety and efficacy of endoscopic ultrasound-guided ablation therapy for solid pancreatic tumors: a systematic review. *Scand J Gastroenterol* 2020; **55**: 1121-1131 [PMID: 32730715 DOI: 10.1080/00365521.2020.1797870]

91 **Testoni SGG**, Healey AJ, Dietrich CF, Arcidiacono PG. Systematic review of endoscopy ultrasound-guided thermal ablation treatment for pancreatic cancer. *Endosc Ultrasound* 2020; **9**: 83-100 [PMID: 32295966 DOI: 10.4103/eus.eus_74_19]

92 **Jürgensen C**, Schuppan D, Neser F, Ernstberger J, Junghans U, Stölzel U. EUS-guided alcohol ablation of an insulinoma. *Gastrointest Endosc* 2006; **63**: 1059-1062 [PMID: 16733126 DOI: 10.1016/j.gie.2005.10.034]

93 **Levy MJ**, Thompson GB, Topazian MD, Callstrom MR, Grant CS, Vella A. US-guided ethanol ablation of insulinomas: a new treatment option. *Gastrointest Endosc* 2012; **75**: 200-206 [PMID: 22078104 DOI: 10.1016/j.gie.2011.09.019]

94 **Qin SY**, Lu XP, Jiang HX. EUS-guided ethanol ablation of insulinomas: case series and literature review. *Medicine (Baltimore)* 2014; **93**: e85 [PMID: 25255024 DOI: 10.1097/MD.0000000000000085]

95 **Choi JH**, Park DH, Kim MH, Hwang HS, Hong SM, Song TJ, Lee SS, Seo DW, Lee SK. Outcomes after endoscopic ultrasound-guided ethanol-lipiodol ablation of small pancreatic neuroendocrine tumors. *Dig Endosc* 2018; **30**: 652-658 [PMID: 29575213 DOI: 10.1111/den.13058]

96 **Lakhtakia S**, Ramchandani M, Galasso D, Gupta R, Venugopal S, Kalpala R, Reddy DN. EUS-guided radiofrequency ablation for management of pancreatic insulinoma by using a novel needle electrode (with videos). *Gastrointest Endosc* 2016; **83**: 234-239 [PMID: 26394384 DOI: 10.1016/j.gie.2015.08.085]

97 **Limmer S**, Huppert PE, Juette V, Lenhart A, Welte M, Wietholtz H. Radiofrequency ablation of solitary pancreatic insulinoma in a patient with episodes of severe hypoglycemia. *Eur J Gastroenterol Hepatol* 2009; **21**: 1097-1101 [PMID: 19685572 DOI: 10.1097/meg.0b013e328323d70e]

98 **Rossi S**, Viera FT, Ghittoni G, Cobianchi L, Rosa LL, Siciliani L, Bortolotto C, Veronese L, Vercelli A, Gallotti A, Ravetta V. Radiofrequency ablation of pancreatic neuroendocrine tumors: a pilot study of feasibility, efficacy, and safety. *Pancreas* 2014; **43**: 938-945 [PMID: 24717825 DOI: 10.1097/MPA.0000000000000133]

99 **Barthet M**, Giovannini M, Lesavre N, Boustiere C, Napoleon B, Koch S, Gasmi M, Vanbiervliet G, Gonzalez JM. Endoscopic ultrasound-guided radiofrequency ablation for pancreatic neuroendocrine tumors and pancreatic cystic neoplasms: a prospective multicenter study. *Endoscopy* 2019; **51**: 836-842 [PMID: 30669161 DOI: 10.1055/a-0824-7067]

100 **Oleinikov K**, Dancour A, Epshtein J, Benson A, Mazeh H, Tal I, Matalon S, Benbassat CA, Livovsky DM, Goldin E, Gross DJ, Jacob H, Grozinsky-Glasberg S. Endoscopic Ultrasound-Guided Radiofrequency Ablation: A New Therapeutic Approach for Pancreatic Neuroendocrine Tumors. *J Clin Endocrinol Metab* 2019; **104**: 2637-2647 [PMID: 31102458 DOI: 10.1210/jc.2019-00282]

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MINIREVIEWS

Efficacy of cytapheresis in patients with ulcerative colitis showing insufficient or lost response to biologic therapy

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Abstract

For the optimal management of refractory ulcerative colitis (UC), secondary loss of response (LOR) and primary non-response to biologics is a critical issue. This article aimed to summarize the current literature on the use of cytapheresis (CAP) in patients with UC showing a poor response or LOR to biologics and discuss its advantages and limitations. Further, we summarized the efficacy of CAP in patients with UC showing insufficient response to thiopurines or immunomodulators (IM). Eight studies evaluated the efficacy of CAP in patients with UC with inadequate responses to thiopurines or IM. There were no significant differences in the rate of remission and steroid-free remission between patients exposed or not exposed to thiopurines or IM. Three studies evaluated the efficacy of CAP in patients with UC showing an insufficient response to biologic therapies. Mean remission rates of biologics exposed or unexposed patients were 29.4 % and 44.2%, respectively. Fourteen studies evaluated the efficacy of CAP in combination with biologics in patients with inflammatory bowel disease showing a poor response or LOR to biologics. The rates of remission/response and steroid-free remission in patients with UC ranged 32%-69% (mean: 48.0%, median: 42.9%) and 9%-75% (mean: 40.7%, median: 38%), respectively. CAP had the same effectiveness for remission induction with or without prior failure on thiopurines or IM but showed little benefit in patients with UC refractory to biologics. Although heterogeneity existed in the efficacy of the combination therapy with CAP and biologics, these combination therapies induced clinical remission/response and steroid-free remission in more than 40% of patients with UC refractory to biologics on average. Given the excellent safety profile of CAP, this combination therapy can be an alternative therapeutic strategy for UC refractory to biologics. Extensive prospective studies are needed to understand the efficacy of combination therapy with CAP and biologics.

Key Words: Ulcerative colitis; Inflammatory bowel disease; Cytapheresis; Granulocyte and monocyte adsorptive apheresis; Anti-tumor necrosis factor-α antibody; Combination therapy

Core Tip: Management of refractory ulcerative colitis (UC) experiencing primary non-response or loss of response to biologics is a critical issue. We first summarized the efficacy of cytapheresis (CAP) for such patients. Although CAP tended to have lower effects for induction of remission in patients with UC who were refractory to biologics, combination therapies with CAP and biologics induced clinical remission or response in more than 40% of such patients with UC on average. Given the excellent safety profile of CAP, we believe that this combination therapy can be an alternative therapeutic strategy for such refractory UC patients.

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease (IBD) of the colon characterized by a relapsing and remittent course[1,2]. Multiple factors, such as genetic background, environmental and luminal factors, and mucosal immune dysregulation, have been suggested to contribute to UC pathogenesis[1]. Several treatments for UC are available to induce and maintain the clinical remission of the disease. For patients with mild to moderate UC, 5-aminosalicylic acid (5-ASA) is generally used, and more than 90% of patients receive 5-ASA within 1 year of diagnosis[3]. Corticosteroids (CSs) are the first-line treatment to induce remission in moderate to severe UC[2]. It was reported that immediate outcomes from CSs were complete remission in 54%, partial remission in 30%, and no response in 16% of patients[4]. Despite the effectiveness of CSs in patients with UC, it has been reported that the rate of steroid dependence was 17%-22% at 1 year following treatment with the initial CS therapy and increased to 38% mostly within 2 years[4-8].

Thiopurines have been conventionally used for the treatment of steroid-dependent UC[9-14]. The rates of the induction of CS-free remission with thiopurines in steroid-dependent patients with UC were reported to be 44% and 53%, respectively[13,14]. However, it has also been reported that thiopurine therapy has failed in approximately 25% of IBD patients within 3 mo after treatment initiation, mostly due to drug intolerance or toxicity[11].

Along with the recent advancements in the treatment for UC, effective treatments, including biologics [anti- tumor necrosis factor-α (TNF-α) antibodies[15-25], anti-integrin monoclonal antibody[26]], Janus kinase (JAK) inhibitor[27] and tacrolimus[28,29], have been developed for refractory UC. A meta-analysis showed that anti-TNF-α antibodies had more clinical benefits than placebo as evidenced by the former's increased frequency of clinical, steroid-free, and endoscopic remission and decreased frequency of colectomy[30]. It has been reported that the rates of induction of steroid-free remission in refractory patients with UC with anti-TNF-α antibodies ranged from 40.0%-76.5%[2,16,18,21,22,24]. Vedolizumab (VDZ) is an anti-integrin monoclonal antibody. Studies on VDZ showed that clinical response and remission were achieved in 51% and 30% of patients with UC by week 14, respectively [31]. However, despite the significant efficacy of biologics for UC, secondary loss of response (LOR) is a common clinical problem. It was reported that the rate of LOR to anti-TNF-α antibodies in UC ranged from 23%-46% at 12 mo after anti-TNF-α initiation[32]. It was also reported that the incidence rates of LOR to adalimumab (ADA) and infliximab (IFX) were 58.3% and 59.1% during maintenance therapy, respectively (mean follow-up: 139 and 158.8 wk, respectively)[33]. Recent data from a systematic review showed that the pooled incidence rates of LOR to VDZ were 47.9 and 39.8 per 100 person-years of follow-up among patients with Crohn's disease (CD) and UC, respectively[34]. Considering these results, secondary LOR as well as primary non-response to biologics, are a critical issue for the optimal management of refractory UC. In this context, recent studies have shown the efficacy of use of cytapheresis (CAP) in such patients with UC[35-51].

CAP is a non-pharmacological extracorporeal therapy and has been developed as a treatment for UC [52-58]. CAP is performed using two methods, namely, granulocyte and monocyte adsorptive apheresis (GMA), which uses cellulose acetate beads (Adacolumn, JIMRO Co., Ltd., Takasaki, Japan), and leukocytapheresis (LCAP), which uses polyethylene phthalate fibers (Cellsorba., Asahi Kasei Medical Co., Ltd., Tokyo, Japan)[42,52]. GMA selectively depletes elevated levels of granulocytes and monocytes

from the patients' circulation, but spares most of the lymphocytes[52]. LCAP exerts its anti-inflammatory effects by removing activated leukocytes or platelets from the peripheral blood through extracorporeal circulation[42]. It has been reported that CAP is an effective therapeutic strategy for active refractory UC with fewer adverse effects[52-59]. In addition, it is notable that there have been no reports showing LOR to CAP during the treatment.

As described above, recent studies have shown the efficacy of the use of CAP in patients with UC showing a poor response or LOR to biologics, but the results of these studies have not been summarized to date. The purpose of this article is to summarize the current literature on the use of CAP as an alternative therapeutic strategy for patients with UC showing insufficient response or LOR to biologics and discuss the advantages and limitations of this strategy. We also summarized the efficacy of CAP for patients with UC showing insufficient response to thiopurines or immunomodulators (IM)[36,37,42,58-62].

LITERATURE SEARCH STRATEGY

Electric search for studies published before December 2021 was performed in the PubMed databases. The search terms used were as follows; ulcerative colitis, inflammatory bowel disease, cytapheresis, GMA, biologics, loss of response, anti-TNF-α antibody, infliximab, adalimumab, golimumab, vedolizumab, ustekinumab, combination therapy, thiopurine, and immunomodulator. Reference lists of all relevant articles were searched for further studies. The search was restricted to articles in the English language and included prospective studies, retrospective studies, case series, case reports, and randomized control studies. Subsequently, we generated a state-of-the-art comprehensive review by summarizing the data on the efficacy of CAP in patients with UC (or IBD) showing insufficient or lost response to biologic therapy, and efficacy of CAP in patients with UC showing insufficient response to thiopurines or IM.

EFFICACY OF CAP IN PATIENTS WITH UC SHOWING INSUFFICIENT RESPONSE TO THIOPURINES OR IM

There were eight studies that evaluated the efficacy of CAP in patients having UC with insufficient response to thiopurines (Table 1)[36,37,42,58-62]. These studies include three prospective studies, two retrospective studies, one historical cohort study, one single-arm, open-label, multicentre trial, and one multicenter cohort study. Among them, four studies showed remission rates, and four studies showed steroid-free remission rates in CAP therapy in patients with UC concomitantly treated with thiopurines or IM. Although the background of the patients in these studies were different, the remission rates in CAP therapy ranged from 40.3%-73% (mean ± SD: 56.25 ± 16.03%, median: 55.85%, interquartile range: 41.475%-71.425%) and the steroid-free remission rates in CAP therapy ranged from 36%-56.3% (mean ± SD: 47.25 ± 9.99%, median: 48.35%, interquartile range: 37.425%-55.975%) (Figure 1). Among these studies, four[37,42,58,59] compared the rates of clinical remission between the patients exposed to thiopurines or IM and the patients unexposed to them. In all four studies significant differences were not observed in the rates of remission between patients exposed to thiopurines or IM and control. In three of the four studies, the remission rates in patients with UC concomitantly exposed to thiopurines or IM ranged from 45%-73% (mean ± SD: 61.57 ± 14.69%, median: 66.7%), and those in patients unexposed to thiopurines or IM ranged from 48%-71% (mean ± SD: 62.7 ± 12.8%, median: 69.1%) (Figure 2A).

Specifically, Yokoyama *et al*[42] used LCAP in their study and demonstrated that the clinical remission rate of the patients concomitantly using thiopurines was 73% and that of the patients without using thiopurines was 71%. They showed that in univariate analysis, concomitant use of thiopurine did not show statistically significant differences between the remission and nonremission groups (P = 0.623). Yamamoto *et al*[37] used GMA in their study and showed that the clinical remission rate of patients exposed to immunosuppressants was 45%, and that of the patients unexposed to immunosuppressants was 48%. They showed that in the univariate analysis, exposure to immunosuppressants did not affect the likelihood of clinical remission in the treatment of GMA (P = 0.61).

Regarding the rate of steroid-free remission, two studies[59,62] compared the rates of steroid-free remission between the patients concomitantly treated with thiopurines or IM and the patients treated without them. These studies showed that significant differences were not observed between the patients concomitantly treated with thiopurines or IM and patients treated without them. The steroid-free remission rates in patients with UC concomitantly treated with thiopurines or IM were 41.7% and 56.3% (mean: 49%) and the rates of steroid-free remission in patients with UC treated without thiopurines or IM were 45.5% and 53.5% (mean: 49.5%), respectively (Figure 2B). Specifically, Ishiguro *et al*[62] showed that in the univariate analysis, IM therapy was not associated with remission induction rate by GMA (P = 1.00). However, they also showed that in the multivariate analysis, only IM therapy was associated with an increased risk of relapse (OR: 37.6877, 95%CI: 2.4178-587.4632; P = 0.0013).

Table 1 Efficacy of cytapheresis in patients with ulcerative colitis showing insufficient response to thiopurine or immunomodulators

Ref.	Study type	Total number of patients included in the study	Number of patients insufficient response to thiopurine or IM	Regimen of CAP	Rate of remission	Rate of steroid- free remission
Cabriada *et al*[60], 2010	Prospective study	18 (SD)	18	GMA or LCAP (5-10 sessions, 1 session/wk)		55%
Takayama *et al*[58], 2013	Historical cohort study	90	14	GMA or LCAP (5-10 sessions, 1-2/wk)	49% (total Pts), pre-use of IM had little effects on the response to therapy	
Yokoyama *et al*[42], 2014	Prospective Observation Study	623 (for efficacy assessment)	196	LCAP (5-10 sessions, mean 8.4), intensive LCAP was performed in > 70% of Pts	73% (Pts concomitantly treated with thiopurine), 71% (Pts treated without thiopurine), $P = 0.623$	
Imperiali *et al*[61], 2017	Prospective multicenter study	33 (SD)	33	GMA (5 sessions, 1 session/wk)		36%
Yamamoto *et al*[37], 2018	Retrospective study	593	159	GMA (5 sessions, 1 to 5 sessions/wk), 5 or 6 GMA were added in Pts who did not achieve clinical remission	45% (Pts exposed to IM), 48% (Pts unexposed to IM), $P = 0.61$	
Dignass *et al* [36], 2016	Single-arm, open-label, multicentre trial	86 (SD)	83	GMA (5-10 sessions, 1 session/wk)	40.3%	
Ishiguro *et al*[62], 2020	Multicenter cohort study	102, SD or SR UC Pts were not included	16	GMA (mean number of GMA 9.9 sessions, 1-3 sessions/wk)		56.3% (Pts concomitantly treated with IM), 53.5% (Pts treated without IM), $P = 1.00$
Iizuka *et al* [59], 2021	Retrospective study	55 (SD: 33, SR: 21)	12	GMA or LCAP [5-20 sessions (mean 8.8), 1-2 sessions/wk (in principle)]	66.7% (Pts concomitantly treated with thiopurine), 69.1% (all Pts), no significant differences	41.7% (Pts concomitantly treated with thiopurine), 45.5% (all Pts), no significant differences

CAP: Cytapheresis; GMA: Granulocyte and monocyte adsorptive apheresis; LCAP: Leukocytapheresis; IM: Immunomodulators (or immunosuppressants); Pts: Patients; SD: Steroid dependent patients; SR: Steroid refractory patients; Intensive LCAP: Defined as performing ≥ 4 leukocytapheresis treatment within the first 2 wk.

In summary, it was suggested that CAP has the same effectiveness for induction of remission in patients with UC with and without prior failure to thiopurines or IM.

EFFICACY OF CAP IN PATIENTS WITH UC SHOWING PREVIOUS BIOLOGICS FAILURE

Three studies have evaluated the efficacy of CAP in patients with UC showing an insufficient response to anti-TNF-α therapy or exposure to biologics compared with biologic naïve patients with UC[35-37] (Table 2). These studies include two retrospective studies and one single-arm open-label multicentre trial. Among these studies, Yamamoto *et al*[37] showed that the clinical remission rate of the patients exposed to biologics was 31%, and that of the patients unexposed to biologics was 48% ($P = 0.01$). They showed that in the univariate analysis, biologic naïve patients responded well to GMA ($P = 0.01$). In multivariate analysis, exposure to biologics was an independent significant factor affecting the clinical efficacy of GMA ($P = 0.01$). Dignass *et al*[36] conducted a study on a large cohort of steroid-dependent patients with UC refractory to immunosuppressant and /or biologic treatment to provide additional clinical data regarding the safety and efficacy of Adacalumn (GMA). They showed that remission was achieved at week 12 in 31/77 [40.3% (95%CI: 29.2, 52.1)] of patients who failed on immunosuppressants, 10/36 [27.8% (95%CI: 14.2, 45.2)] of patients who failed on anti-TNF-α treatment, and 9/30 [30.0% (95%CI: 14.7, 49.4)] of patients who failed on both immunosuppressants and anti-TNF-αtreatment. Their results suggested that the remission rate using Adacalumn tended to be lower in patients who failed on anti-TNF-α treatment or on both immunosuppressants and anti-TNF-α treatment compared to that of the patients who failed on immunosuppressants. The remission rates in patients with UC exposed to anti-TNF-α treatment in the two studies were 27.8% and 31% (mean: 29.4%), and the remission rates in

Table 2 Efficacy of cytapheresis in patients with ulcerative colitis showing previous biologics failure

Ref.	Study type	Biologics exposure	Number of patients (total number of patients in the study)	Regimen of CAP	Rate of remission	Rate of steroid-free remission
Cabriada *et al*[35], 2012	Retrospective study (results of nationwide Spanish registry)	IFX	33 (total: 142 SD)	GMA (95% of the Pts), 1-10 sessions (median 5 sessions)		37% (all Pts), no differences in clinical remission were found among those Pts with previous thiopurine or IFX failure
Dignass *et al* [36], 2016	Single-arm, open-label, multicentre trial	TNF-α	37 (total: 86 SD)	GMA (5-10 sessions, 1 session/wk)	27.8% (Pts who failed on TNF-α), 40.3% (Pts who failed on immunosup-pressants	
Yamamoto *et al*[37], 2018	Retrospective study	(1) IFX; and (2) ADA	(1) 31; and (2) 36 (total: 593)	GMA (5 sessions, 1 to 5 sessions/wk), 5 or 6 GMA were added in Pts who did not achieve clinical remission	31% (Pts exposed to biologics), 48% (Pts unexposed to biologics), P = 0.01	

CAP: Cytapheresis; SD: Steroid dependent patients; GMA: Granulocyte and monocyte adsorptive apheresis; Pts: Patients; IFX: Infliximab; TNF-α: Tumor necrosis factor-α; ADA: Adalimumab.

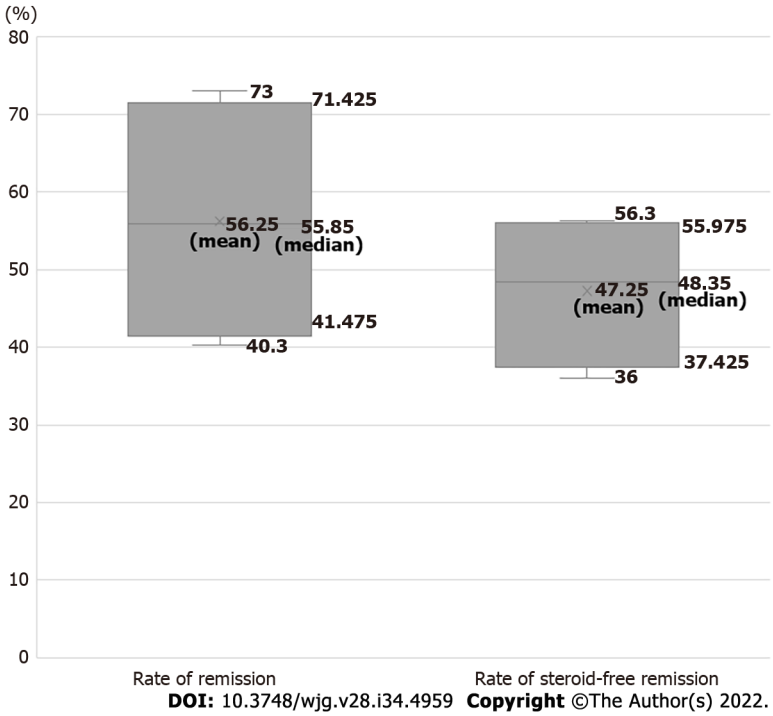

Figure 1 Remission and steroid-free remission rates in cytapheresis therapy in patients with ulcerative colitis concomitantly treated with thiopurines or immunomodulators. Box plot shows that, in cytapheresis therapy, the remission rates range from 40.3%-73% (mean: 56.25%, median: 55.85%, interquartile range: 41.475%-71.425%) and the rates of steroid-free remission range from 36%-56.3% (mean: 47.25%, median: 48.35%, interquartile range: 37.425%-55.975%).

patients with UC unexposed to anti-TNF-α treatment were 40.3% and 48% (mean: 44.15%), respectively (Figure 3).

Cabriada *et al*[35] conducted a clinical study including 142 steroid-dependent patients with UC [previous thiopurines failure 98 (69%), previous IFX failure 33 (23%)] to evaluate the short and long-term effectiveness and safety of leukocytapheresis therapy by means of a nationwide registry of clinical practice. Although the rate of remission in patients who were refractory to thiopurines or IFX was not described in the paper, no differences in clinical remission were found among those with previous thiopurine or IFX failure.

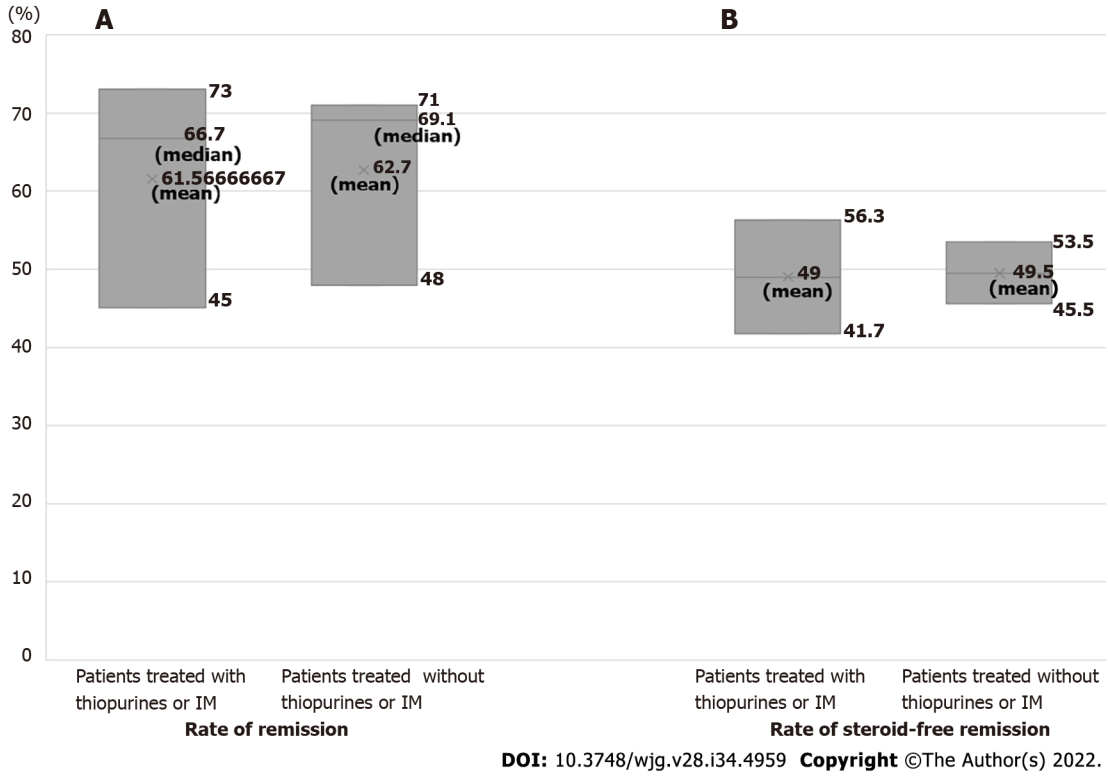

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Figure 2 Remission and steroid-free remission rates in cytapheresis therapy in patients with ulcerative colitis concomitantly treated with thiopurines or immunomodulators and in those treated without thiopurines or immunomodulators. A: Box plot shows that the rates of remission in patients concomitantly treated with thiopurines or immunomodulators (IM) and in those treated without thiopurines or IM range from 45%-73% (mean: 61.57%, median: 66.7%) and 48%-71% (mean: 62.7%, median: 69.1%), respectively; B: The rates of steroid-free remission in patients with ulcerative colitis (UC) concomitantly treated with thiopurines or IM are 41.7% and 56.3% (mean: 49%), and those in patients with UC treated without thiopurines or IM are 45.5% and 53.5% (mean: 49.5%), respectively.

In summary, it is controversial whether CAP has a similar clinical effect in patients with UC who failed biologics treatment and in biologic naïve patients with UC due to limited studies. However, based on these studies, it is suggested that CAP tended to have less efficacy for induction of clinical remission in patients with UC who failed on anti-TNF-α treatment compared to biologic naïve patients with UC.

EFFICACY OF COMBINATION THERAPY WITH CAP AND BIOLOGICS IN IBD PATIENTS SHOWING INSUFFICIENT RESPONSE OR LOSS OF RESPONSE TO BIOLOGICS

Efficacy of the combination therapy with CAP and biologics

There have been 14 studies that evaluated the efficacy of the combination therapy of CAP and biologics in IBD patients that were refractory to biologics[38-51] (Table 3). These studies include two prospective studies, four retrospective studies, one preliminary study, and seven case reports. These studies include eight studies evaluating combination therapy with GMA or LCAP and anti-TNF-α (IFX: 6 studies; ADA: 1 study; IFX, ADA, golimumab: 1 study), four studies with GMA and VDZ, one study with GMA and ustekinumab, and one exceptional study with GMA and a pan-JAK inhibitor tofacitinib. Among these 14 studies, seven studies[42,44,45,47-50] examined the efficacy of combination therapies in patients with UC, five studies[38-41,46] examined its efficacy for CD patients, and two studies[43,51] examined its efficacy for both UC and CD patients.

As shown in Table 3, there were differences in the background of the patients and methods of combination therapies among the studies, and heterogeneity existed in the efficacy of the combination therapies with CAP and biologics among the studies. The rates of remission or response to combination therapies in IBD (UC and CD) patients in these studies excluding seven case reports ranged from 32%-100% (mean ± SD: 62.72 ± 26.65%, median: 57.85%, interquartile range: 40.175%-89.275%) and the rates of steroid-free remission ranged from 9%-75% (mean ± SD: 43 ± 27.4%, median: 44%, interquartile range: 16.25%-68.75%), respectively (Figure 4). Regarding the efficacy of the combination therapies in patients with UC, three studies showed the rates of remission or response, and three studies showed steroid-free remission in the combination therapies of CAP and biologics in patients with UC refractory to biologics. The rates of remission or response ranged from 32%-69% (mean ± SD: 47.97 ± 19.0%, median: 42.9%),

Table 3 Efficacy of combination therapy with cytapheresis and biologics in inflammatory bowel disease patients showing insufficient response or loss of response to biologics

Ref.	Study type	Biologics to which insufficient response or LOR was shown	Number of patients	Methods of combination therapy	Regimen of CAP	Rate of remission	Rate of response	Rate of steroid-free remission	Rate of AE (%)
González Carro *et al* [38], 2006	Case report	IFX (LOR)	CD 1	IFX + GMA	GMA 1 session/8 wk, 12 mo	100%			
Fukunaga *et al*[39], 2010	Case report	IFX (LOR)	CD 1	IFX + GMA	GMA 1 sessions/wk, 3 consecutive weeks × 3 courses and maintenance therapy	100%			0/1 (0%)
Sono *et al* [40], 2012	Prospective study	IFX (LOR)	CD 15	IFX + GMA	GMA 1 session/wk, 5 consecutive wk		46.7%; a fall in CDAI by more than 15%		
Ozeki *et al* [41], 2012	Case report	(1) IFX (failure); (2) ADA (failure); (3) Steroid refractory and *etc.*	(1) CD 1; (2) CD 1; and (3) CD 3	ADA + GMA	GMA 2 sessions/wk, 5 consecutive wk	100%			0/5 (0%)
Yokoyama *et al*[42], 2014	Prospective observational study	IFX	UC 42	IFX + LCAP	LCAP 5-10 sessions (mean 8.4), intensive LCAP was performed in > 70% of Pts	69.0% (Pts concomitantly treated with IFX)			
Yokoyama *et al*[43], 2018	Case report	IFX (LOR)	UC 2; CD 1	IFX + GMA	GMA 1 session/wk, 3 consecutive wk or more	UC 100%, CD 100%			
Scrivo *et al* [44], 2018	Case report	VDZ (primary nonresponse to VDZ; Previous LOR to IFX; Primary non-response to ADA)	UC 1	VDZ + GMA	GMA 1 session/wk,5 wk			100%	0/1 (0%)
Sáez-González *et al* [45], 2018	Case report	VDZ (primary nonresponse to VDZ; Primary nonresponse to ADA and IFX)	UC 1	VDZ + GMA	GMA 2 sessions/wk, 5 wk + 14 monthly maintenance sessions			100%	
Tanida *et al* [46], 2018	Retrospective study	(1) IFX (LOR); (2) ADA (LOR); (3) Steroid refractory	(1) CD 1; (2) CD 1; and (3) CD 1	UST + GMA	GMA: 2 sessions/wk, for 5 consecutive wk	100%		50%	0/3 (0%)
Rodríguez-Lago *et al* [47], 2019	Retrospective multicenter study	Anti-TNF therapy (IFX 23, ADA 18, GLM 6); Primary nonresponse 49%, LOR 51%	UC 47	Anti-TNF therapy + GMA	GMA 1 sessions/wk 45%, 2 sessions/wk 55%; 5-10 sessions 51%, > 10 sessions 19% (median of 10 sessions)		32%	9%	2/47 (4%)
Rodríguez-Lago *et al* [48], 2019	Retrospective multicentre pilot study	VDZ (primary nonresponse 25%, secondary LOR 75%); All Pts had previously received anti-TNF agents (IFX 88%, ADA 50%, GLM 38%)	UC 8	VDZ + GMA	GMA: 5-38 sessions (median 15), biweekly 75%, weekly 25%; maintenance GMA 75%, monthly 38%, every 2 wk 25%		Partial Mayo score decreased ($P = 0.01$)	38%	0/8 (0%)
Nakamura *et al*[49], 2020	Case report	VDZ (primary nonresponse to VDZ; Serious	UC 1	VDZ + GMA	semiweekly GMA, 4 wk	100%			

		allergy to IFX)						
Tanida *et al* [50], 2020	Retrospective study	(1) IFX(LOR); (2) ADA (LOR); (3) Steroid refractory or dependent	(1) UC 2; (2) UC 2; and (3) UC 3	TOF + GMA	GMA: 2 sessions/wk, total 10 sessions		75%	3/7 (43%)
Yokoyama *et al*[51], 2020	Preliminary study	IFX (LOR)	UC 7; CD 7	IFX + GMA	1 or 2 sessions/wk, for 5 consecutive wk, Pts who did not achieved remission by week 8 underwent another GMA (1 session/wk, 5 consecutive wk)	All IBD 64.3%, UC 42.9%, CD 85.7%		0/14 (0%)

LOR: Loss of response; CAP: Cytapheresis; CD: Crohn's disease; GMA: Granulocyte and monocyte adsorptive apheresis; UC: Ulcerative colitis; LCAP: Leukocytapheresis; AE: Adverse events; IFX: Infliximab; ADA: Adalimumab; Pts: Patients; VED: Vedolizumab; UST: Ustekinumab; TNF-α: Tumor necrosis factor-α; GLM: Golimumab; TOF: Tofacitinib; Intensive LCAP: Defined as performing ≥ 4 leukocytapheresis within the first 2 wk.

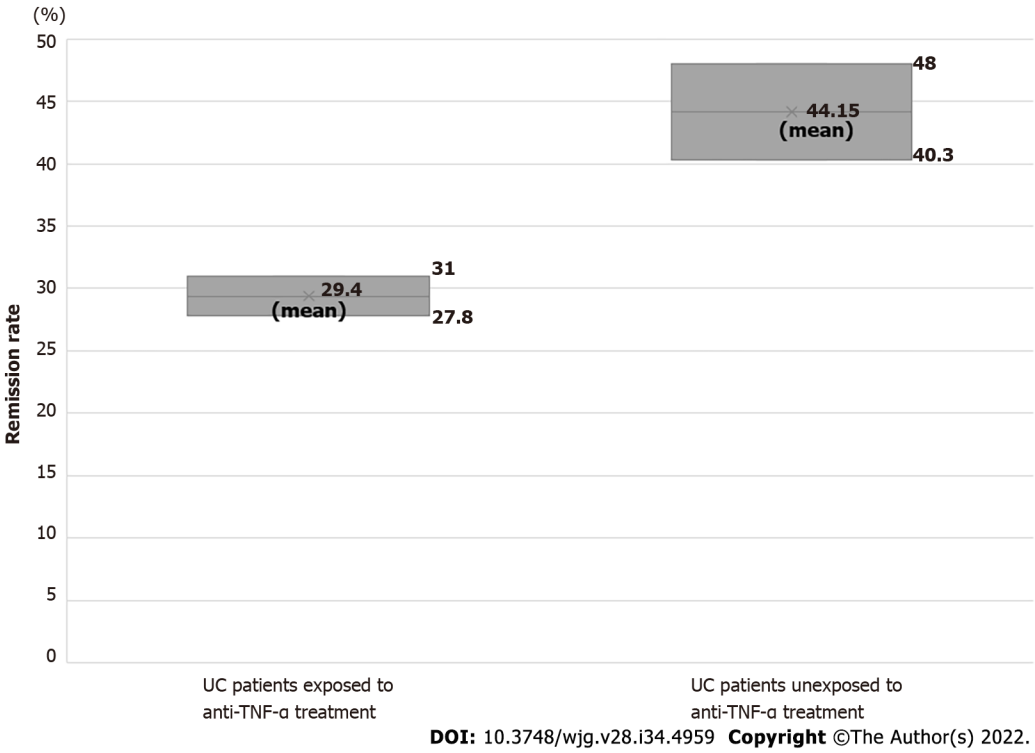

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Figure 3 Remission rates in cytapheresis therapy in patients with ulcerative colitis exposed to anti-tumor necrosis factor-α treatment and in those unexposed to anti-tumor necrosis factor-α treatment. The remission rates in patients with ulcerative colitis (UC) exposed to anti-tumor necrosis factor-α (TNF-α) treatment are 27.8% and 31% (mean: 29.4%), and those in patients with UC unexposed to anti-TNF-α treatment are 40.3% and 48% (mean: 44.15%), respectively.

and the rates of steroid-free remission in patients with UC ranged from 9%-75% (mean ± SD: 40.7 ± 33.1%, median: 38%), respectively (Figure 5). On the other hand, the rates of remission or response in CD patients ranged from 46.7%-100% (mean ± SD: 77.5 ± 27.6%, median 85.7%), and the rate of steroid-free remission in CD patients was 50%.

Regarding the efficacy of the combination therapy, Rodríguez-Lago *et al*[47] found no differences in the efficacy depending on the type of anti-TNF received during the combination therapy. They also reported that the response to the combination therapy was inversely proportional to the number of previous anti-TNF agents, but it was not influenced by the presence of primary non-response or secondary LOR. Another important aspect being considered is the regimen of CAP prescribed because an intensified regimen with longer and biweekly sessions has demonstrated rapid and higher efficacy rates without increasing the number of adverse events (AEs)[47,57]. In Table 3, it seems that the studies using a higher frequency of biweekly CAP or intensive CAP tended to demonstrate good clinical efficacy.

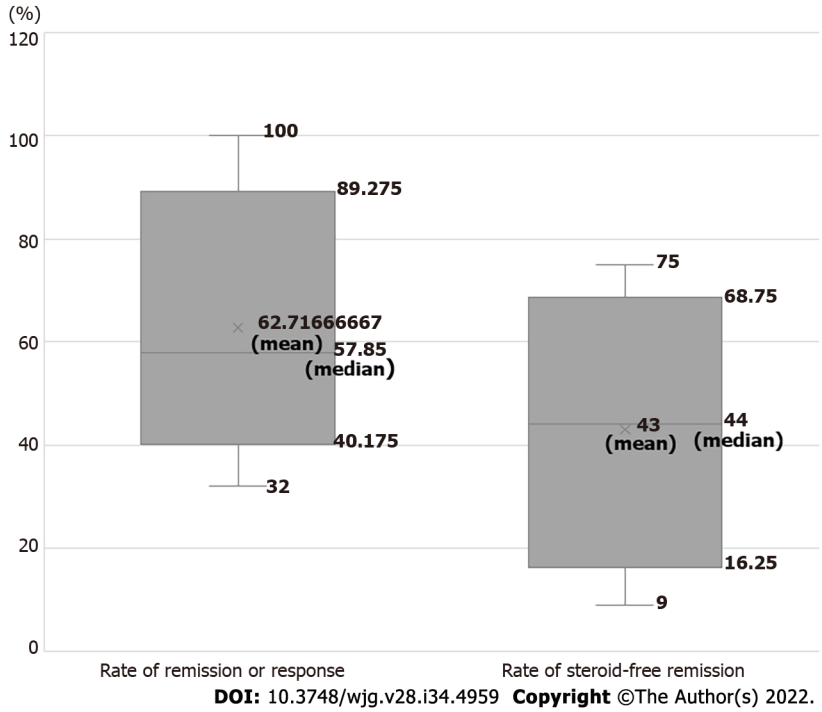

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Figure 4 Rates of remission or response and steroid-free remission in the combination therapies of cytapheresis and biologics in inflammatory bowel disease patients showing insufficient response or loss of response to biologics. Box plot shows that the remission rates in the combination therapies of cytapheresis and biologics range from 32%-100% (mean: 62.72%, median: 57.85%, interquartile range: 40.175%-89.275%), and the rates of steroid-free remission in the combination therapies range from 9%-75% (mean: 43%, median: 44%, interquartile range: 16.25%-68.75%).

Safety of the combination therapy

One of the strengths of CAP is its safety profile[63]. Of note, several studies have reported the excellent safety of CAP[36,42,52,56,63]. Among these studies, Hibi *et al*[56] evaluated the safety of Adacolumn in 697 patients with UC in 53 medical institutions. They reported that no serious AEs were observed, and only mild to moderate adverse events were observed in 7.7% of patients. In addition, Motoya *et al*[52] showed that the incidence of AEs among elderly patients was similar in all patients. Regarding the safety of the combination therapy with CAP and biologics, eight out of 14 studies listed in Table 3 reported the rate of AEs[39,41,44,46-48,50,51]. Six of the eight studies reported no adverse events. On the other hand, Rodríguez-Lago *et al*[47] reported 4% (2/47) AEs related to the technique (anxiety and headache), and Tanida *et al*[50] reported 43% (3/7) AEs (one had orolabial herpes, one had a transient increase in creatinine phosphokinase due to intense physical exercise, and one had triglyceride increase). However, Tanida *et al*[50] described that AEs observed in their study were consistent with the AEs reported in the oral clinical trials for tofacitinib in ulcerative colitis induction 1 and 2 trials, suggesting that AEs observed in their study were due to AEs from tofacitinib. In summary, AEs were observed in five out of 86 patients (5.8%) in the eight studies.

Based on these results, combination therapy with CAP and biologics is safe and well- tolerated.

Possible mechanisms of the efficacy of the combination therapy with CAP and biologics

Regarding the mechanism of the efficacy of the combination therapy of CAP and biologics, Rodriguez-Largo *et al*[47] suggested that the benefit may be related to multiple mechanisms of action. They suggested that GMA could reduce the circulating inflammatory burden in addition to direct improvement in disease activity, thus allowing the anti-TNF to restore its response. They also suggested an alternative hypothesis that states that the benefits come from the possible interaction between both treatments. This interaction could be an improvement in blood trough levels of the drug, a reduction of anti-drug antibodies, or both. In this context, several studies supported their hypothesis. Soluble TNF receptors are known to neutralize TNF without invoking a TNF-like response. Saniabadi *et al*[64] reported that blood levels of soluble TNF-α receptors I and II increased in IBD patients who underwent Adacolumn therapy. Hanai *et al*[65] also showed that soluble TNF-α receptor I/II, which are believed to have potent anti-inflammatory actions, were significantly increased in the peripheral blood at the end of the GMA session. Sono *et al*[40] showed an increase in plasma IL-10 and a decrease in circulating immune complexes and anti-nuclear antibodies during GMA therapy in GMA-responder CD patients with LOR to IFX. Furthermore, Yokoyama *et al*[43] showed that upon GMA therapy, the average plasma trough IFX increased from 0.91 μg/mL to 1.46 μg/mL, with concomitant decreases in C-reactive protein, IL-6, and IL-17A in IBD patients experiencing LOR to IFX. In their recent study, Yokoyama *et al*[51]

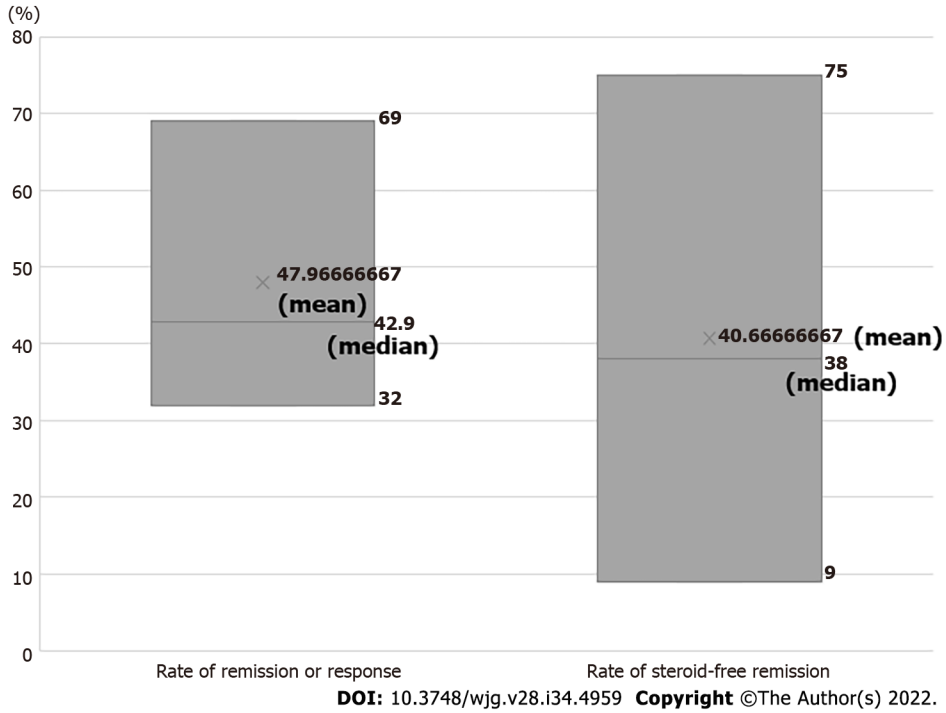

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Figure 5 Rates of remission or response and steroid-free remission in the combination therapies of cytapheresis and biologics in patients with ulcerative colitis showing insufficient response or loss of response to biologics. Box plot shows that the remission rates in the combination therapies of cytapheresis and biologics range from 32%-69% (mean: 47.97%, median: 42.9%), and the rates of steroid-free remission in the combination therapies range from 9%-75% (mean: 40.7%, median: 38%).

showed that the levels of antibodies to IFX in patients with LOR to IFX were significantly elevated compared with those indicating a sustained clinical remission. They also showed that in patients who received IFX + GMA combination therapy, the IBD symptoms significantly improved together with a decrease in antibodies to IFX. These studies suggest the possibility that GMA therapy can decrease IFX antibodies and increase plasma trough IFX in patients with LOR to IFX.

Regarding combination therapy with GMA and VDZ, it was hypothesized that this strategy might target the migration of leukocytes into the inflamed tissue by combining their mechanism of action. The peripheral inflammatory cells affected by VDZ may be removed by the ability of GMA to adsorb multiple immune cells[48]. Nakamura *et al*[49] also suggested that VDZ and GMA were able to strengthen the suppression of the migration of leukocytes into the inflamed tissue by combining their mechanisms of action. Since the migration of peripheral inflammatory cells from the blood vessels is blocked by VDZ, multiple immune cells-including the congested ones in the peripheral blood- can be removed by GMA. Thus, considering the mechanism of action of GMA and VDZ, it is suggested that this combination therapy can synergically strengthen the therapeutic effects of each therapy.

Summary of the combination therapies with CAP and biologics

In summary, combination therapies of CAP and biologics can safely induce clinical remission or response and steroid-free remission in 32%-100% (mean: 62.72%, median: 57.85%) and 9%-75% (mean: 43%, median: 44%) of the IBD patients and in 32%-69% (mean: 47.97%, median: 42.9%) and 9%-75% (mean: 40.7%, median: 38%) of patients with UC refractory to biologics, respectively. In addition, it is a strong point of CAP that there have been no reports showing LOR to CAP during treatment. Given the excellent safety profile of CAP, these results suggest that this combination therapy can be an effective and alternative therapeutic strategy for patients with UC that experienced primary non-response or LOR to biologics. The economic burden of GMA may also be considered in decision-making[63]. A recent study showed that the availability of biosimilars had reduced the costs of anti-TNF agents, but GMA still has a cost slightly below the new biologicals (*i.e.*, ustekinumab and vedolizumab) with an even better safety profile[63]. In this context, Tominaga *et al*[66] evaluated the efficacy, safety, and treatment cost of prednisolone (PSL) and GMA in 41 patients with active UC who had achieved remission with GMA or with orally administered PSL. They showed that adverse events were reported in 12.5% of the GMA group and 35.3% of the PSL group. The average medical cost was 12739.4€/patient in the GMA group and 8751.3€ in the PSL group ($P < 0.05$). From these results, they concluded that the higher cost of GMA is offset by its good safety profile.

CONCLUSION

Summarizing the results of previous studies, it is suggested that CAP has the same effectiveness for induction of remission in patients having UC with or without prior failure of thiopurines or IM. It is controversial whether CAP has a similar clinical effect in patients with UC that failed on previous biologics therapy and in biologic naïve patients. However, it seems that CAP tended to be less effective for induction of clinical remission in patients with UC that were refractory to biologics therapy. Although there was heterogeneity in the efficacy of the combination therapy with CAP and biologics in patients with IBD refractory to biologics, it is notable that combination therapies with CAP and biologics induced clinical remission or response and steroid-free remission in more than 40% of patients with UC that failed on previous biologics therapy on average. Given the excellent safety profile of CAP, it is suggested that this combination therapy can be an alternative therapeutic strategy for patients with UC that were refractory to biologics. However, the number of studies examining this combination therapy has been small and limited to date. Larger prospective studies are needed to better understand the efficacy of the combination therapy of CAP and biologics for refractory patients with UC.

FOOTNOTES

Author contributions: Iizuka M was responsible for the conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version; Etou T and Sagara S was responsible for the critical revision and final approval of the final version.

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REFERENCES

1 **Kobayashi T**, Siegmund B, Le Berre C, Wei SC, Ferrante M, Shen B, Bernstein CN, Danese S, Peyrin-Biroulet L, Hibi T. Ulcerative colitis. *Nat Rev Dis Primers* 2020; **6**: 74 [PMID: 32913180 DOI: 10.1038/s41572-020-0205-x]
2 **Khan HM**, Mehmood F, Khan N. Optimal management of steroid-dependent ulcerative colitis. *Clin Exp Gastroenterol* 2015; **8**: 293-302 [PMID: 26648749 DOI: 10.2147/CEG.S57248]
3 **Nguyen NH**, Fumery M, Dulai PS, Prokop LJ, Sandborn WJ, Murad MH, Singh S. Comparative efficacy and tolerability of pharmacological agents for management of mild to moderate ulcerative colitis: a systematic review and network meta-analyses. Lancet Gastroenterol Hepatol 2018;3:742-753 [DOI: 10.1016/S2468-1253(18)30231-0]
4 **Faubion WA Jr**, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001; **121**: 255-260 [PMID: 11487534]
5 **Ho GT**, Chiam P, Drummond H, Loane J, Arnott ID, Satsangi J. The efficacy of corticosteroid therapy in inflammatory bowel disease: analysis of a 5-year UK inception cohort. *Aliment Pharmacol Ther* 2006; **24**: 319-330 [PMID: 16842459]
6 **Edwards FC**, Truelove SC. The course and prognosis of ulcerative colitis. *Gut* 1963; **4**: 299-315 [PMID: 14084741]
7 **Farmer RG**, Easley KA, Rankin GB. Clinical patterns, natural history, and progression of ulcerative colitis. A long-term follow-up of 1116 patients. *Dig Dis Sci* 1993; **38**: 1137-1146 [PMID: 8508710]
8 **Khan N**, Abbas A, Williamson A, Balart L. Prevalence of corticosteroids use and disease course after initial steroid exposure in ulcerative colitis. *Dig Dis Sci* 2013; **58**: 2963-2969 [PMID: 23812861 DOI: 10.1007/s10620-013-2748-0]
9 **Park SK**, Yang SK, Ye BD, Kim KJ, Yang DH, Jung KW, Park SH, Kim JW, Byeon JS, Myung SJ, Kim JH. The long-term efficacy of azathioprine in steroid-dependent ulcerative colitis. *Scand J Gastroenterol* 2013; **48**: 1386-1393 [PMID: 24164382 DOI: 10.3109/00365521.2013.845908]
10 **Bär F**, Sina C, Fellermann K. Thiopurines in inflammatory bowel disease revisited. *World J Gastroenterol* 2013; **19**: 1699-1706 [PMID: 23555158 DOI: 10.3748/wjg.v19.i11.1699]
11 **Jharap B**, Seinen ML, de Boer NK, van Ginkel JR, Linskens RK, Kneppelhout JC, Mulder CJ, van Bodegraven AA.

Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis* 2010; **16**: 1541-1549 [PMID: 20155846 DOI: 10.1002/ibd.21221]

12 **van Gennep S**, de Boer NK, D'Haens GR, Löwenberg M. Thiopurine Treatment in Ulcerative Colitis: A Critical Review of the Evidence for Current Clinical Practice. *Inflamm Bowel Dis* 2017; **24**: 67-77 [PMID: 29272488 DOI: 10.1093/ibd/izx025]

13 **Ardizzone S**, Maconi G, Russo A, Imbesi V, Colombo E, Bianchi Porro G. Randomised controlled trial of azathioprine and 5-aminosalicylic acid for treatment of steroid dependent ulcerative colitis. *Gut* 2006; **55**: 47-53 [PMID: 15972298]

14 **Rosenberg JL**, Wall AJ, Levin B, Binder HJ, Kirsner JB. A controlled trial of azathioprine in the management of chronic ulcerative colitis. *Gastroenterology* 1975; **69**: 96-99 [PMID: 1097295]

15 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

16 **Armuzzi A**, Pugliese D, Danese S, Rizzo G, Felice C, Marzo M, Andrisani G, Fiorino G, Sociale O, Papa A, De Vitis I, Rapaccini GL, Guidi L. Infliximab in steroid-dependent ulcerative colitis: effectiveness and predictors of clinical and endoscopic remission. *Inflamm Bowel Dis* 2013; **19**: 1065-1072 [PMID: 23448790 DOI: 10.1097/MIB.0b013e3182802909]

17 **Sjöberg M**, Magnuson A, Björk J, Benoni C, Almer S, Friis-Liby I, Hertervig E, Olsson M, Karlén P, Eriksson A, Midhagen G, Carlson M, Lapidus A, Halfvarson J, Tysk C; Swedish Organization for the Study of Inflammatory Bowel Disease (SOIBD). Infliximab as rescue therapy in hospitalised patients with steroid-refractory acute ulcerative colitis: a long-term follow-up of 211 Swedish patients. *Aliment Pharmacol Ther* 2013; **38**: 377-387 [PMID: 23799948 DOI: 10.1111/apt.12387]

18 **Barreiro-de Acosta M**, Lorenzo A, Mera J, Dominguez-Muñoz JE. Mucosal healing and steroid-sparing associated with infliximab for steroid-dependent ulcerative colitis. *J Crohns Colitis* 2009; **3**: 271-276 [PMID: 21172286 DOI: 10.j.crohns]

19 **Mocciaro F**, Orlando A, Scimeca D, Cottone M. [Infliximab in moderate to severe steroid-dependent or steroid-refractory ulcerative colitis]. *Recenti Prog Med* 2007; **98**: 560-564 [PMID: 18044405]

20 **Gavalas E**, Kountouras J, Stergiopoulos C, Zavos C, Gisakis D, Nikolaidis N, Giouleme O, Chatzopoulos D, Kapetanakis N. Efficacy and safety of infliximab in steroid-dependent ulcerative colitis patients. *Hepatogastroenterology* 2007; **54**: 1074-1079 [PMID: 17629042]

21 **Sandborn WJ**, van Assche G, Reinisch W, Colombel JF, D'Haens G, Wolf DC, Kron M, Tighe MB, Lazar A, Thakkar RB. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2012; **142**: 257-65.e1 [PMID: 22062358 DOI: 10.1053/j.gastro.2011.10.032]

22 **Italian Group for the Study of Inflammatory Bowel Disease**, Armuzzi A, Biancone L, Daperno M, Coli A, Pugliese D, Annese V, Aratari A, Ardizzone S, Balestrieri P, Bossa F, Cappello M, Castiglione F, Cicala M, Danese S, D'Incà R, Dulbecco P, Feliciangeli G, Fries W, Genise S, Gionchetti P, Gozzi S, Kohn A, Lorenzetti R, Milla M, Onali S, Orlando A, Papparella LG, Renna S, Ricci C, Rizzello F, Sostegni R, Guidi L, Papi C. Adalimumab in active ulcerative colitis: a "real-life" observational study. *Dig Liver Dis* 2013; **45**: 738-743 [PMID: 23683530 DOI: 10.1016/j.dld.2013.03.018]

23 **Sandborn WJ**, Feagan BG, Marano C, Zhang H, Strauss R, Johanns J, Adedokun OJ, Guzzo C, Colombel JF, Reinisch W, Gibson PR, Collins J, Järnerot G, Hibi T, Rutgeerts P; PURSUIT-SC Study Group. Subcutaneous golimumab induces clinical response and remission in patients with moderate-to-severe ulcerative colitis. *Gastroenterology* 2014; **146**: 85-95; quiz e14 [PMID: 23735746 DOI: 10.1053/j.gastro.2013.05.048]

24 **Bosca-Watts MM**, Cortes X, Iborra M, Huguet JM, Sempere L, Garcia G, Gil R, Garcia M, Muñoz M, Almela P, Maroto N, Paredes JM. Short-term effectiveness of golimumab for ulcerative colitis: Observational multicenter study. *World J Gastroenterol* 2016; **22**: 10432-10439 [PMID: 28058024 DOI: 10.3748/wjg.v22.i47.10432]

25 **Tursi A**, Allegretta L, Buccianti N, Della Valle N, Elisei W, Forti G, Faggiani R, Gallina S, Hadad Y, Larussa T, Lauria A, Luzza F, Lorenzetti R, Mocci G, Penna A, Polimeni N, Pranzo G, Ricciardelli C, Zampaletta C, Picchio M. Effectiveness and Safety of Golimumab in Treating Outpatient Ulcerative Colitis: A Real-Life Prospective, Multicentre, Observational Study in Primary Inflammatory Bowel Diseases Centers. *J Gastrointestin Liver Dis* 2017; **26**: 239-244 [PMID: 28922435 DOI: 10.15403/jgld.2014.1121.263.trs]

26 **Feagan BG**, Rutgeerts P, Sands BE, Hanauer S, Colombel JF, Sandborn WJ, Van Assche G, Axler J, Kim HJ, Danese S, Fox I, Milch C, Sankoh S, Wyant T, Xu J, Parikh A; GEMINI 1 Study Group. Vedolizumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2013; **369**: 699-710 [PMID: 23964932 DOI: 10.1056/NEJMoa1215734]

27 **Sandborn WJ**, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, Danese S, Feagan BG, Reinisch W, Niezychowski W, Friedman G, Lawendy N, Yu D, Woodworth D, Mukherjee A, Zhang H, Healey P, Panés J; OCTAVE Induction 1, OCTAVE Induction 2, and OCTAVE Sustain Investigators. Tofacitinib as Induction and Maintenance Therapy for Ulcerative Colitis. *N Engl J Med* 2017; **376**: 1723-1736 [PMID: 28467869 DOI: 10.1056/NEJMoa1606910]

28 **Ogata H**, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, Hibi T. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. *Gut* 2006; **55**: 1255-1262 [PMID: 16484504 DOI: 10.1136/gut.2005.081794]

29 **Baumgart DC**, Pintoffl JP, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease--a long-term follow-up. *Am J Gastroenterol* 2006; **101**: 1048-1056 [PMID: 16573777]

30 **Lv R**, Qiao W, Wu Z, Wang Y, Dai S, Liu Q, Zheng X. Tumor necrosis factor alpha blocking agents as treatment for ulcerative colitis intolerant or refractory to conventional medical therapy: a meta-analysis. *PLoS One* 2014; **9**: e86692 [PMID: 24475168 DOI: 10.1371/journal.pone.0086692]

31 **Engel T**, Ungar B, Yung DE, Ben-Horin S, Eliakim R, Kopylov U. Vedolizumab in IBD-Lessons From Real-world Experience; A Systematic Review and Pooled Analysis. *J Crohns Colitis* 2018; **12**: 245-257 [PMID: 29077833 DOI: 10.1093/ecco-jcc/jjx143]

32 **Ben-Horin S**, Kopylov U, Chowers Y. Optimizing anti-TNF treatments in inflammatory bowel disease. *Autoimmun Rev* 2014; **13**: 24-30 [PMID: 23792214]

33 **Ma C**, Huang V, Fedorak DK, Kroeker KI, Dieleman LA, Halloran BP, Fedorak RN. Outpatient Ulcerative Colitis Primary

Anti-TNF Responders Receiving Adalimumab or Infliximab Maintenance Therapy Have Similar Rates of Secondary Loss of Response. *J Clin Gastroenterol* 2015; **49**: 675-682 [PMID: 25389599 DOI: 10.1097/MCG.0000000000000265]

34 **Peyrin-Biroulet L**, Danese S, Argollo M, Pouillon L, Peppas S, Gonzalez-Lorenzo M, Lytras T, Bonovas S. Loss of Response to Vedolizumab and Ability of Dose Intensification to Restore Response in Patients With Crohn's Disease or Ulcerative Colitis: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2019; **17**: 838-846.e2 [PMID: 29935327 DOI: 10.1016/j.cgh.2018.06.026]

35 **Cabriada JL**, Domènech E, Ibargoyen N, Hernández V, Clofent J, Ginard D, Gutiérrez-Ibarluzea I, Hinojosa J. Leukocytapheresis for steroid-dependent ulcerative colitis in clinical practice: results of a nationwide Spanish registry. *J Gastroenterol* 2012; **47**: 359-365 [PMID: 22105230 DOI: 10.1007/s00535-011-0499-2]

36 **Dignass A**, Akbar A, Hart A, Subramanian S, Bommelaer G, Baumgart DC, Grimaud JC, Cadiot G, Makins R, Hoque S, Bouguen G, Bonaz B. Safety and Efficacy of Granulocyte/Monocyte Apheresis in Steroid-Dependent Active Ulcerative Colitis with Insufficient Response or Intolerance to Immunosuppressants and/or Biologics [the ART Trial]: 12-week Interim Results. *J Crohns Colitis* 2016; **10**: 812-820 [PMID: 26818659 DOI: 10.1093/ecco-jcc/jjw032]

37 **Yamamoto T**, Iida T, Ikeya K, Kato M, Matsuura A, Tamura S, Takano R, Tani S, Osawa S, Sugimoto K, Shimoyama T, Hanai H. A multicenter retrospective study aiming to identify patients who respond well to adsorptive granulomonocytapheresis in moderately to severely active ulcerative colitis. *Clin Transl Gastroenterol* 2018; **9**: 170 [PMID: 29977035 DOI: 10.1038/s41424-018-0037-0]

38 **González Carro P**, Pérez Roldán F, Roncero García Escribano O, Lafuente R, Legaz Huidobro ML, Amigo Echenagusía A. Case report: combination therapy with granulocyte apheresis and infliximab for refractory Crohn's disease. *J Clin Apher* 2006; **21**: 249-251 [PMID: 16607627 DOI: 10.1002/jca.20093]

39 **Fukunaga K**, Yokoyama Y, Kamikozuru K, Yoshida K, Kikuyama R, Nagase K, Nakamura S, Takei Y, Miwa H, Matsumoto T. Selective depletion of peripheral granulocyte/monocyte enhances the efficacy of scheduled maintenance infliximab in Crohn's disease. *J Clin Apher* 2010; **25**: 226-228 [PMID: 20544712 DOI: 10.1002/jca.20242]

40 **Sono K**, Yamada A, Yoshimatsu Y, Takada N, Suzuki Y. Factors associated with the loss of response to infliximab in patients with Crohn's disease. *Cytokine* 2012; **59**: 410-416 [PMID: 22633084 DOI: 10.1016/j.cyto.2012.04.026]

41 **Ozeki K**, Tanida S, Mizoshita T, Tsukamoto H, Ebi M, Mori Y, Kataoka H, Kamiya T, Joh T. Combination Therapy with Intensive Granulocyte and Monocyte Adsorptive Apheresis plus Adalimumab: Therapeutic Outcomes in 5 Cases with Refractory Crohn's Disease. *Case Rep Gastroenterol* 2012; **6**: 765-771 [PMID: 23341799 DOI: 10.1159/000346312]

42 **Yokoyama Y**, Matsuoka K, Kobayashi T, Sawada K, Fujiyoshi T, Ando T, Ohnishi Y, Ishida T, Oka M, Yamada M, Nakamura T, Ino T, Numata T, Aoki H, Sakou J, Kusada M, Maekawa T, Hibi T. A large-scale, prospective, observational study of leukocytapheresis for ulcerative colitis: treatment outcomes of 847 patients in clinical practice. *J Crohns Colitis* 2014; **8**: 981-991 [PMID: 24556083 DOI: 10.1016/j.crohns.2014.01.027]

43 **Yokoyama Y**, Kamikozuru K, Watanabe K, Nakamura S. Inflammatory bowel disease patients experiencing a loss of response to infliximab regain long-term response after undergoing granulocyte/monocyte apheresis: A case series. *Cytokine* 2018; **103**: 25-28 [PMID: 29291447 DOI: 10.1016/j.cyto.2017.12.030]

44 **Scrivo B**, Vitello A, Cappello M. Response to "The combination therapy with cytapheresis plus vedolizumab in a corticosteroid-dependent patient with ulcerative colitis and previous Anti-TNF alfa failure". *Dig Liver Dis* 2018; **50**: 1103-1104 [PMID: 29871799 DOI: 10.1016/j.dld.2018.05.001]

45 **Sáez-González E**, Aguas M, Huguet JM, Nos P, Beltrán B. Combination therapy with cytapheresis plus vedolizumab in a corticosteroid-dependent patient with ulcerative colitis and previous ANTI-TNF-α drug failure. *Dig Liver Dis* 2018; **50**: 415-417 [PMID: 29397323 DOI: 10.1016/j.dld.2018.01.124]

46 **Tanida S**, Mizoshita T, Ozeki K, Katano T, Tanaka M, Nishie H, Shimura T, Okamoto Y, Kubota E, Kataoka H, Joh T. Combination Therapy With Intensive Granulocyte and Monocyte Adsorptive Apheresis Plus Ustekinumab in Patients With Refractory Crohn's Disease. *Ther Apher Dial* 2018; **22**: 295-300 [PMID: 29790276 DOI: 10.1111/1744-9987.12697]

47 **Rodríguez-Lago I**, Sempere L, Gutiérrez A, Núñez A, Leo Carnerero E, Hinojosa E, Mora M, Cañete F, Mañosa M, Herrera C, Beltrán B, Forés A, Arjona D, Barreiro-de Acosta M, Khorrami S, Aguirre U, Ginard D, Cabriada JL. Granulocyte-monocyte apheresis: an alternative combination therapy after loss of response to anti-TNF agents in ulcerative colitis. *Scand J Gastroenterol* 2019; **54**: 459-464 [PMID: 30982369 DOI: 10.1080/00365521.2019.1600715]

48 **Rodríguez-Lago I**, Benítez JM, Sempere L, Sáez-González E, Barreiro-de Acosta M, de Zárate JO, Cabriada JL. The combination of granulocyte-monocyte apheresis and vedolizumab: A new treatment option for ulcerative colitis? *J Clin Apher* 2019; **34**: 680-685 [PMID: 31518013 DOI: 10.1002/jca.21746]

49 **Nakamura M**, Yamamura T, Maeda K, Sawada T, Mizutani Y, Ishikawa E, Ohashi A, Kajikawa G, Furukawa K, Ohno E, Honda T, Kawashima H, Ishigami M, Fujishiro M. Refractory Ulcerative Colitis Improved by Scheduled Combination Therapy of Vedolizumab and Granulocyte and Monocyte Adsorptive Apheresis. *Intern Med* 2020; **59**: 3009-3014 [PMID: 32727993 DOI: 10.2169/internalmedicine.5302-20]

50 **Tanida S**, Ozeki K, Mizoshita T, Kitagawa M, Ozeki T, Tanaka M, Nishie H, Shimura T, Kubota E, Kataoka H. Combination Therapy With Tofacitinib Plus Intensive Granulocyte and Monocyte Adsorptive Apheresis as Induction Therapy for Refractory Ulcerative Colitis. *J Clin Med Res* 2020; **12**: 36-40 [PMID: 32010420 DOI: 10.14740/jocmr4037]

51 **Yokoyama Y**, Sawada K, Aoyama N, Yoshimura N, Sako M, Hirai F, Kashiwagi N, Suzuki Y. Efficacy of Granulocyte and Monocyte Adsorptive Apheresis in Patients With Inflammatory Bowel Disease Showing Lost Response to Infliximab. *J Crohns Colitis* 2020; **14**: 1264-1273 [PMID: 32166331 DOI: 10.1093/ecco-jcc/jjaa051]

52 **Motoya S**, Tanaka H, Shibuya T, Osada T, Yamamoto T, Hongo H, Mizuno C, Saito D, Aoyama N, Kobayashi T, Ito H, Tanida S, Nojima M, Kokuma S, Hosoi E. Safety and effectiveness of granulocyte and monocyte adsorptive apheresis in patients with inflammatory bowel disease in special situations: a multicentre cohort study. *BMC Gastroenterol* 2019; **19**: 196 [PMID: 31752695 DOI: 10.1186/s12876-019-1110-1]

53 **Yamamoto T**, Umegae S, Matsumoto K. Mucosal healing in patients with ulcerative colitis during a course of selective leukocytapheresis therapy: a prospective cohort study. *Inflamm Bowel Dis* 2010; **16**: 1905-1911 [PMID: 20310015 DOI: 10.1002/ibd.21260]

54 **Lindberg A**, Eberhardson M, Karlsson M, Karlén P. Long-term follow-up with Granulocyte and Monocyte Apheresis re-treatment in patients with chronically active inflammatory bowel disease. *BMC Gastroenterol* 2010; **10**: 73 [PMID: 20604939 DOI: 10.1186/1471-230X-10-73]

55 **Sandborn WJ**. Preliminary data on the use of apheresis in inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12** Suppl 1: S15-S21 [PMID: 16378006]

56 **Hibi T**, Sameshima Y, Sekiguchi Y, Hisatome Y, Maruyama F, Moriwaki K, Shima C, Saniabadi AR, Matsumoto T. Treating ulcerative colitis by Adacolumn therapeutic leucocytapheresis: clinical efficacy and safety based on surveillance of 656 patients in 53 centres in Japan. *Dig Liver Dis* 2009; **41**: 570-577 [PMID: 19211314 DOI: 10.1016/j.dld]

57 **Sakuraba A**, Motoya S, Watanabe K, Nishishita M, Kanke K, Matsui T, Suzuki Y, Oshima T, Kunisaki R, Matsumoto T, Hanai H, Fukunaga K, Yoshimura N, Chiba T, Funakoshi S, Aoyama N, Andoh A, Nakase H, Mizuta Y, Suzuki R, Akamatsu T, Iizuka M, Ashida T, Hibi T. An open-label prospective randomized multicenter study shows very rapid remission of ulcerative colitis by intensive granulocyte and monocyte adsorptive apheresis as compared with routine weekly treatment. *Am J Gastroenterol* 2009; **104**: 2990-2995 [PMID: 19724269 DOI: 10.1038/ajg.2009.453]

58 **Takayama T**, Kanai T, Matsuoka K, Okamoto S, Sujino T, Mikami Y, Hisamatsu T, Yajima T, Iwao Y, Ogata H, Hibi T. Long-term prognosis of patients with ulcerative colitis treated with cytapheresis therapy. *J Crohns Colitis* 2013; **7**: e49-e54 [PMID: 22633997 DOI: 10.1016/j.crohns.2012.05.005]

59 **Iizuka M**, Etou T, Shimodaira Y, Hatakeyama T, Sagara S. Cytapheresis re-induces high-rate steroid-free remission in patients with steroid-dependent and steroid-refractory ulcerative colitis. *World J Gastroenterol* 2021; **27**: 1194-1212 [PMID: 33828394 DOI: 10.3748/wjg.v27.i12.1194]

60 **Cabriada JL**, Ibargoyen N, Hernández A, Bernal A, Castiella A. Sustained remission after steroids and leukocytapheresis induced response in steroid-dependent ulcerative colitis: results at 1 year. *Dig Liver Dis* 2010; **42**: 432-435 [PMID: 19833566 DOI: 10.1016/j.dld.2009.09.001]

61 **Imperiali G**, Amato A, Terpin MM, Beverina I, Bortoli A, Devani M, Viganò C; Study Group on IBD (GSMII). Granulocyte-Monocyte Apheresis in Steroid-Dependent, Azathioprine-Intolerant/Resistant Moderate Ulcerative Colitis: A Prospective Multicenter Study. *Gastroenterol Res Pract* 2017; **2017**: 9728324 [PMID: 29403531 DOI: 10.1155/2017/9728324]

62 **Ishiguro Y**, Ohmori T, Umemura K, Iizuka M. Factors associated with the outcomes in ulcerative colitis patients undergoing granulocyte and monocyte adsorptive apheresis as remission induction therapy: A multicenter cohort study. *Ther Apher Dial* 2021; **25**: 502-512 [PMID: 33029920 DOI: 10.1111/1744-9987.13594]

63 **Domènech E**, Grífols JR, Akbar A, Dignass AU. Use of granulocyte/monocytapheresis in ulcerative colitis: A practical review from a European perspective. *World J Gastroenterol* 2021; **27**: 908-918 [PMID: 33776362 DOI: 10.3748/wjg.v27.i10.908]

64 **Saniabadi AR**, Hanai H, Suzuki Y, Ohmori T, Sawada K, Yoshimura N, Saito Y, Takeda Y, Umemura K, Kondo K, Ikeda Y, Fukunaga K, Nakashima M, Beretta A, Bjarnason I, Lofberg R. Adacolumn for selective leukocytapheresis as a non-pharmacological treatment for patients with disorders of the immune system: an adjunct or an alternative to drug therapy? *J Clin Apher* 2005; **20**: 171-184 [PMID: 15892107 DOI: 10.1002/jca.20046]

65 **Hanai H**, Watanabe F, Yamada M, Sato Y, Takeuchi K, Iida T, Tozawa K, Tanaka T, Maruyama Y, Matsushita I, Iwaoka Y, Saniabadi A. Correlation of serum soluble TNF-alpha receptors I and II levels with disease activity in patients with ulcerative colitis. *Am J Gastroenterol* 2004; **99**: 1532-1538 [PMID: 15307873 DOI: 10.1111/j.1572-0241.2004.30432.x]

66 **Tominaga K**, Nakano M, Hoshino M, Kanke K, Hiraishi H. Efficacy, safety and cost analyses in ulcerative colitis patients undergoing granulocyte and monocyte adsorption or receiving prednisolone. *BMC Gastroenterol* 2013; **13**: 41 [PMID: 23452668 DOI: 10.1186/1471-230X-13-41]

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ORIGINAL ARTICLE

Basic Study

Long noncoding RNA ZNFX1-AS1 promotes the invasion and proliferation of gastric cancer cells by regulating LIN28 and CAPR1N1

Zhong-Ling Zhuo, Hai-Peng Xian, Yu-Jing Sun, Yan Long, Chang Liu, Bin Liang, Xiao-Tao Zhao

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Abstract

BACKGROUND

Long noncoding RNA (lncRNA) ZNFX1-AS1 (ZFAS1) is a newly discovered lncRNA, but its diagnostic value in gastric cancer is unclear.

AIM

To investigate the potential role of ZFAS1 in gastric cancer and to evaluate the clinical significance of ZFAS1 as a biomarker for gastric cancer screening.

METHODS

Quantitative real-time polymerase chain reaction (qRT-PCR) was used to screen for gastric cancer-associated lncRNAs in gastric cancer patients, gastric stromal tumor patients, gastritis or gastric ulcer patients, and healthy controls. Correlations between ZFAS1 expression and clinicopathological features were analyzed. The biological effects of ZFAS1 on the proliferation, migration, and invasion of gastric cancer cells were studied by MTT, colony formation, and transwell migration assays. The potential mechanism of ZFAS1 was demonstrated using enzyme-linked immunosorbent assay and qRT-PCR. The relationship between ZFAS1 and tumorigenesis was demonstrated using *in vivo* tumor formation assays.

RESULTS
The plasma level of lncRNA ZFAS1 was significantly higher in preoperative patients with gastric cancer than in individuals in the other 4 groups. Increased expression of ZFAS1 was significantly associated with lymph node metastasis, advanced TNM stage, and poor prognosis. ZFAS1 regulated the proliferation, migration, and invasion of gastric cancer cells and regulated the growth of gastric cancer cells *in vivo*. LIN28 and CAPRIN1 were identified as key downstream mediators of ZFAS1 in gastric cancer cells.

CONCLUSION
LncRNA ZFAS1 promoted the invasion and proliferation of gastric cancer cells by modulating LIN28 and CAPRIN1 expression, suggesting that ZFAS1 can be used as a potential diagnostic and prognostic biomarker in gastric cancer.

Key Words: Long noncoding RNA; ZNFX1-AS1; Gastric cancer; Biomarker; Invasion; Proliferation

Core Tip: Long noncoding RNA (lncRNA) ZNFX1-AS1 (ZFAS1) is a newly discovered lncRNA, but its diagnostic value in gastric cancer is unclear. This study aimed to investigate the potential role of ZFAS1 in gastric cancer and to evaluate the clinical significance of ZFAS1 as a biomarker for gastric cancer screening. LncRNA ZFAS1 promoted invasion and proliferation of gastric cancer cells by modulating LIN28 and CAPRIN1, suggesting that ZFAS1 can be used as a potential biomarker for the diagnosis and prognosis of gastric cancer.

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INTRODUCTION

Gastric carcinoma is the 2nd primary cause of death due to carcinoma and the 6th most common cancer worldwide. Approximately 1000000 new cases of gastric cancer are diagnosed each year, and approximately 800000 patients die from the disease, accounting for approximately 8.2% of global cancer-related mortality[1]. The progression-free survival (PFS) rate and prognosis of gastric carcinoma highly depend on the TNM stage of the disease. The high mortality is associated with the faultiness of standard screening protocols and lack of overt early symptoms[1]. Therefore, early detection and optimal treatment are necessary to improve the prognosis of gastric carcinoma. However, current biologic markers, such as carbohydrate antigen 199 (CA-199) and carcinoembryonic antigen (CEA), have low sensitivity and specificity[2]. Therefore, there is an urgent need to find novel biomarkers for early diagnosis of gastric cancer.

Long noncoding RNAs (lncRNAs) are a class of non-protein-coding RNA molecules > 200 nucleotides in length[3]. Recently, some studies have reported that lncRNAs are involved in protein modification and gene expression and that their dysregulation leads to a variety of genetic diseases[4-6]. Published articles have also shown that lncRNAs exhibit significant regulatory effects on transcription patterns in malignant tumors, some of which involve tumor cell invasion and metastasis, with a poor prognosis[6, 7]. For example, H19 and SUMO1 pseudogene 3 (SUMO1P3) are upregulated in gastric cancer, while gastric cancer-associated transcript 1 is downregulated[7-11]. Additionally, HOTAIR overexpression may be associated with tumor escape mechanisms[12]. These studies strongly suggest that lncRNAs underlie the molecular etiology of gastric carcinoma. It is worth noting that the detection of circulating lncRNAs provides a new gastric biomarker for gastric cancer that is expected to be useful for monitoring and screening patients with gastric cancer[13].

Here, we selected 15 candidate cancer-associated lncRNAs. Among these lncRNAs, lncRNA ZNFX1-AS1 (ZFAS1) was found to be upregulated in the plasma of preoperative gastric cancer patients compared with healthy controls, and expression of ZFAS1 was significantly associated with lymphatic invasion, advanced TNM stage, and poor prognosis. We then investigated the biological effects of ZFAS1 on the survival, proliferation, and migration of gastric cancer cells. The underlying mechanism of ZFAS1 and the relationship between ZFAS1 and tumorigenesis were identified. These results showed that lncRNA ZFAS1 is a potential biomarker for gastric cancer.

MATERIALS AND METHODS

Sample collection and ethics statement

All samples were obtained from Peking University People's Hospital between July 2015 and June 2016. Seventy-five matched (pre- and postoperative) whole blood and serum samples were collected from patients who underwent gastrointestinal surgery for gastric resection. In addition, intraperitoneal free cancer cell (IFCC) samples were collected from 60 of these patients. Other whole blood and serum specimens were collected from 60 gastric stromal tumor patients (before surgery), 60 gastritis or ulcer patients (before treatment), and 75 healthy controls. All specimens were collected with the corresponding patient information, such as age, sex, and collection time. In addition, the tumor size, tumor location (evaluated according to the National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology, Version II[14]), pathological type, degree of differentiation (based on the World Health Organization Classification of Tumors of the Digestive System, 4th edition[15]), TNM stage and lymphatic invasion status (based on the American Joint Committee on Cancer Staging Manual, 7th edition[16]) of the corresponding patients were collected.

Plasma and serum samples were collected in BD vacutainer ethylenediamine tetraacetic acid tubes and BD vacutainer somatostatin tubes (BD Biosciences, NJ, United States), respectively. Preoperative samples of patients with gastric cancer were obtained at least 2 mo before surgery or after radiotherapy or chemotherapy. Postoperative samples from these patients were collected 7-10 d after surgery. Samples from patients with gastric stromal tumor or gastritis/peptic ulcer were collected before any treatment was administered. The healthy control samples were randomly collected from normal subjects. For plasma samples, a special centrifugation protocol (2348 × g for 30 min at 4 °C; 4696 × g for 5 min at 4 °C; 10733 × g for 5 min at 4 °C) was performed to prevent contamination with cellular nucleic acids. Plasma and IFCC samples were stored at -80 °C in 3 volumes of TRIzol® reagent (Qiagen, CA, United States) for further analysis. Serum samples were analyzed directly[17]. This study was approved by the Research Ethics Committee of Peking University People's Hospital. Patient data and samples were treated according to the ethical and legal criteria adopted in the 2013 Declaration of Helsinki. Written informed consent for ethical approval and patient consent was obtained from all participants.

RNA extraction and quantitative real-time polymerase chain reaction

This method is the same as that described in our previously published article[17]. LncRNA was extracted from plasma using a Direct-zol™ RNA MiniPrep R2050i Kit (Zymo Research, CA, United States) according to the manufacturer's protocol. The concentration and purity of total RNA were measured using a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, MA, United States). Total RNA was then solubilized using RNase-free water, and reverse transcription was immediately performed using PrimeScript RT Master Mix (Takara Bio, Kyushu, Japan) according to the manufacturer's instructions. After reverse transcription, quantitative polymerase chain reaction (qPCR) was performed in a LightCycler 480 Instrument II (Roche Diagnostics, Basel, Switzerland) using TransStart Green qPCR SuperMix (TransGen, Beijing, China) according to the supplier's instructions. The primers used for qPCR were designed with Primer Premier V 5.0 (Premier Biosoft International, CA, United States) and were synthesized by Beijing Sunbiotech Co., Ltd. (Beijing, China). The sequences of all primers, including those specific for GAPDH, are listed in Table 1. PCR was carried out with initial denaturation at 95 °C for 3 min followed by 40 cycles of 1 min at 95 °C and 1 min at 60 °C with subsequent detection. Due to the stability of GAPDH in plasma, it was chosen as the internal control for data standardization. For the calculations, the equation $\Delta C_q = C_{q\ \text{selected lncRNA}} - C_{q\ \text{GAPDH lncRNA}}$ was used, where ΔC_q was defined as the difference in the quantification cycle (C_q) values. Every specimen was analyzed in triplicate, and the experiment was repeated 3 times.

Cell culture

The human gastric cancer cell lines BGC-823 and SGC-7901 were a gift from Peking University People's Hospital Gastrointestinal Surgery Laboratory and were maintained in RPMI 1640 medium (Invitrogen, CA, United States) supplemented with 10% fetal bovine serum (FBS) (Gibco, CA, United States). The flask was incubated in a humidified incubator at 37 °C under 5% CO_2 in air.

LncRNA silencing and overexpression

ZFAS1-siRNA against ZFAS1 was synthesized by GenePharma (Shanghai, China). BGC-823 and SGC7901 cells were grown in 6-well plates (2 × 10^5cells/well) and transfected for 36 h using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, CA, United States). The ZFAS1 siRNA sequences were as follows: ZFAS1 siRNA1, 5′-AGACGCGAAAGAACGAAUGTT-3′; ZFAS1 siRNA2, 5′-UUACAAGGCAGACUGAAUCTT-3′; and ZFAS1 siRNA3, 5′-UAUGCAGGUAGGCAGUUAGTT-3′. The GAPDH siRNA and negative control siRNA sequences were as follows: GAPDH siRNA, 5'-ACGUGACACGUUCGGAGAATT-3' and negative control siRNA, 5'-ACGUGCAC AGUACUAGGAATT-3'.

Table 1 List of all primers used to screen gastric cancer-associated long noncoding RNAs

Name	Forward-primer	Reverse-primer
GAPDH	ACCCACTCCTCCACCTTTGAC	TGTTGCTGTAGCCAAATTCGTT
H19	TACAACCACTGCACTACCTG	TGGAATGCTTGAAGGCTGCT
CCAT1	CATTGGGAAAGGTGCCGAGA	ACGCTTAGCCATACAGAGCC
HOTAIR	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
LINC00152	CTCCAGCACCTCTACCTGTTG	GGACAAGGGATTAAGACACACA
ZNFX1-AS1	CCAGTTCCACAAGGTTAC	GCAGGTAGGCAGTTAGAA
PVT1	CTTGAGAACTGTCCTTACG	CAGATGAACCAGGTGAAC
GAS5	CACAGGCATTAGACAGAA	AGGAGCAGAACCATTAAG
SNHG12	GACTTCCGGGGTAATGACAG	GCCTTCTGCTTCCCATAGAG
TUG1	TAGCAGTTCCCCAATCCTTG	CACAAATTCCCATCATTCCC
CHET1	CCCCACAAATGAAGACACT	TTCCCAACACCCTATAAGAT
SUMP1P3	ACTGGGAATGGAGGAAGA	TGAGAAAGGATTGAGGGAAAAG
GACAT3	GGGGGCTTGTTTCTTTGTGTAG	CATTCGGCTCTGACCTCTCAC
ABHD11-AS1	GAACGGGATGAAGCCATTG	GCTGATTCTGGACCTGCTG
GACAT2	TGGATGCTTACAAAGGACTGG	CTGCAATTACGGAAAGAGCTG
uc001lsz	GACGGCACCTACTACACCTT	GCTGACCACCTTGTTGTTGAA

The ZFAS1 overexpression short hairpin RNA (ZFAS1-shRNA1), sh-ZFAS1 directed against ZFAS1 short hairpin RNA (ZFAS1-shRNA2), and NC-shRNA short hairpin RNA constructs were synthesized by GenePharma (Shanghai, China). The sequences of ZFAS1-shRNA1, ZFAS1-shRNA2, and NC-shRNA are shown in Table 2. BGC-823 and SGC7901 cells were grown in 6-well plates (2×10^5 cells/well) and transduced for 36 h using Polybrene (GenePharma, REVG0001) and Enhanced Infection Solution (GenePharma, REVG0002).

MTT assay

After transduction with NC-shRNA, ZFAS1-shRNA1, and ZFAS1-shRNA2, BGC823 and SGC7901 cells were trypsinized, and six replicates of 12×10^3 cells per well were seeded in 96-well plates, with wells without cells used as blank wells. Proliferation was measured by an MTT (Sigma Aldrich, St. Louis, MO, United States) transformation assay. Briefly, 20 mL of MTT solution (5 mg/mL) was added to each well, and cells were grown for 4 h at 37 °C. After adding 100 mL of the dissolved solution (10% SDS in 0.01 M HCl solution), cells were further cultured at 37 °C for 3 h. The specific optical density of all wells was then measured at 490 nm.

Transwell migration assay

BGC823 and SGC7901 cells were suspended in serum-free medium after transduction with NC-shRNA, ZFAS1-shRNA1, and ZFAS1-shRNA2 and inoculated into transwell chambers with inserts of 8 μm pore size. Medium containing 10% FBS was placed in the bottom chamber. After 36 h of incubation, cells that migrated through the membrane to the lower surface were fixed with paraformaldehyde, stained with crystal violet, counted, and photographed. The results are shown as the average of three independent experiments.

Colony formation assay

BGC823 and SGC7901 cells transduced with NC-shRNA, ZFAS1-shRNA1, and ZFAS1-shRNA2 were seeded into 6-well plates (1000 cells per well) and cultured in a humidified incubator at 37 °C in 5% CO_2 for 10 d. The medium was changed every 3 d. At the end of the incubation period, the cultured cells were fixed with 4% paraformaldehyde and stained with crystal violet to assess the number of colonies. The results are shown as the average of three independent experiments.

Prediction and expression of ZFAS1 binding proteins

Binding proteins of lncRNA ZFAS1 were predicted based on the starBase V2.0 database (http://starbase.sysu.edu.cn). The primers specific for the mRNA sequences of the genes encoding the binding proteins were designed according to the nucleic acid sequence information for the corresponding proteins in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) with a primer design tool (

Table 2 The sequences of ZNFX1-AS1-shRNA1, ZNFX1-AS1-shRNA2 and NC-shRNA

	Sequence
ZFAS1-shRNA1	ACCGGTCGGGGGCCCAGGGTGGAGAGCACGAGGGCCTGGCCCAGGCACGGCCGGCGCCTCCGCCCTCGA GGAGGGCGTCACCTCAGCTCCCCCCGGCGGCGGAGCCGGCGGGCTCAGGCGGGCGCGGCTGAGGGGAGCGGAC CGCGGGGGGCGGGAGATGACTGCGCCCAAGGCCTTTGCGGGCCTCAGCCGGCCCCAGAGGAAGGGGAACCCGT CGAGCGGTTTGGTGCGTGTGAAGCGCGACATGGCGAGGAAGCGGACAAGCCCGGGTGGCCCGGCGTGTAGAGG GAAGGGGGCGGGGCTAGACGCGGCCTGGACAACTACTAGAGCGCCTCGGGCTGTGCTGCTCGAGACTACATTT CCCAGAGCGACGCGCGCGGAGCGGGCGGGAAAGAGAGCGTTTCGGGTCCAGTGCGCAGGTGCGAAAGCCATCT TTGGTTATATAAGGGAGGTTCAGGAAGCCATTCGTTCTTTCGCGTCTGCGGTGCCCGGAGTGTGGTACTTCTC CTAGTTGCAGTCAGGCTTCATACGCTATTGTCCTGCCCGTTAGAGCAGCCAGCGGGTACAGAATGGATTTTGG AAGAGGGAGTCACCACTGGACCTCCAAGGAAGCCACGTGCAGACATCTACAACCTTCGATCTCCTGACGAGTT TATTGTTGGCCAAAACCAGGCTTTGATTGAACCAGGATGAATGCGGGTGTTGGAAGTAGAATATATATATACA TATAAAATTGGTTGGGAGCCACGTGTACCAGTGTGTGTTGATCTTGGCTTGATTCAGTCTGCCTTGTAACAGA AACTGGCGATGGAATATGAGAGGAGCCCTCTGGAAAGAAAAGGACAGACCCTGTGCTTTCATGAAAGTGAAGA TCTGGCTGAACCAGTTCCACAAGGTTACTGTATACATAGCCTGAGTTTAAAAGGCTGTGCCCACTTCAAGAAT GTCATTGTTAGACTTTGAAATTTCTAACTGCCTACCTGCATAAAGAAAATAAAATCTTTTAAATCAAAAGCTAGC
ZFAS1-shRNA2	TTACAAGGCAGACTGAATCTT
NC-shRNA	TTCTCCGAACGTGTCACGT

ZFAS1: ZNFX1-AS1.

http://www.ncbi.nlm.nih.gov/tools/primer-Blast). The sequences of all primers, including those specific for GAPDH, are listed in Table 3.

NC-shRNA, ZFAS1-shRNA1, and ZFAS1-shRNA2 were transduced into BGC823 and SGC7901 cells, and mRNA was then reverse transcribed. The mRNA expression levels of the genes encoding the candidate lncRNA ZFAS 1 binding proteins were determined by quantitative real-time-PCR (qRT-PCR) using the primers listed in Table 3. The experimental method was the same as that described previously.

Protein extraction and enzyme-linked immunosorbent assay

Stably transfected cells, as well as tumor/lymph node tissue from BALB/c nude mice, were lysed with NP40 cell lysis buffer (Invitrogen, CA, United States) supplemented with SigmaFAST™ protease inhibitor tablets (Sigma Aldrich, St. Louis, MO, United States). LIN28, CAPRIN1, CEA, and CK20 were detected by enzyme-linked immunosorbent assay (ELISA) using a Mouse Protein lin-28 homolog A (Lin28a) ELISA Kit (ELISAGenie, Dublin, Ireland), a Human Caprin 1 (CAPRIN1) ELISA Kit (Abbexa, Cambridge, United Kingdom), a CEA ELISA Kit (Bioss, United Kingdom) and CK20 ELISA kits (FKBIO, Chengdu, China), respectively.

In vivo tumor formation assay

Four-week-old female athymic BALB/c nude mice were maintained under specific pathogen-free conditions and operated on according to the protocol approved by the Beijing Medical Laboratory Animal Management Committee. NC-shRNA-, ZFAS1-shRNA1-, and ZFAS1-shRNA2-transduced BGC823 cells were harvested. For tumor formation assays, 10^7 cells were injected subcutaneously into one side of each mouse. Tumor growth was examined every week, and the tumor volume was calculated using the equation V = 0.5 × D × d2 (V, volume; D, longitudinal diameter; d, latitudinal diameter). The expression of lncRNA ZFAS1 in BALB/c mouse blood was detected by qRT-PCR every week, and the primer sequences are listed in Table 1. The expression of LIN28 and CAPRIN1 proteins in BALB/c nude mouse tumors and the expression of CEA and CK20 in BALB/c nude mouse lymph nodes were detected by ELISA after four weeks. This study was conducted in strict accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The program was approved by the Animal Experiment Ethics Committee of Peking University People's Hospital.

Statistical analysis

The formula $2^{-\Delta\Delta Ct}$ were used to calculate the levels of relative lncRNA expression in plasma[18]. Based on this formula, all relative lncRNA expression levels in plasma were evaluated. Continuous variables were described by the mean ± SD (normal distribution), or the median and interquartile range M (P25, P75) (non-normal distribution). The *t*-test or Mann-Whitney *U* test was used according to the data distribution to identify statistically significant differences. For categorical variables, the percentages of patients in each category were calculated using the χ^2 test or Fisher's exact test. In addition, receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic value of circulating lncRNA and traditional tumor markers in distinguishing between gastric carcinoma patients and healthy subjects. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, United States). *P* values less than 0.05 were considered significant.

Table 3 List of primers used to detect mRNA expression of long noncoding RNA binding proteins

Name	Forward-primer (5' to 3')	Reverse-primer (5'to 3')
GAPDH	ACCCACTCCTCCACCTTTGAC	TGTTGCTGTAGCCAAATTCGTT
UPF1	GGTCCCTGATAATTATGGCGATG	ACGGCATAAACCTGGGAGTG
eIF4AIII	GGCATCTACGCTTACGGTTT	CAGCCAACTCTCTTGTGGGA
IGF2BP1	AGCTCCTTTATGCAGGCTCC	GAGCCTTGAATTGGGCCTCT
FMRP	CTCAAGGCTTGGCAGGGTATG	CCGTGCCCCCTATTTCTGTA
LIN28	AGATCAAAAGGAGACAGGTGCT	AATAGCCCCCACCCATTGTG
IGF2BP2	ACCCTCTCGGGTAAAGTGGA	GTTGACAACGGCGGTTTCTG
FUS	GCAAGATGGATTCCAGGGGTG	TCCAGGAAAGTGAAAAGGGGG
ZC3H7B	TGTGCAAAGGAGGAGATCGAC	ACAGACGGAGAGTCCTTGGT
IGF2BP3	CCTGGTGAAGACTGGCTACG	CCAGCACCTCCCACTGTAAAT
CAPRIN1	CTGCTGGCTGGCTAAGTCC	GGCCGAGGGCATCGTG

RESULTS

Relative expression of a selected subgroup of lncRNAs (plasma) from 15 gastric cancer patients and 15 healthy control subjects

Fifteen matched samples from preoperative and postoperative gastric cancer patients and samples from 15 healthy control subjects were used as the sample cohort. The general characteristics and clinicopathological features of all subjects are provided in Table 4. The relative expression of the 15 lncRNAs, namely, uc001 Lsz/HOTAIR/CCAT1/H19/GACAT2/ABHD11-AS1/GACAT3 /SUMO1P3/CHET1/TUG1/SNHG12/GAS5/PVT1/LINC00152, and ZFAS1, was evaluated in the plasma of all subjects. As mentioned in Figure 1, the levels of lncRNA ZFAS1 in preoperative patient plasma were significantly higher than those in postoperative patient and healthy control subject plasma ($P < 0.01$).

Relative levels of the lncRNA ZFAS1 in the plasma of healthy control subjects, gastritis/peptic ulcer patients, GIST patients, and preoperative and postoperative gastric cancer patients

The general characteristics and clinicopathological features of 60 healthy control subjects, 60 gastritis/peptic ulcer patients, 60 GIST patients, and 60 patients with matched preoperative and postoperative data are summarized in Table 5. The relative levels of ZFAS1 in the plasma of all subjects were assessed by qRT-PCR. As shown in Figure 2A, the levels of lncRNA ZFAS1 in preoperative patient plasma were significantly higher than those in plasma from the individuals in the other four groups ($P < 0.01$).

Relative levels of lncRNA ZFAS1 (plasma) in preoperative gastric carcinoma patients (different TNM stages)

We used the ΔΔCt formula to calculate the relative levels of lncRNA ZFAS1 in the samples (plasma) of patients with different TNM stages, including 20 patients with stage I and II disease and 40 patients with stage III and IV disease. As mentioned in Figure 2B, the median relative levels of lncRNA ZFAS1 in healthy controls, patients with stage I and II disease, and patients with stage III and IV disease were 0.81, 1.61, and 2.52, respectively. The relative levels in early- and advanced-stage patients were significantly higher than that in healthy controls ($P < 0.01$).

Relative levels of lncRNA ZFAS1 in 60 matched plasma samples from pre-operative and post-operative gastric carcinoma patients

Sixty matched pre-operative and post-operative samples (plasma) were included in our research. We found that the median relative levels of ZFAS1 before and after surgery were 2.22 and 1.01, respectively. The level decreased in 55 of the 60 gastric cancer patients (92%) approximately 10 d after surgery ($P < 0.01$; Figure 2C).

Correlation between the relative level of lncRNA ZFAS1 in IFCC and plasma samples

Sixty matched preoperative patient plasma and IFCC samples were collected. We used the ΔΔCt method to evaluate the relative levels of ZFAS1 in plasma and IFCC. As shown in Figure 2D, the relative levels of ZFAS1 in plasma and IFCC were strongly positively correlated ($R^2 = 0.76$, $P < 0.01$).

Table 4 General characteristics and clinicopathological factors of gastric cancer patients and healthy control subjects

Variables	Gastric cancer (*n* = 15)	Healthy control (*n* = 15)
Gender		
Male	12	12
Female	3	3
Age (yr)		
Mean	61	62
Range	48-70	47-80
Tumor site[1]		
Upper third	3	-
Middle third	4	-
Lower third	8	-
Tumor size		
≥ 5 cm	6	-
< 5 cm	9	-
Pathological differentiation[2]		
Undifferentiated or poorly differentiated	12	-
Others	3	-
TNM classification[3]		
I + II	4	-
III + IV	11	-
Lymphatic invasion[3]		
Positive	11	-
Negative	4	-

[1]According to National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Version I, 2016.
[2]According to World Health Organization Classification of Tumors of the Digestive System: 4th edition.
[3]According to the American Joint Committee on Cancer Staging Manual: 7th edition.

Diagnostic accuracy of plasma ZFAS1 and traditional serum biomarkers

ROC analysis revealed that the area under the curve (AUC) value of plasma ZFAS1 for discriminating gastric cancer patients from healthy control subjects was 0.85 (Figure 2E). The highest AUC value of any traditional serum biomarker was 0.63, while the AUC value of the other traditional biomarkers was lower than that of CEA. The highest accuracy of plasma ZFAS1 was obtained at a cutoff level of 1.00, where its sensitivity and specificity for identifying gastric carcinoma patients were 0.82 and 0.72, respectively.

ZFAS1 amplification is correlated with poor prognosis in gastric cancer

To explore the correlations between the relative ZFAS1 level and gastric cancer clinicopathological characteristics, we divided the patients into groups by sex, age, tumor site/size/lymphatic invasion/pathological differentiation status and TNM classification (Table 6). Data analysis indicated that the relative ZFAS1 expression level was strongly associated with tumor size (P = 0.01), TNM classification (P = 0.02), and lymphatic invasion (P = 0.03). We then divided patients into two groups by the median relative ZFAS1 level. Kaplan-Meier survival analysis was used to assess the potential correlation between the relative ZFAS1 expression level and patient prognosis. As shown in Figures 2F and G, patients with a high ZFAS1 level had shorter overall survival (OS) and PFS times than those with a low ZFAS1 level. This result suggests that ZFAS1 is a potential prognostic biomarker in gastric cancer patients.

ZFAS1 knockdown inhibited the viability, migration, and proliferation of gastric cancer cells

The expression level of ZFAS1 was positively correlated with lymph node metastasis, suggesting that ZFAS1 may be involved in tumor metastasis. Therefore, we studied the effect of ZFAS1 knockdown on

Table 5 General characteristics and clinicopathological factors of gastric cancer patients and healthy control subjects

Variables	Healthy control (*n* = 60)	Gastritis/peptic ulcer (*n* = 60)	GIST (*n* = 60)	Gastric cancer (*n* = 60)
Gender				
Male	46	37	35	46
Female	14	23	25	14
Age (yr)				
Mean	61	60	60	61
Range	37-91	37-89	30-85	37-91
Tumor site[1]				
Upper third	-	-	-	13
Middle third	-	-	-	14
Lower third	-	-	-	33
Tumor size				
≥ 5 cm	-	-	-	29
< 5 cm	-	-	-	31
Pathological differentiation[2]				
Undifferentiated or poorly differentiated	-	-	-	45
Others	-	-	-	15
TNM classification[3]				
I + II	-	-	-	20
III + IV	-	-	-	40
Lymphatic invasion[3]				
Positive	-	-	-	43
Negative	-	-	-	17

[1]According to National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Version I, 2016.
[2]According to World Health Organization Classification of Tumors of the Digestive System: 4th edition.
[3]According to the American Joint Committee on Cancer Staging Manual: 7th edition.
GIST: Gastric stromal tumor.

gastric cancer cells. To verify the efficiency of ZFAS1 knockdown and prevent off-target effects, we first transfected three targeted siRNAs, ZFAS1-siRNA1, ZFAS1-siRNA2, and ZFAS1-siRNA3, into BGC823 and SGC7901 cells. Fluorescence microscopy was used to test the transfection efficiency. As shown in Figure 3, all three siRNAs significantly reduced the expression level of ZFAS1 in transfected BGC823 and SGC7901 cells. Next, we constructed ZFAS1-shRNA2 (with a sequence based on that of ZFAS1-siRNA2) and transduced it into BGC823 and SGC7901 cells. The MTT and colony formation assay results showed that compared to control cells transduced with NC-shRNA, BGC823 and SGC7901 cells with ZFAS1 knockdown showed significant decreases in viability and proliferation (Figures 4 and 5). The transwell migration assay showed that ZFAS1 knockdown in BGC823 and SGC7901 cells significantly inhibited cell migration (Figure 6). In summary, our data suggest that knockdown of ZFAS1 reduces the viability, proliferation, and migration of gastric cancer cells.

ZFAS1 overexpression enhanced the viability, migration, and proliferation of gastric cancer cells

To further investigate the function of ZFAS1 in gastric cancer, we transduced ZFAS1-shRNA1 into BGC823 and SGC7901 cells to overexpress ZFAS1 and evaluated the transduction efficiency by fluorescence microscopy. As shown in Figure 7, the expression level of ZFAS1 was significantly increased in transduced BGC823 and SGC7901 cells. The MTT and colony formation assay results showed that compared to control cells transfected with NC-shRNA, BGC823 and SGC7901 cells overexpressing ZFAS1 showed significant increases in viability and proliferation (Figures 4 and 5). The transwell migration assay showed that ZFAS1 overexpression in BGC823 and SGC7901 cells significantly promoted cell migration (Figure 6). In summary, our data indicate that overexpression of ZFAS1 enhances the viability, proliferation, and migration of gastric cancer cells.

Table 6 Correlation between the relative expression of long noncoding RNA HULC or ZNFX1-AS1 and clinicopathologic factors

Variables	Number of cases	*P* value
Gender		
Male	46	0.99
Female	14	
Age (yr)		
≤ 61	28	0.26
> 61	32	
Tumor site[1]		
Upper third	13	0.12
Middle third	14	
Lower third	33	
Tumor size		
≥ 5 cm	29	0.01
< 5 cm	31	
Pathological differentiation[2]		
Undifferentiated or poorly differentiated	45	0.55
Others	15	
TNM classification[3]		
I + II	20	0.02
III + IV	40	
Distant metastasis[3]		
Positive	5	0.97
Negative	55	
Lymphatic invasion[3]		
Positive	43	0.03
Negative	17	

[1]According to National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Version I, 2016.
[2]According to World Health Organization Classification of Tumors of the Digestive System: 4th edition.
[3]According to the American Joint Committee on Cancer Staging Manual: 7th edition.

LIN28 and CAPRIN1 are the key downstream mediators of ZFAS1 in gastric cancer cells

To further investigate the mechanism by which lncRNA ZFAS1 affects the aggressiveness of gastric cancer, we used the starBase V2.0 database to analyze binding proteins of lncRNA ZFAS1[19]. The results showed that a total of 10 proteins had more than three binding sites for lncRNA ZFAS1. These proteins were UPF1, eIF4AIII, IGF2BP1, FMRP, LIN28, IGF2BP2, FUS, ZC3H7B, IGF2BP3, and CAPRIN1. We then analyzed the mRNA expression levels of these proteins in BGC823 and SGC7901 cells transduced with ZFAS1-shRNA1 and ZFAS1-shRNA2. Our goal was to determine which proteins exhibited changes in expression consistent with the change in ZFAS1 expression. The qRT-PCR results showed that ZFAS1 overexpression significantly increased the expression of LIN28 and CAPRIN1 ($P < 0.05$) and that ZFAS1 knockdown significantly decreased the expression of LIN28 and CAPRIN1 ($P < 0.05$) (Figure 8). ELISA showed the same results (Figure 9).

Association of ZFAS1 with the tumorigenesis of gastric cancer cells in vivo

To further investigate whether ZFAS1 overexpression or knockdown affects tumor growth *in vivo*, BGC823 cells stably transduced with NC-shRNA, ZFAS1-shRNA1 and ZFAS1-shRNA2 were inoculated into 4-wk female athymic BALB/c nude mice. The expression of lncRNA ZFAS1 in nude mice was detected weekly by qRT-PCR. The results showed that the expression of lncRNA ZFAS1 in the three groups of nude mice increased over time. The expression level in the ZFAS1-shRNA1 group was higher

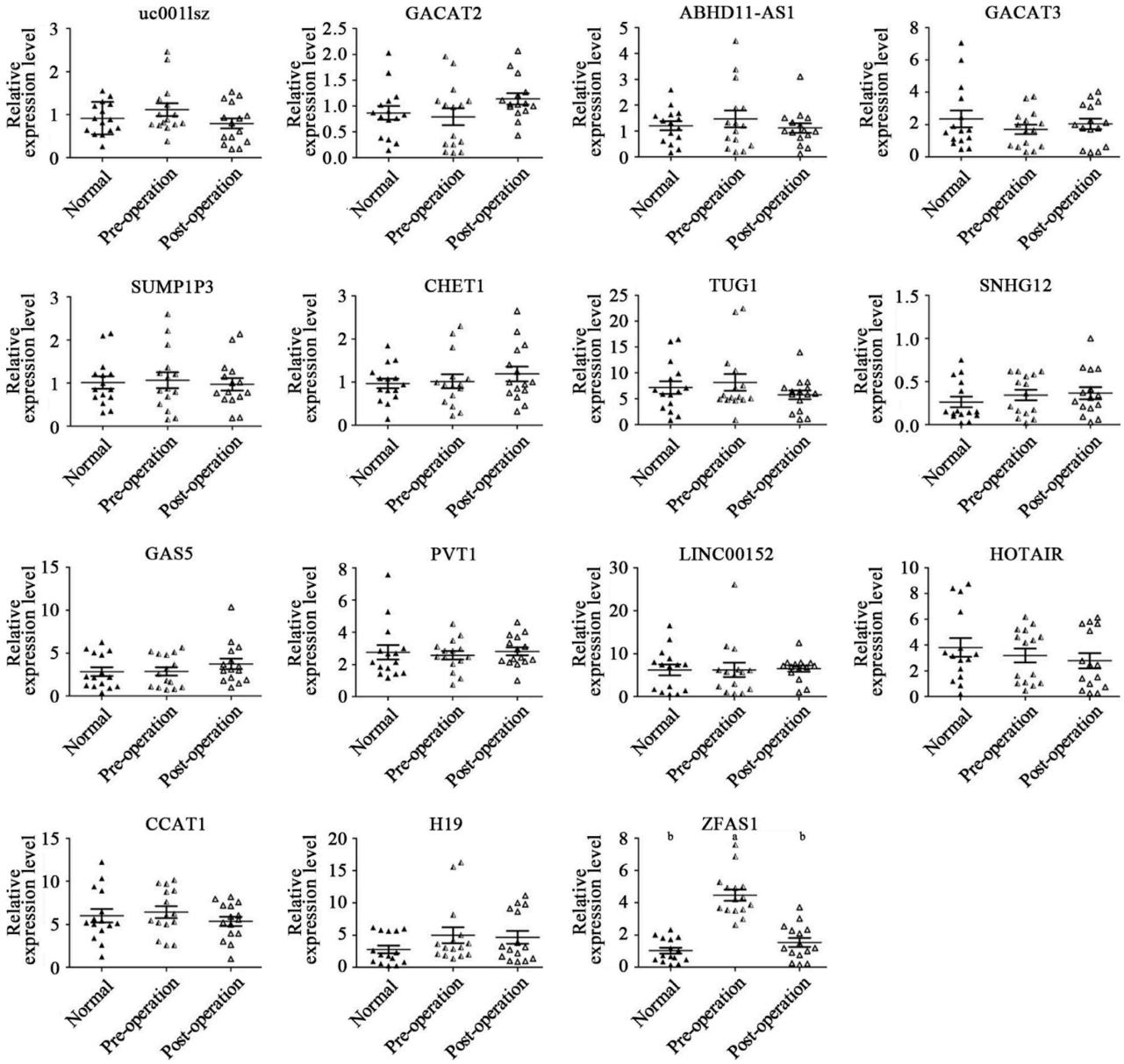

Figure 1 Relative expression levels of a selected subset of plasma long noncoding RNAs in 15 matched preoperative and postoperative gastric cancer patients and 15 healthy control subjects. Scatter plot of long noncoding RNA relative expression levels in the plasma of preoperative patients (preoperative; *n* = 15), postoperative patients (postoperative; *n* = 15), and healthy control subjects (normal; *n* = 15), as assessed by quantitative polymerase chain reaction. The upper and lower bars indicate the ± SD values, and the middle bar indicates the median value. *P* values ($^{a}P < 0.05$; $^{b}P < 0.01$) were determined using the *t*-test.

than that in the control group, and the expression level in the ZFAS1-shRNA2 group was lower than that in the control group (Figure 10A). Images of tumors from nude mice are shown in Figures 10B and C. Tumor size and weight were measured weekly; compared with tumors in the control group, tumors in the ZFAS1-shRNA1 group were significantly larger and heavier, and tumors in the ZFAS1-shRNA2 group were significantly smaller and lighter (Figures 10D and E). Next, in tumor tissue, ELISA confirmed that the expression of LIN28 and CAPRIN1 was significantly increased in tumors in the ZFAS1-shRNA1 group compared with those in the control group ($P < 0.05$), while the expression of LIN28 and CAPRIN1 was significantly decreased in tumors in the ZFAS1-shRNA2 group ($P < 0.05$) (Figure 10F). In addition, ELISA showed significant increases in CEA and CK20 expression in lymph nodes in the ZFAS1-shRNA1 group compared with the control group ($P < 0.05$) and significant decreases in CEA and CK20 expression in lymph nodes in the ZFAS1-shRNA2 group ($P < 0.05$) (Figure 10G), confirming the relationship between lncRNA ZFAS1 expression and lymph node metastasis in gastric cancer cells. In conclusion, our results indicate that ZFAS1 overexpression promotes the growth of gastric cancer cells *in vivo* and that ZFAS1 knockdown inhibits the growth of gastric cancer cells *in vivo*.

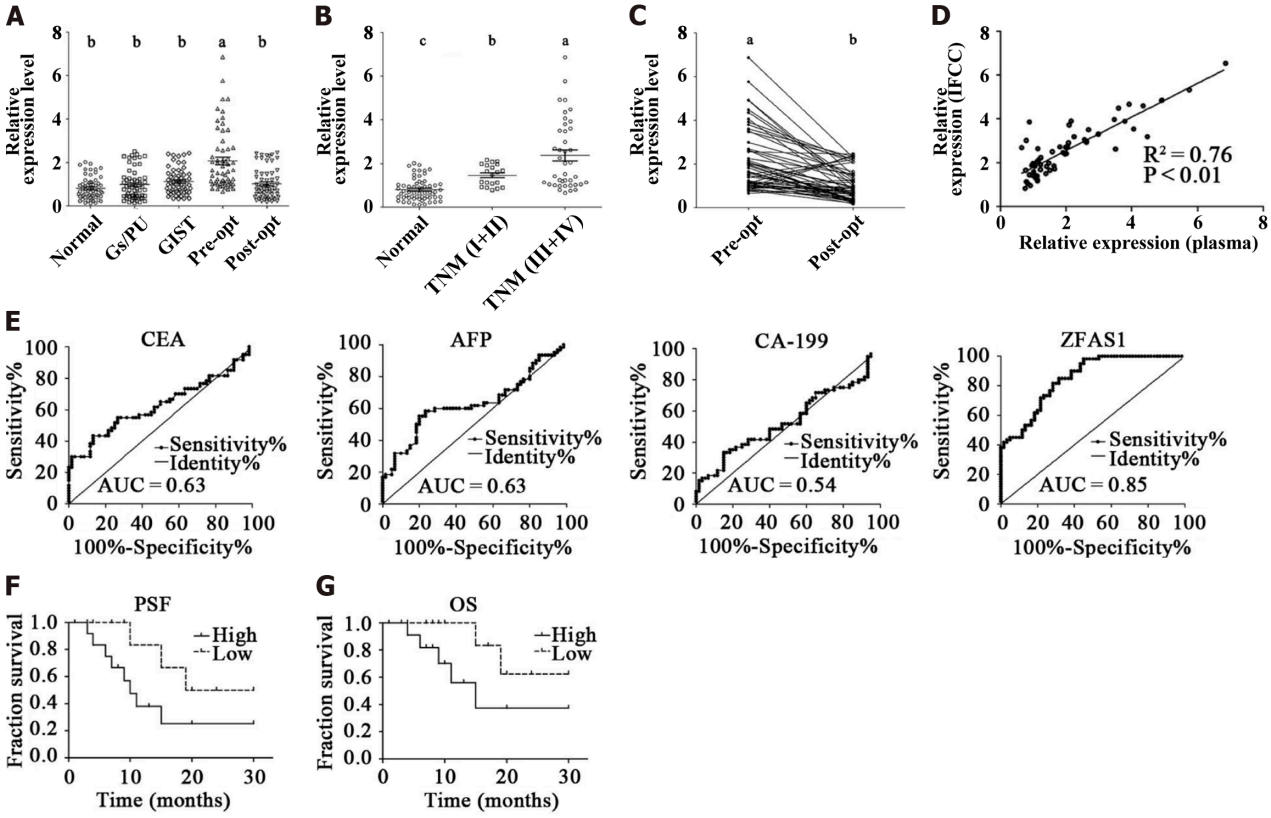

Figure 2 ZNFX1-AS1 is a potential diagnostic and prognostic biomarker. A: Scatter plot of long noncoding RNA (lncRNA) relative expression levels in the plasma of healthy control subjects (normal; *n* = 60), gastritis/peptic ulcer patients (Gs/PU patients; *n* = 60), gastric stromal tumor patients (GIST; *n* = 60), preoperative patients (pre-opt; *n* = 60), and postoperative patients (post-opt; *n* = 60), as assessed by real-time polymerase chain reaction (PCR); B: Scatter plot of lncRNA relative expression levels in the plasma of healthy control subjects (normal; *n* = 60), early-stage patients (TNM I + II; *n* = 20), and advanced-stage patients (TNM III + IV; *n* = 40), as assessed by real-time PCR; C: The endpoints indicate the relative expression levels of lncRNA ZNFX1-AS1 (ZFAS1) in preoperative (pre-opt) and postoperative (post-opt) patient plasma, while the lines connecting the pairs of endpoints indicate the trends in the relative expression levels in the matched preoperative and postoperative patient plasma; D: The linear correlations between the relative expression of lncRNAs in plasma and infrequent clonal complexes were analyzed. *P* values ($^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$) were determined using the *t*-test; E: Receiver operating characteristic curves showing the area under the curve values of plasma ZFAS1 and traditional serum biomarkers; F: Progression-free survival times indicating the potential prognostic value of ZFAS1 in gastric cancer patients; G: Overall survival times indicating the potential prognostic value of ZFAS1 in gastric cancer patients. Gs/PU: Gastritis/peptic ulcer patients; GIST: Gastric stromal tumor; AUC: Area under the curve; CEA: Carcinoembryonic antigen; AFP: Alpha fetoprotein; CA-199: Carbohydrate antigen 199; OS: Overall survival; PFS: Progression-free survival.

DISCUSSION

Gastric cancer is a common malignancy that is particularly difficult to diagnose early[20]. However, traditional biomarkers have low specificity and low sensitivity. As lncRNA research has intensified, many lncRNAs have been determined to be involved in the development, progression, and prognosis of gastric cancer[21], but their diagnostic value as gastric cancer markers needs further study. By reviewing the literature, we selected 15 candidate lncRNAs that have high expression in gastric tumor tissues. Next, the relative levels of these 15 candidate lncRNAs were evaluated in the plasma of 15 normal subjects and 15 matched samples from preoperative and postoperative patients with gastric cancer. The results showed that the plasma level of only ZFAS1 was significantly higher in preoperative gastric cancer patients than in normal subjects and postoperative gastric cancer patients (Figure 1). Therefore, we selected ZFAS1 as a follow-up research object. Moreover, we confirmed that lncRNAs can exist stably in peripheral blood, consistent with the findings of Li *et al*[22]. Therefore, lncRNA ZFAS1 may be a potential plasma biomarker for gastric cancer.

We recollected plasma from 60 healthy control subjects, 60 gastritis/peptic ulcer patients, 60 GIST patients, and 60 matched preoperative and postoperative patients to further validate the aforementioned results. Our data indicated that the expression of ZFAS1 in the peripheral blood of patients with gastric cancer was significantly higher than that of healthy controls and patients with benign gastrointestinal disease (Figure 2A), consistent with previous reports. Our data also showed that ZFAS1 is significantly associated with advanced TNM stage and lymphatic invasion (Figure 2B). There was a sharp decrease in the relative level of ZFAS1 in peripheral blood after surgery (Figure 2C). We also evaluated traditional serological markers such as CEA, CA-199, and alpha fetoprotein in the 60 preoperative patients. Compared with traditional biomarkers, plasma lncRNA ZFAS1 had higher

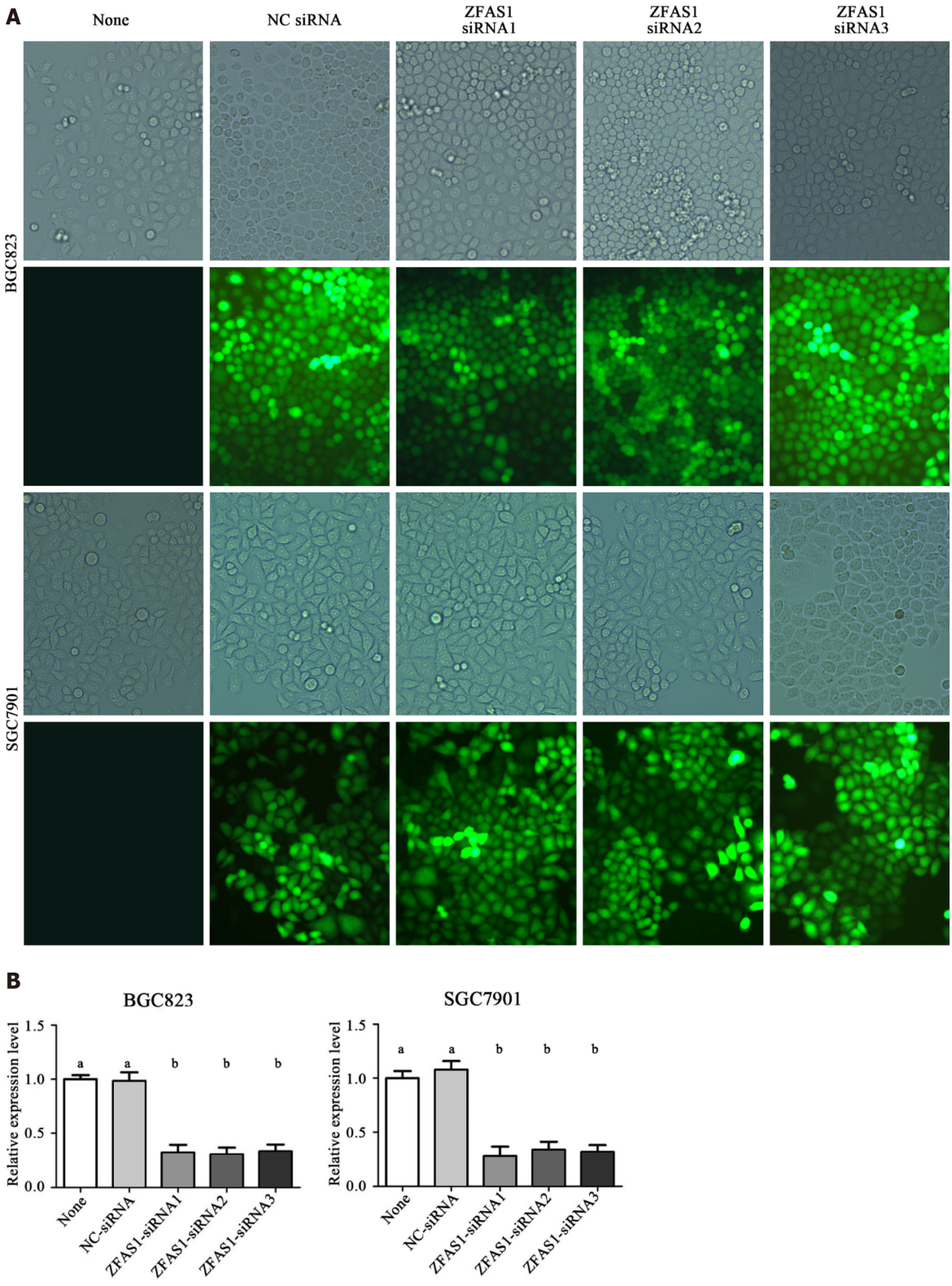

Figure 3 Fluorescence microscopy was used to verify the transfection efficiency of ZNFX1-AS1-siRNA1, ZNFX1-AS1-siRNA2 and ZNFX1-AS1-siRNA3. A: The same position of BGC823 cells and SGC7901 cells under a normal microscope and fluorescence microscope, respectively; B: The relative expression of long noncoding RNA ZNFX1-AS1 (ZFAS1) in five groups of cells (None, NC-siRNA, ZFAS1-siRNA1, ZFAS1-siRNA2, ZFAS1-siRNA3) was evaluated by quantitative reverse transcription polymerase chain reaction. ZFAS1: ZNFX1-AS1. $^{a}P < 0.05$; $^{b}P < 0.01$.

sensitivity and specificity (Figure 2E). In addition, the relative level of ZFAS1 in plasma was significantly correlated with that in IFCCs (Figure 2D). This indicates that lncRNA ZFAS1 is released into the peripheral blood by gastric tumors. This suggests that ZFAS1 may promote the progression of gastric cancer. Both the PFS and OS analysis results showed (Figures 2F and G) that a high level of lncRNAs in peripheral blood was not favorable for patients. Thus, lncRNAs may be used not only for early diagnosis but also for postoperative evaluation of patients.

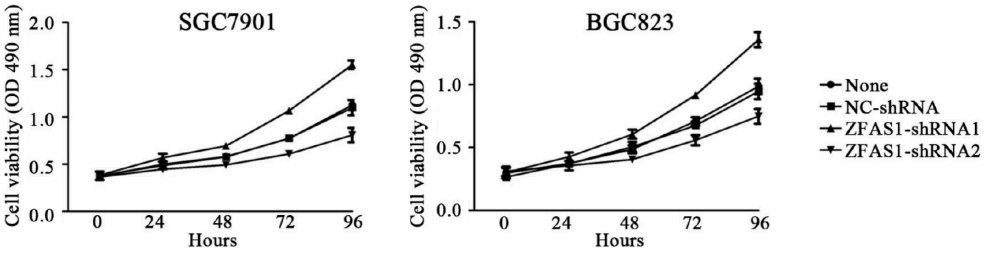

Figure 4 MTT assays were performed to determine the viability of ZGCS1-shRNA1- and ZNFX1-AS1-shRNA2-transduced BGC823 and SGC7901 cells. OD: Optical density.

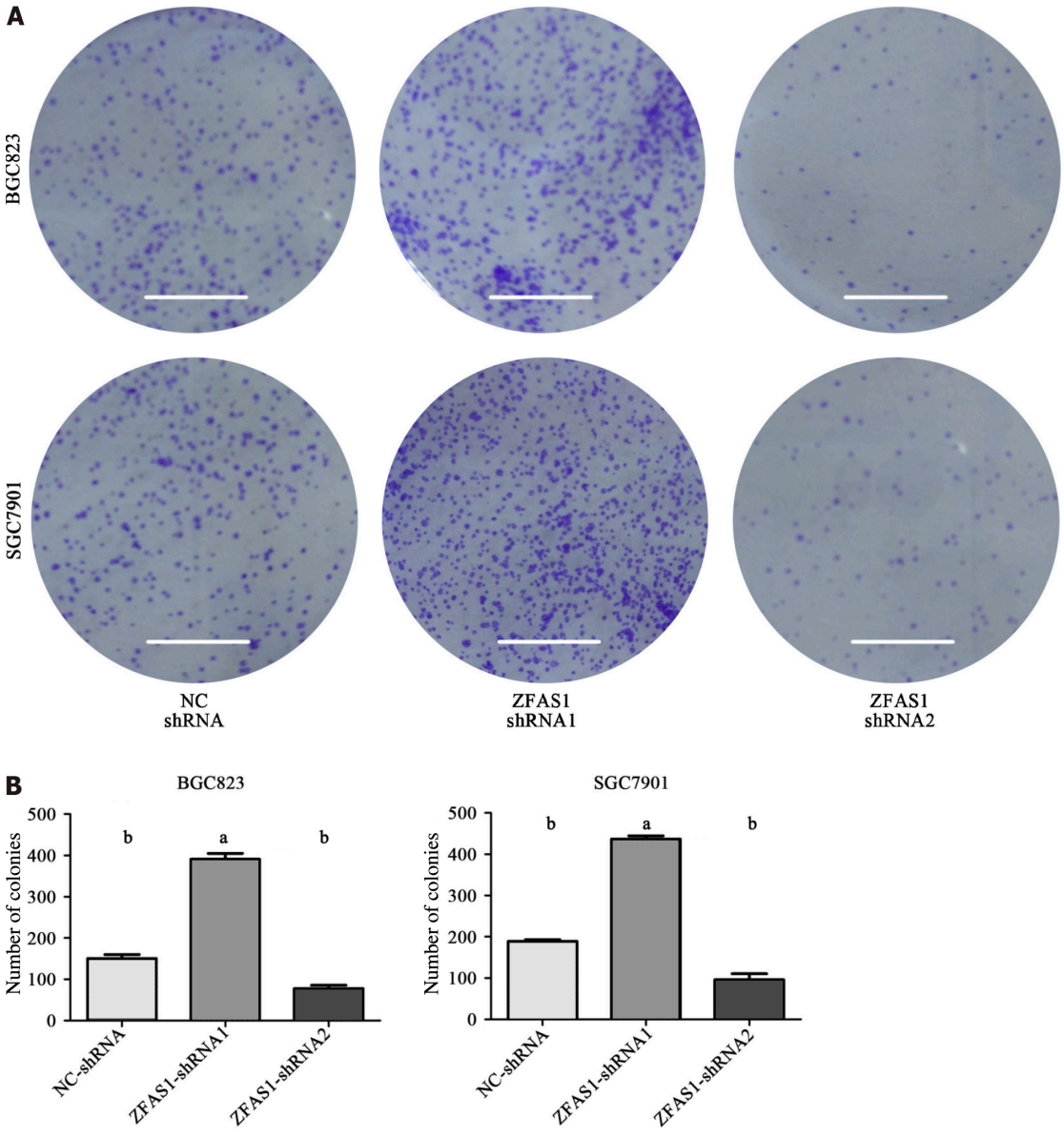

Figure 5 Colony formation assays were performed to determine the proliferation of BGC823 and SGC7901 cells transduced with ZNFX1-AS1-shRNA1 and ZNFX1-AS1-shRNA2. A: BGC823 cells and SGC7901 cells were subjected to colony formation assay after transduction, and the number of spots was analyzed; B: The number of spots for the three groups [NC-shRNA, ZNFX1-AS1 (ZFAS1)-shRNA1, ZFAS1-shRNA2] in the colony formation assays were compared. ZFAS1: ZNFX1-AS1. $^{a}P < 0.05$; $^{b}P < 0.01$.

In addition, ZFAS1 is reported to be involved in metabolism, prognosis, and cell proliferation in liver cancer[23,24]. It has been reported that the expression of ZFAS1 in gastric cancer tissues is significantly higher than that in adjacent tissues and that ZFAS1 expression is related to the prognosis of gastric cancer patients[25,26]. siRNA transfection techniques were used to determine the appropriate lncRNA ZFAS1 interference sequence. We used three types of siRNAs (ZFAS1 siRNA1, ZFAS1 siRNA2, and

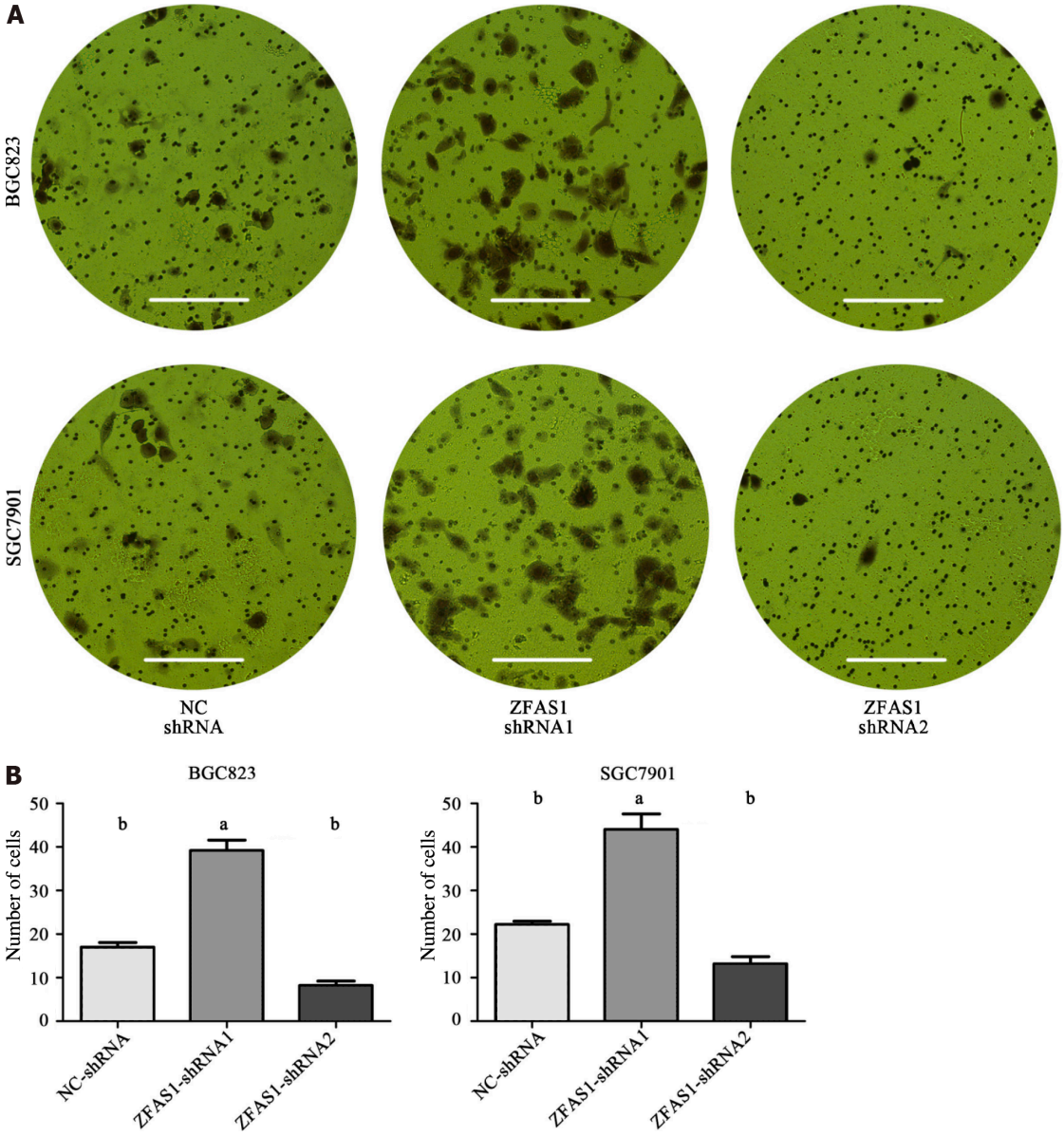

Figure 6 Transwell migration assays were performed to determine the migration ability of ZNFX1-AS1-shRNA1- and ZNFX1-AS1-shRNA2-transduced BGC823 and SGC7901 cells. A: BGC823 cells and SGC7901 cells were subjected to transwell migration assay after transduction, and the number of cell was calculated; B: The number of cells for the three groups [NC-shRNA, ZNFX1-AS1 (ZFAS1)-shRNA1, ZFAS1-shRNA2] in the transwell migration assays were compared. $^{a}P < 0.05$; $^{b}P < 0.01$. ZFAS1: ZNFX1-AS1.

ZFAS1 siRNA3) to interfere with the expression of ZFAS1 in cell lines to prevent off-target effects. All these siRNAs significantly reduced the expression of lncRNA ZFAS1 in BGC823 cells and SGC7901 cells (Figure 3). We finally selected the ZFAS1 siRNA2 sequence to construct ZFAS1-shRNA2 and successfully generated BGC823 cells and SGC7901 cells with low expression of lncRNA ZFAS1. BGC823 cells and SGC7901 cells with stable knockdown of lncRNA ZFAS1 were also successfully cultured. We further employed loss-of-function and gain-of-function studies to assess the role of ZFAS1 in gastric cancer cell proliferation and migration by MTT, transwell, and colony formation assays (Figures 4, 5 and 6). We observed that ZFAS1 knockdown inhibited gastric cancer cell proliferation and migration, whereas ZFAS1 overexpression had the opposite effects.

We then studied the mechanism by which ZFAS1 affects the proliferation and invasion of gastric cancer cells. The starBase V2.0 database was used to analyze the lncRNA ZFAS1 binding proteins[19]. Only 10 proteins had more than three binding sites for lncRNA ZFAS1. We found that the changes in the mRNA expression of LIN28 and CAPRIN1 were consistent with the change in ZFAS1 expression in gastric cancer cells (Figure 8). The LIN28 protein is thought to play an important role in gastrointestinal tumors[27,28]. In colorectal cancer, LIN28 has been shown to enhance tumor cell invasion *via* the Wnt pathway, whereas overexpression of LIN28 also recruits the microRNA let-7 to enhance tumor cell metastasis[28]. In gastric cancer, LIN28 affects the human epidermal growth factor receptor 2 level through posttranscriptional regulation, which in turn affects the invasive ability of gastric cancer cells.

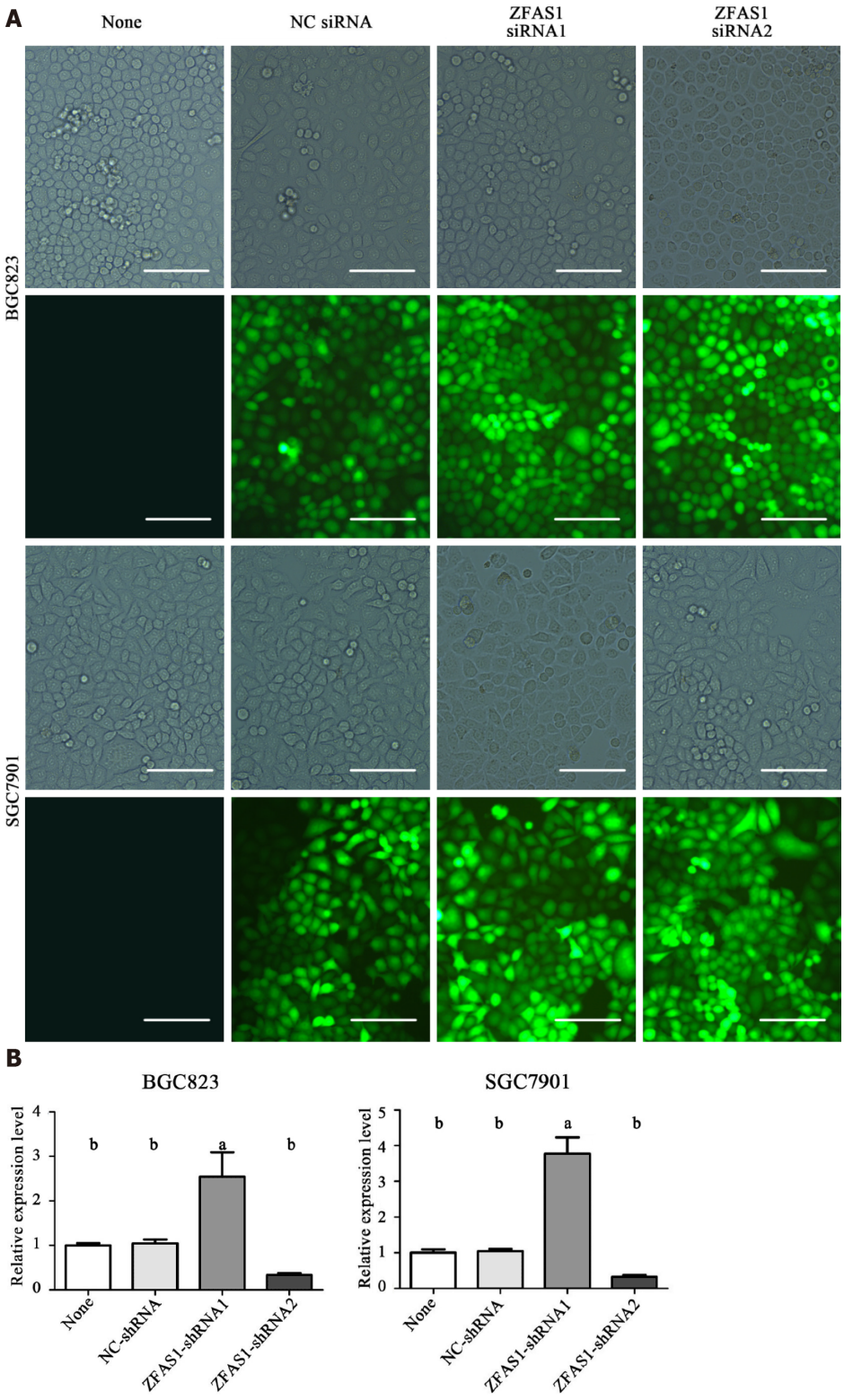

Figure 7 Fluorescence microscopy was used to test the transduction efficiency of ZNFX1-AS1-shRNA1 and ZNFX1-AS1-shRNA2. A: The same position of BGC823 cells and SGC7901 cells under a normal microscope and fluorescence microscope, respectively; B: The relative expression of long noncoding RNA ZNFX1-AS1 (ZFAS1) in four groups of cells (None, NC-shRNA, ZFAS1-shRNA1, ZFAS1-shRNA2) was evaluated by quantitative reverse transcription polymerase chain reaction. ZFAS1: ZNFX1-AS1. $^{a}P < 0.05$; $^{b}P < 0.01$.

In addition, LIN28 is an independent risk factor for the prognosis of gastric cancer. This result suggests that LIN28 may be a key protein downstream of lncRNA ZFAS1 and that lncRNA ZFAS1 may enhance the proliferation and invasion of gastric cancer cells by regulating LIN28. CAPRIN1 is thought to be involved in cell invasion in a variety of tumors[29-31]. In osteosarcoma, CAPRIN1 has been shown to cause cisplatin resistance and abnormal apoptosis of tumor cells *via* the Akt pathway and the ERK1/2 pathway[31], and it can also be used as an independent risk predictor for breast cancer. The changes in

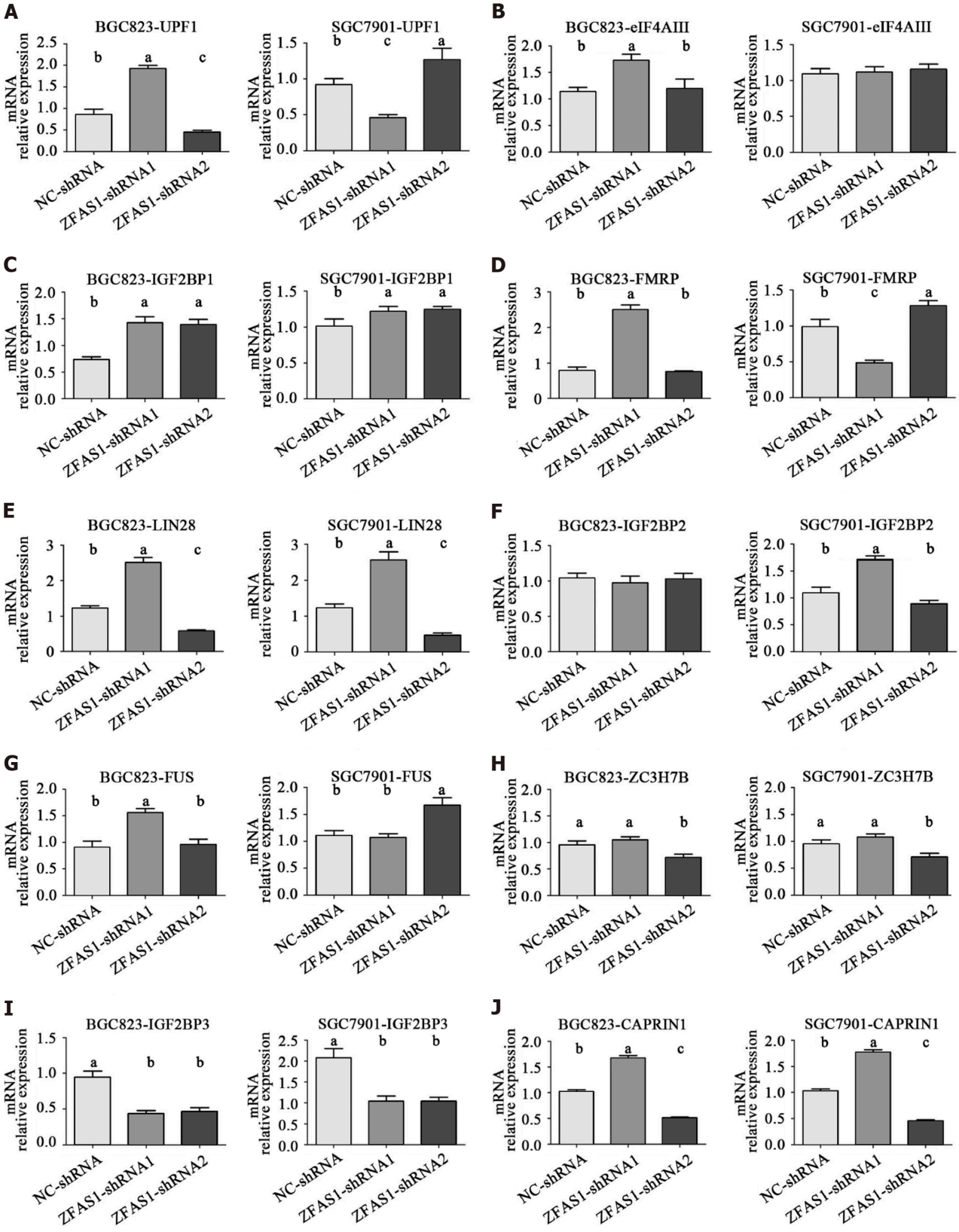

Figure 8 The relative mRNA levels of genes encoding ZNFX1-AS1 binding proteins in transduced BGC823 and SGC7901 cells were determined by quantitative reverse transcription polymerase chain reaction. A: UFP1; B: eIF4AIII; C: IGF2BP1; D: FMRP; E: LIN28; F: IGF2BP2; G: FUS; H: ZC3H7B; I: IGF2BP3; J: CAPRIN1. AFAS1: ZNFX1-AS1. $^{a}P < 0.05$; $^{b}P < 0.01$.

the mRNA expression of LIN28 and CAPRIN1 were consistent with knockdown and overexpression of ZFAS1, whereas LIN28 and CAPRIN1 were significantly associated with tumor invasion, which explains the mechanism connecting ZFAS1 with lymphatic invasion in tumor patients. ZFAS1 has been shown to silence the expression of Kruppel-like factor 2 and naked cuticle homolog 2 by binding to

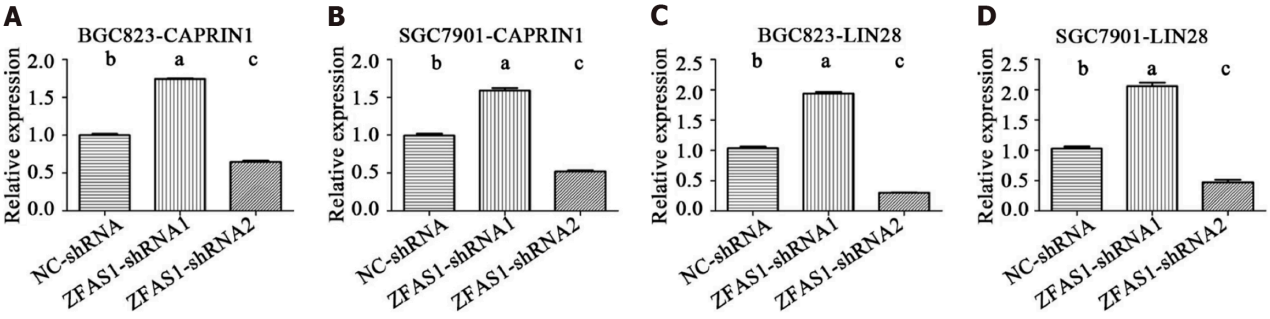

Figure 9 The relative protein levels of LIN28 and CAPRIN1 in transduced BGC823 and SGC7901 cells were determined by enzyme-linked immunosorbent assay. A: The levels of CAPRIN1 in transduced BGC823 cells; B: The levels of CAPRIN1 in transduced SGC7901 cells; C: The levels of LIN28 in transduced BGC823 cells; D: The levels of LIN28 in transduced SGC7901 cells. $^{a}P < 0.05$; $^{b}P < 0.01$.

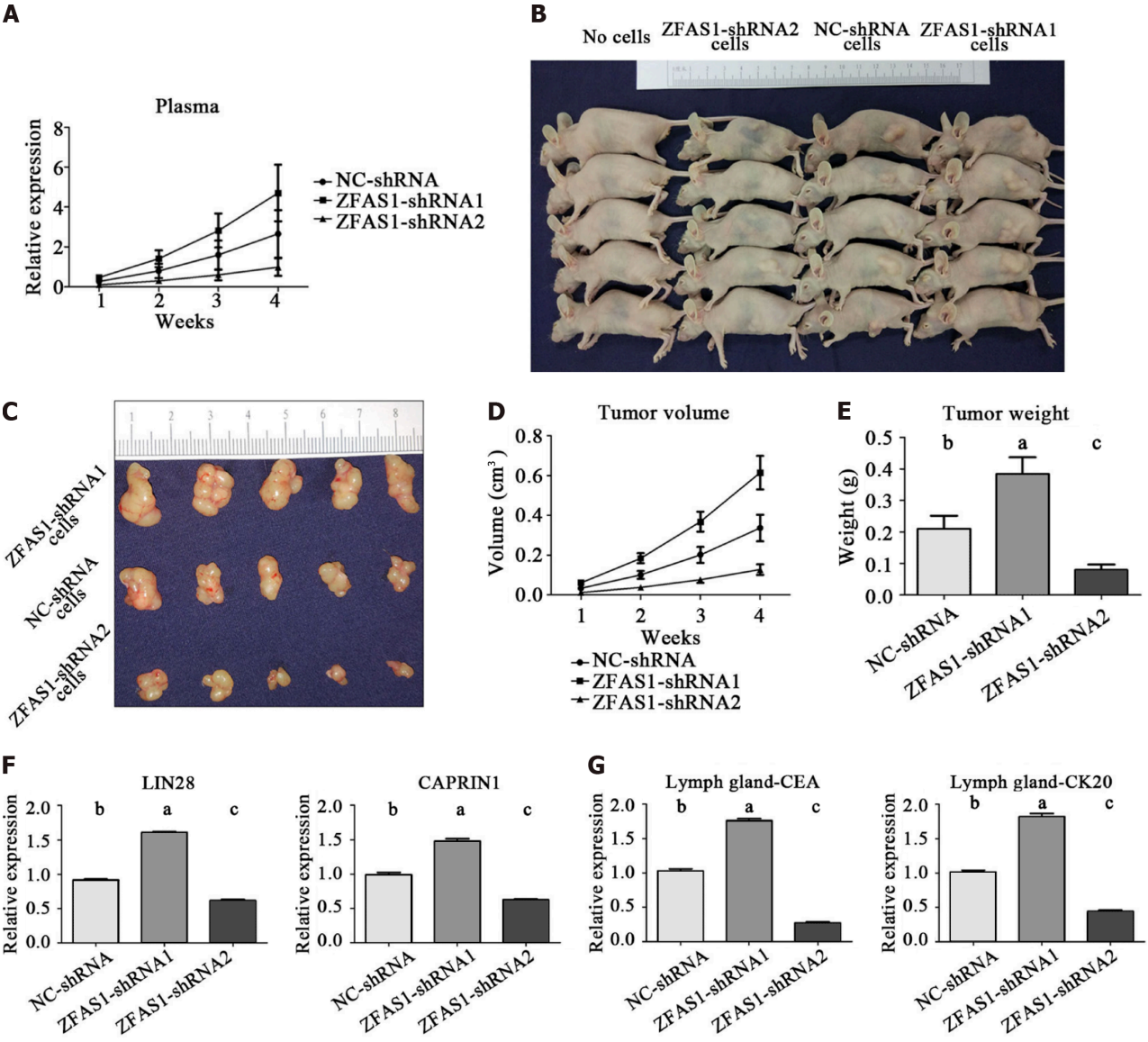

Figure 10 Effect of ZNFX1-AS1 on gastric cancer cell tumorigenesis *in vivo*. A: The expression of long noncoding RNA ZNFX1-AS1 in BALB/c nude mice was detected weekly by quantitative reverse transcription polymerase chain reaction; B and C: Images of tumors from BALB/c nude mice; D and E: Tumor size and weight were measured weekly; F: Expression of LIN28 and CAPRIN1 in tumors of BALB/c nude mice; G: Expression of carcinoembryonic antigen and CK20 in lymph nodes of BALB/c nude mice. CEA: Carcinoembryonic antigen; ZFAS1: ZNFX1-AS1. $^{a}P < 0.05$; $^{b}P < 0.01$; $^{c}P < 0.001$.

polycomb repressive complex 2 and lysine-specific demethylase 1, leading to the development of gastric cancer[25]. In addition, it promotes the development of liver cancer tumor cells[23]. CAPRIN1 may play a key role in the regulation of tumor cell proliferation and invasion *via* lncRNA ZFAS1.

In this study, we validated the above hypothesis by establishing tumor-bearing mice. The expression level of lncRNA ZFAS1 in the plasma of mice injected with tumor cells with high expression of lncRNA ZFAS1 was significantly higher than that in mice in the other two groups (Figure 10A). The *in vivo* experiments also indicated that ZFAS1 overexpression promoted gastric cancer cell proliferation and migration, whereas ZFAS1 knockdown had the opposite effect (Figures 10B-E). The data also demonstrated that LIN28 and CAPRIN1 are still regulated by lncRNA ZFAS1 in mice (Figure 10F). CK20 and CEA are classical biomarkers of gastric cancer cells in tissues. The CEA and CK20 levels in the lymph nodes of nude mice injected with tumor cells with high expression of lncRNA ZFAS1 were significantly higher than those in mice in the other two groups (Figure 10G). This further demonstrated that high expression of lncRNA ZFAS1 may enhance the invasion of gastric cancer cells.

CONCLUSION

In conclusion, we demonstrated in this study that the lncRNA ZFAS1 level is increased in the plasma of gastric cancer patients. LncRNA ZFAS1 promotes the invasion and proliferation of gastric cancer cells by modulating LIN28 and CAPRIN1 expression. These findings indicate that ZFAS1 plays an oncogenic role in gastric cancer and can be used as a potential diagnostic biomarker and a new therapeutic target for gastric cancer.

ARTICLE HIGHLIGHTS

Research background

Long noncoding RNA (lncRNA) ZNFX1-AS1 (ZFAS1) is a newly discovered lncRNA, but its diagnostic value in gastric cancer is unclear.

Research motivation

We investigated the biological effects of ZFAS1 on the survival, proliferation, and migration of gastric cancer cells.

Research objectives

This study aimed to investigate the potential role of ZFAS1 in gastric cancer and to evaluate the clinical significance of ZFAS1 as a biomarker for gastric cancer screening.

Research methods

RNA extraction, quantitative realtime polymerase chain reaction, lncRNA silencing and overexpression, MTT assay, transwell migration assay, Colony formation assay, protein extraction, immunosorbent assay, and *in vivo* tumor formation assay were performed in this study.

Research results

ZFAS1 amplification was correlated with poor prognosis in gastric cancer. ZFAS1 knockdown inhibited the viability, migration, and proliferation of gastric cancer cells. ZFAS1 overexpression enhanced the viability, migration, and proliferation of gastric cancer cells. LIN28 and CAPRIN1 were the key downstream mediators of ZFAS1 in gastric cancer cells. ZFAS1 was associated with the tumorigenesis of gastric cancer cells *in vivo*.

Research conclusions

LncRNA ZFAS1 is a potential biomarker for gastric cancer.

Research perspectives

ZFAS1 plays an oncogenic role in gastric cancer and can be used as a potential diagnostic biomarker and a new therapeutic target for gastric cancer.

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FOOTNOTES

Author contributions: Zhuo ZL analyzed the experimental data and completed the draft of the manuscript; Xian HP completed all of the experiments; Sun YJ, Long Y, and Liu C collected all of the clinical data; Liang B and Zhao XT are responsible for designing the work and for final approval of the version to be published.

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Institutional review board statement: This study was approved by the Research Ethics Committee of Peking University People's Hospital. Patient data and samples were treated according to the ethical and legal criteria adopted in the 2013 Declaration of Helsinki. Written informed consent for ethical approval and patient consent was obtained from all participants.

Institutional animal care and use committee statement: This study was approved by the Peking University People's Hospital Animal Use Protocol & Ethic Review.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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REFERENCES

1 **Bray F**, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018; **68**: 394-424 [PMID: 30207593 DOI: 10.3322/caac.21492]
2 **Riquelme I**, Ili C, Roa JC, Brebi P. Long non-coding RNAs in gastric cancer: mechanisms and potential applications. *Oncotarget* 2016 [DOI: 10.18632/oncotarget.9396]
3 **Sun J**, Song Y, Chen X, Zhao J, Gao P, Huang X, Xu H, Wang Z. Novel long non-coding RNA RP11-119F7.4 as a potential biomarker for the development and progression of gastric cancer. *Oncol Lett* 2015; **10**: 115-120 [PMID: 26170986 DOI: 10.3892/ol.2015.3186]
4 **Vance KW**, Sansom SN, Lee S, Chalei V, Kong L, Cooper SE, Oliver PL, Ponting CP. The long non-coding RNA Paupar regulates the expression of both local and distal genes. *EMBO J* 2014; **33**: 296-311 [PMID: 24488179 DOI: 10.1002/embj.201386225]
5 **Wapinski O**, Chang HY. Long noncoding RNAs and human disease. *Trends Cell Biol* 2011; **21**: 354-361 [DOI: 10.1016/j.tcb.2011.04.001]
6 **Qi P**, Xu MD, Ni SJ, Huang D, Wei P, Tan C, Zhou XY, Du X. Low expression of LOC285194 is associated with poor prognosis in colorectal cancer. *J Transl Med* 2013; **11**: 122 [PMID: 23680400 DOI: 10.1186/1479-5876-11-122]
7 **Mei D**, Song H, Wang K, Lou Y, Sun W, Liu Z, Ding X, Guo J. Up-regulation of SUMO1 pseudogene 3 (SUMO1P3) in gastric cancer and its clinical association. *Med Oncol* 2013; **30**: 709 [PMID: 23996296 DOI: 10.1007/s12032-013-0709-2]
8 **Yang F**, Bi J, Xue X, Zheng L, Zhi K, Hua J, Fang G. Up-regulated long non-coding RNA H19 contributes to proliferation of gastric cancer cells. *FEBS J* 2012; **279**: 3159-3165 [PMID: 22776265 DOI: 10.1111/j.1742-4658.2012.08694.x]
9 **Sun W**, Wu Y, Yu X, Liu Y, Song H, Xia T, Xiao B, Guo J. Decreased expression of long noncoding RNA AC096655.1-002 in gastric cancer and its clinical significance. *Tumour Biol* 2013; **34**: 2697-2701 [PMID: 23645148 DOI: 10.1007/s13277-013-0821-0]
10 **Xiao B**, Guo J. Long noncoding RNA AC096655.1-002 has been officially named as gastric cancer-associated transcript 1, GACAT1. *Tumour Biol* 2013; **34**: 3271 [PMID: 23754450 DOI: 10.1007/s13277-013-0916-7]
11 **Song H**, Sun W, Ye G, Ding X, Liu Z, Zhang S, Xia T, Xiao B, Xi Y, Guo J. Long non-coding RNA expression profile in human gastric cancer and its clinical significances. *J Transl Med* 2013; **11**: 225 [PMID: 24063685 DOI:

10.1186/1479-5876-11-225]
12 **Song B**, Guan Z, Liu F, Sun D, Wang K, Qu H. Long non-coding RNA HOTAIR promotes HLA-G expression *via* inhibiting miR-152 in gastric cancer cells. *Biochem Biophys Res Commun* 2015; **464**: 807-813 [PMID: 26187665 DOI: 10.1016/j.bbrc.2015.07.040]
13 **Arita T**, Ichikawa D, Konishi H, Komatsu S, Shiozaki A, Shoda K, Kawaguchi T, Hirajima S, Nagata H, Kubota T, Fujiwara H, Okamoto K, Otsuji E. Circulating long non-coding RNAs in plasma of patients with gastric cancer. *Anticancer Res* 2013; **33**: 3185-3193 [PMID: 23898077]
14 **Levy MH**, Back A, Benedetti C, Billings JA, Block S, Boston B, Bruera E, Dy S, Eberle C, Foley KM, Karver SB, Knight SJ, Misra S, Ritchie CS, Spiegel D, Sutton L, Urba S, Von Roenn JH, Weinstein SM. NCCN clinical practice guidelines in oncology: palliative care. *J Natl Compr Canc Netw* 2009; **7**: 436-473 [PMID: 19406043 DOI: 10.6004/jnccn.2009.0031]
15 **Nagtegaal ID**, Odze RD, Klimstra D, Paradis V, Rugge M, Schirmacher P, Washington KM, Carneiro F, Cree IA; WHO Classification of Tumours Editorial Board. The 2019 WHO classification of tumours of the digestive system. *Histopathology* 2020; **76**: 182-188 [PMID: 31433515 DOI: 10.1111/his.13975]
16 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]
17 **Xian HP**, Zhuo ZL, Sun YJ, Liang B, Zhao XT. Circulating long non-coding RNAs HULC and ZNFX1-AS1 are potential biomarkers in patients with gastric cancer. *Oncol Lett* 2018; **16**: 4689-4698 [PMID: 30197680 DOI: 10.3892/ol.2018.9199]
18 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]
19 **Li JH**, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* 2014; **42**: D92-D97 [PMID: 24297251 DOI: 10.1093/nar/gkt1248]
20 **Ferlay J**, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**: E359-E386 [PMID: 25220842 DOI: 10.1002/ijc.29210]
21 **Shao Y**, Ye M, Jiang X, Sun W, Ding X, Liu Z, Ye G, Zhang X, Xiao B, Guo J. Gastric juice long noncoding RNA used as a tumor marker for screening gastric cancer. *Cancer* 2014; **120**: 3320-3328 [PMID: 24986041 DOI: 10.1002/cncr.28882]
22 **Li Q**, Shao Y, Zhang X, Zheng T, Miao M, Qin L, Wang B, Ye G, Xiao B, Guo J. Plasma long noncoding RNA protected by exosomes as a potential stable biomarker for gastric cancer. *Tumour Biol* 2015; **36**: 2007-2012 [PMID: 25391424 DOI: 10.1007/s13277-014-2807-y]
23 **Li T**, Xie J, Shen C, Cheng D, Shi Y, Wu Z, Deng X, Chen H, Shen B, Peng C, Li H, Zhan Q, Zhu Z. Amplification of Long Noncoding RNA ZFAS1 Promotes Metastasis in Hepatocellular Carcinoma. *Cancer Res* 2015; **75**: 3181-3191 [PMID: 26069248 DOI: 10.1158/0008-5472.CAN-14-3721]
24 **Askarian-Amiri ME**, Crawford J, French JD, Smart CE, Smith MA, Clark MB, Ru K, Mercer TR, Thompson ER, Lakhani SR, Vargas AC, Campbell IG, Brown MA, Dinger ME, Mattick JS. SNORD-host RNA Zfas1 is a regulator of mammary development and a potential marker for breast cancer. *RNA* 2011; **17**: 878-891 [PMID: 21460236 DOI: 10.1261/rna.2528811]
25 **Nie F**, Yu X, Huang M, Wang Y, Xie M, Ma H, Wang Z, De W, Sun M. Long noncoding RNA ZFAS1 promotes gastric cancer cells proliferation by epigenetically repressing KLF2 and NKD2 expression. *Oncotarget* 2017; **8**: 38227-38238 [PMID: 27246976 DOI: 10.18632/oncotarget.9611]
26 **Pan L**, Liang W, Fu M, Huang ZH, Li X, Zhang W, Zhang P, Qian H, Jiang PC, Xu WR, Zhang X. Exosomes-mediated transfer of long noncoding RNA ZFAS1 promotes gastric cancer progression. *J Cancer Res Clin Oncol* 2017; **143**: 991-1004 [PMID: 28285404 DOI: 10.1007/s00432-017-2361-2]
27 **Wang Q**, Zhou J, Guo J, Teng R, Shen J, Huang Y, Xie S, Wei Q, Zhao W, Chen W, Yuan X, Chen Y, Wang L. Lin28 promotes Her2 expression and Lin28/Her2 predicts poorer survival in gastric cancer. *Tumour Biol* 2014; **35**: 11513-11521 [PMID: 25128063 DOI: 10.1007/s13277-014-2481-0]
28 **Tu HC**, Schwitalla S, Qian Z, LaPier GS, Yermalovich A, Ku YC, Chen SC, Viswanathan SR, Zhu H, Nishihara R, Inamura K, Kim SA, Morikawa T, Mima K, Sukawa Y, Yang J, Meredith G, Fuchs CS, Ogino S, Daley GQ. LIN28 cooperates with WNT signaling to drive invasive intestinal and colorectal adenocarcinoma in mice and humans. *Genes Dev* 2015; **29**: 1074-1086 [PMID: 25956904 DOI: 10.1101/gad.256693.114]
29 **Bidet K**, Dadlani D, Garcia-Blanco MA. G3BP1, G3BP2 and CAPRIN1 are required for translation of interferon stimulated mRNAs and are targeted by a dengue virus non-coding RNA. *PLoS Pathog* 2014; **10**: e1004242 [PMID: 24992036 DOI: 10.1371/journal.ppat.1004242]
30 **Gong B**, Hu H, Chen J, Cao S, Yu J, Xue J, Chen F, Cai Y, He H, Zhang L. Caprin-1 is a novel microRNA-223 target for regulating the proliferation and invasion of human breast cancer cells. *Biomed Pharmacother* 2013; **67**: 629-636 [PMID: 23953883 DOI: 10.1016/j.biopha.2013.06.006]
31 **Sabile AA**, Arlt MJ, Muff R, Husmann K, Hess D, Bertz J, Langsam B, Aemisegger C, Ziegler U, Born W, Fuchs B. Caprin-1, a novel Cyr61-interacting protein, promotes osteosarcoma tumor growth and lung metastasis in mice. *Biochim Biophys Acta* 2013; **1832**: 1173-1182 [PMID: 23528710 DOI: 10.1016/j.bbadis.2013.03.014]

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ORIGINAL ARTICLE

Basic Study

Oxidized low-density lipoprotein stimulates CD206 positive macrophages upregulating CD44 and CD133 expression in colorectal cancer with high-fat diet

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Abstract

BACKGROUND

Oxidized low-density lipoprotein (ox-LDL), which is abnormally increased in the serum of colorectal cancer (CRC) patients consuming a high-fat diet (HFD), may be one of the risk factors for the development of CRC. Ox-LDL exerts a regulatory effect on macrophages and may influence CRC through the tumor microenvironment. The role of ox-LDL in CRC remains unclear.

AIM

To investigate the role of ox-LDL through macrophages in HFD associated CRC.

METHODS

The expression of ox-LDL and CD206 was detected in colorectal tissues of CRC patients with hyperlipidemia and HFD-fed mice by immunofluorescence. We stimulated the macrophages with 20 μg/mL ox-LDL and assessed the expression levels of CD206 and the cytokines by cell fluorescence and quantitative polymerase chain reaction. We further knocked down LOX-1, the surface receptor of

ox-LDL, to confirm the function of ox-LDL in macrophages. Then, LoVo cells were co-cultured with the stimulated macrophages to analyze the CD44 and CD133 expression by western blot.

RESULTS

The expression of ox-LDL and the CD206 was significantly increased in the stroma of colorectal tissues of CRC patients with hyperlipidemia, and also upregulated in the HFD-fed mice. Moreover, an increased level of CD206 and decreased level of inducible nitric oxide synthase were observed in macrophages after ox-LDL continuous stimulation. Such effects were inhibited when the surface receptor LOX-1 was knocked down in macrophages. Ox-LDL could induce CD206+ macrophages, which resulted in high expression of CD44 and CD133 in co-cultured LoVo cells.

CONCLUSION

Ox-LDL stimulates CD206+ macrophages to upregulate CD44 and CD133 expression in HFD related CRC.

Key Words: Oxidized low-density lipoprotein; CD206 positive macrophages; CD44; CD133

Core Tip: Obesity increases the risk of colorectal cancer (CRC), but the mechanism remains unknown. CD206+ macrophages promote CRC. It has been established that the prevalence of CRC was higher in people consuming a high-fat diet (HFD) and HFD fed mice with up-regulated CD206+ macrophages levels in colorectal tissue. Oxidized low-density lipoprotein (ox-LDL) is a lipid peroxide which has been found to be increased in serum of CRC patients. Importantly, ox-LDL exerts a regulatory effect on macrophages and may regulate CRC through the tumor microenvironment. Our study showed that ox-LDL stimulates CD206+ macrophages to up-regulate CD44 and CD133 expression in HFD associated CRC.

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INTRODUCTION

Obesity is a widespread health condition. There is ample evidence to suggest that obesity increases the risk of colorectal cancer (CRC)[1]. A prospective cohort study of 85256 women found that obese individuals had a 1.93 times higher risk of CRC by age 50 than normal-weight individuals[2]. Excessive intake of dietary fat, *e.g.*, a high-fat diet (HFD), is the main cause of obesity and one of the important reasons for the increased incidence of CRC[3]. However, the specific mechanism of CRC remains unknown. It is currently believed that HFD promotes intestinal cancer by increasing the number and malignant potential of intestinal stem cells[4,5].

Interestingly, it has been shown that HFD could activate systemic inflammation and increase the malignant potential of intestinal tumors by upregulating the expression of macrophages[6,7]. It has been established that macrophages exhibit multiple phenotypes, like those positive for CD206, CD163, or inducible nitric oxide synthase (iNOS)[8]. Importantly, some types of macrophages promote cell repair and proliferation, like CD206+ macrophages[9]. An increasing body of evidence suggests that CD206+ macrophages in the tumor microenvironment promote CRC development, and a positive correlation has been documented between the level of CD206+ macrophages and the degree of tumor malignancy[10]. Moreover, metastasis can be promoted through the interactions of CD206+ macrophages with CRC cells [11]. Liu *et al*[7] found that the prevalence of CRC was higher in people on a HFD with upregulated CD206+ macrophage levels in colon tissue. Moreover, when mice with intestinal microflora disorder were fed an HFD, it was found that CD206+ macrophages in colon tissues correlated with the number of colon tumors and the degree of malignancy, suggesting that the increased CD206+ macrophage level induced by an HFD could exert a significant promoting effect on CRC.

HFD can cause low-grade inflammation and oxidative stress in the whole body, leading to increased lipid levels such as cholesterol and low-density lipoprotein[6]. Oxidized low-density lipoprotein (ox-LDL) is a lipid peroxide produced under oxidative stress that can be used to assess oxidative stress and lipid metabolism in the body. Several studies on obese people have found that the serum ox-LDL level in CRC patients was higher than that in the control group with a healthy intestinal tract[12-14].

Importantly, ox-LDL exerts a regulatory effect on macrophages and may regulate CRC through the tumor microenvironment[15]. However, the mechanisms underlying macrophage regulation in CRC by ox-LDL remain unclear. In addition, the regulatory role of macrophages on tumors may be related to tumor stem cells. In this regard, Yang *et al*[16] found that CD44 levels were gradually increased in lung cancer tissue with an increase in the level of CD206+ macrophages. Lv *et al*[17] also found that CD133 levels were upregulated in thyroid cancer with the increasing CD206+ macrophages. CD206+ macrophages share a similar relationship with CD44 and CD133 in CRC. In this study, the expression and corresponding effects of ox-LDL in colorectal tissue from hyperlipidemia patients were studied to explore the regulatory effects of ox-LDL on macrophages and the tumor stem cell markers CD44 and CD133 in the colorectal stroma. Our findings will provide a new mechanism of increased CRC susceptibility with HFD.

MATERIALS AND METHODS

Patient samples

Colonoscopy was performed on hyperlipidemia patients with CRC, with no prior radiotherapy, chemotherapy, or surgery. Healthy colorectal tissues were collected from volunteers who underwent colonoscopy in the physical examination. CRC (cancer, n = 16, male:female = 10:6) and normal colorectal tissues (normal, n = 20, male:female = 11:9) were collected. The average age of all patients was 57 ± 6 years old. All tissue samples were examined by experienced pathologists. All the CRC tissues that we collected were adenocarcinoma. After sampling, tissue samples were fixed in 4% paraformaldehyde. All patients were treated at the Guangdong Provincial People's Hospital, and the tissue samples were collected by the same endoscopist. The human study was approved by the Ethics Committee of Guangdong Provincial People's Hospital. Informed consent was obtained from all patients before the beginning of the study.

Animal model

Six C57/BL6 mice aged 4 wk assigned to an HFD group were fed an HFD (#H10141, China) for a total of 12 wk. Another six C57/BL6 mice aged 4 wk were assigned to a control group and fed a normal diet (normal grade, #02, China) for the same duration. After 12 wk, the mice were sacrificed for colorectal tissue harvesting. All tissues were immediately transferred to 4% paraformaldehyde. Animal experiments were also approved by the Ethics Committee of Guangdong Provincial People's Hospital.

Specimen processing

After the above clinical and animal specimens were fixed at room temperature for 24 h, tissue sections were prepared as follows. The tissues were first dehydrated and then embedded with paraffin. The paraffin-embedded tissues were cut into 3 μm-thick sections and cross-sections of the intestinal tissue were observed.

Animal histological analysis

Three- micron-thick mice tissue slices were cut and dried in a 65 °C oven for 120 min. The slices were stained with hematoxylin and eosin, dehydrated, and cleared. Finally, the slices were dried and sealed with neutral gum. All samples were examined by experienced pathologists.

Immunofluorescence staining

The specimens were dried in a 65 °C oven for 2 h for dewaxing and dehydration, and then soaked in deionized water for 5 min. The tissue specimens were soaked in sodium citrate solution overnight in a 60 °C water bath to expose the antigen. The sections were immersed in phosphate buffer solution (PBS) and then incubated with 10% goat serum at 37 °C for 1 h for antigen blocking. The slices were incubated at 4 °C for 12 h with the corresponding primary antibody, rewarmed at room temperature, and washed with PBS. The cells were incubated with 100 μL/well working solution containing Alexa Fluor 594-conjugated goat anti-rabbit secondary antibody at room temperature for 1 h in the dark. 4,6-diamino-2-phenylindole (DAPI; Thermo Fisher Science, United States) was used for nuclear counterstaining. The stained slides were imaged using an inverted fluorescence microscope (magnification, × 400; Olympus Corporation).

For calculation of the rate of ox-LDL positive cells in immunofluorescence (IF) staining, three fields were randomly selected from each section to observe the stroma of colorectal tissue under a 400 × microscope. For iNOS-F4/80 or CD206-F4/80 double staining, the number of iNOS, CD206, and F4/80 positive cells and total cells was counted, and the rate of iNOS and F/480, or CD206 and F4/80 positive cells was calculated. For ox-LDL-CD206 or LOX-1-CD206 double staining, the number of positive cells and total cells was counted, and their rate was calculated. The mean value of the rates from three fields was the positive cell rate of each section. All the analyses were double-blind and graded by two or more observers.

Cell experiments

Cell lines and culture: The human colorectal adenocarcinoma cell line (LoVo) and mouse monocyte-macrophage leukemia cell line (RAW 264.7) were purchased from the American Center for Typical Culture Preservation (ATCC, United States). The human monocytic leukemia cell line was purchased from Wuhan Penoside Company (THP-1, #CL-0233, China). The maintenance medium for cell culture was Dulbecco modified Eagle medium (DMEM, Gibco) containing glucose (4.5 g/L), 10% fetal bovine serum, 100 U/mL penicillin, and 100 mg/mL streptomycin. All cells were cultured under standard cell culture conditions of 37 °C, 5% CO_2, and 95% humidity.

Lipoprotein induction and RNA extraction: THP-1 cells were inoculated in 6-well plates, and 50 μg/mL ox-LDL was added 24 h and 72 h after sample collection. The confluence of cells in both groups was maintained at 60%-70% at the beginning of treatment, while the control group did not receive any treatment. The samples were centrifuged (800 rpm, 3 min) and washed with sterile PBS, and re-centrifuged (800 rpm, 3 min). Then, total RNA was extracted from tissues or cells using TRIzol reagent, according to the manufacturer's instructions. The RNA samples were stored at -80 °C.

Cell fluorescence: RAW264.7 cells were inoculated in 12-well plates and treated with 50 μg/mL ox-LDL for 72 h when the cell confluence reached 60%-70%, while the control group did not receive any treatment. After 72 h, the culture medium was discarded, and the cells were treated with 4% paraformaldehyde for 15 min. The cells were washed with PBS, blocked with 10% sheep serum at 37 °C for 1 h, and incubated with PBS solution dissolved in 1% BSA and 0.1% TritonX-100 followed by primary antibody [rabbit anti-CD206 antibody (1:100, #18704-1-AP, United States)] at 4 °C for 12 h. The cells were then washed with PBS, and incubated with the AlexaFluor594 conjugated secondary antibody (1:500) at room temperature in the dark for 1 h. The cells were washed with PBS, one drop of DAPI was added to each well, and images were obtained under the corresponding fluorescence channel using an inverted fluorescence microscope. The method used to calculate the IF-positive cell rate was the same as above.

Co-culture and protein extraction: LoVo cells and THP-1 cells were inoculated in the lower and upper chambers of 12-well transwell plates, respectively. The cell density was 60%-70%, and cells were incubated with ox-LDL (#S24879, China) for 72 h. There were no THP-1 cells in the upper compartment of the culture plate in the control group, and the other conditions were the same as those for the treatment group. The culture medium was discarded 72 h later, and cells were gently moistened with PBS. Total protein of LoVo cells in each group was extracted with RIPA lysis buffer on ice. Protein concentration was detected by the BCA method, and protein samples were stored at -80 °C.

Quantitative polymerase chain reaction

RNA concentration was determined first, and the samples were diluted to ensure that the concentrations were consistent. The RNA was reverse-transcribed to cDNA in a 20 μL system, and the total RNA content was kept below 1000 ng. Then, the cDNA was used as the template for quantitative polymerase chain reaction (qPCR) amplification. ABI 7300 real-time PCR software was used to analyze the PCR results and detect the Ct value of the sample. *GAPDH* was used as the internal reference gene, and the relative quantitative analysis was carried out by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used are shown in Table 1.

LOX-1 small interfering RNA transfection

Small interfering RNA (siRNA) was used to inhibit the expression of LOX-1 in macrophages. LOX-1 siRNA or siRNA negative control (GenePharma, GenePharma, Suzhou, China) was introduced into THP-1 cells *via* Liposome 3000 (3 μL/mL) for 24 h. The sequence of LOX-1 siRNA used is shown in Table 2.

Western blot analysis

Protein samples (10 μg) were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a PVDF membrane. The membrane was then blocked with 5% skim milk. One hour later, anti-CD44 antibody (1:5000, # AB189524, United Kingdom) and anti-GAPDH antibody (1:10000, # AB9458, United Kingdom) were added. After incubating with the primary antibody, the membrane was washed with TBST. The samples were then incubated with horseradish peroxidase-labeled secondary antibody (1:3000) for 1 h at room temperature. After washing with TBST, immune reactive bands were analyzed after protein band visualization with enhanced chemiluminescence reagents.

Statistical analysis

Microsoft Excel 2019 worksheet was used to summarize the experimental results and generate tables. Adobe Photoshop 2020 software was used to process the images, and GraphPad Prism 8.0.2 software was used for statistical analyses. The Chi-square test or unpaired *t*-test was performed to compare the difference between two groups. One-way ANOVA test was performed to compare the difference among

Table 1 Forward and reverse primer sequences of *GAPDH*, inducible nitric oxide synthase, *CD206*, and *LOX-1*

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
GAPDH	CTGTTCGACAGTCAGCCGCATC	GCGCCCAATACGACCAAATCCG
INOS	TTCAGTATCACAACCTCAGCAAG	TGGACCTGCAAGTTAAAATCCC
CD206	TGGAGAGGGAAGAGAGTGAACA	GCCCATAAGTGTGCTCTGAA
LOX-1	GTGGCATGGAGAAAACTGTTAC	CATCCAAAGACAAGCACTTCTC

iNOS: Inducible nitric oxide synthase.

Table 2 Sequence of LOX-1 small interfering RNA

si-LOX-1	Forward sequence (5'-3')	Reverse sequence (5'-3')
1	UUUGCUACUCUCUUCAGUGTT	CACUGAAGAGAGUAGCAAATT
2	UUGCUUGCUGGAUGAAGUCTT	GACUUCAUCCAGCAAGCAATT
3	UUUCUGACUCCUGUGAAGCTT	GCUUCACAGGAGUCAGAAATT

more than two groups, and the Dunnett *post-hoc* test was used for multiple comparisons. The data were assessed for normality before conducting the unpaired *t*-test and one-way ANOVA test. A P value < 0.05 was considered statistically significant. All tests were carried out more than three times.

RESULTS

Ox-LDL expression is increased in the stroma of CRC tissue

To explore the distribution and expression of ox-LDL in CRC, we collected tissue samples from CRC patients with hyperlipidemia (n = 16) and normal subjects (n = 20) and performed IF staining for ox-LDL. The results showed that compared with normal colorectal tissues, the expression of ox-LDL was significantly increased in the stroma of CRC tissues (Figures 1A and 1E). It has been reported that LOX-1 is the surface receptor of ox-LDL in macrophages[12]. LOX-1-positive cells were also abundantly detected in CRC tissues (Figures 1C and 1G).

CD206+ macrophages are increased in the stroma of CRC tissue

Double IF for CD206-F4/80 or iNOS-F4/80 showed that the number of CD206+ macrophages increased significantly in the stroma of CRC tissue (Figures 1I-L). Besides, double IF also showed the number of CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells increased abundantly in CRC tissue (Figures 1B, 1D, 1F, and 1H).

Establishment of HFD mouse model

Four-week-old C57 mice were fed an HFD, and mice of the same age were fed a normal diet as controls. After 20 wk, the mice were sacrificed, and colorectal tissue specimens were harvested (n = 6). The colorectal length of control mice and HFD-fed mice was measured, respectively. We found that the colorectal length of HFD mice was shorter (Figures 2A and 2B). Subsequently, the tissue samples were sectioned and stained with hematoxylin and eosin. The staining results showed that glandular nuclei and interstitial cells in the colorectal tissues of HFD mice were slightly enlarged but to a milder degree than those usually observed with intraepithelial neoplasia (Figure 2C).

HFD increases ox-LDL expression and CD206 positive macrophages in the colorectal stroma of mice

After establishing the HFD-fed mouse model, we performed double IF staining for CD206-F4/80 or iNOS-F4/80 in the colorectal tissue sections of the control and HFD-fed mice. We found that the number of CD206 positive macrophages was significantly increased in the colorectal stroma of HDF-fed mice (Figures 3A, 3B, 3E, and 3F). The number of CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells also increased significantly in the colorectal stroma of HDF-fed mice (Figures 3C, 3D, 3G, and 3H).

Continuous ox-LDL stimulation promotes CD206+ macrophages

To explore whether ox-LDL is related to macrophage polarization, we stimulated human monocytic leukemia cells (THP-1) with ox-LDL for 24 h and 72 h *in vitro*. qPCR results showed that the expression level of *CD206* gradually increased with increased stimulation time. However, the expression level of

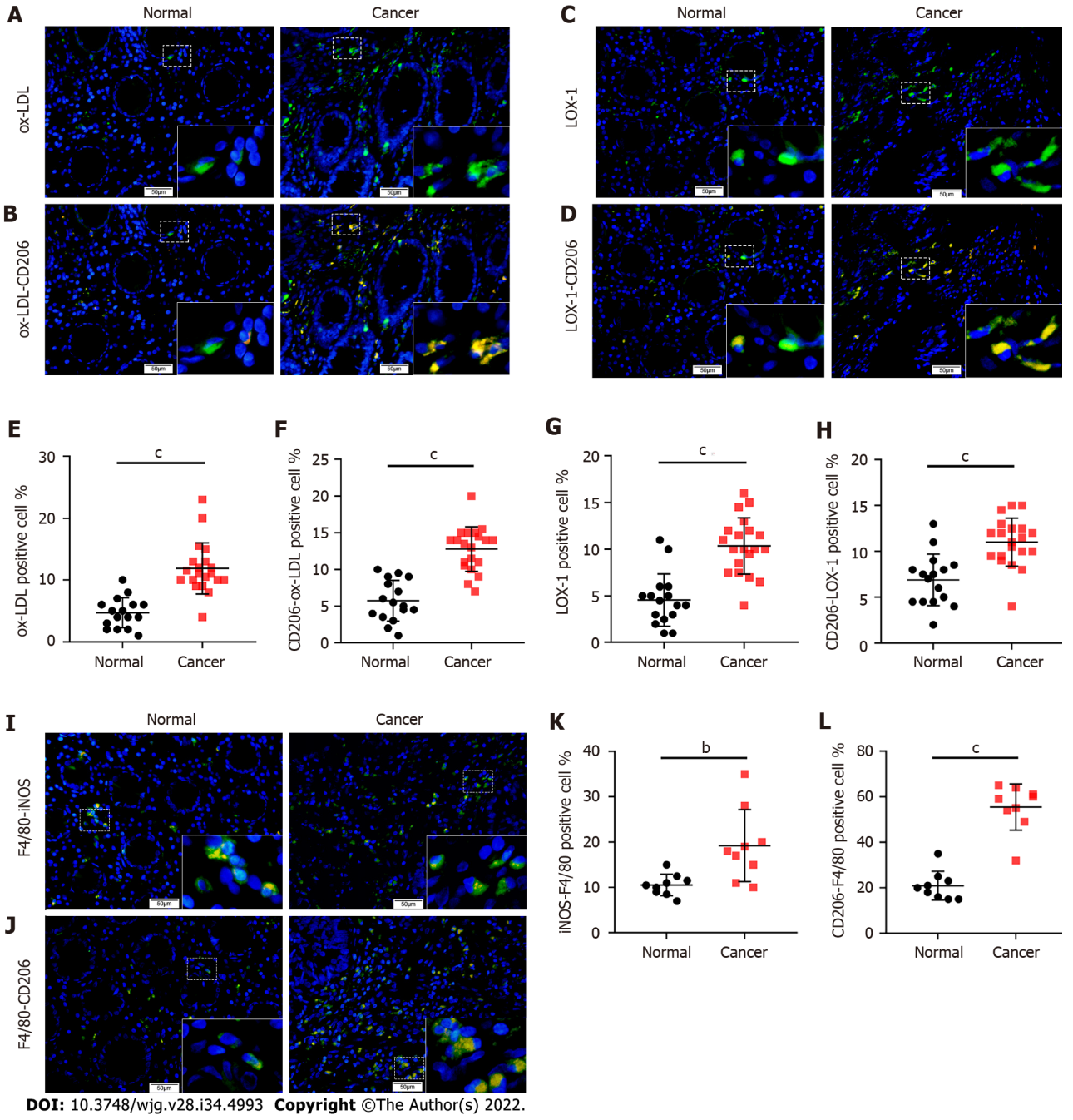

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Figure 1 Increased expression of oxidized low-density lipoprotein and CD206 in the stroma of colorectal cancer tissue. A: Immunofluorescence (IF) images of oxidized low-density lipoprotein (ox-LDL) expression in colorectal tissues (scale bars represent 50 μm); B: Double IF images of CD206-ox-LDL expression in colorectal tissues (scale bars represent 50 μm); C: IF images of LOX-1 expression in colorectal tissues (scale bars represent 50 μm); D: Double IF images of CD206-LOX-1 expression in colorectal tissues (scale bars represent 50 μm); E: Histogram of ox-LDL expression in colorectal tissues based on IF results in Figure 1A; F: Quantification of CD206 and ox-LDL positive cells in colorectal tissues based on IF results in Figure 1B; G: Histogram of LOX-1 expression in colorectal tissues based on IF results in Figure 1C; H: Quantification of CD206 and LOX-1 positive cells in colorectal tissues based on IF results in Figure 1D; I: Double IF images of inducible nitric oxide synthase (iNOS)-F4/80 expression in colorectal tissues (scale bars represent 50 μm); J: Double IF images of CD206-F4/80 expression in colorectal tissues (scale bars represent 50 μm); K: Quantification of iNOS positive macrophages in colorectal tissues based on IF results in Figure 1I; L: Quantification of CD206 positive macrophages in colorectal tissues based on IF results in Figure 1J. Data are shown as the mean ± SD. Statistical analyses were conducted using an unpaired *t*-test. The boxed area is enlarged in the bottom right corner. $^{b}P < 0.01$, $^{c}P < 0.001$. ox-LDL: Oxidized low-density lipoprotein; iNOS: Inducible nitric oxide synthase.

iNOS increased in a short period and then decreased significantly below the initial level (Figure 4A). In addition, ox-LDL was used to stimulate mouse leukemic monocyte/macrophage cell line (RAW 264.7) for 72 h, and IF detection for CD206 was conducted. The results showed that CD206+ macrophages significantly increased in RAW 264.7 cells 3 d after ox-LDL stimulation (Figures 4B and 4C). Overall, we found that CD206+ macrophages gradually increased with continuous stimulation with ox-LDL, while iNOS+ macrophages initially increased then decreased in the later stages.

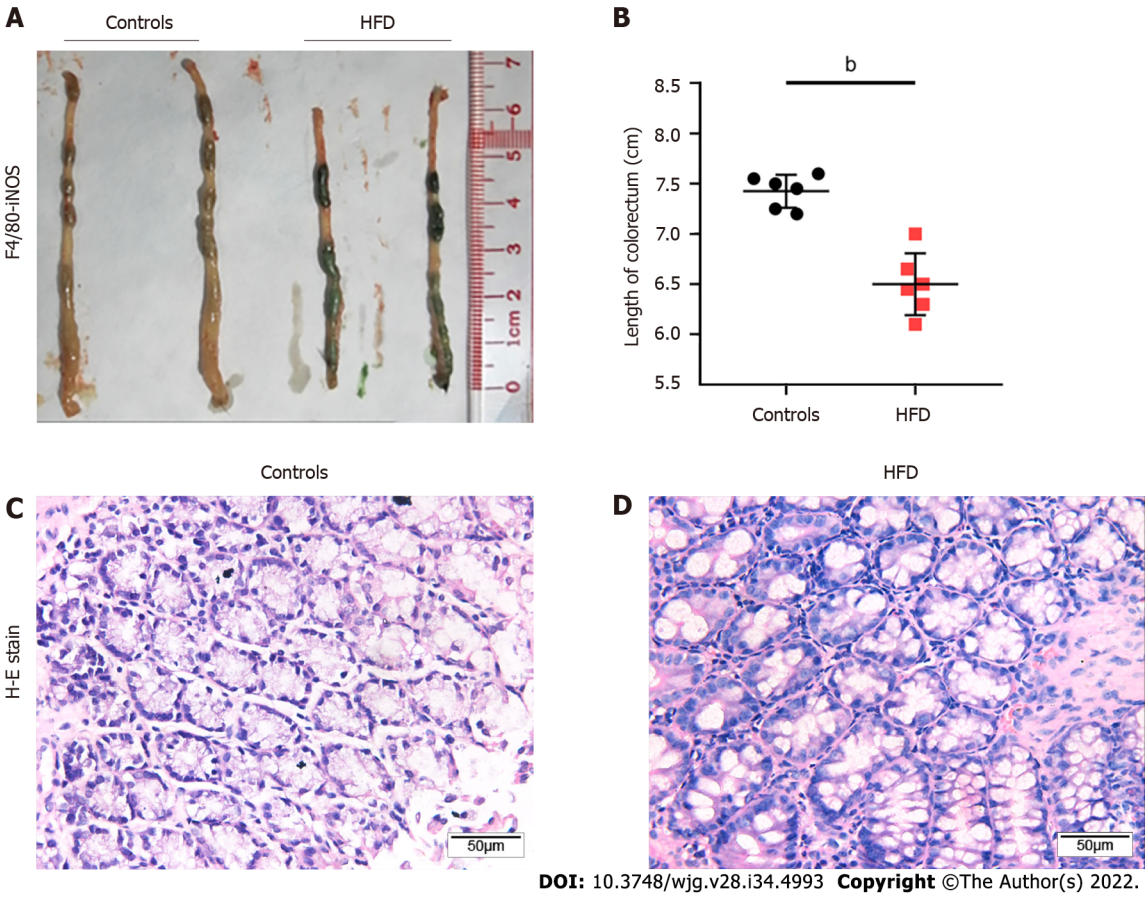

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Figure 2 Establishment of a high-fat diet mouse model. A: Length of colorectal segment of controls and high-fat diet (HFD) mice; B: Quantification of the length of colorectal segment of controls and HFD mice; C: Histopathological colorectal tissue sections of controls and HFD mice (scale bars represent 50 μm). Data are shown as the mean ± SD. Statistical analyses were conducted using an unpaired *t*-test. $^{b}P < 0.01$. HFD: High-fat diet; H-E stain: Hematoxylin-eosin staining; iNOS: Inducible nitric oxide synthase.

In order to confirm the relationship between ox-LDL and CD206+ cells, we transfected LOX-1 siRNA into THP-1 cells to inhibit the expression of LOX-1, the specific receptor of ox-LDL (Figure 4D). After 72 h of ox-LDL stimulation, the inhibition of iNOS expression and the promotion of CD206 expression were significantly weakened in THP-1 cells transfected with LOX-1 siRNA (Figure 4E). Further examination of the function of CD206+ macrophages showed that after 72 h of ox-LDL stimulation, the levels of the CD206+ macrophage-related cytokines interleukin IL-4, IL-10, and tumor necrosis factor TNF-β increased significantly in THP-1 cells except IL-13 (Figure 4F).

Macrophages promote the expression of tumor stem cell markers CD44 and CD133 in an ox-LDL-stimulated high-fat microenvironment

To investigate whether the occurrence of CRC is related to macrophages in the colorectal stroma and a high-fat microenvironment, we simultaneously inoculated human colorectal adenocarcinoma cells (LoVo) and THP-1 cells into transwell culture plates supplemented with 20 μg/mL ox-LDL for 72 h (Figure 5A). Thus, a high-lipid microenvironment was established to stimulate macrophages *in vitro* (n = 5). Western blot analysis showed that CD44 and CD133 expression was significantly increased in LoVo cells co-cultured with THP-1+ ox-LDL compared with ox-LDL alone (Figures 5C and 5D). We further transfected LOX-1 siRNA or siRNA negative control into THP-1 cells, and provided ox-LDL stimulation and co-cultured with LoVo cells again (Figure 5B). After ox-LDL stimulation, the levels of CD44 and CD133 in LoVo cells were inhibited when we knocked down LOX-1 in THP-1 cells (Figures 5E and 5F).

DISCUSSION

An increasing body of evidence suggests that HFD increases the risk of CRC. Importantly, studies have demonstrated the relationship between HFD and cancer by establishing animal models. HFD is often associated with abnormal oxidative stress and elevated lipid levels. Ox-LDL is a metabolite that reflects oxidative stress and lipid metabolism and is associated with various tumors. Ma *et al*[18] hypothesized

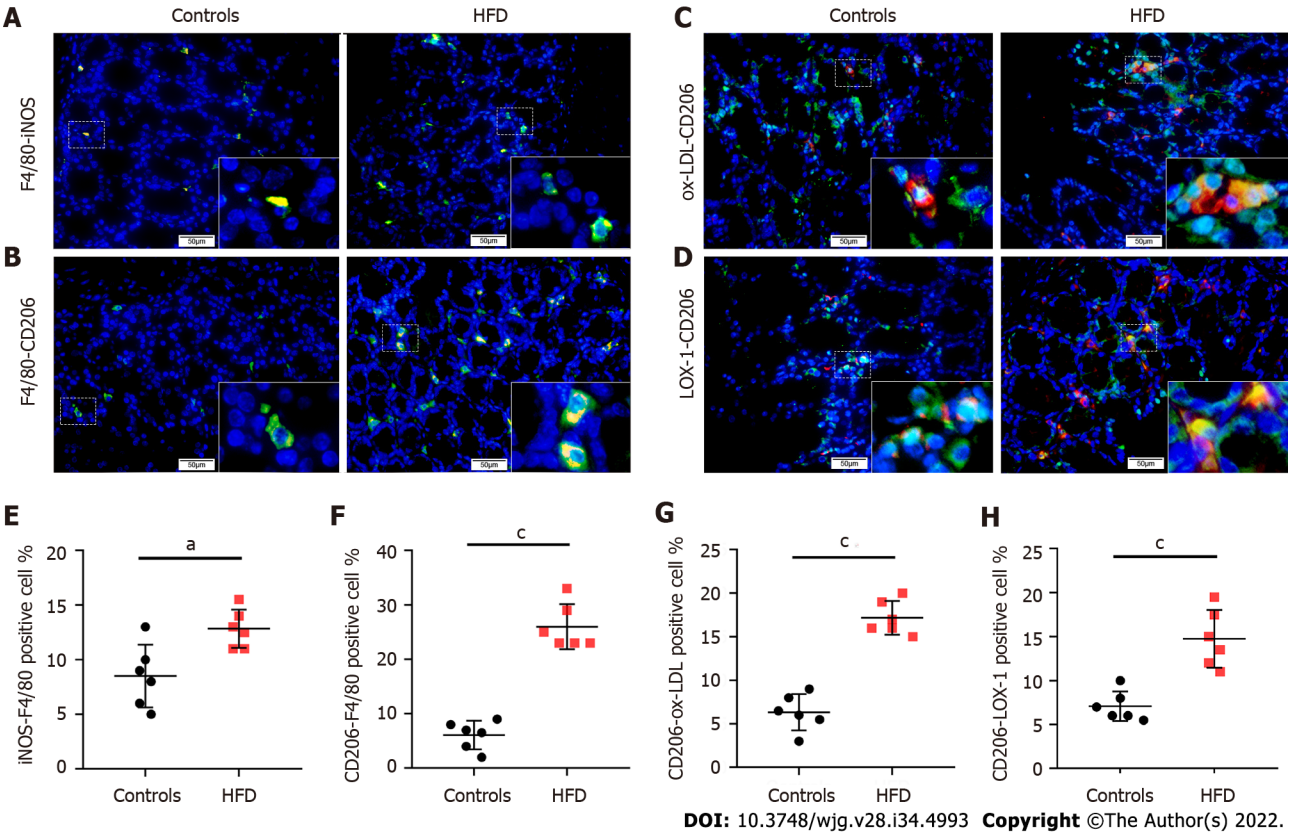

Figure 3 High-fat diet leads to increased expression of oxidized low-density lipoprotein and an increase of CD206 positive macrophages in mouse colorectal stroma. A: Double immunofluorescence (IF) images of inducible nitric oxide synthase (iNOS)-F4/80 expression in colorectal tissues of controls and high-fat diet (HFD)-fed mice (scale bars represent 50 μm); B: Double IF images of inducible nitric oxide synthase CD206-F4/80 expression in colorectal tissues of controls and HFD-fed mice (scale bars represent 50 μm); C: Double IF images of CD206-oxidized low-density lipoprotein (ox-LDL) expression in colorectal tissues of controls and HFD-fed mice (scale bars represent 50 μm); D: Double IF images of CD206-LOX-1 expression in colorectal tissues of controls and HFD-fed mice (scale bars represent 50 μm); E: Quantification of iNOS positive macrophages in colorectal tissues based on IF results in Figure 3A; F: Quantification of CD206 positive macrophages in colorectal tissues based on IF results in Figure 3B; G: Quantification of CD206 and ox-LDL positive cells in colorectal tissues based on IF results in Figure 3C; H: Quantification of CD206 and LOX-1 positive cells in colorectal tissues based on IF results in Figure 3D. Data are shown as the mean ± SD. Statistical analyses were conducted using an unpaired *t* test. The boxed area is enlarged in the bottom right corner. $^{a}P < 0.05$, $^{c}P < 0.001$. ox-LDL: Oxidized low-density lipoprotein; HFD: High-fat diet; iNOS: Inducible nitric oxide synthase.

that ox-LDL could promote gastric cancer metastasis and demonstrated that ox-LDL could promote vascular proliferation and lymphatic metastasis in gastric cancer by activating the nuclear factor-kappa B signaling pathway in animal and cell experiments. Ox-LDL may play a potential role in promoting HFD associated CRC. It has been reported that ox-LDL is correlated with CRC in patients with dyslipidemia, and the serum ox-LDL level of CRC patients is higher than that in subjects without tumors[19]. A study from Egypt found that the serum ox-LDL level of obese colon cancer patients was higher than that in obese patients with a healthy intestine; a positive correlation was found between the serum ox-LDL level and the degree of tumor malignancy[13]. Furthermore, it was found that the serum ox-LDL level of patients after surgery was significantly lower than that before surgery[14]. These findings suggest that ox-LDL is a potential predictor and prognostic biomarker of CRC. However, the above experiments were conducted using blood serum tests of CRC patients. To the best of our knowledge, no study has documented ox-LDL expression in colorectal tissue. Herein, we provided compelling evidence that ox-LDL was abnormally expressed in CRC patients with hyperlipidemia at the tissue level. In this regard, the ox-LDL level was upregulated in the cancer tissue of CRC patients, especially in the stroma. Besides, we demonstrated that LOX-1, the surface receptor of ox-LDL, was also upregulated in CRC tissues. Consistently, increased ox-LDL and LOX-1 levels were documented in the colorectal tissues of HFD-fed mice, suggesting the regulatory role of ox-LDL in the tumor microenvironment in CRC.

Macrophages, the most important immune cells in the tumor microenvironment, exhibit multiple phenotypes and exert various function[8]. It has been shown that iNOS+ macrophages inhibit tumor progression mainly by playing a pro-inflammatory role[20], while CD206+ macrophages promote cell repair and cell proliferation and growth, thus promoting tumor progression[9]. Different macrophages exhibit dynamic changes in the tumor microenvironment, and CD206+ macrophages are closely related to CRC development[21]. Existing clinical studies have reported that the CD206+ macrophage level is

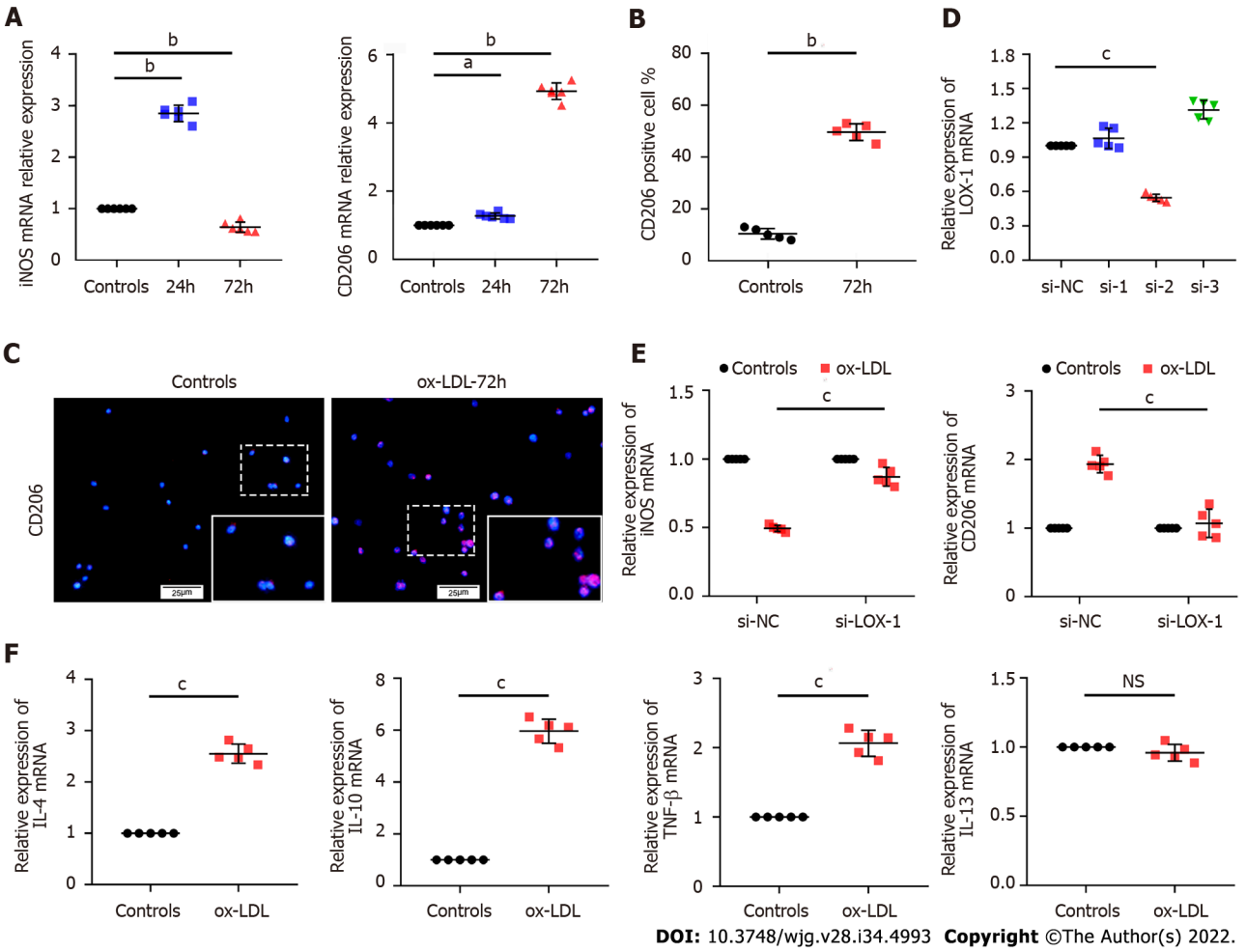

Figure 4 Continuous stimulation with oxidized low-density lipoprotein promotes CD206+ macrophages. A: Quantitative polymerase chain reaction (qPCR) detection of mRNA expression of *CD206* and inducible nitric oxide synthase (*iNOS*) in THP-1 cells after oxidized low-density lipoprotein (ox-LDL) stimulation; B: Quantification of CD206-positive cells in RAW 264.7 cells based on IF results in Figure 4C; C: Cell fluorescence images of CD206 expression in RAW 264.7 cells after ox-LDL stimulation (scale bars represent 25 μm); D: qPCR detection of the expression of *LOX-1* mRNA in THP-1 cells after transfection with *LOX-1* small interfering RNA of different sequences; E: qPCR detection of the expression of *iNOS* and *CD206* mRNA in THP-1 cells with low expression of *LOX-1* stimulated with ox-LDL; F: qPCR detection of the expression of interleukin *IL-4, IL-10, IL-13*, and tumor necrosis factor-β mRNA in THP-1 cells after 72 h of ox-LDL stimulation. Data are shown as the mean ± SD. Statistical analyses were conducted using an unpaired *t* test (Figure 4A, 4D, 4E, and 4F) or one-way analysis of variance followed by Dunnett's multiple comparison test (Figure 4B). The boxed area is enlarged in the bottom right corner. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. ox-LDL: Oxidized low-density lipoprotein; iNOS: Inducible nitric oxide synthase; IL: Interleukin; TNF: Tumor necrosis factor; si-NC: Small interfering RNA negative control.

positively correlated with the TNM stage, the number of metastasized lymph nodes, and the degree of vascular invasion[22], and high CD206+ macrophage expression suggests a poor prognosis. Han *et al* [11] found that CD206+ macrophages in the tumor microenvironment could promote CRC metastasis through interaction with CRC cells. CD206+ macrophages have been reported to be elevated in colorectal tissues in patients with hyperlipidemia. A higher prevalence of CRC was detected in subjects with an HFD in a retrospective cohort study conducted by Liu *et al*[7]. In the present study, CD206+ macrophages were upregulated in colorectal tissues in patients with hyperlipidemia. In addition, animal experiments also confirmed that an increase in the number of intestinal tumors in HFD-fed mice was accompanied by a higher level of CD206+ macrophages, which correlated with the degree of malignancy of the tumor[7]. Our study demonstrated that CD206+ macrophages were highly expressed in the stroma of CRC tissues compared with normal tissues. These results suggest that the elevated level of CD206+ macrophages induced by HFD exerted a stimulatory effect on CRC. We also found that CD206+/ox-LDL+ cells and CD206+/LOX-1+ cells were highly expressed in the colorectal stroma of HDF-fed mice and CRC patients. In addition, studies confirmed that ox-LDL exert a regulatory effect on macrophages, and iNOS+ macrophages increased 24 h after ox-LDL induction *in vitro*[15]. Our study further found that CD206+ macrophages occurred in THP-1 cells under continuous ox-LDL stimulation. A high level of CD206+ macrophages was observed in RAW264.7 cells after ox-LDL stimulation for 72 h. Further examination of the function of CD206+ macrophages showed that after ox-LDL stimulation for 72 h, the levels of CD206+ macrophage-related cytokines IL-4, IL-10, and TNF-β increased significantly in THP-1 cells. Our study demonstrated that following the knockdown of LOX-1, the number of CD206+ macrophages mediated by ox-LDL was significantly depressed. Based on our results, we hypothesized

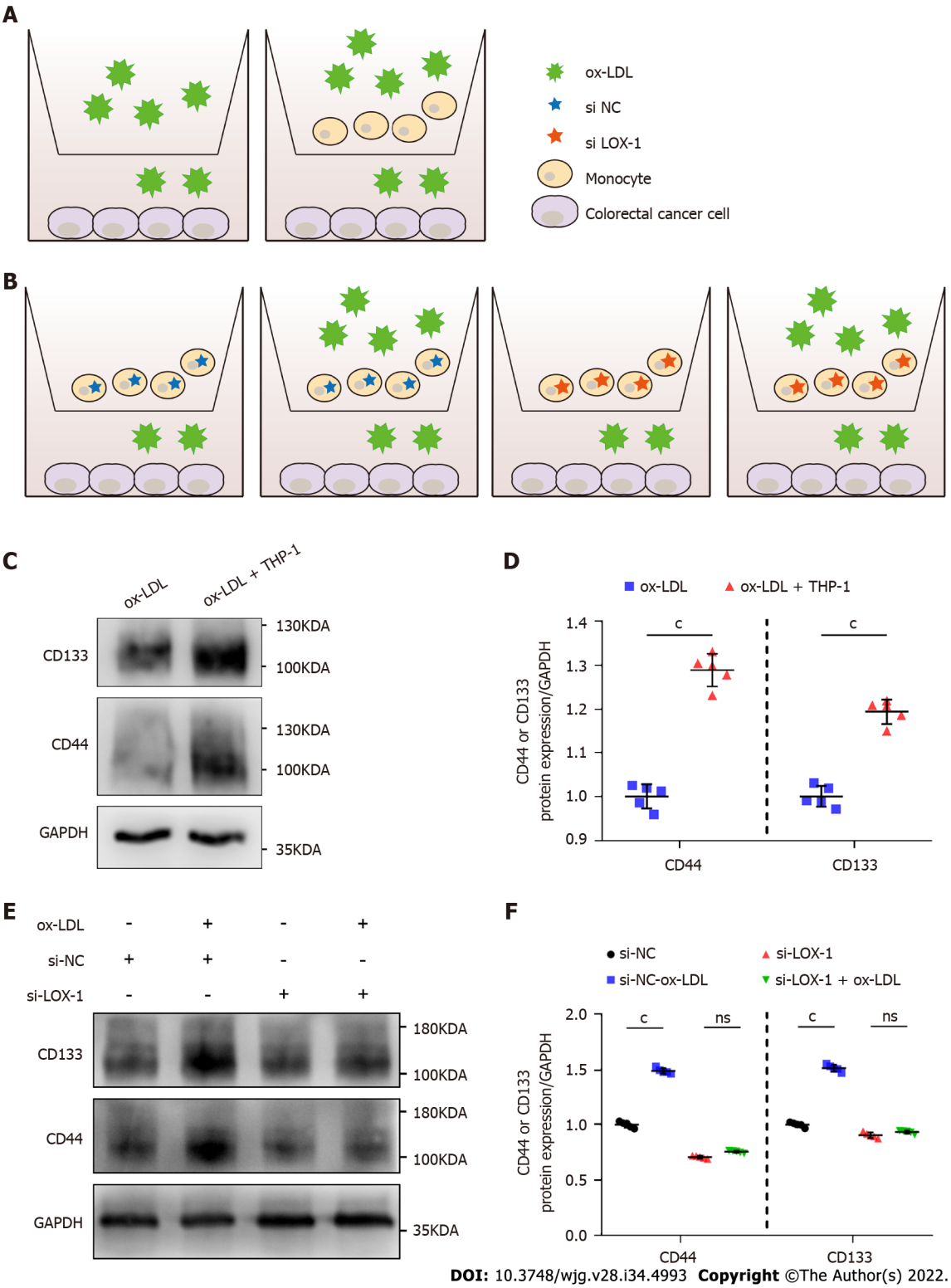

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Figure 5 Macrophages promote the expression of CD44 in LoVo cells in an oxidized low-density lipoprotein-stimulated hyperlipidemic microenvironment. A: Flow diagram of the experimental result in Figure 5C; B: Flow diagram of the experimental result in Figure 5E; C: Protein expression of CD44 and CD133 in LoVo cells after oxidized low-density lipoprotein (ox-LDL) or THP-1 + ox-LDL stimulation detected by western blot; D: Quantification of CD44 and CD133 expression in LoVo cells based on western blot results in Figure 5C; E: Protein expression of CD44 and CD133 in LoVo cells after THP-1 + ox-LDL stimulation detected by western blot. The THP-1 cells were transfected with si-LOX-1 or small interfering RNA negative control; F: Quantification of CD44 and CD133 expression in LoVo cells based on western blot results in Figure 5E. Data are shown as the mean ± SD. Statistical analyses were conducted using an unpaired *t* test. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. ox-LDL: Oxidized low-density lipoprotein; si-NC: Small interfering RNA negative control.

that ox-LDL could promote the progression of CRC by continuous stimulation of macrophages to induce CD206+ macrophages.

In addition, ox-LDL is also associated with tumor stem cells. Yang *et al*[23] found that ox-LDL could increase the malignancy of tumor stem cells in bladder cancer, thus promoting the development of bladder cancer. Active cell proliferation has been documented in gastrointestinal tissue from HFD-fed mice, with increased malignancy of tumor stem cells, which has been attributed to the inflammatory environment in colorectal tissue[4,5,24]. The high level of CD206+ macrophages in HFD-fed mice may play a certain role in this process. After THP-1 cells were stimulated with ox-LDL for 72 h and co-cultured with LoVo cells, the levels of the tumor stem cell markers CD44 and CD133 significantly increased in LoVo cells. Further, when we knocked down LOX-1 in macrophages, the increase in the levels of CD44 and CD133 was not that obvious in CRC cells, confirming that ox-LDL mediated the CD206+ macrophages to upregulate CD44 and CD133 expression in colon cancer cells.

CONCLUSION

In this study, we hypothesized that HFD could induce ox-LDL and its surface receptor LOX-1 accumulation in CRC tissue, suggesting the regulatory role of ox-LDL in the microenvironment of CRC. Furthermore, continuous stimulation of ox-LDL on macrophages induced CD206+ macrophages, which could further promote the increase of CD44 and CD133 levels in CRC cells. However, there are many limitations to our study. First of all, this study was a single-center study, and the sample size of included clinical specimens was relatively small. Larger sample size and multi-center prospective studies are needed to confirm our findings. Moreover, the mechanism underlying the effects of ox-LDL and the relevant signaling pathways were not explored, warranting further studies. In conclusion, we demonstrated that HFD causes ox-LDL accumulation in the colorectal tissue and upregulates CD44 and CD133 expression in colorectal cells by inducing CD206+ macrophages. These findings provide evidence for a new mechanism of increased CRC susceptibility with HFD.

ARTICLE HIGHLIGHTS

Research background

Oxidized low-density lipoprotein (ox-LDL), abnormally increased in the serum of patients with colorectal cancer (CRC) associated with a high-fat diet (HFD), may be one of the risk factors for CRC. Ox-LDL exerts a regulatory effect on macrophages, is associated with cancer stem cells, and may regulate CRC through the tumor microenvironment. The role of ox-LDL in CRC remains unclear. It is essential to explore the function of ox-LDL to explore the pathogenesis of HFD associated CRC.

Research motivation

The expression of ox-LDL in human colorectal cancerous tissues and colorectal tissues of hyperlipidemic mice was detected, and the function of ox-LDL in the macrophages in the tumor microenvironment was explored. Our key point is that ox-LDL up-regulates CD44 and CD133 in HFD associated CRC, which is mediated by macrophages. Our study will provide a new idea for the mechanism of HFD associated CRC.

Research objectives

The study aimed to investigate the role of ox-LDL through macrophage in HFD associated CRC.

Research methods

The expression of ox-LDL and CD206 was detected in colorectal tissues of CRC patients with hyperlipidemia and HFD-fed mice by immunofluorescence. We stimulated macrophages with 20 ug/mL ox-LDL and assessed the expression levels of CD206 and cytokines by cell fluorescence and quantitative polymerase chain reaction. We further knocked down LOX-1, the surface receptor of ox-LDL, to confirm the function of ox-LDL in macrophages. Then, LoVo cells were co-cultured with the stimulated macrophages to analyze the CD44 and CD133 expression by western blot.

Research results

The expression of ox-LDL and CD206 was significantly increased in the stroma of colorectal tissues of CRC patients with hyperlipidemia, and also upregulated in the HFD-fed mice. Moreover, an increased level of CD206 and decreased level of inducible nitric oxide synthase were observed in macrophages after the continuous stimulation of ox-LDL. Such effects were inhibited when the surface receptor LOX-1 was knocked down in macrophages. Ox-LDL could induce CD206+ macrophages, which resulted in high expression of CD44 and CD133 in co-cultured LoVo cells.

Research conclusions

Our study found that HFD could induce ox-LDL accumulation in CRC tissue, suggesting the regulatory role of ox-LDL in the microenvironment of CRC. Continuous stimulation of macrophages with ox-LDL induced CD206+ macrophages, which could further promote the increase of CD44 and CD133 levels in CRC cells.

Research perspectives

Our future study will collect more samples. We look forward to make a convincing analysis to identify the correlation between ox-LDL and progression and survival of the enrolled patients in the near future. We will explore the potential signal pathways related to ox-LDL promoting M2-type macrophages by using the technology of single cell sequencing and/or RNA-Seq assay. We are confident that it will provide exciting data in the near future.

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FOOTNOTES

Author contributions: Zheng SM, Sha WH, Chen H, and Ma J designed and coordinated the study; Zheng SM, Chen XF, Yin JB, Zhu XB, and Zheng ZW performed the experiments; Zheng SM and Yin JB acquired and analyzed the data; Chen XF, Zhu XB, and Zheng ZW interpreted the data; Zheng SM, Sha WH, and Chen H wrote the manuscript; and all authors approved the final version of the article.

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Institutional review board statement: The institutional review board of the Guangdong General Hospital approved the study protocol. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Institutional animal care and use committee statement: The animal protocol was designed to minimize pain or discomfort to the animals. The animals were acclimatized to laboratory conditions (23 °C, 12 h/12 h light/dark, 50% humidity, ad libitum access to food and water) for 2 wk prior to experimentation.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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REFERENCES

1 **Sung H**, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J Clin* 2021; **71**: 209-249 [PMID: 33538338 DOI: 10.3322/caac.21660]

2 **Liu PH**, Wu K, Ng K, Zauber AG, Nguyen LH, Song M, He X, Fuchs CS, Ogino S, Willett WC, Chan AT, Giovannucci EL, Cao Y. Association of Obesity With Risk of Early-Onset Colorectal Cancer Among Women. *JAMA Oncol* 2019; **5**: 37-44 [PMID: 30326010 DOI: 10.1001/jamaoncol.2018.4280]

3 **Keum N**, Giovannucci E. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol* 2019; **16**: 713-732 [PMID: 31455888 DOI: 10.1038/s41575-019-0189-8]

4 **Mana MD**, Hussey AM, Tzouanas CN, Imada S, Barrera Millan Y, Bahceci D, Saiz DR, Webb AT, Lewis CA, Carmeliet P, Mihaylova MM, Shalek AK, Yilmaz ÖH. High-fat diet-activated fatty acid oxidation mediates intestinal stemness and tumorigenicity. *Cell Rep* 2021; **35**: 109212 [PMID: 34107251 DOI: 10.1016/j.celrep.2021.109212]

5 **van Driel MS**, van Neerven SM, Vermeulen L. High-Fat Diet Impacts on Tumor Development in the Gut. *Trends Cancer* 2021; **7**: 664-665 [PMID: 34219052 DOI: 10.1016/j.trecan.2021.06.005]

6 **Lin HY**, Weng SW, Shen FC, Chang YH, Lian WS, Hsieh CH, Chuang JH, Lin TK, Liou CW, Chang CS, Lin CY, Su YJ, Wang PW. Abrogation of Toll-Like Receptor 4 Mitigates Obesity-Induced Oxidative Stress, Proinflammation, and Insulin Resistance Through Metabolic Reprogramming of Mitochondria in Adipose Tissue. *Antioxid Redox Signal* 2020; **33**: 66-86 [PMID: 31950846 DOI: 10.1089/ars.2019.7737]

7 **Liu T**, Guo Z, Song X, Liu L, Dong W, Wang S, Xu M, Yang C, Wang B, Cao H. High-fat diet-induced dysbiosis mediates MCP-1/CCR2 axis-dependent M2 macrophage polarization and promotes intestinal adenoma-adenocarcinoma sequence. *J Cell Mol Med* 2020; **24**: 2648-2662 [PMID: 31957197 DOI: 10.1111/jcmm.14984]

8 **Murray PJ**, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, Gordon S, Hamilton JA, Ivashkiv LB, Lawrence T, Locati M, Mantovani A, Martinez FO, Mege JL, Mosser DM, Natoli G, Saeij JP, Schultze JL, Shirey KA, Sica A, Suttles J, Udalova I, van Ginderachter JA, Vogel SN, Wynn TA. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* 2014; **41**: 14-20 [PMID: 25035950 DOI: 10.1016/j.immuni.2014.06.008]

9 **Li R**, Zhou R, Wang H, Li W, Pan M, Yao X, Zhan W, Yang S, Xu L, Ding Y, Zhao L. Gut microbiota-stimulated cathepsin K secretion mediates TLR4-dependent M2 macrophage polarization and promotes tumor metastasis in colorectal cancer. *Cell Death Differ* 2019; **26**: 2447-2463 [PMID: 30850734 DOI: 10.1038/s41418-019-0312-y]

10 **Noy R**, Pollard JW. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* 2014; **41**: 49-61 [PMID: 25035953 DOI: 10.1016/j.immuni.2014.06.010]

11 **Han L**, Wang S, Wei C, Fang Y, Huang S, Yin T, Xiong B, Yang C. Tumour microenvironment: a non-negligible driver for epithelial-mesenchymal transition in colorectal cancer. *Expert Rev Mol Med* 2021; **23**: e16 [PMID: 34758892 DOI: 10.1017/erm.2021.13]

12 **Khaidakov M**, Mehta JL. Do atherosclerosis and obesity-associated susceptibility to cancer share causative link to oxLDL and LOX-1? *Cardiovasc Drugs Ther* 2011; **25**: 477-487 [PMID: 21881851 DOI: 10.1007/s10557-011-6330-8]

13 **Keshk WA**, Zineldeen DH, Wasfy RE, El-Khadrawy OH. Fatty acid synthase/oxidized low-density lipoprotein as metabolic oncogenes linking obesity to colon cancer *via* NF-kappa B in Egyptians. *Med Oncol* 2014; **31**: 192 [PMID: 25173531 DOI: 10.1007/s12032-014-0192-4]

14 **Salehi SS**, Mirmiranpour H, Rabizadeh S, Esteghamati A, Tomasello G, Alibakhshi A, Najafi N, Rajab A, Nakhjavani M. Improvement in Redox Homeostasis after Cytoreductive Surgery in Colorectal Adenocarcinoma. *Oxid Med Cell Longev* 2021; **2021**: 8864905 [PMID: 34381561 DOI: 10.1155/2021/8864905]

15 **Pei C**, Zhang Y, Wang P, Zhang B, Fang L, Liu B, Meng S. Berberine alleviates oxidized low-density lipoprotein-induced macrophage activation by downregulating galectin-3 *via* the NF-κB and AMPK signaling pathways. *Phytother Res* 2019; **33**: 294-308 [PMID: 30402951 DOI: 10.1002/ptr.6217]

16 **Yang P**, Rhea PR, Conway T, Nookala S, Hegde V, Gagea M, Ajami NJ, Harribance SL, Ochoa J, Sastry JK, Cohen L. Human Biofield Therapy Modulates Tumor Microenvironment and Cancer Stemness in Mouse Lung Carcinoma. *Integr Cancer Ther* 2020; **19**: 1534735420940398 [PMID: 32975128 DOI: 10.1177/1534735420940398]

17 **Lv J**, Liu C, Chen FK, Feng ZP, Jia L, Liu PJ, Yang ZX, Hou F, Deng ZY. M2like tumourassociated macrophagesecreted IGF promotes thyroid cancer stemness and metastasis by activating the PI3K/AKT/mTOR pathway. *Mol Med Rep* 2021; **24** [PMID: 34184083 DOI: 10.3892/mmr.2021.12249]

18 **Ma C**, Xie J, Luo C, Yin H, Li R, Wang X, Xiong W, Zhang T, Jiang P, Qi W, Zhou T, Yang Z, Wang W, Ma J, Gao G, Yang X. OxLDL promotes lymphangiogenesis and lymphatic metastasis in gastric cancer by upregulating VEGFC expression and secretion. *Int J Oncol* 2019; **54**: 572-584 [PMID: 30483757 DOI: 10.3892/ijo.2018.4648]

19 **Suzuki K**, Ito Y, Wakai K, Kawado M, Hashimoto S, Toyoshima H, Kojima M, Tokudome S, Hayakawa N, Watanabe Y, Tamakoshi K, Suzuki S, Ozasa K, Tamakoshi A; Japan Collaborative Cohort Study Group. Serum oxidized low-density lipoprotein levels and risk of colorectal cancer: a case-control study nested in the Japan Collaborative Cohort Study. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 1781-1787 [PMID: 15533907]

20 **Lee JW**, Lee SM, Chun J, Im JP, Seo SK, Ha N, Il Choi Y, Kim JS. Novel Histone Deacetylase 6 Inhibitor CKD-506 Inhibits NF-κB Signaling in Intestinal Epithelial Cells and Macrophages and Ameliorates Acute and Chronic Murine Colitis. *Inflamm Bowel Dis* 2020; **26**: 852-862 [PMID: 31895948 DOI: 10.1093/ibd/izz317]

21 **Inagaki K**, Kunisho S, Takigawa H, Yuge R, Oka S, Tanaka S, Shimamoto F, Chayama K, Kitadai Y. Role of tumor-associated macrophages at the invasive front in human colorectal cancer progression. *Cancer Sci* 2021; **112**: 2692-2704 [PMID: 33964093 DOI: 10.1111/cas.14940]

22 **Ma X**, Gao Y, Chen Y, Liu J, Yang C, Bao C, Wang Y, Feng Y, Song X, Qiao S. M2-Type Macrophages Induce Tregs Generation by Activating the TGF-β/Smad Signalling Pathway to Promote Colorectal Cancer Development. *Onco Targets Ther* 2021; **14**: 5391-5402 [PMID: 34908844 DOI: 10.2147/OTT.S336548]

23 **Yang L**, Sun J, Li M, Long Y, Zhang D, Guo H, Huang R, Yan J. Oxidized Low-Density Lipoprotein Links

Hypercholesterolemia and Bladder Cancer Aggressiveness by Promoting Cancer Stemness. *Cancer Res* 2021; **81**: 5720-5732 [PMID: 34479964 DOI: 10.1158/0008-5472.CAN-21-0646]

24 **Beyaz S**, Mana MD, Roper J, Kedrin D, Saadatpour A, Hong SJ, Bauer-Rowe KE, Xifaras ME, Akkad A, Arias E, Pinello L, Katz Y, Shinagare S, Abu-Remaileh M, Mihaylova MM, Lamming DW, Dogum R, Guo G, Bell GW, Selig M, Nielsen GP, Gupta N, Ferrone CR, Deshpande V, Yuan GC, Orkin SH, Sabatini DM, Yilmaz ÖH. High-fat diet enhances stemness and tumorigenicity of intestinal progenitors. *Nature* 2016; **531**: 53-58 [PMID: 26935695 DOI: 10.1038/nature17173]

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ORIGINAL ARTICLE

Basic Study

Ji-Chuan decoction ameliorates slow transit constipation *via* regulation of intestinal glial cell apoptosis

Xiu-Min Wang, Li-Xia Lv, Yue-Si Qin, Yu-Zhu Zhang, Ni Yang, Shu Wu, Xiu-Wen Xia, Hong Yang, Hong Xu, Ying Liu, Wei-Jun Ding

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Abstract

BACKGROUND

Slow transit constipation (STC) is a common intestinal disease with increasing incidence. STC results from various factors, such as the enteric nervous system and metabolic changes. As a classical formula of traditional Chinese medicine, Ji-Chuan decoction (JCD) has been extensively and effectively used in STC treatment, yet its pharmacological mechanism remains unclear.

AIM

To explore the integrated regulatory pattern of JCD against STC through hyphenated techniques from metabolism, network pharmacology and molecular methods.

METHODS

STC model mice were generated by intragastric administration of compound diphenoxylate (10 mg/kg/d) for 14 d. The STC mice in the low dose of JCD (3.04 g/kg), middle dose of JCD (6.08 g/kg) and high dose of JCD (12.16 g/kg) groups

were orally administered JCD solution once a day for 2 wk. The acetylcholine (ACH) level was examined by enzyme-linked immunosorbent assay. The pathological features of colon tissue were observed by hematoxylin and eosin staining. The differentially expressed metabolites and metabolic pathways were tested by nontargeted metabolomics. The main targets and core ingredients of JCD were identified by network pharmacology, and the expression of AKT was confirmed by immunohistochemistry. Finally, the pathways involved in JCD treatment were predicted using a combination of differentially expressed metabolites and targets, and intestinal glial cell apoptosis was demonstrated by immunofluorescence.

RESULTS

JCD significantly promoted intestinal motility, increased the levels of the excitatory neurotransmitter ACH and reduced intestinal inflammation in STC mice. Untargeted metabolomics results showed that JCD significantly restored metabolic dysfunction and significantly affected taurine and hypotaurine metabolism. Network pharmacology and molecular experiments showed that JCD regulates AKT protein expression, and the core component is quercetin. Combined analysis demonstrated that apoptosis may be an important mechanism by which JCD relieves constipation. Further experiments showed that JCD reduced enteric glial cell (EGC) apoptosis.

CONCLUSION

This work demonstrated that reducing EGC apoptosis may be the critical mechanism by which JCD treats STC. These findings call for further molecular research to facilitate the clinical application of JCD.

Key Words: Slow-transit constipation; Ji-Chuan decoction; Taurine and hypotaurine metabolism; AKT; Enteric glial cell; Apoptosis

Core Tip: Slow transit constipation (STC) model mice, which were established with compound diphenoxylate, were effectively treated with Ji-Chuan decoction (JCD). The results show that JCD can promote intestinal motility, increase acetylcholine content, reduce enteric inflammation, improve metabolic dysfunction, and reduce enteric glial cell apoptosis. This work demonstrated that reducing enteric glial cell apoptosis may be the critical mechanism by which JCD treats STC. These findings call for further molecular research to facilitate the clinical application of JCD.

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INTRODUCTION

Chronic constipation is a common complaint and can generally be divided into defecatory disorder, mixed constipation, normal transit constipation and slow transit constipation (STC). STC is the major type of chronic constipation characterized by a substantial increase in bowel transit time. STC has become an epidemic that particularly impacts the quality of life of elderly patients[1]. Despite high morbidity worldwide, the etiology of STC is poorly understood. An accumulation of publications indicates that multiple factors have been documented in the pathogenesis of STC[2-4]. Consequently, an interdisciplinary approach is necessary for exploring its pathological characteristics and developing therapies based on multiple components and multiple targets, such as traditional Chinese medicine (TCM)[5,6]. The active components of Ma-Zi-Ren-Wan could safely and effectively relieve the severity of functional constipation. Based on network pharmacology[7], the anti-STC mechanism of Gui-ren-Run-chang granules, another TCM formula, is associated with repairing the SCF/c-kit pathway and reducing aquaporin-4 expression in the colon[8].

Ji-Chuan decoction (JCD) is a representative TCM formula that originated from Jing-Yue-Zhang in the Ming Dynasty. It has been extensively used for STC and other gastroenteric disorders for hundreds of years[9]. JCD, as a typical TCM prescription, is composed of six herbs and includes multiple bioactive ingredients and complex targets. Studies have shown that JCD can effectively shorten colonic transit

time, improve anorectal dynamics, regulate gastrointestinal neurotransmitters, alleviate constipation symptoms in STC patients, and improve the quality of life of STC patients[9,10]. After 1 mo of continuous use, only a few patients experienced adverse reactions such as dizziness (1.7%) and dry mouth (1.7%)[10]. Our clinical practice confirmed that JCD was a useful formula for STC therapy. *Cistanche deserticola* (*C. deserticola*) is the monarch drug for JCD, and the combination of geniposide and Lactobacillus plantarum KSFY06 has been shown to have anti-montmorillonite-induced constipation effects in Kunming mice[11,12]. However, direct experimental study of the effects of JCD against STC remains to be performed. In this work, we attempt to assess the laxative effect of JCD against STC through a multiomics approach, including metabolomics and TCM network pharmacology, to explore the integrated therapeutic mechanism of JCD.

MATERIALS AND METHODS

Reagents and JCD preparation

Rabbit anti-AKT monoclonal antibody (No: bs-0115R) was provided by Bioss. The biotin secondary antibody anti-rabbit working solution (Goat, SP-9001) was provided by Beijing Zhongshan Jinqiao Biological Technology Co., Ltd. Glial fibrillary acidic protein (GFAP) (mouse, code: ab4648) and Caspase3 (rabbit, code: ab4051) were purchased from Abcam. CY3-labeled goat anti-mouse immunoglobulin G (IgG) (code: 202110) and fluorescein isothiocyanate-labeled goat anti-rabbit IgG (code: GB22303) were purchased from Servicebio.

The JCD solution was prepared according to the protocol described by Cui *et al*[13]. A clinical packet of JCD is composed of six Chinese herbs, *i.e.*, *Angelica sinensis* (15 g), *C. deserticola* (9 g), *Achyranthes bidentata* (*A. bidentata*) (6 g), *Fructus aurantii* (*F. aurantii*) (4.5 g), *Alisma orientalis* (3 g) and *Cimicifuga heracleifuga* (3 g). Six packets of JCD were purchased from Chengdu Hospital of Integrated TCM and Western Medicine and identified by Dr. Wan Lin from Chengdu University of TCM. These herbs were then soaked in 2430 mL ddH_2O (w/v 1:10) for 30 min and decocted for 20 min. Combined solutions following three decoctions were filtered by a 0.22-μm filter, concentrated to 0.81 g of crude herb per milliliter by a vapor evaporator, and stored at -20 °C for further use. In the past, scholars have studied the hyphenated to liquid chromatography characteristic fingerprints of JCD substances[14].

STC model-made, JCD treatment and sample collection

This experiment was approved by the Animal Ethics Committee, Chengdu University of TCM (license 2016-16). Thirty C57BL/6J male mice, aged 7 wk and weight 20.21 ± 2.10 g, were purchased from Chongqing Evansville Laboratory Animal Co., Ltd. (Chongqing, China). The animals were adaptively fed for 7 d in an environment with relative humidity of 45%-55%, a 12-h light/dark cycle and temperature of 22 ± 2 °C. The mice were then randomly divided into six groups, with five animals in each group: Healthy control (HC), STC model (STC), positive drug treatment (mosapride, MSP), low dose of JCD (JCDL), middle dose of JCD (JCDM) and high dose of JCD (JCDH). Mice in the HC group were orally administered normal saline (0.1 mL/10 g/d) as a negative control. The other mice were induced as the STC models by oral administration of compound diphenoxylate (10 mg/kg/d) for 14 d. After model identification, mice in the MSP group were orally administered with MSP (2.5 mg/kg); mice in the JCDL (3.04 g/kg), JCDM (6.08 g/kg) and JCDH (12.16 g/kg) groups were orally administered with JCD; and normal saline (0.1 mL/10 g/d) was gavaged in the HC and STC groups. Each mouse was administered treatments once a day for 14 d. Body weight, food intake and water intake were monitored every week. At the end of experiment, the feces of each mouse were collected under sterile procedures, frozen in liquid nitrogen and stored at -80 °C for further use.

The number of defecation particles within 6 h was counted, and the wet weight of the stool samples was evaluated. Then, the dry weight of the stool was weighed after drying at 60 °C for 12 h in a desiccator, and the moisture content of the stool was calculated. The colonic samples were harvested after euthanasia[15]. The acetylcholine (ACH) concentrations were detected by enzyme-linked immunosorbent assay.

Evaluation of the intestinal propulsive rate

The intestinal propulsive rate was measured after the last administration as follows[16]: All mice were fasted for 12 h and allowed free access to water. Then, mice were fed charcoal powder in 10% acacia gum. After 30 min, the abdomen was opened and the intestines were removed. The length from the pylorus to the ileocecal junction, as well as the charcoal transport distance were measured. The intestinal propulsive rate was calculated by the following formula[17]: Charcoal transit ratio (%) = distance of charcoal transport (cm)/length from pylorus to ileocecal junction (cm) × 100%.

Hematoxylin and eosin and immunofluorescence staining

The collected tissue samples were fixed with 10% formalin, dehydrated with alcohol, and embedded in paraffin wax. The embedded tissues were then sliced into 5-μm slices using a microtome (Leica, Buffalo

Grove, United States) and stained with hematoxylin and eosin (HE). The pathological features were imaged by a digital microscope (Xiamen, China), and the optical densities were quantified using Image-Pro Plus 6.0.

Ultrahigh-pressure liquid chromatography coupled with tandem mass spectrometry analysis

Metabolomics was performed with a Vanquish UHPLC (Thermo, Germany) coupled with a Q Exactive™ HF (Thermo, Germany) platform (Novogene, Beijing, China) as previously described. Fecal samples (100 mg) were ground in liquid nitrogen, incubated on ice for 5 min, and centrifuged at 15000 × g for 20 min at 4 °C. The supernatant was diluted with liquid chromatography-mass spectrometry grade water to a final concentration of 53% methanol. After another centrifugation step, the supernatant was injected into the liquid chromatography-tandem mass spectrometry system for analysis. Samples were injected into a Hypesil Gold column (C18) using a 17-min linear gradient at a flow rate of 0.2 mL/min. The eluents in positive polarity mode were Eluent A (0.1% FA in water) and Eluent B (methanol). The eluents for negative polarity mode were Eluent A (5 mmol/L ammonium acetate, pH 9.0) and Eluent B (methanol). The solvent gradient was set as follows: 2% B, 1.5 min; 2%-100% B, 12.0 min; 100% B, 14.0 min; 100%-2% B, 14.1 min; and 2% B, 17 min. The Q Exactive™ HF mass spectrometer was operated in positive/negative mode with a spray voltage of 3.2 kV, a capillary temperature of 320 °C, a sheath gas flow of 40 arb, and an auxiliary gas flow of 10 arb. Then, the data were matched to the mzCloud, mzVault, and MassList databases for accurate qualitative and relative quantitative results. The threshold for differential metabolites was set as variable importance in the projection (VIP) > 1.0, fold change (FC) > 1.5 or < 0.667 and P value < 0.05. The metabolic functions and relevant metabolic pathways were enriched by MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/).

Network pharmacology analysis and immunohistochemical validation

TCMSP (https://tcmsp-e.com/) was applied to find the chemical components and corresponding targets of JCD, with OB ≥ 30% and DL ≥ 0.18. The UniProt database (https://www.UniProt.org/) was used to correct potential targets. The chemical composition and corresponding targets of JCD were checked by BAT-MAN (http://bionet.ncpsb.org.cn/batman-tcm/index.php), with a score cutoff over 500. Then, the targets obtained from TCMSP and BAT-MAN were combined and deduplicated. Taking "slow transfer constipation" as the keyword, the target points with a score greater than 5.0 were collected from the GeneCard database (https://www.genecards.org/), and all the targets collected from OMIM (https://omim.org/) were included in the follow-up study. The common genes of drug targets and disease genes were put into the STRING11.0 (https://string-db.org/) database to construct a protein-protein interaction (PPI) network. The relevant data were imported into Cytoscape (3.7.1) software in tsv format, and the cytoHubba plug-in was used to display the top 30 nodes in the betweenness algorithm as Hub nodes and to display them in Excel. The distribution pattern and expression levels of AKT, which is one of the most important hub nodes in colonic tissues, were analyzed by immunohistochemistry. Rabbit anti-AKT antibody (Bioss; 1:100) was applied overnight at 4 °C, followed by horseradish peroxidase-conjugated goat anti-rabbit IgG incubation at room temperature for 30 min, and diaminobenzidine was used for staining. The graphic processing software Image-Pro plus 6.0 was used to quantify the expression of AKT. Five fields of each slide were randomly observed, and the optical density values were determined. The components acting on AKT are considered core components of JCD.

Integrated analysis of differentially expressed metabolites and target genes

The HGNC database (https://www.genenames.org/) was applied to transform the obtained JCD target genes into mouse genes. The Joint-Pathway analysis of MetaboAnalyst 5.0 was used to enrich differentially expressed metabolites (DEMs) and target genes associated with JCD. Except for those nominated by other diseases, the top 25 pathways were selected for integrated analysis. The degree of apoptosis of enteric glial cells (EGCs) in colon tissue was analyzed by immunofluorescence. Rabbit anti-caspase3 antibody (Abcam; 1:100) was applied overnight at 4 °C, and mouse anti-GFAP antibody (Abcam; 1:100) was stained at 37 °C for 30 min followed by 4',6-diamidino-2-phenylindole for 10 min at room temperature. ImageJ graphics processing software was used to calculate the apoptosis of EGCs. Five fields of each slide were randomly observed, and densitometric values were estimated.

Statistical analysis

Data processing and statistical analyses were performed by Graph Pad Prism 7 (GraphPad, La Jolla, CA, United States). Data are expressed as the mean ± SD. One-way analysis of variance was used to compare the data of six groups. Student's t test was used for the pairwise comparison of data. The results were considered significant when P < 0.05.

RESULTS

JCD promoted intestinal motility, increased excitatory neurotransmitters and reduced intestinal inflammation in STC mice

General restoration of STC symptoms was observed after JCD treatment. The food intake, water intake and body weight in the JCDH and JCDM groups were similar to those in the HC group (Supplementary Table 1). Compared with HC mice, the average number of stool particles collected from STC mice was markedly reduced. After JCD administration, the average number of stool particles collected from the JCDL, JCDM and JCDH groups was significantly increased and was similar to the level of the positive control drug MSP (Figure 1A). Compared with the HC group, the dry weight, wet weight, and water content of the feces in the STC group were significantly decreased, and the dry weight, wet weight, and water content of the feces in the MSP, JCDM, and JCDH groups were significantly increased (Table 1). Compared with the HC group, the intestinal propulsion rate was significantly decreased in the STC mice, whereas the intestinal propulsion rate was significantly increased in the JCDM, JCDH, and MSP groups (Figure 1B). Finally, the concentrations of excitatory neurotransmitters (colon ACH) in STC mice were significantly reduced, while those in the three JCD groups were significantly increased (Figure 1C).

In addition, pathological observations demonstrated that JCD significantly reduced constipation-associated intestinal inflammation. Pathological observation demonstrated typical pathology of intestinal inflammation in STC mice, with inflammatory infiltration and necrosis in the distal colon. JCD repaired the colonic injuries in a dose-dependent manner (Figure 2). Briefly, the results showed that JCD exerts its effects by enhancing intestinal motility, promoting excitatory neurotransmitters and inhibiting intestinal inflammation in STC mice. Therefore, it is considered that the modeling was successful.

JCD beneficially regulated taurine and hypotaurine metabolism

Forty-two DEMs (Supplementary Table 2) (negative ion mode) between the HC and STC groups and 86 DEMs (Supplementary Table 3) between the STC and JCDH groups were identified (the sample chromatogram in negative ion mode is shown in Supplementary Figure 1). Among the DEMs, eighteen were altered by the modeling process but recovered after JCDH treatment (Supplementary Table 4). As shown in Figure 3A, the closely focused cluster of quality control (QC) samples indicated the reproducibility of the experiments. Figures 3B and C display the fecal DEMs observed after modeling and JCDH treatment. Compared with the STC group, the R2 (evaluation of the modeling ability) and Q2 (description of the predictive ability) of the HC and JCDH groups indicated that the orthogonal partial least squares-discrimination analysis model had high predictability and reliability. Compared with the STC group, the R2 intercepts of the HC and JCDH groups were 0.94 and 0.92, respectively, and the Q2 intercepts were 0.72 and 0.88, respectively (Figures 3D and E). Figures 3F and G show the DEMs by volcano maps. Furthermore, taurine and hypotaurine metabolism were the main pathways impacted by JCDH treatment (Figure 3H).

The core target of JCD is AKT, and the core component is quercetin

To further determine the therapeutic mechanism of JCD in STC, we performed a network pharmacology analysis. After data screening, 45 active pharmaceutical ingredients (Supplementary Table 2), 280 related targets, and 1372 disease targets were obtained. Comparing the goals related to JCD and STC, 89 common goals were identified and registered in the STRING database. Cytoscape 3.7.2 software was used to construct a PPI network, including 132 nodes and 2492 interactive edges (Figure 4A). According to the betweenness centrality value, the top 30 hub nodes were identified (Figures 4B and C), and AKT was considered one of the important targets. Immunohistochemistry confirmed that colonic AKT expression was significantly suppressed in STC mice compared with the HC group, whereas JCD significantly reversed this effect (Figures 4D and E). The chemical that acts on AKT is considered to be the core component of JCD, *i.e.,* the quercetin from *A. bidentata* and *C. deserticola,* baicalein, kaempferol and wogonin from *A. bidentata*, and naringenin from *F. aurantii* (Table 2).

JCD inhibited EGC apoptosis

To comprehensively probe the pharmacological mechanism of JCD, a joint-pathway analysis was performed based on DEMs and common genes from network pharmacology. Twenty-five remarkable pathways are presented in Figure 5A. Apoptosis may be an important signaling pathway in the treatment of STC by JCD. GFAP is an EGC marker. Immunofluorescence double-labeling of GFAP and caspase3 showed that compared with the HC group, the apoptotic rate of EGCs in the STC group was significantly increased, while those in the JCDH and JCDM groups were significantly decreased (Figures 5B and C), confirming the predicted results.

Table 1 Dry weight, wet weight and moisture content of feces of mice in each group

Grouping/feces index	Wet weight (g/mou)	Dry weight (g/mou)	Moisture content (%/mou)
HC	0.26 ± 0.02	0.18 ± 0.01	0.30 ± 0.04
STC	0.12 ± 0.01[a]	0.10 ± 0.01[c]	0.18 ± 0.01[c]
JCDL	0.25 ± 0.01	0.18 ± 0.01	0.29 ± 0.02
JCDM	0.26 ± 0.02[a]	0.18 ± 0.02[c]	0.30 ± 0.02[c]
JCDH	0.26 ± 0.02[a]	0.18 ± 0.01[c]	0.31 ± 0.02[c]
MSP	0.27 ± 0.03[b]	0.19 ± 0.02[c]	0.30 ± 0.01[c]

[a]$P < 0.05$: Compared with the healthy control group.
[b]$P < 0.01$: Compared with the healthy control group.
[c]$P < 0.05$: Compared with the slow transit constipation group.
Data are expressed as the mean ± SD. HC: Healthy control; STC: Slow transit constipation; JCD: Ji-Chuan decoction; JCDL: Low-dose Ji-Chuan decoction treatment; JCDM: Middle-dose Ji-Chuan decoction treatment; JCDH: High-dose Ji-Chuan decoction treatment; MSP: Mosapride treatment.

Table 2 The core ingredients of Ji-Chuan decoction

Molecular name	Oral bioavailability (%)	Drug like
Baicalein	33.52	0.21
Kaempferol	41.88	0.24
Naringenin	59.29	0.21
Quercetin	46.43	0.28
Wogonin	30.68	0.23

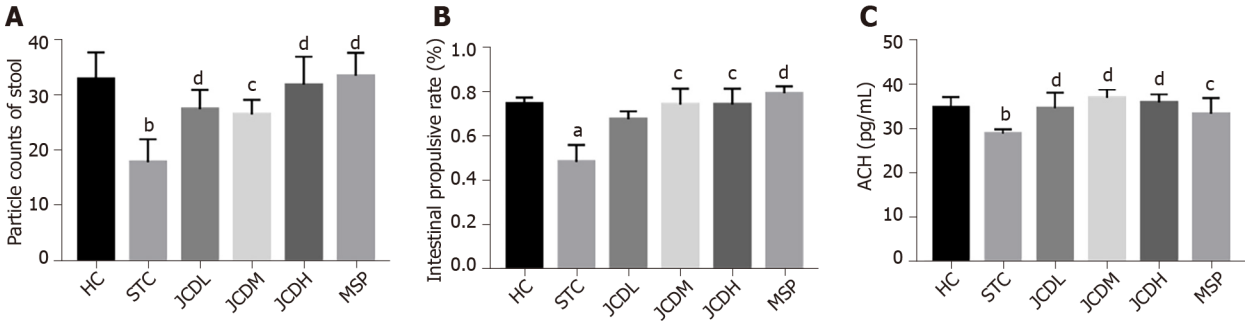

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Figure 1 Results of the constipation-related index. A: Particle counts of stool; B: Intestinal propulsive rate; C: The expression levels of colonic acetylcholine. ACH: Acetylcholine; HC: Healthy control; STC: Slow transit constipation; JCD: Ji-Chuan decoction; JCDL: Low-dose Ji-Chuan decoction treatment; JCDM: Middle-dose Ji-Chuan decoction treatment; JCDH: High-dose Ji-Chuan decoction treatment; MSP: Mosapride treatment. Compared with the healthy control group: [a]$P < 0.05$, [b]$P < 0.01$. Compared with the slow transit constipation group: [c]$P < 0.05$, [d]$P < 0.01$.

DISCUSSION

JCD is an established TCM formula that is a particularly effective therapy for STC differentiated by TCM as Spleen and Kidney Yang deficiency syndrome[9], yet its pharmaceutical mechanism remains unclear. We explored the therapeutic mechanism of JCD in a dose-dependent manner and observed that the best effect was achieved by JCDH. The compound diphenoxylate is widely used to induce STC in animal models and can inhibit the peristaltic reflex of intestinal mucosa. It is easier to induce constipation than the commonly used loperamide and is more available than morphine[17]. It is easier to induce constipation than other loperamides and easier to obtain than morphine[17]. The holistic view is shared between systems biology and TCM[18]; therefore, we used key disciplines of systems biology, such as metabolomics and network pharmacology, to effectively reveal the integrated mechanism of JCD for STC.

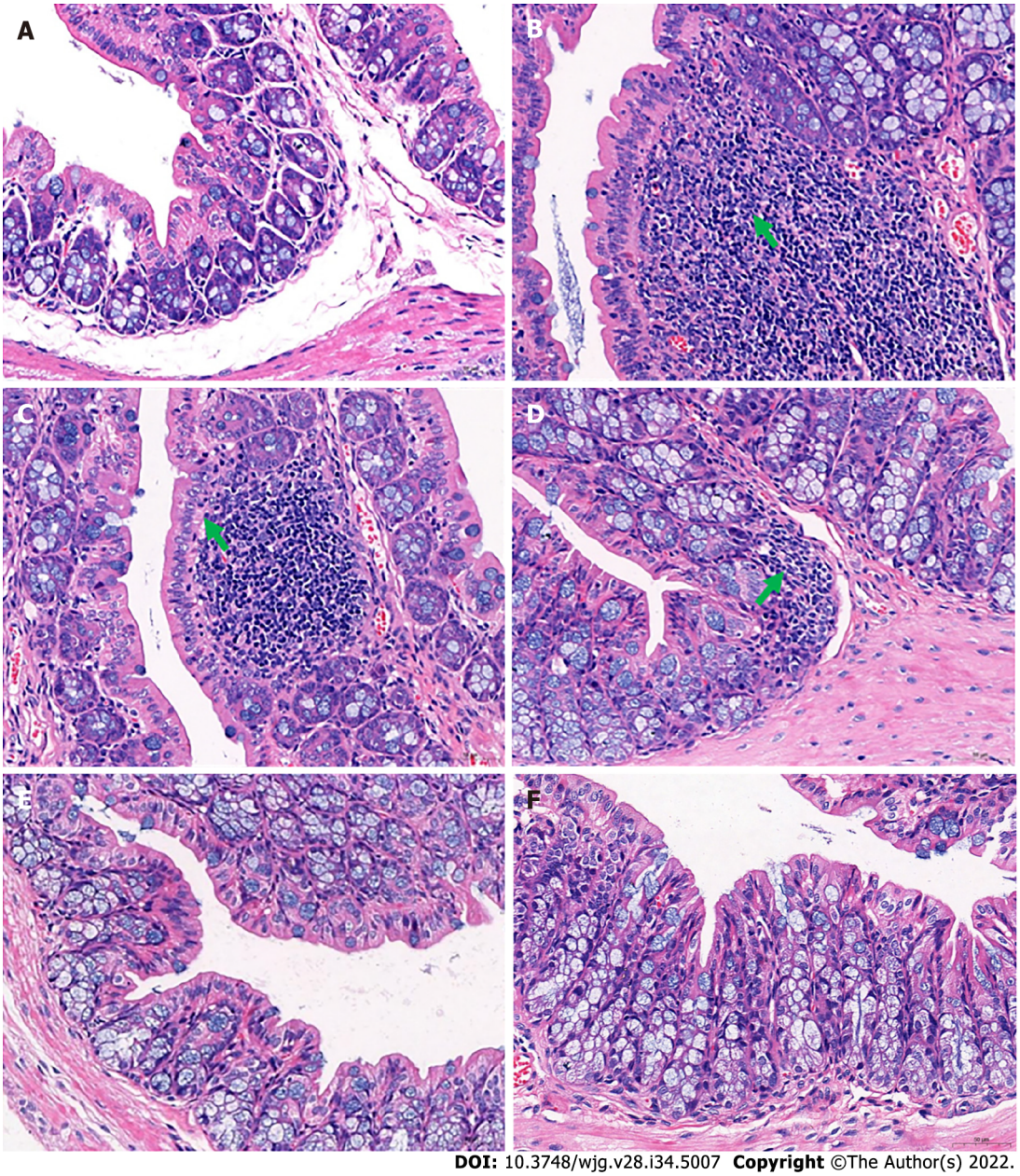

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Figure 2 Histological changes in the distal colon. A: Distal colon of the healthy control group; B: In the distal colons of mice in the slow transit constipation group, more severe inflammatory infiltration was observed; C: In the distal colons of mice in the low-dose Ji-Chuan decoction (JCD) treatment group, more severe inflammatory infiltration was observed; D: In the distal colon of mice in the middle-dose JCD group, there was reduced inflammation; E: High-dose JCD treatment restored the normal tissue morphology of the distal colon of constipated mice; F: Mosapride treatment restored the normal tissue morphology of the distal colon of constipated mice. Sections were observed at 400 × using a light microscope.

JCD substantially improved the manifestation of STC in mice

Constipation refers to laborious defecation, dry and hard stools and low stool volume. Effective drugs will increase key indices of constipation, such as the quantity and water content of the feces and the intestinal propulsive rate[19,20]. In this study, we observed that JCD substantially improved almost all indices tested in this work (Figures 1A and B, Table 1). The intestinal propulsive rates, extensively used to evaluate the pathological degree and curative effect of constipation, were significantly decreased after diphenoxylate treatment. Similar anti-constipation effects were obtained among the JCDM, JCDH and the positive reagent MSP groups (Figure 1B). In addition, the three JCD doses could statistically increase the concentration of excitatory neurotransmitter (ACH) (Figure 1C), suggesting that the increase in excitatory neurotransmitter is a critical approach for anti-constipation by JCD[3]. Our colonic pathological observation showed that JCD significantly reduced inflammatory infiltration and repaired pathological damage caused by the compound diphenoxylate in a dose-dependent manner (Figure 2), confirming previous studies[21]. Therefore, our work indicates an integrated effect of JCD on STC by promoting excitatory neurotransmitters, inhibiting gastroenteric inflammation and accelerating intestinal motility.

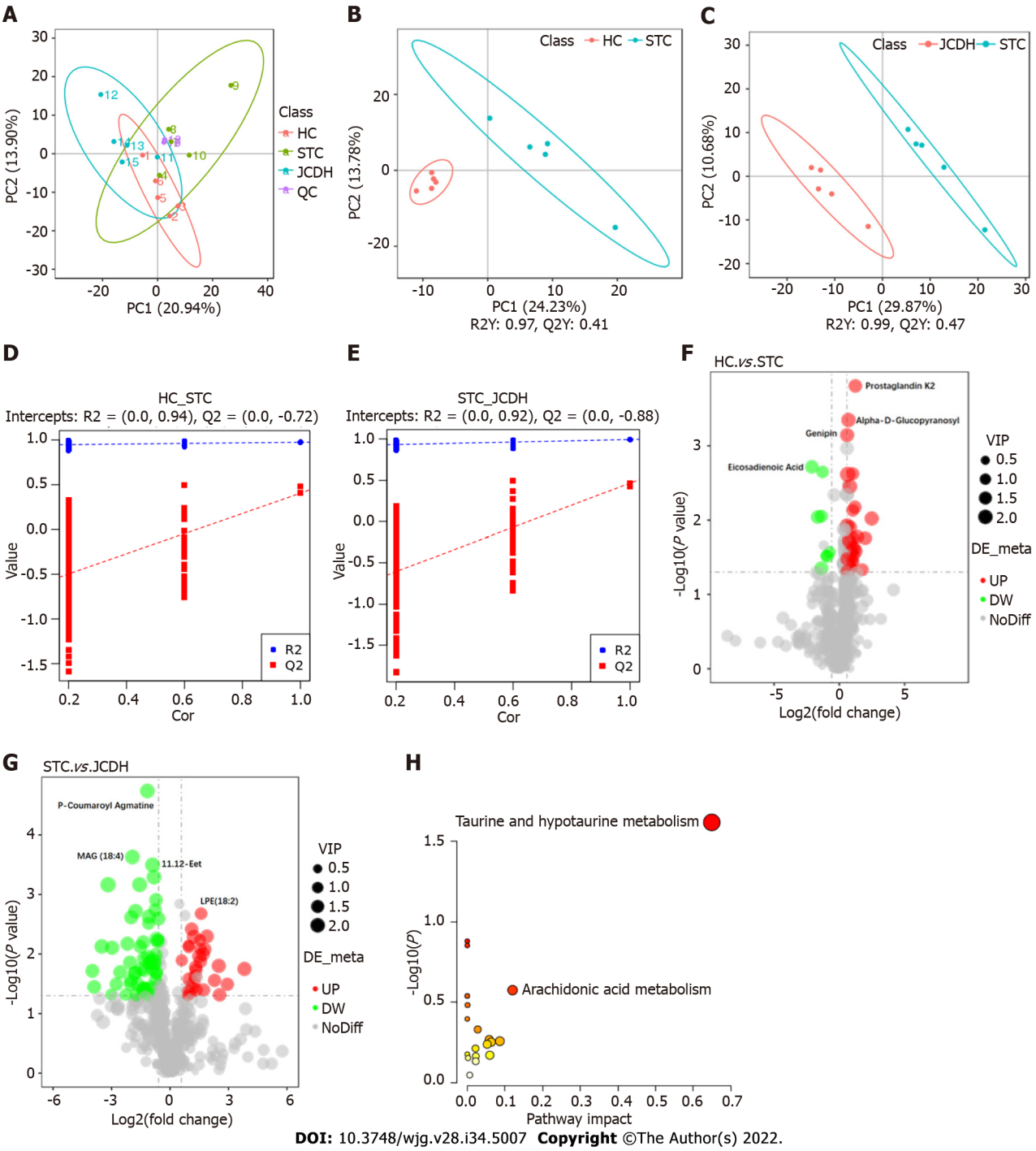

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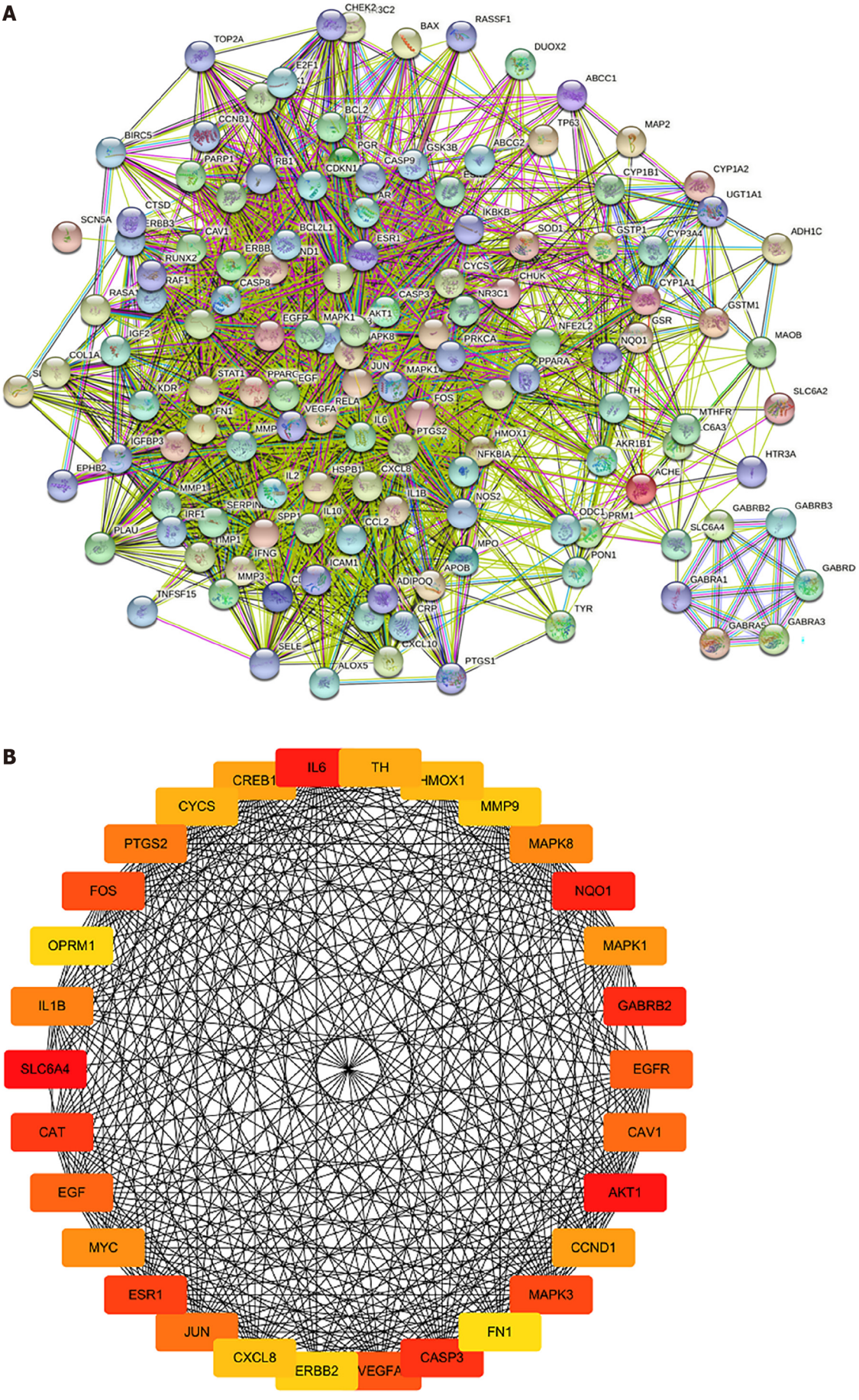
Figure 3 Different intestinal metabolites detected by ultrahigh-pressure liquid chromatography coupled with tandem mass spectrometry.
A: Principal component analysis; B: Orthogonal partial least squares discrimination analysis score plots of intestinal metabolic profiling in the healthy control (HC) *vs* slow transit constipation (STC) groups; C: STC *vs* high-dose Ji-Chuan decoction (JCDH) treatment groups under negative ion mode; D: Permutation test in negative ion mode of the HC *vs* STC groups; E: Permutation test in negative ion modes of the STC *vs* JCDH groups; F: Volcano plot of the different metabolites between the STC and HC groups; G: Volcano plot of the different metabolites between the JCDH and STC groups; H: Critical metabolic pathways of the STC *vs* JCDH groups. HC: Healthy control; STC: Slow transit constipation; JCD: Ji-Chuan decoction; JCDL: Low-dose Ji-Chuan decoction treatment; JCDM: Middle-dose Ji-Chuan decoction treatment; JCDH: High-dose Ji-Chuan decoction treatment; MSP: Mosapride treatment; MAG: 1-(6Z,9Z,12Z,15Z-Octadecatetraenoyl)-sn-glycerol; 11.12-EET: (5Z,8Z,14Z)-11,12-Epoxyeicosa-5,8,14-trienoic acid; LPE: 2-(9Z,12Z-octadecadienoyl)-sn-glycero-3-phosphoethanolamine; QC: Quality control.

JCD altered critical enteric metabolites, especially taurine and hypotaurine

The metabolome results showed that the pathways involving taurine and hypotaurine metabolism were important for the anti-constipation effect of JCD. Taurine is a sulfur-containing α-amino acid with anti-inflammatory properties that can be sequentially converted into homocysteine, cystathionine, cysteine and/or hypotaurine[22,23]. The concentration of taurine was significantly reduced in loperamide-induced constipation rats, and red liriope ameliorated constipation and increased the level of taurine[24, 25]. Our work also showed that the contents of taurine and its metabolite hypotaurine were negatively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GSK3B
ABCG2
CYP1B1
CYP1A2
UGT1A1
CTSD
ERBB3
SCN5A
CAV1
BCL2L1
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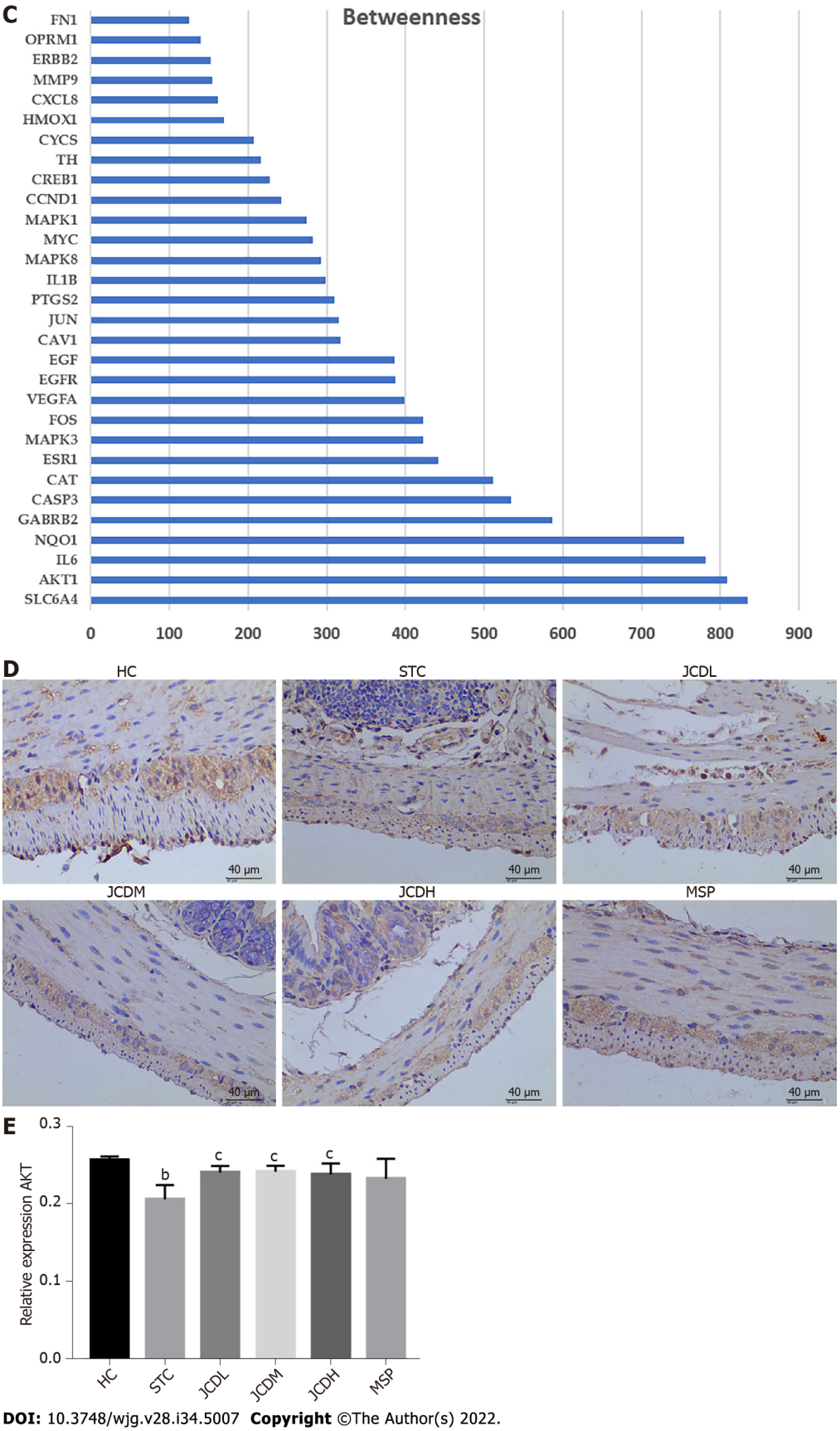

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Figure 4 Network construction, screening and validation of the core targets for Ji-Chuan decoction treatment of slow transit constipation. A: Protein-protein interaction (PPI) network; B: Top 30 protein targets with the largest betweenness in the PPI network; C: Betweenness analysis of the top 30 protein targets; D and E: The relative expression of AKT as assessed by immunofluorescent staining. AKT: Serine/threonine-protein kinase; HC: Healthy control; STC: Slow transit constipation; JCD: Ji-Chuan decoction; JCDL: Low-dose Ji-Chuan decoction treatment; JCDM: Middle-dose Ji-Chuan decoction treatment; JCDH: High-dose Ji-Chuan decoction treatment; MSP: Mosapride treatment; SLC6A4: Sodium-dependent serotonin transporter; AKT1: RAC-alpha serine/threonine-protein

kinase; IL: Interleukin; NQO1: NAD(P)H dehydrogenase (quinone)1; GABRB2: Gammaaminobutyric acid receptor subunit beta-2; CASP3: Caspase-3; CAT: Catalase; ESR1: Estrogen receptor; MAPK3: Mitogen-activated protein kinase 3; FOS: Proto-oncogene c-Fos; VEGFA: Vascular endothelial growth factor A; EGFR: Epidermal growth factor receptor; EGF: Pro-epidermal growth factor; CAV1: Caveolin-1; JUN: Transcription factor AP-1; PTGS2: Prostaglandin G/H synthase 2; MAPK8: Mitogen-activated protein kinase 8; MYC: Myc proto-oncogene protein; MAPK1: Mitogen-activated protein kinase 1; CCND1: G1/S-specific cyclin-D1; CREB1: Cyclic AMP-responsive element-binding protein 1; TH: Thyroid hormone; CYCS: Cytochrome c; HMOX1: Heme oxygenase 1; MMP9: Matrix metalloproteinase-9; ERBB2: Receptor tyrosine-protein kinase erbB-2; FN1: Fibronectin. Data are expressed as the mean ± SD. Compared with the healthy control group: $^{b}P < 0.01$. Compared with the slow transit constipation group: $^{c}P < 0.05$, $^{d}P < 0.01$.

associated with the manifestation of STC and positively associated with intestinal transit rates[26]. However, the molecular mechanism of taurine on gastrointestinal motility and the regulatory mechanisms of JCD remain to be further explored.

JCD restored the levels of AKT, a key protein involved in apoptosis regulation

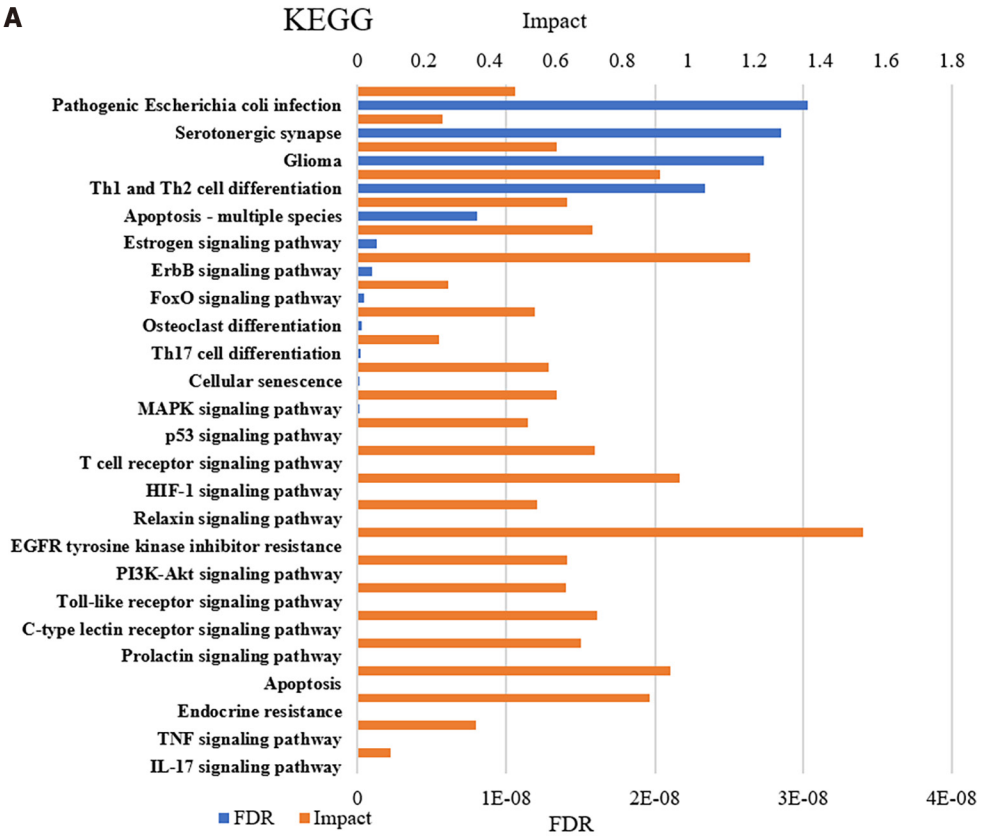
PPI network prediction showed that AKT is the core target of JCD for STC therapy. In our experiments, STC mice showed downregulated expression of AKT, while all three doses of JCD treatment reversed the abnormally reduced expression levels. AKT plays an important role in cell survival and apoptosis. Previous studies have shown that high glucose levels can induce EGC apoptosis through the AKT pathway, which is closely related to intestinal motility[27]. Another study showed that EGCs could protect the nervous system from hyperglycemia-induced damage by activating the Akt/GSK-3β pathway[28]. Therefore, we speculate that the treatment of STC with JCD may be related to EGC apoptosis.

JCD rescued excessive EGC apoptosis

Experimental evidence in humans and animals suggests that EGCs play a key role in regulating gastrointestinal motility and transit[29-32]. A cohort study of twenty-six STC patients showed reduced EGCs compared with ten healthy volunteers[33]. EGC can increase the expression of Akt and ZO-1 by releasing glial cell-derived neurotrophic factor, indirectly regulating the integrity of the intestinal epithelial barrier, reducing intestinal inflammation and improving delayed colonic transit[34,35]. Another study showed that enteric glial LPAR1 signaling regulates gastrointestinal motility through EGCs and may contribute to chronic intestinal pseudo-obstruction in humans[36]. Activation of opioid receptors in EGCs may be associated with morphine-induced constipation[37]. Previous studies have shown that the key components of JCD are associated with neural apoptosis, and many of them also involve changes in AKT protein. For example, wogonin, a key component of JCD identified in this paper, can prevent hippocampal injury after brain trauma through antioxidation and anti-apoptosis, which has been shown to occur through the PI3K/Akt/nuclear factor E2-related factor 2 (Nrf2)/HO-1 pathway[38]. Baicalein reduces sevoflurane-induced neurodegeneration, improves learning and memory retention in rats, and modulates the PI3/Akt/GSK-3β and JNK/ERK signaling pathways[39]. Kaempferol prevents cerebral ischemia-reperfusion injury by interfering with oxidative and inflammatory stress-induced apoptosis[40]. Quercetin is involved in neuroprotection by regulating Nrf2, paraoxonase 2, JNK, tumour necrosis factor alpha, PGC-1α, MAPKs, CREB and PI3K/Akt[41]. Naringenin can effectively inhibit Aβ25-35-induced neuronal injury in PC12 cells by regulating the ER and PI3K/Akt pathways[42]. To our knowledge, the current work is the first to demonstrate that JCD can improve constipation by reducing EGC apoptosis. Our work applied a multiomics strategy to explore the therapeutic mechanism of JCD in the treatment of STC and found some interesting evidence that remains to be elucidated in more detail in the future. The JCDH group of mice exhibited a better effect, suggesting that a suitable dose needs to be further evaluated.

CONCLUSION

This work demonstrated that reduced enteric EGC apoptosis may be the critical mechanism of JCD in STC therapy. These findings call for further molecular research to facilitate the clinical application of JCD.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Serotonergic synapse
Glioma
Th1 and Th2 cell differentiation
Apoptosis - multiple species
Estrogen signaling pathway
ErbB signaling pathway
FoxO signaling pathway
Osteoclast differentiation
Th17 cell differentiation
Cellular senescence
MAPK signaling pathway
p53 signaling pathway
T cell receptor signaling pathway
HIF-1 signaling pathway
Relaxin signaling pathway
EGFR tyrosine kinase inhibitor resistance
PI3K-Akt signaling pathway
Toll-like receptor signaling pathway
C-type lectin receptor signaling pathway
Prolactin signaling pathway
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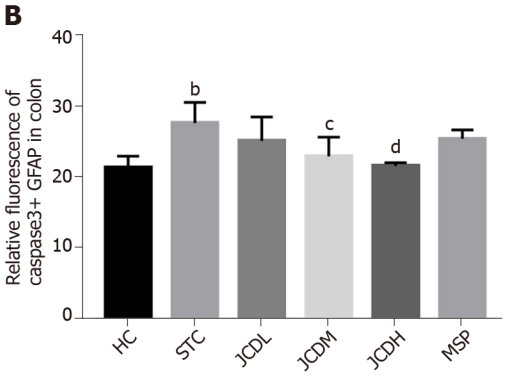
B
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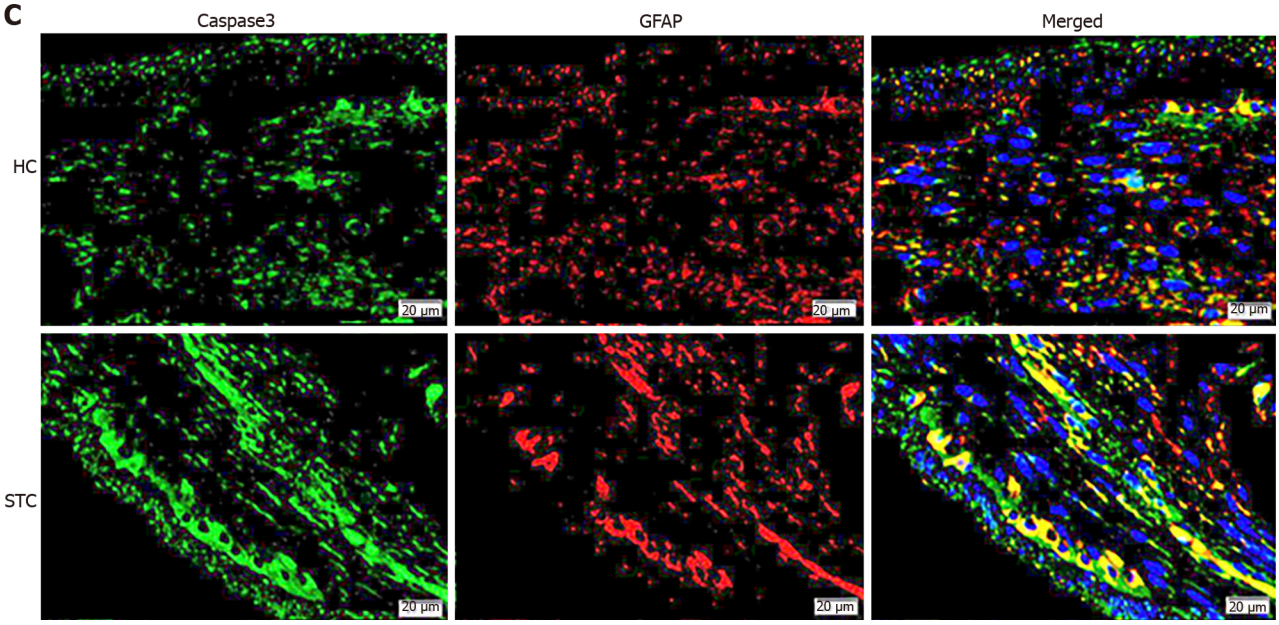
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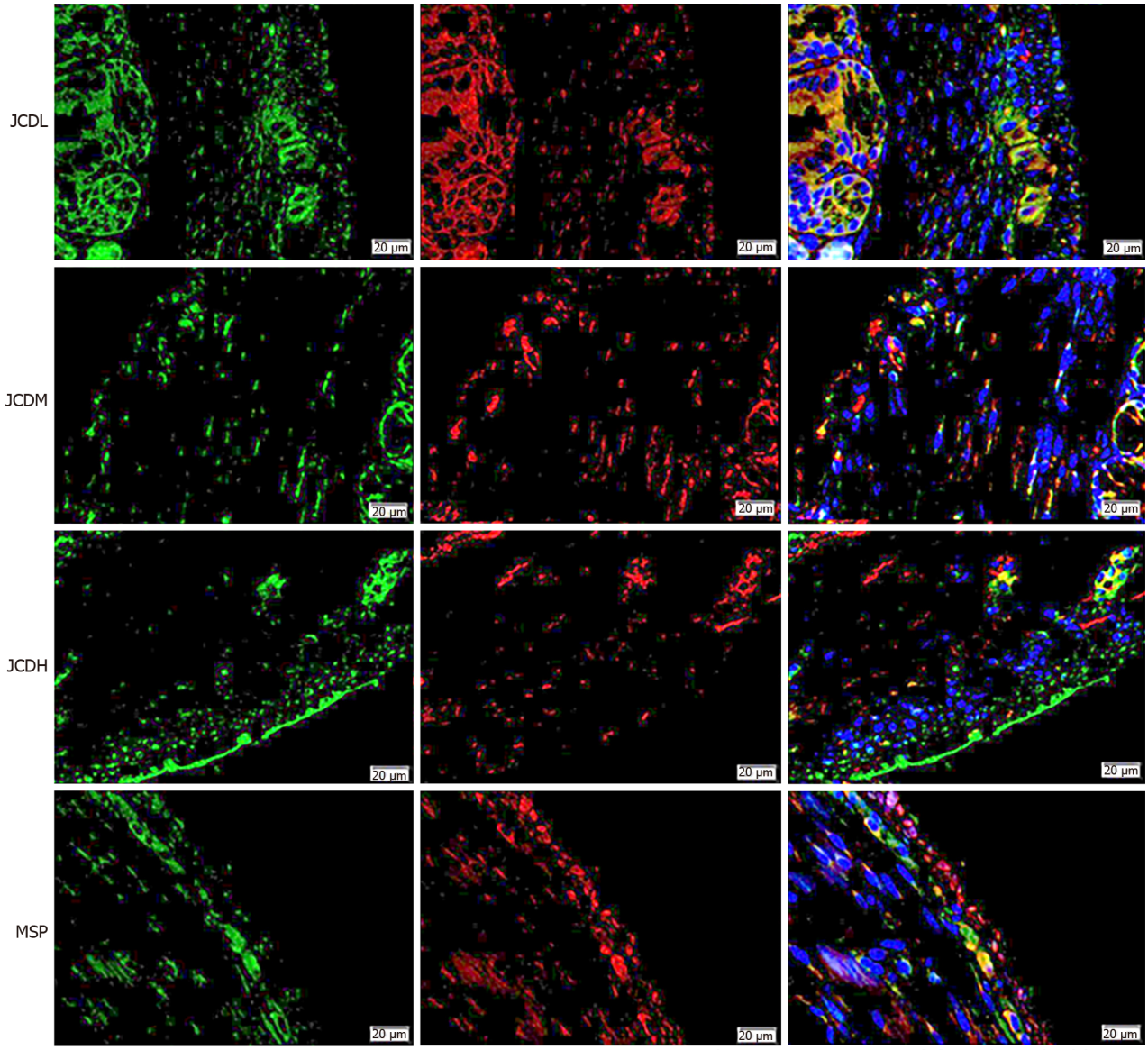

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Figure 5 Integrative analysis and experimental validation of improved network pharmacology and metabolites. A: Integration analysis between the modified network pharmacology and metabolites. The ordinate stands for pathways, the primary abscissa stands for minus false discovery rates, and the secondary abscissa stands for impact; B and C: The relative expression of Caspase3+ Glial fibrillary acidic protein was assessed by immunofluorescent staining. GFAP: Glial fibrillary acidic protein; HC: Healthy control; STC: Slow transit constipation; JCD: Ji-Chuan decoction; JCDL: Low-dose Ji-Chuan decoction treatment; JCDM: Middle-dose Ji-Chuan decoction treatment; JCDH: High-dose Ji-Chuan decoction treatment; MSP: Mosapride treatment; FDR: False discovery rates; KEGG: Kyoto Encyclopedia of Genes and Genomes; Th: Thyroid hormone; EGFR: Epidermal growth factor receptor; MAPK: Mitogen-activated protein kinase; IL: Interleukin; TNF: Tumor necrosis factor; HIF: Hypoxia-inducible factor. Data are expressed as the mean ± SD. Compared with the healthy control group: $^{a}P < 0.05$, $^{b}P < 0.01$. Compared with the slow transit constipation group: $^{c}P < 0.05$, $^{d}P < 0.01$.

ARTICLE HIGHLIGHTS

Research background

Slow transit constipation (STC) is a common intestinal disorder without an effective therapeutic regimen. Ji-Chuan Decoction (JCD) is an established formula for STC. However, its pharmacological mechanism is still unclear.

Research motivation

To determine the ingredients and mechanism of JCD for STC treatment.

Research objectives

To explore the integrated regulatory pattern of JCD against STC through hyphenated techniques from metabolism, network pharmacology and molecular methods.

Research methods

STC model mice were generated by gavage of diphenoxylate for 14 d. STC mice in the low- (3.04 g/kg), medium- (6.08 g/kg) and high-dosage (12.16 g/kg) JCD groups were orally administered. The acetylcholine (ACH) level was detected by enzyme-linked immunosorbent assay. AKT expression and enteric glial cell (EGC) apoptosis were demonstrated by immunofluorescence. The differentially expressed metabolites were tested by nontargeted metabolomics. The targets and core ingredients were identified by network pharmacology.

Research results

JCD significantly promotes intestinal motility, increases colonic ACH content and reduces inflammation in STC mice. It markedly restores the misaligned metabolites, including taurine/hypotaurine, and rescues AKT expression with quercetin. Inhibition of EGC apoptosis is a potential mechanism by which JCD relieves constipation.

Research conclusions

Regulating gut metabolites and reducing EGC apoptosis in STC mice may be the key mechanism of JCD for STC treatment.

Research perspectives

Further investigation into the molecular interactions among the JCD ingredients and metabolites, intestinal microbiota and host response in STC mice is necessary.

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FOOTNOTES

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REFERENCES

1 **Nelson AD**, Camilleri M, Chirapongsathorn S, Vijayvargiya P, Valentin N, Shin A, Erwin PJ, Wang Z, Murad MH. Comparison of efficacy of pharmacological treatments for chronic idiopathic constipation: a systematic review and network meta-analysis. *Gut* 2017; **66**: 1611-1622 [PMID: 27287486 DOI: 10.1136/gutjnl-2016-311835]

2 **Yeh KM**, Johansson O, Le H, Rao K, Markus I, Perera DS, Lubowski DZ, King DW, Zhang L, Chen H, Liu L. Cystic fibrosis transmembrane conductance regulator modulates enteric cholinergic activities and is abnormally expressed in the enteric ganglia of patients with slow transit constipation. *J Gastroenterol* 2019; **54**: 994-1006 [PMID: 31392489 DOI: 10.1007/s00535-019-01610-9]

3 **Russell JP**, Mohammadi E, Ligon C, Latorre R, Johnson AC, Hoang B, Krull D, Ho MW, Eidam HS, DeMartino MP, Cheung M, Oliff AI, Kumar S, Greenwood-Van Meerveld B. Enteric RET inhibition attenuates gastrointestinal secretion and motility *via* cholinergic signaling in rat colonic mucosal preparations. *Neurogastroenterol Motil* 2019; **31**: e13479 [PMID: 30311722 DOI: 10.1111/nmo.13479]

4 **Kim JE**, Park JW, Kang MJ, Choi HJ, Bae SJ, Choi Y, Lee YJ, Seo S, Hong JT, Hwang DY. Laxative Effect of Spicatoside A by Cholinergic Regulation of Enteric Nerve in Loperamide-Induced Constipation: ICR Mice Model. *Molecules* 2019; **24** [PMID: 30836659 DOI: 10.3390/molecules24050896]

5 **Zhong LLD**, Zheng G, Da Ge L, Lin CY, Huang T, Zhao L, Lu C, Lu AP, Bian ZX. Chinese herbal medicine for constipation: zheng-based associations among herbs, formulae, proprietary medicines, and herb-drug interactions. *Chin Med* 2016; **11**: 28 [PMID: 27347002 DOI: 10.1186/s13020-016-0099-4]

6 **Whiting RL**, Ford AC. Efficacy of traditional chinese medicine in functional constipation. *Am J Gastroenterol* 2011; **106**: 1003; author reply 1003-1003; author reply 1004 [PMID: 21540906 DOI: 10.1038/ajg.2011.14]

7 **Huang T**, Ning Z, Hu D, Zhang M, Zhao L, Lin C, Zhong LLD, Yang Z, Xu H, Bian Z. Uncovering the Mechanisms of Chinese Herbal Medicine (MaZiRenWan) for Functional Constipation by Focused Network Pharmacology Approach. *Front Pharmacol* 2018; **9**: 270 [PMID: 29632490 DOI: 10.3389/fphar.2018.00270]

8 **Ali MZ**, Mehmood MH, Haneef M, Saleem M, Ishrat G, Siddiqi HS, Gilani AU, Ahmed M. A flavonoid driven phyto-pharmacological effects of Capparis decidua Edgew. in rodents. *Pak J Pharm Sci* 2020; **33**: 333-342 [PMID: 32122866]

9 **Dai L**, Zhong LL, Ji G. Irritable bowel syndrome and functional constipation management with integrative medicine: A systematic review. *World J Clin Cases* 2019; **7**: 3486-3504 [PMID: 31750331 DOI: 10.12998/wjcc.v7.i21.3486]

10 **Zhang SX**, Zhang XA, An YK. Effect of Jichuanjian on Gastrointestinal Function□Serum Intestinal Neurotransmitters andIntestinal Flora in Elderly with Chronic Functional Constipation. *Chin J ETMF* 2018; **24**: 169-174 [DOI: 10.13422/j.cnki.syfjx.20182131]

11 **Gan Y**, Liang J, Diao W, Zhou X, Mu J, Pang L, Tan F, Zhao X. *Lactobacillus plantarum* KSFY06 and geniposide counteract montmorillonite-induced constipation in Kunming mice. *Food Sci Nutr* 2020; **8**: 5128-5137 [PMID: 32994973 DOI: 10.1002/fsn3.1814]

12 **Zhang Y**, Wang Y, Yang S, Xiao Y, Guan H, Yue X, Wang X, Li X. The Difference of Chemical Components and Biological Activities of the Raw Products slices and the Wine Steam-Processed Product from Cistanche deserticola. *Evid Based Complement Alternat Med* 2019; **2019**: 2167947 [PMID: 31007699 DOI: 10.1155/2019/2167947]

13 **Cui H**, Li Y, Wang Y, Jin L, Yang L, Wang L, Liao J, Wang H, Peng Y, Zhang Z, Liu X. Da-Chai-Hu Decoction Ameliorates High Fat Diet-Induced Nonalcoholic Fatty Liver Disease Through Remodeling the Gut Microbiota and Modulating the Serum Metabolism. *Front Pharmacol* 2020; **11**: 584090 [PMID: 33328987 DOI: 10.3389/fphar.2020.584090]

14 **Shi C**, Li X, Feng J, Huang Y, Wang Y, Chen J, Li S. Study on HPLC characteristic fingerprint of substance benchmark of classical famou sprescription of Jichuan Decoction. *Zhong Cao Yao* 2020; **51**: 3930-3936

15 **Lee HY**, Kim JH, Jeung HW, Lee CU, Kim DS, Li B, Lee GH, Sung MS, Ha KC, Back HI, Kim SY, Park SH, Oh MR, Kim MG, Jeon JY, Im YJ, Hwang MH, So BO, Shin SJ, Yoo WH, Kim HR, Chae HJ, Chae SW. Effects of Ficus carica paste on loperamide-induced constipation in rats. *Food Chem Toxicol* 2012; **50**: 895-902 [PMID: 22178225 DOI: 10.1016/j.fct.2011.12.001]

16 **Nagakura Y**, Naitoh Y, Kamato T, Yamano M, Miyata K. Compounds possessing 5-HT3 receptor antagonistic activity inhibit intestinal propulsion in mice. *Eur J Pharmacol* 1996; **311**: 67-72 [PMID: 8884238 DOI: 10.1016/0014-2999(96)00403-7]

17 **Yang A**, Yan Y, He H, Gao Y, He K, Shi H, Liu M. Comparative study on four models for preparing functional constipation in mice. *China Med Eng* 2019; **27**: 1-4 [DOI: 10.19338/j.issn.1672-2019.2019.03.001]

18 **Meng X**, Ma J, Kang AN, Kang SY, Jung HW, Park YK. A Novel Approach Based on Metabolomics Coupled With Intestinal Flora Analysis and Network Pharmacology to Explain the Mechanisms of Action of Bekhogainsam Decoction in the Improvement of Symptoms of Streptozotocin-Induced Diabetic Nephropathy in Mice. *Front Pharmacol* 2020; **11**: 633 [PMID: 32508632 DOI: 10.3389/fphar.2020.00633]

19 **Qian Y**, Zhao X, Kan J. Preventive effect of resistant starch on activated carbon-induced constipation in mice. *Exp Ther Med* 2013; **6**: 228-232 [PMID: 23935751 DOI: 10.3892/etm.2013.1096]

20 **Gibson GR**, Probert HM, Loo JV, Rastall RA, Roberfroid MB. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr Res Rev* 2004; **17**: 259-275 [PMID: 19079930 DOI: 10.1079/NRR200479]

21 **Ren X**, Liu L, Gamallat Y, Zhang B, Xin Y. Enteromorpha and polysaccharides from enteromorpha ameliorate loperamide-induced constipation in mice. *Biomed Pharmacother* 2017; **96**: 1075-1081 [PMID: 29198923 DOI: 10.1016/j.biopha.2017.11.119]

22 **Obeid OA**, Johnston K, Emery PW. Plasma taurine and cysteine levels following an oral methionine load: relationship with coronary heart disease. *Eur J Clin Nutr* 2004; **58**: 105-109 [PMID: 14679374 DOI: 10.1038/sj.ejcn.1601755]

23 **Wójcik OP**, Koenig KL, Zeleniuch-Jacquotte A, Costa M, Chen Y. The potential protective effects of taurine on coronary heart disease. *Atherosclerosis* 2010; **208**: 19-25 [PMID: 19592001 DOI: 10.1016/j.atherosclerosis.2009.06.002]

24 **Kim JE**, Lee YJ, Kwak MH, Jun G, Koh EK, Song SH, Seong JE, Kim JW, Kim KB, Kim S, Hwang DY. Metabolomics approach to serum biomarker for loperamide-induced constipation in SD rats. *Lab Anim Res* 2014; **30**: 35-43 [PMID:

24707303 DOI: 10.5625/lar.2014.30.1.35]
25 **Kim JE**, Lee YJ, Ryu SH, Park JW, Kang MJ, Choi HJ, Bae SJ, Choi Y, Kang HG, Kim KB, Kim S, Lim Y, Hwang DY. Metabolomics approach to serum biomarker for laxative effects of red *Liriope platyphylla* in loperamide-induced constipation of SD rats. *Lab Anim Res* 2019; **35**: 9 [PMID: 32257897 DOI: 10.1186/s42826-019-0009-x]
26 **Lee DS**, Jo HG, Kim MJ, Lee H, Cheong SH. Laxative Effects of Taurine on Loperamide-Induced Constipation in Rats. *Adv Exp Med Biol* 2019; **1155**: 261-271 [PMID: 31468405 DOI: 10.1007/978-981-13-8023-5_25]
27 **Chen Y**, Liu G, He F, Zhang L, Yang K, Yu H, Zhou J, Gan H. MicroRNA 375 modulates hyperglycemia-induced enteric glial cell apoptosis and Diabetes-induced gastrointestinal dysfunction by targeting Pdk1 and repressing PI3K/Akt pathway. *Sci Rep* 2018; **8**: 12681 [PMID: 30140011 DOI: 10.1038/s41598-018-30714-0]
28 **Luo P**, He WX, Li C, Chang MJ. Enteric glial cells exert neuroprotection from hyperglycemia-induced damage *via* Akt/GSK3β pathway. *Neuroreport* 2021; **32**: 875-881 [PMID: 34029286 DOI: 10.1097/WNR.0000000000001670]
29 **Neunlist M**, Rolli-Derkinderen M, Latorre R, Van Landeghem L, Coron E, Derkinderen P, De Giorgio R. Enteric glial cells: recent developments and future directions. *Gastroenterology* 2014; **147**: 1230-1237 [PMID: 25305504 DOI: 10.1053/j.gastro.2014.09.040]
30 **Ochoa-Cortes F**, Turco F, Linan-Rico A, Soghomonyan S, Whitaker E, Wehner S, Cuomo R, Christofi FL. Enteric Glial Cells: A New Frontier in Neurogastroenterology and Clinical Target for Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 433-449 [PMID: 26689598 DOI: 10.1097/MIB.0000000000000667]
31 **Rao M**, Rastelli D, Dong L, Chiu S, Setlik W, Gershon MD, Corfas G. Enteric Glia Regulate Gastrointestinal Motility but Are Not Required for Maintenance of the Epithelium in Mice. *Gastroenterology* 2017; **153**: 1068-1081.e7 [PMID: 28711628 DOI: 10.1053/j.gastro.2017.07.002]
32 **Grubišić V**, Gulbransen BD. Enteric glia: the most alimentary of all glia. *J Physiol* 2017; **595**: 557-570 [PMID: 27106597 DOI: 10.1113/JP271021]
33 **Bassotti G**, Villanacci V, Maurer CA, Fisogni S, Di Fabio F, Cadei M, Morelli A, Panagiotis T, Cathomas G, Salerni B. The role of glial cells and apoptosis of enteric neurones in the neuropathology of intractable slow transit constipation. *Gut* 2006; **55**: 41-46 [PMID: 16041063 DOI: 10.1136/gut.2005.073197]
34 **Zhang DK**, He FQ, Li TK, Pang XH, Cui DJ, Xie Q, Huang XL, Gan HT. Glial-derived neurotrophic factor regulates intestinal epithelial barrier function and inflammation and is therapeutic for murine colitis. *J Pathol* 2010; **222**: 213-222 [PMID: 20632386 DOI: 10.1002/path.2749]
35 **Liu GX**, Yang YX, Yan J, Zhang T, Zou YP, Huang XL, Gan HT. Glial-derived neurotrophic factor reduces inflammation and improves delayed colonic transit in rat models of dextran sulfate sodium-induced colitis. *Int Immunopharmacol* 2014; **19**: 145-152 [PMID: 24462388 DOI: 10.1016/j.intimp.2014.01.008]
36 **Ahmadzai MM**, McClain JL, Dharshika C, Seguella L, Giancola F, De Giorgio R, Gulbransen BD. LPAR1 regulates enteric nervous system function through glial signaling and contributes to chronic intestinal pseudo-obstruction. *J Clin Invest* 2022; **132** [PMID: 35166239 DOI: 10.1172/JCI149464]
37 **Gao H**, Zhang Y, Li Y, Chang H, Cheng B, Li N, Yuan W, Li S, Wang Q. μ-Opioid Receptor-Mediated Enteric Glial Activation Is Involved in Morphine-Induced Constipation. *Mol Neurobiol* 2021; **58**: 3061-3070 [PMID: 33624141 DOI: 10.1007/s12035-021-02286-0]
38 **Feng Y**, Ju Y, Yan Z, Ji M, Yang M, Wu Q, Wang L, Sun G. Protective role of wogonin following traumatic brain injury by reducing oxidative stress and apoptosis *via* the PI3K/Nrf2/HO1 pathway. *Int J Mol Med* 2022; **49** [PMID: 35179214 DOI: 10.3892/ijmm.2022.5109]
39 **Wang S**, Zhou Y. Baicalein Inhibits Neuroapoptosis Via Pathways in Sevoflurane Induced Rats. *Transl Neurosci* 2018; **9**: 88-98 [PMID: 30042862 DOI: 10.1515/tnsci-2018-0015]
40 **Wang J**, Mao J, Wang R, Li S, Wu B, Yuan Y. Kaempferol Protects Against Cerebral Ischemia Reperfusion Injury Through Intervening Oxidative and Inflammatory Stress Induced Apoptosis. *Front Pharmacol* 2020; **11**: 424 [PMID: 32351385 DOI: 10.3389/fphar.2020.00424]
41 **Grewal AK**, Singh TG, Sharma D, Sharma V, Singh M, Rahman MH, Najda A, Walasek-Janusz M, Kamel M, Albadrani GM, Akhtar MF, Saleem A, Abdel-Daim MM. Mechanistic insights and perspectives involved in neuroprotective action of quercetin. *Biomed Pharmacother* 2021; **140**: 111729 [PMID: 34044274 DOI: 10.1016/j.biopha.2021.111729]
42 **Zhang N**, Hu Z, Zhang Z, Liu G, Wang Y, Ren Y, Wu X, Geng F. Protective Role Of Naringenin Against $A\beta_{25-35}$-Caused Damage *via* ER and PI3K/Akt-Mediated Pathways. *Cell Mol Neurobiol* 2018; **38**: 549-557 [PMID: 28699113 DOI: 10.1007/s10571-017-0519-8]

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ORIGINAL ARTICLE

Retrospective Cohort Study

Pregnancy and fetal outcomes of chronic hepatitis C mothers with viremia in China

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Abstract

BACKGROUND

Data that assess maternal and infant outcomes in hepatitis C virus (HCV)-infected mothers are limited.

AIM

To investigate the frequency of complications and the associated risk factors.

METHODS

We performed a cohort study to compare pregnancy and fetal outcomes of HCV-viremic mothers with those of healthy mothers. Risk factors were analyzed with logistic regression.

RESULTS

Among 112 consecutive HCV antibody-positive mothers screened, we enrolled 79 viremic mothers. We randomly selected 115 healthy mothers from the birth

registry as the control. Compared to healthy mothers, HCV mothers had a significantly higher frequency of anemia [2.6% (3/115) *vs* 19.0% (15/79), $P < 0.001$] during pregnancy, medical conditions that required caesarian section [27.8% (32/115) *vs* 48.1% (38/79), $P = 0.004$], and nuchal cords [9.6% (11/115) *vs* 34.2% (27/79), $P < 0.001$]. In addition, the mean neonatal weight in the HCV group was significantly lower (3278.3 ± 462.0 *vs* 3105.1 ± 459.4 gms; $P = 0.006$), and the mean head circumference was smaller (33.3 ± 0.6 *vs* 33.1 ± 0.7 cm; $P = 0.03$). In a multivariate model, HCV-infected mothers were more likely to suffer anemia [adjusted odds ratio (OR): 18.1, 95% confidence interval (CI): 4.3-76.6], require caesarian sections (adjusted OR: 2.6, 95%CI: 1.4-4.9), and have nuchal cords (adjusted OR: 5.6, 95%CI: 2.4-13.0). Their neonates were also more likely to have smaller head circumferences (adjusted OR: 2.1, 95%CI: 1.1-4.3) and lower birth weights than the average (≤ 3250 gms) with an adjusted OR of 2.2 (95%CI: 1.2-4.0). The vertical transmission rate was 1% in HCV-infected mothers.

CONCLUSION

Maternal HCV infections may associate with pregnancy and obstetric complications. We demonstrated a previously unreported association between maternal HCV viremia and a smaller neonatal head circumference, suggesting fetal growth restriction.

Key Words: Hepatitis C virus viremia; Mother-to-child transmission; Pregnancy complications; Maternal health; Infant hepatitis C virus infection

Core Tip: Although hepatitis C virus (HCV) affects a significant number of pregnant women, there is limited data regarding the impact of HCV active infection on pregnancy and infant outcomes. The current cohort study compared maternal complications and fetal development of HCV mothers with detectable levels of HCV RNA with those of healthy mothers. The study demonstrates a previously unreported association between maternal HCV viremia and a smaller neonate head circumference. In addition, HCV viremia was an independent predictor for negative maternal outcomes including anemia during pregnancy, medical conditions that required caesarian section, and nuchal cords. These findings increase the need for close antenatal surveillance in HCV mothers with viremia for maternal complications and delayed fetal development.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a common infectious disease that affects the liver and remains a significant global health burden[1]. Although spontaneous viral clearance may occur in approximately 15% of patients who have acute HCV infection, the majority develop a chronic HCV infection. Among patients who have chronic hepatitis C, approximately 10%-15% will progress to cirrhosis within the first 20 years of infection, which eventually becomes decompensated without appropriate therapy and places them at high risk of developing liver cancer[2]. The prevalence of antibodies to HCV (HCV-Ab) in pregnant women is 0.1% to 2.4%, although it is much higher in some endemic areas[3]. The proportion of pregnant women with HCV-Ab positivity and active infection with viremia is approximately 60% to 70%[3].

Globally, up to 8% of pregnant women are infected with HCV in highly endemic areas[4]. In the United States, surveillance published in 2017 revealed a nationwide increase in HCV infection among pregnant women, which is an increasing but potentially modifiable threat to maternal and child health [5]. The proportion of infants born to HCV-infected women is also increasing in the United States[6]. It has been reported that vertical transmission is the most common mechanism of HCV infection in children, occurring in approximately 6% of infants born to women with HCV infection[7]. The risk of HCV vertical transmission increases if the maternal serum HCV viral load is above 10^5 copies/mL[8,9]. In addition, published studies have suggested that vertical transmission encompasses several potential transmission routes from an infected woman to her newborn, including intrauterine, intrapartum, and postnatal routes[10-13]. According to the American Association for the Study of Liver Diseases

guidelines, all pregnant women should be tested for HCV infections, ideally at the time of initiation of prenatal care[14].

Although HCV affects a significant number of pregnant women, there are limited data regarding the impact of HCV active infection on pregnancy and infant outcomes. Prior studies of HCV and pregnancy have focused on the vertical transmission rates of HCV infection using limited assessments of the effects of chronic HCV infections on maternal health, complications during delivery, and fetal complications [15]. Therefore, there are data gaps in supporting strategies for the clinical management of mothers with HCV infections during pregnancy. Additionally, the identification of adverse consequences could improve current perinatal care and monitoring recommendations. With that in mind, we conducted a retrospective cohort study to compare the frequency and severity of adverse maternal outcomes during pregnancy, as well as fetal and infant outcomes, between mothers with HCV viremia and healthy mothers.

MATERIALS AND METHODS

Study design, setting, and patient selection

This is a single-center retrospective observational cohort study conducted at a tertiary referral university hospital located in Shijiazhuang city of Hebei province in China, which receives referrals from different levels of community medical clinics and health facilities in the city. The study site mainly included mothers with infectious diseases, including hepatitis B, hepatitis C, and human immunodeficiency virus (HIV). The Institutional Review Board approved the study, and the need for informed consent was waived. Local standards of care for prenatal care include regular clinic visits approximately every 4 to 6 wk during pregnancy for mothers who are infected with chronic viral hepatitis. Mothers received a symptom-directed physical exam, blood tests, and ultrasonography exams from the early second trimester to delivery. Viral hepatitis and HIV screening were performed at the first prenatal visit (often during the first or early second trimester), and hospital delivery was mandated in the entire province except in an emergency event.

In the current study, patients who attended the services in the prenatal care clinic from November 1, 2011 to May 31, 2020 were screened for eligibility. Adult patients (age > 18 years old) who had a diagnosis of HCV-Ab positivity for at least six months and detectable levels of HCV RNA (> 15 IU/mL) during prenatal screening were eligible for enrollment. Major exclusion criteria were the following: Coinfection with hepatitis B virus, hepatitis D virus, or HIV; current or history of intravenous drug use or sexually transmitted diseases; liver cancer; autoimmune liver disease; primary biliary cirrhosis; and alcohol-related liver diseases (consumption of more than 20 g/day of alcohol for > 5 years). Patients with other liver diseases, including inherited liver diseases and drug-induced liver injury, were also excluded. For each patient included in the HCV group, a healthy mother was identified and selected from the Delivery Suite Registry at random. The selection was based on their infants' date of birth (± 30 d) matched to those of the HCV mothers with similar baseline values (matched for gestational days and parity). Based on the ratio of approximately 1:1 to match the number of subjects enrolled in the HCV group, a similar number of subjects was included in the healthy mother group. All patients included in the study were not smoking, drinking alcohol, or using any recreational drugs since these variables may affect the infants' outcomes.

Laboratory measurements for subjects in the study were all performed by the central laboratory in the medical center. HCV-Ab was tested by a chemiluminescent microparticle immunoassay (Autobio, Zhengzhou, China). Serum HCV RNA levels were measured with the real-time quantitative polymerase chain reaction method by using the Cobas TaqMan polymerase chain reaction assay according to the laboratory manuals (Roche Diagnostics, United States). An undetectable level was defined as below the lowest level of quantitation = 15 IU/mL. The comprehensive chemistry panel was tested using a HITACHI 7600 fully automatic biochemical analyzer, with the ULN of alanine aminotransferase (ALT) set at 40 U/L (Wako Pure Chemical Industries, Ltd. Japan).

Patient data collection and outcome assessment

Using an electronic medical record system and paper charts, we collected the following maternal data: Patients' demographic information and pertinent clinical data, including a history of liver disease or hepatocellular carcinoma, pregnancy or obstetric complications; medication lists; positive physical findings, including pelvimetry, labor outcomes, and modes of delivery; laboratory results of completed blood count, coagulation tests, chemistry panels with ALT, and virological tests; and imaging results if available. Pertinent data were assessed at all visits starting from gestational week 12 with a four-week interval before delivery, at delivery, and at postpartum weeks 12, 24, and 36. Perinatal information for fetal development, including birth weight, height, Apgar scores, gestational age, and perinatal complications such as birth trauma and neonatal jaundice, was extracted from the neonatal records. Infant outcomes, such as intrauterine growth restriction, birth defects, macrosomia, low birth weight, and meconium staining stool, were collected.

The primary assessment was to analyze the frequency of maternal complications (both pregnancy and obstetrics complications) and negative fetal outcomes in HCV-infected mothers with viremia *vs* those in healthy mothers. In addition, vertical transmission rates were analyzed among mothers with HCV infection. Secondary assessments were the association between demographic or clinical features and negative maternal or fetal outcomes in a multivariable logistic regression analysis. The current study used the following criteria to define the vertical transmission of HCV and considered the transmission confirmed if any of the following occurred: (1) Detection of HCV RNA in an infant who is 3 to 6 months old; (2) Detection of HCV RNA in the infant on at least 2 occasions; (3) Finding elevated serum aminotransferase levels in an HCV-Ab positive child (ULN = 40 U/mL); or (4) Confirming an identical viral genotype between mother and child[3].

Statistical analysis

Data analyses were performed using the Statistical Package for Social Science for Windows, Version 25.0 (SPSS Inc., IBM, New York, United States). Frequencies and percentages were used to summarize the categorical variables. Fisher's exact tests or chi-squared tests were used when comparing data between and within groups. Depending on the underlying distribution of the data, descriptive values are expressed as the means ± SD or medians and interquartile ranges. The student's t-test was used to assess continuous variables between groups. The maternal outcomes or infant outcomes were calculated *per* pregnant mother and/or *per* infant when appropriate. The baseline demographic or characteristic variables were analyzed as independent variables, whereas the negative maternal or infant outcomes were considered dependent variables. Risk factors identified from the univariate analysis (P value < 0.05) were further analyzed in the multivariate logistic regression model. The aforementioned risk factors associated with negative outcomes are presented with crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs). All tests were two-tailed with a 95%CI, and a P value < 0.05 was considered significant.

RESULTS

Patient characteristics

Among the 122 consecutive HCV antibody-positive pregnant women screened, 30 were not eligible due to undetectable levels of HCV RNA throughout the pregnancy. In addition, 13 patients were excluded because they were coinfected with HIV (n = 6) or hepatitis B virus (n = 7). As a result, seventy-nine patients who had HCV viremia during pregnancy were eligible for the HCV group. In addition, 115 healthy mothers were identified and selected from the Delivery Suite Registry at random (delivery date ± 30 d matched to those cases in the HCV-infected group with similar baseline variables). As a result, our cohort consisted of 194 pregnant women with 79 and 115 mothers in the HCV group and a healthy mother (noninfected) group, respectively. The patient selection process is shown in Figure 1. All patients in the HCV group had no clinical indicator for liver decompensation. The clinical characteristics of the study patients are presented in Table 1. The demographic characteristics were well matched between the two groups in the majority of variables, including pre-pregnancy mean BMI, the number of parities or pluralities, mean gestational days, and mean ALT at delivery. However, mothers in the healthy group had a significantly older mean age (29.4 ± 4.9 *vs* 25.8 ± 4.7 years, P < 0.001), a low frequency of intertuberous diameter < 8.5 cm (29.6% *vs* 48.1%, P = 0.009) and were taller (160.9 ± 4.0 *vs* 159.6 ± 3.8 cm, P < 0.001) than those in the HCV group.

Maternal outcomes

Data from gestational week 12 to delivery about pregnancy or obstetric complications and maternal laboratory abnormalities were analyzed. The following pregnancy and obstetric complications were identified in both groups (Table 2): Preterm labor, preeclampsia, eclampsia, gestational hypertension, anemia, abnormal renal or thyroid function, oligohydramnios, gestational diabetes, nuchal cord, umbilical cord prolapses, postpartum hemorrhage, premature rupture of membranes, and cesarean section due to medical needs. When comparing the aforementioned outcomes or laboratory abnormalities between the HCV-infected and healthy individuals, a significantly higher frequency of anemia during pregnancy was observed in the HCV group [19.0% (15/79) *vs* 2.6% (3/115), P < 0.001]. In addition, a significantly higher frequency of nuchal cords [34.2% (27/79) *vs* 9.6% (11/115); P < 0.001] and cesarean sections due to medical needs [48.1% (38/79) *vs* 27.8% (32/115); P = 0.004] was reported in the HCV group. The frequencies of other pregnancy or obstetric complications did not differ between the two groups (Table 2).

Fetal and infant outcomes

When comparing the fetal and infant outcomes between the HCV-infected and healthy mother groups (Table 3), we observed a significantly lower mean ± SD body weight in neonates who were born to HCV-infected mothers (3105.1 ± 459.4 *vs* 3278.3 ± 462.0 gms; P = 0.006). However, the frequency of low

Table 1 The demographic and clinical characteristics of mothers and infants

Variables presented with *n* (%), mean ± SD, or specified	HCV mothers with viremia (*n* = 79)[1]	Healthy mothers (*n* = 115)[1]	*P* value
Maternal characteristics			
Age (yr)	25.8 ± 4.7	29.4 ± 4.9	< 0.001
< 20	2/79 (2.5)	0/115 (0.0)	0.003
20-34	73/79 (92.4)	94/115 (81.7)	
≥ 35	4/79 (5.1)	21/115 (18.3)	
Height, cm	159.6 ± 3.8	160.9 ± 4.0	0.02
Pre-pregnancy BMI	27.4 ± 3.4	27.5 ± 3.1	0.80
< 23	4/79 (5.1)	3/115 (2.6)	0.44
23-29	53/79 (67.1)	86/115 (74.8)	
≥ 29	22/79 (27.8)	26/115 (22.6)	
History of miscarriage	1/79 (1.3)	1/115 (0.9)	> 0.99
History of stillbirth	2/79 (2.5)	2/115 (1.7)	> 0.99
Previous uterine surgery	28/79 (35.4)	35/115 (30.4)	0.46
Parity			
0-1	47/79 (59.5)	62/115 (53.9)	0.44
≥ 2	32/79 (40.5)	53/115 (46.1)	
Plurality			
0-1	47/79 (59.5)	62/115 (53.9)	0.44
> 2	32/79 (40.5)	53/115 (46.1)	
Gestational (days)	273.8 ± 9.5	276.0 ± 9.1	0.11
Interspinous distance, median (IRQ), cm	25.0 (24.0, 25.0)	25.0 (24.0, 25.0)	0.23
Intercristal distance, median (IRQ), cm	27.0 (27.0, 28.0)	27.0 (27.0, 28.0)	0.40
External conjugate, median (IRQ), cm	20.0 (20.0, 21.0)	20.0 (20.0, 21.0)	0.33
Intertuberous diameter, median (IRQ), cm	8.5 (8.0, 8.5)	8.5 (8.0, 8.5)	0.006
< 8.5	38/79 (48.1)	34/115 (29.6)	0.009
≥ 8.5	41/79 (51.9)	81/115 (70.4)	
Hemoglobin, median (IRQ), g/L	111.0 (100.0, 119.0)	110.0 (100.0, 119.0)	0.88
< 110	37/79 (46.8)	51/115 (44.3)	0.73
≥ 110	42/79 (53.2)	64/115 (55.7)	
Platelet, median (IRQ), × 109/L	207.0 (166.0, 242.0)	201.0 (167.0, 242.0)	0.95
Prothrombin time, median (IRQ), seconds	10.6 (10.1, 11.1)	10.5 (10.0, 11.1)	0.40
ALT at delivery, U/L	26.3 ± 20.1	16.6 ± 56.7	0.15
HCV RNA at delivery, IU/mL	19, 217, 509 ± 35, 745, 723	-	-

[1]All mothers in the study had a singleton pregnancy.

HCV: Hepatitis C virus; ALT: Alanine aminotransferase; IRQ: Interquartile range; BMI: Body mass index.

birth weight (< 2500 g) did not differ between the two groups [8.9% (7/79) *vs* 3.5% (4/115); $P = 0.20$]. The other variables did not differ between the two groups. In addition, neonates in the HCV group had a significantly smaller mean head circumference (33.1 ± 0.7 *vs* 33.3 ± 0.6 cm; $P = 0.03$). The other measurements did not differ between the two groups, which included gestational weeks, the percentage of neonates that reached full-term or small for gestational age at delivery, and the mean height at birth. There were no miscarriages, stillbirths, birth defects, or Apgar scores < 7 at 5 min after birth in the entire cohort.

Table 2 Maternal outcomes in the hepatitis C virus viremic group *vs* the control group

Variables, *n* (%)	HCV-infected group (*n* = 79), %	Healthy mother group (*n* = 115), %	*P* value
Pregnancy complications			
Preterm (< 37 wk)	1/79 (1.3)	0/115 (0.0)	> 0.99
Preeclampsia	3/79 (3.8)	0/115 (0.0)	0.07
Eclampsia	0/79 (0.0)	2/115 (2.5)	0.17
Gestational hypertension	0/79 (0.0)	4/115 (3.5)	0.15
Abnormal thyroid function	4/79 (5.1)	1/115 (0.9)	0.16
Gestational diabetes	4/79 (5.1)	4/115 (3.5)	0.72
Oligohydramnios	6/79 (7.6)	3/115 (2.6)	0.16
Abnormal renal function	49/79 (62.0)	64/115 (55.7)	0.38
Anemia during pregnancy	15/79 (19.0)	3/115 (2.6)	< 0.001
Obstetric complications			
Rates of cesarean section[1]	38/79 (48.1)	32/115 (27.8)	0.004
Nuchal cord	27/79 (34.2)	11/115 (9.6)	< 0.001
Umbilical cord prolapses	1/79 (1.3)	0/115 (0.0)	0.41
Postpartum hemorrhage	1/79 (1.3)	0/115 (0.0)	0.41
Premature rupture of membranes	3/79 (3.8)	4/115 (3.5)	> 0.99

[1]The rates of cesarean section were calculated based on the number of cases performed due to medical needs. Patients who requested a cesarean section without medical indications were not included.

Among infants who were born to HCV-infected mothers, all were tested HCV-Ab positive at birth, and one had a detectable level of HCV RNA (2165 IU/mL). All infants in the study cohort were breastfed. Their HCV-Ab became negative beyond six months, except for the one who had HCV viremia at birth. This infant continued to have HCV antibodies and detectable levels of HCV RNA measured at the ages of three months and nine months, meeting the criteria of chronic hepatitis C infection. The HCV transmission rate in our study was 1.3% (n = 1/79). In the review of maternal characteristics, the mother was 25 years old with a maternal HCV RNA level of 2.58 × 5 Log_{10} IU/mL at delivery. She had a history of blood transfusion and was diagnosed with chronic HCV infection during prenatal screening. Her pregnancy was uneventful, with normal levels of ALT throughout the entire pregnancy. She delivered a girl with normal physical development at gestational week 39 plus 5 d.

Risk factors associated with negative outcomes

When comparing the pregnancy and obstetric complications between the two groups, we found that a significantly higher frequency of anemia, nuchal cord, and cesarean section due to medical needs occurred among HCV-infected mothers. The crude and adjusted ORs with 95%CIs of each risk factor are presented in Table 4. The analyses indicated that HCV infection was the only factor associated with anemia (adjusted OR: 18.1, 95%CI: 4.3-76.6), increased numbers of C sections due to medical needs (adjusted OR: 2.6, 95%CI: 1.4-4.9), and nuchal cords during pregnancies (adjusted OR: 5.6, 95%CI: 2.4-13.0). Since a significantly smaller head circumference and lower mean birth weight were the only two negative fetal outcomes identified in infants from HCV-infected mothers, we analyzed the maternal risk factors (Table 5) and found that maternal HCV infection was associated with these negative outcomes. The adjusted ORs of maternal HCV infection associated with a smaller head circumference and birth weight ≤ 3250 gms were 2.1 (95%CI: 1.1-4.3) and 2.2 (95%CI: 1.2-4.0), respectively.

DISCUSSION

Although HCV vertical transmission can occur in up to 5.8% of mother-infant pairs[16], many children can clear HCV infection spontaneously[17]. The disease can also be cured with oral antiviral therapy starting at the age of 3[17]. Therefore, the clinical landscape of managing HCV-infected mothers has recently shifted from addressing HCV vertical transmission to the assessment and management of negative pregnancy or neonatal outcomes. Published studies have linked several negative pregnancy outcomes to maternal HCV infection, including intrahepatic cholestasis[18-20], gestational diabetes[21-

Table 3 The fetal and infant outcomes in the two study groups

Infant characteristics at birth[1] variables presented with *n* (%) or mean ± SD	Infants from HCV-infected mothers (*n* = 79)	Infants from healthy mothers (*n* = 115)	*P* value
Gestational weeks	39.1 ± 1.4	39.4 ± 1.3	0.11
Full-term birth	74/79 (93.7)	107/115 (93.0)	0.86
Meconium staining positive	8/79 (10.1)	11/115 (9.6)	> 0.99
Height at birth, cm	49.0 ± 1.6	49.4 ± 1.0	0.05
Head circumference, cm	33.1 ± 0.7	33.3 ± 0.6	0.03
Weight at birth, gms	3105.1 ± 459.4	3278.3 ± 462.0	0.006
≤ 3250 gms	49/79 (62.0)	52/115 (45.2)	0.02
< 2500 gms	7/79 (8.9)	4/115 (3.5)	0.20
Small for gestational age[2]	1/79 (1.3)	1/115 (0.9)	> 0.99
Birth defects	0/79 (0.0)	0/115 (0.0)	> 0.99
Apgar score < 7 at 5 min	0/79 (0.0)	0/115 (0.0)	> 0.99
HCV-Ab (+) at birth, *n* (%)	79/79 (100)	-	-
Detectable HCV RNA, *n* (%)	1/79 (1.27)	-	-

[1]All cases in the study were singleton.
[2]Small for gestational age; defined as a birth weight below the 10th percentile for each gestational age using sex-specific criteria.
HCV: Hepatitis C virus.

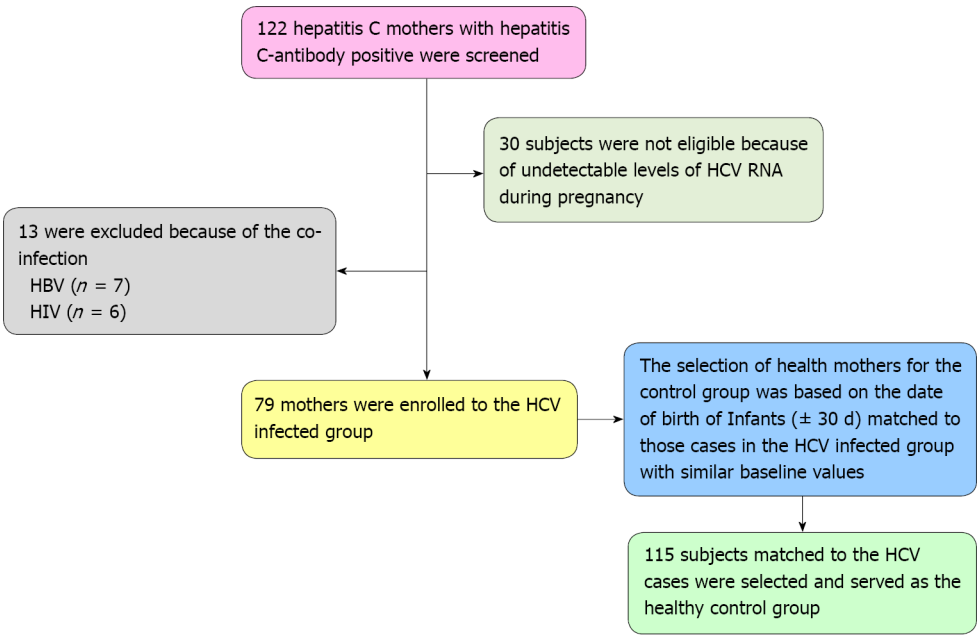

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Figure 1 Flow Chart for patients enrolled in the study. Data analyses included 194 patients. Among the 122 patients with hepatitis C antibody positive evaluated, 79 were eligible and enrolled. A control group with healthy uninfected patients (*n* = 115) was selected to match the infected patients. Patients were divided into two groups: a hepatitis C patient group and a healthy control group comparison. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

23], the premature rupture of the membranes[24,25], the requirement for cesarean delivery[24,25], preterm delivery[23], small for gestational age[26], and low birth weight[26]. However, its effect on restriction or disturbance of intrauterine fetal growth remains inconclusive[21,23,26-28].

Our study assessed both maternal and fetal outcomes in viremic mothers with HCV infections. To our knowledge, this is the first study from China to assess pregnancy outcomes in HCV viremic mothers. We found that HCV might be associated with a higher frequency of nuchal cords and a smaller neonatal

Table 4 Risk factors associated with obstetric complications

Clinical variables	Case/exposed[1]	Crude OR (95%CI); *P* value	Adjusted OR (95%CI); *P* value
C-section[2]			
Age, *n* (%)			
< 35	59/194 (30.4)	1	1
≥ 35	11/194 (5.7)	1.5 (0.6-3.4); *P* = 0.38	1.5 (0.6-3.9); *P* = 0.42
Nulliparity, *n* (%)			
No	37/194 (19.1)	1	1
Yes	33/194 (17.0)	0.6 (0.3-1.0); *P* = 0.06	0.6 (0.3-1.1); *P* = 0.08
BMI, *n* (%)			
< 30	57/194 (29.4)	1	1
≥ 30	13/194 (6.7)	1.3 (0.6-2.7); *P* = 0.56	1.2 (0.5-2.7); *P* = 0.65
HCV infection, *n* (%)			
No	32/194 (16.5)	1	1
Yes	38/194 (19.6)	2.4 (1.3-4.4); *P* = 0.004	2.6 (1.4-4.9); *P* = 0.003
Intertuberous diameter, *n* (%)			
≥ 8.5	41/194 (21.1)	1	1
< 8.5	29/194 (14.9)	1.3 (0.7-2.2); *P* = 0.41	2.6 (1.4-4.9); *P* = 0.65
Nuchal cord			
Age, *n* (%)			
< 35	33/194 (17.0)	1	1
≥ 35	5/194 (2.6)	1.0 (0.4-2.9); *P* = 0.96	2.8 (0.8-10.3); *P* = 0.11
Nulliparity, *n* (%)			
No	13/194 (6.7)	1	1
Yes	25/194 (12.9)	1.6 (0.8-3.5); *P* = 0.19	2.0 (0.9-4.8); *P* = 0.11
BMI, *n* (%)			
< 30	32/194 (16.5)	1	1
≥ 30	6/194 (3.1)	0.9 (0.4-2.5); *P* = 0.90	0.7 (0.2-1.9); *P* = 0.44
HCV infection, *n* (%)			
No	11/194 (5.7)	1	1
Yes	27/194 (13.9)	4.9 (2.3-10.7); *P* < 0.001	5.6 (2.4-13.0); *P* < 0.001
Intertuberous diameter, *n* (%)			
≥ 8.5	20/194 (10.3)	1	1
< 8.5	18/194 (9.3)	1.3 (0.7-2.4); *P* = 0.35	1.3 (0.6-2.8); *P* = 0.51
Maternal anemia during pregnancy			
Age, *n* (%)			
< 35	19/194 (9.8)	1	1
≥ 35	3/194 (1.5)	1.1 (0.3-3.9); *P* = 0.91	2.5 (0.5-13.3); *P* = 0.28
Nulliparity, *n* (%)			
No	13/194 (6.7)	1	1
Yes	9/194 (4.6)	0.5 (0.2-1.2); *P* = 0.13	0.5 (0.2-1.4); *P* = 0.18
BMI, *n* (%)			
< 30	20/194 (10.3)	1	1

≥ 30	2/194 (1.0)	0.5 (0.1-2.1); $P = 0.33$	0.4 (0.1-1.8); $P = 0.21$
HCV infection, *n* (%)			
No	3/194 (1.5)	1	1
Yes	19/194 (9.8)	11.8 (3.4-41.6); $P < 0.001$	18.1 (4.3-76.6); $P < 0.001$
Intertuberous diameter, *n* (%)			
≥ 8.5	13/194 (6.7)	1	1
< 8.5	9/194 (4.6)	1.2 (0.5-3.0); $P = 0.35$	0.8 (0.3-2.3); $P = 0.72$

[1]Case/exposed: The case refers to the number of patients who presented with the specified variable and the Exposed refers to the total number of patients in the entire study cohort.
[2]The rates of cesarean section were calculated based on the number of cases performed due to medical needs. Patients who requested a cesarean section without medical indications were not included.
BMI: Body mass index; OR: Odds ratio; HCV: Hepatitis C virus.

head circumference, which has not been reported in the literature before. We also found that HCV viremia was linked to pregnancy anemia, cesarean sections due to medical needs, and low gestational weight in neonates. In addition, we observed that lower birth weight was associated with maternal infection, which was consistent with published data from the United States and Europe[23,26]. In the context of all infants in our study being breastfed, the HCV vertical transmission rate was 1.27%, which was within the range (0.2%-6%) of the already published studies[16,29].

Early studies by Jaffery *et al*[30] and Bohman *et al*[31] found that fetal outcomes did not differ between HCV-positive mothers and healthy controls[30,31]. However, in a study by Salemi *et al*[29], the risk of an adverse neurological outcome was higher in infants born to HCV mothers, including feeding difficulties (OR: 1.32, 95%CI: 1.06-1.64) and neonatal seizures (OR: 1.74, 95%CI: 0.98-3.10)[29]. The aforementioned studies have limitations of lacking a well-defined study population because the diagnosis of HCV was based on the HCV antibody, and HCV RNA was not always tested. Paternoster *et al*[24] observed that intrahepatic cholestasis was more common in HCV-RNA-positive mothers than in HCV-RNA-negative mothers, suggesting that HCV viremia may lead to different outcomes[19]. In addition, cofounders such as intravenous drug use or sexually transmitted diseases may not be adjusted in studies based on pregnancy registries[26,32]. These factors could contribute to the discrepancies among the study findings. In the context of the paucity of data and infrequency of fetal negative events, Huang *et al*[33] performed a meta-analysis and found that low birth weight was linked to maternal HCV infection (OR: 1.97, 95%CI: 1.43-2.71)[33].

In our study, birth weight ≤ 3250 gms was associated with HCV exposure. There was also a trend of HCV-exposed neonates with a birth weight of < 2500 gms. More importantly, our cohort demonstrates a previously unreported association between maternal HCV viremia and a smaller neonatal head circumference. Our findings provide new evidence supporting the intrauterine restriction of fetal growth in a well-defined HCV population, which enrolled only HCV-infected mothers with detectable levels of HCV RNA who had no history of intravenous drug use or sexually transmitted diseases.

Although the mechanism of fetal growth restriction is not fully understood, several studies have suggested that HCV-induced inflammation in the placenta may cause fetal development restriction. In an *in vitro* study, HCV infected a human cytotrophoblast and changed its ultrastructure dramatically upon infection[33]. In addition, Hurtado *et al*[34] observed that the cytotoxicity of natural killer cells and natural killer T cells was enhanced in the placenta, and placental natural killer T-cell cytotoxicity was further increased by HCV infections[34]. Several population-based retrospective cohort studies reported higher rates of gestational diabetes in HCV-infected mothers than in noninfected mothers[35,36], but the association was limited to women with excessive weight gain during pregnancy. Our study did not show such complications, which is likely because our patients are Asians with a much lower body mass index than those in other studies[21,24,26].

In this cohort study, some limitations should be addressed. Being a single-center retrospective design, this study has a limited capacity when adjusting or balancing all covariates between the HCV-exposed and HCV nonexposed groups. Additionally, we did not have HCV genotype data. However, published studies in China indicated that the majority of Chinese patients with HCV had genotype 1[36]. Second, cohort data about HCV antibody-positive but nonviremic mothers are limited: These mothers were not enrolled in our study due to the small number of patients in our center ($n = 30$, Figure 1). Further studies in this subgroup will add to the understanding of pregnancy outcomes. Third, the liver fibrosis stages for patients with HCV infection were not assessed in this study, although all patients had no clinical indications of liver decompensation. Therefore, future studies might be needed to investigate whether HCV-infected patients with advanced fibrosis have negative maternal and fetal outcomes. Last, the frequency of negative events in HCV-infected mothers could be underestimated due to the maternal mean age being younger than that of healthy mothers.

Table 5 Risk factors associated with fetal negative outcomes

Clinical variables	Case/exposed[1]	Crude OR (95%CI)	Adjusted OR (95%CI)
Head circumference ≤ 33 cm at birth			
Maternal age, *n* (%)			
< 35	118 /194 (60.8)	1	1
≥ 35	18 /194 (9.3)	1.1 (0.4-2.8); *P* = 0.82	2.0 (0.7-5.7); *P* = 0.19
Nulliparity, *n* (%)			
No	53/194 (27.3)	1	1
Yes	83 /194 (42.8)	1.9 (1.0-3.6); *P* = 0.04	2.4 (1.2-4.9); *P* = 0.01
BMI, *n* (%)			
< 30	120/194 (61.9)	1	1
≥ 30	16/194 (8.2)	0.4 (0.2-0.8); *P* = 0.008	0.3 (0.1-0.7); *P* = 0.003
HCV infection, *n* (%)			
No	75/194 (38.7)	1	1
Yes	61/194 (31.4)	1.8 (0.9-3.5); *P* = 0.08	2.1 (1.1-4.3); *P* = 0.03
Intertuberous diameter, *n* (%)			
≥ 8.5	85/194 (43.8)	1	1
< 8.5	51/194 (26.3)	1.1 (0.6-2.0); *P* = 0.86	1.0 (0.5-2.0); *P* = 0.75
Weight at birth ≤ 3250 gms			
Age, *n* (%)			
< 35	88/194 (45.4)	1	1
≥ 35	13/194 (6.7)	1.0 (0.4-2.3); *P* > 0.99	1.7 (0.6-4.3); *P* = 0.29
Nulliparity, *n* (%)			
No	39/194 (20.1)	1	1
Yes	62/194 (32.0)	1.6 (0.9-2.8); *P* = 0.13	1.8 (0.9-3.4); *P* = 0.07
BMI, *n* (%)			
< 30	88/194 (45.4)	1	1
≥ 30	13 /194 (6.7)	0.6 (0.3-1.2); *P* = 0.16	0.5 (0.2-1.1); *P* = 0.09
HCV infection HCV, *n* (%)			
No	52/194 (26.8)	1	1
Yes	49/194 (25.3)	2.0 (1.1-3.5); *P* = 0.02	2.2 (1.2-4.0); *P* = 0.01
Intertuberous diameter, *n* (%)			
≥ 8.5	61/194 (31.4)	1	1
< 8.5	40/194 (20.6)	1.2 (0.7-2.2); *P* = 0.46	1.1 (0.6-2.1); *P* = 0.66

[1]Case/exposed: The case refers to the number of patients who presented with the specified variable and the Exposed refers to the total number of patients in the entire study cohort.
BMI: Body mass index; OR: Odds ratio.

CONCLUSION

In conclusion, our study demonstrates a previously unreported association between maternal HCV viremia and a smaller neonatal head circumference. Given our new findings on the intrauterine restriction of fetal growth from HCV exposure, screening all mothers during pregnancy for HCV should be a mandatory practice. More importantly, our findings indicate a need for close antenatal surveillance for maternal complications and delayed fetal development in HCV mothers with viremia. Last, our data support that preconception health management should include HCV screening, so HCV infection can be treated before pregnancy to improve the health of both the mothers and infants.

ARTICLE HIGHLIGHTS

Research background

Hepatitis C virus (HCV) infection remains a significant global health burden, and there is a high proportion of women with antibodies to HCV positive whose active infection with viremia. In addition, HCV infection among pregnant women is an increasing but potentially modifiable threat to maternal and child health.

Research motivation

Although HCV affects a significant number of pregnant women, there is limited data regarding the impact of HCV active infection on pregnancy and infant outcomes. Therefore, there are data gaps in supporting strategies for clinical management of mothers with HCV infections during pregnancy.

Research objectives

We conducted a retrospective cohort study to compare the frequency and severity of adverse maternal outcomes during pregnancy, as well as fetal and infant outcomes between mothers with HCV viremia and healthy mothers.

Research methods

A retrospective observational cohort study was conducted to compare pregnancy and fetal outcomes of HCV-viremic mothers with those of healthy mothers. After HCV mothers with viremia and healthy mothers were enrolled, we collected their demographic information and pertinent clinical data using an electronic medical record system and paper charts. Perinatal information for fetal development and infant outcomes were extracted from the neonatal records. Data analyses were performed using the Statistical Package for Social Science for Windows, Version 25.0 (SPSS Inc., IBM, New York, United States).

Research results

Our study enrolled 79 viremic mothers and 115 healthy mothers. Compared to healthy mothers, HCV mothers had a significantly higher frequency of anemia, caesarian section, and nuchal cords during pregnancy. In addition, the mean neonatal weight and head circumference in the HCV group was significantly lower. In a multivariate model, similar results were found.

Research conclusions

Our study demonstrates the association between maternal HCV viremia and a smaller neonate head circumference. We also confirmed the high frequency of pregnancy and obstetric complications in HCV viremic mothers.

Research perspectives

Multi-center and large sample studies are needed to verify these results in the future and to investigate if HCV-infected patients with advanced fibrosis have negative maternal and fetal outcomes.

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FOOTNOTES

Author contributions: Pan CQ provided the concept and designed the study, wrote the manuscript, communicated with the journal, and addressed comments from reviewers; Dai EH and Zhu BS obtained the funding and supervised the study; Pan CQ, Zhang XH, and Dai EH performed data analyses; All other authors contributed to the data collection.

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REFERENCES

1 National Institutes of Health Consensus Development Conference Statement: Management of hepatitis C 2002 (June 10-12, 2002). *Gastroenterology* 2002; **123**: 2082-2099 [PMID: 12454863 DOI: 10.1053/gast.2002.1232082]
2 **Chen SL**, Morgan TR. The natural history of hepatitis C virus (HCV) infection. *Int J Med Sci* 2006; **3**: 47-52 [PMID: 16614742 DOI: 10.7150/ijms.3.47]
3 **Roberts EA**, Yeung L. Maternal-infant transmission of hepatitis C virus infection. *Hepatology* 2002; **36**: S106-S113 [PMID: 12407583 DOI: 10.1053/jhep.2002.36792]
4 **Spera AM**, Eldin TK, Tosone G, Orlando R. Antiviral therapy for hepatitis C: Has anything changed for pregnant/Lactating women? *World J Hepatol* 2016; **8**: 557-565 [PMID: 27134703 DOI: 10.4254/wjh.v8.i12.557]
5 **Patrick SW**, Bauer AM, Warren MD, Jones TF, Wester C. Hepatitis C Virus Infection Among Women Giving Birth - Tennessee and United States, 2009-2014. *MMWR Morb Mortal Wkly Rep* 2017; **66**: 470-473 [PMID: 28493860 DOI: 10.15585/mmwr.mm6618a3]
6 **Koneru A**, Nelson N, Hariri S, Canary L, Sanders KJ, Maxwell JF, Huang X, Leake JA, Ward JW, Vellozzi C. Increased Hepatitis C Virus (HCV) Detection in Women of Childbearing Age and Potential Risk for Vertical Transmission - United States and Kentucky, 2011-2014. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 705-710 [PMID: 27442053 DOI: 10.15585/mmwr.mm6528a2]
7 **Benova L**, Mohamoud YA, Calvert C, Abu-Raddad LJ. Vertical transmission of hepatitis C virus: systematic review and meta-analysis. *Clin Infect Dis* 2014; **59**: 765-773 [PMID: 24928290 DOI: 10.1093/cid/ciu447]
8 **Ceci O**, Margiotta M, Marello F, Francavilla R, Loizzi P, Francavilla A, Mautone A, Impedovo L, Ierardi E, Mastroianni M, Bettocchi S, Selvaggi L. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. *J Pediatr Gastroenterol Nutr* 2001; **33**: 570-575 [PMID: 11740231 DOI: 10.1097/00005176-200111000-00011]
9 **Hayashida A**, Inaba N, Oshima K, Nishikawa M, Shoda A, Hayashida S, Negishi M, Inaba F, Inaba M, Fukasawa I, Watanabe H, Takamizawa H. Re-evaluation of the true rate of hepatitis C virus mother-to-child transmission and its novel risk factors based on our two prospective studies. *J Obstet Gynaecol Res* 2007; **33**: 417-422 [PMID: 17688606 DOI: 10.1111/j.1447-0756.2007.00582.x]
10 **Resti M**, Azzari C, Mannelli F, Moriondo M, Novembre E, de Martino M, Vierucci A. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. *BMJ* 1998; **317**: 437-441 [PMID: 9703524 DOI: 10.1136/bmj.317.7156.437]
11 **Mok J**, Pembrey L, Tovo PA, Newell ML; European Paediatric Hepatitis C Virus Network. When does mother to child transmission of hepatitis C virus occur? *Arch Dis Child Fetal Neonatal Ed* 2005; **90**: F156-F160 [PMID: 15724041 DOI: 10.1136/adc.2004.059436]
12 **Arshad M**, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period--are they opportunities for treatment? *J Viral Hepat* 2011; **18**: 229-236 [PMID: 21392169 DOI: 10.1111/j.1365-2893.2010.01413.x]
13 **Pembrey L**, Newell ML, Tovo PA; EPHN Collaborators. The management of HCV infected pregnant women and their children European paediatric HCV network. *J Hepatol* 2005; **43**: 515-525 [PMID: 16144064 DOI: 10.1016/j.jhep.2005.06.002]
14 **AASLD**. HCV Guidance: Recommendations for Testing, Managing, and Treating Hepatitis C. [cited 10 February 2022].

Available from: https://wwwhcvguidelinesorg/evaluate/testing-and-linkage

15 **Society for Maternal-Fetal Medicine (SMFM)**; Hughes BL, Page CM, Kuller JA. Hepatitis C in pregnancy: screening, treatment, and management. *Am J Obstet Gynecol* 2017; **217**: B2-B12 [PMID: 28782502 DOI: 10.1016/j.ajog.2017.07.039]

16 **Duan Z**, Jia JD, Hou J, Lou L, Tobias H, Xu XY, Wei L, Zhuang H, Pan CQ. Current challenges and the management of chronic hepatitis C in mainland China. *J Clin Gastroenterol* 2014; **48**: 679-686 [PMID: 24921215 DOI: 10.1097/MCG.0000000000000109]

17 **Reddick KL**, Jhaveri R, Gandhi M, James AH, Swamy GK. Pregnancy outcomes associated with viral hepatitis. *J Viral Hepat* 2011; **18**: e394-e398 [PMID: 21692952 DOI: 10.1111/j.1365-2893.2011.01436.x]

18 **Connell LE**, Salihu HM, Salemi JL, August EM, Weldeselasse H, Mbah AK. Maternal hepatitis B and hepatitis C carrier status and perinatal outcomes. *Liver Int* 2011; **31**: 1163-1170 [PMID: 21745298 DOI: 10.1111/j.1478-3231.2011.02556.x]

19 **Pergam SA**, Wang CC, Gardella CM, Sandison TG, Phipps WT, Hawes SE. Pregnancy complications associated with hepatitis C: data from a 2003-2005 Washington state birth cohort. *Am J Obstet Gynecol* 2008; **199**: 38.e1-38.e9 [PMID: 18486089 DOI: 10.1016/j.ajog.2008.03.052]

20 **Money D**, Boucoiran I, Wagner E, Dobson S, Kennedy A, Lohn Z, Krajden M, Yoshida EM. Obstetrical and neonatal outcomes among women infected with hepatitis C and their infants. *J Obstet Gynaecol Can* 2014; **36**: 785-794 [PMID: 25222357 DOI: 10.1016/S1701-2163(15)30480-1]

21 **Dibba P**, Cholankeril R, Li AA, Patel M, Fayek M, Dibble C, Okpara N, Hines A, Ahmed A. Hepatitis C in Pregnancy. *Diseases* 2018; **6** [PMID: 29702563 DOI: 10.3390/diseases6020031]

22 **Epstein RL**, Espinosa C. Hepatitis C Virus in Neonates and Infants. *Clin Perinatol* 2021; **48**: 343-357 [PMID: 34030818 DOI: 10.1016/j.clp.2021.03.007]

23 **Geenes V**, Williamson C. Intrahepatic cholestasis of pregnancy. *World J Gastroenterol* 2009; **15**: 2049-2066 [PMID: 19418576 DOI: 10.3748/wjg.15.2049]

24 **Paternoster DM**, Fabris F, Palù G, Santarossa C, Bracciante R, Snijders D, Floreani A. Intra-hepatic cholestasis of pregnancy in hepatitis C virus infection. *Acta Obstet Gynecol Scand* 2002; **81**: 99-103 [PMID: 11942897]

25 **Locatelli A**, Roncaglia N, Arreghini A, Bellini P, Vergani P, Ghidini A. Hepatitis C virus infection is associated with a higher incidence of cholestasis of pregnancy. *Br J Obstet Gynaecol* 1999; **106**: 498-500 [PMID: 10430202 DOI: 10.1111/j.1471-0528.1999.tb08305.x]

26 **Rac MW**, Sheffield JS. Prevention and management of viral hepatitis in pregnancy. *Obstet Gynecol Clin North Am* 2014; **41**: 573-592 [PMID: 25454991 DOI: 10.1016/j.ogc.2014.08.004]

27 **Prasad MR**, Honegger JR. Hepatitis C virus in pregnancy. *Am J Perinatol* 2013; **30**: 149-159 [PMID: 23389935 DOI: 10.1055/s-0033-1334459]

28 **Floreani A**. Hepatitis C and pregnancy. *World J Gastroenterol* 2013; **19**: 6714-6720 [PMID: 24187446 DOI: 10.3748/wjg.v19.i40.6714]

29 **Salemi JL**, Whiteman VE, August EM, Chandler K, Mbah AK, Salihu HM. Maternal hepatitis B and hepatitis C infection and neonatal neurological outcomes. *J Viral Hepat* 2014; **21**: e144-e153 [PMID: 24666386 DOI: 10.1111/jvh.12250]

30 **Jaffery T**, Tariq N, Ayub R, Yawar A. Frequency of hepatitis C in pregnancy and pregnancy outcome. *J Coll Physicians Surg Pak* 2005; **15**: 716-719 [PMID: 16300710]

31 **Bohman VR**, Stettler RW, Little BB, Wendel GD, Sutor LJ, Cunningham FG. Seroprevalence and risk factors for hepatitis C virus antibody in pregnant women. *Obstet Gynecol* 1992; **80**: 609-613 [PMID: 1407881]

32 **Berkley EM**, Leslie KK, Arora S, Qualls C, Dunkelberg JC. Chronic hepatitis C in pregnancy. *Obstet Gynecol* 2008; **112**: 304-310 [PMID: 18669727 DOI: 10.1097/AOG.0b013e318180a4f3]

33 **Huang QT**, Hang LL, Zhong M, Gao YF, Luo ML, Yu YH. Maternal HCV infection is associated with intrauterine fetal growth disturbance: A meta-analysis of observational studies. *Medicine (Baltimore)* 2016; **95**: e4777 [PMID: 27583932 DOI: 10.1097/MD.0000000000004777]

34 **Nie QH**, Gao LH, Cheng YQ, Huang XF, Zhang YF, Luo XD, Wang JQ, Wang YY. Hepatitis C virus infection of human cytotrophoblasts cultured in vitro. *J Med Virol* 2012; **84**: 1586-1592 [PMID: 22930506 DOI: 10.1002/jmv.23380]

35 **Hurtado CW**, Golden-Mason L, Brocato M, Krull M, Narkewicz MR, Rosen HR. Innate immune function in placenta and cord blood of hepatitis C--seropositive mother-infant dyads. *PLoS One* 2010; **5**: e12232 [PMID: 20814429 DOI: 10.1371/journal.pone.0012232]

36 **Trevisanuto D**, Peruzzetto C, Cavallin F, Vedovato S, Cosmi E, Visentin S, Chiarelli S, Zanardo V. Fetal placental inflammation is associated with poor neonatal growth of preterm infants: a case-control study. *J Matern Fetal Neonatal Med* 2013; **26**: 1484-1490 [PMID: 23560517 DOI: 10.3109/14767058.2013.789849]

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ORIGINAL ARTICLE

Retrospective Study

Trends in hospitalization for alcoholic hepatitis from 2011 to 2017: A USA nationwide study

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Abstract

BACKGROUND

Severe alcoholic hepatitis (AH) is one of the most lethal manifestations of alcohol-associated liver disease. In light of the increase in alcohol consumption worldwide, the incidence of AH is on the rise, and data examining the trends of AH admission is needed.

AIM

To examine inpatient admission trends secondary to AH, along with their clinical outcomes and epidemiological characteristics.

METHODS

The National Inpatient Sample (NIS) database was utilized, and data from 2011 to 2017 were reviewed. We included individuals aged ≥ 21 years who were admitted with a primary or secondary diagnosis of AH using the International Classification of Diseases (ICD)-9 and its correspondent ICD-10 codes. Hepatitis not related to alcohol was excluded. The national estimates of inpatient admissions were obtained using sample weights provided by the NIS.

RESULTS

AH-related hospitalization demonstrated a significant increase in the USA from 281506 (0.7% of the total admission in 2011) to 324050 (0.9% of the total admi-ssion

in 2017). The median age was 54 years. The most common age group was 45–65 years (range 57.8%–60.7%). The most common race was white (63.2%–66.4%), and patients were predominantly male (69.7%–71.2%). The primary healthcare payers were Medicare (29.4%–30.7%) and Medicaid (21.5%–32.5%). The most common geographical location was the Southern USA (33.6%–34.4%). Most patients were admitted to a tertiary care center (50.2%–62.3%) located in urban areas. Mortality of AH in this inpatient sample was 5.3% in 2011 and 5.5% in 2017. The most common mortality-associated risk factors were acute renal failure (59.6%–72.1%) and gastrointestinal hemorrhage (17.2%–20.3%). The total charges were noted to range between $25242.62 and $34874.50.

CONCLUSION

The number of AH inpatient hospitalizations significantly increased from 2011 to 2017. This could have a substantial financial impact with increasing healthcare costs and utilization. AH-mortality remained the same.

Key Words: Alcoholic hepatitis; Cirrhosis; Fatty liver disease; Alcohol abuse

Core tip: This study demonstrated a significant increase in the number of hospitalizations due to alcoholic-associated hepatitis (AH) throughout the USA, with an overall increase in the cost and financial burden of the disease. These trends were in line with the increase in the incidence of alcohol misuse across the years. This study provides potential data for future prospective research to help trigger more aggressive screening and prevention methods for alcohol abuse to prevent AH. Additionally, there is a need for the development of novel therapeutic agents targeting the disease since AH treatment is limited.

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INTRODUCTION

Alcohol-associated liver disease (ALD) comprises a spectrum of liver diseases ranging from reversible fatty liver to severe alcohol-associated hepatitis (AH) and cirrhosis, to acute-on-chronic liver failure[1]. All manifestations of ALD may overlap and can develop after heavy alcohol consumption for at least 6 mo[2]. AH is a clinical syndrome with acute onset jaundice in the presence of heavy alcohol misuse[2]. Mild AH patients with alcohol abstinence can have a good outcome. However, those with severe disease defined by Maddrey's discriminant function ≥ 32, or model for end-stage liver disease score ≥ 20, have a high 30-d mortality rate[1]. ALD is one of the leading causes of cirrhosis and it is the second leading indication for liver transplantation in the USA according to the Scientific Registry of Transplant Recipients data[3]. Alcohol consumption continues to be on the rise, with global data extrapolated from the World Health Organization showing the average annual per capita alcohol consumption has risen from 5.5 L in 2005 to 6.4 L in 2018[4]. Consequently, global alcohol-attributable mortality in 2016 has increased to 38.8 per 100000 people and 1759 disability-adjusted life-years per 100000 people[4]. According to a study from Denmark, during the study period (1999–2008), the annual incidence rate of AH was reported to increase for both men and women from 37 to 46 per 100000 and from 24 to 34 per 100 000, respectively[5]. The 5-year overall mortality in this study was found to be 56% and was significantly higher in patients with cirrhosis compared to patients without cirrhosis (69% *vs* 47%, respectively)[5]. In the USA, a study evaluating the United States National Inpatient Sample (NIS) database showed that out of 325 000 hospital admissions in 2010, 0.8% were AH-related[6]. With the availability of highly effective direct-acting antivirals therapy for chronic hepatitis C, the burden of chronic liver disease (CLD) is shifting towards ALD and non-ALD[7,8]. Studies have demonstrated that there is an increasing trend in consuming alcohol in the USA[9-12], with the initiation of alcohol at younger ages[13-15], and binging representing the most frequent pattern of alcohol consumption[16]. As a consequence of this upward trend in alcohol use disorder, it is anticipated that the correlating rise in ALD will have significant health, social and economic burdens accredited to the increase in hospitalization rate, as well as the elevated support required for these patients in an outpatient setting[8].

According to the 2007 NIS database, a significant healthcare cost and use of resources were reported [17]. Another study using the NIS database from 2012 to 2016, reported a higher admissions rate for CLD with a 26.2% increase in hospitalization costs and an $18.8 billion economic burden in 2016[18]. Despite the advances in medicine, the currently available therapeutics for severe AH are scarce and only limited to corticosteroids, which contributes to the high mortality of this disease[1].

Given the increased prevalence of alcohol misuse in the USA, this study aimed to provide a relatively recent descriptive analysis of trends in AH hospitalization within the USA. Data were obtained using the NIS database from 2011 to 2017.

MATERIALS AND METHODS

Data source

The Healthcare Cost and Utilization Project (HCUP) is a collection of databases and contains the NIS database[19]. The NIS is the largest publicly available inpatient database that encompasses a range of different data encoded by International Classification of Diseases (ICD) codes from more than 1000 hospitals, constituting a 20% sample data of all US hospitals. The database enables data extraction on a broad range of health conditions and specific populations, including cost and quality of health services, medical practice patterns, and outcomes of treatments on a national level.

Study design and study population

This was a retrospective analysis. All subjects in the database ≥ 21 years old who were hospitalized with a discharge diagnosis of AH from 2011 to 2017 were included. To minimize ascertainment bias, we classified hospitalization as AH-related if it was associated with a primary admission diagnosis of AH or a primary discharge diagnosis. A secondary AH-related hospitalization was classified as having the AH diagnosis anywhere in the admission diagnosis (25 diagnoses) or anywhere in the discharge diagnoses. We excluded all non-alcohol-related hepatitis diagnoses using ICD-9 and ICD-10. This included autoimmune hepatitis, acute and chronic viral hepatitis A–E, and nonalcoholic steatohepatitis. All data were weighted using discharge level values, to produce an accurate estimate of the patient population nationwide. AH-related hospitalization was identified by ICD-9 and ICD-10 discharge diagnosis codes. The ICD codes included were alcohol-associated hepatitis, alcoholic fatty liver disease, and alcohol-associated cirrhosis (Table 1). Previous validation studies have shown that the discharge diagnosis captures the cause of hospitalization accurately, such as in primary biliary cholangitis[20], coronary artery disease[21], and hepatitis B and C[11]. Other outcomes examined were inpatient mortality. We identified known risk factors related to mortality in patients with the following: acute renal failure (ARF), gastrointestinal bleeding, and sepsis (Table 2). Patient age, sex, household income, race, and geographic region (Northeast, Midwest, South and West) were obtained. The primary payer for the hospitalization was categorized as Medicare, Medicaid, private insurance, self-pay, or others. The types of hospitals were categorized into teaching, non-teaching community, and rural hospitals. Hospitalization characteristics were presented separately where the primary reason for hospitalization was AH *versus* AH presenting as a secondary condition. These characteristics included in-hospital mortality, length of stay, and discharge disposition.

Statistical analysis

Patients were first screened and selected based on the presence of an AH diagnosis. The temporal trend of the AH-related hospitalization was tested by Cochran–Armitage test. The patient demographics, additional clinical characteristics, and clinical outcome measures were then summarized using the median with interquartile range for continuous variables, or frequencies with percentages for categorical variables. To compare these variables among different years, Kruskal–Wallis and χ^2 tests were used. These demographics and variables were tested against the year. Overall and between-categorical group P values were reported for categorical variables. Prevalence of AH-related diagnosis among HCUP dataset and mortality-related risk factors among AH patients who died during hospitalization in 2011–2017 were tested using the χ^2 test. A multivariable logistic model was fitted and resulting odds ratios (OR) with 95% confidence intervals were used to test for association between the risk factors and the primary outcome of in-hospital mortality, further adjusting for demographics and additional clinical characteristics. Due to the desire to have population-level interpretations, survey weights were applied to all patient-level observations as provided in the NIS database. All reported P values were two-sided, and the significance cut-off was set at 0.05. The Bonferroni adjustment was used to adjust for multiple testing in between-categorical χ^2 test and logistic regression. Data analyses were completed in SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) and R studio (R version 3.6.0, R Foundation for Statistical Computing, Vienna, Austria).

Table 1 Diagnosis to be included International Classification of Diseases-9 and its correspondent International Classification of Diseases-10

ICD-9	Correspondent ICD-10
571.0 alcoholic fatty liver	K.70 alcoholic fatty liver
571.1 acute alcoholic hepatitis	K.70.11 alcoholic hepatitis with ascites
	K.70.10 alcoholic hepatitis without ascites
571.2 alcoholic cirrhosis of liver	Correspondent ICD-10 found
571.3 alcoholic liver damage, unspecified	K70.40 alcoholic hepatic failure without coma
	K70.41 alcoholic hepatic failure with coma
	K70.9 alcoholic liver disease, unspecified

ICD: International Classification of Diseases.

Table 2 Trend of alcoholic hepatitis-related hospitalization 2011 to 2017

	2011	2012	2013	2014	2015	2016	2017	*P*
Total admission, *n*	38590733	36484846	35597792	35358818	35769942	35675421	35798453	
AH-related admission, *n* (%)	281506 (0.7)	274365 (0.8)	278580 (0.8)	291435 (0.8)	308765 (0.9)	313235 (0.9)	324050 (0.9)	< 0.001
Admission when primary admission diagnosis is AH, *n* (%)	47140 (0.1)	45710 (0.1)	46715 (0.1)	48395 (0.1)	54955 (0.2)	66170 (0.2)	71290 (0.2)	< 0.001

AH: Alcoholic hepatitis.

RESULTS

AH-related hospitalization showed an increase from 281506 (0.7% of the total in 2011) to 324050 (0.9% of the total in 2017; $P < 0.01$) (Figure 1). This included all AH-related admissions when AH was not the primary or secondary diagnosis. There was also an increase in the number of admissions when the primary admission diagnosis was AH (47140 in 2011 to 71290 in 2017) ($P < 0.01$; Table 2).

Results from demographic observations showed that the median age of patients hospitalized with AH was 54 years. The most common age group was 45–65 years (58.4%–60.7%; $P < 0.01$); the most common race was white (63.2%–66.4%; $P < 0.01$); patients were predominantly male (69.7%–71.2%; $P < 0.01$); and the primary healthcare payers were Medicare (29.4%–30.7%; $P < 0.01$) and Medicaid (21.5%–32.5%; $P < 0.01$). The most common geographical location was the southern region of the USA (33.6%–34.4%; $P = 0.017$). Most patients were admitted to tertiary hospitals (50.2%–62.3%; $P < 0.01$) in urban areas. The most common presenting diagnosis was AH (63.5%–69%; $P < 0.01$). The most common outcome was routine discharge (60%–63.3%; $P < 0.01$). The median length of stay was 5.98 d (SD = 7.11) in 2011 which increased to 6.14 d (SD = 7.43) in 2017 ($P < 0.01$). Total charge for hospitalized patients with AH ranged between $46507.47 (SD = 87193.29) and $63574.52 (SD = 108850.63; $P < 0.01$) (Table 3).

The mortality of AH-related hospitalizations was 5.3% in 2011 and 5.5% in 2017 ($P < 0.01$). Risk factors that could be associated with AH mortality were sepsis (increased from 7.4% in 2011 to 48.3% in 2017; $P < 0.01$), renal failure (59.6% in 2011 to 72.1% in 2018; $P < 0.01$), and gastrointestinal (GI) hemorrhage (17.2% in 2011 to 20.3% in 2017; $P = 0.048$) (Table 4). Multivariable regression analysis on death during hospitalization was performed and demonstrated higher odds of mortality in the following group: age > 65 years (OR 3.55; $P < 0.0001$), female (OR 1.13; $P < 0.0001$), large academic hospital (OR 1.3; $P < 0.0001$), sepsis (OR 3.33; $P < 0.0001$), GI hemorrhage (OR 2.31; $P < 0.0001$), and ARF (OR 7.19; $P < 0.0001$) (Table 5).

DISCUSSION

ALD is a spectrum of diseases ranging from hepatic steatosis to fibrosis and eventually cirrhosis, with continued alcohol use[22]. Alcohol consumption has been on the rise in the USA. Previously, a large survey conducted by the National Epidemiologic Survey showed a prevalence of alcohol use over any 12-mo period to rise from 65% to 72%, with an overall increase in alcohol consumption between 2001

Table 3 Summary statistics of demographic of hospitalized alcoholic hepatitis patients, 2011 to 2017

Characteristic	AH-related admission							*P*[1]	
	2011	2012	2013	2014	2015	2016	2017	Overall	Between-group
n	281506	274365	278580	291435	308765	313235	324050		
Age in years at admission [median (Q1, Q3)]	54.0 (46.0, 61.0)	54.0 (46.0, 61.0)	54.0 (46.0, 62.0)	54.0 (46.0, 62.0)	54.0 (46.0, 62.0)	54.0 (46.0, 62.0)	55.0 (46.0, 62.0)	< 0.001	
Age group (yr)								< 0.001	
< 25, *n* (%)	1545 (0.5)	1215 (0.4)	1135 (0.4)	1295 (0.4)	1320 (0.4)	1635 (0.5)	1535 (0.5)		0.011
25-44, *n* (%)	57738 (20.5)	57040 (20.8)	58155 (20.9)	61335 (21.0)	66670 (21.6)	69165 (22.1)	70935 (21.9)		< 0.001
45-64, *n* (%)	170876 (60.7)	166030 (60.5)	167810 (60.2)	174765 (60.0)	181845 (58.9)	182905 (58.4)	187350 (57.8)		< 0.001
≥65, *n* (%)	51348 (18.2)	50080 (18.3)	51480 (18.5)	54040 (18.5)	58930 (19.1)	59530 (19.0)	64230 (19.8)		< 0.001
Gender								< 0.001	
Male, *n* (%)	198491 (70.5)	195350 (71.2)	196820 (70.7)	203735 (69.9)	216650 (70.2)	218295 (69.7)	227080 (70.1)		< 0.001
Female, *n* (%)	83016 (29.5)	79000 (28.8)	81725 (29.3)	87615 (30.1)	92075 (29.8)	94810 (30.3)	96965 (29.9)		< 0.001
Missing, *n* (%)	0 (0.0)	15 (0.0)	35 (0.0)	85 (0.0)	40 (0.0)	130 (0.0)	5 (0.0)		
Race								< 0.001	
White, *n* (%)	178031 (63.2)	180550 (65.8)	183125 (65.7)	193560 (66.4)	202205 (65.5)	207155 (66.1)	214760 (66.3)		< 0.001
Black, *n* (%)	27402.8 (9.7)	27045 (9.9)	26625 (9.6)	27630 (9.5)	29145 (9.4)	28025 (8.9)	29290 (9.0)		< 0.001
Hispanic, *n* (%)	37280 (13.2)	36670 (13.4)	38575 (13.8)	38905 (13.3)	43390 (14.1)	45870 (14.6)	49135 (15.2)		< 0.001
Asian or Pacific Islander, *n* (%)	2235 (0.8)	2370 (0.9)	2680 (1.0)	2855 (1.0)	3565 (1.2)	3520 (1.1)	3840 (1.2)		< 0.001
Native American, *n* (%)	5056 (1.8)	5985 (2.2)	5720 (2.1)	5910 (2.0)	6725 (2.2)	7140 (2.3)	7565 (2.3)		< 0.001
Other, *n* (%)	7134 (2.5)	7760 (2.8)	6940 (2.5)	7785 (2.7)	7780 (2.5)	8305 (2.7)	9085 (2.8)		0.030
Missing, *n* (%)	24368.5 (8.7)	13985 (5.1)	14915 (5.4)	14790 (5.1)	15955 (5.2)	13220 (4.2)	10375 (3.2)		
Median household income national quartile for patient ZIP code								< 0.001	
$1-$38999, *n* (%)	79993 (28.4)	83510 (30.4)	80530 (28.9)	85525 (29.3)	95660 (31.0)	94940 (30.3)	95785 (29.6)		< 0.001
$39000-$47999, *n* (%)	69137 (24.6)	66095 (24.1)	71125 (25.5)	76895 (26.4)	72860 (23.6)	77800 (24.8)	83130 (25.7)		< 0.001
$48000-$62999, *n* (%)	68313 (24.3)	62360 (22.7)	65010 (23.3)	65695 (22.5)	72025 (23.3)	73690 (23.5)	75805 (23.4)		< 0.001
$63000 or more, *n* (%)	56576 (20.1)	53795 (19.6)	53035 (19.0)	54360 (18.7)	59515 (19.3)	58905 (18.8)	60560 (18.7)		< 0.001
Missing, *n* (%)	7487.7 (2.7)	8605 (3.1)	8880 (3.2)	8960 (3.1)	8705 (2.8)	7900 (2.5)	8770 (2.7)		
Primary expected payer								< 0.001	
Medicare, *n* (%)	82774 (29.4)	81905 (29.9)	85335 (30.6)	88860 (30.5)	93460 (30.3)	94160 (30.1)	99510 (30.7)		< 0.001
Medicaid, *n* (%)	60601 (21.5)	61975 (22.6)	62490 (22.4)	86040 (29.5)	96230 (31.2)	100255 (32.0)	105305 (32.5)		< 0.001
Private including HMO, *n* (%)	71261 (25.3)	65885 (24.0)	65405 (23.5)	70445 (24.2)	76095 (24.6)	75795 (24.2)	76200 (23.5)		< 0.001
Self-pay, *n* (%)	45272 (16.1)	43865	42785 (15.4)	31705	28095 (9.1)	27930 (8.9)	28890 (8.9)		< 0.001

		(16.0)		(10.9)				
No charge, *n* (%)	4980.6 (1.8)	3455 (1.3)	5330 (1.9)	3235 (1.1)	3195 (1.0)	2995 (1.0)	2610 (0.8)	< 0.001
Other, *n* (%)	15296.9 (5.4)	16515 (6.0)	16670 (6.0)	10555 (3.6)	11150 (3.6)	11625 (3.7)	10805 (3.3)	< 0.001
Missing, *n* (%)	1320 (0.5)	765 (0.3)	565 (0.2)	595 (0.2)	540 (0.2)	475 (0.2)	730 (0.2)	
Bed size of hospital								< 0.001
Small, *n* (%)	32567 (11.6)	36820 (13.4)	37640 (13.5)	54105 (18.6)	54870 (17.8)	57875 (18.5)	64035 (19.8)	< 0.001
Medium, *n* (%)	73628 (26.2)	77055 (28.1)	76680 (27.5)	86355 (29.6)	94260 (30.5)	91795 (29.3)	97445 (30.1)	< 0.001
Large, *n* (%)	175312 (62.3)	160490 (58.5)	164260 (59.0)	150975 (51.8)	159635 (51.7)	163565 (52.2)	162570 (50.2)	< 0.001
Region of hospital								0.001
Northeast, *n* (%)	53252 (18.9)	51120 (18.6)	51855 (18.6)	53795 (18.5)	56100 (18.2)	57590 (18.4)	59600 (18.4)	0.16
Midwest, *n* (%)	61095 (21.7)	59575 (21.7)	60330 (21.7)	64160 (22.0)	67170 (21.8)	68655 (21.9)	72150 (22.3)	0.42
South, *n* (%)	95441 (33.9)	94365 (34.4)	95705 (34.4)	98035 (33.6)	105260 (34.1)	105730 (33.8)	108555 (33.5)	0.017
West, *n* (%)	71718 (25.5)	69305 (25.3)	70690 (25.4)	75445 (25.9)	80235 (26.0)	81260 (25.9)	83745 (25.8)	0.046
Location teaching status of hospital								< 0.001
Rural, *n* (%)	28721 (10.2)	27135 (9.9)	26909.9 (9.7)	23195 (8.0)	23990 (7.8)	24250 (7.7)	25035 (7.7)	< 0.001
Urban non-teaching, *n* (%)	127976 (45.5)	113625 (41.4)	112610 (40.4)	84150 (28.9)	88965 (28.8)	87650 (28.0)	77930 (24.0)	< 0.001
Urban teaching, *n* (%)	124809 (44.3)	133605 (48.7)	139060 (49.9)	184090 (63.2)	195810 (63.4)	201335 (64.3)	221085 (68.2)	< 0.001

[1]Missing data is excluded from the *P* value calculation.
AH: Alcoholic hepatitis; HMO: Health Maintenance Organization.

Table 4 Mortality-related risk factors in alcoholic hepatitis patients who expired while hospitalized, 2011 to 2017

	2011	2012	2013	2014	2015	2016	2017	*P*
n	15002	14845	15310	15885	16385	17435	17785	
Sepsis, *n* (%)	1111 (7.4)	950 (6.4)	980 (6.4)	1170 (7.4)	2850 (17.4)	8145 (46.7)	8595 (48.3)	< 0.001
GI hemorrhage, *n* (%)	2582 (17.2)	2755 (18.6)	2715 (17.7)	3230 (20.3)	3025 (18.5)	3355 (19.2)	3395 (19.1)	0.042
Acute renal failure, *n* (%)	8941 (59.6)	9450 (63.7)	9780 (63.9)	10485 (66.0)	11265 (68.8)	12325 (70.7)	12825 (72.1)	< 0.001

GI: Gastrointestinal.

and 2012[23].

Our study included all AH-related admissions from 2011 to 2017 in the NIS database. Out of the 38.5 million admissions in 2011, about 281 506 (0.7%) were due to AH. This number increased to 324050 (0.9%) out of 35.7 million admissions in 2017 ($P < 0.001$; Figure 1). Moreover, the number of admissions due to a primary diagnosis of AH increased by almost 1.5 times between 2011 and 2017 from 47140 (0.1%) to 71290 (0.2%) ($P < 0.001$). These are alarming figures, and they match the results of increased alcohol consumption and binge drinking in the USA[8,12]. This has major consequences as AH continues to cause significant morbidity and mortality. Additionally, we found that each AH-related admission costs on average $46000 to $63000 with an average in-hospital length of stay of 4 d per admission. About 60% of those patients had Medicare or Medicaid insurance as the primary expected payer. These increasing admissions, as reported previously, escalates the burden on the healthcare system and the public payer funded by tax dollars[24].

Table 5 Multivariable logistic regression on death during hospitalization among alcoholic hepatitis-related admission[1] from 2011 to 2017

Variables	Levels	Odds ratio		
		Estimate	95% confidence interval	*P* after Bonferroni correction[2]
Age groups (yr)	< 25	1.0		Reference
	25-44	1.89	1.28-2.79	0.039
	45-64	2.85	1.93-4.21	< 0.0001
	65/65+	3.55	2.4-5.25	< 0.0001
Sex	Male	Reference		
	Female	1.13	1.09-1.17	< 0.0001
Race	White	1.0		Reference
	Black	0.82	0.77-0.86	< 0.0001
	Hispanic	0.94	0.9-0.98	0.20
	Asian or Pacific Islander	0.92	0.8-1.07	1.00
	Native American	1.04	0.94-1.15	1.00
	Other	1.12	1.02-1.22	0.36
Primary expected payer	Medicare	1.0		Reference
	Medicaid	1.12	1.07-1.18	0.00020
	Private including HMO	1.11	1.06-1.17	0.00025
	Self-pay	1.37	1.29-1.45	< 0.0001
	No charge	0.92	0.78-1.09	1.00
	Other	1.45	1.34-1.57	< 0.0001
Median household income national quartile for patient ZIP code	$1-$38999	1.0		Reference
	$39000-$47999	0.98	0.94-1.02	1.00
	$48000-$62999	0.95	0.92-1	0.84
	$63000 or more	0.9	0.86-0.94	0.00021
Bed size of hospital	Small	1.0		Reference
	Medium	1.2	1.14-1.26	< 0.0001
	Large	1.3	1.25-1.37	< 0.0001
Region of hospital	Northeast	1.0		Reference
	Midwest	0.91	0.87-0.96	0.0062
	South	1	0.96-1.05	1.00
	West	1.08	1.03-1.14	0.023
Location/teaching status of hospital	Rural	1.0		Reference
	Urban non-teaching	1.03	0.97-1.1	1.00
	Urban teaching	1.08	1.02-1.15	0.28
Sepsis	Yes	3.33	3.2-3.47	< 0.0001
GI hemorrhage	Yes	2.31	2.22-2.4	< 0.0001
Acute renal failure	Yes	7.19	6.96-7.42	< 0.0001

[1]Outcome is death during hospitalization (n = 1901436).
[2]P value adjusted by Bonferroni correction to account for multiple testing.

GI: Gastrointestinal; HMO: Health Maintenance Organization.

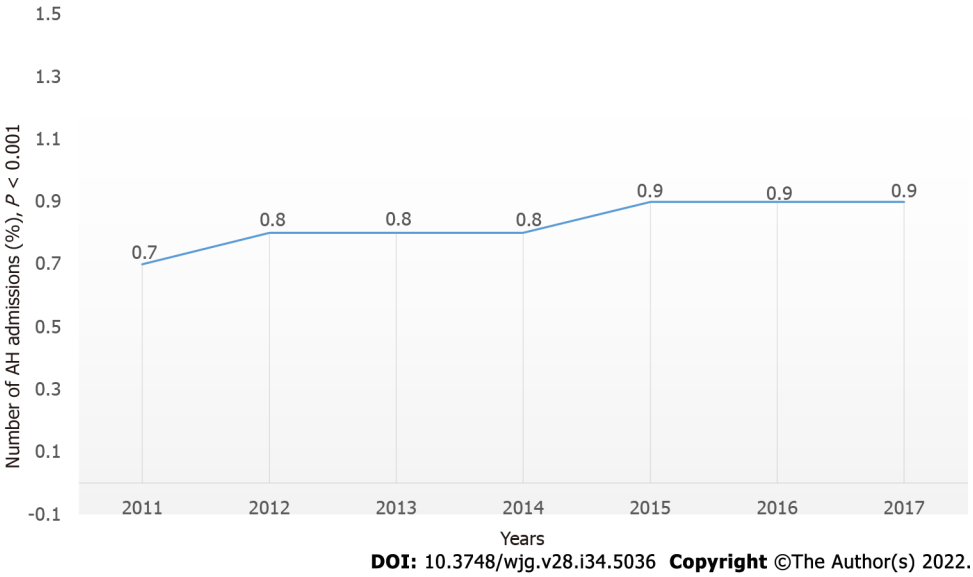

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Figure 1 Trends in alcoholic hepatitis-related admissions from 2011 to 2017.

The increase in AH burden has a huge impact on trends of liver transplantation and consequently, organ allocation. Recent data showed that in select patients with severe AH, early liver transplantation resulted in high survival rates in the early transplant of AH at 6 mo *vs* no transplant (77% *vs* 23%)[25, 26]. Based on these results, many liver transplant centers around the USA are performing liver transplants for select severe AH patients. Consequently, this has resulted in ALD surpassing chronic hepatitis C virus infection as the leading indication for liver transplantation[27].

Our study showed that the majority of AH hospitalized individuals were middle-aged white men. This is not surprising, as more men consume alcohol above the recommended safety levels compared to women[24]; although women are more susceptible to developing AH within a shorter period and less exposure to alcohol compared to men[28].

We observed that the mortality of hospitalized patients with AH was about 5%, which has remained similar from 2011 to 2017. We also examined some of the major mortality-related risk factors among AH patients who expired during hospitalization. ARF was the most common finding in these patients and increased from 59.6% in 2011 to 72% in 2017. A possible explanation of these results may include the lack of therapy for severe AH with ARF since the benefit of steroids is unknown in this patient population as the steroids or pentoxifylline for AH trial excluded patients with ARF defined as creatinine > 5.7 mg/dL[29]. In addition, hepatorenal syndrome is associated with high mortality, which could be as high as 80% at 2 wk[30]. Sepsis and GI bleeding were also noted as mortality-risk factors in almost 48% and 19%, respectively, in patients who died from AH in 2017. Sepsis had a significant increase from 7.4% in 2014 to 46.7% in 2016 and 48.3% in 2017. This likely resulted from implementing the conversion of ICD-9 to ICD-10 on October 1, 2015. Sepsis is coded for by one code in ICD-9 (995.91), in contrast, there are 26 codes for different types of sepsis in ICD-10, which could potentially explain the sharp rise in sepsis rate. Additionally, AH patients usually present with fever and leukocytosis meeting Systemic Inflammatory Response Syndrome, criteria leading clinicians to code for sepsis even without an active infection present.

A regression analysis was performed that showed that advanced age, female gender, ARF, sepsis and GI bleeding were the most prominent risk factors. These mortality risk factors do not establish causality, since there is no etiology of death in the NIS database. Moreover, there is no information as to whether these risk factors were resolved or not during hospitalization since it was captured by the ICD codes used.

One limitation of this study was the accuracy of the NIS database in capturing ICD-9 and ICD-10 codes. For instance, some AH-related admissions could have been reported as jaundice or hepatic failure, which would exclude those patients from our analysis. There was also no information regarding the outcomes of AH patients once they were discharged. This has resulted in a lower in-hospital mortality rate (about 5%) compared to the established high 30-d mortality of AH patients (30%–50%) [31]. The mortality rate in our study is the percentage of patients who died while hospitalized only.

CONCLUSION

We observed that AH-related hospitalization continued to increase during the study period. This could have a substantial impact on increasing healthcare costs and utilization among patients hospitalized for AH. Mortality remained the same throughout the study period. These findings are alarming and should trigger more aggressive screening and prevention of alcohol abuse to prevent the increasing cases of AH and its consequences.

ARTICLE HIGHLIGHTS

Research background

Alcoholic hepatitis (AH) is a significant healthcare issue with rising alcohol use in the USA. Alcohol-associated liver disease is the second leading indication for liver transplantation after surpassing chronic hepatitis C infection.

Research motivation

With increasing alcohol consumption there is a need to measure the magnitude of AH effects.

Research objectives

The study aimed to examine the trends in hospitalization of AH patients across the USA. The second aim was to look at the mortality of hospitalized patients, along with the risk factors associated with death while hospitalized.

Research methods

Data were extracted from National Inpatient Sample database using discharge diagnosis codes of International Classification of Diseases (ICD)-9 and their corresponding ICD-10. We included hospitalization for the years from 2011 to 2017.

Research results

AH inpatient hospitalization increased from 0.7% of total admissions to 0.9% of total admissions. Mortality for admitted patients remained the same.

Research conclusions

There has been an increase in AH hospitalization that could affect the healthcare system. Acute renal failure, sepsis and gastrointestinal hemorrhage are highly associated with increased mortality in AH patients.

Research perspectives

New studies should focus on finding new therapeutic targets of AH. New studies should look for improved strategies to limit alcohol misuse.

FOOTNOTES

Author contributions: Wakil A contributed to the manuscript writing, methodology, editing, project administration; Mohamed M contributed to the manuscript writing and editing; Tafesh, Z, Olivo R and Niazi M contributed to the reviewing and editing; Greenberg P and Xia W contributed to the statistical analysis, data extraction; Pyrsopoulos N contributed to the supervision, reviewing and editing; all authors have read and approved the final manuscript.

Institutional review board statement: This study was done using the NIS database which does not require approval from the IRB, thus no IRB approval was needed for this study.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data using NIS database which contains no identifying patient information and does not require informed consent to use the data.

Conflict-of-interest statement: All authors have no relevant conflict of interest.

Data sharing statement: No additional data are available.

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REFERENCES

1 **Gao B**, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology* 2011; **141**: 1572-1585 [PMID: 21920463 DOI: 10.1053/j.gastro.2011.09.002]
2 **Singal AK**, Kamath PS, Gores GJ, Shah VH. Alcoholic hepatitis: current challenges and future directions. *Clin Gastroenterol Hepatol* 2014; **12**: 555-64; quiz e31 [PMID: 23811249 DOI: 10.1016/j.cgh.2013.06.013]
3 **Kwong AJ**, Kim WR, Lake JR, Smith JM, Schladt DP, Skeans MA, Noreen SM, Foutz J, Booker SE, Cafarella M, Snyder JJ, Israni AK, Kasiske BL. OPTN/SRTR 2019 Annual Data Report: Liver. *Am J Transplant* 2021; **21** Suppl 2: 208-315 [PMID: 33595192 DOI: 10.1111/ajt.16494]
4 **World Health Organization**. Global Status Report on Alcohol and Health 2018. Poznyak V, Rekve D, editor. Geneva: World Health Organization, 2018
5 **Sandahl TD**, Jepsen P, Thomsen KL, Vilstrup H. Incidence and mortality of alcoholic hepatitis in Denmark 1999-2008: a nationwide population based cohort study. *J Hepatol* 2011; **54**: 760-764 [PMID: 21126790 DOI: 10.1016/j.jhep.2010.07.016]
6 **Singal AK**, Kuo YF, Anand BS. Hepatitis C virus infection in alcoholic hepatitis: prevalence patterns and impact on in-hospital mortality. *Eur J Gastroenterol Hepatol* 2012; **24**: 1178-1184 [PMID: 22735607 DOI: 10.1097/MEG.0b013e328355cce0]
7 **Williams R**. The pervading influence of alcoholic liver disease in hepatology. *Alcohol Alcohol* 2008; **43**: 393-397 [PMID: 18385413 DOI: 10.1093/alcalc/agn013]
8 **Neff GW**, Duncan CW, Schiff ER. The current economic burden of cirrhosis. *Gastroenterol Hepatol (N Y)* 2011; **7**: 661-671 [PMID: 22298959]
9 **Yörük BK**. Legalization of Sunday alcohol sales and alcohol consumption in the United States. *Addiction* 2014; **109**: 55-61 [PMID: 24103041 DOI: 10.1111/add.12358]
10 **Xuan Z**, Nelson TF, Heeren T, Blanchette J, Nelson DE, Gruenewald P, Naimi TS. Tax policy, adult binge drinking, and youth alcohol consumption in the United States. *Alcohol Clin Exp Res* 2013; **37**: 1713-1719 [PMID: 23711219 DOI: 10.1111/acer.12152]
11 **Niu B**, Forde KA, Goldberg DS. Coding algorithms for identifying patients with cirrhosis and hepatitis B or C virus using administrative data. *Pharmacoepidemiol Drug Saf* 2015; **24**: 107-111 [PMID: 25335773 DOI: 10.1002/pds.3721]
12 **Shirazi F**, Singal AK, Wong RJ. Alcohol-associated Cirrhosis and Alcoholic Hepatitis Hospitalization Trends in the United States. *J Clin Gastroenterol* 2021; **55**: 174-179 [PMID: 32520887 DOI: 10.1097/MCG.0000000000001378]
13 **Siegel M**, DeJong W, Naimi TS, Heeren T, Rosenbloom DL, Ross C, Ostroff J, Jernigan DH. Alcohol brand preferences of underage youth: results from a pilot survey among a national sample. *Subst Abus* 2011; **32**: 191-201 [PMID: 22014249 DOI: 10.1080/08897077.2011.601250]
14 **Miller TR**, Levy DT, Spicer RS, Taylor DM. Societal costs of underage drinking. *J Stud Alcohol* 2006; **67**: 519-528 [PMID: 16736071 DOI: 10.15288/jsa.2006.67.519]
15 **Siegel MB**, Naimi TS, Cremeens JL, Nelson DE. Alcoholic beverage preferences and associated drinking patterns and risk behaviors among high school youth. *Am J Prev Med* 2011; **40**: 419-426 [PMID: 21406275 DOI: 10.1016/j.amepre.2010.12.011]
16 **Bor J**, Basu S, Coutts A, McKee M, Stuckler D. Alcohol use during the great recession of 2008-2009. *Alcohol Alcohol* 2013; **48**: 343-348 [PMID: 23360873 DOI: 10.1093/alcalc/agt002]
17 **Jinjuvadia R**, Liangpunsakul S; Translational Research and Evolving Alcoholic Hepatitis Treatment Consortium. Trends in Alcoholic Hepatitis-related Hospitalizations, Financial Burden, and Mortality in the United States. *J Clin Gastroenterol* 2015; **49**: 506-511 [PMID: 25198164 DOI: 10.1097/MCG.0000000000000161]
18 **Hirode G**, Saab S, Wong RJ. Trends in the Burden of Chronic Liver Disease Among Hospitalized US Adults. *JAMA Netw Open* 2020; **3**: e201997 [PMID: 32239220 DOI: 10.1001/jamanetworkopen.2020.1997]
19 **Myers RP**, Shaheen AA, Fong A, Wan AF, Swain MG, Hilsden RJ, Sutherland L, Quan H. Validation of coding algorithms for the identification of patients with primary biliary cirrhosis using administrative data. *Can J Gastroenterol* 2010; **24**: 175-182 [PMID: 20352146 DOI: 10.1155/2010/237860]
20 **Rawson NS**, Malcolm E. Validity of the recording of ischaemic heart disease and chronic obstructive pulmonary disease in the Saskatchewan health care datafiles. *Stat Med* 1995; **14**: 2627-2643 [PMID: 8619104 DOI: 10.1002/sim.4780142404]

21 **Agency for Healthcare Research and Quality**. Overview of the National (Nationwide) Inpatient Sample (NIS). Sep 13, 2021. [cited Feb 6, 2022]. Available from: http://www.hcup-us.ahrq.gov/nisoverview.jsp

22 **O'Shea RS**, Dasarathy S, McCullough AJ; Practice Guideline Committee of the American Association for the Study of Liver Diseases; Practice Parameters Committee of the American College of Gastroenterology. Alcoholic liver disease. *Hepatology* 2010; **51**: 307-328 [PMID: 20034030 DOI: 10.1002/hep.23258]

23 **Grant BF**, Chou SP, Saha TD, Pickering RP, Kerridge BT, Ruan WJ, Huang B, Jung J, Zhang H, Fan A, Hasin DS. Prevalence of 12-Month Alcohol Use, High-Risk Drinking, and DSM-IV Alcohol Use Disorder in the United States, 2001-2002 to 2012-2013: Results From the National Epidemiologic Survey on Alcohol and Related Conditions. *JAMA Psychiatry* 2017; **74**: 911-923 [PMID: 28793133 DOI: 10.1001/jamapsychiatry.2017.2161]

24 **Mathurin P**, Moreno C, Samuel D, Dumortier J, Salleron J, Durand F, Castel H, Duhamel A, Pageaux GP, Leroy V, Dharancy S, Louvet A, Boleslawski E, Lucidi V, Gustot T, Francoz C, Letoublon C, Castaing D, Belghiti J, Donckier V, Pruvot FR, Duclos-Vallée JC. Early liver transplantation for severe alcoholic hepatitis. *N Engl J Med* 2011; **365**: 1790-1800 [PMID: 22070476 DOI: 10.1056/NEJMoa1105703]

25 **Crabb DW**, Im GY, Szabo G, Mellinger JL, Lucey MR. Diagnosis and Treatment of Alcohol-Associated Liver Diseases: 2019 Practice Guidance From the American Association for the Study of Liver Diseases. *Hepatology* 2020; **71**: 306-333 [PMID: 31314133 DOI: 10.1002/hep.30866]

26 **Goldberg D**, Ditah IC, Saeian K, Lalehzari M, Aronsohn A, Gorospe EC, Charlton M. Changes in the Prevalence of Hepatitis C Virus Infection, Nonalcoholic Steatohepatitis, and Alcoholic Liver Disease Among Patients With Cirrhosis or Liver Failure on the Waitlist for Liver Transplantation. *Gastroenterology* 2017; **152**: 1090-1099.e1 [PMID: 28088461 DOI: 10.1053/j.gastro.2017.01.003]

27 **Liangpunsakul S**. Clinical characteristics and mortality of hospitalized alcoholic hepatitis patients in the United States. *J Clin Gastroenterol* 2011; **45**: 714-719 [PMID: 21085006 DOI: 10.1097/MCG.0b013e3181fdef1d]

28 **Roerecke M**, Vafaei A, Hasan OSM, Chrystoja BR, Cruz M, Lee R, Neuman MG, Rehm J. Alcohol Consumption and Risk of Liver Cirrhosis: A Systematic Review and Meta-Analysis. *Am J Gastroenterol* 2019; **114**: 1574-1586 [PMID: 31464740 DOI: 10.14309/ajg.0000000000000340]

29 **Thursz MR**, Richardson P, Allison M, Austin A, Bowers M, Day CP, Downs N, Gleeson D, MacGilchrist A, Grant A, Hood S, Masson S, McCune A, Mellor J, O'Grady J, Patch D, Ratcliffe I, Roderick P, Stanton L, Vergis N, Wright M, Ryder S, Forrest EH; STOPAH Trial. Prednisolone or pentoxifylline for alcoholic hepatitis. *N Engl J Med* 2015; **372**: 1619-1628 [PMID: 25901427 DOI: 10.1056/NEJMoa1412278]

30 **Ginès A**, Escorsell A, Ginès P, Saló J, Jiménez W, Inglada L, Navasa M, Clària J, Rimola A, Arroyo V. Incidence, predictive factors, and prognosis of the hepatorenal syndrome in cirrhosis with ascites. *Gastroenterology* 1993; **105**: 229-236 [PMID: 8514039 DOI: 10.1016/0016-5085(93)90031-7]

31 **Lucey MR**, Mathurin P, Morgan TR. Alcoholic hepatitis. *N Engl J Med* 2009; **360**: 2758-2769 [PMID: 19553649 DOI: 10.1056/NEJMra0805786]

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ORIGINAL ARTICLE

Retrospective Study

Analysis of invasiveness and tumor-associated macrophages infiltration in solid pseudopapillary tumors of pancreas

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Abstract

BACKGROUND

Solid pseudopapillary tumor (SPT) is a rare pancreatic tumor. Considering its malignant behaviors, SPT has been classified as a low-grade malignant tumor. Indeed, only 9.2% of all SPT patients are initially diagnosed as malignant with invasion or metastasis. Thus, one of the challenges in managing SPT patients is predicting malignant behavior.

AIM

To investigate the malignant behavior and tumor-associated macrophage (TAM) infiltration between different histopathologic features of SPT patients.

METHODS

Twenty-five formalin-fixed paraffin-embedded tissue samples from 22 patients pathologically diagnosed with an SPT between 2009 and 2019 at West China Hospital were included in this retrospective study. Integrity of the capsule and growth pattern of the tumor cells was assessed microscopically in hematoxylin-eosin (HE)-stained sections. Based on the histopathological features, the SPT patients were divided into two groups: capsule or invasion. Clinical features, malignant behavior, and TAM infiltration were compared between the two groups.

RESULTS

Among the 22 SPT patients, 11 were identified for each group, having either a capsule or invasion histopathologic feature. Malignant behavior was more frequent in the invasion group, including 2 patients who had peripheral organ

invasion, 3 with liver metastasis, and 1 with both lymph node and spleen metastases (P= 0.045). Ki-67 index of more than 3% was also more frequent in the invasion group (P = 0.045). Immunohistochemical analysis showed that the invasion group had a significant increase of CD68-positive TAMs in intratumor and peritumor sites in comparison with the capsule group (all P < 0.0001). Similarly, CD163-positive M2-like macrophages were also markedly increased in the intratumor and peritumor sites in the invasion group (all P < 0.0001). At the liver metastasis site, both intratumor and peritumor tissues showed relatively high-level CD68-positive TAMs and CD163-positive M2-like macrophages infiltration. However, the differences between the intratumor, peritumor and normal hepatic tissues did not reach statistical significance (all P > 0.05).

CONCLUSION

SPT patients with invasion evident under microscope were more likely to exhibit malignant behavior and TAM infiltration, especially M2-like macrophages. This finding can help in future investigations of the underlying mechanism of TAM-mediated SPT malignant behavior.

Key Words: Pancreatic neoplasms; Solid pseudopapillary tumors; Malignancy; Tumor-associated macrophages; Histological labeling; Immunohistochemistry

Core Tip: In the present study, we reviewed 22 solid pseudopapillary tumor (SPT) patients to investigate the malignant behavior and tumor-associated macrophage (TAM) infiltration between different histopathologic features. The results showed that invasion detected under microscope was associated with the malignant behavior and TAM infiltration. The phenomenon of increased infiltration of TAMs, especially M2-like macrophages in the invasion feature, might help us to investigate the underlying mechanism of TAM-mediated SPT malignant behavior, as well as to potentially treat recurrent and metastatic SPT by targeting TAMs.

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INTRODUCTION

Solid pseudopapillary tumor (SPT) is a rare pancreatic tumor, first reported by Frantz in 1959[1]. Considering its malignant behaviors, including the invasion of peripheral organs and metastasis at diagnosis or recurrence after surgical resection, the World Health Organization (WHO) has classified SPT as a low-grade malignant tumor[2,3]. The overall prognosis of SPT patients is good, with 5-year and 10-year survival rates up to 89.5% and 86.3%, respectively[4]. Reportedly, among all SPT patients, only 9.2% are initially diagnosed as malignant with invasion or metastasis[3]. Thus, one of the challenges in managing SPT patients lies in predicting malignant behavior.

Currently, clinical features are being studied to predict the malignant behaviors of SPT. For instance, patients over 40 years of age have been found to be more likely to develop malignant SPT[5,6]. The computed tomography (CT) imaging features of multiple and geographic uptake type and progressive enhancement during the delayed phase have shown potential predictive power[6,7]. One of the most frequently mentioned predictive factors is capsule or incomplete capsule of SPT, with the latter being significantly correlated with malignancy[1,5,8-10]. However, the capsule or incomplete capsule state is primarily assessed on CT imaging. Assessment of the integrity of a capsule and growth pattern of tumor cells, especially on SPT margins under the microscope, might reflect the real biological behavior of SPT.

Macrophages that infiltrate or surround the tumor microenvironment are termed as tumor-associated macrophages (TAMs)[11]. TAMs, especially those of the M2-like macrophage subtype, could enhance tumor invasion and metastasis[12]. However, only limited studies are available regarding the role of TAMs in the SPT microenvironment and their relationship with malignant behavior of SPT. Herein, we classified SPT patients in either a capsule group or invasion group based on the integrity of capsule and growth pattern of tumor cells determined microscopically, and compared the clinical features and malignant behaviors between the two groups. Furthermore, we used immunohistochemical (IHC) staining to analyze the infiltration of TAMs, especially the M2-like macrophages, in primary SPT and

liver metastasis sites.

MATERIALS AND METHODS

Patients and tissues

Twenty-five formalin-fixed paraffin-embedded tissue samples (including 22 tumors and peritumor tissues, and 3 metastatic sites) from 22 patients pathologically diagnosed with an SPT according to WHO 2010 criteria between 2009 and 2019 at West China Hospital were included in this retrospective study. All the samples were obtained by surgery and reviewed by two expert pathologists blinded to the original data. This study was approved by the biomedical ethics committee from our hospital with written informed consent from all subjects (2014 Trial No. 37).

Data collection and definition

Based on the integrity of the capsule and growth pattern of tumor cells, especially on tumor margins, evident in hematoxylin-eosin (HE)-stained sections under a microscope, SPT patients were divided into two groups: the capsule or invasion group. The invasion feature was defined as a tumor with an incomplete fibrous capsule with tumor cells invasively present in normal pancreatic tissue, as determined microscopically. On the other hand, a capsule feature is considered when the tumor has an intact fibrous envelope (Figure 1).

The malignant behaviors of SPT at diagnosis, including deep infiltration into the surrounding tissue and lymph node or distant organ metastasis, were recorded. The Ki-67 index was measured based on a manual visual counting method[13], and 3% was chosen as the cut-off value, according to findings from a recent study[14].

Clinical data was collected, including sex, age, serum tumor marker CA19-9, CA125, carcinoembryonic antigen, and neuron-specific enolase (NSE). Tumor size (largest diameter of the tumor), tumor location (head and neck or body and tail), component of the tumor (solid and cystic or solid), capsule or incomplete capsule, and calcification were also measured based on the CT imaging data and surgical specimens.

IHC analysis

IHC staining was used to evaluate the intratumoral and peritumoral presence of TAMs (CD68-positive) and M2-like macrophages (CD163-positive), a subtype of TAM, in primary and metastasis site tissues. Five micrometer-thick sequential tissue sections were obtained from paraffin-embedded tissue samples and used for the IHC analysis as described in previous studies[15,16]. The primary antibodies used in IHC staining were antibodies against CD68 (rabbit anti-human, D4B9C, dilution 1:400; Cell Signaling Technology, Danvers, MA, United States) and CD163 (rabbit anti-human, D6U1J, dilution 1:500; Cell Signaling Technology). Anti-rabbit IgG, horseradish peroxidase-linked antibody was used as the secondary antibody. For the assessment of expression of CD68- and CD163-positive cells, the tissue sections were screened using each immunohistochemistry slide at the low power fields (× 4), and the hot spots were selected. Immune cell staining was scored by counting the number of stained immune cells in three high power fields (× 400) in the hot spots. IHC sections were independently assessed by three authors (Yang J, Chen YH, and Zhou L).

Statistical methods

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) Version 24 (IBM Corp., Armonk, NY, United States). Categorical variables are expressed as percentages (%), and continuous variables are expressed as medians. Normality and homogeneity of the data were assessed by Shapiro–Wilk's and Levene's tests, respectively. Fisher's exact test and Mann–Whitney test were used to detect di□erences in categorical and continuous variables between groups of patients. A P value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Among the 22 SPT patients, 11 were identified for each group based on the capsule or invasion histopathologic feature, respectively. The clinical and pathological characteristics between the capsule and invasion groups are shown in Table 1. The patients included 4 males and 18 females, ranging in age from 12 years to 65 years (median age of 35 years) and showed no difference between the two groups (P = 0.586 and P= 0.146, respectively). Only 3 patients showed CA19-9 or CA125 elevation in the invasion group. Interestingly, 2 patients in the capsule group and 5 in the invasion group showed positivity for NSE, although the difference did not reach statistical significance (P = 0.361). All 22 patients received surgical treatment, which included 4 pancreaticoduodenectomies, 10 distal pancreatectomies, 7 central

Table 1 Clinical and pathological characteristics between capsule and invasion solid pseudopapillary tumor patients

	Capsule group, *n* = 11	Invasion group, *n* = 11	*P* value
Age, yr (Median, range)	26 (12, 46)	36 (15, 65)	0.146
Sex (Female)	10	8	0.586
Positive serum tumor marker			
CA19-9	0	1	1.000
CA125	0	2	0.476
CEA	0	0	-
NSE	2	5	0.361
Surgical procedure			-
Pancreaticoduodenectomy	1	3	
Distal pancreatectomy	5	5	
Total pancreatectomy	1	0	
Central pancreatectomy	4	3	
Combined segmental hepatectomy	0	3	
Tumor size (Median, range)	4.8 (2.0-8.1)	4.0 (1.3-8.8)	0.834
Tumor location			1.000
Head and neck	6	5	
Body and tail	5	6	
Component of the tumor			0.387
Solid	3	6	
Solid and cystic	8	5	
Calcification	3	2	1.000
Capsule assessed by CT imaging	9	2	0.004
Peripheral organ invasion	0	2	0.476
Metastasis	0	4[a]	0.045
Lymph node	0	1	
Liver	0	3	
Spleen	0	1	
Ki-67 Index > 3%	0	4	0.045

[a]1 patient had both spleen and lymph node metastasis.
CEA: Carcino-embryonic antigen; CT: Computed tomography; NSE: Neuron-specific enolase.

pancreatectomies, and 1 total pancreatectomy. All 3 patients with liver metastasis received combined segmental hepatectomy.

No difference was evident in tumor size, location, component, nor calcification between the two groups. However, compared with the capsule group, malignant behavior of SPTs was more frequent in the invasion group, including in 2 patients with peripheral organ invasion, 3 with liver metastasis, and 1 with lymph node and spleen metastases (P = 0.045). Furthermore, Ki-67 index more than 3% was also more frequent in the invasion group (P = 0.045).

TAM infiltration in primary tumor site

Since the invasion group had increased malignant behavior compared with the capsule group, we compared the infiltration of TAMs in the SPT microenvironment between the two groups. All primary tumor samples of the 22 patients showed positivity for macrophage and M2-like macrophage infiltration in IHC analysis. Although the intratumoral macrophage infiltration was increased in both groups compared with normal pancreatic tissue, the invasion group had a significant increase of intratumoral macrophage infiltration compared with the capsule group (all P < 0.0001; Figure 2A-C). A similar trend

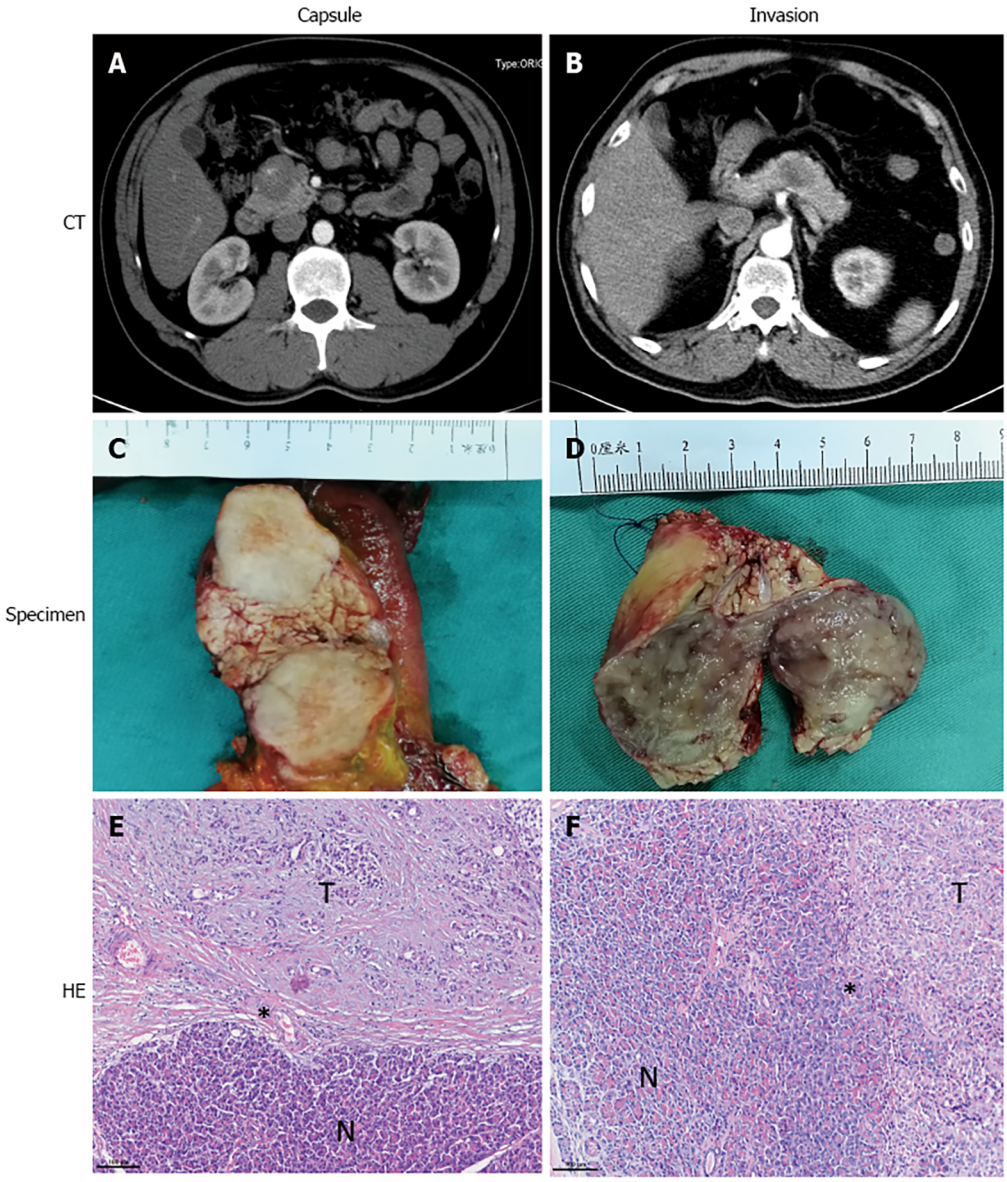

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Figure 1 Computed tomography imaging, specimen and histopathologic features of capsulized and invasive solid pseudopapillary tumors. A, C and E: For the capsulized solid pseudopapillary tumors (SPTs), computed tomography (CT) imaging (A) and specimen (C) showed a clear distinction from surrounding pancreatic tissue, and an intact fibrous envelope on the tumor margin on hematoxylin and eosin (HE)-stained section (E); B, D and F: Invasive SPT was indistinguishable from surrounding normal pancreatic tissue on CT imaging (B) and in the specimen (D), and tumor cells showed infiltrative growth into normal pancreatic tissue on HE-stained section (F). N: Normal pancreas tissue; T: Tumor. *Tumor margin.

was observed for infiltrated peritumor macrophages. The TAM infiltration in peritumoral tissue, especially in the tumor margin, was markedly increased in the invasion group compared with capsule group ($P < 0.0001$; Figure 2D-F).

As a subtype of TAMs, the expression of CD163-positive M2-like macrophages was relatively low compared with CD68-positive TAMs. Elevation in both intratumor and peritumor sites in both the invasion group and capsule group compared with the normal pancreas tissue was evident by IHC (all $P < 0.0001$; Figure 3). Similarly, the expression of CD163-positive M2-like macrophages was also markedly increased in the intratumor and peritumor sites in the invasion group in comparison to the capsule group (all $P < 0.0001$; Figure 3).

TAM infiltration in liver metastasis site

Tumors in the liver metastasis sites also showed invasion features. Tumor cells were observed as invasively grown into normal hepatic cells, and no complete fibrous capsule was found on HE-stained sections (Figure 4A and B). Liver metastasis of 3 SPT patients showed positivity for macrophage and M2-like macrophage infiltration. Like the primary tumor site of the invasion group, both intratumor and peritumor areas of the metastasis site had relatively high-level CD68-positive TAM infiltration.

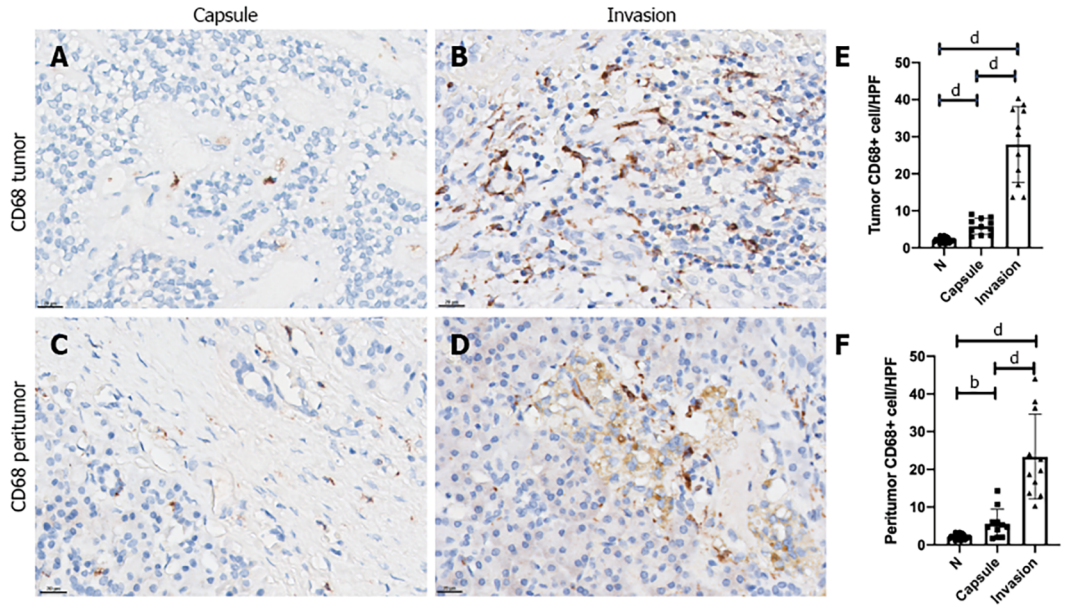

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Figure 2 Invasion group showed increased tumor-associated CD68-positive macrophage infiltration compared with capsule group by immunohistochemical analysis. A-F: Immunohistochemical-stained imaging and analysis of tumor (A-C) and peritumor (D-F) infiltrated with CD68-positive macrophages in representative patients from the capsule (A, D) and invasion (B, E) groups. N: Normal pancreas tissue. $^{b}P < 0.01$; $^{d}P < 0.0001$.

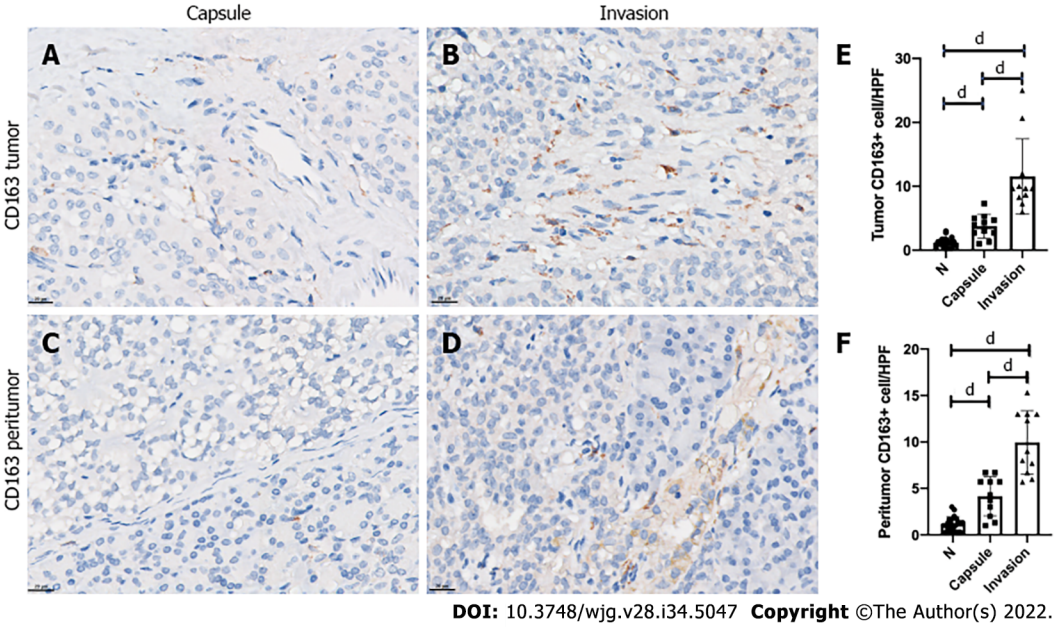

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Figure 3 Invasion group showed increased tumor-associated CD163-positive M2-like macrophage infiltration compared with capsule group by immunohistochemical analysis. A-F: Immunohistochemical-stained images and analysis of tumor (A-C) and peritumor (D-F) tissues infiltrated with CD163-positive M2-like macrophages in representative patients from the capsule (A, D) and invasion (B, E) groups. N: Normal pancreas tissue. $^{d}P < 0.0001$.

However, there was no difference between the intratumor, peritumor and normal hepatic tissues. The CD163-positive M2-like macrophages had relatively high expression in both the intratumor and peritumor metastasis sites. No statistically significant difference was found upon comparison of the CD163-positive M2-like macrophage infiltration of intratumor, peritumor and normal hepatic tissues.

DISCUSSION

In the present study, we found that the invasion feature evident under a microscope was associated

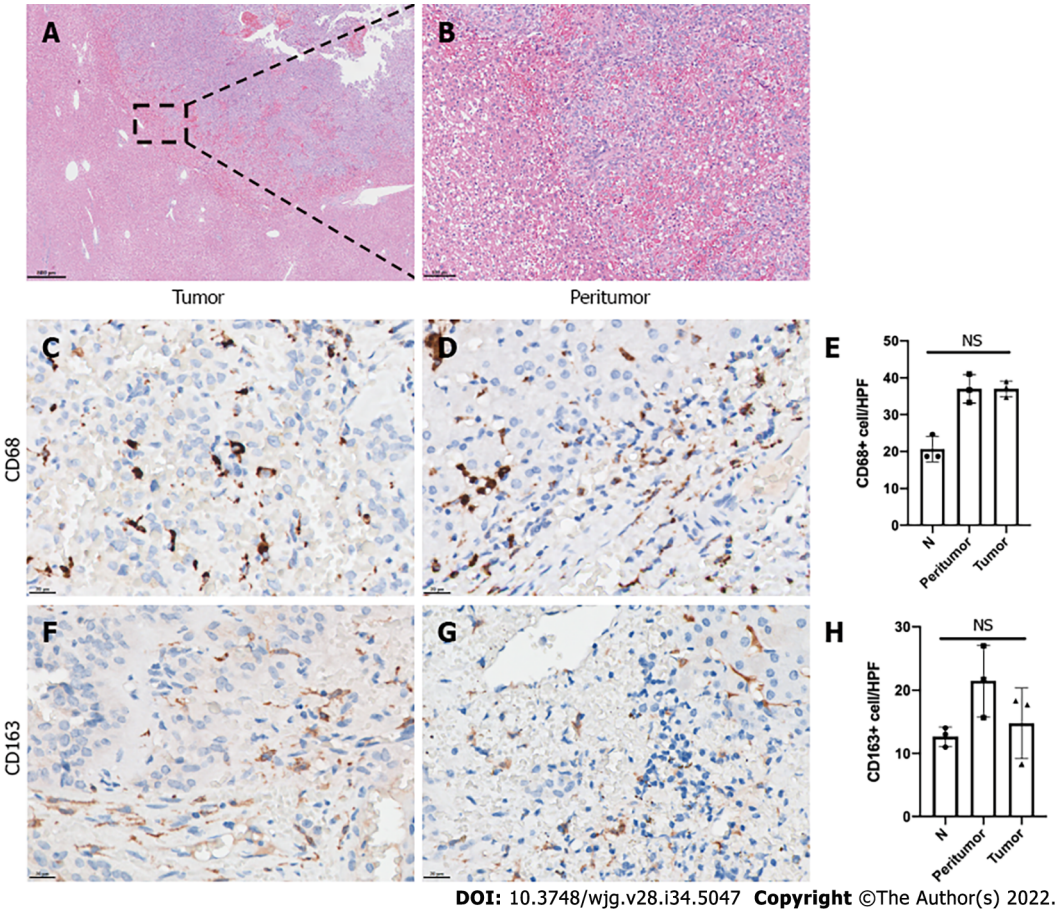

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Figure 4 Tumor-associated macrophage infiltration in liver metastasis sites of solid pseudopapillary tumor patients. A and B: Hematoxylin-eosin staining of the liver metastasis site of representative solid pseudopapillary tumor (SPT) patients; C-H: Immunohistochemical-stained imaging and analysis of intratumor and peritumor CD68 (C-E) and CD163 (F-H) expression in the liver metastasis site of representative SPT patients. N: Normal hepatic tissue; NS: No significance.

with malignant behavior of SPT. Additionally, we elaborated the spatial distribution of TAMs in the SPT tumor microenvironment and showed distinct expression of TAMs and M2-like macrophages between tissues with invasion and capsule features. Furthermore, we also described the intratumor and peritumor distribution of TAMs and M2-like macrophages in the liver metastases site. Considering the malignant behavior of SPTs, this study may provide a histopathological basis for identification of malignant SPTs. The phenomenon of increased infiltration of TAMs, especially M2-like macrophages, in the invasion feature might help in subsequent investigations of the underlying mechanism of TAM-mediated SPT malignant behavior, as well as to potentially treat recurrent and metastatic SPT by targeting TAMs.

The incomplete capsule feature assessed by CT imaging has been widely used and is considered able to predict malignant behavior of SPT[4]. Ye *et al*[1] showed this incomplete capsule group had a larger tumor size, an exogenous growth pattern, a malignant tendency during intraoperative frozen biopsy, and more likely to have invasion into the vasculature or organs. Indeed, in our study, the capsule or incomplete capsule measured by CT imaging was consistent with the capsule or invasion feature detected upon evaluation under a microscope. However, 2 patients in each group had distinct results. Considering the increased malignant behaviors of these SPT patients, we are concerned that assessment by CT imaging might miss patients with invasion features that could be detected with a microscope.

Other tumor types, such as small renal cell carcinoma, colorectal cancer and urinary bladder carcinoma, also have histopathological features of capsule or invasion as growth patterns, and the invasive growth feature has been shown as associated with worse prognosis of patients[17-19].

The intact fibrous envelope in extracellular matrix (ECM) might serve as a barrier for tumor cell invasion[20]. In our study, none of the 11 SPT patients with a capsule feature showed malignant behavior, such as invasion to surrounding tissues and distant metastasis. TAMs can secrete several proteolytic enzymes, including matrix metalloproteinases (MMPs, such as MMP7, MMP2, and MMP9), which can mediate the ECM degradation[21]. Particularly, M2-like macrophages could produce chitinase 3-like protein 1 to upregulate MMP expression and promote the invasiveness of gastric and breast cancer cells[22]. The expression of TAMs and M2-like macrophages in peritumor tissues of patients in the invasion group were significantly higher than those in the capsule group, which may be

related to the pathological characteristics of incomplete fibrous capsule and tumor cells' invasive growth pattern, and thus the malignant behaviors. Furthermore, the epithelial-mesenchymal transition hotpot, another mechanism associated with tumor cell invasiveness that mainly occurred at the peritumor site, was also accompanied by TAM infiltration in abundance in hepatocellular carcinoma patients[23]. Of interest, a recent study found that lipid-loaded TAMs could also sustain invasiveness in prostate cancer[24].

Metastasis to the liver through the portal venous system is a common route for the metastasis of pancreatic tumors. Indeed, 3 SPT patients in our study had liver metastasis. TAMs were involved in vascularization in a tumor environment and intravasation of tumor cells, two major mechanisms for tumor metastasis[11,25]. Vascular endothelial growth factor-A (VEGFA) is one of the primary factors driving expansion of the tumor vascular vascularization[26]. Infiltration of TAMs was associated with increased VEGFA expression and vascular density in tumor microenvironment[27,28]. This is probably because the TAMs could promote the expression of VEGFA in vascular endothelial cells by producing WNT7B[29]. Indeed, elevated expressions of fibroblast growth factor, placental growth factor, and anti-inflammatory cytokines, such as transforming growth factor-beta (TGF-β) and IL-10 in M2-like macrophages, could potentially enhance the vascularization[30,31]. Furthermore, tumor-associated M2-like macrophages could directly facilitate tumor metastasis by binding tumor cells and mediating their intravasation[32]. Therefore, when an SPT has an invasion feature, the increase of TAMs, especially M2-like macrophages, might facilitate metastasis compared with an SPT that has a capsule feature.

In addition to the vascularization and intravasation in primary tumor sites, the metastasis process includes the formation of a pre-metastatic niche followed by the growth phase[33]. TAMs in the pre-metastatic niche show an immunosuppressive phenotype characterized by elevated programmed death ligand-1 and nitric oxide synthase expression[34]. Macrophages are recruited during expansion of the metastatic site in the liver, through the CCL2–CCR2 signaling pathway[35]. Blocking the CCL2–CCR2 signaling axis was shown to reduce macrophage infiltration and metastatic expansion[36]. Few studies have reported the polarization of macrophages within or around liver metastasis sites[37]. In our study, we observed relatively more infiltration of TAMs, especially M2-like macrophages, in both intratumor and peritumor sites after the formation of SPT liver metastases. More importantly, to understand the role of TAMs in the formation of liver metastases, dynamic observation of the temporal and spatial changes of TAMs from a pre-metastatic niche to a pro-metastasis site is required.

Recently, Yang *et al*[14] classified SPT patients by the Ki-67 index based on their similar biological behavior in the 2017 WHO's pancreatic neuroendocrine tumor grade. In that study, the Ki-67 index was significantly associated with recurrence of SPT[14]. Regarding the Ki-67 index in the malignant behavior of SPT, in our research, a Ki-67 index more than 3% was observed in 4 patients in the invasion group, including 1 patient with peripheral organ invasion, 1 with both lymph node and spleen metastases, and 2 with liver metastasis. However, the Ki-67 index was only 1% in the remaining patient with peripheral organ invasion and liver metastasis. Indeed, previous meta-analyses that evaluated the Ki-67 index to predict malignant behavior of SPTs demonstrated distinct results, which might be due to the limited number of patients included and differences in the methods used to assess the Ki-67 index[38,39]. A multicenter study is needed to reveal the role of Ki-67 index in SPT patients. TAMs might be involved in the regulation of tumor cell Ki-67 expression. Lindsten *et al*[40] showed that increased infiltration of macrophages was associated with decreased progesterone receptor (commonly known as PR) expression and increased Ki-67 expression in breast tumor cells. Considering the regular expression of PR in SPT[41], the potential influence of TAMs on Ki-67 index in SPT needs further investigation.

Limitations of the present study should be noted. One of the limitations of this study was that the retrospective design and the paucity of the number of patients did not allow us to investigate causal associations between the TAMs and the invasiveness of SPT. In addition, a single immunohistochemical marker to identify target cells might not reflect the real proportion of M2-like macrophages in TAMs, so we did not compare this proportion between invasion and capsule groups in our study. The dichotomy of M1 and M2 classification of human TAM polarizations was not as obvious as in mouse, so we used M2-like macrophages instead. Further analysis of the specific distribution and function of TAMs in SPT patients using flow cytometry and a single-cell sequencing method might be used.

CONCLUSION

SPT patients with the invasion feature were more likely to demonstrate malignant behavior. These patients also showed increased infiltration of TAMs, especially M2-like macrophages, in both intratumor and peritumor sites compared with patients with the capsule feature.

ARTICLE HIGHLIGHTS

Research background

Solid pseudopapillary tumor (SPT) has been classified as a low-grade malignant tumor, and indeed only 9.2% patients of all SPT patients are initially diagnosed as malignant with invasion or metastasis. Thus, one of the challenges in managing SPT patients is predicting malignant behavior. One of the most frequently mentioned predictive factors is whether the tumor had a capsule or incomplete capsule as assessed by computed tomography imaging. However, assessing the integrity of the capsule and growth pattern of tumor cells, especially on SPT margins, using a microscope might reflect the real biological behavior of SPT. Tumor-associated macrophages (TAMs), especially the M2-like macrophages subtype, could enhance tumor invasion and metastasis; however, only limited studies are available regarding the role of the TAMs in the SPT microenvironment and their relationship with malignant behaviors of SPT.

Research motivation

The present study suggests that an invasiveness feature, as determined using a microscope is a good predictive factor for the malignant behavior of an SPT and illustrates the macrophage infiltration in the tumor microenvironment of an SPT.

Research objectives

Instead of assessing the integrity of the capsule on computed tomographic imaging, we divided SPT patients into two groups, either with invasion or capsule, based on the integrity of capsule and growth pattern of the tumor cells, especially on the tumor margins, under the microscope; we then investigated differences in the malignant behavior and TAM infiltration between these two groups.

Research methods

Hematoxylin-eosin-stained sections of SPT patients were used to assess the integrity of the capsule and growth pattern of tumor cells, especially on tumor margins. Immunohistochemical staining was used to evaluate the intratumoral and peritumoral presence of TAMs (CD68-positive) and M2-like macrophages (CD163-positive).

Research results

Malignant behavior was present more frequently in the invasion group, including in 2 patients with peripheral organ invasion, 3 with liver metastasis, and 1 with both lymph node and spleen metastases. Immunohistochemical analysis found that the invasion group had a significant increase of CD68-positive TAMs and CD163-positive M2-like macrophages in intratumoral and peritumoral sites in comparison with the capsule group.

Research conclusions

SPT patients with invasion detected using a microscope were more likely to have a tumor that demonstrated malignant behavior and TAM infiltration, especially of M2-like macrophages.

Research perspectives

This study may provide a histopathological basis for identification of malignant SPT. The phenomenon of increased infiltration of TAMs, especially M2-like macrophages in the invasion feature, might help us to investigate the underlying mechanism of TAM-mediated SPT malignant behavior, as well as to potentially treat recurrent and metastatic SPT by targeting TAMs.

FOOTNOTES

Author contributions: Yang J, Tan CL, Long D, Liang Y, Zhou Li, Liu XB, and Chen YH designed the research study; Yang J, Tan CL, Liu XB, and Chen YH performed the research; Yang J, Long D, Liang Y, Zhou Li, and Chen YH analyzed the data and wrote the manuscript; all authors have read and approved the final manuscript.

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REFERENCES

1 **Ye J**, Ma M, Cheng D, Yuan F, Deng X, Zhan Q, Shen B, Peng C. Solid-pseudopapillary tumor of the pancreas: clinical features, pathological characteristics, and origin. *J Surg Oncol* 2012; **106**: 728-735 [PMID: 22688864 DOI: 10.1002/jso.23195]
2 **Papavramidis T**, Papavramidis S. Solid pseudopapillary tumors of the pancreas: review of 718 patients reported in English literature. *J Am Coll Surg* 2005; **200**: 965-972 [PMID: 15922212 DOI: 10.1016/j.jamcollsurg.2005.02.011]
3 **Yu PF**, Hu ZH, Wang XB, Guo JM, Cheng XD, Zhang YL, Xu Q. Solid pseudopapillary tumor of the pancreas: a review of 553 cases in Chinese literature. *World J Gastroenterol* 2010; **16**: 1209-1214 [PMID: 20222163 DOI: 10.3748/wjg.v16.i10.1209]
4 **You L**, Yang F, Fu DL. Prediction of malignancy and adverse outcome of solid pseudopapillary tumor of the pancreas. *World J Gastrointest Oncol* 2018; **10**: 184-193 [PMID: 30079144 DOI: 10.4251/wjgo.v10.i7.184]
5 **Wang J**, Chen X, Wang C, Cui W, Ren S, Wang Z, Li H. Differentiation of aggressive from non-aggressive pancreatic solid pseudopapillary neoplasms using computed tomography. *Abdom Radiol (NY)* 2019; **44**: 2448-2458 [PMID: 30850890 DOI: 10.1007/s00261-019-01969-6]
6 **Kim JS**, Hao EI, Rho SY, Hwang HK, Lee WJ, Yoon DS, Kang CM. Clinical Pattern of Preoperative Positron Emission Tomography/Computed Tomography (PET/CT) Can Predict the Aggressive Behavior of Resected Solid Pseudopapillary Neoplasm of the Pancreas. *Cancers (Basel)* 2021; **13** [PMID: 33925678 DOI: 10.3390/cancers13092119]
7 **Rastogi A**, Assing M, Taggart M, Rao B, Sun J, Elsayes K, Tamm E, Bhosale P. Does Computed Tomography Have the Ability to Differentiate Aggressive From Nonaggressive Solid Pseudopapillary Neoplasm? *J Comput Assist Tomogr* 2018; **42**: 405-411 [PMID: 29287021 DOI: 10.1097/RCT.0000000000000698]
8 **Park JK**, Cho EJ, Ryu JK, Kim YT, Yoon YB. Natural history and malignant risk factors of solid pseudopapillary tumors of the pancreas. *Postgrad Med* 2013; **125**: 92-99 [PMID: 23816775 DOI: 10.3810/pgm.2013.03.2634]
9 **Song H**, Dong M, Zhou J, Sheng W, Zhong B, Gao W. Solid Pseudopapillary Neoplasm of the Pancreas: Clinicopathologic Feature, Risk Factors of Malignancy, and Survival Analysis of 53 Cases from a Single Center. *Biomed Res Int* 2017; **2017**: 5465261 [PMID: 29094047 DOI: 10.1155/2017/5465261]
10 **Song H**, Dong M. The Prognostic Factors of Preoperative Prognostic Nutritional Index and Radiological Findings of Solid Pseudopapillary Tumors of Pancreas: A Single-Center Experience of 14 Years. *Cancer Manag Res* 2020; **12**: 5689-5699 [PMID: 32765072 DOI: 10.2147/CMAR.S256650]
11 **Lin Y**, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J Hematol Oncol* 2019; **12**: 76 [PMID: 31300030 DOI: 10.1186/s13045-019-0760-3]
12 **Qian BZ**, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell* 2010; **141**: 39-51 [PMID: 20371344 DOI: 10.1016/j.cell.2010.03.014]
13 **Reid MD**, Bagci P, Ohike N, Saka B, Erbarut Seven I, Dursun N, Balci S, Gucer H, Jang KT, Tajiri T, Basturk O, Kong SY, Goodman M, Akkas G, Adsay V. Calculation of the Ki67 index in pancreatic neuroendocrine tumors: a comparative analysis of four counting methodologies. *Mod Pathol* 2015; **28**: 686-694 [PMID: 25412850 DOI: 10.1038/modpathol.2014.156]
14 **Yang F**, Wu W, Wang X, Zhang Q, Bao Y, Zhou Z, Jin C, Ji Y, Windsor JA, Lou W, Fu D. Grading Solid Pseudopapillary Tumors of the Pancreas: the Fudan Prognostic Index. *Ann Surg Oncol* 2021; **28**: 550-559 [PMID: 32424583 DOI: 10.1245/s10434-020-08626-z]
15 **Rahmati M**, Rashno A. Automated image segmentation method to analyse skeletal muscle cross section in exercise-induced regenerating myofibers. *Sci Rep* 2021; **11**: 21327 [PMID: 34716401 DOI: 10.1038/s41598-021-00886-3]
16 **Rahmati M**, Taherabadi SJ. The effects of exercise training on Kinesin and GAP-43 expression in skeletal muscle fibers of STZ-induced diabetic rats. *Sci Rep* 2021; **11**: 9535 [PMID: 33953268 DOI: 10.1038/s41598-021-89106-6]
17 **Krüger S**, Noack F, Böhle A, Feller AC. Histologic tumor growth pattern is significantly associated with disease-related survival in muscle-invasive transitional cell carcinoma of the urinary bladder. *Oncol Rep* 2004; **12**: 609-613 [PMID: 15289845]

18 **Morikawa T**, Kuchiba A, Qian ZR, Mino-Kenudson M, Hornick JL, Yamauchi M, Imamura Y, Liao X, Nishihara R, Meyerhardt JA, Fuchs CS, Ogino S. Prognostic significance and molecular associations of tumor growth pattern in colorectal cancer. *Ann Surg Oncol* 2012; **19**: 1944-1953 [PMID: 22189472 DOI: 10.1245/s10434-011-2174-5]

19 **Li G**, Xiao T, Wang K, Zhang R, Wang A, Yan C, Wang C. Histopathological validation of safe margin for nephron-sparing surgery based on individual tumor growth pattern. *World J Surg Oncol* 2021; **19**: 255 [PMID: 34454535 DOI: 10.1186/s12957-021-02375-3]

20 **Mierke CT**. The matrix environmental and cell mechanical properties regulate cell migration and contribute to the invasive phenotype of cancer cells. *Rep Prog Phys* 2019; **82**: 064602 [PMID: 30947151 DOI: 10.1088/1361-6633/ab1628]

21 **Kessenbrock K**, Plaks V, Werb Z. Matrix metalloproteinases: regulators of the tumor microenvironment. *Cell* 2010; **141**: 52-67 [PMID: 20371345 DOI: 10.1016/j.cell.2010.03.015]

22 **Chen Y**, Zhang S, Wang Q, Zhang X. Tumor-recruited M2 macrophages promote gastric and breast cancer metastasis *via* M2 macrophage-secreted CHI3L1 protein. *J Hematol Oncol* 2017; **10**: 36 [PMID: 28143526 DOI: 10.1186/s13045-017-0408-0]

23 **Fu XT**, Dai Z, Song K, Zhang ZJ, Zhou ZJ, Zhou SL, Zhao YM, Xiao YS, Sun QM, Ding ZB, Fan J. Macrophage-secreted IL-8 induces epithelial-mesenchymal transition in hepatocellular carcinoma cells by activating the JAK2/STAT3/Snail pathway. *Int J Oncol* 2015; **46**: 587-596 [PMID: 25405790 DOI: 10.3892/ijo.2014.2761]

24 **Masetti M**, Carriero R, Portale F, Marelli G, Morina N, Pandini M, Iovino M, Partini B, Erreni M, Ponzetta A, Magrini E, Colombo P, Elefante G, Colombo FS, den Haan JMM, Peano C, Cibella J, Termanini A, Kunderfranco P, Brummelman J, Chung MWH, Lazzeri M, Hurle R, Casale P, Lugli E, DePinho RA, Mukhopadhyay S, Gordon S, Di Mitri D. Lipid-loaded tumor-associated macrophages sustain tumor growth and invasiveness in prostate cancer. *J Exp Med* 2022; **219** [PMID: 34919143 DOI: 10.1084/jem.20210564]

25 **Fu LQ**, Du WL, Cai MH, Yao JY, Zhao YY, Mou XZ. The roles of tumor-associated macrophages in tumor angiogenesis and metastasis. *Cell Immunol* 2020; **353**: 104119 [PMID: 32446032 DOI: 10.1016/j.cellimm.2020.104119]

26 **Claesson-Welsh L**, Welsh M. VEGFA and tumour angiogenesis. *J Intern Med* 2013; **273**: 114-127 [PMID: 23216836 DOI: 10.1111/joim.12019]

27 **Valković T**, Dobrila F, Melato M, Sasso F, Rizzardi C, Jonjić N. Correlation between vascular endothelial growth factor, angiogenesis, and tumor-associated macrophages in invasive ductal breast carcinoma. *Virchows Arch* 2002; **440**: 583-588 [PMID: 12070596 DOI: 10.1007/s004280100458]

28 **Laoui D**, Van Overmeire E, Di Conza G, Aldeni C, Keirsse J, Morias Y, Movahedi K, Houbracken I, Schouppe E, Elkrim Y, Karroum O, Jordan B, Carmeliet P, Gysemans C, De Baetselier P, Mazzone M, Van Ginderachter JA. Tumor hypoxia does not drive differentiation of tumor-associated macrophages but rather fine-tunes the M2-like macrophage population. *Cancer Res* 2014; **74**: 24-30 [PMID: 24220244 DOI: 10.1158/0008-5472.CAN-13-1196]

29 **Yeo EJ**, Cassetta L, Qian BZ, Lewkowich I, Li JF, Stefater JA 3rd, Smith AN, Wiechmann LS, Wang Y, Pollard JW, Lang RA. Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. *Cancer Res* 2014; **74**: 2962-2973 [PMID: 24638982 DOI: 10.1158/0008-5472.CAN-13-2421]

30 **Jetten N**, Verbruggen S, Gijbels MJ, Post MJ, De Winther MP, Donners MM. Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo. *Angiogenesis* 2014; **17**: 109-118 [PMID: 24013945 DOI: 10.1007/s10456-013-9381-6]

31 **Cui X**, Morales RT, Qian W, Wang H, Gagner JP, Dolgalev I, Placantonakis D, Zagzag D, Cimmino L, Snuderl M, Lam RHW, Chen W. Hacking macrophage-associated immunosuppression for regulating glioblastoma angiogenesis. *Biomaterials* 2018; **161**: 164-178 [PMID: 29421553 DOI: 10.1016/j.biomaterials.2018.01.053]

32 **Wang J**, Cao Z, Zhang XM, Nakamura M, Sun M, Hartman J, Harris RA, Sun Y, Cao Y. Novel mechanism of macrophage-mediated metastasis revealed in a zebrafish model of tumor development. *Cancer Res* 2015; **75**: 306-315 [PMID: 25492861 DOI: 10.1158/0008-5472.CAN-14-2819]

33 **Tsilimigras DI**, Brodt P, Clavien PA, Muschel RJ, D'Angelica MI, Endo I, Parks RW, Doyle M, de Santibañes E, Pawlik TM. Liver metastases. *Nat Rev Dis Primers* 2021; **7**: 27 [PMID: 33859205 DOI: 10.1038/s41572-021-00261-6]

34 **Morrissey SM**, Zhang F, Ding C, Montoya-Durango DE, Hu X, Yang C, Wang Z, Yuan F, Fox M, Zhang HG, Guo H, Tieri D, Kong M, Watson CT, Mitchell RA, Zhang X, McMasters KM, Huang J, Yan J. Tumor-derived exosomes drive immunosuppressive macrophages in a pre-metastatic niche through glycolytic dominant metabolic reprogramming. *Cell Metab* 2021; **33**: 2040-2058.e10 [PMID: 34559989 DOI: 10.1016/j.cmet.2021.09.002]

35 **Grossman JG**, Nywening TM, Belt BA, Panni RZ, Krasnick BA, DeNardo DG, Hawkins WG, Goedegebuure SP, Linehan DC, Fields RC. Recruitment of CCR2^{+} tumor associated macrophage to sites of liver metastasis confers a poor prognosis in human colorectal cancer. *Oncoimmunology* 2018; **7**: e1470729 [PMID: 30228938 DOI: 10.1080/2162402X.2018.1470729]

36 **Zhao L**, Lim SY, Gordon-Weeks AN, Tapmeier TT, Im JH, Cao Y, Beech J, Allen D, Smart S, Muschel RJ. Recruitment of a myeloid cell subset (CD11b/Gr1 mid) *via* CCL2/CCR2 promotes the development of colorectal cancer liver metastasis. *Hepatology* 2013; **57**: 829-839 [PMID: 23081697 DOI: 10.1002/hep.26094]

37 **Brodt P**. Role of the Microenvironment in Liver Metastasis: From Pre- to Prometastatic Niches. *Clin Cancer Res* 2016; **22**: 5971-5982 [PMID: 27797969 DOI: 10.1158/1078-0432.Ccr-16-0460]

38 **Zou C**, Yang F, Wu W, Fu D. Ki-67 and malignancy in solid pseudopapillary tumor of the pancreas: A systematic review and meta-analysis. *Pancreatology* 2020; **20**: 683-685 [PMID: 32247627 DOI: 10.1016/j.pan.2020.03.015]

39 **Yepuri N**, Naous R, Meier AH, Cooney RN, Kittur D, Are C, Jain A, Dhir M. A systematic review and meta-analysis of predictors of recurrence in patients with Solid Pseudopapillary Tumors of the Pancreas. *HPB (Oxford)* 2020; **22**: 12-19 [PMID: 31350105 DOI: 10.1016/j.hpb.2019.06.005]

40 **Lindsten T**, Hedbrant A, Ramberg A, Wijkander J, Solterbeck A, Eriksson M, Delbro D, Erlandsson A. Effect of macrophages on breast cancer cell proliferation, and on expression of hormone receptors, uPAR and HER-2. *Int J Oncol* 2017; **51**: 104-114 [PMID: 28498427 DOI: 10.3892/ijo.2017.3996]

41 **Zalatnai A**, Kis-Orha V. Solid-pseudopapillary Neoplasms of the Pancreas is still an Enigma: a Clinicopathological Review. *Pathol Oncol Res* 2020; **26**: 641-649 [PMID: 31209654 DOI: 10.1007/s12253-019-00671-8]

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ORIGINAL ARTICLE

Observational Study

Impact of adalimumab on disease burden in moderate-to-severe ulcerative colitis patients: The one-year, real-world UCanADA study

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Abstract

BACKGROUND

A gap remains in documenting the impact of anti-tumor necrosis factor therapy on disease burden in ulcerative colitis (UC) patients treated in a real-world setting. The use of patient-reported outcomes (PROs) has been discussed as a primary endpoint in the context of the FDA PRO Guidance, for labelling purposes. Specifically, the efficacy and safety of adalimumab have been demonstrated in pivotal trials; however, data are needed to understand how clinical results translate into improvements in key aspects of the daily lives of UC patients, such as symptoms, health-related quality of life (HRQoL), and disability.

AIM

To assess real-world effectiveness of adalimumab on PRO measures in patients with moderate-to-severe UC.

METHODS

UCanADA was a single arm, prospective, 1-year multicenter Canadian post-marketing observational study in which multiple PRO questionnaires were completed—with psychologic distress/depression symptoms as the primary endpoint—by patients with moderate-to-severe UC. Assessments were performed during patients' routine care visit schedule, which was at the initiation of adalimumab (baseline), after induction (approximately 8 wk), and 52 wk after baseline. Additional optional assessments between weeks 8 and 52 were collected at least once but no more than two times during this period. Serious safety events and per-protocol adverse events were collected.

RESULTS

From 23 Canadian centres, 100 patients were enrolled and 48 completed the study. Measured with the Patient Health Questionnaire–9 items at week 52, 61.5% (40/65) [95% confidence interval (CI): 49.7%-73.4%] of the patients improved in psychologic distress/depression symptoms, which was slightly higher in completers [65.9% (29/44); 95%CI: 51.9%-79.9%)]. At week 52, clinical response and clinical remission were achieved respectively by 65.7% (44/73) and 47.8% (32/73) of the patients. The odds of improving depressive symptoms for those achieving a clinical remission at week 52 was 7.94 higher compared with those not achieving a clinical remission (CI: 1.42, 44.41; P = 0.018). Significant changes from baseline to weeks 8 and 52 were observed in disability, HRQoL, and fatigue. Meaningful improvement was reported in work impairment.

CONCLUSION

At week 52, over 60% of the UCanADA patients had depressive symptoms significantly reduced, as well as HRQoL, fatigue symptoms, and work impairment improved. No new safety signals were detected.

Key Words: Disease burden; Patient-reported outcome; Depressive symptoms; Ulcerative colitis; Adalimumab; Real-world data

Core Tip: In real-world at week 52, over 60% of patients with moderate-to-severe ulcerative colitis treated with adalimumab had their depressive symptoms improved, as well as their quality of life, fatigue symptoms, and work impairment. No new safety signals were detected.

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INTRODUCTION

Ulcerative colitis (UC)—an intermittent, idiopathic, and chronic disease of the colon—has a worldwide incidence of 1 to 20 *per* 100000 individuals and a prevalence of 5 to 500 *per* 100000 individuals[1,2]. As of 2018 in Canada, it is estimated that 120000 individuals live with this disease.

The burden of UC has been recognized to extend beyond its clinical signs and physical symptoms such as bloody diarrhea and abdominal pain, including the development of anxiety/depression, a decreased health-related quality of life (HRQoL), an impact on work productivity and social interactions, and impairments in sexual function[3-6]. As such, having access to multidisciplinary, collaborative, chronic disease models of care improves patients' HRQoL[7].

Around 30% of patients with inflammatory bowel disease (IBD) experience psychiatric disorders, and depression/anxiety in these patients have been shown to be three times greater than in the general population[8]. In UC patients, the prevalence of depression symptoms and disorders have been estimated as 16.7% [95% confidence interval (CI): 12.0%-21.4%][9]. Assessing the severity of depression in over 158000 IBD patients, a Patient Health Questionnaire–9 items (PHQ-9) pooled mean score of 7.6 (95%CI: 6.3-8.8, on a 0-27 scale) has been reported, which can be interpreted as a mild depression[9,10].

Among physical symptoms, fatigue, which is not relieved by rest and implies limitations of daily activities[11], has been reported by 42% to 47% of UC patients at diagnosis[12]. Fatigue has been shown to impact IBD patients' QoL and is experienced by those of all ages, with some studies suggesting a

greater burden in women[13-17]. In a recent review on IBD, Nocerino *et al*[18] documented strong associations between fatigue and sleep disturbance and inadequate sleep, highlighting a proportion of more than 50% of both active and inactive IBD patients reporting sleep deficiency.

Consistent with the increasing inclusion of patient's voice in all aspects of health care as in UC therapies[19] and aiming to align with the FDA guidance[20], the use of patient-reported outcome (PRO) questionnaires are more and more used as clinical endpoints in IBD studies[21]. The use of PRO instruments helps understand patients' preferences, which in turn has been shown to be associated with treatment acceptance and adherence[22-25].

In IBD populations, depression/anxiety has been measured with a wide range of tools, including the PHQ-9[9,26-29]. The Inflammatory Bowel Disease Questionnaire (IBDQ) and its short version (SIBDQ) as well as the EuroQol 5-Dimentions, 5 Levels (EQ-5D-5L) questionnaire have been used to assess HRQoL[30-34], fatigue has been assessed by the Functional Assessment Chronic Illness Therapy–Fatigue (FACIT-F) questionnaire[18,35-37], and work productivity with the Work Productivity and Activity Impairment (WPAI) questionnaire[26,30,38-40].

Developed and validated in 2012 in a population-based cohort, the inflammatory bowel disease disability index (IBD-DI) is specific to assess disability in IBD patients[41-43]. On a 0-100 scale, the mean (interquartile range) value of the IBD-DI was 35.3 (Q1 = 19.6; Q3 = 51.8). Higher IBD-DI values were associated with female gender ($P < 0.001$), clinical disease activity ($P < 0.0001$), and disease duration ($P = 0.02$)[42].

To provide real-world data on improvements in daily lives of UC patients, the overall goal of the UCanADA study was to gather evidence on effectiveness, quality of life, disability, and work productivity during an adalimumab treatment. The primary objective was to evaluate psychological distress/depression symptoms using change from baseline in the PHQ-9 after 1 year of a real-world adalimumab treatment in moderate-to-severe UC patients.

MATERIALS AND METHODS

Study design and patients

In this prospective, single arm, 1-year multicenter Canadian post-marketing observational study, adults (≥ 18 years) with a confirmed diagnosis of UC and a moderate-to-severe disease activity–evidenced by either a Mayo endoscopic subscore (MES) of 2 or 3 from endoscopic investigation in the previous 3-mo closest to the baseline visit, or a Mayo rectal bleeding subscore ≥ 2 and a calprotectin value greater than 250 mg/g–were enrolled if they were prescribed adalimumab as part of their treatment by their treating physician. If a patient was previously treated with vedolizumab or any anti-tumor necrosis factor (TNF) agent (except adalimumab), an appropriate washout period took place *per* routine practice, which period varied usually from 2 to 3 mo.

Excluded from the study were patients who either previously received adalimumab, used infliximab or any anti-TNF agent and did not clinically respond at any time unless they experienced a treatment limiting reaction; had a history of subtotal colectomy with ileorectostomy or colectomy with ileoanal pouch, Kock pouch, or ileostomy for UC or planned bowel surgery; had a current diagnosis of indeterminate colitis, ulcerative proctitis only, or with a current diagnosis and/or have a history of Crohn's disease; had other TNF immune-modulated disease; OR had a significant history of renal, neurologic, psychiatric, endocrinologic, metabolic, immunologic, cardiovascular, or hepatic disease that in the opinion of the investigator would adversely affect his/her participating in this study. Also, we excluded from the study pregnant or breast-feeding female patients and patients currently participating in another prospective study including controlled clinical trials.

Eligible patients were approached to participate in the study after a decision to change the patient's therapy for adalimumab was already made by the treating physician. To participate in the study and to disclose personal health information, all patients were required to sign a patient authorization form (or written informed consent), which was approved by an Independent Ethics Committee/Institutional Review Board (ClinicalTrials.gov Identifier: NCT02506179). The study was conducted between July 2015 and December 2019 in 23 Canadian sites, with approximately half of the sites being community based and the other half academic based.

Assessments

Patients were followed for 52 wk post initiation of adalimumab treatment (baseline). The assessments were performed during patients' routine care visits schedule, coinciding approximately to 8 and 52 wk after baseline, in accordance with the Canadian approved label (product monograph) and as *per* regional requirements. The completers population was defined as patients who received at least one dose of treatment, and at least one follow-up appointment and did not terminate the study early or discontinue.

During these visits, the patients' medical history and changes in medical conditions, previous and concomitant medications, and disease severity and activity [clinical response defined as simple clinical colitis activity index (SCCAI)[44] decrease from baseline of ≥ 2, clinical remission defined as SCCAI

score ≤ 2, endoscopic evaluation (MES), assessment of rectal bleeding (Mayo Rectal bleeding Subscore), and the physician's global assessment (PGA)] were assessed.

Also, patients were required to fill, on paper at the physician's office, eight PRO questionnaires for evaluating: The presence and severity of depression (PHQ-9 using a 0-27 scale)[10], the entire spectrum of limitations in functioning in patients (IBD-DI questionnaire evaluating 4 domains of body functions, activity and participation, body structures, and environmental factors)[41], HRQoL (EQ-5D-5L questionnaire comprising mobility, self-care, usual activities, pain/discomfort, and anxiety/depression using a 0-1 scale)[45], SIBDQ questionnaire assessing the social, emotional, bowel, and systemic domains on a 1-7 scale[46], fatigue (FACIT-F questionnaire having a fatigue subscale score with a range from 0 to 52)[47], sleep related outcomes [Medical Outcomes Study Sleep scale (MOS Sleep) 12-item questionnaire including sleep disturbance, sleep awakening short of breath or with headache, sleep adequacy, somnolence, and quantity of sleep/optimal sleep][48], work related outcomes [WPAI: UC V2.0[49] presenting percentages of absenteeism (work time missed), presenteeism (impairment while working), an overall work impairment (overall productivity loss, accounting for both absenteeism and presenteeism), and activity impairment (impairment in activities outside work)], and Valuation of Lost Productivity (VOLP) questionnaire[50], assessing the impact of health conditions on lost productivity in monetary units. The order by which the PRO questionnaires were filled was varied to limit the potential of missing data that would systemically be found for a particular instrument.

Safety assessments included serious adverse events (AEs), any non-serious event of malignancy in patients 30 years of age and younger[51], unusual failure in efficacy, and AEs leading to discontinuation. These were coded using Medical Dictionary for Regulatory Activities version 17.1.

Study size and statistical methods

The sample size was calculated assuming a proportion of 15% of patients would improve their PHQ-9 score compared with baseline and change severity category. Using an alpha of 0.05 with a lower CI of 6%, a sample size of 72 patients would be needed. To account for a potential 25% attrition over the course of one year, the sample size was increased to 100 patients. It was anticipated that up to 30% of the 100 moderate-to-severe UC patients newly treated with adalimumab would have prior experience with biologics.

The primary effectiveness endpoint—the proportion of patients with a change in depressive symptoms using the PHQ-9 score from baseline following initiation of adalimumab and after 1 year of treatment—was calculated, and the 95%CIs were estimated. Changes in PHQ-9 scores from baseline were tested by paired sample *t*-test. Least-square mean (LS mean) of the changes were also estimated by the mixed effect repeated measures models where baseline values were included as a covariate. Changes in severity categories were tested by Bowker's test (*kxk* table where $k > 2$) or McNemar's test (2 × 2 table).

To understand the independent effect of clinical effectiveness on the probability of improving in PHQ-9 at week 52, a logistic regression analysis was conducted to examine the effect of clinical response and clinical remission adjusting for the baseline PHQ-9 score and other potential prognostic factors. A similar analysis was conducted to assess the association between clinical effectiveness on changes of PHQ-9 scores from baseline. LS mean of the changes associated with clinical response and clinical remission was estimated by the mixed effect repeated measures models using all follow-up visits.

For secondary outcomes, the IBD-DI, EQ-5D-5L, SIBDQ, FACIT-F, and MOS Sleep, scores at baseline, week 8, and week 52 were summarized, and changes in scores from baseline were tested by paired sample *t*-tests. LS mean of the changes were also estimated by the mixed effect repeated measures models where the baseline value was included as a covariate. Productivity outcomes (WPAI and VOLP) at baseline, week 52, and changes in outcome from baseline were summarized. The 95%CIs for the changes were estimated by bootstrapped percentile CIs based on samples of 10000.

The sensitivity to change for the IBD-DI was evaluated using the effective size (ES) and the standardized response mean (SRM). For both statistics, values of 0.020, 0.50, and 0.80 or greater were used to represent small, moderate, and large, respectively. The association between the change in PROs (EQ-5D-5L, SIBDQ, FACIT-F, and MOS Sleep) and clinical response/remission (effectiveness) were assessed using a mixed model for repeated measures using observations from all follow-up visits with the baseline value included in the model as a covariate. All models with repeated measures included a random intercept with the effectiveness variable (fixed, forced-in), visit (fixed, forced-in), baseline value of the PRO measure (fixed, forced-in) and other covariates. Cross-sectional regression models included an intercept with the effectiveness variable (forced-in), baseline value of the PRO measure (fixed) and other covariates. Least squares means, *P* value and 2-sided 95%CI of the difference between the two groups defined by the clinical effectiveness were determined. Additional details on the statistical analysis used to determine the correlation between effectiveness (clinical response and remission) rates and PRO measures are provided in the Supplementary material section.

Missing data were imputed only for the sensitivity analysis of the primary outcome. For missing responses on PRO questionnaire items, missing data were handled *per* the imputation solutions provided in the coding of the PRO instruments. To assess the impact of missing data on the primary endpoint estimate, the sensitivity analysis was performed using two imputation methods: Non-responder imputation (NRI), defined as patients who did not provide week 52 effectiveness data or

dropped out of the study prior to week 52 were considered as no improvement; and last observation carried forward (LOCF) defined as the last effectiveness assessment prior to week 52 was used for those missing week 52 assessment.

All calculations and analyses were performed using SAS version 9.4 (Cary, NC: SAS Institute Inc.) under the Windows 10 Enterprise operating system at the Centre for Health Evaluation and Outcome Sciences, in Vancouver, Canada.

RESULTS

Patients

One hundred patients from 23 Canadian sites were included in the study (Figure 1). Respectively, 94, 48, and 98 patients were included in the effectiveness population [intent-to-treat (ITT) population], the completers population, and the safety population. Patients in the ITT population had a mean age (SD) of 42.5 (15.3) years and a mean body mass index of 25.4 (4.5) kg/m^2 (Table 1). The majority was White (93.6%) and male (59.6%). The mean age at UC diagnosis was 34.5 (15.3) years, and the mean duration of disease was 7.9 (8.0) years. Forty-eight (48%) patients completed the study.

Impact on psychological distress/depression symptoms

Following routine care treatment with adalimumab, the proportion of patients who improved in psychological distress/depressive symptoms using the PHQ-9 total score at week 52—defined as a change in PHQ-9 total score from baseline, the study primary endpoint—was 61.5% (40/65) (95%CI: 49.7%-73.4%) for the ITT population and 65.9% (29/44) (95%CI: 51.9%-79.9%) for the completers population (Figure 2A). To assess the impact of missing data, the sensitivity analyses conducted on the primary endpoint showed that the proportions of patients who improved in psychological distress/depressive symptoms, using the NRI and LOCF imputation methods, were similar to the proportion obtained using the original non-imputed data analysis, with the ITT population (Supplementary Table 1).

Overall, changes from baseline in the PHQ-9 total score were significant at weeks 8 and 52 (P = 0.010), with changes slightly higher for the completers population [-2.5 (6.1) and -3.4 (6.8) at weeks 8 and 52] than in the ITT population [-2.2 (6.1) -2.4 (7.1)] on a 0-27 scale (Table 2). The proportion of patients with a PHQ-9 total score 10 (yellow flag category, *i.e.*, moderate or more severe depression) was 25.6% (21/82) at week 8, which slightly increased to 29.2% (19/65) at week 52 for the ITT population. These proportions were lower and stable over time (18%-19%) for the completers population. For patients with severe depressive symptoms (red flag category), the proportions improved from 19.1% (18/94) and 16.7% (8/48) at baseline to 12.3% (8/65) and 2.3% (1/44) at week 52 for the ITT population and completers population, respectively.

The PHQ-9 questionnaire items that showed the highest improvement from baseline were 'Poor appetite or overeating' (response 'Not at all' increased by 23.3% from baseline to week 52, and response 'Nearly every day' decreased by 20.3% from baseline to week 52), 'Little interest or pleasure in doing things' (response 'Not at all' increased by 15.3% from baseline to week 52, and response 'Nearly every day' decreased by 12.1% from baseline to week 52), and 'Feeling tired or having little energy' (response 'Not at all' increased by 8.2% from baseline to week 52, and response 'Nearly every day' decreased by 20.3% from baseline to week 52) (Supplementary Table 2).

Clinical endpoints

The proportions of patients who achieved a clinical response (decrease from baseline ≥ 2 in SCCAI score) remained similar throughout the study [64.2% (52/81) at week 8 and 65.7% (44/67) at week 52], and the proportion who achieved clinical remission (SCCAI score ≤ 2) slightly increased [41.5% (34/82) at week 8 and 47.8% (32/67) at week 52] in the ITT population (Figure 2B). For the completers population, these proportions increased during the study for both the clinical response and clinical remission, reaching 85.4% (35/41) and 73.2% (30/41), respectively, at week 52 (Supplementary Table 3).

Similarly, the proportions of patients who achieved endoscopic healing remained constant between weeks 8 and 52 [42.9% (6/14) had a Mayo endoscopic score of 0 or 1 and 11.5% (3/26), had a fecal calprotectin concentration < 50 μg/g] in the ITT population (Figure 2C), whereas in the completers population, the proportions increased over time [71.4% (5/7) and 80.0% (8/10), measured with the Mayo endoscopic score and 7.7% (1/13) and 11.8% (2/17) measured with the fecal calprotectin concentration, at weeks 8 and 52, respectively] (Supplementary Table 3).

No major changes were observed over time in the extracolonic feature, with the majority of patients not having arthritis at both baseline and follow-up visit [81.5% (66/81) at week 8 and 82.1% (55/67) at week 52] (Figure 2D). The proportion of patients who had arthritis at baseline and none at follow-up was 8.6% (7/81) at week 8 and 9.0% (6/67) at week 52. Similar proportions were observed in the completers population (Supplementary Table 3).

Table 1 Baseline patient and disease characteristics

Characteristics		*N*	*n* (%) or mean ± SD
Age		94	42.5 (15.3)
Gender	Male	94	56 (59.6)
BMI (kg/m^2)		91	25.4 (4.5)
Race	American Indian/Alaska Native	94	1 (1.1)
	Asian		5 (5.3)
	White		88 (93.6)
Employment status	Disability		2 (2.1)
	Employed (fulltime, part time < 35 h/week)		63 (67.0)
	Homemaker		3 (3.2)
	Retired		12 (12.8)
	Student		6 (6.4)
	Temporary leave of absence		1 (1.1)
	Unemployed		6 (6.4)
	Unknown		1 (1.1)
Tobacco use	Current smoker	94	2 (2.1)
	Former smoker		34 (36.2)
	Never smoked		55 (58.5)
	Unknown		3 (3.2)
Alcohol use	Non-drinker	94	20 (21.3)
	Ex-drinker		5 (5.3)
	Light (less than 2 drinks *per* day)		61 (64.9)
	Moderate (2-4 drinks *per* day)		5 (5.3)
	Unknown		3 (3.2)
Age at UC diagnosis (yr)		98	34.5 (15.3)
Disease duration (yr)		98	7.9 (8.0)
Family history of UC	No	98	59 (60.2)
	Yes		20 (20.4)
	Unknown		19 (19.4)
Montreal classification of extent of UC	E2	97	55 (56.7)
(prior 3 mo)	E3		42 (43.3)
Mayo Endoscopic Subscore	1	90	1 (1.1)
(prior 3 mo)	2		66 (73.3)
	3		23 (25.6)
Endoscopy (prior 6 mo)	Yes	98	91 (92.9)
UC-related ED visit (prior 6 mo)	Yes	98	13 (13.3)
UC-related hospitalization (prior 6 mo)	Yes	98	12 (12.2)
Previous biologic use	Yes	44	5 (11.4)
Medication use:	Corticosteroids	98	63 (64.3)
Since UC diagnosis to prior 6 mo	Imuran (azathioprine)		37 (37.8)
	6-MP		8 (8.2)
	5-ASA		83 (84.7)

	Methotrexate		5 (5.1)
	Cyclosporine		1 (1.0)
Medication use:	Corticosteroids	98	61 (62.2)
Since prior 6 mo to current	Imuran (azathioprine)		39 (39.8)
	6-MP		5 (5.1)
	5-ASA		67 (68.4)
	Methotrexate		8 (8.2)
	Cyclosporine		0 (0.0)

Montreal classification of extent of UC: E2 = left sided (distal) ulcerative colitis, E3 = extensive (pancolitis) ulcerative colitis; Mayo endoscopic subscore: 0 = normal or inactive disease, 1 = mild disease, 2 = moderate disease, 3 = severe disease; 5-ASA: 5-aminosalicylic acid; 6-MP: Mercaptopurine; BMI: Body mass index; ED: Emergency department; UC: Ulcerative colitis.

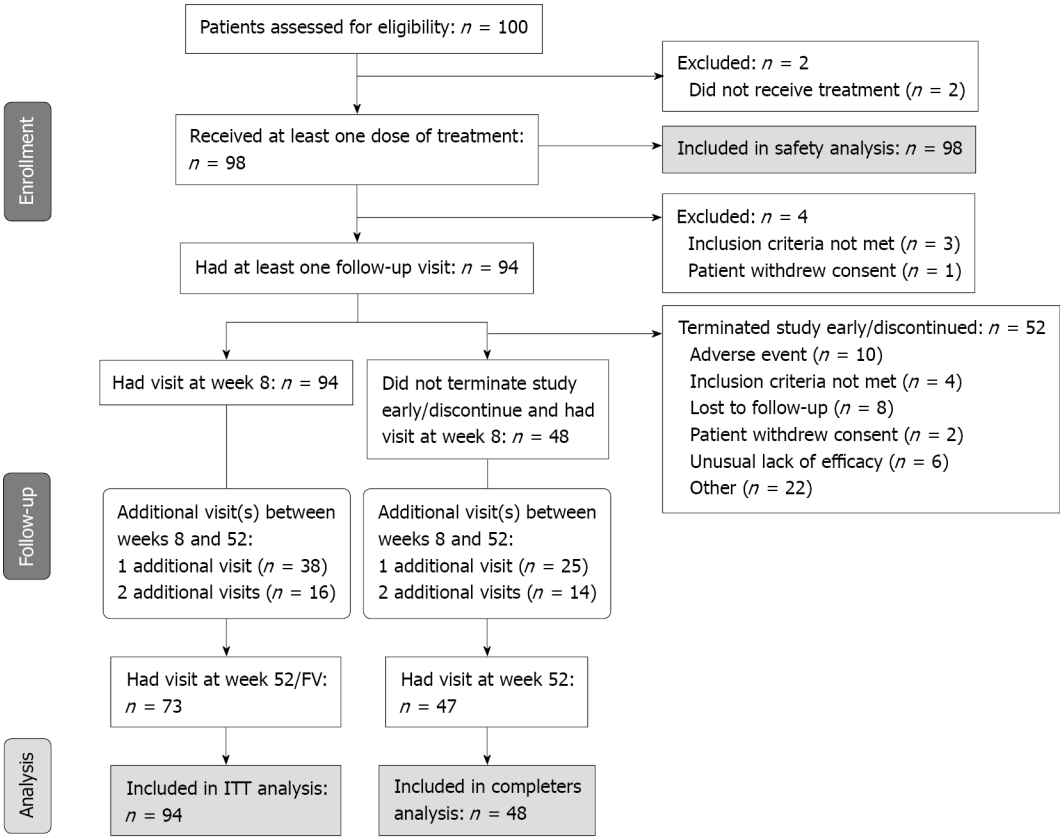

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Figure 1 UCanADA study flow diagram. The intent-to-treat population was defined as patients who received at least one dose of treatment and had at least one follow-up appointment. The completers population was defined as patients who received at least one dose of treatment, and at least one follow-up appointment and did not terminate the study early or discontinue. The safety population was defined as patients who received at least one dose of treatment. FV: Final visit; ITT: Intent-to-treat.

The proportion of patients who were PGA responders, defined as a decrease from baseline of ≥ 1 point, varied from 66.7% (50/75) to 61.5% (40/65) for the ITT population (Figure 2E) and from 73.2% (30/41) to 83.7% (36/43) for the completers population, from week 8 to week 52, respectively (Supplementary Table 3). While at baseline the majority of patients had moderate disease [73.9% (68/92)], at week 8 the highest proportion of patients had mild disease [35.5% (27/76)], and at week 52, 50.0% (33/66) of patients were assessed as normal (Figure 2E). The proportion of patients assessed with severe disease remained comparable between week 8 [5.3% (4/76)] and week 52 [6.1% (4/66)]. Similar results were reported for the completers population (Supplementary Table 3).

A proportion of 5.5% (4/73) of patients reported complications including hospitalization and surgery at week 52 in the ITT population (Supplementary Table 4), and none were reported among the completers population (Supplementary Table 3). The proportion of patients with current steroid use

Table 2 Patient Health Questionnaire–9 items at week 8 and week 52/final visit

Outcome	ITT			Completers		
	Baseline (*n* = 94)	Week 8 (*n* = 94)	Week 52/final visit (*n* = 73)	Baseline (*n* = 48)	Week 8 (*n* = 48)	Week 52/final visit (*n* = 47)
PHQ-9 total score						
n	94	82	65	48	43	44
mean ± SD	8.8 (6.3)	6.8 (5.3)	6.5 (5.6)	8.2 (6.7)	5.8 (5.6)	4.6 (4.3)
Median	8.0	6.0	5.0	5.0	5.0	3.0
Min, Max	0.0, 26.0	0.0, 26.0	0.0, 22.0	1.0, 26.0	0.0, 26.0	0.0, 15.0
Change from baseline						
n		82	65		43	44
mean ± SD		-2.2 (6.1)	-2.4 (7.1)		-2.5 (6.1)	-3.4 (6.8)
P value		0.002	0.008		0.010	0.002
PHQ-9 category, *n* (%)						
Minimal	35 (37.2)	31 (37.8)	28 (43.1)	23 (47.9)	21 (48.8)	25 (56.8)
Mild	23 (24.5)	30 (36.6)	18 (27.7)	8 (16.7)	14 (32.6)	11 (25.0)
Moderate	18 (19.1)	14 (17.1)	11 (16.9)	9 (18.8)	4 (9.3)	7 (15.9)
Moderately severe	11 (11.7)	4 (4.9)	7 (10.8)	4 (8.3)	2 (4.7)	1 (2.3)
Severe	7 (7.4)	3 (3.7)	1 (1.5)	4 (8.3)	2 (4.7)	0 (0.0)
P value		0.280	0.681		0.610	0.542
PHQ-9: Yellow flag category, *n* (%)						
	36 (38.3)	21 (25.6)	19 (29.2)	17 (35.4)	8 (18.6)	8 (18.2)
P value		0.028	0.083		0.021	0.059
PHQ-9: Red flag category, *n* (%)						
Yes	18 (19.1)	7 (8.5)	8 (12.3)	8 (16.7)	4 (9.3)	1 (2.3)
P value		0.013	0.134		0.180	0.034

The yellow flag category was defined as the proportion of patients with a Patient Health Questionnaire–9 total score ≥ 10, and the red flag category was defined as patients with severe depressive symptoms. ITT: Intent-to-treat; Max: Maximum; Min: Minimum; PHQ-9: Patient Health Questionnaire–9 items.

decreased between week 8 and week 52 from 29.4% (25/85) to 19.2% (14/73) in the ITT population and from 22.2% (10/45) to 12.8% (6/47) in the completers population.

Improvement in IBD-DI

The mean change from baseline in IBD-DI increased from -10.7 (17.21) at week 8 to -13.8 (22.24) at week 52 ($P < 0.001$), with proportions of patients who improved disability increasing from 72.6% (53/73) to 74.1% (40/54) over time in the ITT population (Figure 2F). Results from the completers population were slightly higher [mean change from baseline = -14.69 (17.99) and -20.09 (17.74) and improved disability = 81.4% (35/43) and 88.9% (32/36) at weeks 8 and 52, respectively] (Supplementary Table 5).

The IBD-DI was moderately sensitive to change for the ITT population, varying from -0.61 to -0.77 for ES and had a SRM of -0.62 (Supplementary Table 6). For the completers population, the IBD-DI was greatly sensitive to change. The ES varied from -0.75 to 1.08 and the SRM varied from -0.82 to -1.13

Correlations between PHQ-9 and clinical outcomes

A correlation analysis showed that at week 52 an improvement in PHQ-9 total score was associated with the baseline PHQ-9 score, with a higher baseline score predicting a greater improvement on the PHQ-9 (Table 3). No associations were detected between an improvement in PHQ-9 and clinical response; however, an association was measured with clinical remission in the ITT analysis (OR: 7.94; 95%CI: 1.42-44.41; $P = 0.018$), though this was not statistically significant in the completer analysis.

Table 3 Association between clinical response/remission and improvement in Patient Health Questionnaire–9 items total score at week 52/final visit–intent-to-treat and completers populations

Analysis population	Parameter	Model coefficient (SE)	Est. odds ratio (95%CI)	*P* value
Clinical response				
ITT	Baseline PHQ-9 total score	0.17 (0.06)	1.19 (1.05-1.34)	0.005
	Clinical response at week 52: Yes versus No	0.30 (0.65)	1.35 (0.38-4.84)	0.648
	UC duration (years)	0.11 (0.05)	1.11 (1.00-1.24)	0.051
Completers	Baseline PHQ-9 total score	0.20 (0.10)	1.22 (1.00-1.48)	0.049
	Clinical response at week 52: Yes versus No	-1.29 (1.29)	0.28 (0.02-3.43)	0.317
	UC duration (years)	0.14 (0.08)	1.15 (0.98-1.35)	0.079
Clinical remission				
ITT	Baseline PHQ-9 total score	0.28 (0.09)	1.33 (1.11-1.59)	0.002
	Clinical remission at week 52: Yes versus No	2.07 (0.88)	7.94 (1.42-44.41)	0.018
	UC duration (years)	0.11 (0.06)	1.11 (1.00-1.24)	0.054
Completers	Baseline PHQ-9 total score	0.22 (0.11)	1.24 (1.00-1.53)	0.045
	Clinical remission at week 52: Yes versus No	1.39 (1.07)	4.00 (0.50-32.37)	0.193
	UC duration (years)	0.14 (0.08)	1.15 (0.98-1.36)	0.095

Clinical response based on simple clinical colitis activity index (SCCAI): decrease from baseline of ≥ 2; Clinical remission: SCCAI ≤ 2; CI: Confidence interval; ITT: Intent-to-treat; PHQ-9: Patient Health Questionnaire–9 Items; SE: Standard error.

A regression analysis between the PHQ-9 total score and clinical response/remission at week 52 showed an association at week 52 using the ITT population ($P < 0.001$), but not in the completer population ($P \geq 0.098$) (Supplementary Table 7).

Impact on other PROs and correlation with clinical outcomes

For the other PRO tools used to assess the impact of the adalimumab treatment, changes from baseline at weeks 8 and 52 were significant for the EQ-5D-5L utility score, SIBDQ total score, FACIT-F total score, MOS Sleep Problems Index I and Sleep Problems Index II ($P = 0.049$) measured in the ITT population (Table 4) and the completers population (Supplementary Tables 8 and 9). A regression analysis showed that changes in the PRO measures between baseline and week 52 were all significantly associated with clinical outcomes ($P < 0.001$), except for the MOS Sleep measures ($P \geq 0.064$) (Supplementary Table 10).

The SIBDQ items of social function and bowel symptoms improved the most from baseline [mean change at week 52, 1.09 (2.11) and 0.93 (1.66), respectively on a 1-7 scale] ($P < 0.001$) (Table 4). Observing the FACIT-F measures, patients reported gaining more over time from the physical fatigue [3.86 (7.11)] than from the functional fatigue [2.58 (6.36)], emotional fatigue [2.06 (5.26)], and social impact of fatigue [1.32 (4.60)], at week 52 ($P = 0.024$). For the MOS Sleep subscales, only the sleep adequacy subscale significantly improved over time, with a mean change from baseline of 11.69 (5.26) at week 52 ($P < 0.001$).

All the WPAI scores improved from baseline, and the ones that improved the most were activity impairment [-16.9% (29.8) at week 8 and -16.7% (33.6) at week 52], *i.e.*, activities performed outside of work, and overall work impairment [-16.2% (30.2) at week 8 and -14.5% (34.4) at week 52], which combines absenteeism and presenteeism at work (Table 4). Similar results were reported for the completers (Supplementary Table 11).

For the VOLP, the most important changes were reported at week 52 and were related to paid work [paid work productivity loss in the past x months = -42.2 (115.7) hours and any paid work productivity loss in the past x months = -17% (-16.7%)] and lost productivity [any costs of lost productivity in the past x month = -15% (11.6%) hours and total costs of lost productivity in the past x months ($) = -1998 (-7299.7) $]. Study completers reported slightly higher results (Supplementary Table 11).

Safety

The safety profile was consistent with the known safety profile of adalimumab. During the study, 18 (18.4%) patients experienced at least one AE (Table 5). The AEs reported by more than 1% of patients

Table 4 Change from baseline in other patient-reported outcomes at weeks 8 and 52–intent-to-treat population

PRO measure	Baseline	Change from baseline				
	n (%) or mean ± SD	*N*	Week 8, *n* (%) or mean ± SD	*P* value	*N*	Week 52, *n* (%) or mean ± SD	*P* value
EQ-5D-5L	0.78 (0.17)	83	0.05 (0.17)	0.021	65	0.06 (0.24)	0.049
SIBDQ							
Total score	4.26 (1.08)	83	0.60 (1.08)	< 0.001	65	0.71 (1.24)	< 0.001
Social function	4.40 (1.91)	82	0.93 (1.78)	< 0.001	64	1.09 (2.11)	< 0.001
Emotional function	4.32 (0.78)	83	0.23 (0.82)	0.013	65	0.20 (0.93)	0.093
Bowel symptoms	4.25 (1.33)	83	0.69 (1.50)	< 0.001	65	0.93 (1.66)	< 0.001
Systemic symptoms	4.11 (1.63)	83	0.63 (1.33)	< 0.001	65	0.68 (1.56)	< 0.001
FACIT-F							
Fatigue subscale	30.10 (13.76)	83	3.78 (12.29)	0.006	65	5.41 (13.87)	0.003
Physical fatigue	17.67 (6.59)	83	2.44 (6.27)	< 0.001	65	3.86 (7.11)	< 0.001
Social impact of fatigue	20.69 (5.02)	82	0.52 (3.97)	0.234	65	1.32 (4.60)	0.024
Emotional fatigue	15.37 (4.71)	83	1.10 (4.24)	0.021	65	2.06 (5.26)	0.002
Functional fatigue	15.65 (5.54)	83	2.07 (5.43)	< 0.001	65	2.58 (6.36)	0.002
Trial outcome index	63.42 (24.02)	83	8.29 (21.79)	< 0.001	65	11.85 (24.85)	< 0.001
FACT-G total score	69.38 (16.99)	82	6.16 (15.58)	< 0.001	65	9.82 (18.58)	< 0.001
FACIT-F total score	99.48 (29.04)	82	9.99 (26.34)	< 0.001	65	15.23 (30.64)	< 0.001
MOS Sleep							
Sleep problems index I	40.46 (19.16)	83	-4.14 (15.89)	0.020	65	-6.56 (16.21)	0.002
Sleep problems index II	42.30 (19.69)	83	-3.47 (15.66)	0.047	65	-4.92 (16.75)	0.021
Sleep disturbance scale	41.52 (25.58)	83	-3.67 (20.82	0.112	65	-4.22 (22.30)	0.132
Snoring scale	30.22 (33.08)	81	0.00 (24.49)	1.000	63	2.54 (25.78)	0.437
Short of breath scale	11.06 (19.37)	83	2.41 (21.50)	0.310	65	3.08 (19.12)	0.199
Sleep adequacy	42.13 (26.96)	83	8.07 (26.01)	0.006	65	11.69 (26.31)	< 0.001
Somnolence scale	43.12 (27.35)	83	-1.37 (22.42)	0.581	65	-4.00 (25.24)	0.206
Sleep quantity	6.73 (1.41)	82	0.13 (1.29)	0.371	63	0.34 (1.31)	0.042
WPAI[1]							
Work time missed (%)	18.9 (31.1)	48	-6.7 (30.9)		37	-9.4 (35.0)	
Work impairment while working (%)	39.5 (28.5)	46	-14.8 (33.1)		38	-14.5 (35.8)	
Overall work impairment (%)	44.2 (30.1)	42	-16.2 (30.2)		35	-14.5 (34.4)	
Activity impairment (%)	46.0 (31.9)	83	-16.9 (29.8)		64	-16.7 (33.6)	
VOLP							
Any paid work productivity loss in the past x months (%)	45 (76.3)	58	-5 (-4.6)		47	-17 (-16.7)	
Paid work productivity loss in the past x months (hours)	98.5 (122.7)	51	13.5 (127.8)		42	-42.2 (115.7)	
Any unpaid work productivity loss in the past 7 d (%)	20 (29.0)	61	-8 (-9.3)		48	-7 (-1.9)	
Unpaid work productivity loss in the past 7 d (hours)	3.9 (11.6)	61	-3.4 (12.4)		48	-1.9 (14.8)	
Any costs of lost productivity in the past x month (%)	47 (79.7)	58	-5 (-7.3)		47	-15 (-11.6)	

Total costs of lost productivity in the past x months ($)	6075.8 (8890.9)	51	-1328 (5594.9)	42	-1998 (7299.7)

[1]Due to health.

EQ-5D-5L: EuroQol 5-Dimensions, 5 Levels; CI: Confidence interval; FACIT-F: Functional Assessment Chronic Illness Therapy-Fatigue; MOS: Medical Outcomes Study; PRO: Patient-reported outcome; SIBDQ: Short Quality of Life in Inflammatory Bowel Disease Questionnaire.

Table 5 Overview of adverse events–safety population

	Events (*n* = 55)	Patients (*n* = 98)
All AEs	55	18 (18.4%)
Severe AEs	10 (18.2%)	5 (5.1%)
AEs related to study drug	17 (30.9%)	12 (12.2%)
Mild	5 (29.4%)	4 (4.1%)
Moderate	6 (35.3%)	6 (6.1%)
Severe	4 (23.5%)	2 (2.0%)
Not provided	2 (11.8%)	2 (2.0%)
Serious AEs	27 (49.1%)	7 (7.1%)
Number of patients with AEs		
Resulting in hospitalization		6 (6.1%)
Resulting in study drug discontinuation		15 (15.3%)
Malignancy in patients ≤ 30 yr	0	0
Death	0	0

Percentages of patients were calculated based on the total number of patients. Percentages of events were based on the total number of events.
AE: Adverse event.

were: Colitis ulcerative [6 (6.1%) patients], drug ineffective [6 (6.1%) patients], haematochezia [2 (2.0%) patients], and arthralgia [2 (2.0%) patients]. Each of the severe AEs was experienced by only one (1.0%) patient, which included one event each of anal fissure, colitis, dysphagia, and mouth ulceration. One patient experienced two events of severe oesophagitis.

Two (2.0%) patients experienced serious treatment-related AEs that were assessed by the investigator to be reasonably possibly related to adalimumab: 1 (1.0%) patient experienced two events of severe oesophagitis that led to hospitalization and prolongation of hospitalization, and one event of severe aggravated colitis that led to hospitalization; 1 (1.0%) patient experienced severe injection site pain. There was one report of cutaneous basal cell cancer in a 63-year-old male. Monitored as *per* protocol safety variable, there were no reports of malignancy in patients 30 years of age and younger. No death was reported during the study, and no new signal or unexpected trend was identified for the patient population.

DISCUSSION

To fill an information gap on Canadian real-world data on the effectiveness of adalimumab on PRO measures in moderate-to-severe UC patients, and consistent with the FDA guidelines on the use of PRO measures to support labelling claims[20], the UCanADA study enrolled 100 patients from 23 centres using as a primary endpoint the change from baseline in depressive symptoms at week 52, measured by the PHQ-9 questionnaire.

The PHQ-9 measures in study UCanADA showed that over 60% of the study population improved in psychological distress/depression symptoms during the real-world adalimumab treatment, with most gains observed at week 52 in the completers population (65.9%). Significant changes from baseline were observed at week 8, which were maintained at week 52 and were slightly higher for the completers population (P = 0.010). Despite this improvement, these scores may be interpreted as a remaining mild depression in patients[10] and not necessarily a clinically meaningful change[52-54], which indicates a potential relevance to offer psychological support to this population[7,55]. In the present observational

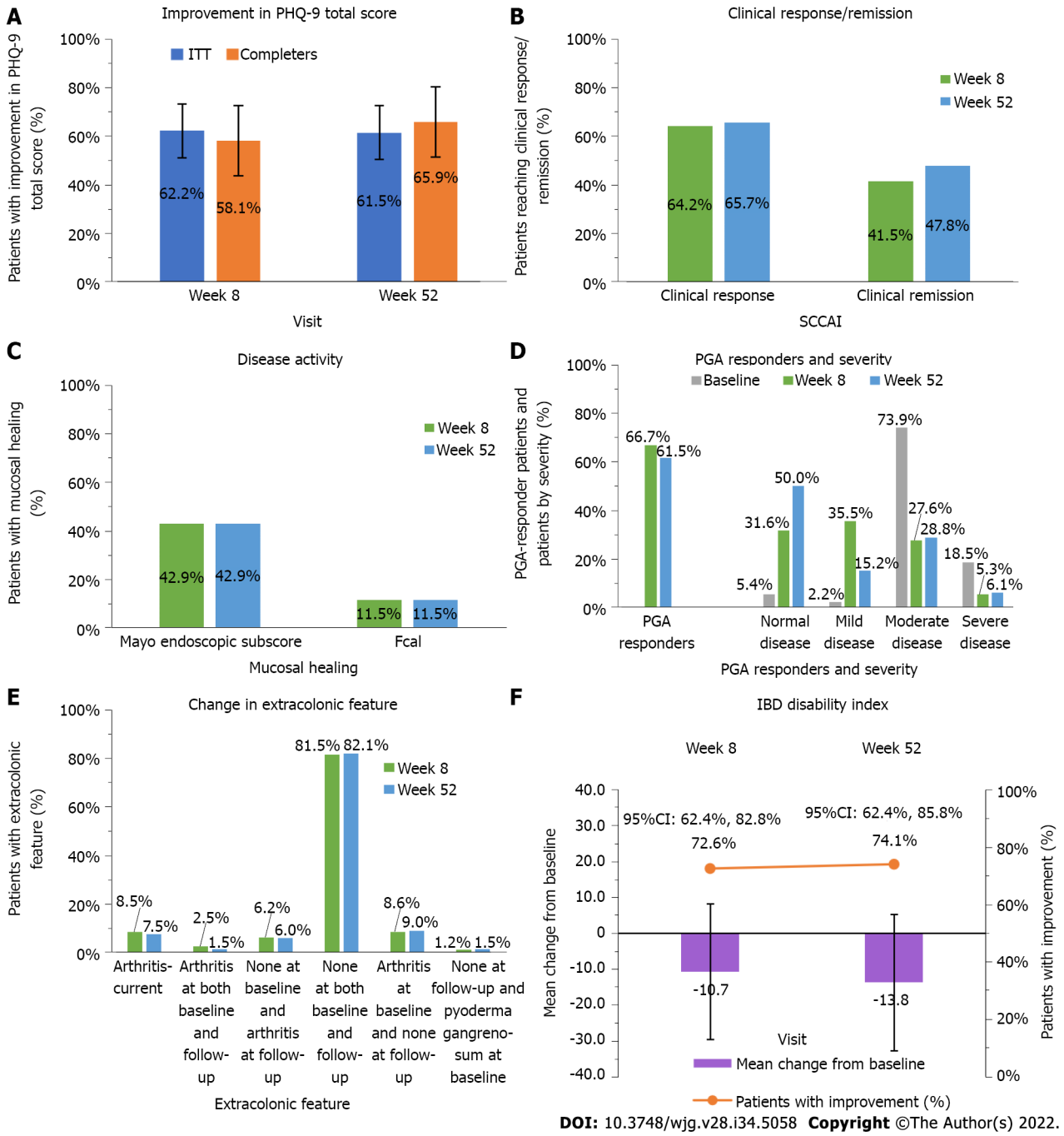

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Figure 2 Effect of a real-world adalimumab treatment on effectiveness and patient-reported outcomes in moderate-to-severe ulcerative colitis patients. A: Proportion of patients who improved in Patient Health Questionnaire–9 items total score at week 52 in the intent-to-treat and completers populations (%), improvement defined as change from baseline, bars show 95% confidence intervals; B: Proportion of patients achieving clinical response/remission at weeks 8 and 52 (%), measured by the simple clinical colitis activity index (SCCAI) ≤ 2; C: Proportion of patients with endoscopic healing at weeks 8 and 52 (%), healing measured as a Mayo endoscopic subscore of 0 or 1 or a fecal calprotectin concentration < 50 μg/g; D: Proportion of patients with extracolonic feature at weeks 8 and 52 (%), measured by the SCCAI; E: Proportion of physician's global assessment responders and severity at weeks 8 and 52 (%), responders defined as with a decrease from baseline of ≥ 1 point; F: inflammatory bowel disease index mean change from baseline and proportion of patients who improved (%). Bars represent standard deviations. PRO: Patient-reported outcome; UC: Ulcerative colitis; PHQ-9: Patient Health Questionnaire–9; IBD: Inflammatory bowel disease; SCCAI: Simple clinical colitis activity index; ITT: Intent-to-treat; PGA: Physician's global assessment.

study, while patients who had a preliminary failure to biologics were excluded, patients with secondary failures were included, which may have led to the inclusion of patients with greater psychological burden than biologic-naïve patients.

Our results show a significant change from baseline in PHQ-9 score earlier during treatment than those of a recently reported cohort of 1804 UC outpatients, who were included regardless of treatment assignment or disease activity[26]. However, a similar decrease in PHQ-9 score was measured in UC patients at least 30 d after being initiated on an anti-TNF therapy (including infliximab, adalimumab, or

certolizumab) and/or immunomodulator therapy (methotrexate or azathioprine)[29].

At week 52, clinical response and clinical remission were achieved respectively by 65.7% and 47.8% of the ITT population, and 85.4% and 73.2% of the completers population. These results are comparable to those from the InspirADA study at week 8, in which 463 moderate-to-severe UC patients from 92 international sites were treated with adalimumab following usual clinical practice[40].

To our knowledge, UCanADA is the first study reporting associations between PHQ-9 scores and clinical response/remission in UC patients in a real-world setting. A regression analysis showed that in the ITT population, the odds of improving depressive symptoms for those achieving a clinical remission at week 52 was 7.94 higher compared to those not achieving a clinical remission (OR: 7.94; 95%CI: 1.42-44.41; $P = 0.018$).

These results–as well as the significant associations measured between the PHQ-9 total score and clinical response/remission at week 52 ($P < 0.001$) and between clinical response/remission and IBD-DI, EQ-5D-5L, SIBDQ total score, and FACIT-F fatigue subscale ($P = 0.002$)–are consistent with the other findings showing a relationship between disease activity and HRQoL[34,35,56]. In a 6-mo study including 199 UC patients, a consistent and almost linear relationship was demonstrated between SCCAI values and the EQ-5D-5L index values (correlation: ρ: -0.53; $P < 0.001$)[34].

Other studies conducted in UC patients reported correlations between disease activity indexes and HRQoL measures. Aniwan *et al*[35] reported a good correlation between the SIBDQ and the combination of self-rated rectal bleeding and stool frequency using the 6-point partial Mayo score (ClinPRO2) and MES, from a study on 90 UC patients ($r = -0.70$; $P < 0.01$). Assessed on 110 UC patients, a significant correlation has been reported between SIBDQ and SCCAI and MES alone ($r = −0.79$ and $r = −0.58$, respectively)[56]. Consistent with our findings, these support an association between clinical remission and improved HRQoL.

To the PHQ-9 item 'Feeling tired or having little energy' between baseline and week 52, the proportion of patients feeling it 'Nearly every day' decreased by 20%, and those feeling it 'Not at all' increased by 8%. These results are in line with the significant decrease in fatigue shown in the FACIT-F total score and fatigue subscale ($P = 0.006$).

As fatigue has been reported to be strongly associated with sleep disturbances in IBD patients[18], not surprisingly our scores from the MOS sleep problems indexes I and II also significantly improved during the study, as well as sleep adequacy and sleep quantity subscores ($P = 0.042$ at week 52). Using the NIH PROMIS questionnaire in 160 patients with IBD using either and anti-TNF or vedolizumab, Stevens *et al*[57] reported significant and meaningful improvement in sleep quality by week 6 ($P = 0.009$), which was paralleled by a significant reduction in depression ($P < 0.05$), as measured in the UCanADA study population. These results reinforce the need to assess sleep disorders a part of an algorithmic approach for the systemic workup of fatigue[18].

Also related to fatigue, in a study including 1185 IBD patients (462 with UC), Williet *et al*[58] reported a strong correlation between FACIT-F score and IBD-DI measure ($r = -0.78$) as well as overall work impairment ($r = -0.70$). Similar trends were observed in UCanADA, *i.e.*, a significant improvement in fatigue (FACIT-F total score; $P < 0.001$) mirrored by a significant improvement in IBD-DI measures ($P < 0.001$) as well as a meaningful improvement in overall work impairment during the study. Our IBD-DI measures are similar to those reported from other UC populations[43]. In a meta-analysis including 3167 IBD patients, Lo *et al*[43] reported a significant lower disease disability rates in patients on biological treatment than those on corticosteroids ($P < 0.01$).

At week 8 and week 52, there was a gain between 15% and 17% in work impairment, overall work impairment, and activity impairment in the UCanADA study population. These represent less gains than those reported from the InspirADA population at week 26; however, they were twice as high as the minimal clinically important difference of 7% in WPAI outcome[59].

Limitations of the research methods used in this study are related to, but may not have been limited to, the observational nature of the study with regards to missing data, which has been alleviated with the use of sensitivity analyses for the primary endpoint. This study consisted of a small cohort of patients, and only 48 (48%) patients completed the study. However, the results between the ITT population and completers population were fairly consistent. The PRO questionnaires being self-administered provide subjective data as opposed to objective data. The collection of secondary PROs data may be subject to a recall bias.

CONCLUSION

At week 52 in a real-world setting, adalimumab was effective in reducing depressive symptoms in patients with UC, with more than 60% of the patients achieving an improvement the PHQ-9 with a mean improvement of 2.4 points. A broad range of PROs including HRQoL and work productivity also significantly improved during the study. The safety profile was consistent with the known safety profile of adalimumab, and no new signal or unexpected trend was identified for the patient population.

ARTICLE HIGHLIGHTS

Research background

The efficacy and safety of adalimumab have been demonstrated in pivotal trials, but there remained a need to assess more holistically how the clinical results translate into concrete improvements in key aspects of the daily lives of ulcerative colitis (UC) patients, such as symptoms, health-related quality of life (HRQoL), and disability.

Research motivation

Although some patient-reported outcomes (PROs) from existing studies may have items capturing some of these aspects, limited data was available for adalimumab in UC, specifically on psychological distress/depression, disability, fatigue, and pain or sleep quality in real-life setting.

Research objectives

The overarching goal for the UCanADA study was to assess the real-life effectiveness of adalimumab on PRO measures, while taking the opportunity to use the inflammatory bowel disease disability index to assess the impact of adalimumab on key components of patients' functioning when affected with moderate-to-severe UC.

Research methods

UCanADA was a single arm, prospective, 1-year multicenter Canadian post-marketing observational study in which multiple PRO questionnaires were completed—with psychologic distress/depression symptoms as the primary endpoint—by patients with moderate-to-severe UC. Assessments were performed during patients' routine care visit schedule, which was at the initiation of adalimumab (baseline), after induction (approximately 8 wk), and 52 wk after baseline. Additional optional assessments between weeks 8 and 52 were collected at least once but no more than two times during this period. Serious safety events and per-protocol adverse events were collected.

Research results

One hundred patients were included in this final analysis, with 94 (94%) patients included in the efficacy population (identified as the intent-to-treat (ITT) population), 48 (48%) patients included in the completers' population, and 98 (98%) patients included in the safety population. The primary endpoint–the proportion of patients who achieved a change from baseline, defined as an improvement in total severity score relative to baseline, in the Patient Health Questionnaire–9 items (PHQ-9) measure at week 52–was 61.5% [40/65 patients; 95% confidence interval (CI): 49.7%-73.4%] for the ITT population and 65.9% (29/44 patients; 95%CI: 51.9%-79.9%) for completers. The safety profile was consistent with the known safety profile of adalimumab, and no new signal or unexpected trend was identified for the patient population.

Research conclusions

At week 52, adalimumab, used in a real-life study, was effective in reducing depressive symptoms in patients with UC, with more than 60% of the patients achieving an improvement the PHQ-9 with a mean improvement of 2.4 points. Thus, the treatment with adalimumab contributed to reducing the depressive symptoms frequently experienced in patients with UC as well as improving a broad range of PROs such as HRQoL and work productivity, as assessed with PRO instruments. The safety profile was consistent with the known safety profile of adalimumab, and no new signal or unexpected trend was identified for the patient population.

Research perspectives

Improvements in PHQ-9 were associated with clinical remission. Beyond the PHQ-9, significant improvements in several PROs were observed suggesting an improvement in HRQoL and work productivity as well. The population in the study, as well as the inclusion and exclusion criteria, was representative of the target population. In addition, coinciding the study visits with the patient's routine care visit schedule helped increase generalizability of the PRO instruments by decreasing the impact on real life.

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FOOTNOTES

Author contributions: Bessissow T was involved in study design, coordinating the data collection, interpretation of the results, and review and revision of the manuscript; Ruel J, Nguyen GC, and Tarabain O were involved in coordinating the data collection, interpretation of the results, and review and revision of the manuscript; Foucault N and McHugh K were involved in study design, interpretation of the results, and review and revision of the manuscript.

Institutional review board statement: All sites had the study reviewed and approved by an ethics committee. While CEC Advarra IRB Services (Aurora, Ontario, Canada) reviewed and approved for some sites, other sites were approved through local/multi centres ethics committees (Dr. Everett Chalmers Regional Hospital, MUHC-Royal Victoria Hospital, CHUM-Hôpital Maisonneuve-Rosemont, CHAU-Hôpital Hôtel-Dieu de Levis, Royal University Hospital, Centre Hospitalier de l'Université de Sherbrooke, CHUM-Hôpital St-Luc, Zeidler Ledcor Centre, Ottawa Hospital-General Campus, Mount Sinai Hospital, London Health Sciences Centre, Centre Hospitalier de l'Université Laval (CHUL), University of Calgary Gastrointestinal Research Group).

Informed consent statement: All patients were required to sign a patient authorization form (or written informed consent) to participate in the study, and to disclose personal health information.

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REFERENCES

1 **Ford AC**, Moayyedi P, Hanauer SB. Ulcerative colitis. *BMJ* 2013; **346**: f432 [PMID: 23386404 DOI: 10.1136/bmj.f432]
2 **Danese S**, Fiocchi C. Ulcerative colitis. *N Engl J Med* 2011; **365**: 1713-1725 [PMID: 22047562 DOI: 10.1056/NEJMra1102942]
3 **McCormick JB**, Hammer RR, Farrell RM, Geller G, James KM, Loftus EV Jr, Mercer MB, Tilburt JC, Sharp RR. Experiences of patients with chronic gastrointestinal conditions: in their own words. *Health Qual Life Outcomes* 2012; **10**: 25 [PMID: 22401607 DOI: 10.1186/1477-7525-10-25]
4 **Mikocka-Walus A**, Knowles SR, Keefer L, Graff L. Controversies Revisited: A Systematic Review of the Comorbidity of Depression and Anxiety with Inflammatory Bowel Diseases. *Inflamm Bowel Dis* 2016; **22**: 752-762 [PMID: 26841224 DOI: 10.1097/MIB.0000000000000620]
5 **Regueiro M**, Greer JB, Szigethy E. Etiology and Treatment of Pain and Psychosocial Issues in Patients With Inflammatory Bowel Diseases. *Gastroenterology* 2017; **152**: 430-439.e4 [PMID: 27816599 DOI: 10.1053/j.gastro.2016.10.036]
6 **Yarlas A**, Rubin DT, Panés J, Lindsay JO, Vermeire S, Bayliss M, Cappelleri JC, Maher S, Bushmakin AG, Chen LA, DiBonaventura M. Burden of Ulcerative Colitis on Functioning and Well-being: A Systematic Literature Review of the SF-36® Health Survey. *J Crohns Colitis* 2018; **12**: 600-609 [PMID: 29718244 DOI: 10.1093/ecco-jcc/jjy024]
7 **Jones JL**, Nguyen GC, Benchimol EI, Bernstein CN, Bitton A, Kaplan GG, Murthy SK, Lee K, Cooke-Lauder J, Otley AR. The Impact of Inflammatory Bowel Disease in Canada 2018: Quality of Life. *J Can Assoc Gastroenterol* 2019; **2**: S42-S48 [PMID: 31294384 DOI: 10.1093/jcag/gwy048]
8 **Zhang CK**, Hewett J, Hemming J, Grant T, Zhao H, Abraham C, Oikonomou I, Kanakia M, Cho JH, Proctor DD. The influence of depression on quality of life in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2013; **19**: 1732-1739 [PMID: 23669400 DOI: 10.1097/MIB.0b013e318281f395]
9 **Neuendorf R**, Harding A, Stello N, Hanes D, Wahbeh H. Depression and anxiety in patients with Inflammatory Bowel Disease: A systematic review. *J Psychosom Res* 2016; **87**: 70-80 [PMID: 27411754 DOI: 10.1016/j.jpsychores.2016.06.001]
10 **Kroenke K**, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* 2001; **16**: 606-613 [PMID: 11556941 DOI: 10.1046/j.1525-1497.2001.016009606.x]
11 **Barsevick AM**, Cleeland CS, Manning DC, O'Mara AM, Reeve BB, Scott JA, Sloan JA; ASCPRO (Assessing Symptoms of Cancer Using Patient-Reported Outcomes). ASCPRO recommendations for the assessment of fatigue as an outcome in clinical trials. *J Pain Symptom Manage* 2010; **39**: 1086-1099 [PMID: 20538190 DOI: 10.1016/j.jpainsymman.2010.02.006]
12 **Grimstad T**, Norheim KB, Isaksen K, Leitao K, Hetta AK, Carlsen A, Karlsen LN, Skoie IM, Gøransson L, Harboe E, Aabakken L, Omdal R. Fatigue in Newly Diagnosed Inflammatory Bowel Disease. *J Crohns Colitis* 2015; **9**: 725-730 [PMID: 25994356 DOI: 10.1093/ecco-jcc/jjv091]
13 **Jelsness-Jørgensen LP**, Bernklev T, Henriksen M, Torp R, Moum BA. Chronic fatigue is associated with impaired health-related quality of life in inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **33**: 106-114 [PMID: 21083587 DOI: 10.1111/j.1365-2036.2010.04498.x]
14 **Romberg-Camps MJ**, Bol Y, Dagnelie PC, Hesselink-van de Kruijs MA, Kester AD, Engels LG, van Deursen C, Hameeteman WH, Pierik M, Wolters F, Russel MG, Stockbrügger RW. Fatigue and health-related quality of life in inflammatory bowel disease: results from a population-based study in the Netherlands: the IBD-South Limburg cohort. *Inflamm Bowel Dis* 2010; **16**: 2137-2147 [PMID: 20848468 DOI: 10.1002/ibd.21285]
15 **Cohen BL**, Zoëga H, Shah SA, Leleiko N, Lidofsky S, Bright R, Flowers N, Law M, Moniz H, Merrick M, Sands BE. Fatigue is highly associated with poor health-related quality of life, disability and depression in newly-diagnosed patients with inflammatory bowel disease, independent of disease activity. *Aliment Pharmacol Ther* 2014; **39**: 811-822 [PMID: 24612278 DOI: 10.1111/apt.12659]
16 **Graff LA**, Vincent N, Walker JR, Clara I, Carr R, Ediger J, Miller N, Rogala L, Rawsthorne P, Lix L, Bernstein CN. A population-based study of fatigue and sleep difficulties in inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 1882-1889 [PMID: 21830266 DOI: 10.1002/ibd.21580]
17 **Jonefjäll B**, Simrén M, Lasson A, Öhman L, Strid H. Psychological distress, iron deficiency, active disease and female gender are independent risk factors for fatigue in patients with ulcerative colitis. *United European Gastroenterol J* 2018; **6**: 148-158 [PMID: 29435325 DOI: 10.1177/2050640617703868]
18 **Nocerino A**, Nguyen A, Agrawal M, Mone A, Lakhani K, Swaminath A. Fatigue in Inflammatory Bowel Diseases: Etiologies and Management. *Adv Ther* 2020; **37**: 97-112 [PMID: 31760611 DOI: 10.1007/s12325-019-01151-w]
19 **Daperno M**, Armuzzi A, Danese S, Fries W, Liguori G, Orlando A, Papi C, Principi M, Rizzello F, Viscido A, Gionchetti P. Unmet Medical Needs in the Management of Ulcerative Colitis: Results of an Italian Delphi Consensus. *Gastroenterol*

Res Pract 2019; **2019**: 3108025 [PMID: 31565051 DOI: 10.1155/2019/3108025]
20 **Food and Drug Administration**. Guidance for industry patient-reported outcome measures: Use in medical product development to support labeling claims. In: Services USDoHaH, editor, 2009: 39
21 **Bojic D**, Bodger K, Travis S. Patient Reported Outcome Measures (PROMs) in Inflammatory Bowel Disease: New Data. *J Crohns Colitis* 2017; **11**: S576-S585 [PMID: 27797917 DOI: 10.1093/ecco-jcc/jjw187]
22 **Bastemeijer CM**, Voogt L, van Ewijk JP, Hazelzet JA. What do patient values and preferences mean? *Patient Educ Couns* 2017; **100**: 871-881 [PMID: 28043713 DOI: 10.1016/j.pec.2016.12.019]
23 **Zhang Y**, Coello PA, Brożek J, Wiercioch W, Etxeandia-Ikobaltzeta I, Akl EA, Meerpohl JJ, Alhazzani W, Carrasco-Labra A, Morgan RL, Mustafa RA, Riva JJ, Moore A, Yepes-Nuñez JJ, Cuello-Garcia C, AlRayees Z, Manja V, Falavigna M, Neumann I, Brignardello-Petersen R, Santesso N, Rochwerg B, Darzi A, Rojas MX, Adi Y, Bollig C, Waziry R, Schünemann HJ. Using patient values and preferences to inform the importance of health outcomes in practice guideline development following the GRADE approach. *Health Qual Life Outcomes* 2017; **15**: 52 [PMID: 28460638 DOI: 10.1186/s12955-017-0621-0]
24 **Postmus D**, Mavris M, Hillege HL, Salmonson T, Ryll B, Plate A, Moulon I, Eichler HG, Bere N, Pignatti F. Incorporating patient preferences into drug development and regulatory decision making: Results from a quantitative pilot study with cancer patients, carers, and regulators. *Clin Pharmacol Ther* 2016; **99**: 548-554 [PMID: 26715217 DOI: 10.1002/cpt.332]
25 **Ho M**, Saha A, McCleary KK, Levitan B, Christopher S, Zandlo K, Braithwaite RS, Hauber AB; Medical Device Innovation Consortium's Patient Centered Benefit-Risk Steering Committee. A Framework for Incorporating Patient Preferences Regarding Benefits and Risks into Regulatory Assessment of Medical Technologies. *Value Health* 2016; **19**: 746-750 [PMID: 27712701 DOI: 10.1016/j.jval.2016.02.019]
26 **Ghosh S**, Sensky T, Casellas F, Rioux LC, Ahmad T, Márquez JR, Vanasek T, Gubonina I, Sezgin O, Ardizzone S, Kligys K, Petersson J, Suzuki Y, Peyrin-Biroulet L. A Global, Prospective, Observational Study Measuring Disease Burden and Suffering in Patients with Ulcerative Colitis Using the Pictorial Representation of Illness and Self-Measure Tool. *J Crohns Colitis* 2020 [PMID: 32722760 DOI: 10.1093/ecco-jcc/jjaa159]
27 **Moon JR**, Lee CK, Hong SN, Im JP, Ye BD, Cha JM, Jung SA, Lee KM, Park DI, Jeen YT, Park YS, Cheon JH, Kim H, Seo B, Kim Y, Kim HJ; MOSAIK study group of the Korean Association for Study of Intestinal Diseases. Unmet Psychosocial Needs of Patients with Newly Diagnosed Ulcerative Colitis: Results from the Nationwide Prospective Cohort Study in Korea. *Gut Liver* 2020; **14**: 459-467 [PMID: 31533398 DOI: 10.5009/gnl19107]
28 **Geiss T**, Schaefert RM, Berens S, Hoffmann P, Gauss A. Risk of depression in patients with inflammatory bowel disease. *J Dig Dis* 2018; **19**: 456-467 [PMID: 29989345 DOI: 10.1111/1751-2980.12644]
29 **Horst S**, Chao A, Rosen M, Nohl A, Duley C, Wagnon JH, Beaulieu DB, Taylor W, Gaines L, Schwartz DA. Treatment with immunosuppressive therapy may improve depressive symptoms in patients with inflammatory bowel disease. *Dig Dis Sci* 2015; **60**: 465-470 [PMID: 25274158 DOI: 10.1007/s10620-014-3375-0]
30 **Armuzzi A**, Tarallo M, Lucas J, Bluff D, Hoskin B, Bargo D, Cappelleri JC, Salese L, daCosta DiBonaventura M. The association between disease activity and patient-reported outcomes in patients with moderate-to-severe ulcerative colitis in the United States and Europe. *BMC Gastroenterol* 2020; **20**: 18 [PMID: 31964359 DOI: 10.1186/s12876-020-1164-0]
31 **Walter E**, Hausberger SC, Groß E, Siebert U. Health-related quality of life, work productivity and costs related to patients with inflammatory bowel disease in Austria. *J Med Econ* 2020; **23**: 1061-1071 [PMID: 32713223 DOI: 10.1080/13696998.2020.1801187]
32 **Teich N**, Grümmer H, Jörgensen E, Liceni T, Holtkamp-Endemann F, Fischer T, Hohenberger S. Golimumab in real-world practice in patients with ulcerative colitis: Twelve-month results. *World J Gastroenterol* 2020; **26**: 2852-2863 [PMID: 32550760 DOI: 10.3748/wjg.v26.i21.2852]
33 **Paschos P**, Katsoula A, Salanti G, Giouleme O, Athanasiadou E, Tsapas A. Systematic review with network meta-analysis: the impact of medical interventions for moderate-to-severe ulcerative colitis on health-related quality of life. *Aliment Pharmacol Ther* 2018; **48**: 1174-1185 [PMID: 30378141 DOI: 10.1111/apt.15005]
34 **Panés J**, Domènech E, Aguas Peris M, Nos P, Riestra S, Juliá de Páramo B, Cea-Calvo L, Romero C, Marín-Jiménez I. Association between disease activity and quality of life in ulcerative colitis: Results from the CRONICA-UC study. *J Gastroenterol Hepatol* 2017; **32**: 1818-1824 [PMID: 28370253 DOI: 10.1111/jgh.13795]
35 **Aniwan S**, Bruining DH, Park SH, Al-Bawardy B, Kane SV, Coelho Prabhu N, Kisiel JB, Raffals LE, Papadakis KA, Pardi DS, Tremaine WJ, Loftus EV Jr. The Combination of Patient-Reported Clinical Symptoms and an Endoscopic Score Correlates Well with Health-Related Quality of Life in Patients with Ulcerative Colitis. *J Clin Med* 2019; **8** [PMID: 31387259 DOI: 10.3390/jcm8081171]
36 **Saraiva S**, Cortez-Pinto J, Barosa R, Castela J, Moleiro J, Rosa I, da Siva JP, Dias Pereira A. Evaluation of fatigue in inflammatory bowel disease - a useful tool in daily practice. *Scand J Gastroenterol* 2019; **54**: 465-470 [PMID: 31012338 DOI: 10.1080/00365521.2019.1602669]
37 **Villoria A**, García V, Dosal A, Moreno L, Montserrat A, Figuerola A, Horta D, Calvet X, Ramírez-Lázaro MJ. Fatigue in out-patients with inflammatory bowel disease: Prevalence and predictive factors. *PLoS One* 2017; **12**: e0181435 [PMID: 28749985 DOI: 10.1371/journal.pone.0181435]
38 **Christiansen LK**, Lo B, Bendtsen F, Vind I, Vester-Andersen MK, Burisch J. Health-related quality of life in inflammatory bowel disease in a Danish population-based inception cohort. *United European Gastroenterol J* 2019; **7**: 942-954 [PMID: 31428419 DOI: 10.1177/2050640619852532]
39 **Yarlas A**, Maher SM, Bayliss MS, Lovley A, Cappelleri JC, DiBonaventura MD. Psychometric validation of the work productivity and activity impairment questionnaire in ulcerative colitis: results from a systematic literature review. *J Patient Rep Outcomes* 2018; **2**: 62 [PMID: 30547275 DOI: 10.1186/s41687-018-0088-8]
40 **Travis S**, Feagan BG, Peyrin-Biroulet L, Panaccione R, Danese S, Lazar A, Robinson AM, Petersson J, Pappalardo BL, Bereswill M, Chen N, Wang S, Skup M, Thakkar RB, Chao J. Effect of Adalimumab on Clinical Outcomes and Health-related Quality of Life Among Patients With Ulcerative Colitis in a Clinical Practice Setting: Results From InspirADA. *J Crohns Colitis* 2017; **11**: 1317-1325 [PMID: 28981846 DOI: 10.1093/ecco-jcc/jjx093]
41 **Peyrin-Biroulet L**, Cieza A, Sandborn WJ, Coenen M, Chowers Y, Hibi T, Kostanjsek N, Stucki G, Colombel JF;

International Programme to Develop New Indexes for Crohn's Disease (IPNIC) group. Development of the first disability index for inflammatory bowel disease based on the international classification of functioning, disability and health. *Gut* 2012; **61**: 241-247 [PMID: 21646246 DOI: 10.1136/gutjnl-2011-300049]

42 **Gower-Rousseau C**, Sarter H, Savoye G, Tavernier N, Fumery M, Sandborn WJ, Feagan BG, Duhamel A, Guillon-Dellac N, Colombel JF, Peyrin-Biroulet L; International Programme to Develop New Indexes for Crohn's Disease (IPNIC) group; International Programme to Develop New Indexes for Crohn's Disease (IPNIC) group. Validation of the Inflammatory Bowel Disease Disability Index in a population-based cohort. *Gut* 2017; **66**: 588-596 [PMID: 26646934 DOI: 10.1136/gutjnl-2015-310151]

43 **Lo B**, Prosberg MV, Gluud LL, Chan W, Leong RW, van der List E, van der Have M, Sarter H, Gower-Rousseau C, Peyrin-Biroulet L, Vind I, Burisch J. Systematic review and meta-analysis: assessment of factors affecting disability in inflammatory bowel disease and the reliability of the inflammatory bowel disease disability index. *Aliment Pharmacol Ther* 2018; **47**: 6-15 [PMID: 28994131 DOI: 10.1111/apt.14373]

44 **Walmsley RS**, Ayres RC, Pounder RE, Allan RN. A simple clinical colitis activity index. *Gut* 1998; **43**: 29-32 [PMID: 9771402 DOI: 10.1136/gut.43.1.29]

45 **Kind P**. The EuroQol instrument - an index of health-related quality of life. In: Spilker B, editor Quality of Life and Pharmacoeconomics in Clinical Trials 2nd ed. Philadelphia, PA: Lippincott-Raven Publishers, 1996

46 **Irvine EJ**, Zhou Q, Thompson AK. The Short Inflammatory Bowel Disease Questionnaire: a quality of life instrument for community physicians managing inflammatory bowel disease. CCRPT Investigators. Canadian Crohn's Relapse Prevention Trial. *Am J Gastroenterol* 1996; **91**: 1571-1578 [PMID: 8759664]

47 **Tinsley A**, Macklin EA, Korzenik JR, Sands BE. Validation of the functional assessment of chronic illness therapy-fatigue (FACIT-F) in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2011; **34**: 1328-1336 [PMID: 21999576 DOI: 10.1111/j.1365-2036.2011.04871.x]

48 **Hays R**, Stewart A. Sleep Measures. In: Stewart A, Ware J, editors. Measuring functioning and well-being; the medical outcomes study approach Duke edition: Duke University Press; 1992: 235–259

49 **Zhang W**, Bansback N, Boonen A, Young A, Singh A, Anis AH. Validity of the work productivity and activity impairment questionnaire--general health version in patients with rheumatoid arthritis. *Arthritis Res Ther* 2010; **12**: R177 [PMID: 20860837 DOI: 10.1186/ar3141]

50 **Zhang W**, Bansback N, Kopec J, Anis AH. Measuring time input loss among patients with rheumatoid arthritis: validity and reliability of the Valuation of Lost Productivity questionnaire. *J Occup Environ Med* 2011; **53**: 530-536 [PMID: 21508868 DOI: 10.1097/JOM.0b013e318218abf1]

51 **U.S. Food and Drug Administration**. FDA Drug Safety Communication: UPDATE on Tumor Necrosis Factor (TNF) blockers and risk for pediatric malignancy. 2018. [cited 10 January 2022]. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-update-tumor-necrosis-factor-tnf-blockers-and-risk-pediatric

52 **Round JM**, Lee C, Hanlon JG, Hyshka E, Dyck JRB, Eurich DT. Changes in patient health questionnaire (PHQ-9) scores in adults with medical authorization for cannabis. *BMC Public Health* 2020; **20**: 987 [PMID: 32576158 DOI: 10.1186/s12889-020-09089-3]

53 **Hudgens S**, Floden L, Blackowicz M, Jamieson C, Popova V, Fedgchin M, Drevets WC, Cooper K, Lane R, Singh J. Meaningful Change in Depression Symptoms Assessed with the Patient Health Questionnaire (PHQ-9) and Montgomery-Åsberg Depression Rating Scale (MADRS) Among Patients with Treatment Resistant Depression in Two, Randomized, Double-blind, Active-controlled Trials of Esketamine Nasal Spray Combined With a New Oral Antidepressant. *J Affect Disord* 2021; **281**: 767-775 [PMID: 33261932 DOI: 10.1016/j.jad.2020.11.066]

54 **Turkoz I**, Alphs L, Singh J, Jamieson C, Daly E, Shawi M, Sheehan JJ, Trivedi MH, Rush AJ. Clinically meaningful changes on depressive symptom measures and patient-reported outcomes in patients with treatment-resistant depression. *Acta Psychiatr Scand* 2021; **143**: 253-263 [PMID: 33249552 DOI: 10.1111/acps.13260]

55 **Moayyedi P**, Andrews CN, MacQueen G, Korownyk C, Marsiglio M, Graff L, Kvern B, Lazarescu A, Liu L, Paterson WG, Sidani S, Vanner S. Canadian Association of Gastroenterology Clinical Practice Guideline for the Management of Irritable Bowel Syndrome (IBS). *J Can Assoc Gastroenterol* 2019; **2**: 6-29 [PMID: 31294724 DOI: 10.1093/jcag/gwy071]

56 **Theede K**, Kiszka-Kanowitz M, Nordgaard-Lassen I, Mertz Nielsen A. The Impact of Endoscopic Inflammation and Mucosal Healing on Health-related Quality of Life in Ulcerative Colitis Patients. *J Crohns Colitis* 2015; **9**: 625-632 [PMID: 25956537 DOI: 10.1093/ecco-jcc/jjv081]

57 **Stevens BW**, Borren NZ, Velonias G, Conway G, Cleland T, Andrews E, Khalili H, Garber JG, Xavier RJ, Yajnik V, Ananthakrishnan AN. Vedolizumab Therapy Is Associated with an Improvement in Sleep Quality and Mood in Inflammatory Bowel Diseases. *Dig Dis Sci* 2017; **62**: 197-206 [PMID: 27796768 DOI: 10.1007/s10620-016-4356-2]

58 **Williet N**, Sarter H, Gower-Rousseau C, Adrianjafy C, Olympie A, Buisson A, Beaugerie L, Peyrin-Biroulet L. Patient-reported Outcomes in a French Nationwide Survey of Inflammatory Bowel Disease Patients. *J Crohns Colitis* 2017; **11**: 165-174 [PMID: 27516406 DOI: 10.1093/ecco-jcc/jjw145]

59 **Binion DG**, Louis E, Oldenburg B, Mulani P, Bensimon AG, Yang M, Chao J. Effect of adalimumab on work productivity and indirect costs in moderate to severe Crohn's disease: a meta-analysis. *Can J Gastroenterol* 2011; **25**: 492-496 [PMID: 21912760 DOI: 10.1155/2011/938194]

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CASE REPORT

Gastrointestinal tumors in transplantation: Two case reports and review of literature

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Abstract

BACKGROUND

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal tract. As most of them harbor a *KIT* mutation (75%), selective kinase inhibitors are the therapeutic option and show a sustained objective response among patients with metastatic or unresectable GISTs. A well-known higher risk of neoplasm has been described among renal transplant recipients (RTRs). Nevertheless, only few cases of GIST onset among transplant patients have been reported in the literature.

CASE SUMMARY

Here, we describe 2 cases of gastric GIST occurring during the follow-up of RTRs. We also review the existing literature concerning GIST occurrence in transplant patients. In total and in association with our 2 cases, 16 patients have been reported. The median age was 59.5 years and 69% were male. With a median tumor size of 45 mm, no patient displayed metastatic dissemination at diagnosis.

Time from transplantation to diagnosis was highly variable between 5 mo and 21 years. Histopathological data mostly revealed high risk of progression (43%). Death increased to 29% during follow-up. Surgical treatment was systematically performed when the tumor was operable (94%). The use of adjuvant therapy was uncommon (19%).

CONCLUSION
GISTs represent rare but potentially severe malignant complication among transplant patients.

Key Words: Gastrointestinal stromal tumors; Imatinib mesylate; Transplantation; Kidney transplantation; Proto-oncogene protein c-KIT; Case report

Core Tip: Although a well-known higher risk of neoplasm has been described among renal transplant recipients (RTRs), few cases of gastrointestinal stromal tumors (GISTs) have been reported. We describe 2 cases of gastric GIST among RTRs and provide a review of the literature. We report 16 patients with a median age of 59.5 years, and 69% were male. No patient displayed metastasis at diagnosis. Time from transplantation to diagnosis varied between 5 mo and 21 years. Histopathology revealed high risk of progression (43%). Death increased to 29%. Surgical treatment was commonly performed (94%). The use of adjuvant therapy was uncommon (19%).

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INTRODUCTION

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the gastrointestinal (GI) tract[1]. GISTs arise from interstitial cells of Cajal (ICC), which are specialized mesenchymal cells located within the muscle of the GI tract. ICC play a critical role in regulating smooth muscle function and GI tract motility[2]. GISTs are mainly located in the stomach (55%) or the small bowel (30%). About 10% to 47% of patients have metastatic disease at diagnosis[3-5]. About 95% of GISTs display positive staining for the receptor tyrosine kinase KIT (or CD117), 75% of these tumors harbor a *KIT* gene mutation and 10% a platelet-derived growth factor receptor A (*PDGFRA*) gene mutation[6]. Among KIT-negative GISTs, immunohistochemical expression of discovered on GIST-1 (DOG-1) was found in 76% of the cases[7]. Consequently, selective tyrosine kinase inhibitors targeting KIT receptor have been used. The first one, imatinib mesylate (Gleevec®; Novartis, Basel, Switzerland), has shown a sustained objective response in a phase III trial among patients with metastatic or unresectable GISTs in immunocompetent patients[8].

In renal transplant recipients (RTRs), an increased risk of cancer has been reported especially for non-melanoma skin cancer, virus-associated cancer and lymphoproliferative disorders[9]. Currently, malignancy represents a major cause of mortality among RTRs[10]. Nonetheless, only few cases of GIST have been reported among transplant patients. Overall, 8 cases of GIST[11-17] and 2 cases of extra GIST (EGIST)[14,18] have previously been reported in RTRs and respectively 3 cases[19-21] and 1 case[22] in liver transplant recipients.

We report 2 cases of GIST occurring in RTRs and provide a review of the existing literature concerning GIST occurrence in transplant patients.

CASE PRESENTATION

Chief complaints

Case 1: A 60-year-old Caucasian man without any symptoms.

Case 2: A 56-year-old Caucasian man presented with upper GI hemorrhage.

History of present illness

Case 1: Hepatic magnetic resonance imaging (MRI) was performed to explore abnormal hepatic tests.

MRI revealed a 32 mm spherical tumor of the lesser curvature of the stomach.

Case 2: The upper GI hemorrhage led to gastric endoscopy, which revealed a spherical gastric tumor in the fundus.

History of past illness

Case 1: He had end-stage renal disease with a kidney biopsy compatible with nephronophthisis despite negative screening for mutation in hepatocyte nuclear factor 1 beta (*HNF1B*) gene. Hemodialysis was initiated in 2016. In October 2019, he received a kidney transplant from a deceased donor. The initial immunosuppressive therapy combined basiliximab, steroids, tacrolimus, and everolimus. Renal function at hospital discharge was 94 μmol/L, (normal range 53 μmol/L to 97 μmol/L). Initial maintenance immunosuppressive therapy associated steroids, tacrolimus, and everolimus. Due to relapsing lymphocele, everolimus was switched to mycophenolate mofetil (MMF). Moreover, a pre-existing mild cytolysis and cholestasis worsened after transplantation leading to the discontinuation of cotrimoxazole and MMF, which were replaced by atovaquone and belatacept (NULOJIX®; Bristol-Myers Squibb, New York, NY, United States), respectively.

Case 2: The patient developed end-stage renal disease of unknown origin. He received a kidney transplantation from a deceased donor. Due to preformed donor specific antibodies (anti-Cw15, mean fluorescence intensity of 6130) on the day of transplantation, induction immunosuppressive therapy combined basiliximab, steroids, MMF, cyclosporine, and intravenous immunoglobulins. At 10 d after surgery, a kidney biopsy was performed due to delayed graft function. It revealed acute tubular necrosis associated with possible acute humoral rejection (g1 cpt0 v0 i0 t0 according to Banff's classification[23,24], C4d immunostaining was negative). A treatment with high dose steroids, five plasma exchanges and rituximab[25] was initiated allowing improvement of renal function with a nadir in serum creatinine level of 170 μmol/L. Maintenance immunosuppressive therapy included steroids, cyclosporine, and MMF.

Personal and family history

Case 1: His other past medical history consisted in nonalcoholic steatohepatitis, Hashimoto's thyroiditis, and hypertension.

Case 2: The patient had no significant personal or family history.

Physical examination

Case 1: On admission, physical examination was unremarkable.

Case 2: Physical examination was unremarkable except for hematemesis.

Laboratory examinations

Case 1: The patient had mild cytolysis and cholestasis without any other biological abnormality.

Case 2: No abnormal blood test was noticed on admission.

Imaging examinations

Case 1: Body computed tomography (CT) scan confirmed the absence of metastatic dissemination.

Case 2: Body CT scan was consistent with local tumor without metastatic localizations.

Initial diagnosis

Case 1: Upper GI endoscopy found a 3 cm submucosal tumor of the lesser curvature of the stomach. Tumor biopsies were performed using endoscopic ultrasound guidance. Cytological examination revealed spindle-shaped cells that showed positive staining for c-KIT and DOG-1 in immunohistochemistry, confirming the diagnosis of GIST (Figure 1).

Case 2: The gastric endoscopy revealed a spherical gastric tumor in the fundus with a typical macroscopic aspect of GIST.

Initial treatment

Case 1: Partial gastrectomy was performed without complication.

Case 2: Partial gastrectomy was performed.

Course of illness in the hospital

Case 1: No complication associated with the GIST of its treatment was noticed.

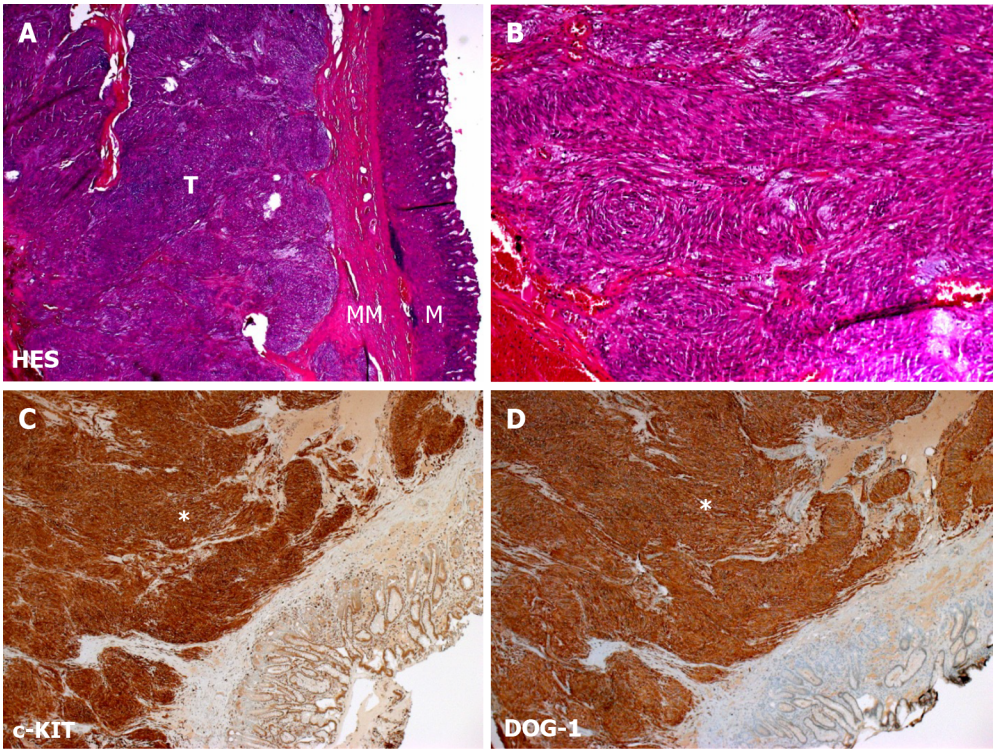

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Figure 1 Histopathological features of case 1 gastrointestinal stromal tumor. A: Hematoxylin and eosin staining showing exophytic development of a tumor from the mucosa musculus (magnification, 2.5 ×); B: Hematoxylin and eosin staining showing spindle cell morphology composed of relatively uniform cells arranged in short fascicles (magnification, 20 ×); C: Immunohistochemistry showing strong positive staining for the protooncogene c-KIT (white asterisk; magnification, 5 ×); D: Immunohistochemistry showing strong positive staining for discovered on gastrointestinal stromal tumor protein 1 (white asterisk; magnification, 5 ×). M: Mucosa; MM: Mucosa musculus; T: Tumor.

Case 2: The patient was rapidly discharged after partial gastrectomy without complication.

FINAL DIAGNOSIS

Case 1

Histopathology revealed a 27 mm stromal tumor strongly positive for KIT and moderately positive for DOG-1 with a mitotic count of 2 mitosis for 5 mm^2. The tumor harbored an exon 11 (p. Val559Ala c.1676T>C) *KIT* mutation[23].

Case 2

Histopathology report described a 51 mm GIST strongly positive for KIT harboring a mitotic count of 10 mitosis for 5 mm^2. Of note, an exon 18 D842V *PDGFRA* mutation was identified.

TREATMENT

Case 1

Regarding the very low risk of progression, no adjuvant therapy was initiated.

Case 2

No adjuvant treatment was initiated at the time of diagnosis.

Table 1 Characteristics of 16 transplanted patients with gastrointestinal stromal tumors

	Overall number	
Male sex, *n* (%)	16	11 (69)
Age (yr), median (min; max)	16	59.5 (23; 74)
Type of organ transplantation, *n* (%)	16	
Kidney		12 (75)
Liver		4 (25)
Location of primitive tumor, *n* (%)	16	
Stomach		9 (56)
Small bowel		3 (19)
Colon		1 (6)
Other: pelvis, perineum, mesentery		3 (19)
Time from transplantation to diagnosis (mo), median (min; max)	16	32 (5; 252)
Metastatic dissemination at diagnosis, *n* (%)	16	0 (0)
Tumor size (mm), median (min; max)	15	45 (10; 230)
Risk of progression according to Joensuu's criteria, *n* (%)	14	
Very low		2 (14)
Low		4 (29)
Intermediate		2 (14)
High		6 (43)
Surgical treatment, *n* (%)	16	15 (94)
Adjuvant treatment, *n* (%)	16	3 (19)
Modification of immunosuppression, *n* (%)	11	9 (82)
Death during follow-up, *n* (%)	14	4 (29)

OUTCOME AND FOLLOW-UP

Case 1

The patient remains in remission at the 1-year follow-up.

Case 2

Two years later, a follow-up MRI revealed hepatic vascular nodules compatible with metastatic lesions. Treatment with imatinib mesylate was initiated. In the absence of a tumor response, imatinib was discontinued 4 mo later and sunitinib (SUTENT©; Bayer, Germany), an anti-angiogenic multikinase inhibitor (anti vascular endothelial growth factor-1, -2, -3, PDGFR-α,-β, c-KIT, fms-like tyrosine kinase 3, and RET) was introduced. Five months later, the onset of thrombopenia, neutropenia, and hepatic cytolysis led to replacement of sunitinib with regorafenib (STIVARGA©; Bayer Pharma AG, Germany), another multikinase inhibitor. Due to side effects and tumor progression, regorafenib was discontinued and dasatinib (SPRYCEL©; Bristol-Myers Squibb, New York, NY, United States) was introduced. Disease progression finally led to stopping all therapies in April 2019. Selective transarterial embolization was performed complicated with artery dissection of the kidney transplant requiring stent implantation. The patient was finally admitted with a clinical presentation of hydrops concomitant with acute renal injury and peritoneal carcinosis. The patient eventually died due to disease progression.

DISCUSSION

GISTs represent an uncommon malignant complication of immunosuppression state in solid organ transplantation. We describe 2 cases of typical GIST occurring early in the course of kidney transplantation. The first patient developed an isolated gastric GIST 5 mo after transplantation and the second 4 years after. Both were nonmetastatic at diagnosis although the second patient developed

Table 2 Clinical features and immunosuppression regimen of 16 transplant patients with gastrointestinal stromal tumor

Ref.	Age (yr)/sex	Transplanted organ	Time from transplantation to diagnosis	Location of primitive GIST	Metastasis at diagnosis	Evolution/delay	Immunosuppression before diagnosis	Immunosuppression after diagnosis
Agaimy and Wünsch [11]	59/F	Kidney	40 mo	Stomach	No	Relapse 68 mo	Not described	Not described
Agaimy and Wünsch [11]	58/F	Kidney	96 mo	Small bowel	No	Not described	Not described	Not described
Saidi *et al* [19]	54/M	Liver	11 mo	Colon	No	Not described	Tac, Aza	Not described
Camargo *et al*[22]	64/M	Liver	7 mo	Perineum	No	Not described	Tac, mycophenolate sodium	Not described
Tu *et al* [18]	57/F	Kidney	6 mo	Pelvis	No	Not described	Steroids, CsA, MMF	CsA and MMF at half dosage; rapamycin-containing regimens-teroids withdrawn
Mulder *et al*[12]	72/M	Kidney	21 yr	Stomach	No	Peritoneal metastasis/24 mo	Steroids, CsA	Steroids, CsA (60% reduction in dosage)
Mrzljak *et al*[20]	53/M	Liver	12 mo	Jejunum	No	No	Tac, MMF	Same
Cimen *et al*[13]	46/F	Kidney	18 yr	Stomach	No	Not described	Steroids, CsA, Aza	Same with reduced dosage of CsA
Cheung *et al*[14]	64/M	Kidney	2 yr	Stomach	No	Yes/2 yr	Steroids, Tac, MMF	Steroids, Tac (reduced dosage), everolimus
Cheung *et al*[14]	48/M	Kidney	1 yr	Mesentery	Multiple tumors	No	CsA, MMF	CsA withdrawal, sirolimus introduction
Patiño *et al*[15]	23/F	Kidney	13 yr	Stomach	No	Local relapse/3 yr	Steroids, Tac, MMF	Not described
Xie *et al* [21]	60/M	Liver	11 mo	Stomach	No	No	Tac, sirolimus, MMF	Same
Elkabets *et al*[17]	74/M	Kidney	7 yr	Stomach	No	No	Steroids, CsA, MMF	Switch CsA to mTOR inhibitor
Takahashi *et al*[16]	64/M	Kidney	72 mo	Small bowel	No	No	Steroids, CsA, MMF	Stop CsA
Stammler *et al*	60/M	Kidney	5 mo	Stomach	No	No	Steroids, Tac, MMF	Switch MMF to belatacept
Stammler *et al*	64/M	Kidney	51 mo	Stomach	No	Yes/23 mo	Steroids, CsA, MMF	Switch CsA to Tac

Aza: Azathioprine; CsA: Cyclosporine; F: Female; GIST: Gastrointestinal stromal tumor; M: Male; MMF: Mycophenolate mofetil; mTOR: Mammalian target of rapamycin; Tac: Tacrolimus.

multiple hepatic metastasis 2 years after complete tumor resection. Of note, the mutation of *PDGFRA* D842V in the second case was associated with resistance to imatinib mesylate.

We looked for previously reported cases of GIST in the literature during the course of transplantation. We searched PubMed and Web of Science databases using the following Medical Subject Headings words: "Gastrointestinal stromal tumors" AND "Kidney transplantation" or "Gastrointestinal stromal tumors" AND "Transplantation." Using these terms, we found 8 and 31 articles, respectively. Only 12 articles were analyzed. From 2007 to 2020, 14 cases of GIST have been reported in transplant recipients [11-22]. We excluded reports of GIST occurring among nontransplant or bone marrow transplant patients. We also excluded article types different than case reports or case series.

Table 1 summarizes the main features of these patients including the 2 cases described in the present manuscript. Tables 2 and 3 give details on the 14 cases reported. In our literature review, the typical patient profile was a male patient with a median age of 59.5-years-old, who developed large nonmetastatic gastric tumors (median size, 45 mm). The delay between transplantation and diagnosis was highly

Table 3 Histopathological features, treatments, and outcome of 16 transplant patients with gastrointestinal stromal tumor

Ref.	Size (mm)	Mitotic count	Fletcher's criteria	Joensuu's criteria	Mutation identified	Resection	Initial adjuvant treatment	Second line treatment	Outcome
Agaimy and Wünsch[11]	35	< 5/50	Low	Low	Not described	Yes	No	Not described	Alive and relapse free at 68 mo
Agaimy and Wünsch[11]	230	14/50	High	High	Not described	Yes	No	Not described	Not described
Saidi *et al* [19]	25	1/50	Low	High	Not described	Yes	No	Not described	Alive and relapse free at 18 mo
Camargo *et al*[22]	50	5/50	Intermediate	High	Not described	Yes	No	Not described	Alive and relapse free at 20 mo
Tu *et al*[18]	45	2-3/50	Low	Low	*PDGFRA* exon 18 V824V	Yes	No	Not described	Alive and relapse free 24 mo
Mulder *et al* [12]	50	> 10/50	High	High	Not described	Yes	No	Imatinib 400 mg/d then 200 mg/d	Died 44 mo
Mrzljak *et al* [20]	10	1/50	Very low	Very low	Not described	Yes	No	No	Died 3 yr after from acute leukemia
Cimen *et al* [13]	150	14/50	High	High	*KIT* T574del	Yes	Imatinib 400 mg/d	Not described	Alive and relapse free at 12 mo
Cheung *et al* [14]	30	9/50	Intermediate	Intermediate	Not described	Yes	No	No	Died from pneumonia at 2 yr
Cheung *et al* [14]	Not described	Not described	Not described	Not described	Not described	No	Imatinib 400 mg/d for 1 yr	Switch CsA to sirolimus	Alive and relapse free at 10 yr
Patiño *et al* [15]	58	Not described	Intermediate or high	Intermediate of high	Not described	Yes	No	Imatinib 400 mg/d	Alive and relapse free/5 yr after imatinib initiation
Xie *et al*[21]	10	< 5/50	Very low	Very low	*KIT* exon 11	Yes	No	No	Not described
Elkabets *et al*[17]	31	Not described	Not described	Not described	Not described	Yes	No	No	Alive and relapse free at 40 mo
Takahashi *et al*[16]	110	20/50	High	High	*KIT* exon 11	Yes	Imatinib 400 mg/d reduced to 3000 mg/d	No	Alive and relapse free at 18 mo
Stammler *et al*	27	2/5	Low	Low	*KIT* exon 11	Yes	No	No	Alive and relapse free at 2 mo
Stammler *et al*	51	10/50	High	High	*PDGFRA* exon 18	Yes	No	Sunitinib then regorafenib then dasatinib	Died 56 mo later

CsA: Cyclosporine.

variable, ranging between 5 mo and 21 years. Histopathological data mostly revealed high risk of progression (42.8%) and death occurred in 29% of the cases during follow-up. Surgical treatment was systematically performed if the tumor features were suitable (94%). The use of adjuvant therapy was uncommon (19%).

Several prognostic classifications have been used to evaluate the risk of recurrence of GIST after surgery. In 2002, Fletcher *et al*[26] claimed size of the tumor and mitotic count, Miettinen and Lasota[27] in 2006 added tumor location and Joensuu *et al*[28] in 2012 adjoined rupture of the tumoral capsule and male gender. Heinrich *et al*[29] demonstrated that *PDGFRA* and *c-KIT* were mutually exclusive proto-oncogenic mutations with similar biologicals consequences, even if associated with different prognostics. Molecular predictors of response to imatinib have been widely studied. Underlying *KIT* or *PDGFRA* mutations are the strongest predictors of imatinib sensitivity[30]. Mutations directly located in the *PDGFRA* binding site of imatinib or inducing variations in tridimensional conformation of the tyrosine kinase receptor and subsequently hiding the binding site, may explain inefficacy of therapy. For instance, *KIT* exon 9 mutation is less sensitive to imatinib and *PDFGRA* exon 18 D842V mutations is associated with imatinib resistance. Nevertheless, these mutations have been correlated with opposite

courses of the disease, indolent for *PDFGRA* exon 18 D842V mutation but aggressive for *KIT* exon 9 mutation[31]. These data should highlight the importance of molecular biomarkers to evaluate prognosis of GIST or EGIST at diagnosis.

Guidelines for the diagnosis, treatment, and follow-up of GIST have recently been published[32]. Management of local or locoregional disease should always aim for complete resection whenever possible. Otherwise, neoadjuvant treatment with imatinib for 6 to 12 mo should be used in case of sensitive mutation with an overall response rate of 50%[30]. Moreover, high-risk patients, as previously described, should receive adjuvant imatinib for a duration of 3 years[33]. Imatinib remains the first-line therapy for metastatic GIST. Several other targeted therapies such as sunitinib or regorafenib have emerged as second- or third-line treatment, and more recently avapritinib and ripretinib. Several biomarkers, such as *KIT* or *PDGFRA* mutations, are used as predictive factors for tumoral response to refine therapeutic strategies[32]. Data are missing concerning the level of tyrosine kinase inhibitors' efficacy in transplanted patients.

Data about the management of immunosuppressive therapy after the diagnosis of GIST are scarce. As both imatinib mesylate and cyclosporin are extensively metabolized by cytochrome P450 3A4, interaction occurrence has been documented[12]. Reduction in the dosage of cyclosporin should be performed if this treatment is maintained. Mammalian target of rapamycin inhibitors (mTORis) have shown antiproliferative properties among transplant patients. Schöffski *et al*[34] highlighted the potential efficacy of association of everolimus and imatinib in imatinib-resistant GIST in a phase II trial. Cheung *et al*[14] reported a case of complete tumoral response with sirolimus in a transplant patient with imatinib-resistant GIST. Among the patients described in Tables 1 and 2, mTORis have been initiated or switched in 4 of them. Three of them were alive and relapse-free at last follow-up and the last patient died from pneumonia 2 years after GIST diagnosis.

This study had several limitations. First, the retrospective analysis of GIST cases impairs the reliability of the data. Very few cases of GIST occurring after solid organ transplantation have been described in the last 15 years reducing the significance of this literature review. Moreover, it was unclear if GIST was a *de novo* feature in our first patient because of the short delay (5 mo) between transplantation and tumor discovery. Unfortunately, the latest available CT scan was performed 7 years before the transplantation. However, some previous cases reported GIST onset within the 1st year following transplantation[14,18-22].

CONCLUSION

To conclude, GISTs represent rare but potentially severe malignant complication among transplant patients. Further analysis of prognosis value of new biomarkers should improve therapeutic strategies.

FOOTNOTES

Author contributions: Stammler R and Lazareth H designed the study; Stammler R, Lazareth H, Anglicheau D, Meatchi T, Ragot E, and Thervet E investigated the patients and collected the data; Stammler R, Lazareth H, and Landi B interpreted the data and wrote the manuscript; all authors revised the manuscript and approved the final version.

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REFERENCES

1 **Rubin BP**, Heinrich MC, Corless CL. Gastrointestinal stromal tumour. *Lancet* 2007; **369**: 1731-1741 [PMID: 17512858 DOI: 10.1016/S0140-6736(07)60780-6]

2 **Sanders KM**, Kito Y, Hwang SJ, Ward SM. Regulation of Gastrointestinal Smooth Muscle Function by Interstitial Cells. *Physiology (Bethesda)* 2016; **31**: 316-326 [PMID: 27488743 DOI: 10.1152/physiol.00006.2016]

3 **Søreide K**, Sandvik OM, Søreide JA, Giljaca V, Jureckova A, Bulusu VR. Global epidemiology of gastrointestinal stromal tumours (GIST): A systematic review of population-based cohort studies. *Cancer Epidemiol* 2016; **40**: 39-46 [PMID: 26618334 DOI: 10.1016/j.canep.2015.10.031]

4 **DeMatteo RP**, Lewis JJ, Leung D, Mudan SS, Woodruff JM, Brennan MF. Two hundred gastrointestinal stromal tumors: recurrence patterns and prognostic factors for survival. *Ann Surg* 2000; **231**: 51-58 [PMID: 10636102 DOI: 10.1097/00000658-200001000-00008]

5 **Emile JF**, Brahimi S, Coindre JM, Bringuier PP, Monges G, Samb P, Doucet L, Hostein I, Landi B, Buisine MP, Neuville A, Bouché O, Cervera P, Pretet JL, Tisserand J, Gauthier A, Le Cesne A, Sabourin JC, Scoazec JY, Bonvalot S, Corless CL, Heinrich MC, Blay JY, Aegerter P. Frequencies of KIT and PDGFRA mutations in the MolecGIST prospective population-based study differ from those of advanced GISTs. *Med Oncol* 2012; **29**: 1765-1772 [PMID: 21953054 DOI: 10.1007/s12032-011-0074-y]

6 **Corless CL**, Barnett CM, Heinrich MC. Gastrointestinal stromal tumours: origin and molecular oncology. *Nat Rev Cancer* 2011; **11**: 865-878 [PMID: 22089421 DOI: 10.1038/nrc3143]

7 **Lopes LF**, West RB, Bacchi LM, van de Rijn M, Bacchi CE. DOG1 for the diagnosis of gastrointestinal stromal tumor (GIST): Comparison between 2 different antibodies. *Appl Immunohistochem Mol Morphol* 2010; **18**: 333-337 [PMID: 20571340 DOI: 10.1097/PAI.0b013e3181d27ec8]

8 **Demetri GD**, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002; **347**: 472-480 [PMID: 12181401 DOI: 10.1056/NEJMoa020461]

9 **Vajdic CM**, McDonald SP, McCredie MR, van Leeuwen MT, Stewart JH, Law M, Chapman JR, Webster AC, Kaldor JM, Grulich AE. Cancer incidence before and after kidney transplantation. *JAMA* 2006; **296**: 2823-2831 [PMID: 17179459 DOI: 10.1001/jama.296.23.2823]

10 **Awan AA**, Niu J, Pan JS, Erickson KF, Mandayam S, Winkelmayer WC, Navaneethan SD, Ramanathan V. Trends in the Causes of Death among Kidney Transplant Recipients in the United States (1996-2014). *Am J Nephrol* 2018; **48**: 472-481 [PMID: 30472701 DOI: 10.1159/000495081]

11 **Agaimy A**, Wünsch PH. Gastrointestinal stromal tumours (GIST) in kidney transplant recipients--a report of two cases. *Nephrol Dial Transplant* 2007; **22**: 1489-1490 [PMID: 17277341 DOI: 10.1093/ndt/gfl807]

12 **Mulder KE**, Egorin MJ, Sawyer MB. Renal dysfunction in a renal transplant patient treated concurrently with cyclosporine and imatinib. *Invest New Drugs* 2012; **30**: 2400-2402 [PMID: 22116657 DOI: 10.1007/s10637-011-9769-3]

13 **Cimen S**, Guler S, Panek R, Alwayn I. Gastrointestinal stromal tumour in a recipient with kidney transplantation. *BMJ Case Rep* 2015; **2015** [PMID: 26032701 DOI: 10.1136/bcr-2014-207178]

14 **Cheung CY**, Lo SH, Chan CK, Li FK, Cheng IK, Chau KF. Gastrointestinal stromal tumors in kidney transplant recipients: Report of two cases and literature review. *Asia Pac J Clin Oncol* 2017; **13**: 104-106 [PMID: 27461055 DOI: 10.1111/ajco.12506]

15 **Seculini Patiño CE**, Tabares AH, Laborie MV, Diller A. [Gastrointestinal stromal tumor and renal transplant]. *Medicina (B Aires)* 2017; **77**: 334-336 [PMID: 28825581 DOI: 10.21037/gist.2018.11.01]

16 **Takahashi R**, Shinoda K, Ishida T, Hamamoto Y, Morita S, Akita H, Kitaoka S, Tamaki S, Asanuma H, Yoshida T, Jinzaki M, Kameyama K, Oya M. Small intestinal perforation due to a huge gastrointestinal stromal tumor in a kidney transplant recipient: a case report and literature review. *BMC Nephrol* 2019; **20**: 120 [PMID: 30943904 DOI: 10.1186/s12882-019-1310-5]

17 Erratum for the Research Article: "mTORC1 Inhibition Is Required for Sensitivity to PI3K p110α Inhibitors in *PIK3CA*-Mutant Breast Cancer" by M. Elkabets, S. Vora, D. Juric, N. Morse, M. Mino-Kenudson, T. Muranen, J. Tao, A. B. Campos, J. Rodon, Y. H. Ibrahim, V. Serra, V. Rodrik-Outmezguine, S. Hazra, S. Singh, P. Kim, C. Quadt, M. Liu, A. Huang, N. Rosen, J. A. Engelman, M. Scaltriti, J. Baselga. *Sci Transl Med* 2018; **10** [PMID: 30429358 DOI: 10.1126/scitranslmed.aav9425]

18 **Tu H**, Li Q, Cai J, Chen Z, Yang H, Jiang H, Mao Y, Shou Z, Chen J. Extragastrointestinal stromal tumor in a kidney transplant recipient. *Clin Exp Nephrol* 2012; **16**: 350-353 [PMID: 22009637 DOI: 10.1007/s10157-011-0550-x]

19 **Saidi RF**, Sepehr A, Cosimi AB, Hertl M. Gastrointestinal stromal tumor in a liver transplant recipient. *Transplantation* 2008; **85**: 1363 [PMID: 18475199 DOI: 10.1097/TP.0b013e31816c7e2f]

20 **Mrzljak A**, Košuta I, Škrtić A, Kardum-Skelin I, Vrhovac R. Metachronous gastrointestinal stromal tumor and acute leukemia after liver transplantation for cholangiocellular carcinoma: is there a link? *Case Rep Oncol* 2013; **6**: 163-168 [PMID: 23626555 DOI: 10.1159/000348817]

21 **Xie M**, Rao W, Zhang P, Zhao Q, Tian Z. Endoscopic full-thickness resection for a gastrointestinal stromal tumor in a liver transplant recipient: A case report. *Medicine (Baltimore)* 2019; **98**: e16669 [PMID: 31374043 DOI: 10.1097/MD.0000000000016669]

22 **Camargo MA**, Boin I, Mainnardi JP, de Lourdes M, Ayrizono S, Coy CS, Leonardi MI, Meirelles L, Leonardi LS, Escanhoela CA. Extragastrointestinal stromal tumor and liver transplantation: case report and review. *Transplant Proc*

2008; **40**: 3781-3783 [PMID: 19100489 DOI: 10.1016/j.transproceed.2008.04.017]

23 **Pai T**, Bal M, Shetty O, Gurav M, Ostwal V, Ramaswamy A, Ramadwar M, Desai S. Unraveling the spectrum of *KIT* mutations in gastrointestinal stromal tumors: An Indian Tertiary Cancer Center Experience. *South Asian J Cancer* 2017; **6**: 113-117 [PMID: 28975118 DOI: 10.4103/sajc.sajc_275_16]

24 **Roufosse C**, Simmonds N, Clahsen-van Groningen M, Haas M, Henriksen KJ, Horsfield C, Loupy A, Mengel M, Perkowska-Ptasińska A, Rabant M, Racusen LC, Solez K, Becker JU. A 2018 Reference Guide to the Banff Classification of Renal Allograft Pathology. *Transplantation* 2018; **102**: 1795-1814 [PMID: 30028786 DOI: 10.1097/TP.0000000000002366]

25 **Roberts DM**, Jiang SH, Chadban SJ. The treatment of acute antibody-mediated rejection in kidney transplant recipients-a systematic review. *Transplantation* 2012; **94**: 775-783 [PMID: 23032865 DOI: 10.1097/TP.0b013e31825d1587]

26 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465 [PMID: 12094370 DOI: 10.1053/hupa.2002.123545]

27 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors: pathology and prognosis at different sites. *Semin Diagn Pathol* 2006; **23**: 70-83 [PMID: 17193820 DOI: 10.1053/j.semdp.2006.09.001]

28 **Joensuu H**, Vehtari A, Riihimäki J, Nishida T, Steigen SE, Brabec P, Plank L, Nilsson B, Cirilli C, Braconi C, Bordoni A, Magnusson MK, Linke Z, Sufliarsky J, Federico M, Jonasson JG, Dei Tos AP, Rutkowski P. Risk of recurrence of gastrointestinal stromal tumour after surgery: an analysis of pooled population-based cohorts. *Lancet Oncol* 2012; **13**: 265-274 [PMID: 22153892 DOI: 10.1016/S1470-2045(11)70299-6]

29 **Heinrich MC**, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299**: 708-710 [PMID: 12522257 DOI: 10.1126/science.1079666]

30 **Kee D**, Zalcberg JR. Current and emerging strategies for the management of imatinib-refractory advanced gastrointestinal stromal tumors. *Ther Adv Med Oncol* 2012; **4**: 255-270 [PMID: 22942908 DOI: 10.1177/1758834012450935]

31 **Palomba G**, Paliogiannis P, Sini MC, Colombino M, Casula M, Manca A, Pisano M, Sotgiu G, Doneddu V, Palmieri G, Cossu A. KIT and PDGFRa mutational patterns in Sardinian patients with gastrointestinal stromal tumors. *Eur J Cancer Prev* 2021; **30**: 53-58 [PMID: 32091431 DOI: 10.1097/CEJ.0000000000000581]

32 **Casali PG**, Abecassis N, Aro HT, Bauer S, Biagini R, Bielack S, Bonvalot S, Boukovinas I, Bovee JVMG, Brodowicz T, Broto JM, Buonadonna A, De Álava E, Dei Tos AP, Del Muro XG, Dileo P, Eriksson M, Fedenko A, Ferraresi V, Ferrari A, Ferrari S, Frezza AM, Gasperoni S, Gelderblom H, Gil T, Grignani G, Gronchi A, Haas RL, Hassan B, Hohenberger P, Issels R, Joensuu H, Jones RL, Judson I, Jutte P, Kaal S, Kasper B, Kopeckova K, Krákorová DA, Le Cesne A, Lugowska I, Merimsky O, Montemurro M, Pantaleo MA, Piana R, Picci P, Piperno-Neumann S, Pousa AL, Reichardt P, Robinson MH, Rutkowski P, Safwat AA, Schöffski P, Sleijfer S, Stacchiotti S, Sundby Hall K, Unk M, Van Coevorden F, van der Graaf WTA, Whelan J, Wardelmann E, Zaikova O, Blay JY; ESMO Guidelines Committee and EURACAN. Gastrointestinal stromal tumours: ESMO-EURACAN Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol* 2018; **29**: iv68-iv78 [PMID: 29846513 DOI: 10.1093/annonc/mdy095]

33 **Joensuu H**, Eriksson M, Sundby Hall K, Reichardt A, Hartmann JT, Pink D, Ramadori G, Hohenberger P, Al-Batran SE, Schlemmer M, Bauer S, Wardelmann E, Nilsson B, Sihto H, Bono P, Kallio R, Junnila J, Alvegård T, Reichardt P. Adjuvant Imatinib for High-Risk GI Stromal Tumor: Analysis of a Randomized Trial. *J Clin Oncol* 2016; **34**: 244-250 [PMID: 26527782 DOI: 10.1200/JCO.2015.62.9170]

34 **Schöffski P**, Reichardt P, Blay JY, Dumez H, Morgan JA, Ray-Coquard I, Hollaender N, Jappe A, Demetri GD. A phase I-II study of everolimus (RAD001) in combination with imatinib in patients with imatinib-resistant gastrointestinal stromal tumors. *Ann Oncol* 2010; **21**: 1990-1998 [PMID: 20507881 DOI: 10.1093/annonc/mdq076]

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CASE REPORT

Spontaneous expulsion of a duodenal lipoma after endoscopic biopsy: A case report

Zhi-Hao Chen, Li-Hong Lv, Wen-Sheng Pan, Yi-Miao Zhu

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Abstract

BACKGROUND

Gastrointestinal (GI) lipomas are benign submucosal tumors of mature adipocytes that arise mainly in the colon and stomach, sometimes in the ileum and jejunum, and rarely in the duodenum. Patients with symptomatic lipomas require endoscopic or surgical treatment. Spontaneous expulsion of lipomas after biopsy is a rare condition that has limited case reports.

CASE SUMMARY

A 56-year-old man presented to our hospital with intermittent postprandial epigastric fullness. Esophagogastroduodenoscopy (EGD) revealed a 10-mm soft yellowish submucosal lesion with the "pillow sign," located in the second portion of duodenum. Endoscopic ultrasonography (EUS) using a 12-MHz catheter probe showed a hyperechoic, homogenous, and round solid lesion (OLYMPUS EUS EU-ME2, UM-DP12-25R, 12-MHz radial miniprobe, Olympus Corporation, Tokyo, Japan). Deep biopsy was performed using the bite-on-bite technique with forceps. Histological examination was compatible with submucosal lipoma. The lesion spontaneously expelled 12 d after the biopsy. Follow-up EUS performed after 2 mo confirmed this condition.

CONCLUSION

Deep biopsy could lead to spontaneous GI lipoma expulsion. This might be the first step in lipoma diagnosis and treatment.

Key Words: Lipoma; Duodenal neoplasms; Spontaneous expulsion; Endoscopic biopsy;

Adipose tissue; Case report

Core Tip: Gastrointestinal lipomas are benign tumors consisting of mature adipocytes. Symptomatic patients may require endoscopic or surgical treatment. Here, we report a case of duodenal papillary lipoma, which was spontaneously expelled 12 d after a bite-on-bite deep biopsy with forceps. The spontaneous expulsion of the lipoma shows the possibility of performing a deep biopsy as a fenestration in the first step as a diagnostic and therapeutic procedure.

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INTRODUCTION

Gastrointestinal (GI) lipomas are benign submucosal tumors of mature adipocytes that are commonly found incidentally on endoscopy and imaging. They arise throughout the GI tract, mainly in the colon (65%-75%), followed by the ileum and jejunum (25%)[1]. The duodenum is a rare site of GI lipomas, with only 4% of lipomas affected, mostly in the second portion of the duodenum[1]. Lipomas are rarely symptomatic and have no malignant potential. However, they occasionally result in hemorrhage, abdominal pain, intestinal intussusception, or obstructive jaundice, which are related to the characteristics of the lipoma, such as its size and location[1,2]. Symptomatic duodenal lipomas require treatment. They can be excised endoscopically or surgically. This report presents a case of duodenal lipoma diagnosed by endoscopic ultrasonography (EUS) that was spontaneously expelled 12 d after a bite-on-bite deep biopsy with forceps.

CASE PRESENTATION

Chief complaints

A 56-year-old Chinese man presented to our gastroenterology department with intermittent postprandial epigastric fullness for half a year.

History of present illness

The patient had early satiety and mild reflux, especially after greasy meals, without abdominal pain, melena, fever, or jaundice. The symptoms were moderate and paroxysmal, which affected his appetite. No weight loss was reported during the past half a year. There was no history of abdominal surgery.

History of past illness

The patient's past medical history was non-contributory.

Personal and family history

The patient has none family history.

Physical examination

On physical examination, the patient's vital signs were stable with a 37.2 °C body temperature on admission. The subject's height was 170 cm and weight was 80 kg, with body mass index of 27.68 kg/m^2. No obvious abnormalities were observed upon pulmonary and cardiac examination. There was no tenderness, mass, unusual bowel sounds, or Murphy's sign upon abdominal examination. No jaundice or superficial lymphadenopathy was observed.

Laboratory examinations

The analyses of complete blood count, blood biochemistry, and serum tumor marker levels were within the normal range.

Imaging examinations

Esophagogastroduodenoscopy (EGD) revealed a 10-mm soft yellowish submucosal lesion with the "pillow sign," located in the second portion of the duodenum, directly upon the duodenal papilla (Figures 1A and B). Contrast-enhanced computed tomography (CT) revealed a suspicious low-density lesion in the periampullary region without enhancement (Figure 1C). Further EUS (OLYMPUS EUS EU-ME2, UM-DP12-25R, 12-MHz radial miniprobe, Olympus Corporation, Tokyo, Japan) revealed a hyperechoic, homogenous, round solid lesion arising from the submucosal layer, accompanied by echo attenuation (Figure 1D).

FINAL DIAGNOSIS

Deep biopsy was performed *via* the bite-on-bite technique using forceps (Micro-Tech Co. Ltd., Nanjing, China) (Figure 1E). Microscopic examination showed a small amount of roundish adipocytes in the submucosa layer, expressing S-100. Tiny lipid droplets were observed in cell cytoplasm. The glands of epithelium were neatly arranged on top. The small fragments of biopsy compatible with a submucosal lipoma. (Figures 1F-H). Based on the classic endoscopic signs, EUS characteristics and histology of the lesion, a diagnosis of duodenal lipoma was made. No bleeding, perforation, or any other complications were observed the patient.

TREATMENT

During endoscopy, the patient underwent neither resection nor hemostatic procedures after the biopsy. Prokinetics and proton pump inhibitor were prescribed to relieve the symptoms. Considering the size and location of the lipoma, we suggested that the patient remain under observation. However, the patient was extremely anxious about the lesion and insisted on its removal; thus, endoscopic resection and prophylactic pancreatic duct stenting were attempted.

OUTCOME AND FOLLOW-UP

The patient reported milder symptoms and fewer episodes after prescription. However, he insisted on resection. On endoscopic resection, which was 12 d after the biopsy, the lipoma had disappeared, with only a red scar and inflammatory mucosa residue in situ of the lesion (Figures 2A and B). Two months after the first EUS, a follow-up EUS was performed, showing that macroscopically the site displayed signs of inflammation and scarring. And the former lesion no longer existed in the surrounding duodenal wall in EUS (Figures 2C-E). After endoscopy, mild symptoms occasionally occurred during the follow-up period, without medications.

DISCUSSION

Duodenal lipoma is a rare benign tumor of the GI tract accounting for 4% of GI lipomas, most of which occur in the second portion of the duodenum[1,2]. Duodenal lipoma can be symptomatic or asymptomatic, depending on the size and location. Moreover, 80% of symptomatic duodenal lipomas have a diameter of > 2 cm, mainly manifesting as intestinal obstruction, hemorrhage, and jaundice[2,3]. The tumor in our case was a submucosal lipoma, which accounts for > 90% of intestinal lipomas, with the others arising from the intermuscular tissue and subserosa[4]. Imaging of lipomas is well recognized. CT shows well-defined border tumors in a uniform low density with fat attenuation of -120 HU to -60 HU, while magnetic resonance imaging shows high-intensity lesion with on T1-weighted, iso-signal intensity on T2-weighted, and low intensity on fat-suppressed T2-weighted imaging[5,6]. The classic endoscopic signs as "pillow sign," "tenting sign," and "naked fat sign" indicate the possibility of lipomas. The typical EUS finding for submucosal lipomas is a homogeneous hyperechoic mass originating from the submucosal layer, with echo attenuation behind and/or inside the rear area[7]. In our case, the duodenal lipoma was present in the second portion of the duodenum, immediately upon the duodenal papilla, with classic imaging on EGD, CT, and EUS, with pathologically confirmation.

Symptomatic lipomas require treatment, including removal *via* endoscopy or surgical excision. There are four techniques for endoscopic excision: Dissection-based resection, endoscopic mucosal resection (EMR), loop-assisted resection, and unroofing[8] (Figure 3). Dissection-based resection defines as endoscopic procedure mainly using dissection-based techniques, such as ESD. Loop-assisted resection is the procedure performed with loop for haemostatic or removal reasons. Because of the poor conduction of electric current in the adipose tissue, endoscopists must apply a higher electrical output during

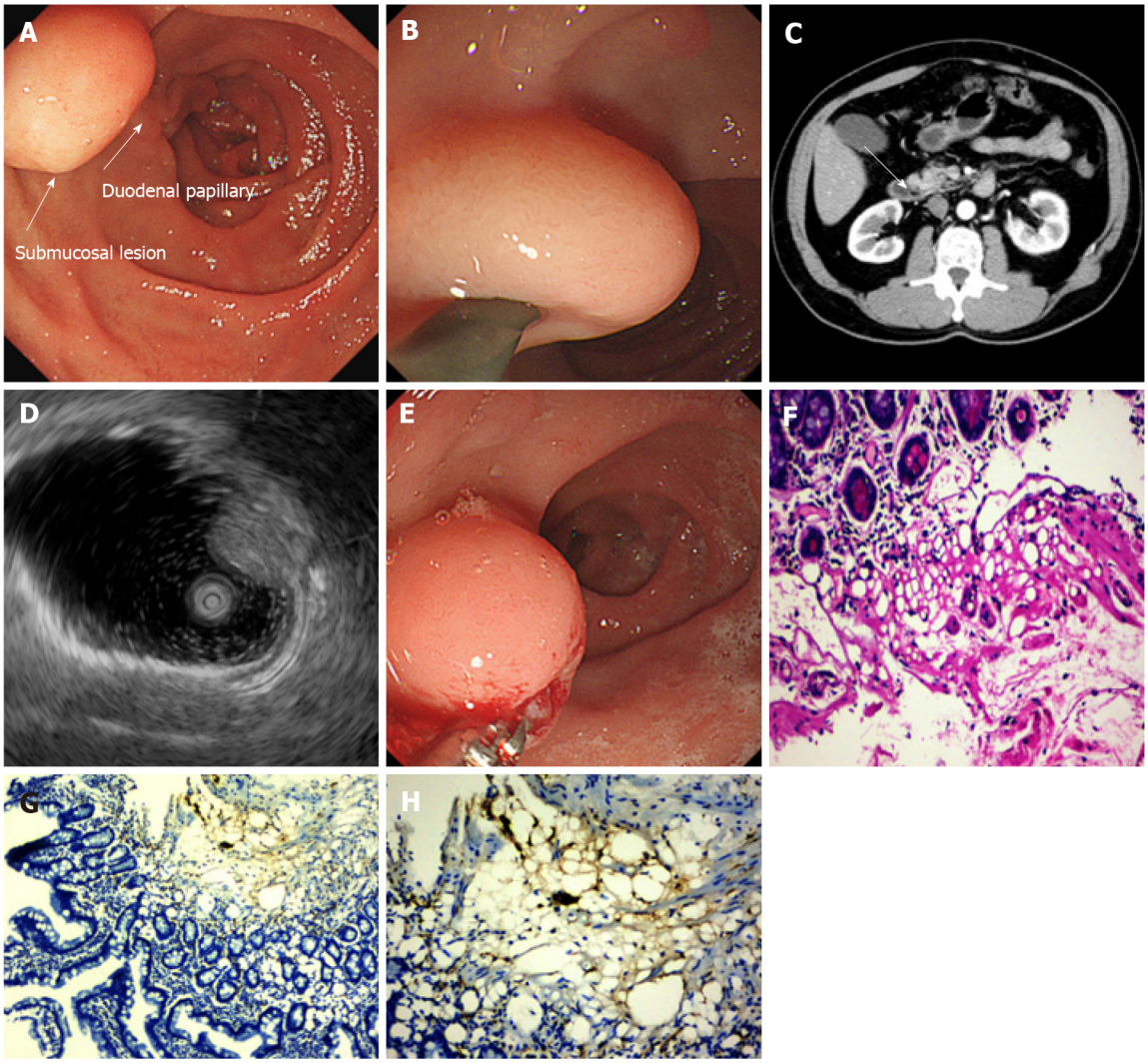

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Figure 1 Imaging findings at the time of diagnosis. A and B: Esophagogastroduodenoscopy revealed a 10-mm soft yellowish submucosal lesion with the "pillow sign," located in the second portion of duodenum, immediately upon the duodenal papillary; C: Abdominal contrast-enhanced computed tomography shows a suspicious low-density lesion in the periampullary region without enhancement (white arrow); D: Endoscopic ultrasonography with a 12-MHz catheter probe showed a hyperechoic, homogenous, and round solid lesion with echo attenuation, arising from the submucosal layer; E: Deep biopsy *via* bite-on-bite technique with forceps was performed; F-H: Microscopic examination showed a small amount of roundish adipocyte in the submucosa layer, expressing S-100. Tiny lipid droplets were observed in cell cytoplasm. The glands of epithelium were neatly arranged on top (F: Hematoxylin and eosin staining, × 200; G: Immunohistochemical S-100 stain, × 100; H: IHC S-100 stain, × 200).

resection, which increases the possibility of complications, such as hemorrhage and perforation, especially for giant lipomas. The unroofing technique, which cuts off the upper half of the lesion, with the remaining adipose tissue extruded from the open surface, was first applied by Mimura *et al*[9]. Its efficacy and safety have been approved by several authors in case reports[10-14]. Bronswijk *et al*[8] collected 24 studies (77 lesions included) of endoscopic treatment for colon lipomas, and a systematic review indicated that endoscopic resolution rates were 60%, 100%, 93.6%, and 93.1% for unroofing, dissection-based resection, EMR, and loop-assisted resection, respectively. The former two techniques reported no adverse events, whereas the latter two showed complication rates of 12.9%-13.8%. Yamamoto *et al*[15] proposed a two-step hybrid technique that combined endoscopic unroofing with EMR for giant lipomas.

Spontaneous disappearance or expulsion of lipomas is an extremely rare manifestation that had limited case reports. There have been some cases of spontaneous expulsion per rectum of submucosal lipomas without any intervention. With a certain volume, these submucosal lipomas cause intussusception, which might lead to ischemia, necrosis, and breakage, and the lipomas were spontaneously expelled[4,16-20]. In our case, the lipoma was spontaneously expelled after deep biopsy *via* the bite-on-bite technique with forceps, which we suspected was the result of fat extravasation from the biopsy site as fenestration over time. There were a few cases of spontaneous expulsion after biopsy of GI lipoma[21-24]. Kurahara *et al*[21] first reported a gastric lipoma that was expelled after biopsy. Ishiyama *et al*[22] reported the expulsion of a colonic lipoma after biopsy. We speculated that, in these cases, three

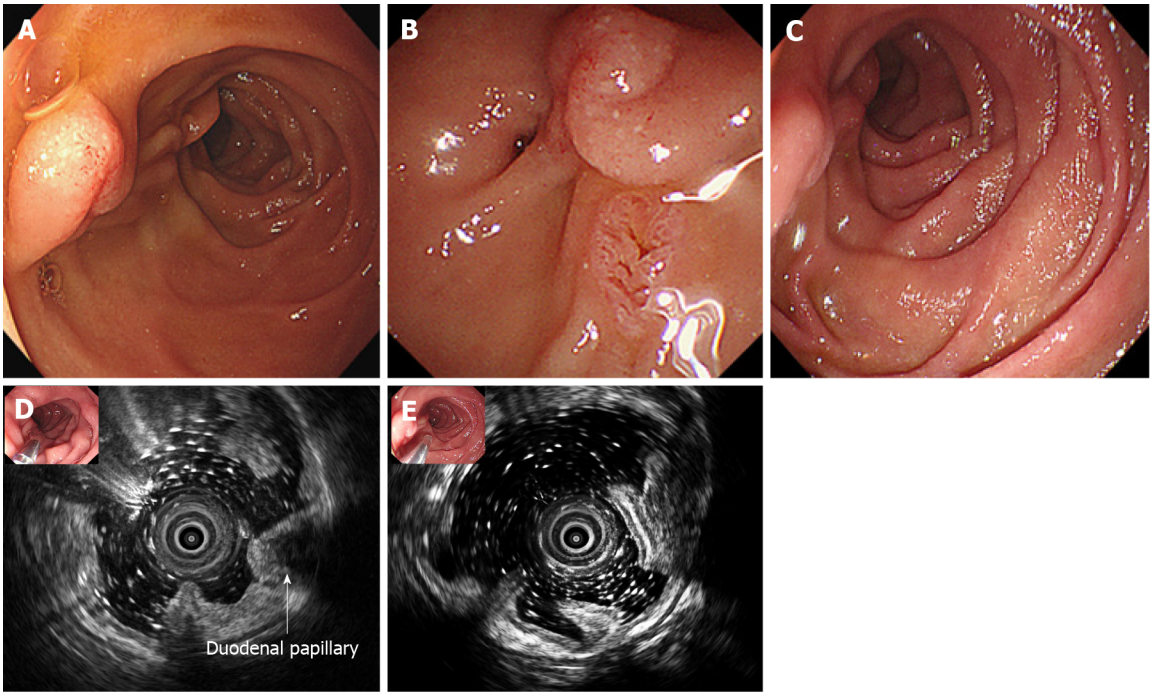

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Figure 2 Follow-up endoscopic view of the lesion. A and B: 12 d after the biopsy, the lipoma was spontaneously expelled, with red scar and inflammatory mucosa residue in situ of the lesion; C-E: Follow-up endoscopic ultrasonography after 2 mo revealed that the *in situ* mucosa was smooth, and the former lesion no longer existed in the surrounding duodenal wall or periduodenal papilla region.

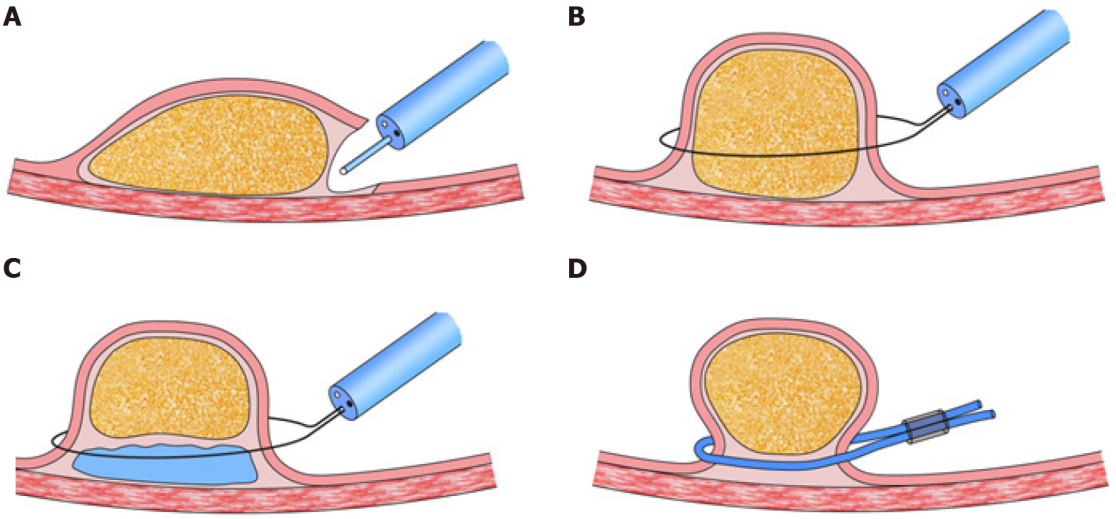

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Figure 3 Techniques for endoscopic excision of gastrointestinal lipomas[14]. A: Dissection-based resection technique; B: Unroofing technique; C: Endoscopic mucosal resection; D: Loop-assisted resection technique.

conditions need to be met for the lipoma to disappear: (1) The overlying mucosa and tumor capsule must be damaged by either acquired opening, such as unroofing and deep biopsy, or spontaneous opening, such as ischemia and ulceration; (2) The lipoma should arise from the submucosal layer; and (3) The extravasation rate, which depends on the tumor volume and size of the fenestration, should be faster than the mucosal healing rate. In this case, the special anatomical position might have facilitated pancreatic lipase and bile digestion of the lipoma to speed up the discharge process.

The treatment techniques and cases of spontaneous expulsion lipomas indicate that we might conduct a deep biopsy based on bite-on-bite techniques or more intense "tunneled biopsy" as fenestration routinely when a lipoma is diagnosed with typical EUS and EGD. Such biopsies could aid in further diagnosis in pathology and reduce the size or even remove the lesion as a treatment, particularly in cases where the lesion is relatively large or located in the small intestine[25]. A simple deep biopsy

during diagnosis might decrease the complications caused by the lesion itself or by the next step of endoscopic or surgical resection. Larger studies are necessary to make conclusion on the efficacy of fenestration on lipoma and algorithm of treatment.

CONCLUSION

In this case, we present the spontaneous expulsion of a duodenal lipoma 12 d after a bite-on-bite deep biopsy. Treatments for benign and slow-growing tumors include endoscopic dissection-based resection, EMR, loop-assisted resection, endoscopic unroofing, and surgical resection. The expulsion of lipoma after biopsy enlightens us the possibility of taking a deep biopsy as a fenestration in the first step as the diagnostic and therapeutic procedure.

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FOOTNOTES

Author contributions: Chen ZH, Lv LH, and Zhu YM assembled, analyzed, and interpreted the patient's data and case presentation; Zhu YM and Pan WS reviewed the literature; Chen ZH and Zhu YM prepared the original manuscript; Zhu YM edited and critically revised the manuscript; all authors contributed to writing the manuscript; and all authors read and approved the final manuscript.

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REFERENCES

1 **O'Riordan BG**, Vilor M, Herrera L. Small bowel tumors: an overview. *Dig Dis* 1996; **14**: 245-257 [PMID: 8843980 DOI: 10.1159/000171556]
2 **Pei MW**, Hu MR, Chen WB, Qin C. Diagnosis and Treatment of Duodenal Lipoma: A Systematic Review and a Case Report. *J Clin Diagn Res* 2017; **11**: PE01-PE05 [PMID: 28892976 DOI: 10.7860/JCDR/2017/27748.10322]
3 **Wilson JM**, Melvin DB, Gray G, Thorbjarnarson B. Benign small bowel tumor. *Ann Surg* 1975; **181**: 247-250 [PMID: 1078626]
4 **Kang B**, Zhang Q, Shang D, Ni Q, Muhammad F, Hou L, Cui W. Resolution of intussusception after spontaneous expulsion of an ileal lipoma per rectum: a case report and literature review. *World J Surg Oncol* 2014; **12**: 143 [PMID: 24884620 DOI: 10.1186/1477-7819-12-143]
5 **Genchellac H**, Demir MK, Ozdemir H, Unlu E, Temizoz O. Computed tomographic and magnetic resonance imaging

findings of asymptomatic intra-abdominal gastrointestinal system lipomas. *J Comput Assist Tomogr* 2008; **32**: 841-847 [PMID: 19204441 DOI: 10.1097/RCT.0b013e318159a4b5]

6 **Kovač JD**, Dunjić MK, Bjelović M, Banko B, Lilić G, Milenković R, Micev M, Maksimović R. Magnetic resonance imaging features of multiple duodenal lipomas: a rare cause of intestinal obstruction. *Jpn J Radiol* 2012; **30**: 676-679 [PMID: 22752443 DOI: 10.1007/s11604-012-0098-z]

7 **Chen HT**, Xu GQ, Wang LJ, Chen YP, Li YM. Sonographic features of duodenal lipomas in eight clinicopathologically diagnosed patients. *World J Gastroenterol* 2011; **17**: 2855-2859 [PMID: 21734794 DOI: 10.3748/wjg.v17.i23.2855]

8 **Bronswijk M**, Vandenbroucke AM, Bossuyt P. Endoscopic treatment of large symptomatic colon lipomas: A systematic review of efficacy and safety. *United European Gastroenterol J* 2020; **8**: 1147-1154 [PMID: 32746773 DOI: 10.1177/2050640620948661]

9 **Mimura T**, Kuramoto S, Hashimoto M, Yamasaki K, Kobayashi K, Kobayashi M, Oohara T. Unroofing for lymphangioma of the large intestine: a new approach to endoscopic treatment. *Gastrointest Endosc* 1997; **46**: 259-263 [PMID: 9378215 DOI: 10.1016/s0016-5107(97)70097-x]

10 **Kim GW**, Kwon CI, Song SH, Jin SM, Kim KH, Moon JH, Hong SP, Park PW. Endoscopic resection of giant colonic lipoma: case series with partial resection. *Clin Endosc* 2013; **46**: 586-590 [PMID: 24143327 DOI: 10.5946/ce.2013.46.5.586]

11 **Lee KJ**, Kim GH, Park DY, Shin NR, Lee BE, Ryu DY, Kim DU, Song GA. Endoscopic resection of gastrointestinal lipomas: a single-center experience. *Surg Endosc* 2014; **28**: 185-192 [PMID: 23996333 DOI: 10.1007/s00464-013-3151-9]

12 **Shin EK**, Kim KJ, Seo JA, Paek JH, Choi IS, Moon W, Park MI, Park SJ. [A case of giant colonic lipoma showing spontaneous resolution after endoscopic partial resection]. *Korean J Gastroenterol* 2007; **50**: 199-202 [PMID: 17885287]

13 **Soares JB**, Gonçalves R, Rolanda C. Endoscopic resection of a large colonic lipoma by unroofing technique. *Endoscopy* 2011; **43** Suppl 2 UCTN: E407 [PMID: 22275025 DOI: 10.1055/s-0030-1256938]

14 **Sugimoto K**, Sato K, Maekawa H, Sakurada M, Orita H, Ito T, Saita M, Ikota M, Yoshida Y, Yamano M. Unroofing technique for endoscopic resection of a large colonic lipoma. *Case Rep Gastroenterol* 2012; **6**: 557-562 [PMID: 22949897 DOI: 10.1159/000342350]

15 **Yamamoto K**, Ikeya T, Shiratori Y. Endoscopic unroofing and mucosal resection for a large colonic lipoma with intussusception: an effective hybrid technique. *VideoGIE* 2021; **6**: 190-192 [PMID: 33898900 DOI: 10.1016/j.vgie.2020.11.018]

16 **Misra SP**, Singh SK, Thorat VK, Gulati P, Malhotra V, Anand BS. Spontaneous expulsion per rectum of an ileal lipoma. *Postgrad Med J* 1988; **64**: 718-719 [PMID: 3251232 DOI: 10.1136/pgmj.64.755.718]

17 **Hamila F**, Elghali MA, Bouriga R, Haj Khlifa MH, Fadhl H, Jarrar MS, Letaief R. Partial spontaneous anal expulsion of the right colon lipoma: An exceptional diagnostic circumstance. *Int J Surg Case Rep* 2017; **41**: 414-416 [PMID: 29546005 DOI: 10.1016/j.ijscr.2017.10.059]

18 **Robertson DA**, Saweirs W, Low-Beer TS. Spontaneous expulsion of a large colonic tumour. *Br Med J (Clin Res Ed)* 1982; **285**: 1084 [PMID: 6812756 DOI: 10.1136/bmj.285.6348.1084]

19 **Zamboni WA**, Fleisher H, Zander JD, Folse JR. Spontaneous expulsion of lipoma per rectum occurring with colonic intussusception. *Surgery* 1987; **101**: 104-107 [PMID: 3492056]

20 **Stebbings WS**, Staunton MD. Spontaneous expulsion of a large submucosal colonic lipoma. *J R Soc Med* 1989; **82**: 624-625 [PMID: 2810300 DOI: 10.1177/014107688908201021]

21 **Kurahara K**, Aoyagi K, Hizawa K, Yao T, Iida M, Fujishima M. Spontaneous disappearance of a gastric lipoma after endoscopic biopsy: report of an unusual case. *Endoscopy* 1999; **31**: S31 [PMID: 10376473]

22 **Ishiyama S**, Tashiro Y, Nagayasu K, Niwa K, Ono S, Sugimoto K, Hata M, Kamiyama H, Komiyama H, Takahashi M, Yaginuma Y, Kojima Y, Goto M, Tanaka M, Sengoku H, Okuzawa A, Tomiki Y, Sakamoto K. Spontaneous disappearance of a giant colonic lipoma after endoscopic biopsy. *Endoscopy* 2011; **43** Suppl 2 UCTN: E16 [PMID: 21271519 DOI: 10.1055/s-0030-1255826]

23 **João M**, Cunha I, Gravito-Soares E, Gravito-Soares M, Amaro P, Figueiredo P. An Atypical Presentation of a Colonic Lipoma: Avoiding Surgery with a Deeper Endoscopic Look. *GE Port J Gastroenterol* 2022; **29**: 45-50 [PMID: 35111963 DOI: 10.1159/000513967]

24 **Sidani SM**, Tawil AN, Sidani MS. Extraction of a large self-amputated colonic lipoma: A case report. *Int J Surg* 2008; **6**: 409-411 [PMID: 18947813 DOI: 10.1016/j.ijsu.2006.05.003]

25 **Koutsoumpas A**, Perera R, Melton A, Kuker J, Ghosh T, Braden B. Tunneled biopsy is an underutilised, simple, safe and efficient method for tissue acquisition from subepithelial tumours. *World J Clin Cases* 2021; **9**: 5822-5829 [PMID: 34368301 DOI: 10.12998/wjcc.v9.i21.5822]

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