ISSN 1948-5204 (online)

World Journal of *Gastrointestinal Oncology*

World J Gastrointest Oncol 2019 December 15; 11(12): 1092-1239

Published by Baishideng Publishing Group Inc

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 11 Number 12 December 15, 2019

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World Journal of Gastrointestinal Oncology

Volume 11 Number 12 December 15, 2019

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Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Paolo Aurello, MD, PhD, Professor, Surgeon, Department of General Surgery, Sapienza University of Rome, Rome 00162, Italy

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NAME OF JOURNAL
World Journal of Gastrointestinal Oncology

ISSN
ISSN 1948-5204 (online)

LAUNCH DATE
February 15, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Monjur Ahmed, Rosa M Jimenez Rodriguez, Pashtoon Kasi

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-5204/editorialboard.htm

EDITORIAL OFFICE
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PUBLICATION DATE
December 15, 2019

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World J Gastrointest Oncol 2019 December 15; 11(12): 1092-1100

DOI: 10.4251/wjgo.v11.i12.1092

ISSN 1948-5204 (online)

OPINION REVIEW

Observation or resection of pancreatic intraductal papillary mucinous neoplasm: An ongoing tug of war

Jan Rune Aunan, Nigel B. Jamieson, Kjetil Søreide

ORCID number: Jan Rune Aunan (0000-0001-6683-4081); Nigel B. Jamieson (0000-0002-9552-4725); Kjetil Søreide (0000-0001-7594-4354).

Author contributions: Aunan JR and Søreide K planned the search and outline of the paper; Aunan JR, Jamieson NB and Søreide K performed the literature search; Aunan JR drafted the first version; Aunan JR, Jamieson NB and Søreide K performed several rounds of revisions; Aunan JR, Jamieson NB and Søreide K accepted the final version for submission.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

Received: May 21, 2019
Peer-review started: May 23, 2019
First decision: August 23, 2019

Jan Rune Aunan, Kjetil Søreide, Department of Gastrointestinal Surgery, HPB Unit, Stavanger University Hospital, Stavanger 4068, Norway

Nigel B. Jamieson, Wolfson Wohl Cancer Research Centre, Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1BD, United Kingdom

Nigel B. Jamieson, West of Scotland Pancreatic Unit, Glasgow Royal Infirmary, Glasgow G4 0SF, United Kingdom

Kjetil Søreide, Gastrointestinal Translational Research Unit, Laboratory for Molecular Medicine, Stavanger University Hospital, Stavanger 4068, Norway

Kjetil Søreide, Department of Clinical Medicine, University of Bergen, Bergen 5003, Norway

Corresponding author: Kjetil Søreide, FRCS (Ed), MD, PhD, Professor, Department of Gastrointestinal Surgery, Stavanger University Hospital, POB 8100, Stavanger 4068, Norway.
ksoreide@mac.com
Telephone: +47-51-518000
Fax: +47-51-519919

Abstract

An increasing number of patients are being referred to pancreatic centres around the world due to often incidentally discovered cystic neoplasms of the pancreas. The evaluation and management of pancreatic cystic neoplasms is a controversial topic and with existing guidelines based on a lack of strong evidence there is discordance between centres and guidelines with regard to when to offer surgery and when to favour surveillance. The frequency, duration and modality of surveillance is also controversial as this is resource-consuming and must be balanced against the perceived benefits and risks involved. While there is consensus that the risk of malignancy should be balanced against the life-expectancy and comorbidities, the indications for surgery and surveillance strategies vary among the guidelines. Thus, the tug of war between surveillance or resection continues. Here we discuss the recommendations from guidelines with further accumulating data and emerging reports on intraductal papillary mucinous neoplasm in the literature.

Key words: Neoplasia; Pancreatic cancer; Pancreatic cyst; Diagnosis; Resection; Surveillance; Mutation; Biomarker

Revised: September 3, 2019
Accepted: September 26, 2019
Article in press: September 26, 2019
Published online: December 15, 2019

P-Reviewer: Cerwenka H
S-Editor: Zhang L
L-Editor: Filipodia
E-Editor: Qi LL

Core tip: For patients with intraductal papillary mucinous neoplasia detected in the pancreas, there is currently debate over the frequency, duration and modality of surveillance in the long-term. Surveillance is resource-consuming and must be balanced against the likely benefits and perceived risks for malignant transformation. Furthermore, the risk of malignancy should be balanced against the overall life-expectancy and comorbidities. Notably, the indications for either surgery or surveillance vary among the available guidelines. Thus, the tug of war between surveillance or resection continues. The recommendations from existing guidelines are highlighted with further accumulating data and emerging reports from the intraductal papillary mucinous neoplasm literature.

Citation: Aunan JR, Jamieson NB, Søreide K. Observation or resection of pancreatic intraductal papillary mucinous neoplasm: An ongoing tug of war. *World J Gastrointest Oncol* 2019; 11(12): 1092-1100
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1092.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1092

INTRODUCTION

An increasing number of patients are being referred to pancreatic centres around the world due to often incidentally discovered cystic neoplasms of the pancreas. High quality radiological imaging is increasingly utilised and although pancreatic cystic neoplasms have most likely always existed, it is only more recently that guidelines for follow-up, diagnosis and management have been issued. These guidelines themselves are based on the scarce data available. Intraductal papillary mucinous neoplasm (IPMN) was first described in 1982 by Ohashi and was, until the turn of the millennium, considered a rare entity but is now among the most commonly discovered and surgically resected pancreatic cystic lesions[2].

The evaluation and management of pancreatic cystic neoplasms is a controversial topic, and the existing guidelines are based on a lack of strong evidence. Thus, there is discordance between practice among centres and the available guidelines with regard to when and to whom surgery should be offered and when to favour surveillance. The frequency, duration and modality of surveillance is also controversial, as this strategy is resource-consuming and must be balanced against the perceived benefits and risks involved.

The first guidelines came after the turn of the millennium, with four guidelines now in place (Table 1), including the International Association of Pancreatology (IAP, also called "Fukuoka") guidelines, European Study Group on Cystic Tumors of the Pancreas[4], American Gastroenterological Association (AGA) and American College of Gastroenterology (ACG) clinical guideline[6]. While there is consensus that the risk of malignancy should be balanced against life-expectancy and comorbidities, the indications for surgery and surveillance strategies vary among the guidelines. Thus, the tug of war between surveillance or resection continues (Figure 1). Here we discuss the recommendations from guidelines with further accumulating data and emerging reports on IPMN in the literature.

IPMN IS A PREMALIGNANT CONDITION

IPMN harbours a malignant potential and is considered a premalignant condition of the pancreas. The underlying mechanisms of cancer progression and malignant evolution is poorly understood, but knowledge is emerging in this field[8-10]. Thus, the main rationale for surgery is to resect lesions that either harbour early invasive cancerous lesions or preferably to remove lesions that contain high-grade dysplasia but have yet to progress into invasive cancer. Based on recent data, approximately one in every four resected IPMN have histologically confirmed malignancy[11].

POPULATION AT RISK FOR IPMN

The exact incidence of IPMN is not known, but it is estimated to be present in 2%-45%

Table 1 Comparison of existing guidelines for intraductal papillary mucinous neoplasia of the pancreas

Guideline (yr)	IAP (2017)	European (2018)	AGA (2015)	ACG (2018)
Resection criteria	≥ 1 high risk stigmata	≥ 1 absolute indication	Solid component and dilated MPD and/or concerning features on EUS-FNA	Decided by multidisciplinary team. Refer if ≥ 1 high risk characteristics
	≥ 1 worrisome feature and ≥ 1 of: definitive mural nodule ≥ 5 mm, MPD involvement, suspicious or positive cytology.	≥ 1 relative indication without significant co-morbidities		
	Consider surgery in young fit patients with cysts > 2 cm	≥ 2 relative indications with significant co-morbidities		
	MD-/MT IPMN if ≥ 1 high risk stigmata	MD-/MT IPMN		
High risk features/surgery indications	High risk stigmata: Jaundice; Enhancing mural nodule > 5 mm; MPD > 10 mm	Absolute criteria: Jaundice; Enhancing mural nodule ≥ 5 mm; MPD ≥ 10 mm; Solid mass; Positive cytology	High risk features: Cyst size ≥ 3 cm; Dilated MPD; Solid component	High-risk characteristics: Jaundice; Mural nodule/solid component; MPD > 5 mm; Abrupt pancreatic duct calibre change with distal atrophy; Cyst size ≥ 3 mm; Cyst growth 3 mm/yr; Positive cytology; Pancreatitis secondary to cyst; Elevated serum Ca19-9
	Worrisome features: Pancreatitis secondary to cyst; Cyst size ≥ 3 cm; Enhancing mural nodule < 5 mm; Enhancing thickened cyst wall; MPD 5-9 mm; Abrupt pancreatic duct calibre change with distal atrophy; Growth ≥ 5 mm/2 yr; Elevated serum Ca19-9	Relative indications: Pancreatitis secondary to cyst; Cyst diameter ≥ 40 mm; Enhancing mural nodule < 5 mm; MPD 5-9 mm; Growth rate > 5 mm/yr; New onset diabetes mellitus; Elevated serum Ca19-9		
Surveillance intervals	< 1 cm: 6 mo, then every 2 yr; 1-2 cm: 6 mo 1st yr, yearly for 2 yr, then every 2 yr; 2-3 cm: 3-6 mo 1st yr, then yearly; > 3 cm 3-6 mo	6 mo 1st yr, then yearly	1, 3 and 5 yr	< 1 cm: Every 2 yr; 1-2 cm: Every 1 yr; 2-3 cm: Every 6-12 mo; > 3 cm Every 6 mo and consider referral to MDT
Surveillance modality	< 2 cm MRI or CT; 2 cm MRI and EUS	MRI and/or EUS Serum Ca19-9	MRI	MRI and/or EUS

IAP: International Association of Pancreatology; AGA: American Gastroenterology Association; ACG: American College of Gastroenterology; MPD: Main pancreatic duct; MRI, Magnetic resonance imaging; EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; IPMN: Intraductal papillary mucinous neoplasm; CT: Computed tomography; MDT: Multidisciplinary team; MD: Main duct; MT: Mixed type.

of the general population[4]. Notably, many IPMNs are incidentally discovered during cross-sectional imaging performed for other unrelated medical reasons. This particularly holds true for the aging population when the prevalence of numerous other medical conditions increase[12], which leads to an increasing number of cross-sectional imaging being undertaken for work-up or surveillance. Thus, for transabdominal scans involving the pancreas, this often leads to the incidental detection of cystic lesions in the pancreas with a request for review by pancreatologists and surgeons. The influence of ageing on loss of functional reserve follows many of the same mechanisms that also lead to risk of premalignant and malignant disease. Thus, finding incidental IPMNs is likely to increase with the widespread use of cross-sectional imaging. Resection *vs* surveillance needs to be tailored to the likely clinical impact and outcome for the particular lesion and person. For one, extended surveillance needs to take into account the life-time risk of malignant development. Furthermore, when considering a need for resection, the type of surgery needs to be taken into account together with patient comorbidities. Notably, the risk profile, including mortality and morbidity is considerably different in pancreatoduodenectomy (mortality 3%-4%) compared to a distal resection (mortality < 1.5%)[14,15]. Furthermore, enucleations and central resections have their own morbidity profile. Lastly, a total pancreatectomy (sometimes indicated for disease affecting the entire gland) needs to be considered carefully as the risk, benefit and long-term consequences are considerable.

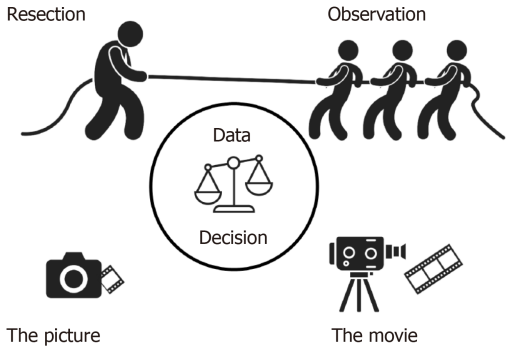

Figure 1 Tug of war between resection and observation in intraductal papillary mucinous neoplasm. Depicted for illustration is the tug of war between strategies for intraductal papillary mucinous neoplasm, either resection or observation. Decision making is based on available data, which currently are conflicting. Reaction on first-event cross-sectional imaging (the Picture) may prevent information obtained from serial, temporal evaluation (the Movie) of how intraductal papillary mucinous neoplasm lesions may change in character, size and context.

CONSIDERING PATIENT FITNESS

An important factor in limiting overtreatment and morbidity from surgery is careful patient selection by limiting surgery in highly comorbid surgical candidates. Progression to cancer, even in the presence of worrisome features, may be less common than previously thought, according to recent observational studies. In one observational group of IPMNs with worrisome features, the 5 year disease specific survival was 96% suggesting that conservative management was appropriate, but high-risk stigmata is associated with a 40% risk of IPMN-related death suggesting surgery as the treatment of choice in fit patients[18]. In a meta-analysis on patients unfit for surgery but with worrisome features and high risk stigmata, the IPMN related mortality was low, especially for branched duct IPMN[19], and death from other causes was much more common. In another observational study of low risk lesions, malignancy occurred rarely (4.2%), but the development of main pancreatic duct dilatation was strongly associated with malignant transformation[20].

RESECTION OR SURVEILLANCE–THE DILEMMA

Surgery is offered to a subset of patients with IPMN, yet pancreatic resection is associated with significant morbidity and even mortality. Thus, it is important to establish a better understanding of the impact of worrisome features so that surgery is offered when justified. The decision to resect an IPMN lesion is often based on any one of the mentioned guidelines, yet all current consensus guidelines are generated from low quality data. Also, even if based on low quality data, the recommendations vary considerably (Table 1). Furthermore, the indications may be "absolute" or "relative" (Table 1) with partial disagreement between guidelines and hence the ongoing debate among pancreatologists and pancreatic surgeons.

Both the European guidelines and the IAP guidelines justify resection if one or more is present: (A) Jaundice due to the IPMN; (B) Enhancing mural nodule over 5 mm; or (C) Main pancreatic duct dilatation over 10 mm (Table 1). These criteria are referred to as absolute indications in the European guidelines and high-risk stigmata in the IAP guidelines (Table 1). The European guidelines also opt for surgery if one or more of several relative indications are present in healthy individuals, which includes pancreatitis, cyst diameter over 40 mm, growth rate over 5 mm/year, new onset diabetes mellitus and elevated Ca19-9 among others. The IAP guidelines refer to many of the same criteria as worrisome features and also recommend surgery but only with some additional basic criteria present, namely main pancreatic duct involvement, mural nodule or positive/suspicious cytology. While the European and IAP guidelines are similar with regards to surgical indications, differences (such as cyst size) are present (Table 1). The European guidelines recommend surgery when cyst size is over 40 mm whereas the IAP guidelines opt for surgery if cyst size is over 20 mm in young fit individuals.

The AGA guidelines for surgical resection require both a solid component AND a dilated pancreatic duct OR a positive cytology on endoscopic ultrasound with fine-needle aspiration. AGA does not mention degree of dilatation of the main pancreatic

duct.

The ACG guidelines fail to provide clear guidance on when to offer surgery but recommend referral to multidisciplinary teams if high risk characteristics are present. This provides opportunity for personalisation of treatment selection according to the teams and their members. For worrisome risk features, models have been proposed to predict risk of malignancy or high-grade dysplasia, with modest prediction values achieved[21].

In a retrospective study evaluating accuracy of resection criteria in patients with pancreatic cysts, the cohort included 75 patients with IPMN[22]. The investigators compared the final pathologic outcome of surgically removed pancreatic cysts with the recommended indications for resection according to three different guidelines (IAP, the European and AGA guidelines). For patients with suspected IPMN (n = 75), resection was justified in 36% of 67% (54%), 36% of 68% (53%) and 32% of 54% (59%) of patients based on the recommendations for surgery in the IAP, European and AGA guidelines, respectively[22]. The AGA guideline would have avoided resection in 21% of 75% (28%) patients, the IAP in 8% of 75% (11%) and the European in 7% of 75% (9%) if the guidelines would have been applied strictly. However, 4% of 33% patients (12%) with high-grade dysplasia or malignancy would have been missed with the AGA guidelines, compared with none if following the IAP or European guidelines[22]. This demonstrates that all guidelines will currently result in a degree of overtreatment, imposing a potential risk to the patient. The AGA guidelines, which represent the more conservative approach towards resection for IPMNs (Table 1), would have missed the surgical indication for two patients with malignancy and two patients with high-grade dysplasia[22]. This indicates that, based on the current guidelines, a degree of overtreatment is necessary in order to capture all malignant and high-grade dysplastic lesions.

IPMNS OF MAIN DUCT, BRANCH DUCT AND MIXED TYPE

Dilatation of the main duct is considered to be a strong risk feature for malignancy, yet controversy persists regarding optimal duct diameter cut-off for resection and the need for either observation or resection in main duct IPMNs[23,24]. The European guidelines recommend resection in all fit individuals, the IAP guidelines require the presence of any high-risk stigmata, the ACG recommends referral to a multidisciplinary team discussion whereas the AGA guidelines do not mention this group with main duct dilatation specifically. A recent study demonstrated a high risk for malignancy with main duct dilatation[23], while another study from Verona did not call main duct dilatation as a risk for malignancy for patients undergoing surveillance. Notably, the bi-institutional series[23] from Johns Hopkins and Karolinska was based on resected IPMNs, thus potentially biasing the results towards patients who underwent resection[23] rather than all patients diagnosed with a dilated main pancreatic duct. However, the Johns Hopkins/Karolinska series found an increased risk of malignancy even in the middle-ranged size of dilated main ducts (5-9 mm). In contrast, the Verona study included both resection and surveillance patients and did not find main duct dilatation alone to be a significant risk factor for malignancy, although it increased risk together with other worrisome features. Thus, we believe the jury is still out on the true risk associated with main duct dilatation in IPMN.

Branch duct IPMN are presumed to have a very low risk of malignancy, yet the risk is definite[20]. Controversy persists regarding criteria for continued observation or resection for these lesions, although the updated international guidelines aim to have better precision in predicting malignancy risk[25]. Initial cysts size (> 40 mm) and annual growth rate may be indicators for risk of malignancy in branch duct lesions observed over time[26]. A French study suggested that branch duct IPMN in men with recent onset diabetes should be considered for resection, as the incidence of malignancy and high-grade dysplasia was higher in this group[27]. This is in line with the evolving evidence that developing fasting blood glucose intolerance is associated with risk of pancreatic malignancy[28,29]. New onset diabetes associated with an IPMN is also a clear indication for resection in healthy individuals when following the European guidelines as it is a "relative indication" that should lead to resection in patients without comorbidities (Table 1). New onset diabetes is not a criterium for resection in the other guidelines, but the ACG guidelines acknowledges its importance and recommend further investigation and tighter surveillance.

INFORMED DECISION-MAKING: A STEPWISE APPROACH

The first step in the decision between surgery or surveillance of a pancreatic cystic lesion is making the correct diagnosis, which is not always straight forward in itself. In addition to evaluating the patient's overall condition and fitness for surgery, information from a variety of modalities can be assimilated in order to enhance the decision-making process.

Transsectional body imaging by magnetic resonance imaging and computed tomography

Magnetic resonance imaging is usually the radiologic modality of choice for classification of IPMN. In the study by Lekkerkerker *et al*[22], preoperative diagnosis was correct in 80% of branch duct IPMNs and 89% of the main/mixed type IPMNs. In other high volume centres the preoperative diagnosis was incorrect in one third of the cases[30,31], and 20% of presumed branch duct IPMN had main duct involvement at postoperative histology. Among the conflicting and debated topics is the decision based on a cross-sectional image. Should one react on the "picture" or the "movie" (Figure 1)? While some argue that worrisome features present at first presentation would warrant resection, others uphold the view of further imaging to get a sense of the "movie" by depicting cystic progression (or stability) over time and thus make a decision to proceed with resection based on this time-dependent information. Obviously, the argument against such an approach is the risk of surveilling patients only to discover a lesion that has progressed to invasive (or even metastatic) carcinoma. Reports on progression to invasive cancer in some apparently innocuous lesions followed for many years is what concerns most pancreatic surgeons[32]. In one series, branch duct cysts that remained ≤ 1.5 cm after 5 years of follow up had a very low risk of progression to cancer[33]. However, in the population with cyst size > 1.5 cm, a reported 7.5% developed malignancy, thus suggesting these should be followed beyond 5 years of surveillance.

Endoscopic ultrasound

Contrast-enhanced endoscopic ultrasound has shown promising results with improved evaluation of mural nodules in cysts and subsequently the differentiation between malignant and non-malignant cysts[34]. Using the same contrast-enhanced endoscopic ultrasound with time intensity curves, blood flow and microvasculature density in IPMN cysts can be evaluated and this correlates with high-grade dysplasia/malignancy and has potential to differentiate them from low-grade dysplasia[35].

Tumour markers

Serum Ca19-9 and CEA are well-established tumour biomarkers that can help in decision making in some cases. Significantly elevated levels of these biomarkers in patients with worrisome lesions (without jaundice) would be suggestive of a higher risk of invasive cancer[36]. However, the overall sensitivity and specificity remains poor[37], and low values do not infer a benign condition. Thus, other biomarkers sampled from cyst fluid or other sources are emerging and of interest. Particular interest is expressed in next generation sequencing of pancreatic cystic fluid[38-41]. Investigators have reported *KRAS/GNAS* mutations to be present in 100% of IPMNs[39] and to be highly sensitive and specific (89% and 100%, respectively) for IPMNs and mucinous cystic lesions[39]. Furthermore, the addition of *TP53/PIK3CA/PTEN* evaluation provided an 88% sensitivity and 97% specificity for IPMNs with advanced neoplasia[39]. Further, evaluation of pancreatic juice mutation concentration by next generation sequencing may help distinguish high-grade from low-grade lesions, and mutant *TP53/SMAD4* concentrations could distinguish patients with malignancy (*e.g.*, invasive cancer or high-grade dysplasia) with a sensitivity and specificity of about 61% and 96%, respectively[42,43]. However, further validation is needed before these methods become integrated within the clinical setting.

In a large meta-analysis, a combination of cytology and immunohistochemical analysis of MUC1 and MUC2 in pancreatic juice samples identified malignant IPMNs with an area under the curve of 0.85 and sensitivity at 85% and specificity at 65%. In a test model, inclusion of cytologic analysis of pancreatic juice in the guideline algorithm significantly increased the specificity of detection of malignant IPMNs[44].

More advanced techniques including targeted mass spectrometry of peptides mucin-5AC, mucin 2 and prostate stem cell antigen could identify high-grade dysplasia/cancer with an accuracy of 96%. Thus, there are several ongoing studies and potential future tools for improved diagnostics and with potential to enhance surveillance algorithms in the future.

FUTURE PERSPECTIVES IN THE TUG OF WAR BETWEEN RESECTION AND OBSERVATION

The future strategy for IPMN treatment should be first and foremost to enhance the evidence that forms the basis of clinical guidelines currently used to guide cystic neoplasm management. Large-scale prospective registries of individuals undergoing cyst surveillance such as the PACYFIC-registry are required to accumulate unbiased data that will ultimately inform evidence-based guidelines. Furthermore, we must improve our understanding of the evolutionary biology underlying pancreatic cystic neoplasms, including IPMNs[9,46]. Improved understanding of the underlying biology will enhance our understanding of risk of malignant transformation beyond the stratification provided by traditional anatomical and radiological subsets of main duct and side branch IPMNs. In addition to the development of molecular assessment of biopsy specimens, the future role of non-invasive assessment techniques, including liquid biopsy and enhanced magnetic resonance imaging may be required in order to detect high-risk lesions early and simultaneously reduce the costs for life-time surveillance strategies. Alas, until this has been achieved the tug of war in management of IPMN lesions will continue.

CONCLUSION

IPMN of the pancreas is increasingly detected in the population, often as an incidental finding. Several guidelines have been proposed to provide criteria for observation or resection. The debate continues as to how to identify the most appropriate surgical candidate. Hence, the tug of war between surveillance and surgery continues until better evidence for decision making has been achieved.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1101-1114

DOI: 10.4251/wjgo.v11.i12.1101

ISSN 1948-5204 (online)

MINIREVIEW

Current status of the genetic susceptibility in attenuated adenomatous polyposis

Víctor Lorca, Pilar Garre

ORCID number: Víctor Lorca (0000-0002-3681-4319); Pilar Garre (0000-0001-8285-4138).

Author contributions: Lorca V generated the figures and wrote the manuscript; Garre P designed the aim of the review and wrote the manuscript.

Conflict-of-interest statement: Authors declare no potential conflicts of interest.

Manuscript source: Invited manuscript

Received: April 17, 2019
Peer-review started: April 18, 2019
First decision: July 31, 2019
Revised: August 18, 2019
Accepted: October 14, 2019
Article in press: October 14, 2019
Published online: December 15, 2019

P-Reviewer: Kadiyska T, Jiang L
S-Editor: Ma YJ

Víctor Lorca, Laboratorio de Oncología Molecular, Grupo de Investigación Clínica y Traslacional en Oncología, Hospital Clínico San Carlos, Madrid 28040, Spain

Pilar Garre, Laboratorio de Oncología Molecular, Servicio de Oncología, Hospital Clínico San Carlos, Madrid 28040, Spain

Corresponding author: Pilar Garre, PhD, Research Scientist, Laboratorio de Oncología Molecular, Servicio de Oncología, Hospital Clínico San Carlos, c/Profesor Martín Lagos s/n, Madrid 28040, Spain. pilar_garre@hotmail.com
Telephone: +34-913-303348
Fax: +34-913-302494

Abstract

Adenomatous polyposis (AP) is classified according to cumulative adenoma number in classical AP (CAP) and attenuated AP (AAP). Genetic susceptibility is the major risk factor in CAP due to mutations in the known high predisposition genes *APC* and *MUTYH*. However, the contribution of genetic susceptibility to AAP is lower and less understood. New predisposition genes have been recently proposed, and some of them have been validated, but their scarcity hinders accurate risk estimations and prevalence calculations. AAP is a heterogeneous condition in terms of severity, clinical features and heritability. Therefore, clinicians do not have strong discriminating criteria for the recommendation of the genetic study of known predisposition genes, and the detection rate is low. Elucidation and knowledge of new AAP high predisposition genes are of great importance to offer accurate genetic counseling to the patient and family members. This review aims to update the genetic knowledge of AAP, and to expound the difficulties involved in the genetic analysis of a highly heterogeneous condition such as AAP.

Key words: Attenuated adenomatous polyposis; Genetic susceptibility; High predisposition gene; Genetic heterogeneity; Colorectal cancer

Core tip: Attenuated adenomatous polyposis (AAP) is a highly genetically and clinically heterogeneous condition in terms of severity, clinical features, heritability, and genetics. The major high predisposition genes *APC* and *MUTYH* explain a small fraction of AAP (10%-20%). Several predisposition genes have been recently proposed, and some of

L-Editor: Filipodia
E-Editor: Ma YJ

them have been validated, but studies addressing their global contribution to AAP genetic predisposition is scarce. Clinicians do not have strong discriminating criteria for the recommendation of genetic testing, and the detection rate is low. Therefore, multigene panel testing and a redefinition of strong clinical criteria could improve the outcome of AAP genetic testing.

Citation: Lorca V, Garre P. Current status of the genetic susceptibility in attenuated adenomatous polyposis. *World J Gastrointest Oncol* 2019; 11(12): 1101-1114
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1101.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1101

INTRODUCTION

Adenomatous polyposis (AP) can be defined as the tendency to develop adenomatous polyps (adenomas) along the large intestine and/or rectum. Although adenomas are benign growths, they are considered the precursor lesions of colorectal carcinoma (CRC)[1]; thus, AP is classified as a cancer risk syndrome with cumulative risks ranging from 40% to 100% depending on the severity of the polyposis (adenoma burden).

AP is usually classified according to the adenoma burden in two major groups: classical AP (CAP) and attenuated AP (AAP). Classical forms are characterized by the detection of hundreds or thousands of adenomas, and have a very low prevalence in the population (1/10000[2]), whereas attenuated forms are defined by the detection of between 10-100 adenomas, and are more prevalent in the adult population. CAP shows aggressive phenotypes, usually triggered during the second decade of life, and with a cumulative absolute cancer risk if adenomas are not removed. Extracolonic manifestations are frequent, and most of the cases show a dominant inheritance pattern[3]. By contrast, AAP is a much more heterogeneous group in terms of polyposis severity and family history[4-6]. Clinical features are distinctive from classical forms; adenoma detection is low or mild, ranging from ten synchronic or 20 cumulative to 100 adenomas, and the polyposis diagnosis age is significantly later than CAP. Cancer risk is also lower and later, ranging from 40% to 80% depending on the adenoma burden. Extracolonic manifestations are uncommon, and a family history of polyposis is frequently absent. AAP is sometimes accompanied by other types of polyps, such as hyperplastic or serrated polyps[3,7].

There are currently two clearly clinically-actionable genes that can lead to AP: *APC* (MIM#611731) and *MUTYH* (MIM#604933). Thus, prevalence and cancer risk estimations are well-defined, allowing accurate genetic counseling and effective high-risk monitoring programs for carriers. Heterozygous germline truncating mutations in the tumor suppressor gene *APC* mainly give rise to CAP, and sometimes to AAP, with dominant inheritance patterns. In contrast, germline biallelic mutations in the DNA repair gene *MUTYH* mainly lead to AAP and less frequently to CAP, with recessive inheritance patterns. In these cases, identification of *APC* or *MUTYH* carriers is important, not only to define the risks and follow-up strategies for the patient, but also to discriminate between high- and low-risk individuals among the family members who could benefit from high-risk follow-up or, on the contrary, avoid unnecessary and invasive monitoring. Ambiguously, even though both genes explain the vast majority of CAP, together they are only able to explain between 10%-20% of AAP.

AAP incidence is significantly increasing in hospital settings, mainly due to the improvement of imaging techniques and the implementation of CRC population screening programs. This increase translates into a problem in the Genetic Counseling Units due to the high heterogeneity of the disease. On the one hand, it is difficult to discriminate not only between sporadic multiple polyposis and real AP in patients with low adenoma burden, but also between attenuated and classical forms in patients with adenoma counts close to 100. On the other hand, family history is not a discriminant criterion for genetic studies due to the high rate of *de novo* mutations described in *APC* (10%-25%)[8,9] and the recessive inheritance pattern of *MUTYH*[10]. Furthermore, only a minority of AAP cases (< 20%) is explained by germline mutations in *APC* or *MUTYH*[11,12], leaving a substantial fraction of AAP cases unexplained. This means that undiscriminating and invasive follow-up programs will be recommended to all first-degree relatives of these patients.

Under this scenario, the elucidation of genetic susceptibility, which could explain the etiology of the disease and improve the accuracy of genetic counseling, has become a priority for scientists and clinicians. Thanks to the advance of sequencing technologies, new genes have been recently associated with primary predisposition to the development of adenomas by genome/exome sequencing studies in unexplained AP cohorts[13-16]. In the same way, other genetic alterations not detected by conventional coding germline DNA sequencing screening strategies have also been described in the *APC* gene, such as mutations in the promoter[17] or introns[18], large inversions[19] or mosaicism phenomes[20]. In addition, the use of wide gene panels for the genetic diagnosis of AP has incidentally revealed an overlap between different polyposis syndromes[21]. However, all these studies together are only able to explain the etiology of a very small fraction of AAP cases, and the unexplained cases are still a major group that needs to be clarified. Most likely, polygenic inheritance models in which the accumulation of multiple low penetrance alleles[22] and lifestyle risk factors such as smoking, alcohol, body mass index, diet and physical activity[23] play a major role in unexplained AAP cases.

Despite the low frequency of high predisposition genes in AAP, their knowledge is important for the detection of carriers, allowing the discrimination of high- and normal-risk individuals among family members, and leading to accurate and cost-effective monitoring programs.

The aim of this review is to describe the current knowledge of the genetic susceptibility of AAP, with emphasis on genes with a primary predisposition to AP that have been described so far, which are either already implemented in clinical practice, in process, or have recently been proposed.

AP PRIMARY PREDISPOSITION ASSOCIATED GENES

Until recently, *APC* and *MUTYH* were the only known AP syndrome predisposition genes. With the advent of next-generation sequencing (NGS) technologies, new AP predisposition genes have emerged. There are currently three new validated genes [*POLE* (MIM#174762), *POLD1* (MIM#174761), *NTHL1* (MIM#602656)], and two more genes that have been described but not validated [*MSH3* (MIM#600887), *MLH3* (MIM#604395)]. The discovery of new AP predisposition genes has allowed for considerable advancement in the biology of AP development and, therefore, in colorectal carcinogenesis. However, the newly described genes are still poorly implemented in clinical practice, mainly because of their low frequency and the lack of accurate risk estimations. Thus, time is needed to increase the number of described cases that allow better prevalence and risk estimations to be obtained.

APC

APC is a tumor suppressor gene closely involved in colorectal carcinogenesis; *APC* somatic mutations are the first event in the canonical CRC carcinogenesis model, which is followed by more than 80% of all CRCs[24]. The *APC* gene encodes a multifunctional protein that is mainly involved in signal transduction, cell adhesion and migration, microtubule assembly and chromosome segregation[25]. Its tumor-suppressing ability relies on its capacity to negatively regulate intracellular β-catenin levels, the main effector of the Wnt pathway. Therefore, inactivation of APC leads to increased β-catenin levels and overexpression of its different target genes involved in cell proliferation, differentiation, migration and apoptosis[26], which histologically correlates with adenoma formation.

APC is located on the long arm of chromosome 5 (5q21), has 15 exons, and encodes a 2,843 amino acid protein[27]. Most of the somatic mutations lie in the mutation cluster region (MCR), which is located between amino acids 1286 and 1513 and overlaps with the β-catenin binding region[28]. Heterozygous *APC* germline mutations have been associated with AP predisposition in a gene location-dependent manner[29]. Most of the germline *APC* mutations are truncating variants lying between codons 178 and 1580, and give rise to stable mutant peptides that exert a dominant-negative effect on the wild-type protein[30,31]. These mutations lead to classical forms of the disease called familial AP (FAP), whereas germline mutations located at both the 5′ and 3′ ends of the transcript, as well as splicing mutations that lead to exon 9 skipping, give rise to attenuated forms of the disease called attenuated familial AP (AFAP) (Figure 1A). Germline mutations at the 3′ end give rise to stable proteins with a certain capability to regulate β-catenin levels[30], and 5′ end mutations upstream of codon 177 produce functional proteins by initiation of translation at codon 184[31,32]. This internal initiation of translation is relatively inefficient, leading to a haploinsufficient phenotype rather than a dominant-negative phenotype. Mutations at the splice donor site in intron 9

lead to inefficient exon skipping with some expression of normal transcript, and therefore with an attenuated form of the disease[33].

Both FAP and AFAP show autosomal dominant inheritance patterns. However, there are some exceptions without any family history. *De novo* mutations have been described in 10%-25% of *APC* carriers[8,9], and recent studies report *APC* mosaicism rates of 20%-50% in unexplained AP cases[20,34]. Whereas *de novo* mutations have been observed in both FAP and AFAP, it is noteworthy that mosaicism carriers present an attenuated form of the disease[20], likely due to the nonubiquitous distribution of the mutant allele.

MUTYH

MUTYH is a DNA repair gene involved in the base excision repair (BER) pathway[35]. It encodes a monofunctional DNA glycosylase responsible for the recognition and excision of the deoxyadenosine misincorporated with 8-hydroxy-2' -deoxyguanosine (8-OHdG) in the DNA molecule. 8-OHdG arises as a consequence of the oxidation of deoxyguanosine, which is a mutagenic base because it has the ability to pair indiscriminately with deoxycytosine or deoxyadenosine, leading to an increase in somatic G>T transversions[36]. Therefore, inactivation of *MUTYH* leads to an increase in the G>T mutation rate, which especially affects known cancer driver genes such as *KRAS* or *APC*[37], both of which are involved in adenoma formation.

MUTYH is located on the short arm of chromosome 1 (1p34.1) and is formed by 6 exons, encoding two major transcripts, which leads to 546 and 535 amino acid isoforms[35]. Biallelic *MUTYH* germline mutations have been associated with AP predisposition, leading to an autosomal recessive syndrome[10]. Because it is a recessive condition, there is no vertical transmission of the disease, and family history is often absent or is presented horizontally (siblings)[38]. *MUTYH*-associated polyposis (MAP) is characterized by the presence of 10–100 adenomatous polyps in the colon rectum resembling AFAP, but in some cases it may be accompanied by hyperplastic or serrated polyps[39]. A minor fraction of MAP presents classical forms of the disease with the detection of more than 100 adenomas. In contrast to *APC*, no relationship has been observed between the location of the mutation and the phenotype of the disease. Mutations located throughout the entire *MUTYH* have been described in MAP, but only two missense mutations, NM_001128425: c.1187G>A p.(Gly396Asp) and c.536A>G p.(Tyr179Cys), are the most prevalent in Caucasians. Other recurrent mutations have been described in more specific populations[40] (Figure 1B).

POLE and POLD1

POLE and *POLD1* encode the catalytic subunits of the polymerase enzyme complexes ε (Polε) and δ (Polδ), respectively, which are the principal leading- and lagging-strand DNA polymerases during S phase[41]. In addition, they also catalyze DNA synthesis in several DNA repair pathways, such as nucleotide excision repair (NER) or mismatch repair (MMR). Both POLE and POLD1 encompass not only a binding DNA region and polymerase domain, but also an exonuclease domain, which confers proofreading capability by the recognition and removal of misincorporated nucleotides during DNA replication[42]. Polymerase proofreading activity, together with high base selectivity and the MMR pathway, are the main cellular mechanisms responsible for minimizing errors during DNA replication[43]. Inactivating point mutations within the exonuclease domains lead to proteins with an active polymerase domain that lack proofreading activity, which causes high genetic instability during DNA replication. Indeed, somatic mutations within the exonuclease domains have been described in human cancer, leading to a high increase in mutational rates[44]. Tumor mutations in the *POLE* exonuclease domain have been identified in 1%-2% of sporadic CRC and in 7%-12% of endometrial cancers, as well as in tumors of the brain, pancreas, ovary, breast and stomach, showing ultramutated and microsatellite-stable tumors[45].

POLE is located on the long arm of chromosome 12 (12q24.33), consists of 49 exons, and encodes a 2,286 amino acid protein. Its exonuclease domain lies between codons 268 and 471[46]. *POLD1* is located on the long arm of chromosome 19 (19q13.33) and consists of 27 exons, encoding an 1,133 amino acid protein. Its exonuclease domain is located between codons 304 and 517[47]. Heterozygous germline mutations within the exonuclease (proofreading) domains of both *POLE* and *POLD1* were recently associated with AAP[13], leading to an autosomal dominant inheritance condition that is characterized by high-penetrance predisposition to multiple colorectal adenomas, large adenomas, early-onset CRC, or multiple CRCs, as well as other extracolonic tumors such as endometrial tumors[48].

Since the first association of *POLE* and *POLD1* with AAP, several studies have validated the results and found new germline mutations in the exonuclease domains[49-55] (Figures 1C and 1D). However, due to the small number of families described so far, accurate risk estimations and the contribution of polymerases to AP

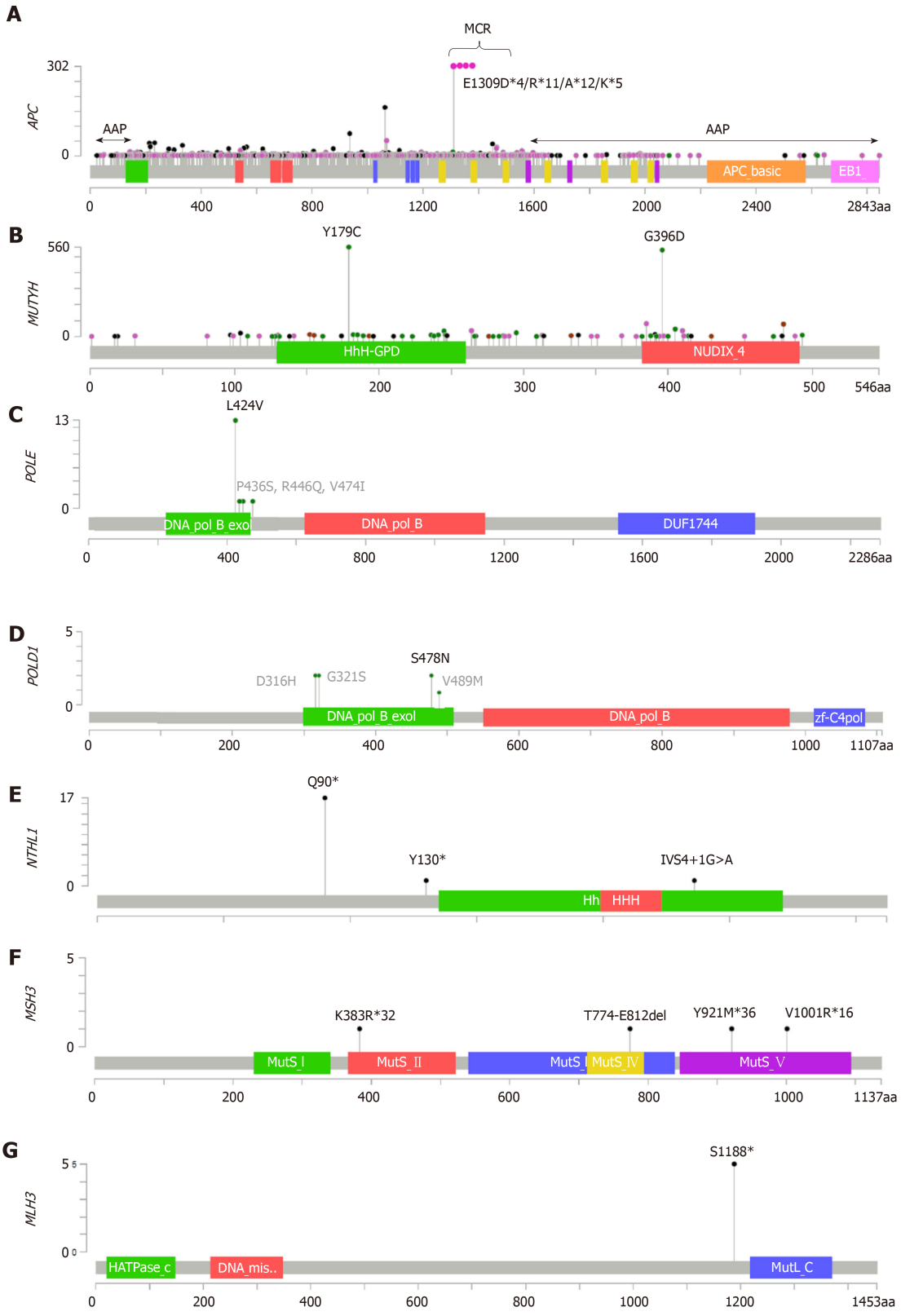

Figure 1 Distribution of germline mutations in attenuated adenomatous polyposis predisposition genes across protein domains. A: *APC* likely pathogenic and pathogenic variants described in the LOVD database[115]. Most of the mutations are truncating variants. Mutations associated with AAP are located at both the 3'-end and 5'-end of the gene (indicated with arrows); B: *MUTYH* likely pathogenic and pathogenic mutations described in the LOVD database[115]. The two most prevalent mutations in Caucasians are shown; C-G: *POLE*, *POLD1*, *NTHL1*, *MSH3* and *MLH3* likely pathogenic and pathogenic mutations described in the literature and associated with AAP. Unclassified variants in the polymerase proofreading POLE and POLD1 domains are in gray. All lollipops were designed with The cBio Cancer Genomics Portal[116,117]. Mutation types are coded as follows: black dots for nonsense variants; pink dots for frameshift and splicing variants; green dots for missense mutations; brown dots for in-frame indels. Reference sequences: *APC*: NM_000038, NP_000029; *MUTYH*: NM_001128425, NP_036354; *POLE*: NM_006231, NP_006222; *POLD1*: NM_001256849, NP_001121897; *NTHL1*: NM_002528, NP_002519; *MSH3*: NM_002439, NP_002430; *MLH3*: NM_001040108, NP_001035197.

are still not well-defined.

NTHL1

Similar to *MUTYH*, *NTHL1* is a DNA repair gene involved in the BER pathway. It encodes a bifunctional N-glycosylase protein that recognizes and removes oxidized pyrimidines, such as 2'-deoxy-5-hydroxycytldine (5-OHdC) and ring-opened purines[56]. 5-OHdC arises as a consequence of the oxidation of deoxycytosine, and it has the ability to pair both deoxyguanosine and deoxyadenine, leading to an accumulation of somatic C>T transitions, which can affect important CRC driver genes such as *APC*, *TP53* or *KRAS*, among others.

NTHL1 is located on the short arm of chromosome 16 (16p13.3), consists of 6 exons, and encodes a 312 amino acid protein[57]. *NTHL1* homozygous or compound heterozygous germline mutations have been recently detected in AAP, delineating an autosomal recessive polyposis syndrome called *NTHL1*-associated polyposis (NAP)[14]. All *NTHL1* biallelic carriers described so far showed AAP, and also frequently showed other extracolonic tumors such as endometrial or breast[58]. One nonsense mutation at codon 90 seems to be involved in nearly all the biallelic carriers described; however, novel pathogenic mutations are arising as new studies emerge[58-62] (Figure 1E).

Theoretical estimations of NAP suggest a prevalence of at least five times lower than that of MAP[62]. Due to the limited number of NAP families described until now, the phenotypic spectrum and cancer risk estimates have not been properly established.

MSH3

MSH3 is one of the six *MMR* genes identified to date in eukaryotic cells[63]. It is involved in the detection of replication errors in microsatellite sequences together with *MSH2* and *MSH6*. *MSH3* encodes an alternative binding partner for MSH2, which is required for the specific detection of insertion or deletion loops of two or more nucleotides[64], as well as for double strand break repair[65]. MSH2 requires the binding of MSH6 or MSH3 to exercise its function. The MSH2-MSH6 dimer recognizes single substitutions and small indel mispairs, whereas MSH2-MSH3 recognizes errors in di- and larger nucleotide repeats[66]. Inactivation of *MSH3* leads to a high microsatellite instability of di- and tetranucleotides (EMAST), which has been associated with a characteristic somatic *APC* mutation spectrum in colorectal adenoma from AAP patients[15].

The *MSH3* gene is located in the long arm of chromosome 5 (5q14.1) and consists of 24 exons, encoding an 1,137 amino acid protein[67]. Biallelic truncating variants in *MSH3* have been recently reported in two patients with AAP, suggesting an additional recessive subtype of colorectal AP[15] (Figure 1F).

Until now, no more studies have validated these results, so its association with AAP and phenotype estimations remain to be defined.

MLH3

MLH3 is a member of the MutL homolog family of MMR proteins[63]. MLH3 dimerizes with MLH1, resulting in the MutLγ complex, which is primarily involved in meiotic recombination rather than in mitotic genetic stability[68].

The *MLH3* gene is located in the long arm of chromosome 14 (14q24.3), consists of 13 exons, and encodes a 1,453 amino acid protein. The homozygous truncating germline variant S1188* was first detected in an unexplained Swedish AAP case[21], and more recently in one more AAP and two CAP subjects from Finland, suggesting a founder effect[16] (Figure 1G). Authors hypothesize the involvement of a defective DNA damage response and/or recombination-related processes in the pathogenesis of these cases[16].

Once again, research on additional cohorts is needed to reinforce the significance of *MLH3* as an AP predisposition gene.

OTHER CANDIDATE GENES SUGGESTED FOR AP PREDISPOSITION

Other candidate genes, including AXIN2 (MIM#604025), *FOCAD* (MIM#614606), *GALNT12* (MIM#610290) and *BUB1* (MIM#602452) /*BUB3* (*MIM*#603719), are involved in the AP predisposition. However, evidence for these genes is not as thorough as those previously discussed.

AXIN2

AXIN2 encodes the Wnt pathway component conductin; it is the scaffold protein of the β-catenin destruction complex and main negative regulator of the pathway[69]. Mutations in this gene have been described in CRC, and similar to *APC*, they increase β-catenin levels and activate β-catenin/T-cell factor signaling, thus promoting CRC development[70]. A*XIN2* is located on the long arm of chromosome 17 (17q24.1), consists of 11 exons, and encodes two major transcripts, which leads to 843 and 778 amino acid isoforms[69]. Deleterious germline mutations have been reported in four families, showing a strong association with oligodontia as well as gastrointestinal neoplasias[71-73]. More recently, a novel missense variant has been described in an AAP family without signs of oligodontia or ectodermal dysplasia, suggesting the possibility of different phenotypes depending on the protein domain affected[74]. Two other works have screened mutations for *AXIN2* in different CRC populations, both in polyposis and nonpolyposis, without any success[75,76]. Therefore, although there is a clear association between *AXIN2* and oligodontia, further studies are needed to clarify its role in CRC syndromes, particularly with AAP.

FOCAD

FOCAD encodes a focal adhesion protein with a potential tumor suppressor function in glyomas[77]. The *FOCAD* gene is located on the short arm of chromosome 9 (9p21.3) and is formed by 46 exons encoding an 1,801 amino acid protein[77]. Two studies identified large deletions and truncating point mutations in a total of five CRC cases: 2/221 cases of unexplained AP[78] and 3/1232 early-onset and familial CRC cases[79]. Altogether, four cases had a diagnosis of AAP. Since *FOCAD* shows high expression levels in colonic epithelial cells and has been involved in cell survival and proliferation, the authors suggest a potential role of this gene in polyposis/CRC susceptibility[79]. Regardless, this association and its contribution to AAP predisposition requires further clarification.

GALNT12

GALNT12 encodes a hexosyltransferase involved in the initial steps of the mucin-type O-glycosylation process[80]. Alterations in this process lead to aberrant glycosylation, which has been associated with alterations in cell growth, differentiation, transformation, adhesion, metastasis and immune surveillance in cancers[81]. *GALNT12* is highly expressed in the digestive tract, and is frequently downregulated in CRC[82]. The *GALNT12* gene is located on the long arm of chromosome 9 (9q22.33), has 10 exons, and encodes a 581 amino acid protein[80]. Evidence for the association between *GALNT12* and CRC has been reported[83], but its association with familial CRC, particularly AP, remains a controversial issue. Partially inactivating variants have been detected in familial CRC along with a mild polyp burden, suggesting the involvement of this gene in CRC predisposition[84]. However, later studies do not support its involvement in nonpolyposis and polyposis CRC predisposition[85,86].

BUB1 and *BUB3 (mitotic checkpoint serine/threonine kinases)*

BUB1 and *BUB3* encode components of the spindle assembly checkpoint complex, which controls chromosome biorientation on the mitotic spindle, delaying the anaphase transition until all kinetochores are properly attached[87]. Alterations in the activity of this complex lead to alterations in chromosome copy number, *i.e*. aneuploidies[88]. The *BUB1* gene is located on the long arm of chromosome 2 (2q13), consists of 25 exons, and encodes a 1,085 amino acid protein, whereas *BUB3* is located on the long arm of chromosome 10 (10q26.13), has 8 exons, and encodes a 328 amino acid protein[89]. Deleterious germline mutations in both genes have been associated not only with increased levels of constitutive aneuploidy, but also with gastrointestinal neoplasms, including adenocarcinomas and adenomas[90,91]. Furthermore, aneuploidy caused by Bub1 insufficiency has been proven to drive colorectal adenoma formation in mice through *APC* loss of heterozygosity (LOH)[92]. Screening of the *BUB1* and *BUB3* genes in familial and AP CRC cohorts has shown functionally relevant germline mutations in a low fraction of patients with CRC who also presented increased levels of constitutive aneuploidy[93,94]. However, the causality of these mutations in CRC/adenoma susceptibility remains unproven.

AAP INCIDENTAL TO OTHER CANCER RISK SYNDROMES

Although phenotypes for related CRC risk syndromes are generally well-defined, there are some overlapping features that can lead to confusion in the clinical suspicion and subsequent misdirection of the genetic testing approach. The cancer risk syndromes prone to phenotypically overlap with AAP are described below.

Lynch syndrome is the main hereditary nonpolyposis colorectal cancer syndrome

caused by heterozygous deleterious mutations in MMR genes (*MSH2*, *MLH1*, *MSH6* and *PMS2*) that can be accompanied by early-onset adenomas[95]. Usually, the adenoma burden does not exceed 10, but it can sometimes mimic AAP.

Constitutional MMR deficiency is due to loss-of-function biallelic germline mutations in the main *MMR* genes. It is an aggressive recessive cancer predisposition syndrome with a wide tumor spectrum, very early age of onset and poor outcome[96]. In addition, nearly 36% of affected subjects develop colorectal AP ranging from a few up to 100 adenomas[97].

Hereditary mixed polyposis syndrome is characterized by multiple colon polyps of mixed pathologic subtypes and an increased risk for CRC[98]. It is caused by large duplications in the 5' regulatory region of *GREM1* (MIM 603054), leading to an excess of coding protein expression[99]. GREM1 is an antagonist of bone morphogenic protein (BMP), so its overexpression can lead to inactivation of the BMP pathway and subsequent hyperproliferation of colonic epithelium[100].

The pathogenesis of polyps in hereditary mixed polyposis syndrome likely overlaps with that of juvenile polyposis syndrome (JPS), which is caused by inactivating mutations in other genes of the BMP pathway, including *BMPR1A* (MIM 601299), *SMAD4* (MIM 600993), *ENG* (MIM 131195) and *BMP4* (MIM 112262)[101-104]. JPS is a hamartomatous polyposis syndrome with an increased risk of CRC as well as other digestive cancers. Cancer risk arises from adenomatous components present in the juvenile polyps, which can sometimes lead to misinterpretations[105].

Germline alterations in genes involved in the PTEN/PI3K/AKT pathway are also associated with hamartomatous polyposis syndromes. Cowden syndrome is caused by heterozygous *PTEN* (MIM 601728) germline mutations, and is characterized by the development of hamartomatous and neoplastic lesions of the skin, mucous membranes, thyroid, breast, endometrium, and brain[106]. Although hamartomatous polyps are the most characteristic gastrointestinal lesions in Cowden syndrome, adenomatous polyps in the colon have been detected in 30% of affected individuals[107].

In contrast, germline heterozygous mutations in *STK11* (MIM 602216) lead to Peutz-Jeghers syndrome (PJS), which is characterized by mucocutaneous pigmentation and diffuse gastrointestinal hamartomas[108]. Similar to other hamartomatous syndromes, polyps with large adenomatous transformation areas and adenomatous polyps have been described in PJS[109].

Currently, thanks to NGS technology and the widespread use of multigene panels for hereditary cancer testing, the detection of overlapping phenotypes between different CRC syndromes is greatly increasing, improving the diagnosis and follow-up of these patients[12,21,110].

CONCLUSION

AAP is a highly heterogeneous disease, covering both moderate and mild forms of AP, as well as hereditary and sporadic forms, recessive and dominant conditions, and the presence or absence of other gastrointestinal or extracolonic manifestations. Thus, the genetic heterogeneity of the syndrome, where several high predisposition genes are involved in the polyposis predisposition of a minor subset of AAP, is not surprising. Two previous studies have investigated the prevalence of pathogenic mutations in large cohorts of AP, detecting approximately 6%-15% of pathogenic mutations in either the *APC* or *MUTYH* genes when analyzing patients with an adenoma burden between 10 and 99[11,12]. These detection rates were decreased (2%-9%, respectively) when only patients between 10 and 19 adenomas were considered, showing that adenoma burden and the likelihood of detecting pathogenic mutations in *APC* and *MUTYH* are directly proportional in AAP. Regarding the prevalence of the new AP predisposition genes, Stanich and collaborators included the analysis of *POLE* and *POLD1* in their cohorts, but the contribution of these genes was scarce (one detection in 2,979 AAP cases), and it did not alter the overall mutation detection rate[12]. The *NTHL1* contribution to AAP has been recently estimated to be five times less prevalent than that of *MUTYH*[62]. Therefore, it seems that the heritability of AAP lies in different predisposition genes, each of which explains a small fraction of the total. Recently, other newly associated genes have been described, but the contribution of genetics to the etiology of the disease, as well as its heritability, are difficult to estimate. The high clinical and genetic heterogeneity, as well as the low prevalence of pathogenic mutations in the described genes, reflects the necessity of multigene panel testing for the effective genetic diagnosis of AAP.

To increase diagnostic sensitivity in such a heterogeneous syndrome, clinical guidelines have been developed with broad criteria, recommending genetic testing in

patients with more than 10 adenomas, even in those patients with oligopolyposis (< 10 adenomas) or early CRC[111-113]. These criteria increase the genetic testing requests in diagnostic laboratories, thus decreasing the mutation detection rate, which makes genetic studies not cost-effective, even if they are performed by multigene panel testing. Furthermore, most of the genetic testing results are not informative, and the probability of unclassified variant detection with multigene panel testing is high, which leads to a major group of patients with anxiety and confusion. Therefore, more stringent clinical criteria, especially in the cumulative number of adenomas, should be redefined to ascertain those patients who are most likely to harbor a hereditary polyposis syndrome. The stricter the recommendation criteria for the genetic study is, the greater the mutation detection rate and lower the ambiguous results. We are in agreement with the last guideline of the American Society of Colon and Rectal Surgeons (ASCRS) that a cutoff of 20 cumulative adenomas should be used to prompt genetic counseling and testing[114].

In conclusion, the contribution of genetics to the etiology of the disease and its heritability are difficult to estimate. The high clinical and genetic heterogeneity, as well as the low prevalence of each AP predisposing gene, reflects the necessity of multigene panel testing for an effective diagnosis of AAP. Nevertheless, the decline in diagnosis rates that comes with the decrease in adenoma burden shows the necessity of stricter clinical criteria when genetic testing is recommended for AAP predisposition genes.

ACKNOWLEDGEMENTS

The authors wish to thank Dra. Trinidad Caldés and Dra. Vanesa Barberán for their critical revision.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1115-1125

DOI: 10.4251/wjgo.v11.i12.1115

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

Improved method for inducing chronic atrophic gastritis in mice

Xian Wei, Xue-Ping Feng, Lu-Yao Wang, Yan-Qiang Huang, Ling-Ling Liang, Xiao-Qiang Mo, Hong-Yu Wei

ORCID number: Xian Wei (0000-0003-0421-114X); Xue-Ping Feng (0000-0003-0725-1067); Lu-Yao Wang (0000-0001-5545-0170); Yan-Qiang Huang (0000-0003-1093-9110); Lin-Ling Liang (0000-0002-1498-2370); Xiao-Qiang Mo (0000-0002-6706-336X); Hong-Yu Wei (0000-0003-1481-741X).

Author contributions: Wei X and Feng XP performed the research and contributed equally to this work; Wang LY, Liang LL, Mo XQ, and Wei HY analyzed the data; Huang YQ designed the research and analyzed the data; all authors drafted the article, made critical revisions related to the intellectual content of the manuscript, and approved the final version of the article to be published.

Supported by National Natural Science Foundation of China, No. 31460023; Science Research and Technology Development Project of Guangxi, No. 1598025-33.

Institutional review board statement: All specimens from the patients were obtained after informed consent and ethical permission were obtained for participation in the study.

Conflict-of-interest statement: The authors report no relevant conflicts of interest.

Xian Wei, Xue-Ping Feng, Lu-Yao Wang, Yan-Qiang Huang, Ling-Ling Liang, Xiao-Qiang Mo, Hong-Yu Wei, Breeding Base of Microbial Infection Key Laboratory in the West Guangxi, Youjiang Medical University for Nationalities, Baise 533000, Guangxi Zhuang Autonomous Region, China.

Corresponding author: Yan-Qiang Huang, MD, PhD, Professor, Breeding Base of Microbial Infection Key Laboratory in the West Guangxi, Youjiang Medical University for Nationalities, No. 98, Countryside Road, Baise 533000, Guangxi Zhuang Autonomous Region, China.
hyq77615@163.com
Telephone: +86-776-2849490
Fax: +86-776-2853272

Abstract

BACKGROUND

Chronic atrophic gastritis (CAG) is a common disease of the digestive system with pathological characteristics of a decreasing number, or disappearance, of inherent glands of the gastric mucosa. CAG has been defined as a precancerous condition of gastric cancer. Intestinal metaplasia or intraepithelial neoplasia accompanying atrophied glands of the stomach is regarded as one of the most important precancerous lesions of gastric cancer. As a common malignant tumour, gastric cancer remains without a satisfactory therapy and its pathogenesis remains unclear, seriously threatening human life. Therefore, some scholars have proposed to prevent the incidence of gastric cancer by avoiding precancerous lesions. If CAG can be reversed, the incidence of gastric cancer can be substantially reduced. To reverse and prevent CAG and study its pathogenesis and therapy, it is necessary to develop an ideal, safe, stable, animal model.

AIM

To study a rapid, stable, and safe method of establishing a mouse model of human CAG.

METHODS

Six-week-old Kunming mice were divided into a phosphate buffered solution control group, a *Helicobacter pylori* (*H. pylori*) group, an N-methyl-N'-nitroguanidine (MNNG) group, an ammonia water group, and a group combining *H. pylori*, MNNG, and ammonia water (hereinafter referred to as the combined group). The mice were administrated with drinking water containing ammonia or infected with *H. pylori* through gavage. At the 30th, 60th, 90th, and 120th day after the last *H. pylori* infection, mice were selected randomly to collect their gastric mucosa for hematoxylin eosin staining, terminal nick-end labelling staining detection, and immunohistochemical staining for Bax and Bcl-2. In

Manuscript source: Unsolicited manuscript

Received: May 16, 2019
Peer-review started: May 21, 2019
First decision: July 31, 2019
Revised: August 5, 2019
Accepted: October 1, 2019
Article in press: October 1, 2019
Published online: December 15, 2019

P-Reviewer: Kay TM, Lin JK, Wittkopf N
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Qi LL

addition, *H. pylori* was isolated, cultured, and identified, and its extent of colonisation calculated. Blood was collected to detect inflammatory factors interleukin (IL)-1β, IL-8, and tumor necrosis factor (TNF)-α and immune function markers CD4 and CD8 to confirm successful establishment of the CAG model.

RESULTS
The combined group showed slight CAG at the 90th day and moderate CAG at the 120th day, while other groups did not show CAG at that time.

CONCLUSION
The combination of *H. pylori*, MNNG, and ammonia is an effective method of developing a mouse model of human CAG.

Key words: Method; Chronic atrophic gastritis; Mice; *Helicobacter pylori*; N-methyl-N'-nitroguanidine; Ammonia water

Core tip: A chronic atrophic gastritis (CAG) model was successfully established at the 90th day after the last *Helicobacter pylori* (*H. pylori*) infection of six-week-old mice, with a success rate reaching 90%. The method overcomes the difficulty in colonising *H. pylori* in mice and the fact that the colonisation time is short. In addition, rats are replaced with mice, and the method is thus simpler and also cheaper. The established models are stable and safe, therefore, the method is a relatively ideal method for establishing a mouse model of CAG. It provides a significant help when exploring the mechanisms of occurrence and prevention CAG.

Citation: Wei X, Feng XP, Wang LY, Huang YQ, Liang LL, Mo XQ, Wei HY. Improved method for inducing chronic atrophic gastritis in mice. *World J Gastrointest Oncol* 2019; 11(12): 1115-1125
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1115.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1115

INTRODUCTION

Chronic atrophic gastritis (CAG) is a common disease of the digestive system with pathological characteristics of a decreasing number, or disappearance, of inherent glands of gastric mucosa[1,2]. As early as 1978, the World Health Organization had announced a close relationship between CAG and the incidence of gastric cancer[3]. CAG has been defined as a precancerous condition of gastric cancer. Intestinal metaplasia or intraepithelial neoplasia accompanying atrophied glands of the stomach is regarded as one of the most important precancerous lesions of gastric cancer. As a common malignant tumour, gastric cancer remains without a satisfactory therapy and its pathogenesis remains unclear, seriously threatening human life[4,5]. Therefore, some scholars have proposed to prevent the incidence of gastric cancer by avoiding precancerous lesions. If CAG can be reversed, the incidence of gastric cancer can be substantially reduced. To reverse and prevent CAG and study its pathogenesis and therapy, it is necessary to develop an ideal, safe, stable, animal model. An ideal animal model of CAG needs to meet the following conditions: (1) The model develops disease to resemble the same condition in humans; (2) Animals are abundant and easily raised; and (3) The model is simple and provides a high success rate, and readily guarantees survival of the animals. In recent years, scholars mainly established animal models by imitating human CAG etiology. Commonly used animals include rats and Mongolian gerbils. Methods based on live *Helicobacter pylori* (*H. pylori*), N-methyl-N'-nitroguanidine (MNNG), ammonia, sodium deoxycholate, sodium salicylate, and immunology are commonly used to establish animal models[6-8]. In addition to this, the animal model can also be developed through combination of diseases and syndromes according to traditional Chinese medicine (TCM)[9]. Regardless of what method is used, the results differ to some extent from the ideal animal model and certain deficiencies therein require to be overcome. It is a challenge to build a CAG model with mice. For example, even if the methods of

chemical mutagens and stimulation of the gastric mucosa with a combination of factors are used for establishing animal models with a high success rate, they retain certain shortcomings. These include various problems, such as the selection of experimental animals, drug combination, drug dosages, and lack of a unified standard for the duration of the modelling. Besides, spontaneous animal models are seldom studied. TCM diagnoses and treats CAG mainly based on an overall analysis of patient condition, rather than the method applied by Western medicine that treats CAG in a general way according to the classification of diseases. Therefore, it is a preferable research approach to establish experimental animals combining diseases and syndromes using TCM to carry out TCM basic and clinical research. Even so, there are also some drawbacks. For instance, there is a lack of a recognised method of developing models and objective evaluation indices thereof. Therefore, it is necessary to improve the animal model of CAG, and explore an ideal method of developing mouse models of CAG. *H. pylori* is the primary pathogenic factor of CAG; the MNNG method is an acknowledged method of making mouse models of CAG; and ammonia simulates the injury of alkaline environment to the gastric mucosa, so it is also a clinically common method for establishing animal models based on etiology. Considering this, this study proposed an improved comprehensive method for establishing an animal model by combining *H. pylori*, MNNG, and ammonia. To our knowledge, the combined modelling method has not yet been reported.

MATERIALS AND METHODS

Animals

Female six-week-old Kunming mice, weighing between 180 and 200g, were purchased from the Experimental Animal Center, Youjiang Medical University for Nationalities. The animals were housed at controlled humidity (50% ± 5%) and temperature (25±2 °C) with a light-dark cycle of 12h. Animal treatment was in accordance with National Institute of Health guidelines and experimental protocols were approved by the Institutional Animal Care and Use Committee of Youjiang Medical University for Nationalities.

Materials

The study involved *H. pylori* strains (Affiliated Hospital of Youjiang Medical University for Nationalities), MNNG (BIDE PHARMATECH Co., ltd), ammonia water (Sinopharm Chemical Reagent Co., Ltd.), IL-1β, IL-8, TNF-α, CD4, CD8, CagA, Bax, a Bcl-2 kit (Beijing Solarbio Science and Technology Co., ltd.), and a DNA kit (TIANGEN, BIOTECH Co., ltd.). All reagents were of analytical grade.

Animal groups

A total of 220 six-week-old Kunming mice were randomly divided into a phosphate buffered solution control group, an *H. pylori* group, an MNNG group, an ammonia water group, and a group combining *H. pylori*, MNNG, and ammonia water (hereinafter referred to as the combined group). Each group contained equal numbers of male and female mice.

Mutagenesis and injury by MNNG

Mice in the MNNG and combined groups were administrated with MNNG (5 mL/kg body mass) at a concentration of 120 μg/mL through daily gavage for seven successive days.

Inducing injury and neutralising gastric acid by ammonia water

Mice were fed drinking water containing 0.02 wt% ammonia throughout. Mice in the control group were fed regularly without intervention.

H. pylori infection

Clinically isolated *H. pylori* strains were used, and they were subjected to Gram staining, urease testing, oxidase testing, and catalase testing, and identified as *H. pylori* through 16S ribosomal RNA sequencing. Strains were CagA and VagA positive and were proliferated and cultured for three days. The concentration of biosynthetic human insulin (BHI) medium was adjusted to 1×10^9 CFU/mL. *H. pylori* was applied to the mice through gavage after intragastric administration of MNNG. The mice were fasted for 12 h before gavage. The mice in the *H. pylori* and combined groups were intragastrically administrated with 5×10^8 CFU of *H. pylori*. Thereafter, the mice were fasted for solids and liquids for 2 h. The administration was conducted every other day, five times in succession. In comparison, 0.5 mL of BHI medium was applied to each mouse in the control group through gavage (also five times).

Confirming modelling results

Five male and five female mice were chosen from each group on the 30th, 60th, 90th, and 120th days. Then, gastric mucosal samples were collected for pathological examination by hematoxylin eosin (HE) staining, terminal nick-end labelling (TUNEL) staining, and immunohistochemical staining for Bax and Bcl-2. In addition, *H. pylori* was isolated, cultured, and identified, and the extent of its colonisation calculated. Blood was collected to detect the inflammatory factors IL-1β, IL-8, and TNF-α, and immune function markers CD4 and CD8. Besides, the liver, kidney, and spleen samples of the modelling groups were detected by HE staining to confirm whether or not CAG has been successfully induced.

RESULTS

HE-stained gastric mucosa

On the 30th day after infection, all of the mice in the combined group were found to have developed acute gastritis, while there were no CAG mice. No significant difference was shown on the 60th day compared with that on the 30th day. On the 90th day, 90% (nine out of ten) of mice had developed slight CAG which developed to moderate CAG on the 120th day, as shown in Figure 1.

TUNEL-stained gastric mucosa

The detection of TUNEL-stained gastric mucosa of mice in the combined group on the 30th, 60th, 90th, and 120th days after infection revealed that epithelial cells of the gastric mucosa all presented significant apoptosis. There was greater cell apoptosis by the 30th day, while at the 90th and 120th days, similar, less severe, apoptosis than that at the 30th day was found, as shown in Figure 2.

Immunohistochemical staining for Bax and Bcl-2

Bax, directly activated by p53, can improve apoptotic sensitivity and promote p53-mediated cell apoptosis. Bcl-2 protein can form a heterodimer with other pro-apoptotic proteins to inhibit cell apoptosis. It also plays an anti-apoptosis effect by interacting with apoptotic protease activating factor or inhibiting the release of cytochrome c, an activator of mitochondrial caspases. Therefore, cell apoptosis can be examined by detecting the expression of Bcl-xL and Bax. The expression of Bax in the combined group was up-regulated at the 90th and 120th day, while that of Bcl-2 was down-regulated, which indicated that the mice in the combined group had undergone significant cell apoptosis, conforming to the characteristics of CAG, as shown in Figure 3.

Identification and colonisation of isolated cultures

According to the postulates of Robert Koch, infecting *H. pylori* has to be isolated and cultured from the modelling groups, so the gastric mucosa was collected for isolation and culture of *H. pylori*, and the cultures were identified. The specific results are described below.

Gram staining: Isolated cultures were selected for Gram staining. The bacteria were Gram negative under a microscope and curving, which is consistent with the morphological characteristics of stained *H. pylori*, as shown in Figure 4.

CagA detection: CagA is a typical virulence factor of *H. pylori*. It had been verified that the infecting *H. pylori* strains contain the CagA gene before *H. pylori* infection through gavage. Therefore, if the isolated bacterium was *H. pylori*, the genome was bound to contain the CagA gene. It was verified that the isolated cultures did contain the CagA gene, as shown in Figure 5.

Detection of oxidase, peroxidase, and urease:*H. pylori* shows the biochemical characteristic of positive oxidase, peroxidase, and urease, so the three factors are commonly used as biochemical indices for identifying *H. pylori*. Detection of the isolated cultures showed that the three were also positive, which accorded with the biochemical characteristics of *H. pylori*, as summarised in Table 1.

Extent of *H. pylori* colonisation: The above identifications revealed that the isolated and cultured bacterium was *H. pylori*, whose population was calculated then to estimate the extent of its colonisation. At the 30th, 60th, 90th, and 120th days after infection, every gram of gastric mucosa of mice in the combined group was found to contain 1×10^4 to 1×10^5 CFU of *H. pylori*, and there was no significant difference across different time periods. This indicated that the colonisation of *H. pylori* did not

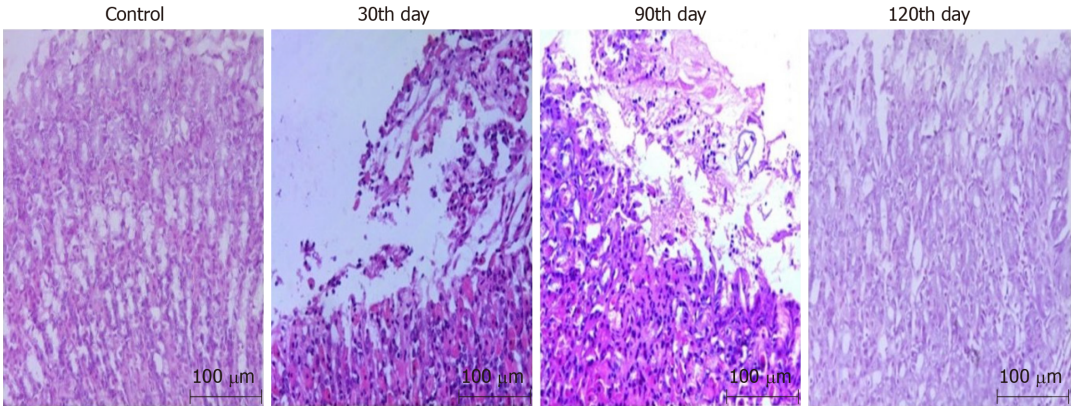

Figure 1 HE-stained gastric mucosal tissues of mice in the combined group (magnification, ×100).

decrease within 120 d after *H. pylori* infection, so *H. pylori* probably played a significant role in the pathogenic process of CAG, as shown in Figure 6.

Detection of inflammatory factors

The incidence and development of CAG can trigger the release of various inflammatory factors such as IL-1β, IL-8, and TNF-α. Inflammatory factors IL-1α, IL-8, and TNF-α were found to have increased expression on the 30th, 90th, and 120th days after mice in the combined group were infected with *H. pylori*. The increase was greater the first month, while the inflammatory factors had equivalent expression at the 90th and 120th days, both being lower than that on the 30th day. The results are consistent with the results of TUNEL staining, also showing the up-regulation of CAG inflammatory factors, as shown in Figure 7.

Detection of immunological functions

The expression of CD4+ and CD8+ immune cells of mice in the combined group were both decreased by the 30th, 90th, and 120th days after infection. The expression was down-regulated significantly by the 30th day, while the two immune cells were expressed in a similar manner on the 90th and 120th days, both having been down-regulated to a lesser extent than that on the 30th day. This was mainly because, by the 30th day, the mice were still affected by the immunosuppressor MNNG, and their immunological functions were impaired. After eliminating the influences of the immunosuppressor, the immunological functions improved while were unable to recover to normal levels. This is also probably because CAG itself is characterised by the impairment of immunological function, as seen from Table 2.

Impairment of other organs

As chemical and biological methods were combined in the study to establish the model, chemical reagents were likely to lead to impairment of organ functions of the mice, and even cancers therein. Therefore, it is necessary to detect the impairment of organs of the mice. By collecting and staining the liver, kidney, and spleen tissues with HE, it revealed that there was no impairment. This indicated that the modelling method did not induce complications of the liver, kidney, and spleen except for the development of CAG, so the method could be deemed safe, as shown in Figure 8.

Statistics of successful mice models

The aforementioned identifications proved that the established animal models were CAG models. Statistical analysis revealed that 90% of mice in the combined group developed CAG at the 90th and 120th day after infection and *H. pylori* colonisation was found in all such mice. In contrast, the *H. pylori* group showed *H. pylori* colonisation but no CAG. Other groups did not exhibit CAG or *H. pylori* colonisation, as can be seen from the data in Table 3.

DISCUSSION

CAG is one of the most common gastrointestinal diseases and an early stage of gastric cancer. CAG is actually one event in a chronic degenerative process beginning with gastritis, proceeding to CAG, then to intestinal metaplasia, dysplasia and, finally, to carcinoma[10]. CAG is hard to be cured with recurrent attacks, severely affecting the

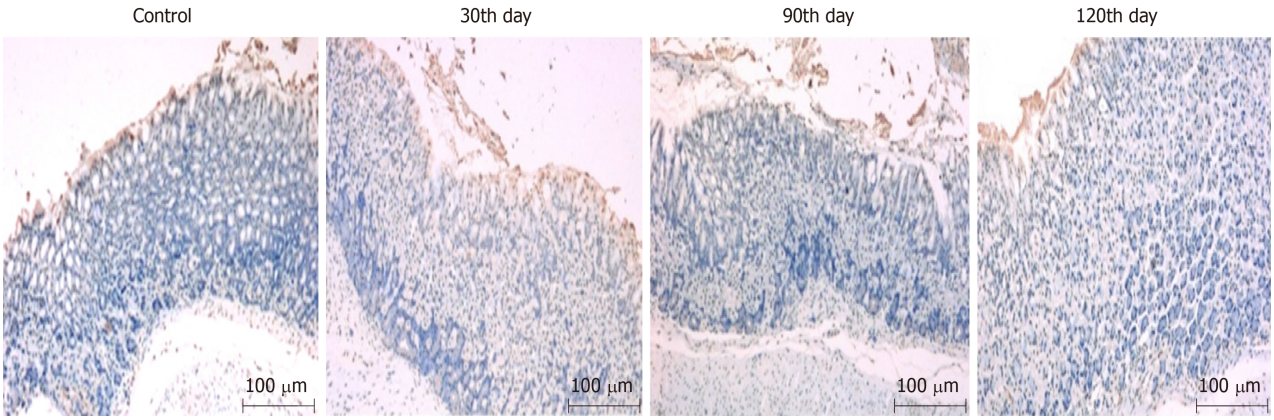

Figure 2 TUNEL-stained gastric mucosal tissues of mice in the combined group (magnification, ×100).

health of human. The research and treatment of CAG are very popular. An animal model of CAG is an important basis for research into chronic gastritis. Although there have been numerous reports on the animal models of CAG, it is still not clear which model is best suited to investigating the mechanism of occurrence of human chronic gastritis and there are no unified standards for the type and age of animals to be selected[11-13]. As the modelling of chronic gastritis takes a long time, there is an urgent need to find a more rapid, stable, safe model. At present, biological modelling, chemical injury, and active immunisation are commonly used methods of establishing animal models, and rats are generally used. MNNG, sodium deoxycholate, and sodium salicylate are commonly model materials with chemical methods. Rats and Mongolian gerbils are commonly used animals to develop CAG models, while mice are unusually used. The reasons may be that mice do not control the dose of the intervention and are prone to other complications or toxic side effects.

H. pylori is a human-specific pathogen, which leads to gastric pathologies including gastritis and gastric ulcers. *H. pylori* is a common risk factor for CAG and can be used to create CAG model. The animal model is due to *H. pylori* infection, more like a human infection model, and beneficial to studying the pathogenesis of CAG. However, *H. pylori* is relatively easy to implant in rats and Mongolian gerbils, and the incidence rate is relatively high, but it is difficult to plant in the mouse. Clinically isolated *H. pylori* strains must be domesticated for adapted planting, and the time of retaining in the stomach is relatively short. After the first month of infection, the maximum number of plants is reached, and it will decrease in the second and third months. The mice will not be able to maintain a relatively high infection rate. Although the C57BL/6 mice and BALB/c mice with *H. pylori* Sydney strain (SS1) can maintain a high amount of plants in the stomach for a long time, the strain was CagA negative and CAG did not appear in 24 wk after the last infection[14]. "*H. pylori* + N-methyl-N-nitrourea can induce CAG in 23.1% of animals after infection for 36 wk, which required a long time, and the success rate is low[15]. As it is difficult to colonise *H. pylori* in the stomach in the long term, and particularly difficult to infect mice for a long time, biological modelling commonly uses rats. However, *H. pylori* does not readily infect rats without injury to the gastric mucosa, therefore, the gastric mucosa of rats has to be impaired before being infected with *H. pylori* in many experiments. So, it is very difficult to establish CAG with *H. pylori* infected mice in a short period of time.

In reference to the methods of Bergin *et al*[16], Nagahara *et al*[17], and Jin *et al*[18], this study established a CAG model with Kunming mice by combining *H. pylori*, MNNG, and ammonia water. The method was proposed on the basis of the fact that infection with *H. pylori* can induce gastritis[19,20], the functions of MNNG as a chemical mutagen and carcinogen[21,22], and the role of ammonia water in neutralising gastric acid and impairing gastric mucosa[23,24]. A CAG model was successfully established by the 90th day after the last *H. pylori* infection of six-week-old mice, with a success rate reaching 90%. The method overcomes the difficulty in colonising *H. pylori* in mice and the fact that the colonisation time is short. In addition, rats are replaced with mice, and the method is thus simpler and also cheaper. The established models are stable and safe, therefore, the method is a relatively ideal method for establishing a mouse model of CAG. It is demonstrated to be possible to build an animal model that is analogous to human CAG, which provides a significant help when exploring the mechanisms of occurrence and prevention of CAG.

Table 1 Identification of cultures isolated from gastric mucosa of mice in the combined group based on biochemical indices

Group	Oxidase	Peroxidase	Urease
Positive control	+	+	+
Negative control	-	-	-
Isolated culture	+	+	+

Table 2 Immunological functionsof mice in the combined group

Group	CD4+			CD8+		
	30th d	90th d	120th d	30th d	90th d	120th d
PBS control	52.56 ± 1.82	51.27 ± 2.05	52.84 ± 1.49	28.13 ± 1.92	29.41 ± 2.39	30.07 ± 1.24
Combined	40.17 ± 1.36	44.38 ± 2.11	43.97 ± 2.08	22.08 ± 1.42	25.53 ± 1.87	25.19 ± 1.53

Table 3 Modelling results of chronic atrophic gastritis

Group	The number of mice developing CAG		The number of mice with Hp colonisation		Success rate
	90th d after infection	120th d after infection	90th d after infection	120th d after infection	
PBS control	0	0	0	0	0
Hp	0	0	90% (9/10)	100% (10/10)	0
MNNG	0	0	0	0	0
Ammonia water	0	0	0	0	0
Combined	90% (9/10)	90 (9/10)	100% (10/10)	100% (10/10)	90%

CAG: chronic atrophic gastritis; MNNG: N-methyl-N'-nitroguanidine.

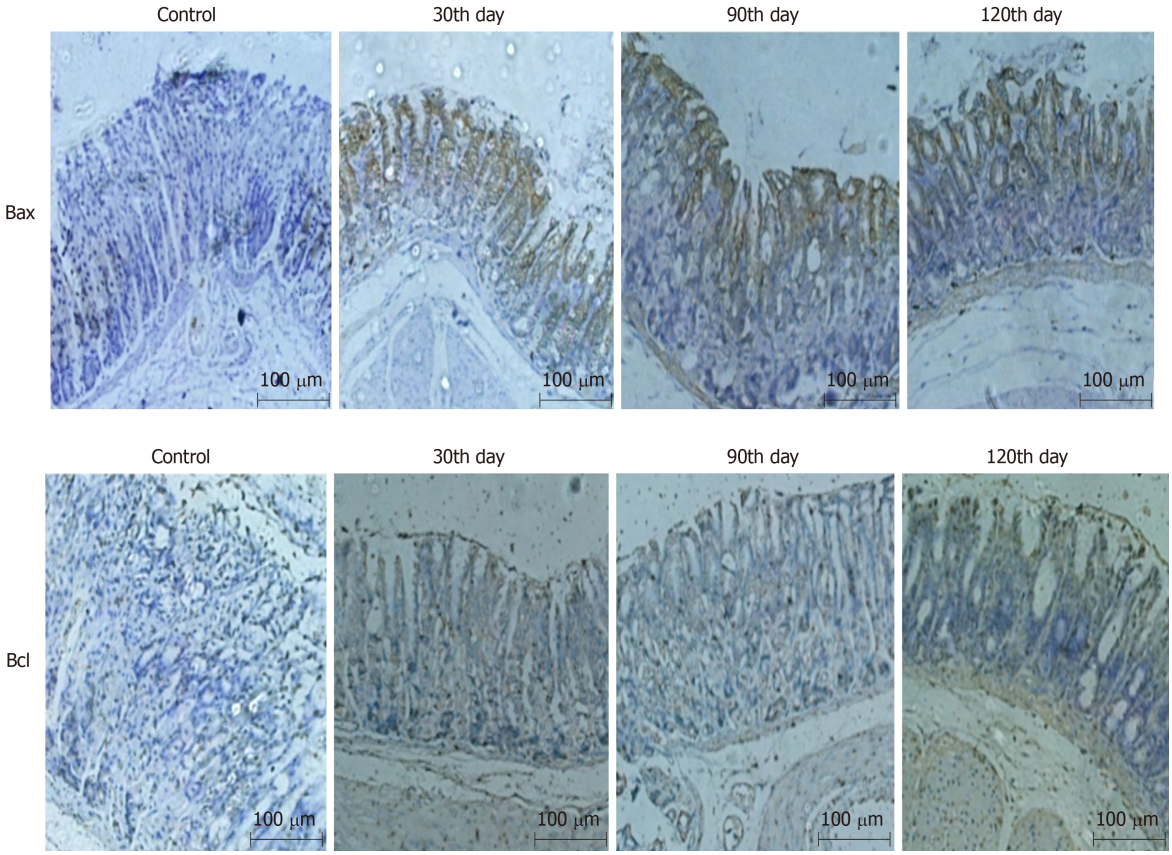

Figure 3 Immunohistochemical staining for Bax and Bcl-2 in the gastric mucosa of mice in the combined group (magnification, ×100).

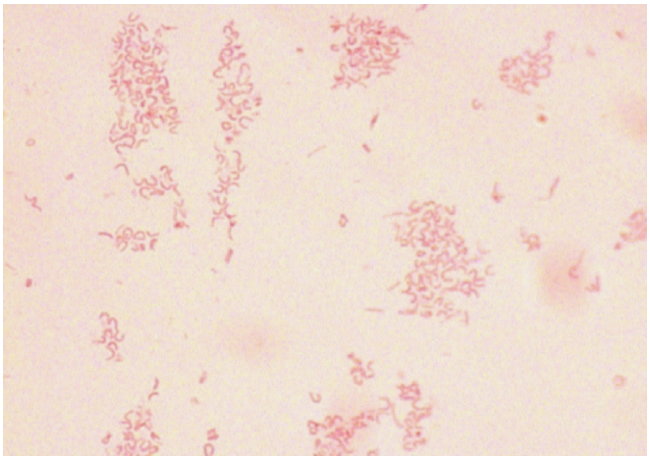

Figure 4 Gram staining of *Helicobacter pylori* cultures isolated from the gastric mucosa of mice in the combined group (magnification, ×100).

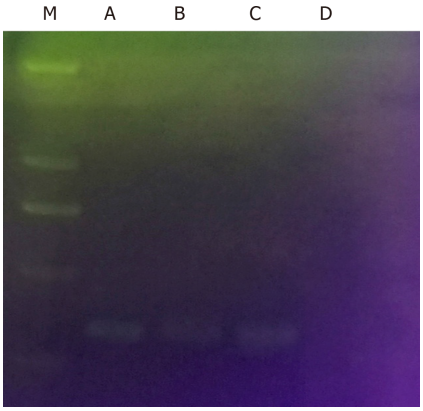

Figure 5 Cag A detection for *Helicobacter pylori* isolated and cultured from the gastric mucosa of mice in the combined group.

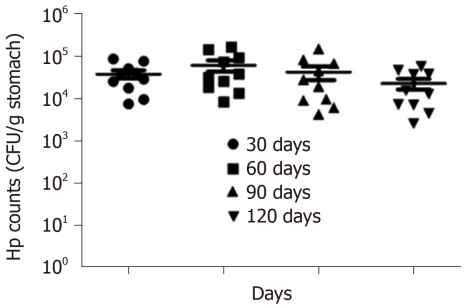

Figure 6 *Helicobacter pylori* colonisation in the gastric mucosa of mice in the combined group.

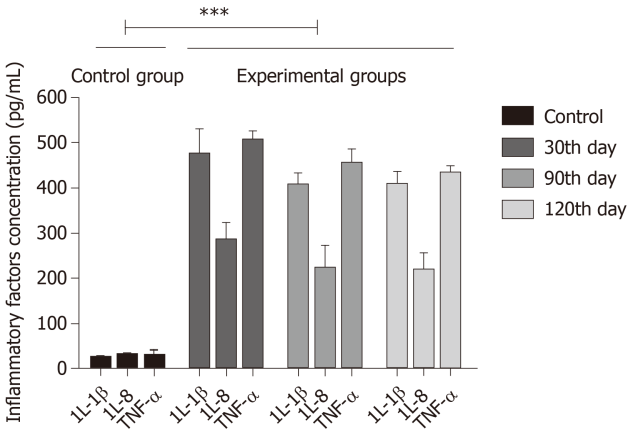

Figure 7 Inflammatory factors in mice in the combined group.

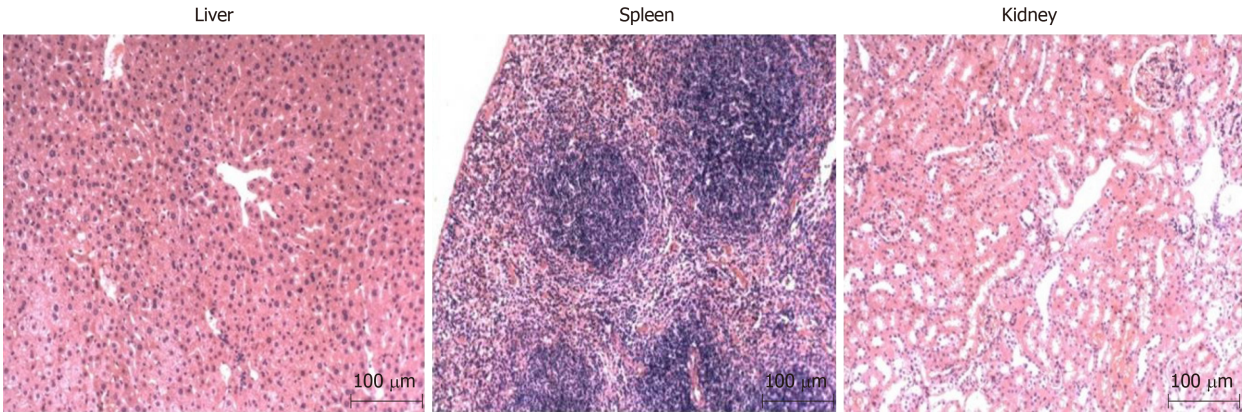

Figure 8 HE-stained liver, kidney, and spleen tissues of mice in the combined group (magnification, ×100).

ARTICLE HIGHLIGHTS

Research background

Chronic atrophic gastritis (CAG) is a precancerous lesion of gastric cancer. If it is effectively treated, GC will be prevented. So the prevention and treatment of CAG are very important. It needs a good animal model, which can provide etiological research and screening of therapeutic drugs. It is an important tool for studying CAG.

Research motivation

Current animal models of chronic atrophic gastritis are not ideal, long cycle, high cost, difficult to operate, and unstable, so better animal models are needed.

Research objectives

To construct a more rapid, safe, stable and efficient chronic atrophic gastritis model with mice.

Research methods

The mice selected for this method were six-week-old Kunming mice, and *Helicobacter pylori*, N-methyl-N'-nitroguanidine, and ammonia water were combined to develop a CAG model.

Research results

The method showed slight CAG at the 90th day and moderate CAG at the 120th day.

Research conclusions

The method presented here is more rapid, safe, stable, and efficient.

Research perspectives

Starting from the etiology of chronic atrophic gastritis, the course of the disease is simulated to explore a simple, fast, convenient, and stable method of modelling.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1126-1140

DOI: 10.4251/wjgo.v11.i12.1126

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Case Control Study

Relationship between cachexia and perineural invasion in pancreatic adenocarcinoma

Livia Petrusel, Ioana Rusu, Daniel Corneliu Leucuta, Radu Seicean, Ramona Suharoschi, Paula Zamfir, Andrada Seicean

ORCID number: Livia Petrusel (0000-0002-1385-3892); Ioana Rusu (0000-0001-9174-6729); Daniel Corneliu Leucuta (0000-0003-4218-8622); Radu Seicean (0000-0003-4519-2352); Ramona Suharoschi (0000-0002-8306-8670); Paula Zamfir (0000-0003-1461-9405); Andrada Seicean (0000-0002-5809-1334).

Author contributions: Petrusel L and Seicean A conceived and designed the study; Petrusel L, Rusu I, Seicean R, Suharoschi R and Zamfir P performed the research; Leucuta DC analysed data; Petrusel L and Seicean A wrote the paper.

Institutional review board statement: This study was reviewed and approved by the Ethics Committee of the Regional Institute of Gastroenterology and Hepatology in Cluj-Napoca.

Conflict-of-interest statement: The authors report no relevant conflicts of interest.

Data sharing statement: Participants gave written informed consent for data sharing.

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

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external

Livia Petrusel, Andrada Seicean, Department of Gastroenterology, Regional Institute of Gastroenterology and Hepatology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca 400162, Romania

Ioana Rusu, Paula Zamfir, Department of Pathology, Regional Institute of Gastroenterology and Hepatology, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca 400162, Romania

Daniel Corneliu Leucuta, Medical Informatics and Biostatistics Department, Iuliu Hatieganu University of Medicine and Pharmacy Cluj-Napoca, Cluj-Napoca 400012, Romania

Radu Seicean, First Surgery Clinic, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca 400006, Romania

Ramona Suharoschi, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca 400372, Romania

Corresponding author: Livia Petrusel, MD, PhD, Doctor, Regional Institute of Gastroenterology and Hepatology, Iuliu Hatieganu University of Medicine and Pharmacy, 19-21 Croitorilor Street, Cluj-Napoca 400162, Romania. livia.cutas@umfcluj.ro
Telephone: +40-72-4402389

Abstract

BACKGROUND

Cachexia is responsible for the low quality of life in pancreatic adenocarcinoma (PDAC). The rapid disease progression and patient deterioration seems related to perineural invasion, but the relationship between cachexia and perineural invasion for the evolution of the disease has been rarely studied. As perineural invasion is difficult to be highlighted, a biomarker such as the neurotrophic factor Midkine (MK) which promotes the neuronal differentiation and the cell migration could be helpful. Also, Activin (ACV) has been described as cachexia related to PDAC. However, their role for assessing and predicting the disease course in daily practice is not known.

AIM

To assess the relationship between perineural invasion and cachexia and their biomarkers, MK and ACV, respectively, and their prognostic value.

METHODS

Manuscript source: Invited manuscript

Received: May 5, 2019
Peer-review started: May 8, 2019
First decision: July 31, 2019
Revised: September 9, 2019
Accepted: October 14, 2019
Article in press: October 14, 2019
Published online: December 15, 2019

P-Reviewer: Mastoraki A
S-Editor: Zhang L
L-Editor: A
E-Editor: Qi LL

This study included prospectively enrolled patients with proven adenocarcinoma and a matched group of controls without any malignancies. Patients with other causes of malnutrition were excluded. The plasma levels of ACV and MK were analyzed using western blotting and were correlated with the clinicopathological features and survival data. These results were validated by immunohistochemical analyses of the pancreatic tumor tissue of the patients included in the study and a supplementary group of surgically resected specimens from patients with a benign disease.

RESULTS

The study comprised 114 patients with PDAC, 125 controls and a supplementary group of 14 benign pancreatic tissue samples. ACV and MK were both overexpressed more frequently in the plasma of patients with PDAC than in the controls (63% *vs* 32% for ACV, $P < 0.001$; 47% *vs* 16% for MK, $P < 0.001$), with similar levels in pancreatic tissue the MK protein expression was closely related to the advanced clinical stage ($P = 0.006$), the presence of metastasis ($P = 0.04$), perineural invasion ($P = 0.03$) and diabetes ($P = 0.002$), but with no influence on survival. No correlation between clinicopathological factors and ACV expression was noted. Cachexia, present in 19% of patients, was unrelated to ACV or MK level. Higher ACV expression was associated with a shorter survival ($P = 0.008$).

CONCLUSION

The MK was a biomarker of perineural invasion, associated with tumor stage and diabetes, but without prognostic value as ACV. Cachexia was unrelated to perineural invasion, ACV level or survival.

Key words: Pancreatic adenocarcinoma; Cachexia; Perineural invasion; Activin; Midkine; Biomarker; Survival; Metastases; Endosonography; Surgery

Core tip: Midkine (MK) is a neurotrophic factor that promotes perineural invasion in pancreatic cancer, neuronal differentiation and cell migration. Our results confirmed that high MK expression is closely correlated with perineural invasion and with advanced tumor stage, diabetes. No relationship with cachexia was found. Activin plays a dominant role in the development and progression of cachexia and in tumor cell growth in pancreatic adenocarcinoma, but this was not confirmed in this study, despite the association with poor survival.

Citation: Petrusel L, Rusu I, Leucuta DC, Seicean R, Suharoschi R, Zamfir P, Seicean A. Relationship between cachexia and perineural invasion in pancreatic adenocarcinoma. *World J Gastrointest Oncol* 2019; 11(12): 1126-1140
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1126.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1126

INTRODUCTION

Pancreatic cancer is currently ranked on the 3rd place in cancer mortality, but is expected to reach the 2nd leading cause of cancer mortality in 2030[1,2]. The unfavorable prognosis of PDAC can be attributed to late diagnosis and aggressive tumor biology, with a very low survival rate (< 6%)[2-4]. A major factor in the low survival rate is the presence of cachexia, which is seen in over 85% of patients with pancreatic cancer[5-7] constituting the highest rate of cachexia in all malignancies.

Cancer cachexia is a complex syndrome, characterized by body-weight loss; muscle, skeleton and adipose tissue wasting; and inflammation, which is often associated with anorexia[8,9].

Cachexia has become an obstacle for the successful treatment of cancer and may significantly contribute to cancer-related death.

The mechanism of cachexia involves the interaction host- tumor through TGFβ *via* the SMAD2/3 pathway which can stimulate tumour growth and inhibits the muscle growth followed by myopenia through myostatin or activin pathway[9,10] or insulin-

growth factor binding protein as part of IGF-1/PI3K/Akt signalling pathway with the inhibition of myogenesis and enhancing myotubule protein degradation[9]. Another mechanism is related to hypercatabolism connected to the production of pro-inflammatory cytokines by the tumor, such as interleukin (IL)-1b, IL-6 and the tumor necrosis factor-α, both of which are involved in lipolysis with white adipose tissue wasting and early occurrence of brown fat tissue with high energy expenditure[11,12], in muscle catabolism too[9].

Astrocyte activation in the spinal cord induces lipolysis in the adipose tissue[13] and muscle atrophy[14,15] and is also involved in cachexia occurrence; this activation may result from the damage to peripheral nerves during perineural invasion in PDAC, with the hypertrophy and thickening of the nerve branches[16,17], but their relationship is not completely understood. The cachexia was associated with the degree of neural invasion, known as responsible for the aggressive behaviour of pancreatic cancer[18,19], with the involvement of the neurotrophic factors[20], such as midkine. It promotes neuronal differentiation and cell migration in peripheral invasion of pancreatic cancer[21]. Midkine was found to be overexpressed in many pancreatic cancers and may represent a target for chemoresistant patients[22], but its relationship with cachexia has not been studied.

The main aim of this study was to determine the relationship between cachexia and perineural invasion in patients with PDAC by using clinico-pathological features and the protein expression levels of Activin and Midkine in plasma and tissue of patients compared to healthy patients. The secondary purpose was to assess the prognostic role of Activin and Midkine in survival and metastasis.

MATERIALS AND METHODS

Patients and sample collection

This study was performed at the Regional Institute of Gastroenterology and Hepatology "O. Fodor" in Cluj-Napoca, Romania. This study was prospectively performed and was approved by the Ethics Committee of the hospital and was registered at clinicaltrials.gov (NCT03042442).

Eligibility criteria

Subjects of the study group were at least 18-years-old, with no previous history of any other cancer in the last 5 years. Written consent was given prior to entry into the study. Patients with pancreatic ductal adenocarcinoma, based on the results of endoscopic ultrasonography (EUS), biopsy, or surgery, were enrolled at the time of diagnosis, before any therapeutic intervention had been given, from January 2015 to September 2017.

The exclusion criteria were obvious malabsorption, major depression, artificial nutrition, hyperthyroidism, and other causes of malnutrition. The final diagnosis was based on the histologic results from endoscopic ultrasonography fine needle aspiration (EUS-FNA) or surgery.

The subjects of the control groups were healthy people who were at least 18-years-old, with no previous history of any cancer and other chronic diseases. For the most part, controls were matched to cases for sex and age (plus/minus 5 years). Age, sex, tumor stage, tumor differentiation, body-mass index (BMI), smoking, and the presence of diabetes were noted.

Nutritional and functional assessment

Current body weight and height were measured at the time of inclusion. Diabetes was diagnosed if fasting glucose values met the ADA criteria[23] and the duration since diabetes onset was recorded.

Cachexia was defined as an involuntary weight loss of more than 5% or a weight loss of more than 2% in individuals with a BMI of less than 20 kg/m^2 over the past 6 mo[24].

Blood sampling

Blood samples were collected at the time of diagnosis. Peripheral venous blood was drawn into a tube containing ethylenediaminetetraacetic acid and was prepared by centrifugation at 5000 × g for 5 min. The plasma samples were stored at −80 °C until use.

Selected proteins were quantified in the plasma using Western blot analyses.

Antibodies

The following antibodies were obtained from Abcam (Cambridge, United Kingdom): rabbit polyclonal antibody to ACV Receptor Type IIB antibody (cat. no. ab128544),

rabbit polyclonal antibody to GAPDH (glyceraldehyde 3-phosphate dehydrogenase) (cat. no. ab37168), and horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG H.L antibody (cat. no. ab97051). The antibodies against midkine, mouse monoclonal IgG1 MK antibody (sc-46701, Santa Cruz Biotechnology, Santa Cruz, CA, United States) and goat anti-mouse IgG-HRP for MK (sc-2005; Santa Cruz Biotechnology, Santa Cruz, CA, United States**)** were obtained from Santa Cruz.

Western blot analyses

Protein concentration was determined using a protein assay kit, Quick Start Bradford Protein Assay (BioRad Laboratories, Inc.). A total of 50 µg of total protein from each plasma sample was loaded per lane onto a 12% polyacrylamide gel. Electrophoresis was performed at 120 mV, and the protein fractions were electrotransferred onto a nitrocellulose membrane at 100 mV for 1 h. The membranes were blocked for 3 h with nonfat dry milk powder (BioRad Laboratories, Inc.) in Trisbuffered saline containing 0.1% Tween20 (TBST) under constant agitation. Subsequently, the membranes were incubated overnight at 4 °C with a rabbit polyclonal antibody to ACV Receptor Type IIB antibody (cat. no. ab128544AbCam) diluted 1: 1000 in TBST, with nonfat dry milk powder and a mouse monoclonal antibody to IgG1 MK antibody (sc-46701; Santa Cruz Biotechnology, Santa Cruz, CA, United States) diluted 1: 100 in TBST with nonfat dry milk powder. For the loading control, a rabbit polyclonal antibody to GAPDH (glyceraldehyde-3-phosphate dehydrogenase) (cat. no. ab37168 AbCam) was used at a concentration of 0.8 µg/mL in nonfat dry milk powder in TBST. Membranes were washed with TBST and incubated at room temperature for 1 h with a horseradish peroxidase (HRP) conjugated goat anti-rabbit IgG H.L antibody (cat. no. ab97051, Abcam) diluted 10000fold in TBST and a goat anti-mouse IgG-HRP antibody for MK (sc-2005; Santa Cruz Biotechnology, Santa Cruz, CA, United States) diluted 5000fold in TBST, with an additional washing step performed prior to detection. For GAPDH determination, an HRPconjugated goat IgG antirabbit IgG antibody (cat. no. ab97051, Abcam) diluted 10000fold in TBST was used. Total protein expression was normalized by dividing each of the protein units by those for GAPDH.

Proteins were detected by using Clarity™ Western ECL (BioRad Laboratories, Inc.) and the membranes were imaged in a Chemidoc Imaging System (BioRad Laboratories, Inc.) and analyzed using ImageLab Software version 5.2.1 for Windows (BioRad Laboratories, Inc.).

Measurement and confirmation of the ACV and MK protein levels is often performed with normalization against "housekeeping proteins", such as glyceraldehyde-3-phosphate dehydrogenase, to correct for protein loading and other factors, such as transfer efficiency.

For protein expression, after densitometry, the integrated density value (IDV) for each protein band (ACV and MK) was determined, and the normalized levels of ACV and MK were calculated by dividing the IDV of a protein band by the IDV of the GAPDH band (arbitrarily assigned a value of 100) in the same sample, thus quantifying the expression of the proteins as high or low expression.

Tissue samples

Tumor tissue samples from endoscopic ultrasound-fine needle aspiration and surgery were fixed with 10% formalin for pathology studies.

In addition, there was a supplementary group of 14 samples containing normal pancreatic tissues from patients who received partial pancreatectomy for benign tumors that were used as normal controls for the immunohistochemical interpretation.

Analysis of perineural invasion

The perineural invasion was assessed only on surgical tissues. The associations of perineural invasion with characteristics of PDAC were assessed on slides stained with hematoxylin-eosin. The presence of perineural invasion was defined by the infiltration of cancer cells into the perineurium or neural fascicles[25]. The evaluation of perineural invasion was assessed mainly in tissue obtained from surgery after curative treatment. The degree of perineural invasion was defined as follows: 0- less than one occurrence per slide; 1 -two to four occurrences per slide; and 2 - more than four occurrences per slide or intraneural invasion.

Immunohistochemistry

For each case a paraffin-embedded block was selected. 3-micron-thick sections were obtained for immunohistochemistry investigation. The primary antibodies used were the following: ACV Receptor Type IIB antibody (cat. no. ab128544) diluted 1: 150 and mouse monoclonal IgG1 MK antibody (sc-46701, Santa Cruz Biotechnology, Santa Cruz, CA, United States) diluted 1: 200. The BONDIII staining instrument (Leica

Biosystems) and Bond Polymer Refine Detection Kit (Leica Biosystems) were used for all antibodies. This analysis was performed with pancreatic tumor and normal tissue samples.

All slides were scored by a pathologist (I.R.) who was blinded to all clinical data. Finally, the tissues were evaluated under a microscope. The intensity of the staining was scored as negative, weak, moderate, or strong (scores of 0, 1, 2, or 3, respectively).

Statistical analysis

Chi square test or Fisher's exact test were used for categorical data. Comparisons between two groups of continuous data were performed with a *t*-test for independent samples for data with a normal distribution or a Wilcoxon rank-sum test otherwise. Univariate and, multivariate Cox proportional hazard models with each protein expression variable adjusted for age ≥ 50 years, gender, stage (T4 *vs* T1-T3), metastasis, tumor size ≥ 3 cm, and diabetes were built. The Cox proportional hazard assumption and multicollinearity assumptions were checked. Similarly, univariate and multivariate logistic regression models were built to predict metastasis. We checked the models for multicollinearity, misspecification and the goodness of fit.

For all statistical tests, a two-tailed *P* value was used, along with a 0.05 significance level. All analyses were performed in the R environment for statistical computing and graphics, version 3.4.4.

RESULTS

Patient characteristics

A total of 114 patients with PDAC and 125 controls with no tumor were enrolled in this study (Table 1).

The mean age of the population was 62.41 years (SD 11.56, range: 27-88 years). There were more males than females (61% *vs* 39%).

A total of 49 (42.98%) patients with PDAC reported a history of diabetes, including new-onset diabetes for 24 (21.05%) patients.

The most common localization of PDAC was in the pancreatic head (66 patients-49%), followed by a localization in the body (33 patients-24%), the isthmus (17 patients-12%), the pancreatic tail (13 patients-10%) and the uncinated process (7 patients-12%).

Nutritional and functional characteristics

Cachexia was more common in women ($P = 0.033$) and in patients with metastasis ($P = 0.011$). The presence of cachexia was unrelated to age ($P = 0.389$), cancer site ($P = 0.611$), tumor staging ($P = 0.148$) and perineural invasion ($P = 0.12$).

Expression of plasma and tissue MK and ACV in pancreatic cancer

MK was expressed in plasma from 54 (47.37%) PDAC patients compared to 20 (16%) controls ($P < 0.001$). Immunohistochemical staining in PDAC tissue was positive in 56 (49.12%) patients, including weak in 34 (29.82%), moderate in 16 (14.04%), and strong in 6 (5.26%), compared to none of the tissue from the supplementary controls ($P = 0.012$) (Figure 1).

ACV was expressed in plasma from 72 (63.16%) PDAC patients compared to 39 (31.2%) controls ($P < 0.001$). Immunohistochemical staining in PDAC tissue was positive in 59 patients (51.8%), including weak in 53 (46.49%) and moderate in 6 (5.26%) compared to none of the tissue from the control group ($P = 0.001$) (Figure 2).

Both proteins were detected in the tumor tissue by immunohistochemistry, and the tissue expression was significantly correlated with the plasma expression ($P = 0.002$ for Activin; and $P = 0.046$ for Midkine).

Relationships of ACV and MK expression with clinicopathological features

Table 2 summarizes the associations of MK and ACV expression with clinicopathological features in pancreatic cancer.

MK was highly expressed in the plasma of patients with an advanced T tumor stage ($P = 0.006$), metastases ($P = 0.046$), long-term diabetes ($P = 0.002$) and nonsmokers ($P = 0.032$). Nevertheless, there was no relationship between clinicopathological factors and high ACV expression. The association between ACV expression and cachexia was not statistically significant ($P = 0.5$).

Relationship between perineural invasion and expression of MK and ACV

The presence of perineural invasion was observed in only 61 PDAC specimens. The MK expression levels in PDAC were significantly higher in patients with perineural invasion than in those without perineural invasion ($P = 0.033$). The ACV expression

Table 1 Patient characteristics in the adenocarcinoma and control groups, *n* (%)

Characteristics	PDAC (*n* = 114)	Control (*n* = 125)
Age (yr), mean (SD)	64.76 (10.58)[b]	60.26 (12.03)
Age > 50 yr	103 (90.35)[b]	89 (71.2)
Sex (female)	47 (41.23)	46 (36.8)
BMI (kg/m^2), median (IQR)	25.09 (22.05-27.25)	25.26 (22.58-28.72)
Weight status		
Underweight	12 (10.53)	5 (4)
Normal	48 (42.11)	53 (42.4)
Overweight	37 (32.46)	44 (35.2)
Obesity	17 (14.91)	23 (18.4)
Smoking	50 (43.86)	69 (55.2)
New-onset diabetes	24 (21.05)[b]	8 (6.4)
Long-term diabetes	24 (21.05)	32 (25.6)
Diabetes	49 (42.98)[a]	38 (30.4)
CA 19-9 (U/mL), median (IQR)	400 (67.75-400)[b]	31.45 (9.58-98.8)
Cachexia	22 (19)	8 (6)
T stage	1-2: 10 (8.85); 3: 65 (57.52); 4: 38 (33.63)	
Histological grade	G1: 8/41 (19.51); G2: 24/41 (58.54); G3: 12/41 (29.27)	
N stage	9 (8.18)	
Metastasis	48 (42.11)	

[a]$P < 0.05$,
[b]$P < 0.01$ *vs* controls. PDAC: Pancreatic ductal adenocarcinoma; BMI: Body-mass index; CA 19-9: Carbohydrate antigen 19-9; QR: Interquartile range; CI: Confidence interval.

level was not significantly associated with perineural invasion ($P = 0.5$).

Prediction of metastasis

To predict the presence of metastasis in patients with adenocarcinoma, we performed uni- and multivariate analysis by creating regression models, where each expression variable (MK and ACV high expression *vs* low expression) in the model was adjusted by age > 50 years, sex, diabetes, stage T4 *vs* T1-3, stage N1 *vs* N0 (Table 3). There were no statistically significant factors.

Survival in PDAC patients

Using data from the 114 patients with PDAC, we further evaluated whether the overexpression of ACV and MK was correlated with patient survival.

The univariate and multivariate Cox proportional survival analysis showed that after adjusting for age, gender, adenopathy, tumor size ≥ 3 cm, and metastases, tumor size > 3 cm, metastasis and ACV expression (Table 4) were independent predictors of poor survival (Figure 3). The survival of patients with high or low levels of MK expression was similar (Figure 4).

DISCUSSION

Our work aimed to study the relationship between perineural invasion and cachexia and their biomarkers, such as MK and ACV, respectively, and their prognostic value in patients with pancreatic cancer.

The survival in PDAC is very low, approximately 6% in 5 years, and one of the reasons is the rich stroma in the tumor tissue and the perineural invasion that extends into the pancreatic nerve plexus[26]. One of the promoters of perineural invasion is a neurite growth factor, MK, which is present on the surface of cells and extracellular matrix that facilitates neural differentiation, cell migration, perineural invasion, neuritis out-growth[27,28], neuronal survival[29,30], carcinogenesis and tumor progression[31,32]. During carcinogenesis in PDAC, it appears that the pancreatic cells bind at the level of the nerve gap or perineurium, then MK binds its receptor through an interaction that involves chondroitin sulfate, which promotes cancer infiltration, migration and the rapid development of peripheral invasion. During the repair of damage to the perineurium, more cells expressing MK are attracted, and a vicious

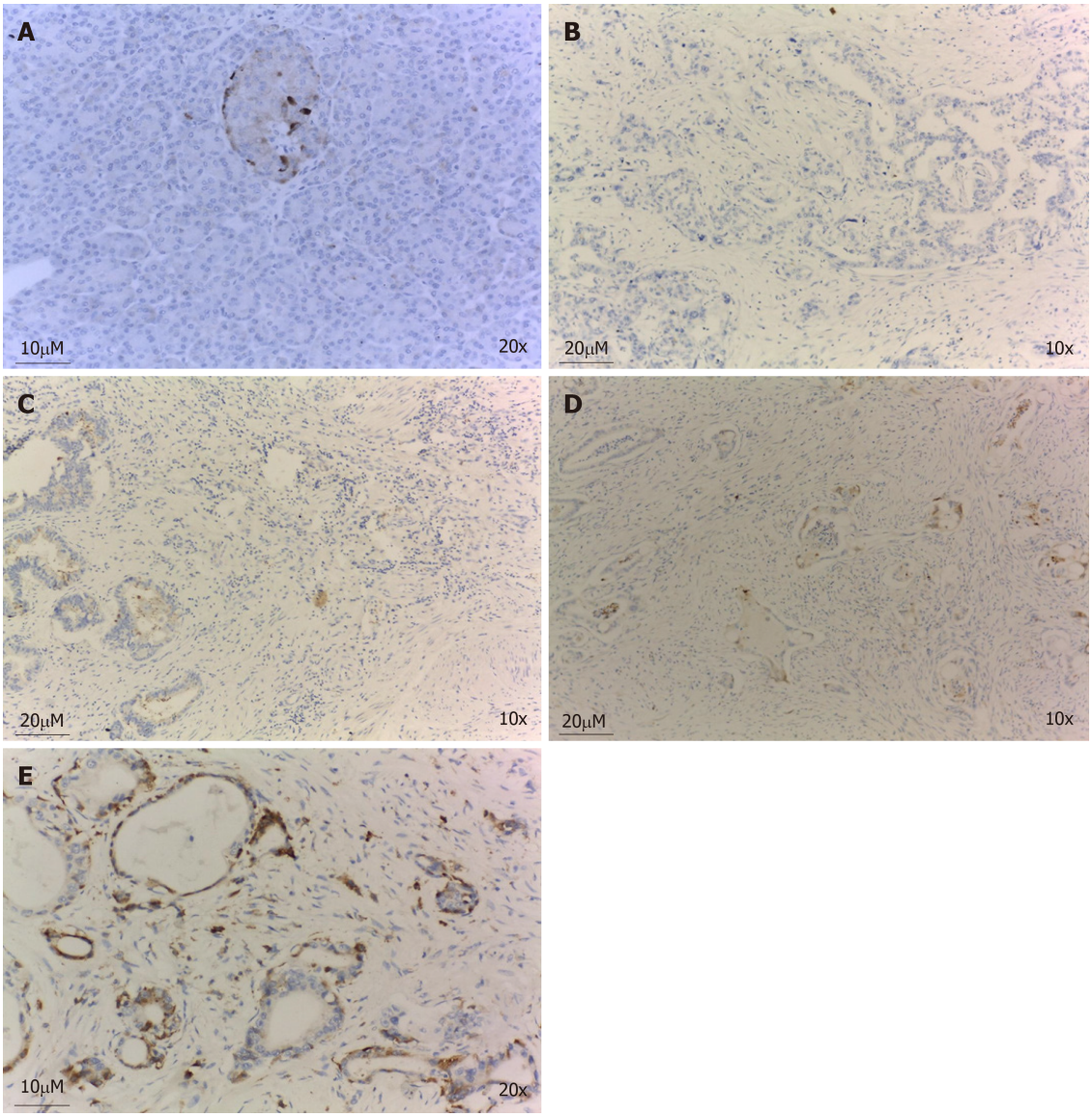

Figure 1 Immunohistochemical staining of Midkine in pancreatic adenocarcinomaand normal pancreatic tissue. A: Negative Midkine staining in normal pancreatic tissue; B: Negative Midkine staining in pancreatic tumor tissue; C: Weak Midkine expression in pancreatic tumor tissue; D: Moderate Midkine expression in pancreatic tumor tissue; E: Strong Midkine expression in pancreatic tumor tissue.

circle is formed[21]. MK expression was not inducible in GEM-treated chemosensitive PDAC cell lines, suggesting that MK is necessary to promote survival during chemotherapy by triggering Notch-2 pathway activation[22].

Increased levels of MK are found in 53% of PDAC, are related to venous invasion, microvessel density, and liver metastasis[22,33] and are involved in biological activities that favor cell growth, survival and angiogenesis[34]. MK had a low expression in the primary tumor in 80% of patients and had moderate expression in 18% of patients[35], similar to our results in which the tissue expression was weak in almost 30%, moderate in 14% and strong in 6% of patients, and the plasma MK protein was strongly positive in PDAC patients compared to controls (47.37%) (Figure 5).

We found that high MK expression was closely correlated with advanced tumor stage, the presence of metastasis, diabetes and perineural invasion, but no relationship with cachexia was found. Similar to a previous study[20], we confirmed the association of the MK protein with the presence of perineural invasion but in a larger group of patients (61 patients with pancreatic adenocarcinoma) than was previously reported.

The high level of MK in plasma was more frequently observed in patients with metastasis, similar to other studies[33], but it did not predict the development of metastasis or survival in multivariate analysis.

Activin A is a member of the TGFβ superfamily and is involved in many pathophysiological processes[36]. ACV exerts most of its biological actions by binding to the membrane ACV type II receptor B[10], a receptor that is shared with Myostatin

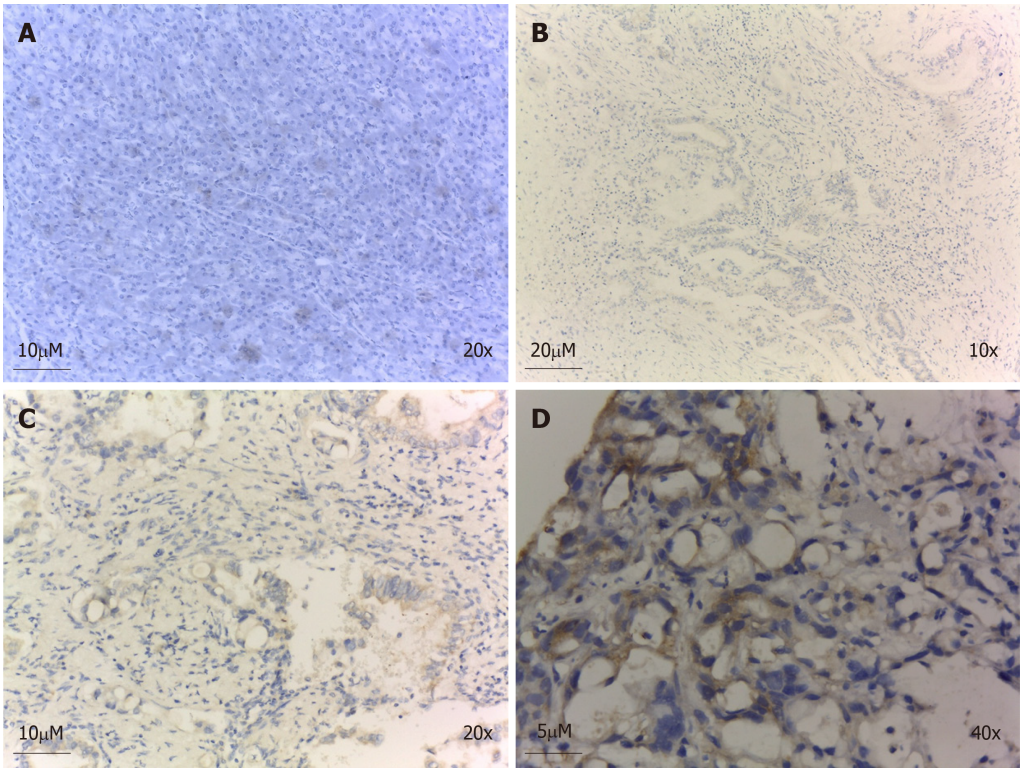

Figure 2 Immunohistochemical staining of Activin in pancreatic adenocarcinoma and normal pancreatic tissue. A: Negative Activin staining in normal pancreatic tissue; B: Weak Activin expression in pancreatic tumor tissue; C: Moderate Activin expression in pancreatic tumor tissue; D: Strong Activin expression in pancreatic tumor tissue.

(another TGF-β superfamily member), and these proteins eventually activate the SMAD pathway, which is involved in pancreatic carcinogenesis[37-39]. ACV receptor B seems to be involved in cancer cachexia[40,41], and its blockade by a soluble form prevents muscle atrophy and increases survival without affecting tumor growth[42,43].

The overexpression of ACV in the tumor tissues[44] and elevated blood ACV levels were found in 38 patients with PDAC, sustaining a non-SMAD (MAPK, PI3K/AKT) pathway[45], which is contradictory to the suppressive role of ACV (by activating the SMAD pathway) that has been previously reported. Further studies are required to confirm the mechanism of ACV in pancreatic cancer.

In our study, the plasma ACV level in PDAC patients was higher than that in controls (63% *vs* 32%) (Figure 5), and the immunohistochemistry was positive in 51% of patients, most of whom had a low level of expression.

Despite the previous association of ACV and cachexia in patients with lung and colorectal cancer[46], in our study, high expression of plasma ACV was not associated with cachexia, but this may be related to the small number of cachectic patients (n = 22). Additionally, in the multivariate analysis, ACV had no role in metastasis. The PDAC patients with high levels of ACV expression had lower survival rates than those of patients with low levels of ACV expression, supporting the prognostic role of ACV, as reported elsewhere[45], together with tumor size and metastatic status.

Cachexia is associated with decreased visceral body fat and is associated with a lower survival in diabetic patients than in nondiabetic patients[47]. In our study, we found no difference regarding survival in patients with or without diabetes.

Also, in our study we did not find a correlation between the presence of the cachexia and the perineural invasion. This may be due to the small number of patients who have undergone surgery (only in these patients it was possible to evaluate perineural invasion).

Patients with newly diagnosed diabetes have a 5-8 fold higher risk of being diagnosed with PDAC in the first 3 years after the development of diabetes, probably due to the direct action of insulin, which facilitates malignant transformation[48]. Diabetes can also be a secondary phenomenon that is induced by PDAC through the destruction of beta cells[49], but in our study, no correlation was noted between new-onset diabetes and the level of ACV or MK. In contrast, the MK level was higher in patients with long-term diabetes, but diabetes and MK again had no prognostic role. Hyperglycemia favors perineural invasion in PDAC by two mechanisms. First, hyperglycemia increases the proliferation of cancer cells, which increases the

Table 2 Protein plasma expression in adenocarcinoma patients and interaction with clinical and biological parameters, *n* (%)

Protein expression	Midkine		Activin	
	Low (*n* = 60)	High (*n* = 54)	Low (*n* = 42)	High (*n* = 72)
Age (yr), mean (SD)	63.02 (10.06)	66.7 (10.91)	63.64 (10.4)	65.42 (10.7)
BMI (kg/m^2), median (IQR)	24.34 (20.71-26.54)	25.79 (23.11-27.52)[a]	25.66 (22.24-27.52)	24.52 (22.05-26.94)
CA 19-9 (U/mL), median (IQR)	400 (53.5-400)	352 (70-400)	400 (53.5-400)	191 (70-400)
Age > 50 yr	53 (88.33)	50 (92.59)	36 (85.71)	67 (93.06)
Sex (female)	27 (45)	20 (37.04)	18 (42.86)	29 (40.28)
Stage (III-IV *vs* I-II)	28 (46.67)	39 (72.22)[b]	20 (47.62)	47 (65.28)
Histological grade				
G1	3 (16.67)	4 (17.39)	3 (16.67)	4 (17.39)
G2	10 (55.56)	12 (52.17)	13 (72.22)	9 (39.13)
G3	5 (27.78)	7 (30.43)	2 (11.11)	10 (43.48)
Metastasis	20 (33.33)	28 (51.85)[a]	14 (33.33)	34 (47.22)
Tumor size ≥ 3 cm	44 (78.57)	43 (87.76)	30 (75)	57 (87.69)
Weight loss of 5% over the past 6 mo	45 (90)	29 (82.86)	11 (26)	23 (32)
Weight				
Underweight	9 (15)	3 (5.56)	3 (7.14)	9 (12.5)
Normal	28 (46.67)	20 (37.04)	16 (38.1)	32 (44.44)
Overweight	15 (25)	22 (40.74)	14 (33.33)	23 (31.94)
Obesity	8 (13.33)	9 (16.67)		8 (11.11)
Smoking	32 (53.33)	18 (33.33)[a]	17 (40.48)	33 (45.83)
New-onset diabetes	16 (26.67)	8 (14.81)	8 (19.05)	16 (22.22)
Long-term diabetes	6 (10)	18 (33.33)[b]	10 (23.81)	14 (19.44)
Diabetes	22 (36.67)	27 (50)	18 (42.86)	31 (43.06)
IHC				
Negative	36 (60)	22 (40.74)[a]	29 (69.05)	26 (36.11)[b]
Weak	18 (30)	16 (29.63)	11 (26.19)	42 (58.33)
Moderate	5 (8.33)	11 (20.37)	2 (4.76)	4 (5.56)
Strong	1 (1.67)	5 (9.26)		
Immunohistochemistry perineural invasion (appearances on slide)	2: 3 (9.09); 0: 17 (51.52); 1: 13 (39.39)	2: 9 (32.14)[a]; 0: 7 (25); 1: 12 (42.86)	2: 5 (17.86); 0: 13 (46.43); 1: 10 (35.71)	2: 7 (21.21); 0: 11 (33.33); 1: 15 (45.45)
Cachexia	16 (73)	6 (27)	15 (26)	7 (32)

[a]$P < 0.05$,
[b]$P < 0.01$. BMI: Body-mass index; CA 19-9: Carbohydrate antigen; IHC: Immunohistochemistry; IQR: Interquartile range; CI: Confidence interval.

expression of cytokines such as the neurotrophic factor (NGF). The overexpression of NGF may increase the interaction between nerve cells and cancerous cells and neurotropism. The second mechanism is that hyperglycemia causes demyelination and axonal nerve degeneration, allowing cancer cells to invade nerve structures. These two mechanisms promote hyperglycemia or diabetes and may have a role in perineural invasion in pancreatic cancer[50]. The intervention of hyperglycemia and signaling between neurons and cancer cells was associated with a higher expression of NGF in PDAC cells and the p75 neurotrophin receptor in nerve fibers in an experimental study[51].

The limitations of this study are that perineural invasion was only examined in patients who underwent surgery (and not in all patients) and survival analysis was performed only for cancer patients. Another limitation of the study is that clinicopathological associations were made based on the expression of plasma proteins rather than in tissue. Further studies are, therefore, warranted to explore the mechanism of ACV and MK function, to investigate their potential as therapeutic targets in PDAC progression and the relationship with diabetes and perineural invasion.

In conclusion, the present data suggest that MK is a useful biomarker for perineural

Table 3 Univariate and multivariate analysis to predict metastasis

	Univariate analyses			Multivariate analyses		
	OR unadjusted	95%CI	*P* value	OR unadjusted	95%CI	*P* value
Age > 50 yr	0.95	0.23-4.16	0.942	1.24	0.33-5.2	0.75
Sex (male *vs* female)	1.25	0.53-2.99	0.617	1.27	0.56-2.91	0.571
N1	2.04	0.53-10.09	0.326	2.27	0.61-10.92	0.251
Tumor size ≥ 3 cm	0.87	0.28-2.8	0.809	1.13	0.4-3.39	0.821
Midkine expression (high-expressed *vs* low-expressed)	2.12	0.92-5	0.08	1.92	0.87-4.33	0.11
Activin expression (high-expressed *vs* low-expressed)	1.94	0.78-5.01	0.157	1.62	0.71-3.81	0.261

OR: Odds ratio; HR: Hazard ratio; CI: Confidence interval.

invasion, being also correlated with advanced tumor stage, the presence of metastasis and diabetes. Unfortunately, the perineural invasion and the expression of activin was not correlated with casexia, but ACV could be an effective biomarker for predicting poor prognosis in PDAC patients, and it might be a novel therapeutic marker for selectively targeting cancer cells.

Table 4 Univariate and multivariate analysis to predict survival

	Univariate analyses			Multivariate analyses		
	HR adjusted	95%CI	*P* value	HR adjusted	95%CI	*P* value
Age > 50 yrs	2.43	1.18-5.01	0.016	1.9	0.86-4.2	0.113
Sex (male *vs* female)	0.51	0.34-0.76	0.001	0.66	0.42-1.05	0.078
N1	1.42	0.74-2.73	0.299	1.13	0.54-2.36	0.745
Tumor size ≥ 3 cm	2.13	1.13-4.01	0.02	2.36	1.21-4.62	0.012
Metastases	2.09	1.4-3.12	< 0.001	1.62	1.01-2.62	0.047
Midkine expression (high-expressed *vs* low-expressed)	1.67	1.12-2.48	0.011	0.76	0.48-1.2	0.24
Activin expression (high-expressed *vs* low-expressed)	3.61	2.34-5.56	< 0.001	1.98	1.2-3.27	0.008

OR: Odds ratio; HR: Hazard ratio; CI: Confidence interval.

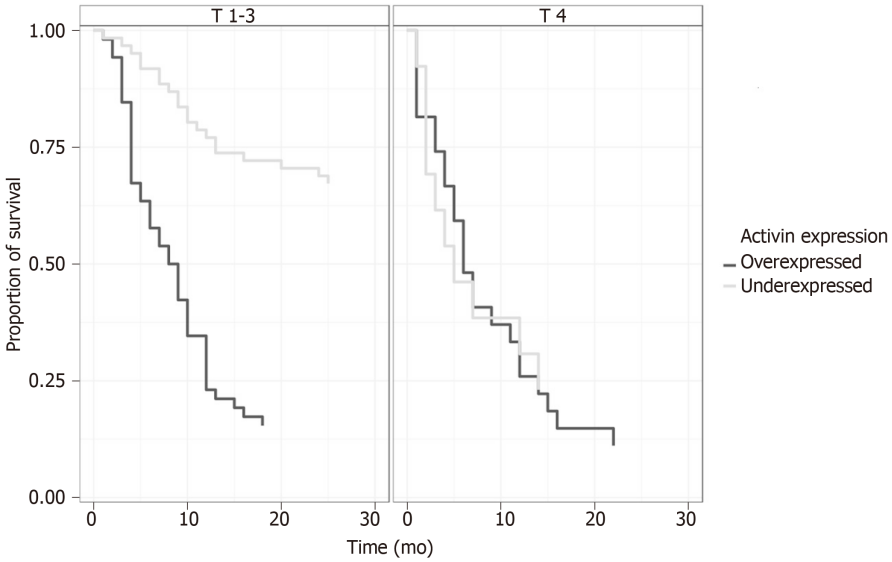

Figure 3 Overall survival comparison between high and low-expressed Activin stratified by tumoral stage T1-3 *vs* T4.

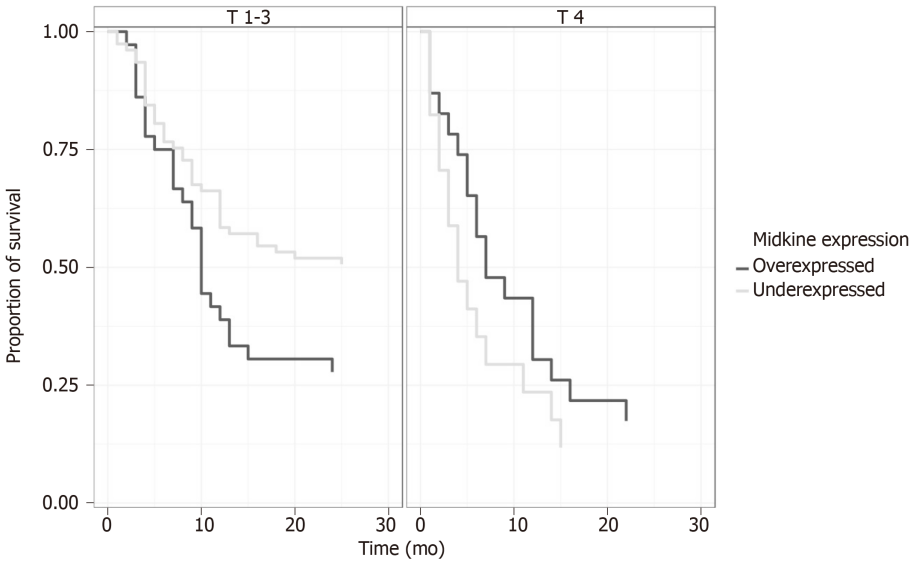

Figure 4 Overall survival comparison between high and low-expressed Midkine stratified by tumoral stage T1-3 *vs* T4.

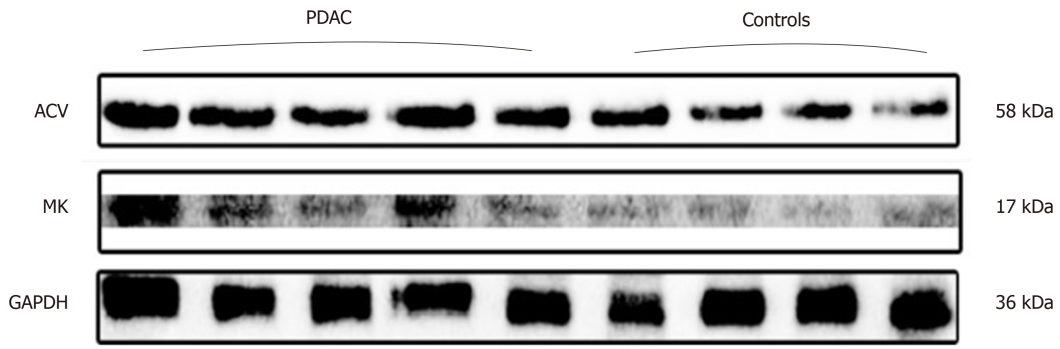

Figure 5 Western blot analyses of Activine and Midkine in patients with pancreatic adenocarcinoma and controls.

ARTICLE HIGHLIGHTS

Research background

Pancreatic cancer has a high mortality rate, due to late diagnosis. Cachexia and perineural invasion have an increased incidence in pancreatic cancer, leading to decreased quality of life. Combating pain and cachexia through optimal treatment options can lead to increased quality of life and improved survival.

Research motivation

We wanted a better understanding of the involvement of cachexia and pain in pancreatic cancer and their relationship with different clinico-pathological factors, constituting a basis for discovering new therapeutic targets.

Research objectives

Defining the profile of cachexia in pancreatic cancer; establishing the degree of perineural invasion in pancreatic cancer; Highlighting the interrelationship between cachexia and perineural invasion in pancreatic cancer.

Research methods

We conducted a prospective study in 114 patients with pancreatic cancer and 125 healthy people as controls. Blood samples were used and pancreatic tissue were collected through EUS-FNA or surgery. The method of determining the biomarkers of cachexia (Activin) and perineural invasion (Midkine) in plasma was western blot, respectively immunohistochemistry in pancreatic tissue.

Research results

The analysis of the data showed an Activin (ACV) and Midkine (MK) proteins overexpression in plasma of patients with pancreatic cancer *vs* control, results that were correlated with the expression of proteins in the pancreatic cancer tissue. MK was also significantly correlated with advanced T stage, metastasis, diabetes and perineural invasion. ACV was significantly correlated with survival.

Research conclusions

MK can be considered a biomarker for perineural invasion, and ACV a prognostic factor for patients with pancreatic cancer.

Research perspectives

This research would open new perspectives in choosing the treatment that involves activin antagonists in order to prolong survival in patients with pancreatic cancer.

ACKNOWLEDGEMENTS

We thank Dr. Cristian Coman, Institute of Biology Research, Cluj-Napoca and Associate Prof. Manuela Banciu, PhD, Faculty of Biology and Geology, University of Babes-Bolyai, Cluj-Napoca for their support. This study was partially funded by the "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, through the Doctoral Research Project-2015 (No. 7690/36/15.04.2016). The financial support allocated from the grant was used for the acquisition of biomarkers and laboratory supplies. The sponsor had no involvement in the study design, collection, analysis and interpretation of data, writing of the manuscript or decision to submit the manuscript for publication.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1141-1150

DOI: 10.4251/wjgo.v11.i12.1141

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Case Control Study

Protein expression trends of DNMT1 in gastrointestinal diseases: From benign to precancerous lesions to cancer

Tian-Miao Ma, Li-Ping Sun, Nan-Nan Dong, Ming-Jun Sun, Yuan Yuan

ORCID number: Tian-Miao Ma (0000-0001-8775-6633); Li-Ping Sun (0000-0003-3637-0305); Nan-Nan Dong (0000-0003-3659-3365); Ming-Jun Sun (0000-0002-8852-5535); Yuan Yuan (0000-0002-7394-9036).

Author contributions: Yuan Y contributed to study design and manuscript revision; Ma TM contributed to data interpretation, performed the experiments, and drafted the manuscript; Sun LP contributed to experiment instruction, data interpretation, and statistical analysis; Dong NN contributed to sample preparation; and Sun MJ contributed to sample collection and experimental instruction.

Institutional review board statement: The study was approved by the ethics committee of the First Hospital of China Medical University (Shenyang, China).

Informed consent statement: All patients gave informed consent.

Conflict-of-interest statement: No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Data sharing statement: Technical appendix, statistical code, and dataset available from the corresponding author at yuanyuan@cmu.edu.cn

Open-Access: This article is an open-access article which was selected by an in-house editor and

Tian-Miao Ma, Ming-Jun Sun, Department of Gastroenterology, the First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China

Li-Ping Sun, Nan-Nan Dong, Yuan Yuan, Tumor Etiology and Screening Department of Cancer Institute and General Surgery, the First Hospital of China Medical University, Shenyang 110001, Liaoning Province, China

Corresponding author: Yuan Yuan, MD, PhD, Professor, Tumor Etiology and Screening Department of Cancer Institute and General Surgery, the First Hospital of China Medical University, No. 155, Nanjingbei Street, Heping District, Shenyang 110001, Liaoning Province, China. yuanyuan@cmu.edu.cn
Telephone: +86-24-83282153

Abstract

BACKGROUND

In recent years, the incidence of gastrointestinal (GI) cancer in China has increased annually. Early detection and appropriate therapy are considered to be the key to treat GI cancer. DNMT1 takes an active part in the advancement of GI cancer, which will change as the disease progresses. But its expression characteristics in the dynamic variations of GI carcinogenesis are still unclear.

AIM

To investigate the expression characteristics of DNMT1 in different GI diseases.

METHODS

We detected the expression of DNMT1 in 650 cases of different GI diseases by immunohistochemistry, including 90 cases of chronic superficial gastritis (CSG), 72 cases of atrophic gastritis with intestinal metaplasia (AG/GIM), 54 cases of low-grade intraepithelial neoplasia (GLIN), 66 cases of high-grade intraepithelial neoplasia (GHIN), 71 cases of early gastric cancer (EGC), 90 cases of normal intestinal mucosa (NIM), 54 cases of intestinal low-grade intraepithelial neoplasia (ILIN), 71 cases of intestinal high-grade intraepithelial neoplasia (IHIN), and 82 cases of early colorectal cancer (ECRC).

RESULTS

In the CSG group, all cases showed weakly positive or negative expression of DNMT1. However, in other four groups (AG/GIM, GLIN, GHIN, and EGC), the positive expression rate gradually increased with the severity of the diseases; the negative or weakly positive cases accounted for 55.56% (40/72), 38.89% (21/54),

Manuscript source: Unsolicited manuscript

Received: March 30, 2019
Peer-review started: April 3, 2019
First decision: July 31, 2019
Revised: September 4, 2019
Accepted: September 12, 2019
Article in press: September 13, 2019
Published online: December 15, 2019

P-Reviewer: Senchukova M, Youssef Mohamed S
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Qi LL

1.52% (1/66), and 1.41% (1/71), respectively. Besides, the moderately positive cases were 44.44% (32/72), 57.41% (31/54), 80.30% (53/66), and 43.66% (31/71), respectively. The strongly positive cases only existed in the GLIN (3.70%, 2/54), GHIN (18.18%, 12/66), and EGC (54.93%, 39/71) groups. The differences between any two groups were statistically significant ($P < 0.05$). Similarly, in the NIM group, cases with weakly positive expression of DNMT1 were predominant (91.11%, 82/90), and the rest were moderately positive cases (8.89%, 8/90). In the ILIN, IHIN, and ECRC groups, the rates of cases with weak or negative expression of DNMT1 were 46.30% (25/54), 12.68% (9/71), and 4.88% (4/82), respectively; with moderately positive expression were 53.70% (29/54), 71.83% (51/71), and 34.15% (28/82), respectively; and with strongly positive expression were 0.00% (0/54), 15.49% (11/71), and 60.98% (50/82), respectively. The differences between any two groups were also statistically significant ($P < 0.05$).

CONCLUSION
The overexpression of DNMT1 protein could effectively predict early GI cancers and severe precancerous lesions, which may have potential clinical application value.

Key words: Gastric cancer; Colorectal cancer; DNMT1; Precancerous lesion; Intraepithelial neoplasia

Core tip: The expression of DNMT1 was significantly effective for screening precancerous lesions and cancers. DNMT1 could be a potential marker for the diagnosis of gastric cancer and colorectal cancer. At the same time, the fluctuation of its expression may suggest the progression of gastrointestinal diseases, which could be instructive for further examination and treatment.

Citation: Ma TM, Sun LP, Dong NN, Sun MJ, Yuan Y. Protein expression trends of DNMT1 in gastrointestinal diseases: From benign to precancerous lesions to cancer. *World J Gastrointest Oncol* 2019; 11(12): 1141-1150
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1141.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1141

INTRODUCTION

The gastrointestinal (GI) tract plays a principal role in the human digestive system, and nearly all the essential nutrients for the human body are absorbed and digested through the GI tract[1]. As a result of unhealthy diet and deteriorating environment, the incidence of GI diseases has increased rapidly[2]. Both gastric cancer (GC) and colorectal cancer (CRC) are malignant GI diseases that require a progressive development process. Early detection and appropriate treatment are the effective measures to delay the GI tumorigenesis[3]. It is well known that common screening methods for GI cancer include gastroscopy, imaging examination, and biomarker examination. Due to the lack of specificity in the early clinical manifestations of GI cancer, the efficiency of imaging examination is not obvious. Endoscopy also has certain limitations due to the invasiveness and the demanding requirements for endoscopists. In comparison, tumor marker detection has several advantages in screening cancers, such as convenient source, good tolerance, and low cost.

DNMT1 is a crucial member during DNA replication, which could copy DNA methylation patterns from the parental DNA strand to the newly synthesized strand[4]. The incidence of cancer cells depends on specific gene silencing mediated by DNA methylation[5], and the primary function of DNMT1 is maintaining and stabilizing gene methylation[6]. When the aberrantly methylated occurs in the mammalian genome, DNMT1 will be transcribed in the nucleus with the expression gradually enhanced, promoting the advancement of DNA methylation and the procession of tumors[7]. Previously, we illustrated that the expression of DNMT1 was significantly higher in GC tissues than in non-GC tissues by meta-analysis[8]. Simultaneously, several studies have also revealed that DNMT1 was strongly expressed in GC and

CRC[9,10], suggesting that DNMT1 could be a potential biomarker in screening of GI cancer.

However, the expression of DNMT1 protein in the dynamic variations of GI carcinogenesis, especially in precancerous lesions, is still unclear. In this study, from the perspective of the evolution process of GI cancer, we measured the protein expression of DNMT1 in different stages of GI diseases (from benign to precancerous to cancerous), investigated its expression characteristics, and further analyzed its clinical application value as a warning biomarker in pre-diagnosis of early GI cancer.

MATERIALS AND METHODS

Study objective

A total of 650 patients with different GI diseases diagnosed by histopathology at the Department of Endoscopy and Anorectal Surgery of the First Hospital of China Medical University from August 2012 to December 2017, who had not received preoperative chemotherapy or radiation, were selected. All the pathological diagnoses were made following the updated Sydney Gastritis Classification and World Health Organization Classification of Tumors of the Digestive System[11,12]. Among them, there were 353 cases of different gastric diseases (210 men and 143 women, with an average age of 60.03 years [range 16-88 years]), including 90 cases of chronic superficial gastritis (CSG), 72 cases of atrophic gastritis with intestinal metaplasia (AG/GIM), 54 cases of low-grade intraepithelial neoplasia (GLIN), 66 cases of high-grade intraepithelial neoplasia (GHIN), and 71 cases of early gastric cancer (EGC). In addition, 297 cases of colorectal disease were also contained in this study (156 men and 141 women, with a mean age of 58.27 years [range 20-85 years]), including 90 cases of normal intestinal mucosa (NIM), 54 cases of intestinal low-grade intraepithelial neoplasia (ILIN), 71 cases of intestinal high-grade intraepithelial neoplasia (IHIN), and 82 cases of early colorectal cancer (ECRC). The study protocol was approved by the Human Ethics Review Committee of the First Hospital of China Medical University. Written informed consent was obtained from each participant.

Immunohistochemical staining and scoring

All biopsies were fixed in 10% formalin and embedded in paraffin. After sectioning at 4-micron thickness and mounting on positive-charged glass slides, these specimens were dewaxed by xylene, rehydrated with gradient alcohol, rinsed through tap water, and soaked in phosphate-buffered saline (PBS) solution (PH 7.4) for 10 min. The tissues were treated in boiling citric acid buffer (PH 6.0) for 1.5 min to complete antigen retrieval. Next, each slide was added with 50 μL of peroxidase blocking solution, and incubated at room temperature (25 ± 2 °C) for 20 min before rinsing with PBS solution. Tissue collagen was blocked by the addition of 50 μL of normal goat serum at room temperature for another 20 min. After draining excess liquid, the sections were incubated with primary antibody against DNMT1 (1: 500, Abcam, ab13537, Cambridge, United Kingdom) for 1 h at 37 °C, and rinsed three times with PBS solution. The buffer was then removed from the coverslip, followed by incubation with 50 μL of goat anti-rabbit antibody and 50 μL of streptomyces avidin-peroxidase for 10 min each. Afterwards, 50 μL of fresh DAB (Maixin, Fujian, China) was added per section for 1-1.5 min. Finally, the sections were washed with PBS, counterstained in hematoxylin, blued in running water, dehydrated with gradient alcohol, cleared with xylene, and mounted with neutral gum.

Two independent pathologists who were blinded to the clinicopathologic characteristics of the patients read and scored these immunohistochemical slides with DNMT1-positive expression. A semi-quantitative method was used to evaluate the area and intensity of the staining results. If the scores given by the two pathologists differ by more than one grade, the results would be reconsidered and discussed to determine the final score. The staining intensity of cells from different tissues was scored as 0-3 (I_{0-3}): I_0 (no staining), I_1 (light yellow), I_2 (dark yellow), and I_3 (brown or dark brown). The percentage of positive cells was scored as 0-3 (P_{0-3}): P_0 (0%); P_1 (>0 but ≤1/3), P_2 (>1/3 but ≤2/3), and P_3 (>2/3). On the basis of semiquantitative scoring, the expression of DNMT1 in the nucleus was assessed using the following formula: IS score = In × Pm. Finally, the protein expression of DNMT1 was graded as follows: negative or weakly positive expression (+), 0-1; moderately positive expression (++), 2-4; and strongly positive expression (+++), 5-9.

Statistical analysis

Statistical analyses were performed in any two gastric groups and intestinal groups separately, by the rank sum test using SPSS vision 22.0 (IBM SPSS statistics, Armonk,

NY, United States) on the basis of DNMT1 positivity, and $P < 0.05$ was considered statistically significant.

RESULTS

DNMT1 expression in different gastric diseases

The representative photomicrographs of immunohistochemical staining for DNMT1 in different gastric disease are shown in Figure 1. DNMT1 was hardly expressed in the CSG group (0/90). The negative or weakly positive expression rates of DNMT1 in the AG/GIM and GLIN groups were separately 55.56% (40/72) and 38.89% (21/54), and the moderately positive expression rates were not very high, being 44.44% (32/72) and 57.41% (31/54), respectively. The strongly positive expression was scarcely in these two groups, with 0.00% (0/72) and 3.70% (2/54), respectively. In the GHIN group, the expression of DNMT1 was mainly moderately-positive, which accounted for 80.30% (53/66). There was still one negative or weakly positive case (1.52%, 1/66), and 12 strongly positive cases (18.18%, 12/66). In the GC group, the number of cases with strongly positive expression was the highest among the five groups (39/71, 54.93%). In addition, this group also had 1 case with negative or weakly positive expression of DNMT1 (1.41%) and 31 cases with moderately positive (43.66%) (Figure 2). The differences between any two groups were statistically significant ($P < 0.05$) (Table 1).

DNMT1 expression in different intestinal diseases

The typical photomicrographs of immunohistochemical staining for DNMT1 in different intestinal diseases are shown in Figure 3. The negative or weakly positive expression rates of DNMT1 protein in the NIM and ILIN groups were 91.11% (82/90) and 46.30% (25/54), and the moderately positive rates were 8.9% (8/90) and 53.7% (29/54), respectively. But there were no strongly positive expression in either group. In the IHIN group, the cases with moderately positive expression were predominant, accounting for 71.83% (51/71). The proportions of weakly positive cases and strongly positive cases in this group were almost the same, being 12.68% (9/71) and 15.49% (11/71), respectively. The majority of cases in the ECRC group had strongly positive expression, accounting for 60.98% (50/82), while those with negative or weakly positive expression accounted for 4.88% (4/82). The remaining part was 34.15% (28/82) (Figure 4). The differences between any two groups were statistically significant ($P < 0.05$) (Table 2).

DISCUSSION

The occurrence of both GC and CRC has a long-term evolving process of "normal-dysplasia-cancer"[13,14]. Accurate identification in early stage of carcinogenesis presents a positive effect on risk intervention and prognostic management[15]. DNA methylation is a kind of pivotal epigenetic modification, which can be apparently altered in precancerous lesions[16]. As a key part in maintaining the process of methylation, the level of DNMT1 expression could be changed to varying degrees in most tumors and even earlier conditions[17]. In this research, we recorded the expression features of DNMT1 protein in different GI diseases, interpreted the dynamic changes of DNMT1 from benign to precancerous to cancer in a full disease chain, and explored its warning role for GI cancer identification.

Considering the evolution of GC, five groups of specimens (including CSG, AG/GIM, GLIN, GHIN, and EGC) were collected to analyze the protein expression of DNMT1 in the present study. In accordance with the results, we could figure out that DNMT1 was hardly expressed in CSG tissues, but its expression was gradually up-regulated in AG/GIM and GLIN tissues, and much higher in the GHIN and EGC groups than in the others. This indicated that the expression level of DNMT1 protein increases with the severity of gastric diseases. Intestinal metaplasia is an important histopathological transformation in atrophic gastritis, which is deemed as a precancerous lesion of GC[18]. Certain abnormalities in tissue structures and physiological functions may turn out in this period. If the interventions can be taken in a timely manner, this situation could be reversed during disease progression[19]. Therefore, the identification of AG/GIM with potential malignant transformation would effectively reduce the possibility of GC. It should be on high alert if DNMT1 has positive expression in order to avoid missing the optimal timing of diagnosis and treatment[20]. Intraepithelial neoplasia (IN), especially high-grade intraepithelial neoplasia (HIN), is a non-invasive intramucosal neoplasia with high cellular and

Table 1 Expression of DNMT1 in different gastric diseases

Group	Cases	(+)		(++)		(+++)		*P* value
		Cases	Ratio (%)	Cases	Ratio (%)	Cases	Ratio (%)	
CSG	90	90	100.00	0	0.00	0	0.00	0.00[a]
AG/GIM	72	40	55.56	32	44.44	0	0.00	0.044[b],0.00[c]
GLIN	54	21	38.89	31	57.41	2	3.70	0.00[d]
GHIN	66	1	1.52	53	80.30	12	18.18	0.00[e]
EGC	71	1	1.41	31	43.66	39	54.93	

[a]*P*: CSG *vs* other groups;
[b]*P*: AG/GIM *vs* GLIN;
[c]*P*: AG/GIM *vs* GHIN and EGC;
[d]*P*: GLIN *vs* GHIN and EGC;
[e]*P*: GHIN *vs* EGC. CSG: Chronic superficial gastritis; AG/GIM: Atrophic gastritis with intestinal metaplasia; GLIN: Gastric low-grade intraepithelial neoplasia; GHIN: Gastric high-grade intraepithelial neoplasia; EGC: Early gastric cancer.

structural atypia, which was defined as carcinoma *in situ* or intramucosal carcinoma in Japan[21]. This study evidenced that the expression of DNMT1 was obviously elevated in the GHIN and EGC groups, testifying that the overexpression of DNMT1 may be an initial event of GC. Our experiments demonstrated that DNMT1 expression had a close relationship with the development of GC, which can be used to monitor the progression of gastric diseases dynamically, and help to distinguish GHIN and GC from benign gastric diseases to some extent.

Studies[22,23] have proved that colon cancer cells cannot survive in the absence of abnormal promoter DNA methylation, and the complete knockdown of DNMT1 would result in the death of a large number of cancer cells. They proposed a strong correlation between DNMT1 and CRC cell viability. We observed the expression of DNMT1 in the NIM, ILIN, IHIN, and ECRC groups. The results showed that the level of DNMT1 protein expression progressively increased with the severity of intestinal lesions. In the NIM group, the positive expression rate of DNMT1 was the lowest. In the ILIN group, there were only weakly or moderately positive cases, and few strongly positive cases. However, when the pathological lesion progressed to the IHIN or ECRC stage, the positive expression rate of DNMT1 was much higher. This trend in intestinal diseases was basically consistent with that in gastric diseases. The precancerous lesions were regarded as the penultimate parts of CRC[24]. Accurate diagnosis, management, and monitoring of the disease at this stage and the adoption of appropriate treatments are very essential to decrease the incidence of cancer[25]. The up-regulation of DNMT1 can usually precede the appearance of DNA methylation[26], which is instructive for estimating the malignancy of precancerous lesions.

The previous classification and assessment of tumors were usually based on the lesion location (like lung cancer, breast cancer, *etc.*) or morphological characteristics (like adenocarcinoma, squamous carcinoma, *etc.*), not reflecting the nature of the disease. Recently, discerning tumors by markers rather than sources has become a theory that more and more scholars advocated, which means the understanding of cancer has risen to a molecular field[27]. In May 2017, the United States Food and Drug Administration approved Keytruda for the treatment of MSI/dMMR solid tumors[28]. This was the first anti-tumor therapy according to genetic biomarker, representing a milestone in cancer treatment. Implementing same or similar diagnosis and follow-up treatment for various tumors based on specific gene targets can provide a new idea for cancer research, and realize the precise medical concept of "diagnose and treat different diseases in same ways"[29]. Changes in genomic DNA methylation status could cause chromosomal instability or abnormal gene expression, leading to tumorigenesis eventually[30]. DNMT1 could be significantly overexpressed in the primary stages of many cancers[31-33], and thus be utilized as a common marker for most tumors and precancerous lesions. Our study simultaneously detected the expression of DNMT1 in various gastric and colorectal diseases from benign to precancerous to cancer, with a consistent trend observed. It gave a proof that DNMT1 could be a common marker for these two cancers, and the fluctuation of its expression may prompt the progression of GI diseases. The quantity of DNMT1 protein ascended with the aggravation of mucosal lesions, which is of vital importance not only for the early diagnosis of GI cancer, but also for further clinical treatment. Targeted therapy for GI cancer with DNMT1 overexpression would become the future research

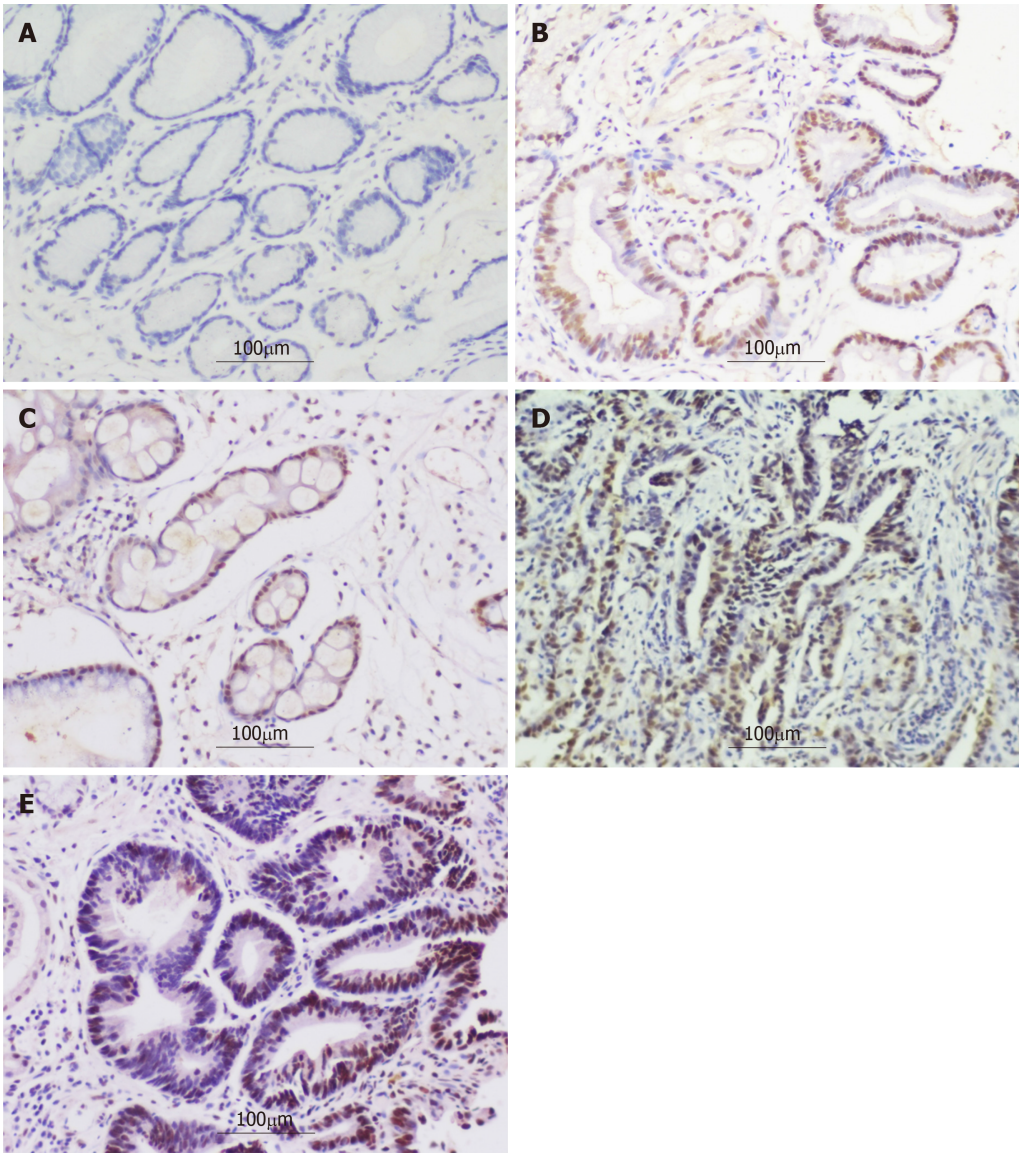

Figure 1 Expression of DNMT1 in different gastric diseases (×20). A: Chronic superficial gastritis [negative expression; score, 0 (+)]; B: Atrophic gastritis with intestinal metaplasia [moderately positive expression; score, 2 (++)]; C: Gastric low-grade intraepithelial neoplasia [moderately positive expression; score, 4 (++)]; D: Gastric high-grade intraepithelial neoplasia [strongly positive expression; score, 6 (+++)]; E: Early gastric cancer [strongly positive expression; score, 9 (+++)].

direction[34].

In summary, this is the first comprehensive study explaining the expression trend of DNMT1 from benign to precancerous to cancer, and discovering the inseparable relationship between DNMT1 overexpression and GI diseases. In cancer or HGIN tissues, DNMT1 is expressed greatly higher than that in non-cancer mucosa or LGIN tissues, which suggests that DNMT1 could discriminate between cancerous tissues and non-cancer tissues, as well as evaluate the extent of precancerous lesions possibly, warning the patients for further examination and treatment. DNMT1 could be used as a potential biomarker for early detection of GI cancer.

Table 2 Expression DNMT1 in different intestinal diseases

Group	Cases	(+)		(++)		(+++)		*P* value
		Cases	Ratio (%)	Cases	Ratio (%)	Cases	Ratio (%)	
NIM	90	82	91.11	8	8.89	0	0.00	0.00[a]
ILIN	54	25	46.30	29	53.70	0	0.00	0.00[b]
IHIN	71	9	12.68	51	71.83	11	15.49	0.00[c]
ECRC	82	4	4.88	28	34.15	50	60.98	

[a]*P*: NIM *vs* other groups;
[b]*P*: ILIN *vs* other groups;
[c]*P*: IHIN *vs* ECRC. NIM: Normal intestinal mucosa; ILIN: Intestinal low-grade intraepithelial neoplasia; IHIN: Intestinal high-grade intraepithelial neoplasia; ECRC: Early colorectal cancer.

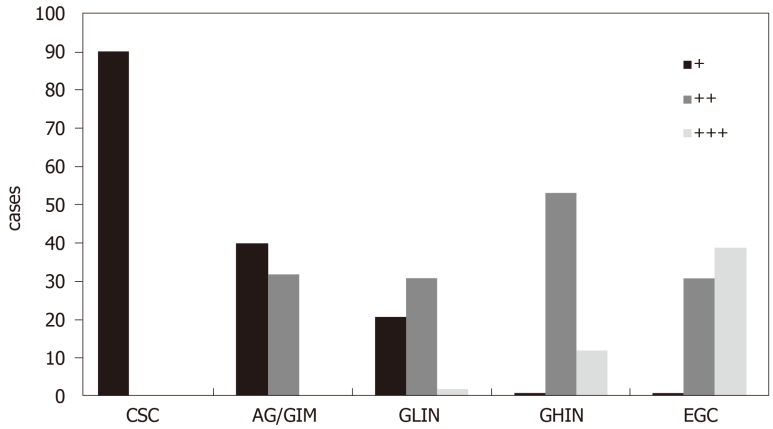

Figure 2 Comparison of DNMT1 expression in different gastric disease groups.

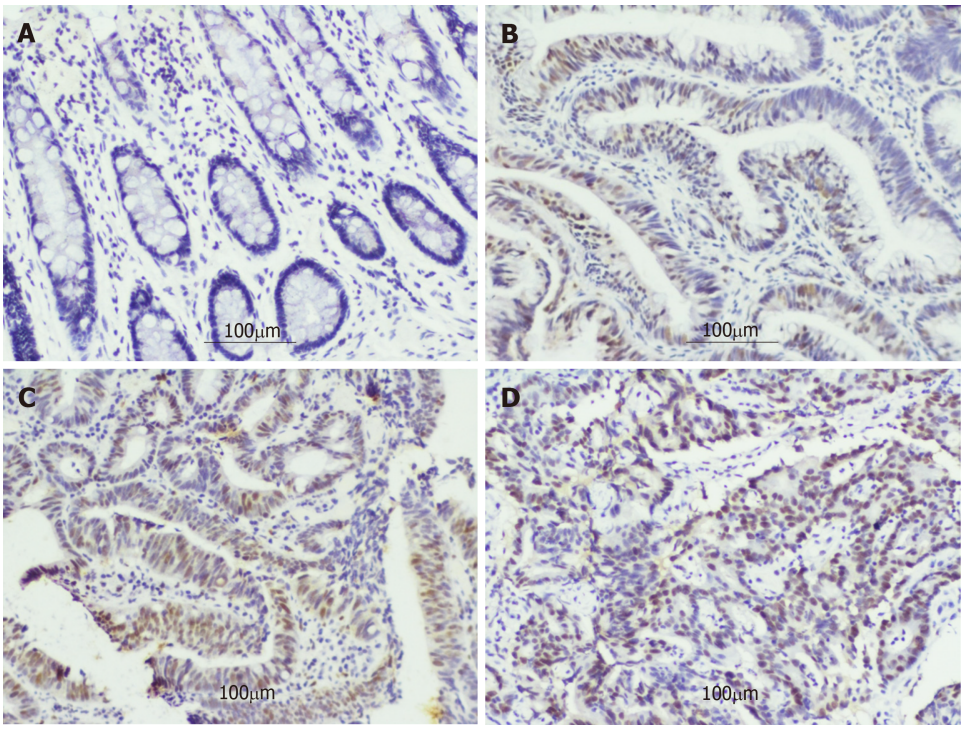

Figure 3 Expression of DNMT1 in different intestinal diseases (×20). A: Normal intestinal mucosa [negative expression; score, 0 (+)]; B: Intestinal low-grade intraepithelial neoplasia [moderate expression; score, 2 (++)]; C: Intestinal high-grade intraepithelial neoplasia [strong expression; score, 6 (+++)]; D: Early colorectal cancer [strong expression; score, 9 (+++)].

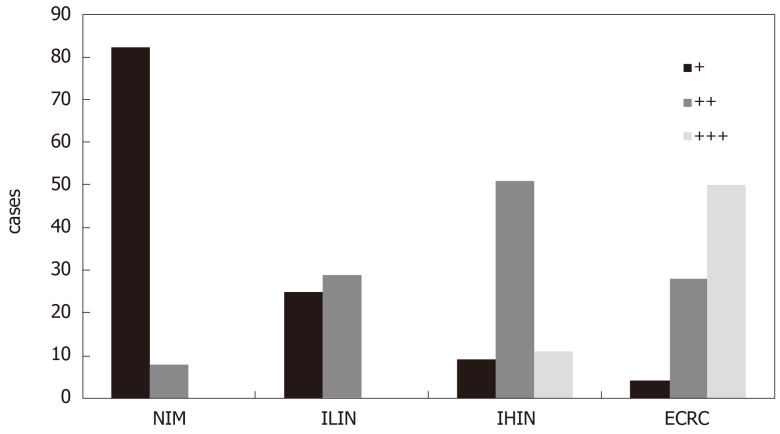

Figure 4 Comparison of DNMT1 expression in different intestinal disease groups.

ARTICLE HIGHLIGHTS

Research background

In recent years, the incidence of gastrointestinal (GI) cancer in China has increased annually. Early detection and appropriate therapy are considered to be the key to treating GI cancer. Unfortunately, at present, the early detection rates of gastric cancer (GC) and colorectal cancer (CRC) are both less than 10% of all diagnosed cases on average. Previously, we illustrated that the expression of DNMT1 was significantly higher in GC tissues than in non-GC tissues by meta-analysis. Simultaneously, several studies have also revealed that DNMT1 is strongly expressed in GC and CRC, suggesting that DNMT1 could be a potential biomarker in screening of GI cancer. However, the expression of DNMT1 protein in the dynamic variations of GI carcinogenesis, especially in precancerous lesions, is still unclear.

Research motivation

In this study, from the perspective of the evolving process of GI cancer, we measured the expression of DNMT1 protein in different stages of GI diseases (from benign to precancerous to cancerous), investigated its expression characteristics, and further analyzed its clinical application value as a warning biomarker in diagnosis of early GI cancer.

Research objectives

Although we only used patient tissues as our samples to detect the dynamic variation of DNMT1 protein expression in different gastrointestinal diseases, we consider DNMT1 may be used as part of a future serological test to assess the severity of patients with gastrointestinal disorders and to prompt them to complete more tests, which could help to discover the disease as soon as possible and take appropriate treatment next step.

Research methods

A total of 650 patients with different gastrointestinal diseases diagnosed pathologically, who had not received preoperative chemotherapy or radiation, were enrolled in our study. Among them, there were 353 cases of different gastric diseases, including 90 cases of chronic superficial gastritis, 72 cases of atrophic gastritis with intestinal metaplasia (AG/GIM), 54 cases of low-grade intraepithelial neoplasia (GLIN), 66 cases of high-grade intraepithelial neoplasia (GHIN), and 71 cases of early gastric cancer (EGC). In addition, 297 cases of colorectal diseases were also contained in our experiment, including 90 cases of normal intestinal mucosa (NIM), 54 cases of intestinal low-grade intraepithelial neoplasia (ILIN), 71 cases of intestinal high-grade intraepithelial neoplasia (IHIN), and 82 cases of early colorectal cancer (ECRC). Immunohistochemistry (IHC) was used to detect the expression of DNMT1 in these cases. Statistical analysis was performed in any two gastric groups and intestinal groups separately, by rank sum test on the basis of DNMT1 positivity, and $P < 0.05$ was considered statistically significant.

Research results

In accordance with the results, we could figure out that DNMT1 was hardly expressed in chronic superficial gastritis tissues, but its expression was gradually up-regulated in atrophic gastritis with intestinal metaplasia and low-grade intraepithelial neoplasia tissues, and much higher in high-grade intraepithelial neoplasia and early gastric cancer. This indicated that the expression level of DNMT1 protein increases with the severity of gastric diseases. In different intestinal diseases, the changes in DNMT1 expression were consistent with the dynamic trend in gastric diseases. In the normal intestinal mucosa group, the positive expression rate of DNMT1 was the lowest. And in the intestinal low-grade intraepithelial neoplasia group, there were many weakly or moderately positive cases, and few strongly positive cases. However, in the high-grade intraepithelial neoplasia or early colorectal cancer stage, the positive expression rate of DNMT1

was much higher. In conclusion, the up-regulation of DNMT1 can usually precede the appearance of DNA methylation, which is instructive for estimating the malignancy of precancerous lesions.

Research conclusions

DNMT1 can not only distinguish between normal tissues and cancerous tissues, but also assess the extent of precancerous lesions. With the aggravation of GI mucosal lesions, the expression of DNMT1 is rapidly increasing, which is of great significance for the early diagnosis of GI cancer. DNMT1 is a gene with significantly differential expression in gastrointestinal diseases, and could be used for early diagnosis of GI cancer and guidance of clinical treatment.

Research perspectives

The present study suggested that the expression level of DNMT1 in the gastrointestinal mucosa significantly correlates with disease progression, thus being used as an early warning sign of cancer. In the future, the feasibility of serum DNMT1 expression detection as an early warning sign of gastrointestinal risks can be investigated.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1151-1160

DOI: 10.4251/wjgo.v11.i12.1151

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Asian Americans have better outcomes of non-metastatic gastric cancer compared to other United States racial groups: A secondary analysis from a randomized study

Omar Abdel-Rahman

ORCID number: Omar Abdel-Rahman (0000-0002-5117-2502).

Author contributions: Abdel-Rahman O made the conception, analysis and writing of the manuscript.

Institutional review board statement: As this manuscript is based on a publicly available database (project data sphere), IRB approval was not required.

Informed consent statement: As this manuscript is based on a publicly available database of de-identified records (project data sphere), informed consent was not required.

Conflict-of-interest statement: The author has no conflict of interest.

Data sharing statement: Dataset of the current study is available upon request from the online platform (Project Data Sphere): https://projectdatasphere.org/projectdatasphere/html/home

Omar Abdel-Rahman, Department of Oncology, University of Alberta and Cross Cancer Institute, Edmonton, Alberta, Canada

Corresponding author: Omar Abdel-Rahman, MBChB, Academic Fellow, Department of Oncology, University of Alberta and Cross Cancer Institute, Edmonton, Alberta T2N4C2, Canada. omar.abdelsalam@ahs.ca
Telephone: +1-780-4328221
Fax: +1-780-4328888

Abstract

BACKGROUND

It has been recognized for a long time that gastric cancer behavior and outcomes might be different between patients living in Asian countries *vs* patients living in Western countries. It is not clear if these differences would persist between patients of Asian ancestry and patients of other racial subgroups within the multiethnic communities of North America. The current study hypothesizes that these differences will present within North American multiethnic communities.

AIM

To evaluate the impact of race on survival outcomes of non-metastatic gastric cancer patients in the United States.

METHODS

This is a secondary analysis of a randomized controlled trial (CALGB 80101 study) that evaluated two adjuvant chemoradiotherapy schedules following resection of non-metastatic gastric cancer. Kaplan-Meier analysis and log-rank testing were utilized to explore the overall and disease-free survival differences according to the race of the patients. Univariate and multivariate Cox regression analyses were then used to explore factors affecting overall and disease-free survivals.

RESULTS

A total of 546 patients were included in the current analysis. Of which, 73.8% have white race (*vs* 12.8% black Americans and 8.2% Asian Americans). Using Kaplan-Meier analysis/log-rank testing, Asian Americans appear to have better overall and disease-free survival outcomes compared to other United States racial groups (White Americans, Black Americans, and other racial groups) ($P = 0.011$; P

Manuscript source: Invited manuscript

Received: March 4, 2019
Peer-review started: March 4, 2019
First decision: July 31, 2019
Revised: September 18, 2019
Accepted: October 14, 2019
Article in press: October 14, 2019
Published online: December 15, 2019

P-Reviewer: Mikulic D
S-Editor: Dou Y
L-Editor: Filipodia
E-Editor: Ma YJ

= 0.010; respectively). Moreover, in an adjusted multivariate model, Asian American race seems to be associated with better overall and disease-free survival (hazard ratio: 0.438; 95% confidence interval: 0.254-0.754), P = 0.003; hazard ratio: 0.460; 95% confidence interval: 0.280-0.755, P = 0.002; respectively).

CONCLUSION
Asian American patients with non-metastatic gastric cancer have better overall and disease-free survival compared to other racial groups in the United States. Further preclinical and clinical research is needed to clarify the reasons behind this observation.

Key words: Race; Asian Americans; Survival; Prognosis; Outcomes

Core tip: Asian American patients with non-metastatic gastric cancer have better overall and disease-free survival compared to other racial groups in the United States. These findings are thought-provoking for the potential biological mechanisms underlying this observation as well as the potential therapeutic implications of these findings.

Citation: Abdel-Rahman O. Asian Americans have better outcomes of non-metastatic gastric cancer compared to other United States racial groups: A secondary analysis from a randomized study. *World J Gastrointest Oncol* 2019; 11(12): 1151-1160
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1151.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1151

INTRODUCTION

Gastric cancer is one of the most common causes of mortality and morbidity ascribed to cancer diagnosis worldwide[1]. A considerable geographical variation exists with regards to its etiology as well as its incidence[2]. Compared to Western countries, many Asian countries have a higher incidence of gastric cancer[3].

It has been recognized for a long time that gastric cancer behavior and outcomes might be different between patients living in Asian countries *vs* patients living in Western countries[4]. Possible reasons for these differences might be related to differences in etiology, biology, stage at presentation, or therapeutic approaches between the two categories of patients[5]. However, it is not yet fully clear if these differences persist between patients of Asian ancestry and patients of other racial subgroups within the multiethnic communities of North America.

A few previously published retrospective studies have suggested a difference between Asian American and other racial groups in the United States[6,7]. These studies were, however, criticized because of the potential -unaccounted for- confounders that frequently accompany retrospective studies. This made their conclusions far from decisive.

To provide a better answer for this question, there is a need to approach this question within the context of a prospectively collected dataset that adequately reports on baseline demographic characteristics of included patients as well as treatments received. Project Data Sphere (PDS) provides an ideal opportunity to tackle this question within the context of a controlled clinical trial[8]. The CALGB 80101 trial (NCT00052910), which evaluated two adjuvant chemoradiotherapy schedules for resected gastric cancer, was downloaded from the PDS platform to tackle this research question.

Objective

The current study aims at evaluating the impact of race on survival outcomes of non-metastatic gastric cancer patients in the United States treated with surgery plus adjuvant treatment.

MATERIALS AND METHODS

About the CALGB 80101 Study

This is a randomized phase III study evaluating two different adjuvant chemoradiotherapy schedules following resection of non-metastatic gastric cancer. These two schedules are: (1) 5-fluorouracil/leucovorin for 5 d on cycles 1,3,4 and 5-fluorouracil continuous intravenous infusion for 5 wk in cycle 2 concurrent with radiation therapy; and (2) Epirubicin/cisplatin/5-fluorouracil for cycles 1,3,4 and 5-fluorouracil continuous intravenous infusion for 5 wk in cycle 2 concurrent with radiation therapy. The start date of the study was in December 2002, and the primary completion date was in June 2012. Patients were included in this study regardless of the extent of nodal dissection. Detailed eligibility, methods, and primary results of this study were published elsewhere. The records of a total of 546 patients were available from the included study[9].

Data collection

The following information was collected (where available) from each of the included participants in the current study: Age at diagnosis, sex, race, ethnicity, performance status, history of prior cancer, T stage, N stage, lymph node ratio (defined as the ratio between positive and examined lymph nodes), histologic grade, primary tumor site, assigned treatment arm, and reason of stoppage of treatment. Type of lymph node dissection (D1 *vs* D2) was not available in the downloaded study datasets.

Primary endpoints for the current study include overall survival (defined as the time from randomization till death of any reason) and disease-free survival (defined as the time from randomization till death or progressive disease diagnosis).

Statistical analyses

Descriptive statistics were initially utilized to explore frequencies and distribution of different baseline parameters in the studied cohort. Chi-Squared testing was then used to explore the distribution of baseline characteristics between Asian Americans and other racial subgroups in the United States.

Kaplan-Meier analysis and log-rank testing were utilized to explore the overall and disease-free survival differences according to the race of the patients. Univariate and multivariate Cox regression analyses were then used to explore factors affecting overall and disease-free survivals. Factors with $P < 0.05$ in univariate analysis were subsequently included into multivariate analysis. SPSS statistics (version 20; IBM; Armonk, NY, United States) was used to execute all statistical procedures. $P < 0.05$ was used as an indicator of statistical significance.

RESULTS

Characteristics of included patients

Among the included patients, 46.2% were ≥ 60-years-old, 67.9% were males, 73.8% have white race (*vs* 12.8% black Americans and 8.2% Asian Americans), and only a minority of patients were of Hispanic ethnicity (9.9%). Almost half of the patients had an Eastern Cooperative Oncology Group performance score of 0, only 4% of patients had a history of another cancer, and most patients had node-positive disease. The study cohort was almost equally divided between the two treatment arms, and most patients (65.8%) completed the planned course of treatment (Table 1).

Comparing Asian Americans with other racial groups, Asian Americans were less likely to have an Eastern Cooperative Oncology Group score of 0 (48.9% *vs* 50.7%; $P < 0.001$). On the other hand, there was no difference between Asian Americans and other racial groups with regards to sex ($P = 0.233$), age group ($P = 0.810$), history of prior cancer ($P = 0.873$), histologic grade ($P = 0.067$), T stage ($P = 0.614$), N stage ($P = 0.867$), or assigned treatment arm ($P = 0.737$) (Table 2).

Survival outcomes according to racial groups

Using Kaplan-Meier analysis/log-rank testing, Asian Americans appear to have better overall and disease-free survival outcomes compared to other United States racial groups (White Americans, Black Americans, and other racial groups) ($P = 0.011$; $P = 0.010$; respectively) (Figure 1).

Univariate analysis was then utilized to explore factors affecting overall and disease-free survival in the studied cohort. The following factors were evaluated in the univariate analysis: Age at diagnosis, race, performance status, sex, ethnicity, history of prior cancer, T stage, lymph node ratio, histologic grade, tumor site, and treatment arm. For both endpoints, the following factors were significant in univariate

Table 1 Baseline characteristics of patients included in the current analysis, 546 patients

Parameter	*n* (%)
Age	
< 60 yr	294 (53.8)
≥ 60 yr	252 (46.2)
Sex	
Male	371 (67.9)
Female	175 (32.1)
Race	
White	403 (73.8)
Black	70 (12.8)
Asian	45 (8.2)
Others	28 (5.1)
Ethnicity	
Hispanic	54 (9.9)
Non-Hispanic	445 (81.5)
Unknown	47 (8.6)
Performance status	
0	276 (50.5)
1	257 (47.1)
2	13 (2.4)
History of prior cancer	
Yes	22 (4)
No	516 (94.5)
Unknown	8 (1.5)
T stage	
T1	33 (6)
T2	219 (40.1)
T3	259 (47.4)
T4	23 (4.2)
Unknown	12 (2.2)
N stage	
N0	77 (14.1)
N1	312 (57.1)
N2	105 (19.2)
N3	34 (6.2)
Unknown	18 (3.3)
Lymph node ratio (mean; SD)	0.32; 0.307
Histologic grade	
Grade 1	15 (2.7)
Grade 2	139 (25.5)
Grade 3	360 (65.9)
Grade 4	17 (3.1)
Unknown	15 (2.8)
Primary tumor site	
GEJ/ cardia/ fundus	208 (38.1)
Body/antrum/ pylorus	161 (29.5)
Others, NOS	177 (32.4)
Treatment arm	
5FU/LCV + RT	280 (51.3)
ECF + RT	266 (48.7)
Completion Of treatment	
Completed as planned	359 (65.8)
Stopped because of adverse events	64 (11.7)

Stopped because of progression/death	33 (6)
Stopped because of other reasons (*e.g.*, consent withdrawal)	90 (16.5)

RT: Radiation therapy; NOS: Not otherwise specified; SD: Standard deviation; GEJ: Gastroesophageal junction.

analysis ($P < 0.05$): T stage, lymph node ratio, primary tumor site, and race. When including these factors in a multivariate analysis for overall survival, the following factors were predictive of better overall survival: Asian American race (hazard ratio (HR) *vs* white race: 0.438; 95% confidence interval (CI): 0.254-0.754 , $P = 0.003$), lower T stage (HR for T4 *vs* T1: 3.807; 95%CI:1.731-8.361; $P = 0.001$), lower lymph node ratio (HR with increasing lymph node ratio: 3.004; 95%CI: 2.154-4.190; $P < 0.001$), and distal site of tumor primary (HR for proximal *vs* distal tumor: 1.523; 95%CI: 1.138-2.037; $P = 0.005$). Likewise, when including these factors in a multivariate analysis for disease-free survival, the following factors were predictive of better disease-free survival: Asian American race (HR *vs* white race: 0.460: 95%CI: 0.280-0.755, $P = 0.002$), lower T stage (HR for T4 *vs* T1: 3.990; 95%CI: 1.885-8.443; $P < 0.001$), lower lymph node ratio (HR with increasing lymph node ratio: 2.471; 95%CI: 1.801-3.390; $P < 0.001$), and distal site of tumor primary (HR for proximal *vs* distal tumor: 1.367; 95%CI: 1.042-1.793; $P = 0.024$) (Table 3).

DISCUSSION

The current study evaluated the impact of racial affiliation on the outcomes of non-metastatic United States gastric cancer patients. It suggests that Asian Americans with non-metastatic gastric cancer have better overall and disease-free survival compared to other racial groups in the United States.

These findings are consistent with previously reported population-based studies that suggested that United States gastric cancer patients of Asian Ancestry have better survival outcomes compared to other racial groups[10,11]. However, the current study is unique in being based on a controlled dataset that was collected in the context of a well-conducted randomized study.

Previous multinational comparisons have also suggested that Asian gastric cancer patients have better survival outcomes compared to other gastric cancer patients from other ethnic backgrounds[12,13]. It was previously postulated that this might be the result of an earlier stage at diagnosis and/or more aggressive surgical approach (particularly with regards to D2 dissection approach)[14]. However, the current study suggests that such differences cannot be explained by these factors alone. Asian American patients were treated in North America (according to the same surgical standard of other racial groups), and the current study does not show clear evidence of a uniquely earlier stage at diagnosis. It is not known, however, if patients labeled in the current study as Asian Americans were of first or subsequent generation of Asian Americans. Thus, one cannot exclude completely the potential contribution of environmental/ social differences between the East and the West. Additional factors that might explain these findings are potential biological differences between Asian and Caucasian patients with gastric cancer. Previous studies suggested that the predominant biological/ topographical subtype of gastric cancer among Asian patients might be different from the one that is prevalent among Caucasian patients[15]. Likewise, the variable role of *Helicobacter Pylori* infection as a carcinogenic factor between different ethnic groups might also be a factor here[16]. Further studies are needed to confirm or refute this hypothesis.

The current study has several limitations that need to be appreciated: Foremost, although the current study is based on a randomized prospective study, the specific question of the impact of race on outcomes was not a priori question within this study but rather an exploratory subsequent question. Thus, the current study is still a retrospective study of a prospectively collected dataset. Second, the current study contains a relatively small number of patients; confirmation of these findings in a larger prospective cohort is needed. These weaknesses need to be weighed against the clear strengths of the current study; most notably, the reliance on controlled well-conducted clinical trial dataset that provides a far more credible comparison compared to traditional retrospective, population-based studies.

The current study might also be informative for future research efforts of gastric cancer both in North America as well as in other parts of the world. Future randomized studies involving gastric cancer patients in North America should include a priori subgroup analysis for Asian Americans *vs* other racial groups.

Table 2 Comparison of baseline demographic/treatment characteristics between Asian Americans and other racial groups, *n* (%)

Variable	Asian Americans, 45 patients	Other patients, 501 patients	*P* value
Age			0.810
< 60 yr	25 (55.6)	269 (53.7)	
≥ 60 yr	20 (44.4)	232 (46.3)	
ECOG Performance score			< 0.001
0	22 (48.9)	254 (50.7)	
1	18 (40)	239 (47.7)	
2	5 (11.1)	8 (1.6)	
Sex			0.233
Male	27 (60)	344 (68.7)	
Female	18 (40)	157 (31.3)	
History of prior cancer			0.873
Yes	2 (4.5)	20 (4)	
No	42 (95)	474 (95.5)	
Unknown	1(0.5)	7 (0.5)	
T stage			0.614
T1-2	20 (44.4)	232 (46.3)	
T3-4	24 (53.4)	258 (51.5)	
Unknown	1 (2.2)	11 (2.2)	
N stage			0.867
N 0	8 (17.8)	69 (13.8)	
N 1-3	35 (77.8)	416 (83)	
Unknown	2 (4.4)	16 (3.2)	
Histologic grade			0.067
Grade 1-2	6 (11.7)	148 (29)	
Grade 3-4	38 (86.3)	339 (68)	
Unknown	1 (2)	14 (3)	
Treatment arm			0.737
5FU/LCV + RT	22 (48.9)	258 (51.5)	
ECF + RT	23 (51.1)	243 (48.5)	

ECOG: Eastern Cooperative Oncology Group; 5FU: 5-Fluorouracil; LCV: Leucovorin; RT: Radiotherapy; ECF: Epirubicin, cisplatin, 5-fluoruracil.

Moreover, additional exploration of the outcomes of first-generation *vs* second and subsequent generation Asian Americans are needed to dissect possible environmental/ social factors from potential biological differences. Likewise, a comparison of the outcomes of Asian Americans *vs* Asians living in Asian countries within a multinational context would also be useful for the same purpose. Moreover, national and international consortia working on the genetic mapping of gastric cancer should also pay additional attention to the possible differences in gastric cancer behavior (and possibly biology) among different ethnic groups.

The current study also further confirms the current practice of requiring confirmation of clinical trial results conducted exclusively on Asian patients (*e.g.*, Japanese trials) prior to generalization to western patients (and vice versa). The clear differences in outcomes of Asian *vs* Caucasian patients (treated within the same clinical trial/ health care system) mandate greater caution prior to the generalization of clinical trial results.

It should also be noted that the term "Asian Americans" includes quite a broad category of ethnicities, and the outcomes of gastric cancer might be variable among these ethnicities as well. Unfortunately, the current study dataset does not differentiate between different Asian American ethnic subgroups. This is another area of research that needs to be tackled in the future.

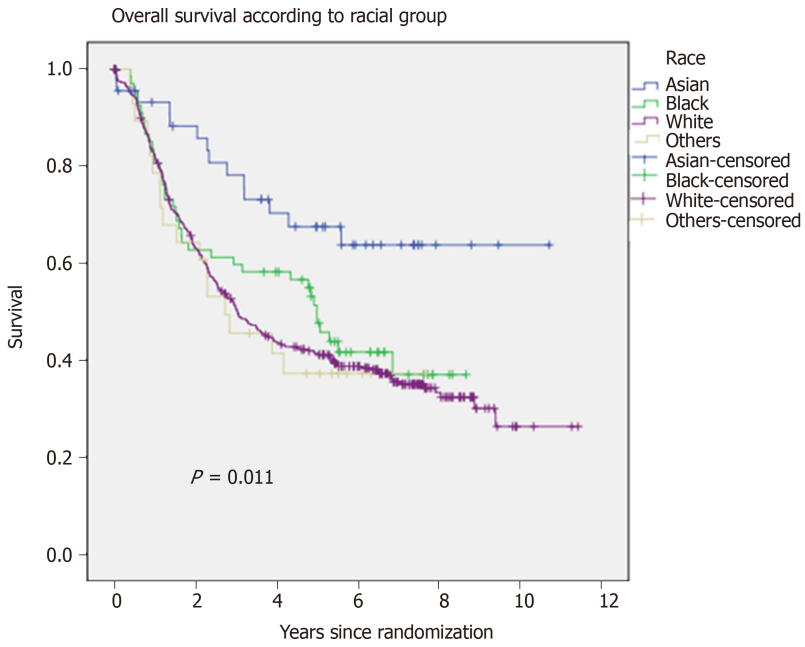
In conclusion, the current study suggests that Asian American patients with non-metastatic gastric cancer have better overall and disease-free survival compared to other racial groups in the United States. Further preclinical and clinical research is needed to clarify the reasons behind this observation.

Table 3 Multivariate Cox-regression analysis for factors affecting overall and disease-specific survival

Parameter	Overall survival		Disease-specific survival	
	Hazard ratio (95%CI)	*P* value	Hazard ratio (95%CI)	*P* value
Race				
White	Reference		Reference	
Black	1.020 (0.717-1.453)	0.911	0.962 (0.684-1.353)	0.825
Asian	0.438 (0.254-0.754)	0.003	0.460 (0.280-0.755)	0.002
Lymph node ratio, continuous	3.004 (2.154-4.190)	< 0.001	2.471 (1.801-3.390)	< 0.001
T stage				
T1	Reference		Reference	
T2	1.680 (0.878- 3.215)	0.117	1.895 (1.020-3.520)	0.043
T3	2.517 (1.321-4.798)	0.005	3.042 (1.643-5.632)	< 0.001
T4	3.807 (1.731-8.361)	0.001	3.990 (1.885-8.443)	< 0.001
Primary site				
Body/antrum/pylorus	Reference		Reference	
GEJ/cardia/fundus	1.523 (1.138-2.037)	0.005	1.367 (1.042-1.793)	0.024

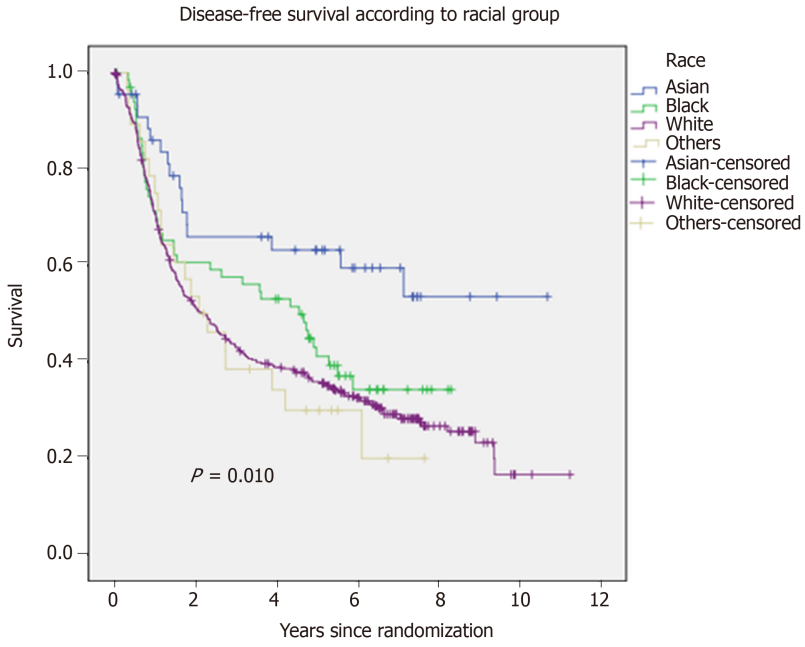
GEJ: Gastroesophageal junction; CI: Confidence interval.

A



Number at risk	0 years	5 years	10 years	15 years
White	401	150	3	0
Black	69	26	0	0
Asian	45	21	1	0
Others	28	8	0	0

B



Number at risk	0 years	5 years	10 years
White	400	130	2
Black	69	22	0
Asian	45	20	1
Others	28	6	0

Figure 1 Kaplan-Meier analysis. A: Kaplan-Meier analysis of the impact of race on overall survival; B: Kaplan-Meier analysis of the impact of race on disease-free survival.

ARTICLE HIGHLIGHTS

Research background

Gastric cancer behavior and outcomes might be different between patients living in Asian

countries *vs* patients living in Western countries. It is not clear if these differences would persist between patients of Asian ancestry and patients of other racial subgroups within the multiethnic communities of North America.

Research motivation
This study hypothesizes that these differences will present within North American multiethnic communities.

Research objectives
To evaluate the impact of race on survival outcomes of non-metastatic gastric cancer patients in the United States.

Research methods
This is a secondary analysis of a randomized controlled trial (CALGB 80101 study) that evaluated two adjuvant chemoradiotherapy schedules following resection of non-metastatic gastric cancer. Kaplan-Meier analysis and log-rank testing were utilized to explore the overall and disease-free survival differences according to the race of the patients. Univariate and multivariate Cox regression analyses were then used to explore factors affecting overall and disease-free survival.

Research results
A total of 546 patients were included in the current analysis. Of which, 73.8% have white race (*vs* 12.8% black Americans and 8.2% Asian Americans). Using Kaplan-Meier analysis/log-rank testing, Asian Americans appear to have better overall and disease-free survival outcomes compared to other United States racial groups (White Americans, Black Americans and other racial groups) (P = 0.011; P = 0.010; respectively). Moreover, in an adjusted multivariate model, Asian American race seems to be associated with better overall and disease-free survival (hazard ratio: 0.438; 95%CI: 0.254-0.754), P = 0.003; hazard ratio: 0.460; 95%CI: 0.280-0.755, P = 0.002; respectively).

Research conclusions
Asian American patients with non-metastatic gastric cancer have better overall and disease-free survival compared to other racial groups in the United States. Further preclinical and clinical research is needed to clarify the reasons behind this observation.

Research perspectives
The findings of this study are thought-provoking for the potential biological mechanisms underlying this observation as well as the potential therapeutic implications of these findings.

ACKNOWLEDGEMENTS

The current study is based on a clinical trial dataset downloaded from Projectdatasphere.org. Neither PDS nor the authors of the original research are responsible for the findings in the current analysis.

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World Journal of Gastrointestinal Oncology

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World J Gastrointest Oncol 2019 December 15; 11(12): 1161-1171

DOI: 10.4251/wjgo.v11.i12.1161

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Gastric partitioning for the treatment of malignant gastric outlet obstruction

Marcus Fernando Kodama Pertille Ramos, Leandro Cardoso Barchi, Rodrigo Jose de Oliveira, Marina Alessandra Pereira, Donato Roberto Mucerino, Ulysses Ribeiro Jr, Bruno Zilberstein, Ivan Cecconello

ORCID number: Marcus Fernando Kodama Pertille Ramos (0000-0003-0200-7858); Leandro Cardoso Barchi (0000-0001-8240-900X); Rodrigo Jose de Oliveira (0000-0002-7066-0210); Marina Alessandra Pereira (0000-0002-6865-0988); Donato Roberto Mucerino (0000-0003-0707-1103); Ulysses Ribeiro Jr (0000-0003-1711-7347); Bruno Zilberstein (0000-0002-1809-8558); Ivan Cecconello (0000-0002-3535-4170).

Author contributions: Ramos MFKP, Barchi LC, Pereira MA designed the study, collected the data, conducted the statistical analysis, and drafted the manuscript; Oliveira RJ and Mucerino DR collected the data and reviewed the manuscript; Ribeiro Jr U, Zilberstein B, and Cecconello C critically analyzed the data and reviewed the manuscript.

Institutional review board statement: The study is part of the database project approved by the hospital ethics committee (NP771/15) and registered online (www.plataformabrasil.com; CAAE:43453515.6.0000.0065).

Informed consent statement: Due to the retrospective, non-interventional, and data analysis-based design of the study, informed consent was waived.

Conflict-of-interest statement: The authors report no relevant conflicts of interest.

Marcus Fernando Kodama Pertille Ramos, Leandro Cardoso Barchi, Rodrigo Jose de Oliveira, Marina Alessandra Pereira, Donato Roberto Mucerino, Ulysses Ribeiro Jr, Bruno Zilberstein, Ivan Cecconello, Department of Gastroenterology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, São Paulo 01249000, Brazil

Corresponding author: Marcus Fernando Kodama Pertille Ramos, FACS, MD, MSc, PhD, Attending Doctor, Department of Gastroenterology, Hospital das Clinicas HCFMUSP, Faculdade de Medicina, Universidade de Sao Paulo, Av Dr Arnaldo 251, São Paulo-SP 01249000, Brazil. marcus.kodama@hc.fm.usp.br
Telephone: +55-11-33932000
Fax: +55-11-33933994

Abstract

BACKGROUND

Gastric outlet obstruction (GOO) is one of the main complications in stage IV gastric cancer patients. This condition is usually managed by gastrojejunostomy (GJ). However, gastric partitioning (GP) has been described as an alternative to overcoming possible drawbacks of GJ, such as delayed gastric emptying and tumor bleeding.

AIM

To compare the outcomes of patients who underwent GP and GJ for malignant GOO.

METHODS

We retrospectively analyzed 60 patients who underwent palliative gastric bypass for unresectable distal gastric cancer with GOO from 2009 to 2018. Baseline clinicopathological characteristics including age, nutritional status, body mass index, and performance status were evaluated. Obstructive symptoms were graded according to GOO score (GOOS). Surgical outcomes evaluated included duration of the procedure, surgical complications, mortality, and length of hospital stay. Acceptance of oral diet after the procedure, weight gain, and overall survival were the long-term outcomes evaluated.

RESULTS

GP was performed in 30 patients and conventional GJ in the other 30 patients. The mean follow-up was 9.2 mo. Forty-nine (81.6%) patients died during that period. All variables were similar between groups, with the exception of worse

Data sharing statement: No additional data are available.

Manuscript source: Invited manuscript

Received: March 25, 2019
Peer-review started: March 26, 2019
First decision: July 31, 2019
Revised: September 3, 2019
Accepted: October 3, 2019
Article in press: October 3, 2019
Published online: December 15, 2019

P-Reviewer: Chiu CC, Lin JM, Jeong KY
S-Editor: Zhang L
L-Editor: Filipodia
E-Editor: Ma YJ

performance status in GP patients. The mean operative time was higher in the GP group (161.2 *vs* 85.2 min, $P < 0.001$). There were no differences in postoperative complications and surgical mortality between groups. The median overall survival was 7 and 8.4 mo for the GP and GJ groups, respectively ($P = 0.610$). The oral acceptance of soft solids (GOOS 2) and low residue or full diet (GOOS 3) were reached by 28 (93.3%) GP patients and 22 (75.9%) GJ patients ($P = 0.080$). Multivariate analysis demonstrated that GOOS 2 and GOOS 3 were the main prognostic factors for survival (hazard ratio: 8.90, 95% confidence interval: 3.38-23.43, $P < 0.001$).

CONCLUSION
GP is a safe and effective procedure to treat GOO. Compared to GJ, it provides similar surgical outcomes with a trend to better solid diet acceptance by patients.

Key words: Stomach neoplasms; Gastric outlet obstruction; Palliative surgery; Gastrojejunostomy; Gastric cancer

Core tip: Gastric partitioning associated with gastrojejunostomy has been employed for the treatment of malignant obstruction. The partitioning creates two separated gastric chambers that may improve gastric emptying, decrease tumor bleeding, and improve survival. We analyzed retrospective data from our center and found that partitioning was as safe and effective as traditional gastrojejunostomy. Postoperative complications and survival were similar between the groups. Acceptance of soft and full diet after the procedure was the most important prognostic variable and was more common after gastric partitioning. A prospective randomized trial is ongoing to further analyze this issue.

Citation: Ramos MFKP, Barchi LC, de Oliveira RJ, Pereira MA, Mucerino DR, Ribeiro Jr U, Zilberstein B, Cecconello I. Gastric partitioning for the treatment of malignant gastric outlet obstruction. *World J Gastrointest Oncol* 2019; 11(12): 1161-1171
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1161.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1161

INTRODUCTION

Gastric cancer (GC) is the fifth most common cancer and third leading cause of cancer-related deaths worldwide[1]. Surgical resection of the tumor with D2 lymphadenectomy is the indicated standard curative treatment[2]. Unfortunately, many patients initially present with advanced disease at the time of diagnosis without the possibility of curative resection. The frequency of patients with clinical stage IV varies according to each country and may reach up to 40% of cases[3-5].

Despite the dismal prognosis, many stage IV GC patients develop complications during the course of disease that require palliative procedures. Among these complications, the following stand out: Tumor bleeding, refractory ascites, intestinal obstruction, and gastric outlet obstruction (GOO).

The incidence of GOO ranges between 5% and 14.9% in patients with distal GC. Palliative resection of the tumor is the procedure of choice in cases of resectable lesions and limited metastatic disease, and in patients with favorable clinical conditions[6].

However, many of these tumors are considered unresectable due to local invasion of adjacent structures or due to patients' unfavorable clinical conditions. Surgical bypass or endoscopic stents are options to restore the gastrointestinal continuity. Endoscopic stents are less invasive and can be deployed out of the operating room. However, concerns regarding its long-term effectiveness still grants an important role for surgery[7,8].

The most traditional surgery performed is gastrojejunostomy (GJ). The procedure is simple and can be accomplished by laparoscopy with low morbidity. However, delayed gastric emptying (DGE) is one of the main postoperative complications, with an incidence that varies between 10% and 26%[9].

In this context, gastric partitioning (GP) associated with GJ, also known as GP, has been considered an option for the treatment of malignant GOO. Initially, it was described in 1925 for complex gastroduodenal ulcers[10]. Currently, is considered a palliative surgery for GOO by the Japanese Gastric Cancer Association guidelines[2].

Our institution has used GP for the past 10 years in such cases. Therefore, the aim of this study was to compare the outcomes of patients who underwent GP and GJ for malignant GOO.

MATERIALS AND METHODS

Patients

A retrospective review of all gastric adenocarcinoma patients who underwent any palliative surgical bypass from 2009 to 2018 was performed from a prospectively collected database.

Inclusion criteria were: Irresectable distal gastric tumor; the presence of obstructive symptoms; and life expectancy superior to 2 mo. Patients with proximal gastric tumors, tumors amenable to palliative resection and associated with small bowel obstruction were excluded.

Clinicopathological characteristics were evaluated as well as laboratory tests to assess nutritional status. Karnofsky performance score (KPS) and Eastern Cooperative Oncology Group (ECOG) scale were used to assign performance status. Tumor spread was evaluated by the presence of distant metastasis and carcinomatosis. Obstructive symptoms were graded according to the GOO score (GOOS) as follows: 0 = no oral intake, 1 = liquids only, 2 = soft solids, 3 = low residue or full diet[11]. Patient's weight in kilograms and body mass index (BMI) were measured prior to surgery and after 30 and 90 d. Maximum weight after surgery and last weight recorded before death were also evaluated.

Postoperative complications were graded according to Clavien-Dindo's classification[12]. Major complications were considered Clavien III-IV-V. Surgical mortality was defined as death within 30 d after surgery or during hospital stay. Survival was evaluated after 30 and 90 d and during follow-up.

Due to limited life expectancy and fragility of the patients, there was no standard postoperative follow-up schedule. An absence in consultations for more than 12 mo was considered loss of follow-up.

Surgical technique

Briefly, GP was performed as follows. Upon confirmation that the tumor was unresectable, the lesser sac was accessed, and the posterior gastric wall was inspected to confirm that there was a tumor-free area for anastomosis. For GP, a point located at least 5 cm proximal to the tumor along the gastric curvatures was chosen. Faucher's tube (32Fr) was positioned along the lesser curvature to ensure a small conduit between the two gastric chambers created by partitioning. It enabled subsequent endoscopic observation of the bypassed tumor. The stomach was partitioned through mechanical linear stapler from the greater curvature towards the Faucher's tube along the lesser curvature. A side to side GJ, 30 cm from the ligament of Treitz, was performed in the proximal part of the stomach (Figure 1). In some cases, Roux-en-Y reconstruction was also performed. Conventional GJ was performed along the posterior gastric curvature hand-sewn or with stapler device, *via* an antecolic or retrocolic route. Deciding which procedure should be performed was not controlled and defined by the surgeon responsible for each case.

Statistical analysis

Descriptive statistics included frequencies by percent for nominal variables and means with standard deviation for continuous variables. Chi-square tests were used for categorical data to evaluate the differences between variables, and the *t*-test was used for continuous data. Overall survival (OS) was estimated using the Kaplan–Meier method, and differences in survival were examined using the log-rank test. The survival period was calculated from the date of surgery until the date of death. Living patients were censored at the date of last personal contact. All tests were two-sided and statistical significance was defined as $P < 0.05$. Analysis was performed using SPSS software, version 18.0 (SPSS Inc, Chicago, IL, United States).

RESULTS

A total of 60 GC patients underwent gastric bypass due to irresectable distal GC. GP

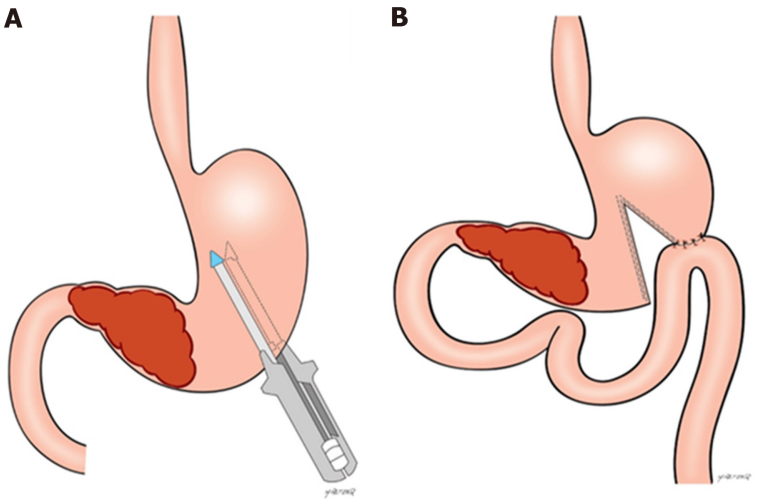

Figure 1 GP and GJ for malignant gastric cancer. A: Mechanical GP along the gastric curvatures; B: GJ performed at the proximal part of the stomach chamber (Image credits: Marcos Retzer/ Department of Gastroenterology, Universidade de São Paulo). GJ: Gastrojejunostomy; GP: Gastric partitioning.

was performed in 30 patients and conventional GJ in the other 30 patients. Initial nutritional variables including hemoglobin, albumin, weight and BMI did not differ between groups (Table 1). KPS and ECOG were worse in GP patients. The presence of distant and peritoneal metastasis was also similar between groups. The complete impossibility of oral ingestion or ingestion of only liquids (GOOS 0-1) were present in 60% of patients in both groups.

Operative outcomes are demonstrated in Table 2. The mean operative time was higher in the partitioning group (161.2 *vs* 85.2 min, $P < 0.001$). Roux-en-Y reconstruction was performed in 16 patients (57.1%) in the GP group and in none of the GJ group. Manual anastomosis was more common in the partitioning group (42.9% *vs* 6.7%, $P = 0.001$). There were no differences regarding postoperative complications and surgical mortality between groups. The mean time in days for ingestion of liquids (GOOS 1), soft diet (GOOS 2), and length of hospital stay was similar between groups. GOOS 2 and 3 were reached by 28 GP patients (93.3%) and 22 GJ patients (75.9%) ($P = 0.080$).

The evolutionary control of weight gain after the procedure evidenced that, after 30 and 90 d, there was no difference between groups (Table 3). Maximum mean weight recorded after surgery was similar between GP and GJ groups (56.6 *vs* 56.8 Kg, $P = 0.966$). A second additional procedure was necessary in four patients in each group to establish nutritional enteral access, which included an enteral feeding tube.

The mean follow-up was 9.2 mo (median of 6.6 mo, standard deviation [SD] ± 9.7) Forty-nine (81.6%) patients died during that period. The median OS of the entire sample was 8 mo (range 0.1–50.5). Regarding the type of surgery, there was no difference in survival between the groups. The median OS was 7 and 8.4 mo for the GP and GJ groups, respectively ($P = 0.610$) (Figure 2).

The multivariate analysis of clinicopathological characteristics and operative outcomes associated with OS demonstrated that only GOOS 2-3 after surgery were statistically significant in improving survival (hazard ratio [HR]: 8.90; 95% confidence interval [CI]: 3.38-23.43, $P < 0.001$) (Table 4). The type of surgery (GJ *vs* GP) was not associated with improvement in OS (HR: 1.16, 95%CI: 0.65-2.07, $P = 0.612$).

DISCUSSION

GP proved to be as effective as GJ for the treatment of GOO. Both procedures had similar results regarding postoperative complications and no difference in survival was found. Nevertheless, there was a trend for GP in promoting better acceptance of soft solids, low residue, and full diets (GOOS 2-3) by the patients ($P = 0.08$). These results reinforce GP as a valid option to be added to GJ in the treatment of GOO.

Recently, endoscopic stents have gained popularity to treat such a condition[13]. It is less invasive and can be performed outside the operating room. The return of oral intake is faster with a shorter length of hospital stay. As a disadvantage, the method presents an acute risk of bleeding, perforation, and stent migration. In the long-term, tumor growth may lead to stent obstruction with the necessity of reinterventions[14].

Table 1 Clinicopathological characteristics of all patients

Variables	GP, *n* = 30	GJ, *n* = 30	*P* value
Gender			0.405
Female	8 (26.7)	11 (36.7)	
Male	22 (73.3)	19 (63.3)	
Age in yr			0.343
mean (SD)	67.5 (13.4)	64.3 (12.7)	
Hemoglobin in g/dL			0.782
mean (SD)	9.7 (2.1)	9.9 (1.8)	
Albumin in g/dL			0.087
mean (SD)	3.6 (0.6)	3.3 (0.6)	
Karnofsky performance status			0.001
60-70	20 (66.7)	7 (23.3)	
80-90	10 (33.3)	23 (76.7)	
ECOG			0.020
0-1	11 (36.7)	20 (66.7)	
2-3	19 (67.9)	10 (33.3)	
Peritoneal metastasis			0.055
P0	17 (60.7)	9 (34.6)	
P1	11 (39.3)	17 (65.4)	
Distant metastasis			0.192
M0	16 (57.1)	12 (40)	
M1	12 (42.9)	18 (60)	
Weight in Kg			0.598
mean (SD)	56.5 (12.1)	54.9 (11.3)	
BMI in Kg/m^2			0.362
mean (SD)	21.8 (5.1)	20.7 (3.9)	
Initial GOOS			1.0
0-1	18 (60)	18 (60)	
2-3	12 (40)	12 (40)	

Total population n = 60. Data presented as n (%). BMI: Body mass index; ECOG: Eastern cooperative oncology group; GJ: Gastrojejunostomy; GOOS: Gastric outlet obstruction score; GP: Gastric partitioning.

According to the multicenter randomized trial SUSTENT, endoscopic stents are mostly indicated for patients with poor status performance, high surgical risk, and life expectancy less than 2 mo[8]. Patients with better clinical conditions and with the possibility of receiving palliative chemotherapy have a potential benefit of definitive surgical gastric derivation[15].

The first report of GP was made by Devine *et al*[10] in 1925, in a patient with obstruction caused by a complex duodenal ulcer. Maingot *et al*[16] in 1936 first reported its use in gastric cancer. In both cases, partitioning of the stomach was complete. This fact led to closed loop syndrome of the distal gastric stump with the risk of a blowout as a consequence of inadequate drainage of the gastric fluids from the excluded stomach. Yet, bleeding from the tumor may occur.

After those initial reports, there were no further publications regarding that method. Nevertheless, its employment gained prominence after Kaminishi's report in 1997. In that original series, 31 unresectable GC patients with GOO underwent either GP or GJ. The rates of acceptance of a regular meal at 2 wk after the operation were 88% in the partitioning group and 31% in the GJ group ($P < 0.05$). Still, the mean survival times for GP and GJ were 13.4 and 5.8 mo, respectively ($P < 0.05$)[17]. The authors presented a modification in the technique, maintaining a small communication between the two gastric chambers created after the partitioning. This communication avoids closed loop syndrome and the risk of a blowout. It also allows the endoscopic access to the tumor and the biliary tree in case of the necessity of biliary drainage. Still, gastric acid entry into the antrum is also maintained, decreasing the stimulation of gastrin and consequent risk of ulcer formation[18]. Regarding GE, the proximal gastric chamber created by partitioning has smaller dimensions in relation to the entire stomach, which is also dilated in many cases. The reduction in organ

Table 2 Surgical outcomes

Variables	Partitioning, *n* = 30	GJ, *n* = 30	*P* value
Operative time in min			< 0.001
mean (SD)	161.2 (76.4)	85.2 (37.7)	
Roux-en-Y reconstruction			< 0.001
No	12 (42.9)	30 (100)	
Yes	16 (57.1)	0 (0)	
Anastomosis			0.001
Manual	12 (42.9)	2 (6.7)	
Stapler	16 (57.1)	28 (93.3)	
Postoperative complication			0.254
No/minor	28 (93.3)	24 (80)	
Major	2 (6.7)	6 (20)	
Surgical mortality			1.0
No	28 (93.3)	27 (90)	
Yes	2 (96.7)	3 (10)	
Days for GOOS ≥ 1			0.375
mean (SD)	3.3 (3.2)	2.6 (1.9)	
Days for GOOS ≥ 2			0.371
mean (SD)	4.7 (3.7)	4 (1.9)	
Length of hospital stay in d			0.201
mean (SD)	8.6 (5.2)	11.1 (9.2)	
Final GOOS			0.080
0-1	2 (6.7)	7 (24.1)	
2-3	28 (93.3)	22 (75.9)	

Data presented as *n* (%). SD: Standard deviation; GJ: Gastrojejunostomy; GOOS: Gastric outlet obstruction score.

dimensions decreases the formation of recesses distal to the anastomosis and in the proximal body and gastric fundus. Thus, it may decrease the recirculation of the ingested food inside the stomach, facilitating its flow to the anastomosis and decreasing the GE time.

Advantages attributed to partitioning include improving GE and reducing tumor bleeding due to less contact of ingested food. Still, it reduces the necessity of blood transfusion. Besides that, improving food intake and reducing bleeding help patients to better tolerate the effects of palliative chemotherapy, which may improve survival[19-21]. Another interesting aspect of such a procedure is the fact that the tumor is isolated in the distal gastric chamber. Subsequently, the possibility of obstruction of the GJ by tumor growth is minimized. In addition, this technique has also been applied for tumors of the biliopancreatoduodenal confluence[9,18].

As a disadvantage, the addition of a stapling line creates new potential sites for postoperative fistula. The operative time may also increase, as shown in the present study. However, the persistent attempt to accomplish tumor resection and the greater proportion of cases with Roux-en-Y reconstruction may have influenced this result. Thus, we believe that after the technique becomes routine, this increment in operative time due to the addition of the partitioning is minimal. There is no consensus regarding the need for Roux-en-Y reconstruction. The presence of biliary flow to the stomach due to the GJ is something that theoretically can impair GE. Moreover, reflux alkaline gastritis and afferent loop syndrome may impair acceptance of diet. The use of a Roux-en-Y reconstruction or the addition of Braun enteroenterostomy may prevent these complications[22]. However, as patients have limited life expectancy, the alleged short and long-term complications of biliary reflux were not observed in our study. Thus, Roux-en-Y reconstruction may not be justified.

Both procedures can be safely performed by laparoscopy[23-25]. They may also be performed during a staging laparoscopy with palliative intent or even to improve nutritional status in a patient with neoadjuvant or conversion therapy indication[26].

When comparing the techniques, the outcomes analyzed vary in literature. Regarding early postoperative results, DGE is widely used but the way of classifying is not standardized and is somewhat subjective. The International Study Group of

Table 3 Control of weight after the procedure

Variables	Partitioning, *n* = 30	GJ, *n* = 30	*P* value
Weight-30 d[1]			0.391
mean (SD)	57.7 (13.4)	54.9 (11.3)	
Weight 90 d[1]			0.132
mean (SD)	59.9 (12,7)	54.6 (10.2)	
Weight-maximum			0.966
mean (SD)	56.6 (11.8)	56.8 (10.0)	
Weight-final[1]			0.343
mean (SD)	51.9 (12.5)	53.9 (12.7)	
Weight variation initial-30 d			0.343
mean (SD)	1,92	- 1.2	
Weight variation initial - 90 d			0.287
mean (SD)	- 0.28	- 0.21	
Weight variation 30 - 90 d			0.140
mean (SD)	- 1.52	0.5	
Weight variation initial - Final			0.383
mean (SD)	- 4.9	- 2.4	
Second procedure			1.0
No	26 (86.7)	26 (86.7)	
Yes	4 (13.3)	4 (13.3)	

[1]Data not available in some cases. Data presented as *n* (%). GJ: Gastrojejunostomy;

Pancreatic Surgery definition has been employed[27]. It conceptually defines DGE when a nasogastric tube is required for 8 d or limited oral intake cannot be tolerated by postoperative day 14. This criterion may be too strict, causing lack of documentation of cases with mild DGE. Ernberg *et al*[9] reported a significantly lower incidence of DGE in GP patients (0%) compared with the GJ (42.9%, $P = 0.024$). Additionally, oral nutrition alone was recorded more often at follow-up in the partitioning group (9/9, 100%) than in the GJ group (4/13, 30.8%) ($P = 0.002$). Thus, in the present study, we decided to evaluate the time to reach GOOS scores 2 and 3. Both groups took the same average of days for diet acceptance and progression. However, the partitioning group had a trend to present higher final values of GOOS. The length of hospital stay is another early outcome commonly used. It indirectly reflects the ability of oral ingestion acceptance. In addition, it is influenced by other factors of interest such as postoperative complications.

Kumagai *et al*[28] published a meta-analysis comparing GP with GJ. Seven studies containing 207 patients were included. GP had a significantly lower risk of DGE (relative risk: 0.32; 95%CI 0.17 to 0.60; $P < 0.001$) and shorter postoperative hospital stay (mean of 6.1 d; $P < 0.001$). Conversely, no significant differences were observed in operative time, blood loss, postoperative complications and anastomotic leak[28].

The main long-term goals of GP are the maintenance of oral intake capacity and survival. What can be verified is that once the GOO is successfully solved, either with GP or GJ, patients maintain the capacity to eat until near death. Only 13.3% of patients did require additional procedures to maintain the alimentary route. When that happened, it was doubtful whether the failure was exclusively due to the initial procedure causing GOO recurrence or due to disease progression. The weight regain was also similar between the two groups, confirming the equal long-term effectiveness of both procedures to maintain oral intake.

Improved OS has been reported with GP[19-21]. The fact that the tumor is excluded in the distal gastric chamber leads to a lower occurrence of tumoral bleeding. Less bleeding associated with improvement in oral intake allows better use of palliative chemotherapy with a beneficial effect on OS. However, we did not verify this result in our study ($P = 0.08$). It could be speculated that, with more patients in the analysis, perhaps some differences among the two techniques would appear. Yet, the GP group had lower values of KPS and ECOG. The selection bias of patients with worse performance and consequently worse OS may have influenced this result. Lastly, multivariate analysis showed that the main prognostic factor in patients with GOO was the ability to eat better after the procedure regardless of the technique used (HR: 8.90, 95%CI: 3.38-23.43, $P < 0.001$).

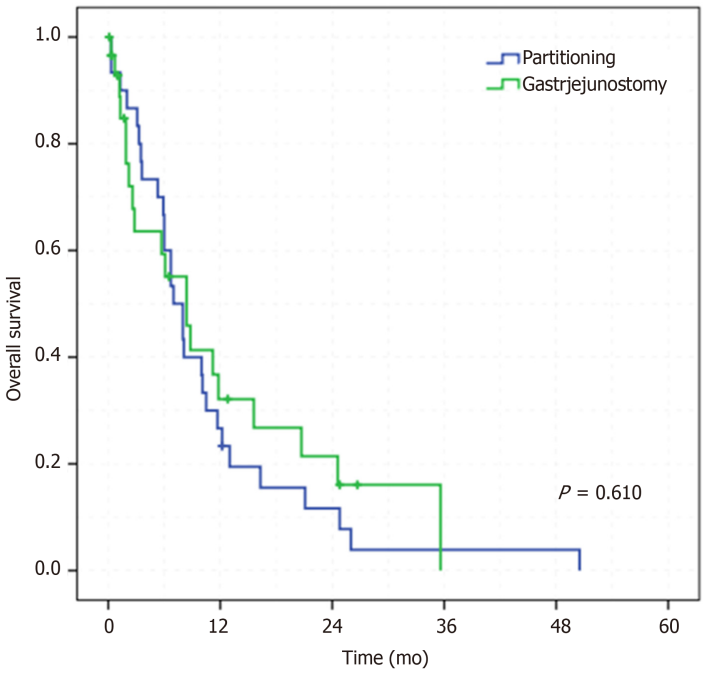

Figure 2 Overall survival for GP and GJ (*P* = 0.610). GJ: Gastrojejunostomy; GP: Gastric partitioning.

Retrospective studies have limitations inherent to their design. The selection of patients for both techniques was not done in an equivalent manner. In the past 10 years, GP has been the procedure of choice for GOO cases in our institution. The decision to perform conventional GJ still takes place as an option, especially in urgent cases or when the surgeon is not familiar with the partitioning technique. This situation allowed us to create a control group. Fortunately, exactly 30 patients were included in the control group in the same period. In order to increase group sampling, it was thoughtful to include patients from previous periods when partitioning was not performed. However, this would bring the disadvantage of not including recent advances in palliative treatment in GC, which would have an impact on survival. Surprisingly, clinicopathological characteristics of patients in both groups were almost similar, with the exception of KPS and ECOG scores, not making the comparison so unequal.

To overcome these limitations, a prospective randomized study comparing GP with GJ was initiated at our institution. Thus, currently, no patient is submitted to any procedure for the treatment of GOO outside the prospective protocol. The study is ongoing recruiting patients and is expected to be completed within the next year (ClinicalTrials.gov: NCT02064803).

In summary, GP proved to be a safe and effective procedure for the treatment of GOO. Compared to conventional GJ, GP has similar early and late outcomes with a trend to better solid diet acceptance by the patients.

Table 4 Univariate and multivariate analyses for survival - Cox regression

Variables[1]	Univariate			Multivariate		*P* value
	HR	95%CI	*P* value	HR	95%CI	
Age 0-64 *vs* > 65 yr	1.05	0.59-1.87	0.866	-	-	-
Female *vs* Male	0.77	0.42-1.40	0.391	-	-	-
BMI ≥ 24.4 *vs* < 24.4	1.07	0.60-1.91	0.819	-	-	-
Initial GOOS 2-3 *vs* 0-1	1.31	0.73-2.34	0.363	-	-	-
Final GOOS 2-3 *vs* 0-1	9.42	3.67-24.20	< 0.001	8.90	3.38 - 23.43	< 0.001
GJ *vs* Partitioning	1.16	0.65-207	0.612	-	-	-
Major POC *vs* Minor/non POC	1.51	1.07-5.90	0.035	1.68	0.65 - 4.36	0.287

[1]The first variable represents the reference category. HR: Hazard ratio; 95%CI: 95% confidence interval; BMI: Body mass index; GOOS: Gastric outlet obstruction score; POC: Postoperative complication.

ARTICLE HIGHLIGHTS

Research background

Gastric outlet obstruction (GOO) is a common complication during gastric cancer treatment. Different treatment modalities have been employed including endoscopic stent placement, surgical resection, and surgical bypass procedures. Surgical bypass may have better results when life expectancy is larger than 2 mo. It may be performed with a simple gastrojejunostomy (GJ) or with the addition of partial gastric partitioning (GP).

Research motivation

GJ has been traditionally performed as bypass procedure for GOO. However, delayed gastric emptying with impaired food ingestion may occur in up to 26% of cases. To overcome this setback, GP has been employed. The partitioning creates two separated gastric chambers that may improve gastric emptying, decrease tumor bleeding, and improve survival.

Research objectives

We compared the surgical results of GJ and GP for the treatment of GOO in patients with unresectable distal gastric cancer.

Research methods

We performed a retrospective analysis of 60 patients submitted to GJ and GP between 2009 and 2018. Clinicopathological characteristics and surgical outcomes were compared.

Research results

GP was performed in 30 patients and conventional GJ in the other 30 patients. Baseline clinicopathological characteristics were similar between groups, with the exception of worse performance status in GP patients. Surgical results related to postoperative complications and surgical mortality did not differ between groups. The median OS was 7 and 8.4 mo for GP and GJ groups, respectively ($P = 0.610$). The oral acceptance of soft solids (GOOS 2) and low residue or full diet (GOOS 3) were reached by 28 (93.3%) GP patients and 22 (75.9%) GJ patients ($P = 0.080$). After multivariate analysis, acceptance of soft solids and low residue or full diet was the main prognostic factors for survival despite the surgical procedure performed (HR: 8.90, 95%CI: 3.38-23.43, $P < 0.001$).

Research conclusions

GP is a safe and effective procedure to treat GOO. Compared to GJ, it provides similar early and late outcomes with a trend to better solid diet acceptance by the patients.

Research perspectives

After this initial experience using GP, a prospective trial was initiated and currently no patient has been submitted to any procedure for the treatment of GOO outside the protocol. The study is ongoing, recruiting patients, and is expected to be completed within the next year (ClinicalTrials.gov: NCT02064803).

ACKNOWLEDGEMENTS

The authors thank other members of the service involved in gastric cancer treatment: Andre R Dias, Claudio JC Bresciani, Marcelo Mester, Osmar K Yagi, Amir Z Charruf, and Fabio P Lopasso.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1172-1181

DOI: 10.4251/wjgo.v11.i12.1172

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Study

Difference in failure patterns of pT3-4N0-3M0 esophageal cancer treated by surgery *vs* surgery plus radiotherapy

Ya Zeng, Wen Yu, Qi Liu, Wei-Wei Yu, Zheng-Fei Zhu, Wei-Xin Zhao, Jun Liu, Jia-Ming Wang, Xiao-Long Fu, Yuan Liu, Xu-Wei Cai

ORCID number: Ya Zeng (0000-0002-3524-5476); Wen Yu (0000-0002-2338-4663); Qi Liu (0000-0002-8770-5470); Wei-Wei Yu (0000-0002-8081-6418); Zheng-Fei Zhu (0000-0002-5516-039X); Wei-Xin Zhao (0000-0001-8553-7429); Jun Liu (0000-0002-8453-2957); Jia-Ming Wang (0000-0001-8842-7110); Xiao-Long Fu (0000-0001-8127-3884); Yuan Liu (0001-6842-9346); Xu-Wei Cai (0000-0001-9673-4188).

Author contributions: All authors participated in this research; Zeng Y and Cai XW contributed to manuscript writing, patient collection, and data analysis; Yu W, Yu WW, Liu Qi, Wang JM, Liu J, Zhu ZF, and Zhao WX contributed to patient collection and follow-up visit; Fu XL and Cai XW contributed to patient collection and research design; and Liu Y reviewed the statistical methods of this study.

Supported by Emerging Advanced Technology Joint Research Project of Shanghai Shenkang Hospital Development Center, No. SHDC12017103; and Shanghai Municipal Education Commission-Gaofeng Clinical Medicine Grant Support, No. 20161433.

Institutional review board statement: Approval was achieved from the Institute Research Ethics Committee of Shanghai Chest Hospital and Shanghai Cancer Center before the patients were enrolled in the relevant studies. So, there was no additional approval for this study.

Ya Zeng, Wen Yu, Jun Liu, Jia-Ming Wang, Xiao-Long Fu, Xu-Wei Cai, Department of Radiation Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China

Wen Yu, Qi Liu, Wei-Wei Yu, Zheng-Fei Zhu, Wei-Xin Zhao, Xiao-Long Fu, Xu-Wei Cai, Department of Radiation Oncology, Fudan University Shanghai Cancer Center, Shanghai 200030, China

Wei-Wei Yu, Department of Radiation Oncology, Shanghai Jiao Tong University Affiliated Shanghai Sixth People's Hospital, Shanghai 200030, China

Yuan Liu, Department of Statistics, Shanghai Chest Hospital, Shanghai Jiao Tong University, Shanghai 200030, China

Corresponding author: Xu-Wei Cai, MD, PhD, Chief Doctor, Department of Radiation Oncology, Shanghai Chest Hospital, Shanghai Jiao Tong University, 241 West Huaihai Road, Shanghai 200030, China. cxw1802@shchest.org
Telephone: +86-18916273163

Abstract

BACKGROUND

There has been no study comparing the difference in the failure patterns between patients with or without postoperative radiotherapy (PORT) after esophagectomy for pT3-4N0-3M0 esophageal squamous cell carcinoma (ESCC).

AIM

To investigate the difference in the failure patterns of stage pT3-4N0-3M0 ESCC patients with or without PORT.

METHODS

Patients with stage pT3-4N0-3M0 ESCC, who underwent surgery with or without PORT, were enrolled in this study. The primary endpoint was to investigate the difference in the failure patterns between patients with or without PORT after esophagectomy. The secondary endpoint was to estimate whether patients with stage pT3-4 ESCC could achieve a disease-free survival (DFS) advantage after receiving adjuvant PORT. Statistical analyses were performed by the Kaplan-Meier method, Cox regression model, and Chi-squared test or Fisher's exact test.

RESULTS

In total, 230 patients with stage pT3-4N0-3M0 ESCC were included in this study. Fifty-six patients who received PORT were screened from a prospective cohort (S

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

Conflict-of-interest statement: None of the authors have any conflict of interest to disclose.

Manuscript source: Unsolicited manuscript

Received: April 26, 2019
Peer-review started: May 9, 2019
First decision: July 31, 2019
Revised: August 8, 2019
Accepted: September 10, 2019
Article in press: September 10, 2019
Published online: December 15, 2019

P-Reviewer: Tarnawski AS
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Qi LL

+ R arm). And 174 patients involving surgery alone were retrospectively selected from July 2006 to October 2014 (S arm). There were no significant differences in the clinical or pathological characteristics of patients between the two arms, except for tumor location (P = 0.031). The failure patterns between the two arms were significantly different (P < 0.001). Patients in the S arm had a significantly higher proportion of locoregional recurrence and a lower proportion of distant metastasis than those in the S + R arm (92.0% *vs* 35.7%, P < 0.001 and 19.0% *vs* 75.0%, P < 0.001, respectively). The difference in the median DFS between the two arms was statistically significant (12.7 *vs* 8 mo, P = 0.048). Univariate analysis and multivariate analysis both demonstrated that the number of lymph node metastases ≥ 3 (HR = 0.572, 95%CI: 0.430-0.762, P < 0.001) was an independent poor prognostic factor for DFS in patients with stage pT3-4N0-3M0 ESCC.

CONCLUSION
PORT could improve DFS and local control of patients with stage pT3-4N0-3M0 ESCC. However, further studies need to be conducted to control hematogenous metastasis after PORT.

Key words: Esophageal squamous cell carcinoma; Postoperative radiotherapy; Failure patterns; Disease-free survival

Core tip: This is the first study to compare the difference in the failure patterns for pT3-4N0-3M0 esophageal squamous cell carcinoma (ESCC) patients with or without postoperative radiotherapy (PORT) after esophagectomy. The result showed that PORT could improve disease-free survival and local control of patients with stage pT3-4N0-3M0 ESCC. However, distant metastasis was the main failure pattern after receiving PORT. Further studies need to be conducted to control hematogenous metastasis in the future.

Citation: Zeng Y, Yu W, Liu Q, Yu WW, Zhu ZF, Zhao WX, Liu J, Wang JM, Fu XL, Liu Y, Cai XW. Difference in failure patterns of pT3-4N0-3M0 esophageal cancer treated by surgery *vs* surgery plus radiotherapy. *World J Gastrointest Oncol* 2019; 11(12): 1172-1181
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1172.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1172

INTRODUCTION

Esophageal cancer is an aggressive malignancy with high morbidity and mortality[1]. The guidelines of the National Comprehensive Cancer Network[2] recommend that esophagectomy is still the primary choice for esophageal cancer. Neoadjuvant chemoradiotherapy followed by surgery is the standard care for patients with locally advanced esophageal squamous cell carcinoma (ESCC)[3]. Nevertheless, many patients tend to choose surgery as their primary treatment because of the traditional concept in China, although neoadjuvant therapy is recommended by surgeons. Furthermore, most patients who were clinically diagnosed with stage T1-2 disease were pathologically confirmed as having stage T3-4 after surgery owing to the less frequent use of ultrasound endoscopy. However, with a poor survival and high rate of recurrence after esophagectomy[4,5], patients with pT3-4 disease require multidisciplinary adjuvant treatments. Additionally, the efficacy of postoperative radiotherapy (PORT) has been investigated widely. Based on a cancer registry database, patients with esophageal cancer could not benefit from PORT[6]. However, many retrospective studies showed that PORT could apparently improve the locoregional control rate and overall survival (OS) rate for patients with locally advanced ESCC (stage III or with positive lymph node)[7-9].

Locoregional recurrence is the most common pattern of failure after esophagectomy[4,10]. Approximately 80% of recurrence sites of ESCC patients after curative esophagectomy are located in the supraclavicular and superior mediastinal lymph nodes, which are called the T-shape field[11-13]. Chen *et al*[7] reported that the locoregional recurrence rate for patients receiving PORT was significantly lower than

that of patients who had undergone surgery alone. However, no study has compared the difference of the failure patterns between stage pT3-4 patients with or without PORT after esophagectomy.

In that case, this study was administered with the intention of investigating the difference in the failure patterns of patients with stage pT3-4N0-3M0 ESCC after complete resection with or without PORT and estimating whether patients could obtain a DFS benefit from PORT.

MATERIALS AND METHODS

Patients

Patients in this study were screened from two treatment centers that included 429 ESCC patients with stage pT1-4N0-3M0. Patients in the S + R arm were screened from a prospective cohort that included 124 patients with stage pT3-T4 ESCC, who had received radiotherapy after surgery[14]. Patients in the S arm were screened from a retrospective cohort including 305 patients who had undergone radical surgery alone[12]. The main eligible inclusion criteria were as follows: (1) A diagnosis of primary thoracic ESCC and pathologically confirmed stage T3-4N0-3M0 disease; (2) Aged 18-75 years; (3) Integrated physical examination, contrast esophagography, enhanced computed tomography of the chest and upper abdomen, and external ultrasonography of the neck or positron emission tomography (PET/CT) conducted before surgery and radiotherapy; (4) Adequate bone marrow, liver and renal functions for patients receiving radiotherapy; and (5) Patients with any patterns of failure during follow-up. The exclusion criteria were as follows: Receiving chemotherapy or radiotherapy before surgery; no failure patterns occurring to the last follow-up; patients with a secondary primary tumor after or during PORT. Additionally, patients who had received adjuvant chemotherapy were not considered in the study. The patient selection procedure is shown in Figure 1.

Radiotherapy

All the patients in the S + R arm received radiotherapy within 8 wk after surgery. The patients underwent CT-based treatment simulation in the supine position with a thermoplastic mask for immobilization. Five-millimeter-thick images were obtained from the entire neck to the upper abdomen. Intensity-modulated radiotherapy with a 6 MV X-ray linear accelerator was used for external beam radiation. The delineation of the clinical tumor volume (CTV) was based on surgery procedure, CT imaging obtained before and after surgery, and gastrointestinal endoscopy obtained before treatment. The CTV included the primary tumor bed and metastatic lymph nodes or plus bilateral supraclavicular and upper-middle mediastinal lymphatic drainage areas (T-shape field). The planning target volume was defined as CTV plus a 0.8 to 1.0 cm margin. The radiation dose to the primary tumor bed and metastatic lymph nodes was 63 Gy (2.25 Gy/day/fraction, 28 fractions) for patients with stage T4 or 60.2 Gy (2.15 Gy/day /fraction, 28 fractions) for patients with stage T3 disease. The lymphatic drainage area was prescribed a dose of 50.4 Gy (1.8 Gy/day/fraction, 28 fractions). The prescription dose was delivered to the target volume mainly through the mediastinum. Normal tissue dose constraints met the usual requirements: (1) Maximum spinal cord dose ≤ 45 Gy; (2) Lung V20 ≤ 25% and mean lung dose ≤ 15 Gy; and (3) Mean heart dose ≤ 30 Gy.

Chemotherapy

After surgery or radiotherapy was completed, chemotherapy was scheduled intravenously with cisplatin (25 mg/m^2/d, d1–3) and 5-fluorouracil (5-Fu, 600 mg/m^2/d, d1–5) or paclitaxel (135-175 mg/m^2/d, d1) for four cycles with an interval of 3-4 wk.

Follow-up

Follow-up visits were administered every 3 mo for the first two years after treatment, every 6 mo for the next three years, and then once a year thereafter. Patients in the S + R arm were followed to March 2017. Follow-up investigations included hematological examination, supraclavicular lymph node ultrasonography, contrast esophagography, and enhanced computed tomography of the chest and upper abdomen. When necessary, PET/CT or bone emission computed tomography was required.

Definitions of failure patterns

This study analyzed the first failure pattern only. The discovery of relapse was mainly based on imaging features. Fine-needle aspiration biopsy and PET/CT were implemented only when necessary. Locoregional recurrence included the tumor bed

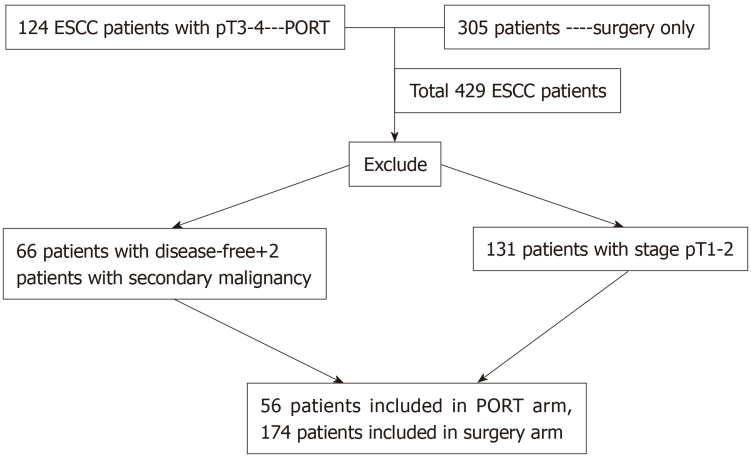

Figure 1 Patient selection flow chart. ESCC: Esophageal squamous cell carcinoma; PORT: Postoperative radiotherapy.

area, anastomotic stoma relapse, and regional lymph nodes, for which the short axis diameter was at least 1 cm in the CT image, according to the tumor location. Distant metastasis was defined as neoplasms occurring in organs or non-regional lymph node metastasis. The definitions of failure patterns were described in detail in our previous report[12]. Recurrence comprising locoregional and distant failure simultaneously was classified as mixed failure.

Statistical analysis

Owing to the retrospective characteristics of the S arm, we did not have access to the OS information of over 10% patients. The primary endpoint of this study was to compare the difference in the failure patterns between the two arms. The secondary endpoint was to evaluate whether patients with locally advanced ESCC could achieve DFS benefit from PORT. DFS was calculated from the date of surgery to the date that any type of failure occurred or the date of the last follow-up. The difference in the failure patterns and clinical characteristics was calculated by the Chi-squared test or Fisher's exact test. The Kaplan-Meier method was used to evaluate the difference in DFS between the two arms. The log-rank test was used to determine the statistical significance of the difference. Univariate analysis and multivariate analysis (Cox proportional hazard regression model) were performed to evaluate the risk factors associated with the prognosis. A P value less than 0.05 (two sided) was considered statistically significant. All statistical analyses were calculated with SPSS (version 22.0 IBM Chicago, United States).

RESULTS

This study included 230 patients. In the prospective cohort of 124 ESCC patients with stage pT3-T4 disease who underwent PORT, 66 patients lived free of disease and 2 patients with secondary primary tumor were excluded. Finally, 56 patients who met the criteria were recruited as the S + R arm from April 2011 to February 2016. We retrospectively screened 305 patients who received surgery alone. Among them, 131 patients with stage pT1-2 disease were excluded and 174 patients with pT3-4 disease suffering failure were enrolled as the S arm from July 2006 and October 2014. Finally, 230 patients were included in this study. All patients in the S + R arm completed the intended radiotherapy treatment plan within 6 wk. There were 60.7% of patients who received sequential chemotherapy after radiotherapy was completed. Others who declined or could not tolerate did not receive chemotherapy. There was no difference in the percentage of patients receiving chemotherapy between the S arm and S + R arm. The baseline clinical and pathologic characteristics of the two arms are shown in Table 1. Differences between the two cohorts in sex, age, pathological differentiation, number of lymph nodes dissected, lymph node involvement, and chemotherapy were not significant, except for tumor location (P = 0.031).

Failure patterns

All patients included in this study developed failure during the follow-up. We compared the difference in the constituent ratio of the failure patterns between the

Table 1 Clinical and pathological characteristics of patients of the two arms, *n* (%)

Variable	S arm (*n* = 174)	S + R arm (*n* = 56)	*P* value
Sex			0.656
Male	159 (91.4)	49 (87.5)	
Female	15 (8.6)	7 (12.5)	
Age (yr)			0.056
<60	106 (60.9)	42 (75.0)	
≥60	68 (39.1)	14 (25.0)	
Pathological differentiation			0.586
Poor	66 (37.5)	1 (1.8)	
Moderate	97 (55.7)	33 (58.9)	
Well	10 (5.7)	22 (39.3)	
Tumor location			0.031a
Upper	15 (8.6)	3 (5.4)	
Middle	98 (56.3)	22 (39.3)	
Lower	61 (35.1)	31 (55.4)	
Lymph node dissection			0.448
2-field resection	139 (79.9)	49 (87.5)	
3-field resection	28 (16.1)	7 (12.5)	
No. of LNs involved			0.740
≤2	113 (64.9)	35 (62.5)	
≥3	61 (35.1)	21 (37.5)	
No. of LNs dissected			0.569
<12	2 (1.1)	1 (1.8)	
≥12	172 (98.9)	55 (98.2)	
T stage			0.075
T3	164 (94.3)	48 (85.7)	
T4	10 (5.7)	8 (14.3)	
Chemotherapy			0.978
Yes	106 (60.9)	34 (60.7)	

[a]$P < 0.05$. S arm: Surgery alone arm; S + R arm: Surgery + postoperative radiotherapy arm; LN: Lymph node.

two arms. As shown in Table 2, the difference in the failure patterns between the two arms was statistically significant ($P < 0.001$). Regarding patients with distant metastasis, locoregional recurrence, and mixed failure, 64.3%, 25.0%, and 10.7%, respectively, were in the S + R arm and 8.0%, 81.0%, and 11.0%, respectively, were in the S arm. Patients in the S arm had a significantly higher proportion of locoregional recurrence than patients in the S + R arm (92.0% *vs* 35.7%, respectively, $P < 0.001$). However, patients in the S + R arm had a much higher proportion of distant metastasis than patients in the S arm (75.0% *vs* 19.0%, $P < 0.001$). The most common regions for locoregional recurrence were the supraclavicular and mediastinal lymph nodes (29.5% and 48.3%, respectively) for the surgery cohort. For the S + R arm, supraclavicular lymph nodes (9/17 patients) were the most common relapse region after PORT. The lungs, liver, and bone [41.7% (14/36 patients), 19.4% (7/36 patients), and 16.7% (6/36 patients), respectively] were the most common metastatic organs for the S + R arm. Distant metastasis mainly occurred in the lungs (7/33), supraclavicular lymph nodes (16/33), and celiac lymph nodes (10/33) for the S arm (Figure 2).

DFS

Recurrence occurred almost within two years for both arms. The 1-, 2-, and 3-year DFS rates of the S arm and S + R arm were 33.7%, 14.5%, and 4.7% and 51.8%, 26.8%, and 8.9%, respectively. The DFS was improved from 8 mo to 12.7 mo after receiving PORT. The difference in DFS for the two arms was marginally significant ($P = 0.048$, Figure 3).

Univariate analysis revealed that only lymph node involvement was associated with DFS for T3-4 ESCC patients ($P < 0.001$, Figure 4 and Table 3). Patients with the number of lymph node metastases fewer than 2 could achieve a better DFS (11.0 *vs* 8.0 mo). DFS was not statistically associated with age, sex, tumor location, pathological

Table 2 Comparison of failure patterns for the two arms, *n* (%)

Type of failure	S arm (*n* = 174)	S + R arm (*n* = 56)	*P* value
Failure patterns			< 0.001
Distant metastasis	14 (8.0)	36 (64.3)	
Locoregional recurrence	141 (81.0)	14 (25.0)	
Mixed failure	19 (11.0)	6 (10.7)	
Distant metastasis			< 0.001
Distant metastasis	33 (19.0)	42 (75.0)	
Others	141 (81.0)	14 (25.0)	
Locoregional failure			< 0.001
Locoregional recurrence	160 (92.0)	20 (78.3)	
Others	14 (8.0)	36 (21.7)	

S arm: Surgery alone arm; S + R arm: Surgery + postoperative radiotherapy arm; LN: Lymph node.

differentiation, lymph node dissection, number of lymph nodes dissected, and adjuvant chemotherapy.

However, multivariate analysis indicated that the number of lymph node metastases ≥ 3 (HR = 1.843, 95%CI: 1.369-2.482; $P < 0.001$), PORT (HR = 0.667, 95%CI: 0.487-0.915; $P = 0.012$), age (HR = 0.712, 95%CI: 0.530-0.957; $P = 0.024$), and chemotherapy (HR = 0.732, 95%CI: 0.552-0.971; $P = 0.031$) were independent prognostic factors for DFS in patients with stage pT3-4N0-3M0 ESCC (Table 3).

DISCUSSION

Although many studies have investigated the characteristics of failure patterns for patients with thoracic esophageal carcinoma after esophagectomy, this study is the first to investigate the difference in the failure patterns for stage pT3-4 ESCC patients with or without PORT after radical surgery. Our study found that radiotherapy following esophagectomy was more effective in improving DFS and decreasing locoregional recurrence of patients with stage pT3-4 ESCC. Distant metastasis was the most common failure after receiving PORT. The number of lymph nodes involved over 3, radiotherapy, age, and chemotherapy were significantly associated with disease progression for patients with stage pT3-4 ESCC.

It is known that neoadjuvant chemoradiotherapy is recommended as the standard care for locally advanced ESCC. However, many patients initially diagnosed with T1-2N0 disease before surgery were finally diagnosed as having pT3-4N0-3M0 disease after surgery. Do these patients need to receive adjuvant treatment after surgery? Xiao *et al*[15] revealed that patients receiving PORT with a prescription dose of 60 Gy could obtain a local control benefit and a higher 5-year OS than patients receiving surgery alone. With the application of 3-dimentional conformality radiotherapy, PORT could bring both local control and OS benefit for patients with stage III and stage T3N0M0 esophageal cancer or patients with positive lymph nodes[16-19]. Additionally, Schreiber *et al*[18] reported a survival benefit for patients with both stage III ESCC and esophageal adenocarcinoma after receiving PORT. Our result also revealed a better DFS for patients in the S + R arm than those in the S arm.

It is common for patients with esophageal cancer to develop relapse after treatment. Locoregional failure is the most common failure pattern for patients with surgery alone; and mediastinal lymph nodes, especially the upper mediastinal lymph nodes and supraclavicular lymph nodes, are the most common recurrence sites[11-13,20]. This study also showed that approximately 80% of cases with locoregional failure occurred in the T-shape field. By comparing the constituent ratio of failure patterns for patients who underwent surgery with or without PORT, it was showed that locoregional recurrence decreased and distant metastasis became the main failure pattern after receiving PORT for patients with stage T3-4 disease, a finding that agrees with the previous study results[7,21]. Distant metastasis after receiving PORT is a new issue that leads to treatment failure. Considering that no difference was found in the survival of patients receiving adjuvant chemotherapy, whether chemotherapy could further decrease the rate of metastasis needs further research. The reasons that more patients showed distant metastasis may be due to the small number of patients, local control increase, or there were inherent factors making patients prone to distant

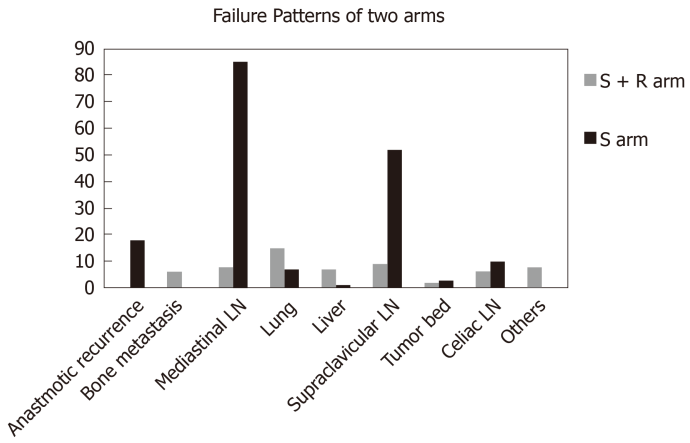

Figure 2 Details of the failure patterns for the two arms. The Y axis represents the number of patients. The most common regions for locoregional recurrence were the supraclavicular and mediastinal lymph nodes (29.5% and 48.3%, respectively) for the surgery cohort. For the S + R arm, supraclavicular lymph nodes (9 of 17 patients) were the most common relapse region after postoperative radiotherapy.

metastasis. Our recent research identified a potential biomarker for metastasis of ESCC[22], and we will study this topic further to clarify the mechanism of increased distant metastasis after PORT.

Both univariate and multivariate analyses demonstrated that lymph node involvement was significantly associated with the DFS of patients with stage T3-4 disease. Adjuvant chemotherapy may be a favorable factor. Meanwhile, many studies have reported that adjuvant chemotherapy could improve local control and OS or DFS, especially for patients with positive lymph nodes[23,24]. Researchers also reported that postoperative chemoradiotherapy could decrease both locoregional and distant recurrence. However, it must be noted that combined chemoradiation therapy may lead to higher toxicity[25]. Thus, we supposed that combined radiation and chemotherapy sequentially may be effective in improving DFS or even OS.

This study possessed some limitations. First, due to the retrospective nature of this study, it is inevitable that some information was uncontrolled or missed and it is difficult to address some biases. A perspective study should be conducted to answer these questions in the future. Second, patients in the S + R arm were fewer than those in the S arm, possibly influencing DFS; a longer follow-up and more enrolled patients are needed. Third, the study compared the constituent ratio of failure patterns because of the respective characteristics of patients.

In conclusion, PORT could improve DFS and decrease the locoregional recurrence of patients with stage pT3-4N0-3M0 ESCC. However, distant metastasis is the main failure pattern in patients after receiving PORT. Further study needs to be conducted to evaluate how to control hematogenous metastasis.

Table 3 Univariate and multivariate analyses for factors affecting survival

	Univariate analysis	Multivariate analysis			
	Median DFS (mo)	χ^2 value	*P* value	HR (95%CI)	*P* value
Sex				0.841 (0.532-1.328)	0.457
Male	9.5	0.309	0.579		
Female	8				
Age (yr)				0.712 (0.530-0.957)	0.024
< 60	9	1.023	0.312		
≥ 60	9.5				
Pathological differentiation				1.128 (0.094-1.934)	0.071
Poor	8	6.377	0.095		
Moderate	10.4				
Well	9.5				
Tumor location				1.423 (0.849-2.386)	0.363
Upper	6.6	3.277	0.194		
Middle	9.9				
Lower	8.8				
Lymph node dissection				0.531 (0.222-1.186)	0.107
2-field resection	9	3.462	0.177		
3-field resection	8				
No. of LNs involved				1.843 (1.369-2.482)	<0.001[a]
≤ 2	11	13.483	< 0.001		
≥ 3	8				
No. of LN dissected				1.460 (0.459-4.644)	0.522
< 12	7	0.656	0.418		
≥ 12	9.5				
Chemotherapy				0.732 (0.552-0.971)	0.031
Yes	8	1.769	0.183		
No	10				
T stage				0.697 (0.441-1.124)	0.132
T3	9	0.087	0.767		
T4	8				
Radiotherapy	-	-	-	0.667 (0.487-0.915)	0.012[a]

[a]*P* < 0.05 in both univariate and multivariate analysis. S arm: Surgery alone arm; S + R arm: Surgery + postoperative radiotherapy arm; LN: Lymph node.

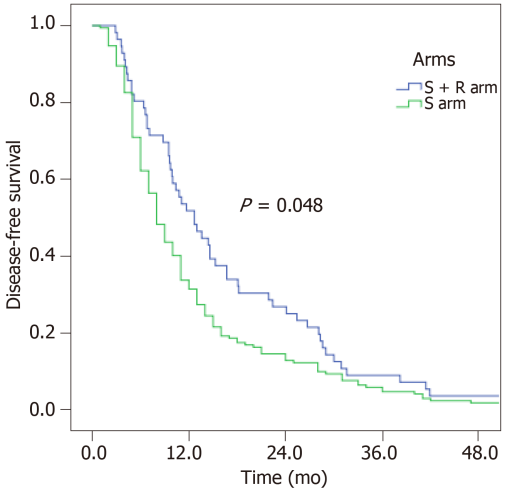

Figure 3 Disease-free survival (174 *vs* 56) of patients in the two arms. The Y axis represents disease-free survival (DFS) rate. The median DFS rates of the S and S + R arm were 8.0 and 12.7 mo, respectively.

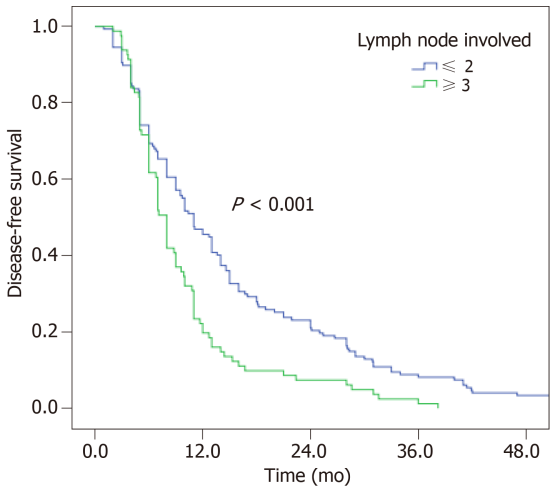

Figure 4 Univariate analysis. The Y axis represents disease-free survival rate. Patients with fewer than two lymph nodes involved could achieve a better disease-free survival.

ARTICLE HIGHLIGHTS

Research background

Postoperative radiotherapy (PORT) could improve the local control of stage T3-4 or lymph node positive esophageal squamous cell carcinoma (ESCC) patients. There was no study comparing the difference of failure patterns after surgery with or without PORT in such patients.

Research motivation

We wanted to investigate the difference of failure patterns in order to guide the following treatment for patients suffering treatment relapse.

Research objectives

To define the difference between patients with stage pT3-4N0-3M0 ESCC with or without PORT after esophagectomy.

Research methods

Patients with pathologically stage T3-4 ESCC who receive PORT after surgery were included in an S + R arm, and the others without PORT were included in an S arm. This study mainly investigated the difference of failure patterns between the two arms.

Research results

This study reported that PORT could decrease locoregional relapse. However, the proportion of distant metastasis in the S + R arm was much more than that in the S arm.

Research conclusions

PORT could improve the local control for patients with stage pT3-4 ESCC. Further studies need to be conducted to control hematogenous metastasis.

Research perspectives

The treatment of locally advanced ESCC is a hot topic. PORT could decrease locoregional lymph node relapse, but distant metastasis after PORT is the main reason that results in treatment failure. It is urgent to find an effective treatment to control this situation. And now we should explain the main failure patterns after undergoing different treatment strategies.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1182-1192

DOI: 10.4251/wjgo.v11.i12.1182

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Impact of regular enteral feeding *via* jejunostomy during neo-adjuvant chemotherapy on body composition in patients with oesophageal cancer

Imran M Mohamed, John Whiting, Benjamin HL Tan

ORCID number: Imran M Mohamed (0000-0003-3831-8594); John Whiting (0000-0001-5432-9838); Benjamin HL Tan (0000-0002-4593-8719).

Author contributions: Mohamed IM, Whiting J and Tan BHL contributed to the completion of the manuscript; Mohamed IM contributed to manuscript writing and data analysis; Whiting J contributed to manuscript writing and review; Tan BHL contributed to conception and design, manuscript writing, data analysis and review; All authors have read and approved the final manuscript.

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

Conflict-of-interest statement: All authors declare no conflicts-of-interest related to this article.

Data sharing statement: No additional data are available.

Imran M Mohamed, John Whiting, Benjamin HL Tan, Department of Upper Gastrointestinal Surgery, Queen Elizabeth Hospital, Birmingham B15 2TH, United Kingdom

Corresponding author: Benjamin HL Tan, MBChB, MD, FRCS, Department of Upper Gastrointestinal Surgery, Queen Elizabeth Hospital, Mindelsohn Way, Birmingham B15 2TH, United Kingdom. benjamin.tan@uhb.nhs.uk

Abstract

BACKGROUND

Malnourishment and sarcopenia are well documented phenomena in oesophageal cancer. Patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy have complex nutritional needs.

AIM

To examine the effect of regular nutritional support *via* feeding jejunostomy on overall body composition in patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy for oesophageal cancer.

METHODS

Retrospective data were collected for 15 patients before and after neo-adjuvant chemotherapy. All patients had feeding jejunostomies inserted at staging laparoscopy prior to neo-adjuvant chemotherapy and underwent regular jejunostomy feeding. Changes in body composition were determined by analysis of computed tomography imaging.

RESULTS

Patient age was 61.3 ± 12.8 years, and 73% of patients were male. The time between start of chemotherapy and surgery was 107 ± 21.6 d. There was no change in weight (74.5 ± 14.1 kg to 74.8 ± 13.1 kg) and body mass index (26.0 ± 3.8 kg/m^2 to 26.1 ± 3.4 kg/m^2). Body composition analysis revealed a statistically significant decrease in lumbar skeletal muscle index despite regular feeding (45.8 ± 8.0 cm^2/m^2 to 43.5 ± 7.3 cm^2/m^2; P = 0.045). The proportion of sarcopenic patients increased (33.3% to 60%). Six patients (40%) experienced dose-limiting toxicity during chemotherapy.

CONCLUSION

Regular jejunostomy feeding during neo-adjuvant chemotherapy can maintain

Manuscript source: Invited manuscript

Received: February 25, 2019
Peer-review started: February 26, 2019
First decision: July 31, 2019
Revised: August 29, 2019
Accepted: November 4, 2019
Article in press: November 4, 2019
Published online: December 15, 2019

P-Reviewer: Aykan NF, Merrett ND, Shi H
S-Editor: Ma YJ
L-Editor: Filipodia
E-Editor: Qi LL

weight and adipose tissue. Feeding alone is not sufficient to maintain muscle mass. Further insight into the underlying processes causing reduced muscle mass in cancer patients may help to provide targeted interventions.

Key words: Body composition; Neo-adjuvant therapy; Oesophageal cancer; Enteral feeding

Core tip: Patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy have complex nutritional needs. Retrospective data were collected for 15 patients before and after neo-adjuvant chemotherapy to examine the effect of regular nutritional support *via* feeding jejunostomy on overall body composition. There was no change in weight and body mass index. Body composition analysis revealed a statistically significant decrease in lumbar skeletal muscle index despite regular feeding. Regular jejunostomy feeding during neo-adjuvant chemotherapy can maintain weight and adipose tissue. Further insight into processes causing reduced muscle mass in cancer patients may provide targeted interventions.

Citation: Mohamed IM, Whiting J, Tan BH. Impact of regular enteral feeding *via* jejunostomy during neo-adjuvant chemotherapy on body composition in patients with oesophageal cancer. *World J Gastrointest Oncol* 2019; 11(12): 1182-1192
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1182.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1182

INTRODUCTION

Oesophageal cancer is the sixth most common cause of cancer death in the United States[1]. Neo-adjuvant chemotherapy is now the mainstay of treatment prior to oesophagectomy and is associated with improved survival compared to surgery alone[2]. However, use of such therapy places additional nutritional burden on patients and has an adverse impact on nutritional status and body composition as measured by the proportions of skeletal muscle and fat[3]. Continued improvement in overall survival has led to increased focus on optimising patients' nutritional status throughout the whole continuum of their management.

Patients with oesophageal cancer have complex nutritional needs. Such patients often experience the onset of a catabolic state induced by the malignancy as well as tumour-associated dysphagia. The use of multiple strategies to manage disease progression including radiation, chemotherapy and surgical resection present an additional nutritional burden to these patients who are often malnourished at presentation. Adequate nutritional reserves are essential to allow patients to tolerate neo-adjuvant chemotherapy as well as subsequent surgery. Malnourishment and sarcopenia are well documented phenomena in oesophageal cancer. Local tumour effects, dysphagia, loss of appetite, physical inactivity as well as psychological factors all impair dietary intake and serve to compound cancer cachexia[2-4]. Malnourishment reduces the potential response of the malignancy to chemoradiotherapy and impairs the patient's ability to tolerate the full course of treatment. Nutritional deficiencies may also contribute to increased perioperative morbidity and mortality.

There are various indicators of nutritional status that predict an individual's ability to tolerate neo-adjuvant chemotherapy and surgery for oesophageal cancer. Lower body mass index (BMI), skeletal muscle depletion, sarcopenia and sarcopenic obesity are associated with dose-limiting toxicity (DLT) during treatment with neo-adjuvant chemotherapy[4-6]. Treatment with neo-adjuvant chemotherapy is also associated with changes in body composition. Indeed, Awad *et al*[7] showed a decrease in fat free mass and increased prevalence of sarcopenia in their observational study after treatment with neo-adjuvant chemotherapy. Tsujimoto *et al*[8] recorded statistically significant decreases in body weight and serum total protein in patients who received neo-adjuvant chemotherapy. Toxicity from neo-adjuvant chemotherapy can also lead to further malnutrition and weight loss. Thus, further changes in body composition can affect tolerability of subsequent treatment, which in turn relates to poorer patient outcomes[3,9].

The period preceding surgery is the ideal time to ensure patients are in the best possible state for surgery. Optimisation of nutrition through nutritional screening and supplementation is an important consideration in the overall peri-operative management of oesophageal cancer, including during treatment with neo-adjuvant chemotherapy.

The use of enteral feeding *via* jejunostomy, particularly in oesophageal cancer, is a reliable method to optimise nutrition. The associated risks of insertion do not significantly outweigh the conferred benefits[8,10,11]. Previous studies of feeding *via* laparoscopically inserted jejunostomy prior to neo-adjuvant chemotherapy demonstrated an increase in weight ranging from 0.4 to 11.8 kg[2]. The present study is the first to our knowledge to examine the changes of body composition from regular enteral feeding *via* jejunostomy during neo-adjuvant therapy for oesophageal cancer[2].

The aim of this study is to examine the effect of regular enteral feeding *via* jejunostomy on overall body composition in a cohort of patients with oesophageal cancer undergoing neo-adjuvant chemotherapy prior to oesophagectomy. The effect of regular jejunostomy feeding on the development of DLT during neo-adjuvant chemotherapy was also examined.

MATERIALS AND METHODS

Study population

Patients having potentially curative, locally advanced oesophageal and oesophago-gastric junctional cancer without evidence of metastasis presenting to the Queen Elizabeth Hospital, Birmingham Upper Gastrointestinal Multidisciplinary Team between March 2014 and June 2017 were considered for this study. All patients were routinely staged with a combination of computed tomography (CT), endoscopic ultrasound and laparoscopy according to the International Union Against Cancer system[12]. Those with locally advanced disease (T2 and greater and/or locoregional lymphadenopathy) and with sufficient physiological reserve to tolerate neo-adjuvant chemotherapy were further examined retrospectively for suitability for inclusion into the present study.

All patients included in this study were reviewed by a Consultant Oesophago-gastric Surgeon after diagnosis and were assessed to have a dysphagia score of 3 (the ability to swallow liquids only) or 4 (complete dysphagia)[13]. These patients had a feeding jejunostomy inserted at the time of staging laparoscopy prior to commencement of neo-adjuvant chemotherapy.

All patients were assessed by a dietician prior to starting regular jejunostomy feeding. After assessment by the dietitian, each patient had the feeding regimen tailored to their individual nutritional needs. The Henry equation[14] was used to estimate basal metabolic rate with 10% added as a stress factor during chemotherapy. Each individuals' activity factor was also considered. Based on these, the calorie intake required per day was calculated. The feeding regiment aims to provide 0.17-0.2 g nitrogen/kg/d. Each individuals' feeding regiment takes into account the amount of oral diet achieved. The feeding regimen aimed to provide 10-12 hr overnight continuous feeding if possible, to limit disruption of daytime activities. All patients were maintained on regular feeding daily throughout their period of receiving neo-adjuvant chemotherapy.

Anthropometric measurements

Weight and height were recorded according to standard methods. Weight was measured with a medical balance beam scale, and height was measured with a stadiometer. BMI was calculated [weight (kg)/height (m^2)].

Image analysis

CT has proven to be accurate for measuring human body composition[15,16]. Regional muscle tissue was measured by CT from electronically stored images, which had been done previously for diagnostic purposes. CT scans were performed at two time points: The first at diagnosis prior to commencement of chemotherapy and the second after completion of neo-adjuvant chemotherapy prior to surgery (Figure 1).

The third lumbar vertebra (L3) was chosen as a landmark, and two consecutive slices were assessed to measure cross-sectional area of muscle and adipose tissue as described[17]. The average value of two images was computed for each patient. Images were analysed using Slice-O-Matic software V4.3 (Tomovision). Cross sectional area for muscle and adipose tissue was normalized for stature (cm^2/m^2) and reported. Patients were classified as sarcopenic according to established cut offs: L3 muscle index < 41 cm^2/m^2 for women and < 43 cm^2/m^2 for men with a BMI < 25 or L3 muscle

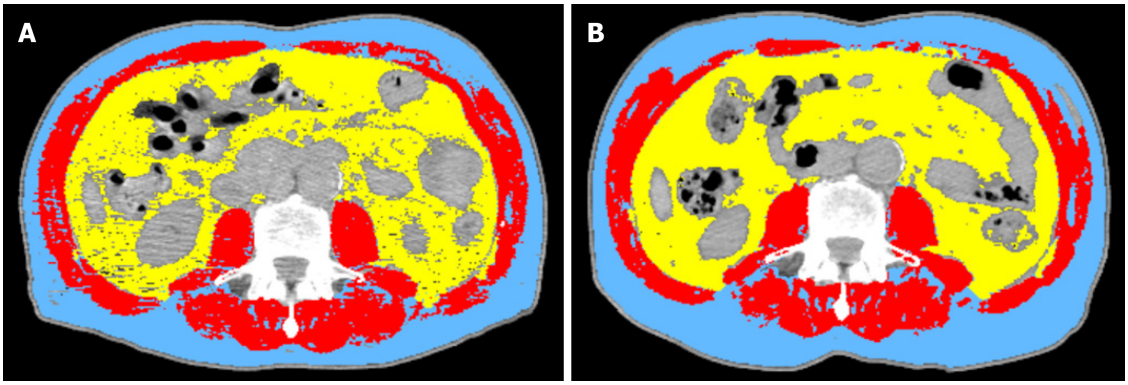

Figure 1 Radiologic image showing a comparison of body composition at diagnosis (A) and post neo-adjuvant (B) chemotherapy (two computed tomography scans of the third lumbar vertebra skeletal mass) for the same patient. Red: Muscle; Yellow: Visceral fat; Blue: Subcutaneous fat.

index < 53 cm^2/m^2 for men with a BMI > 25[18]. The mean Hounsfield unit measurement of all skeletal muscle within the L3 cross-section was recorded as a measure of myosteatosis, which was defined operationally as a mean skeletal muscle radiodensity of < 33 Hounsfield unit in those with a BMI > 25; and < 41 Hounsfield unit in those with a BMI < 25 across the axial orthogonal view[18].

DLT

DLT was defined by intolerable toxicities requiring the postponement of treatment, a drug dose reduction or definitive interruption of drug administration.

Statistical analysis

Data were recorded as mean ± standard deviation unless otherwise stated. Comparison between values at diagnosis and post neo-adjuvant chemotherapy were calculated using the paired *t*-test or McNemar's test. A *P*-value less than 0.05 was considered to be statistically significant. Statistical analysis was done using SPSS 15.0 statistical package (SPSS Inc.).

RESULTS

During the study period, 15 patients underwent regular jejunostomy feeding during neo-adjuvant chemotherapy prior to surgery. Demographic data of the 15 patients are shown in Table 1. The mean age was 61.3 ± 12.8 years, and 73% of patients were male.

The time interval between start of chemotherapy and surgery was 107 ± 21.6 d. During this period, there was no change in weight and BMI (Table 2). However, body composition analysis revealed a statistically significant loss of skeletal muscle despite regular feeding during neo-adjuvant chemotherapy. Lumbar skeletal muscle index decreased from 45.8 ± 8.0 cm^2/m^2 to 43.5 ± 7.3 cm^2/m^2 (P = 0.045). Adipose tissue index remained largely unchanged (Figure 2). The number of sarcopenic patients increased from five (33.3%) at diagnosis to nine (60%) after completion of neo-adjuvant chemotherapy. There was no change in the number of myosteatotic patients before and after neo-adjuvant chemotherapy (Table 2).

Table 3 and Figure 3 show the individual values for changes in lumbar skeletal muscle index (cm^2/m^2) from diagnosis to after neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy. Patients 4 and 8 showed an increase in lumbar skeletal muscle index between diagnosis and after neo-adjuvant chemotherapy. It is interesting to note that both patients were sarcopenic at diagnosis but were no longer sarcopenic after neo-adjuvant chemotherapy.

Table 4 and Figure 4 show individual values for changes in lumbar adipose tissue index (cm^2/m^2) from diagnosis to after neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy. Patients 2, 3, 5, 6, 7 and 9 showed an increase in lumbar adipose tissue index from diagnosis to after neo-adjuvant chemotherapy. The greatest increases were seen in patients 2 and 9. None of these patients were sarcopenic at diagnosis, however patients 2, 3, 6 and 7 became sarcopenic after neo-adjuvant chemotherapy.

Six patients (40%) experienced DLT during chemotherapy. Three patients required a dose reduction, two patients stopped chemotherapy early and one patient experienced a delay in treatment.

Table 1 Patient demographics

	No. of patients, *n* = 15
Age in yr, mean ± SD	61.3 ± 12.8
Sex	
M	11 (73.3)
F	4 (26.7)
Tumour site	
Mid oesophagus	1 (6.6)
Lower oesophagus	4 (26.7)
Oesophageal-gastric junction	10 (66.7)
Histology	
Adenocarcinoma	11 (73.3)
Squamous cell cancer	3 (20.0)
Other	1 (6.7)
Stage	
I	1 (6.6)
II	4 (26.7)
III	10 (66.7)
Neo-adjuvant chemotherapy protocol	
ECX	7 (46.7)
ECF	3 (20)
EOX	1 (6.6)
CX	2 (13.3)
CF	2 (13.3)

Values are number of patients with percentages in parentheses unless indicated otherwise. SD: Standard deviation; M: Male; F: Female; ECX: Epirubicin/cisplatin/capecitabine; ECF: Epirubicin/cisplatin/5-fluorouracil; EOX: Epirubicin/oxaliplatin/capecitabine; CX: Cisplatin/capecitabine; CF: Cisplatin/5-fluorouracil.

DISCUSSION

This observational study of 15 patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy confirmed that formal nutritional intervention provided through the use of feeding jejunostomy had a positive effect on maintaining mean overall weight and BMI.

In concordance with previous studies, this study revealed a small amount of weight gain (0.35 ± 3.0 kg) after regular jejunostomy feeding, though this was negligible in the current study. Interestingly, adipose tissue was maintained but a significant loss in muscle mass remained. The incidence of sarcopenia increased from 33% of patients to 60% of patients after completion of neo-adjuvant chemotherapy.

The percentage of patients (40%) experiencing DLT whilst undergoing neo-adjuvant chemotherapy was similar to a previous study despite regular jejunostomy feeding[5]. This can perhaps be explained by the high prevalence of sarcopenia in oesophageal cancer patients despite regular feeding. Whilst the mechanism that links sarcopenia with increased chemotherapy toxicity in patients is unknown, this association is described in the literature[3,6,7].

Sarcopenia is prevalent in patients diagnosed with oesophageal cancer, and the incidence increases following neo-adjuvant chemotherapy. This is coupled with an overall decrease in body weight, BMI, fat mass and fat-free mass. BMI is often used as an objective marker to assess patients' nutritional status. However, patients can be acutely malnourished and still have a normal or elevated BMI, especially with the increasing prevalence of obesity. Therefore, assessment of specific changes in body composition is perhaps more important than overall changes in weight or BMI.

One of the strengths of this study was the use of the standardised technique of using CT imaging to measure muscle mass using the skeletal muscle index at the level of the L3 vertebra. This has been proven to be a well-established technique for this purpose. Although magnetic resonance imaging and dual energy x-ray absorptiometry are also considered extremely useful to evaluate body composition in clinical practice, CT is performed in all patients with oesophageal cancer. Therefore,

Table 2 Change in body composition from diagnosis to post neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy, *n* = 15

	Diagnosis	Post-chemotherapy	*P* value
Weight, mean ± SD, kg	74.5 ± 14.1	74.8 ± 13.1	0.659[1]
BMI, mean ± SD, kg/m^2)	26.0 ± 3.8	26.1 ± 3.4	0.623[1]
Lumbar skeletal muscle index as cm^2/m^2, mean ± SD	45.8 ± 8.0	43.5 ± 7.3	0.048[1]
Lumbar adipose tissue index as cm^2/m^2, mean ± SD,	124.0 ± 53.7	124.6 ± 47.9	0.878[1]
Sarcopenic	5 (33.3)	9 (60.0)	0.219[2]
Myosteatotic	8 (53.3)	8 (53.3)	1.000[2]

[1]Paired *t*-test;
[2]McNemar's test. Values are number of patients with percentages in round parentheses unless indicated otherwise. SD: Standard deviation; BMI: Body mass index.

analysis of these images is easily available with no additional cost or patient burden[9].

Sarcopenia, myosteatosis and loss of skeletal muscle during neo-adjuvant treatment have been shown to be associated with worse oncological outcomes in surgically treated oesophageal cancer patients[3,7,9]. In sarcopenic patients, the doses of neo-adjuvant therapy may have to be adjusted and even reduced in order to compensate for toxicity associated complications. If the development of sarcopenia can be minimised, patients may benefit not only from longer courses of neo-adjuvant therapy, but the documented poorer outcomes may also be averted. It would therefore seem logical to attempt to maintain or improve skeletal mass during neo-adjuvant chemotherapy.

The use of feeding jejunostomy tubes in this cohort of patients has been shown to be safe. Although the current study did not specifically examine patient tolerance of feeding jejunostomy or complications associated with insertion, a systematic review examining nutritional optimisation in these patients reported no 30-d postprocedural complications in patients undergoing laparoscopically inserted feeding jejunostomy tubes. All such patients showed an increase in overall weight, and available evidence suggests that greater than 90% of patients completed their neo-adjuvant treatment[2]. In light of the relatively few and minor complications associated with jejunostomy, it would make sense to employ this as a feeding technique over nasogastric tubes, which are prone to easily dislodge. The question of whether placement of jejunostomy would increase difficulty of the oesophagectomy is valid. However, with laparoscopic insertion of jejunostomy tubes, intra-abdominal adhesions should be kept to a minimum, and the benefit of the tube to the patient would lend further support to placement of these tubes. Indeed, the jejunostomy tube may aid with early nutrition post-oesophagectomy.

A randomised study has suggested that enteral feed enriched with eicosapentaenoic acid, a compound that modulates the immune system and reduces catabolism in advanced cancer, may contribute to maintaining fat-free mass[19]. However, further research is required to determine the effect of such immuno-enhanced nutrition on overall clinical outcomes in patients with oesophageal cancer.

There are several limitations in the current study. One being the relatively small sample size. There is also heterogeneity in the type of chemotherapy used, which may be a contributory factor towards the changes in body composition. Other factors that may affect changes in weight such as hypoalbuminaemia, oedema and other comorbidities were not specifically examined in this study population. However, by the time of diagnosis, patients with oesophageal cancer are often already suffering from malnutrition and involuntary weight loss with sarcopenia being a major marker of frailty. Thus, addressing malnutrition pre-operatively is a major area to target for pre-optimisation before surgery[4].

It would also be useful to examine data on patients who did not receive feeding *via* feeding jejunostomy and especially those who were maintained on an oral diet. This would allow comparison with the current study group in order to measure any differences in outcome between the two groups and therefore would contribute to more detailed assessment of the effect of feeding in this group of patients. Nevertheless, this study has shown that regular jejunostomy feeding during neo-adjuvant chemotherapy can attenuate the previously shown loss in overall weight and adipose tissue. It is clear, however, that feeding alone is not sufficient to maintain muscle mass in this group of patients. Further insight into the underlying processes causing reduced muscle mass in cancer patients may help to provide targeted interventions.

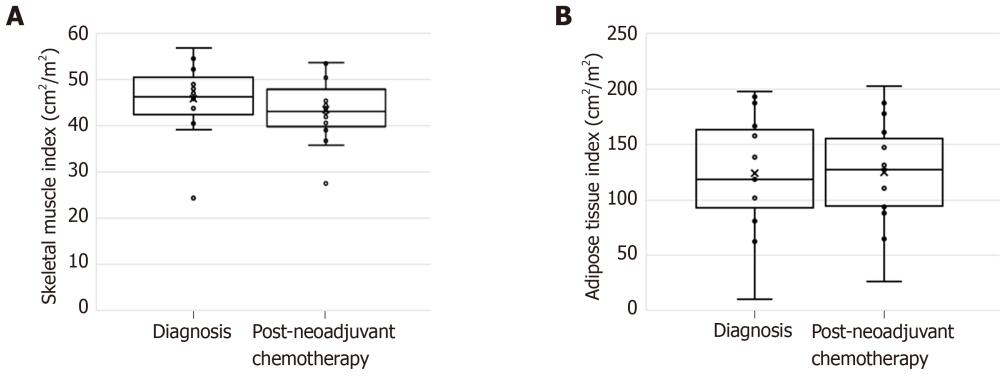

Figure 2 Overall change in body composition from diagnosis to after neo-adjuvant chemotherapy. A: Skeletal muscle index; B: Adipose tissue index.

In the meantime, there is evidence that physical exercise and resistance training has proven efficacy in catabolic conditions including sarcopenia and is advocated as a nonpharmacological intervention in cancer-related skeletal muscle wasting. Interestingly, resistance training during adjuvant breast cancer treatment has been reported to reverse sarcopenic status and lead to higher chemotherapy completion rates[20,21]. Therefore, the potential for such interventions to attenuate DLT in oesophageal cancer warrants further investigation.

This study has shown that nutritional supplementation does serve to maintain overall weight and BMI. Looking more specifically at body composition, skeletal muscle mass decreased despite this nutritional support, and the proportion of sarcopenic patients increased. It is the authors' opinion that multimodal intervention incorporating a combination of regular nutritional support and exercise during the period of neo-adjuvant chemotherapy may lead to improvement in treatment tolerance and optimising surgical candidacy in patients with oesophageal cancer. This would be best investigated by means of a prospective multi-centre, multi-arm randomised controlled trial.

Table 3 Individual values for changes in lumbar skeletal muscle index (cm^2/m^2) from diagnosis to post neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy

Patient ID	Diagnosis	After neo-adjuvant chemotherapy
Patient 1	45.86	43.20
Patient 2	46.27	42.87
Patient 3	44.44	40.65
Patient 4	24.25	27.50
Patient 5	47.97	43.77
Patient 6	54.85	53.80
Patient 7	41.05	35.91
Patient 8	43.79	53.48
Patient 9	47.00	45.39
Patient 10	54.47	50.53
Patient 11	40.50	39.20
Patient 12	39.15	36.71
Patient 13	52.26	44.59
Patient 14	56.74	53.76
Patient 15	48.84	42.05

Table 4 Individual values for changes in lumbar adipose tissue index (cm^2/m^2) from diagnosis to after neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy

Patient ID	Diagnosis	After neo-adjuvant chemotherapy
Patient 1	166.10	160.91
Patient 2	82.71	131.25
Patient 3	102.27	112.29
Patient 4	187.18	177.17
Patient 5	62.08	64.25
Patient 6	192.85	202.49
Patient 7	80.79	88.04
Patient 8	102.87	93.52
Patient 9	10.03	26.22
Patient 10	159.49	146.65
Patient 11	157.76	148.82
Patient 12	197.29	186.82
Patient 13	101.62	93.82
Patient 14	118.47	110.45
Patient 15	137.98	126.63

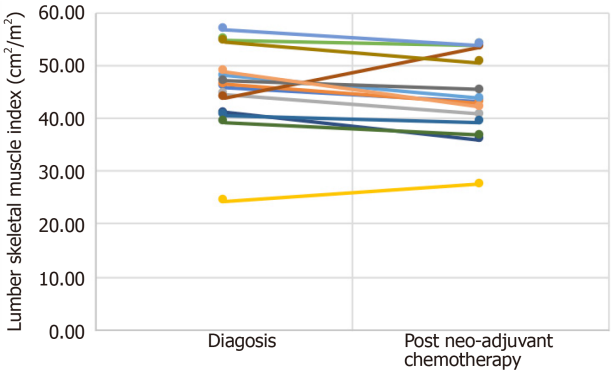

Figure 3 Individual changes in lumbar skeletal muscle index (cm^2/m^2) from diagnosis to after neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy.

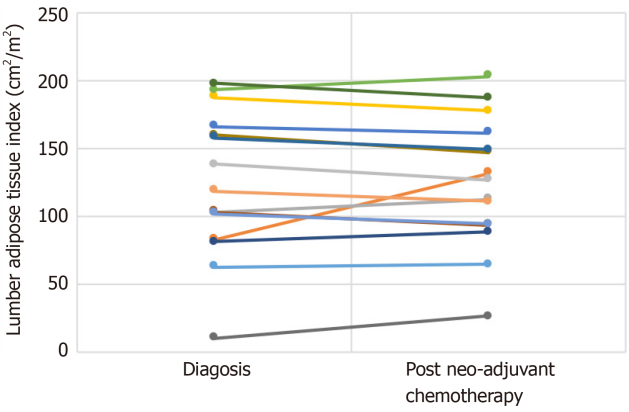

Figure 4 Individual changes in lumbar adipose tissue index (cm^2/m^2) from diagnosis to after neo-adjuvant chemotherapy following regular enteral feeding *via* jejunostomy.

ARTICLE HIGHLIGHTS

Research background

Malnourishment and sarcopenia are well documented phenomena in oesophageal cancer. Neo-adjuvant chemotherapy is now the mainstay of treatment prior to oesophagectomy and is associated with improved survival compared to surgery alone. Patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy have complex nutritional needs. The use of multiple strategies to manage disease progression including radiation, chemotherapy and surgical resection present an additional nutritional burden to these patients who are often malnourished at presentation.

Research motivation

There are various indicators of nutritional status that predict an individual's ability to tolerate neo-adjuvant chemotherapy and surgery for oesophageal cancer. Lower body mass index (BMI), skeletal muscle depletion, sarcopenia and sarcopenic obesity are associated with dose-limiting toxicity during treatment with neo-adjuvant chemotherapy. The period preceding surgery is the ideal time to ensure patients are in the best possible state for surgery. The use of enteral feeding *via* jejunostomy, particularly in oesophageal cancer, is a reliable method to optimise nutrition. The associated risks of insertion do not significantly outweigh the conferred benefits. The present study is the first to our knowledge to examine the changes of body composition from regular enteral feeding *via* jejunostomy during neo-adjuvant therapy for oesophageal cancer.

Research objectives

The aim of this study is to examine the effect of regular enteral feeding *via* jejunostomy on overall body composition in a cohort of patients with oesophageal cancer undergoing neo-adjuvant chemotherapy prior to oesophagectomy. The effect of regular jejunostomy feeding on the development of dose-limiting toxicity during neo-adjuvant chemotherapy was also examined.

Research methods

Patients having potentially curative, locally advanced oesophageal and oesophago-gastric junctional cancer without evidence of metastasis were considered for this study. All patients were routinely staged with a combination of computed tomography (CT), endoscopic ultrasound and laparoscopy according to the International Union Against Cancer system. All patients were assessed by a dietician prior to starting regular jejunostomy feeding. After assessment by the dietitian, each patient had the feeding regimen tailored to their individual nutritional needs. Weight and height were recorded according to standard methods. CT has proven to be accurate for measuring human body composition. Regional muscle tissue was measured by CT from electronically stored images, which had been done previously for diagnostic purposes. CT scans were performed at two time points: The first at diagnosis prior to commencement of chemotherapy and the second after completion of neo-adjuvant chemotherapy prior to surgery. The third lumbar vertebra was chosen as a landmark, and two consecutive slices were assessed to measure cross-sectional area of muscle and adipose tissue as described. The average value of two images was computed for each patient.

Research results

During the study period, 15 patients underwent regular jejunostomy feeding during neo-adjuvant chemotherapy prior to surgery. The time interval between the start of chemotherapy and surgery was 107 ± 21.6 d. During this period, there was no change in weight and BMI. However, body composition analysis revealed a statistically significant loss of skeletal muscle despite regular feeding during neo-adjuvant chemotherapy. Lumbar skeletal muscle index decreased. Adipose tissue index remained largely unchanged.

Research conclusions

This observational study of 15 patients undergoing neo-adjuvant chemotherapy prior to oesophagectomy confirmed that formal nutritional intervention provided through the use of feeding jejunostomy had a positive effect on maintaining mean overall weight and BMI.

Research perspectives

This study has shown that nutritional supplementation does serve to maintain overall weight and BMI. Looking more specifically at body composition, skeletal muscle mass decreased despite this nutritional support, and the proportion of sarcopenic patients increased. It is the authors' opinion that multimodal intervention incorporating a combination of regular nutritional support and exercise during the period of neo-adjuvant chemotherapy may lead to improvement in treatment tolerance and optimising surgical candidacy in patients with oesophageal cancer. This would be best investigated by means of a prospective multi-centre, multi-arm randomised controlled trial.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1193-1205

DOI: 10.4251/wjgo.v11.i12.1193

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Multi-parameter ultrasound based on the logistic regression model in the differential diagnosis of hepatocellular adenoma and focal nodular hyperplasia

Meng Wu, Ru-Hai Zhou, Feng Xu, Xian-Peng Li, Ping Zhao, Rui Yuan, Yu-Peng Lan, Wei-Xia Zhou

ORCID number: Meng Wu (0000-0001-8840-8753); Ru-Hai Zhou (0000-0002-3254-1742); Feng Xu (0000-0001-3379-8480); Xian-Peng Li (0000-0001-1580-5325); Ping Zhao (0000-0002-5689-4358); Rui Yuan (0000-0001-7543-8954); Yu-Peng Lan (0000-0001-4704-3250); Wei-Xia Zhou (0000-0001-8653-0451).

Author contributions: Wu M, Zhou RH, Xu F, Li XP, Zhao P and Yuan R designed research; Wu M, Xu F, Zhao P and Lan YP performed research; Lan YP and Zhou WX contributed new analytic tools; Zhou RH, Li XP, Yuan R and Zhou WX analyzed data; and Wu M, Zhou RH, Xu F, Lan YP and Zhou WX wrote the paper.

Supported by Zhejiang Natural Science Foundation, NO. LY16H160004; Ningbo Yinzhou District Agricultural and Social Development Science and Technology Project, NO. Yinke 2018-74.

Institutional review board statement: The study was approved by the ethics committee of Yinzhou Hospital affiliated to Ningbo University School of Medicine.

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

Meng Wu, Ru-Hai Zhou, Feng Xu, Xian-Peng Li, Ping Zhao, Rui Yuan, Yu-Peng Lan, Wei-Xia Zhou, Department of Ultrasound, Yinzhou Hospital Affiliated to Ningbo University School of Medicine, Ningbo 315000, Zhejiang Province, China

Feng Xu, Xian-Peng Li, Department of Gastroenterology, Yinzhou Hospital Affiliated to Ningbo University School of Medicine, Ningbo 315000, Zhejiang Province, China

Corresponding author: Meng Wu, MD, Doctor, Department of Ultrasound, Yinzhou Hospital affiliated to Ningbo University School of Medicine, No. 251 Baizhang East Road, Ningbo 315040, Zhejiang Province, China. wumengcool@sina.com
Telephone: +86-574-87017240

Abstract

BACKGROUND

Focal nodular hyperplasia (FNH) has very low potential risk, and a tendency to spontaneously resolve. Hepatocellular adenoma (HCA) has a certain malignant tendency, and its prognosis is significantly different from FNH. Accurate identification of HCA and FNH is critical for clinical treatment.

AIM

To analyze the value of multi-parameter ultrasound index based on logistic regression for the differential diagnosis of HCA and FNH.

METHODS

Thirty-one patients with HCA were included in the HCA group. Fifty patients with FNH were included in the FNH group. The clinical data were collected and recorded in the two groups. Conventional ultrasound, shear wave elastography, and contrast-enhanced ultrasound were performed, and the lesion location, lesion echo, Young's modulus (YM) value, YM ratio, and changes of time intense curve (TIC) were recorded. Multivariate logistic regression analysis was used to screen the indicators that can be used for the differential diagnosis of HCA and FNH. A ROC curve was established for the potential indicators to analyze the accuracy of the differential diagnosis of HCA and FNH. The value of the combined indicators for distinguishing HCA and FNH were explored.

RESULTS

Multivariate logistic regression analysis showed that lesion echo (P = 0.000), YM

Conflict-of-interest statement: None of the authors have any conflict of interest to disclose.

Manuscript source: Unsolicited manuscript

Received: June 15, 2019
Peer-review started: June 18, 2019
First decision: July 31, 2019
Revised: August 13, 2019
Accepted: September 10, 2019
Article in press: September 10, 2019
Published online: December 15, 2019

P-Reviewer: Ryan EM, Shaun C, Satya R
S-Editor: Zhang L
L-Editor: Filipodia
E-Editor: Qi LL

value (P = 0.000) and TIC decreasing slope (P = 0.000) were the potential indicators identifying HCA and FNH. In the ROC curve analysis, the accuracy of the YM value distinguishing HCA and FNH was the highest (AUC = 0.891), which was significantly higher than the AUC of the lesion echo and the TIC decreasing slope (P < 0.05). The accuracy of the combined diagnosis was the highest (AUC = 0.938), which was significantly higher than the AUC of the indicators diagnosing HCA individually (P < 0.05). This sensitivity was 91.23%, and the specificity was 83.33%.

CONCLUSION
The combination of lesion echo, YM value and TIC decreasing slope can accurately differentiate between HCA and FNH.

Key words: Hepatocellular adenoma; Focal nodular hyperplasia; Ultrasound; Logistic regression

Core tip: The prognosis of hepatocellular adenoma (HCA) is significantly different from focal nodular hyperplasia (FNH). Accurate identification of HCA and FNH is of great significance. This study explored the accuracy of the combined detection of HCA and FNH by conventional ultrasound, shear wave elastography, and contrast-enhanced ultrasound. The combination of lesion echo, Young's modulus value and time intense curve decreasing slop in multi-parameter ultrasound index based on logistic regression has high clinical guiding value for the differential diagnosis of HCA and FNH.

Citation: Wu M, Zhou RH, Xu F, Li XP, Zhao P, Yuan R, Lan YP, Zhou WX. Multi-parameter ultrasound based on the logistic regression model in the differential diagnosis of hepatocellular adenoma and focal nodular hyperplasia. *World J Gastrointest Oncol* 2019; 11(12): 1193-1205
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1193.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1193

INTRODUCTION

Focal nodular hyperplasia (FNH) is a proliferative lesion caused by vascular malformation, which is very common in the clinic. The potential risk is extremely low, most of FNH will not be malignant, and it has a tendency to spontaneously resolve[1-3]. Hepatocellular adenoma (HCA) is a rare liver tumor, which is prone to secondary hemorrhage and has a tendency towards malignant transformation. Although it can be treated conservatively after diagnosis, it must be closely monitored. Surgical resection is required once it is considered to be malignant, and its prognosis is also significantly different from FNH[4-6]. Therefore, accurate identification of HCA and FNH is essential for clinical treatment. However, patients with HCA or FNH have no obvious clinical symptoms, and tumor markers are not significantly expressed in FNH or HCA[7-9]. Ultrasound is a routine imaging diagnostic tool for diagnosing liver diseases. Although traditional two-dimensional ultrasound and Doppler ultrasound are difficult to distinguish, recent research has revealed that contrast-enhanced ultrasound (CEUS) has a certain value in this identification[10,11]. CEUS can clearly identify FNH and HCA through stellate scars in the central part of small FNH[12]. However, due to the low incidence of HCA, the CEUS performance of FNH is not typical. Hence, the value of CEUS in identifying HCA and FNH is limited currently[13,14]. In recent years, real-time shear wave elastography (SWE) technology can non-invasively and accurately obtain the absolute elasticity value of diseased tissues, and has been applied to the differential diagnosis of benign and malignant liver-occupying lesions[15-17]. To date, however, SWE has less application in identifying HCA and FNH. In the study of Gerber *et al*[18], the hardness of FNH was higher than HCA, but the sample size was insufficient and needed further verification[18]. Therefore, this study recruited HCA and FNH patients as research subjects. It systematically analyzed the different performances of conventional ultrasound, SWE and CEUS between HCA and FNH, and explored the value of ultrasound multi-

parameter indicators in the differential diagnosis of HCA and FNH. Furthermore, a logistic regression model was used to establish a combined diagnosis of ultrasound multi-parameter indicators. The value of multi-parameter combined identification of HCA and FNH was explored to provide useful information for the follow-up treatment of HCA.

MATERIALS AND METHODS

Research objective

Patients diagnosed with HCA or FNH in Yinzhou Hospital affiliated with Ningbo University School of Medicine from January 2017 to September 2019 were recruited. Inclusion criteria were as follows: (1) Diagnosed as HCA or FNH by surgical pathology or biopsy; and (2) Underwent color Doppler ultrasound, SWE, and CEUS examination, as well as obtained complete imaging data before surgery. Patients with malignant tumors or severe liver, kidney, heart, and brain dysfunction were excluded. A total of 31 patients with HCA who met the criteria were divided into the HCA group, including eight males and 23 females. The age range was 20-42 years old, with an average of 27.29 ± 9.87 years. A total of 50 FNH patients were divided into the FNH group, including 24 males and 26 females. The age range was 18-years-old to 48-years-old, with an average of 28.09 ± 10.57 years. The age, gender, history of viral infection, and history of cirrhosis was recorded in the two groups. All patients gave informed consent for the study, and the study was approved by the Ethics Committee of Yinzhou Hospital affiliated with Ningbo University School of Medicine.

Research methods

Liver function and serological examination: Liver function analysis and serological examination were performed on both groups. The patients were kept on a light diet, and fasted for 8 h before examination. Blood was taken from the elbow vein in the morning. Liver function and serological parameters of the two groups were determined using a Bayerl 650 automatic biochemical analyzer. Liver function indicators included alanine aminotransferase (ALT), aspartate aminotransferase (AST), and glutamyl transpeptidase (GGT). Serological indicators included albumin, total bilirubin (Tbil), prothrombin time (PT), and serum ferritin (SF).

Tumor markers: The alpha-fetoprotein (AFP) index of the two groups was examined and recorded by chemiluminescence immunoassay. The kit was purchased from Siemens Medical Diagnostics Products (Shanghai) Co., Ltd. and operated in strict accordance with its instructions. The AFP reference values were as follows: < 20 μg/L indicated normal; 25-100 μg/L indicated a low-level increase; 100-500 μg/L indicated a medium-level increase; > 500 μg/L indicated a high-level increase[19].

Ultrasound examination

Conventional ultrasound: An Esaote MyLab 90 (Esaote Group, Italy) color Doppler ultrasound system with 1-8 MHz frequency was used in this study. The patient was placed in a supine position with arms raised to expose the abdomen. An intercostal space scan was applied with a conventional two-dimensional ultrasound mode, and the image was adjusted after detecting the lesion to avoid rib and lung interference. The best display area of the lesion was located. Lesion location, internal echo, echo uniformity, lesion property, morphology, border, posterior echo, surrounding capsule and microcalcification were recorded. Then, the color Doppler mode was switched to observe the blood supply in the center of and surrounding the lesion.

Ultrasound elastography: SWE studies were performed using the Aixplorer™ ultrasound system (SuperSonic Imagine S.A., Aix-en-Provence, France) with a 6-8 MHz probe. The location of the lesion was determined after routine ultrasound examination. We first set the size of the sampling frame to completely cover the lesion. Meanwhile, we avoided the heart and abdominal large blood vessels. After the sampling frame was set, the patient was asked to hold for 3 s to save the SWE image. In the SWE image, the hard tissue is represented by red, and the soft tissue is represented by blue[20]. The Young's modulus (YM) value (kPa) and YM ratio were measured at the end of the examination.

CEUS examination: An Esaote MyLab 90 (Esaote Group, Italy) color Doppler ultrasound system with 1-4 MHz frequency was used for CEUS. The mechanical index was set to 0.19 and the dynamic range was 80 dB. The contrast agent was used from SonoVue (Braeeo CO., LTD). At the beginning, 25 mg of contrast medium was dissolved in 5 ml of normal saline. A 2.4 mL suspension was then injected *via* the

superficial vein of the elbow. Finally, 5 ml of normal saline was used for washout. The intensive manifestations of lesions in the arterial phase, portal venous phase, and delayed phase were continuously observed in real time. The software can automatically plot the TIC curve and record the following parameters: Background intensity (BI), peak intensity (PI), enhancement time (ET), peak intensity Change (PI-BI), TIC increasing slope, and TIC decreasing slope.

Statistical methods

Analysis was performed using Statistical Product and Service Solutions (SPSS 19.0) software. The measurement data were expressed as x ± s, and comparisons were performed using an independent sample *t* test. The count data were expressed in case or percentage, and the chi-square test was used for comparison. Multivariate logistic regression analysis was used to screen the potential identification indexes of HCA and FNH. An ROC curve was established to analyze the accuracy of the identification of HCA and FNH. The value of the combined indicators based on a logistic regression model for distinguishing HCA and FNH were explored. The difference was considered statistically significant at $P < 0.05$.

RESULTS

In this study, 31 patients in the HCA group included 31 lesions, and 50 patients in the FNH group included 50 lesions. All patients retained complete clinical, pathological, and ultrasound imaging data.

Comparison of clinical data between the HCA and FNH groups

In the HCA group, six patients had a history of viral infection, and four patents had a history of cirrhosis. The GGT was slightly elevated. ALT, AST, Albumin, Tbil, PT, AFP and SF were normal. Compared with the FNH group, the proportion of females was higher than that of the FNH group ($P < 0.05$). The other clinical data were similar with those in the FNH group, and the differences were not statistically significant ($P > 0.05$) (Table 1).

Ultrasound and pathological features of HCA

The ultrasound images of HCA showed solid lesions with round or elliptical shapes, with clear borders and high internal echoes in most cases (Figure 1A). Blood flow signals were common in most HCA lesions (Figure 1B). SWE showed that the hardness of HCA was slightly higher than that of normal surrounding liver tissue (Figure 1C). CEUS showed a rapid and significant increase in the arterial phase, a fast washout in the portal venous phase, and an equal or low enhancement in the delayed phase (Figure 1D). In terms of pathology, the gross specimens found abundant blood vessels on the surface of the tumors. The specimens were dark purple, uniform, and a few specimens were accompanied by necrosis or bleeding in the center. Microscopically, the tumor cells were similar to the surrounding normal liver cells, but there was no portal area, portal vein and small bile duct branches in the HCA. Kupffer cells, nuclear fission phase, and complete bile duct structure were lacking in the HCA (Figure 1E).

Comparison of ultrasound characteristics between the HCA and FNH groups

By comparing the ultrasound features between the HCA and FNH groups, it can be found that the HCA lesions were mostly hyperechoic, while the FNH lesions were mostly hypoechoic. In the SWE feature, the hardness of the HCA lesion was less than that of the FNH lesion. In CEUS performance, the enhancement of HCA was mostly peripheral-centered filling, with low enhancement in the delayed phase, and hemorrhagic necrosis in some HCA lesions. The enhancement of FNH was mostly radioactively filled from the center to the periphery, with slightly high or equal enhancement in the delayed phase. Some stellate scars were found in some FNH lesions. Quantitative analysis of the ultrasound characteristics of the two groups is shown in Table 2. The high echo ratio, solid lesion ratio, and TIC decreasing slope in the HCA group were significantly higher than those in the FNH group. The YM value, YM ratio, and area under the curve (AUC) were significantly lower than those in the FNH group. The differences were statistically significant ($P < 0.05$). The differences in lesion location, lesion boundary, lesion capsule, microcalcification, posterior echo, blood flow, PI-BI, ET, and TIC increasing slope were similar, and there is no statistical significance ($P > 0.05$).

Multivariate logistic regression analysis of HCA

Multivariate logistic regression analysis was performed to explore the potential

Table 1 Comparison of clinical data between hepatocellular adenoma group and focal nodular hyperplasia group

	HCA group, n = 31	FNH group, n = 50	t/χ^2 value	P value
Age	27.29 ± 9.87	28.09 ± 10.57	0.339	0.735
Gender, male/female	8/23	24/26	3.994	0.047
History of viral infection	6%	9%	0.023	0.879
History of cirrhosis	4%	6%	0.014	0.904
ALT, U/L	24.83 ± 12.94	28.76 ± 15.38	1.185	0.239
AST, U/L	29.83 ± 12.85	26.92 ± 13.64	0.954	0.343
GGT, U/L	38.32 ± 14.08	30.84 ± 10.36	1.529	0.183
Albumin, g/L	48.23 ± 3.82	47.93 ± 3.58	0.482	0.528
Tbil, μmol/L	9.38 ± 4.83	10.63 ± 5.63	0.392	0.682
PT, s	12.86 ± 0.86	12.67 ± 0.74	1.055	0.295
AFP, μg/L	14.05 ± 5.97	12.39 ± 4.29	1.454	0.150
SF, μg/L	89.35 ± 35.24	96.32 ± 43.2	0.755	0.452

HCA: Hepatocellular adenoma; FNH: Focal nodular hyperplasia; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Glutamyl transpeptidase; Tbil: Total bilirubin; PT: Prothrombin time; AFP: Alpha-fetoprotein; SF: Serum ferritin.

indicators identifying HCA. The results showed that gender, lesion property and YM ratio had no significant effect on the identification of HCA. The differences were not statistically significant ($P > 0.05$). The lesion echo ($P = 0.000$), YM value ($P = 0.000$) and TIC decreasing slope ($P = 0.000$) were potential indicators for identifying HCA (Table 3).

ROC curve analysis of ultrasound indicators to identify HCA and FNH

The AUC of each ultrasound indicator distinguishing HCA and FNH was different. The prediction accuracy of the YM value was the highest (AUC = 0.891). The sensitivity was 92.16%, but the specificity was low (73.28%). The AUC of TIC decreasing slope and lesion echo were lower than that of YM value, and the differences were statistically significant ($P < 0.05$). The accuracy of the combination of three ultrasound indicators identifying HCA and FNH was the highest (AUC = 0.938), which was significantly higher than the AUCs of the three ultrasound indicators individually identifying HCA and FNH. The differences were statistically significant ($P < 0.05$). The cut-off point was 0.540. Its sensitivity and specificity were 91.23% and 83.33%, respectively (Table 4 and Figure 2).

DISCUSSION

HCA is a clinically rare benign liver tumor, which often needs to be differentiated from FNH, a non-angiogenic liver benign lesion[21]. Due to the fact that the clinical symptoms of HCA and FNH are not obvious, and the level of related tumor markers are not expressed, it is difficult to differentiate them by physical examination. However, their treatments and prognoses are highly distinct[22,23]. FNH is a proliferative lesion caused by vascular malformation, whereas HCA is a dangerous tumor with a tendency to hemorrhage and undergo malignant transformation. Although a needle biopsy can be used for differential diagnosis, it is an invasive procedure and cannot be used as a routine examination. Ultrasound is important for liver examination because of its simple, non-invasive and high diagnostic accuracy. However, gray-scale ultrasound is limited in identifying HCA and FNH[24]. Although CEUS can recognize the stellate scars of FNH and the ischemic necrosis of HCA, the accuracy of CEUS in identifying HCA and FNH is not high due to the low detection rate of stellate scars of FNH[25]. As a new technique that has been widely used in the differential diagnosis of benign and malignant livers[26], SWE can be used for the differential diagnosis of FNH and HCA by detecting their YM. This study explored the accuracy of conventional ultrasound, SWE and CEUS multi-parameter ultrasound indicators to identify HCA and FNH, with an intent to provide useful information for clinical treatment.

HCA is an estrogen-dependent tumor that has been reported in previous studies in young women or men taking steroids[27-29]. In this study, the proportion of female

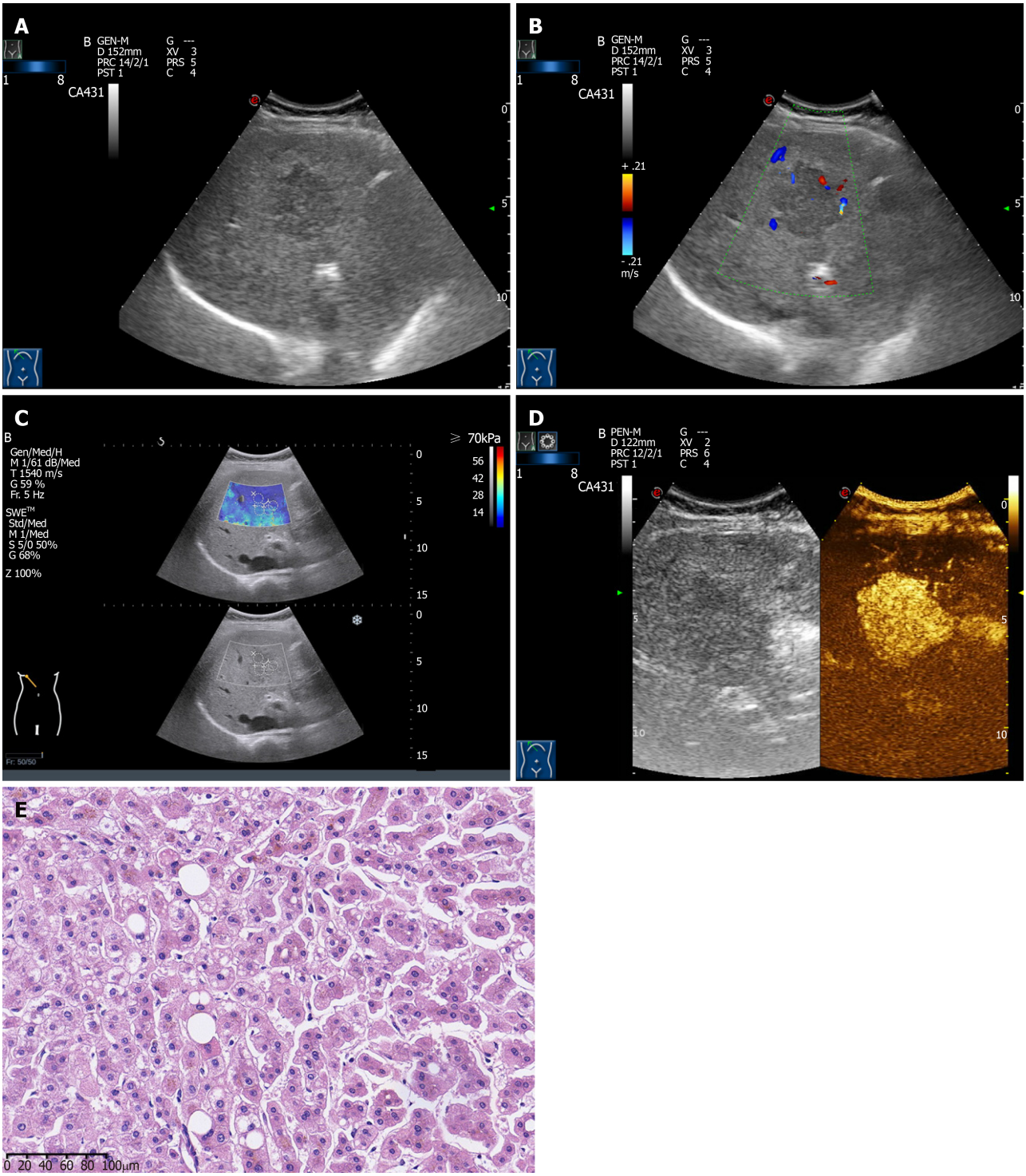

Figure 1 Ultrasonographic and pathological features of hepatocellular adenoma. A: Gray-scale ultrasound image of Hepatocellular adenoma (HCA); B: Color doppler flow Imaging image features of HCA; C: Shear wave elastography image features of HCA; D: Contrast-enhanced ultrasound image features of HCA; E: Pathological features of HCA.

patients with HCA was higher than that of FNH patients. However, there was no significant difference between the two groups in other clinical data, such as the history of viral infection, serological markers for evaluating liver function, and tumor markers (*e.g.*, AFP). It is difficult to identify HCA and FNH solely through clinical data and laboratory examinations[30]. According to pathological diagnostic criteria, we learned that the typical FNH lesions are nodular, with hyperplastic fibrous tissue, small blood vessels and bile duct structures. Stellate scars can be found in some FNH lesions. The tumor cells of HCA are similar to the surrounding normal liver cells. The center of the tumor may be accompanied by necrosis or hemorrhage. Some HCA lesions had scattered and lumen-expanded small blood vessels. However, there is no

Table 2 Comparison of ultrasound characteristics between hepatocellular adenoma group and focal nodular hyperplasia group

		HCA group, *n* = 31	FNH group, *n* = 50	t/χ^2 value	*P* value
Diameter in cm		3.37 ± 1.87	3.29 ± 1.58	0.206	0.837
Lesion location	Liver right lobe	24	36	0.293	0.589
	Liver left lobe	7	14		
Lesion echo	Low	9	31	11.637	0.009
	Equal	5	9		
	High	14	10		
	Mixed	2	0		
Lesion property	Solid	18	16	6.202	0.045
	Cystic	9	23		
	Mixed	3	11		
Lesion morphology	Regular	27	44	0.014	0.904
	Irregular	4	6		
Lesion boundary	Clear	27	47	1.155	0.282
	Unclear	4	3		
Lesion capsule	With capsule	30	46	0.753	0.386
	Without capsule	1	4		
Lesion internal echo	Uniform	26	43	0.069	0.793
	Non-uniform	5	7		
Microcalcification	No	30	48	0.032	0.858
	Yes	1	2		
Posterior echo	No echo attenuation	31	48	1.271	0.260
	Echo attenuation	0	2		
Lesion blood flow	No blood flow	7	20	4.870	0.088
	Spotted blood flow	18	27		
	Strip blood flow	6	3		
SWE	YM value in kPa	14.39 ± 7.28	29.27 ± 12.38	6.065	0.000
	YM ratio	3.73 ± 1.14	4.89 ± 1.99	2.770	0.007
TIC Quantitative analysis	PI-BI, dB	21.84 ± 8.83	20.18 ± 9.38	0.791	0.431
	ET, s	18.02 ± 5.88	20.27 ± 8.39	1.306	0.195
	TIC increasing slop	1.84 ± 0.62	1.72 ± 0.23	1.241	0.218
	TIC decreasing slop	0.31 ± 0.09	0.14 ± 0.07	9.510	0.000

HCA: Hepatocellular adenoma; FNH: Focal nodular hyperplasia; YM: Young's modulus; TIC: Time intense curve; ET: Enhancement time; PI-BI: Peak intensity background intensity; SWE: Shear wave elastography

portal area, portal vein, small bile duct branches, nuclear fission phase, or complete bile duct structures in HCA lesions. The presence of a complete portal system and bile duct structure is a major feature in identifying HCA and FNH. Histopathological examination is an invasive examination and cannot be used as a routine examination, although it can clearly distinguish HCA and FNH. Therefore, it is important to explore a non-invasive and simple imaging examination method to identify HCA and FNH.

This study compared the gray-scale ultrasound, SWE and CEUS indicators between the HCA group and the FNH group in order to explore the potential identification indicators of HCA and FNH. It revealed that the ratio of high echo HCA lesions was higher than that of FNH. We believe that this is due to the fact that HCA tumor cells are rich in glycogen and fat, and the high fat content makes a high echo. The study of Hasab *et al*[31] found that although HCA lesions were mostly in high echo types, their ultrasound performance also varies. Because the blood supply of HCA lesions is from the surrounding large blood vessels rather than the central artery, necrosis or hemorrhage is likely to occur in the tumor, and the site of necrosis or hemorrhage will appear as a no echo area. In addition, due to fibrous tissue hyperplasia, the stellate scars of FNH also represent as high echo, which is easily confused in the differential diagnosis of conventional ultrasound. The present study also found that lesion boundary, morphology, blood flow signals and other indicators of HCA and FNH were not significantly different. It indicated that the identification of HCA and FNH

Table 3 Multivariate logistic regression analysis of hepatocellular adenoma identification

	B	SE	Wald	*P* value	OR	95%CI	
						Lower limit	Upper limit
Gender	0.284	0.354	2.394	0.086	1.328	0.664	2.658
Lesion echo	0.977	0.157	7.967	0.000	2.657	1.953	3.614
Lesion property	0.427	0.964	1.234	0.137	1.532	0.232	10.135
YM value	- 0.289	0.108	6.567	0.000	0.749	0.606	0.926
YM ratio	- 0.154	0.135	1.436	0.134	0.857	0.658	1.117
TIC decreasing slope	1.673	0.284	5.829	0.000	5.328	3.054	9.296

YM: Young's modulus; TIC: Time intense curve.

solely by conventional gray-scale ultrasound was difficult.

The role of CEUS in the diagnosis of FNH has been recognized[32-34]. In the FNH group of the present study, the enhancement mode was mostly radioactively filled from the center to the periphery. The enhancement mode was slightly higher or of equal enhancement in the delayed phase, and some FNH had stellate scars. This is because the FNH blood supply is distributed centrally to the surrounding area, and the blood vessels are derived from the hepatic artery and the portal vein. During the portal vein phase, the contrast agent can be supplemented by the portal venous system, so it is called "fast-forward and slow-out"[35]. The enhancement mode of the HCA group was mostly peripheral-centered filling. Part of the HCA had a filling defect, that is, an ischemic necrotic area. The TIC decreasing slope of the HCA group was significantly higher than that of the FNH group. This is because the HCA tumor is surrounded by abundant peripheral blood vessels, and the branches of the blood vessels are infiltrated into the tumor for feeding. The contrast agent is filled with blood from the periphery to the center. If the lesion is large, the middle region is prone to lack blood supply and become necrotic, forming a filling defect. Because HCA lacks the portal system, its feeding vessels are derived from the hepatic artery. Hence the contrast agent is not supplemented during the portal vein phase. This enhanced mode is called "fast forward and fast out"[36]. In the FNH lesion, the portal vein was absent in the stellate scar, which was formed by fibrous tissue. Thus, the stellate filling defect area would appear in the CEUS. Nevertheless, HCA and FNH can be initially identified based on the difference in the two groups of CEUS findings. Guo *et al*[37] have found that the typical CEUS enhancement of FNH can also be found in HCA. Choi *et al*[38] also reported misdiagnosis in cases of HCA by CEUS[38]. Therefore, it is not accurate to identify HCA and FNH solely by utilizing CEUS.

SWE has been widely used in the differential diagnosis of benign and malignant liver lesions in recent years[39,40]. It was applied in the differential diagnosis of HCA and FNH in this study. The results found that the YM value and YM ratio in the HCA group were significantly lower than those in the FNH group, indicating that the lesion hardness in the HCA group was significantly lower than that in the FNH group. This is because most FNHs have stellate scars formed by coarse fibers. These scars divide the tissue into multiple small nodules. There is hyperplastic fibrous tissue, thickened blood vessel walls and hyperplastic bile ducts among the small nodules. Therefore, the fibrous tissue content in FNH is increased, and the tissue hardness is increased[41]. On the other hand, HCA is mainly composed of hepatocytes rich in glycogen and lipids. The fibrous tissue content is rare. Moreover, HCA lesion lack portal vein structure and bile ducts, often including ischemic necrosis. These all lead to a decrease in the hardness value of the HCA lesion[42-44].

According to the analysis results of the ROC curve in this study, gray-scale ultrasound, SWE and CEUS had their own advantages and disadvantages in identifying HCA and FNH. The lesion echo, YM value, and TIC decreasing slope could be used to identify HCA and FNH. Among them, YM value has the highest accuracy in identifying HCA. The AUC of HCA was 0.891, and the sensitivity was 92.16%. This suggested that the differential diagnosis of HCA and FNH by YM value is not prone to missed diagnosis. However, its specificity is low (73.28%), suggesting that the differential diagnosis of the YM value would result in a certain misdiagnosis rate. The AUC of the three indicators were all < 0.9, indicating that the accuracy of gray-scale ultrasound, SWE and CEUS for individually identifying HCA and FNH is limited. Therefore, this study combined the lesion echo, YM value, and TIC decreasing slope based on the logistic regression model. The combined diagnosis

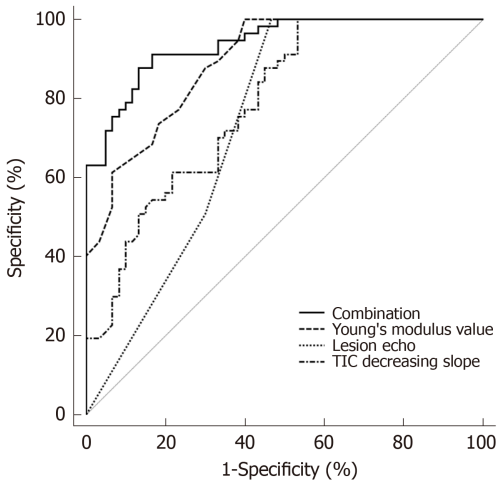

Figure 2 ROC curve analysis of Young's modulus value, time intense curve decreasing slope, lesion echo and their combination in the differential diagnosis of hepatocellular adenoma and focal nodular hyperplasia.

found that the AUC reached 0.938, which was higher than the AUC of these single indicators, indicating that the accuracy of the combined identification of the three indicators was the best. The combination of lesion echo, YM value and TIC decreasing slop in the differential diagnosis of HCA and FNH will hopefully be used in the clinic.

There are still shortcomings in this study. The SWE in this study was susceptible to the depth of the lesion and the operator. Hence, there is an inevitable error in the results. In addition, there is limited attention to this disease in the clinic because HCA is relatively rare and less malignant[45-50]. Thus, several patients in this study were not included because of incomplete clinical data, resulting in a limited sample size. Following this study, we expect to conduct further research on HCA in multiple centers, which will provide more useful information for clinical diagnosis through analyzing more specific data.

In conclusion, the combination of lesion echo, YM value and TIC decreasing slop in multi-parameter ultrasound indicators based on logistic regression has high clinical guiding value for the differential diagnosis of HCA and FNH.

Table 4 ROC curve analysis of multiple ultrasound parameters in the differential diagnosis of hepatocellular adenoma and focal nodular hyperplasia

	AUC	95%CI	Cut-off point	Sensitivity, %	Specificity, %
YM value	0.891[a]	0.820-0.941	23.26	92.16	73.28
TIC decreasing slope	0.785[a]	0.700-0.856	0.23	82.47	66.39
Lesion echo	0.676[a]	0.583-0.759	3	40.35	93.82
Combination	0.938	0.878-0.974	0.540	91.23	83.33

[a]$P < 0.05$. YM: Young's modulus; TIC: Time intense curve.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular adenoma (HCA) is prone to secondary hemorrhage, and has a certain tendency towards malignant transformation. It needs to be closely observed and surgically removed if necessary. Focal nodular hyperplasia (FNH), which often needs to be differentiated, is a vascular malformation lesion, which is not a true tumor and has a tendency to spontaneously resolve, so conservative treatment can be adopted. The treatment methods and prognosis of them are quite different, but they are not easy to clinically identify. Therefore, it is of great significance to explore effective identification methods for them.

Research motivation

Current studies have shown that biochemical indicators do not have obvious advantages in identifying HCA and FNH. In imaging methods, it is difficult to distinguish the difference by using ultrasound. Recent studies have shown that contrast enhanced ultrasound (CEUS) can be used to diagnose HCA, but the diagnostic accuracy of FNH is low. It revealed that the value of differential diagnosis using conventional ultrasound, or CEUS individually, is limited. In recent years, shear wave elastography (SWE) has been widely used in the identification of benign and malignant tumors in the liver, but there are few applications for the differential diagnosis of HCA and FNH. Therefore, methods for identifying HAC and FNH are still lacking in the clinic.

Research objectives

In order to explore effective methods for identifying HCA and FNH, we will analyze the routine clinical indicators, including Doppler ultrasound, CEUS and SWE, in HCA and FNH patients. Logistic regression analysis will be used to analyze the significance of combined diagnosis of multi-parameter ultrasound indicators for improving the differential diagnosis of HCA and FNH.

Research methods

The study included 31 patients with HCA, and 50 patients with FNH. The clinical data of the two groups were recorded, and conventional ultrasound, CEUS, and SWE examinations were performed, and the ultrasound parameters such as lesion position, boundary echo, value and ratio of Young's modulus (YM), slope of TIC curve, *etc* were recorded. Multivariate regression analysis was used to screen potential indicators for the differential diagnosis of HCA and FNH. A ROC curve was used to evaluate the accuracy of potential indicators in differential diagnosis. A logistic regression model was used to establish a combination to explore the accuracy of differential diagnosis.

Research results

Multivariate regression analysis showed that lesion echo ($P = 0.000$), YM value ($P = 0.000$) and TIC decreasing slope ($P = 0.000$) were the potential indicators for identifying HCA and FNH. The accuracy of differential diagnosis of YM value is the highest, but its AUC is still less than 0.9. It is suggested that although the lesion echo, YM value and TIC decreasing slope were the influencing factors of HCA, the accuracy of differential diagnosis using conventional ultrasound, SWE and CEUS alone was limited. Further logistic regression results showed that the accuracy of the combined diagnosis of three indicators (AUC = 0.938) was significantly higher than the AUC of lesion echo (AUC = 0.676), YM value (AUC = 0.891), and TIC decreasing slope (AUC = 0.785). It is suggested that the accuracy of the combination of the three indicators is the best. The combined diagnosis of multi-parameter ultrasound can significantly improve the accuracy of differential diagnosis between HCA and FNH.

Research conclusions

Multi-parameter ultrasound in the differential diagnosis of HCA and FNH plays an important role. The combination of lesion echo, YM value and TIC decreasing slope can significantly improve the accuracy of differential diagnosis.

Research perspectives

In order to avoid the limitation of HCA patient cases, as well as the influence of the depth of the

lesion and operator in SWE defection. This study plans to further develop a multicenter study on HCA to improve diagnosis accuracy. The multi-center large sample study will further reveal the role of multi-parameter ultrasound in the differential diagnosis of HCA and FNH, and further improve the accuracy of the diagnosis.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1206-1217

DOI: 10.4251/wjgo.v11.i12.1206

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

Analysis of factors potentially predicting prognosis of colorectal cancer

Li-Jun Jin, Wei-Bin Chen, Xiao-Yu Zhang, Jie Bai, Hao-Chen Zhao, Zun-Yi Wang

ORCID number: Li-Jun Jin (0000-0002-9818-3815); Wei-Bin Chen (0000-0001-5858-2435); Xiao-Yu Zhang (0000-0002-6363-0435); Jie Bai (0000-0002-9825-5030); Hao-Chen Zhao (0000-0002-06326-8958); Zun-Yi Wang (0000-0002-4318-0813).

Author contributions: Jin LJ, Chen WB, Zhang XY, Bai J, Bai J, and Wang ZY designed the research; Jin LJ, Zhang XY, Bai J, and Wang ZY performed the research; Chen WB and Zhao HC contributed new analytic tools; Jin LJ, Zhao HC, and Wang ZY analyzed the data; and Jin LJ, Chen WB, Zhao HC, and Wang ZY wrote the paper.

Institutional review board statement: The study was approved by the Ethics Committee of Cangzhou Central Hospital.

Informed consent statement: All patients gave informed consent.

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

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

Li-Jun Jin, Xiao-Yu Zhang, Jie Bai, Zun-Yi Wang, Department of Surgical Oncology (Division III), Cangzhou Central Hospital, Cangzhou 061001, Hebei Province, China

Wei-Bin Chen, Department of Radiology, North China University of Science and Technology Affiliated Hospital, Tangshan 063000, Hebei Province, China

Hao-Chen Zhao, Department of Anesthesiology (Division II), Cangzhou Central Hospital, Cangzhou 061001, Hebei Province, China

Corresponding author: Zun-Yi Wang, MD, Doctor, Department of Surgical Oncology (Division III), Cangzhou Central Hospital, No. 16, Xinhua Road West, Cangzhou 061001, Hebei Province, China. czwzy99@163.com
Telephone: +86-317-2075979

Abstract

BACKGROUND

Accurate assessment of the prognosis after colorectal cancer surgery is of great significance in patients with colorectal cancer. However, there is no systematic analysis of factors affecting the prognosis of colorectal cancer currently.

AIM

To systematically analyze the influence of clinical data and serological and histological indicators on the prognosis of patients with colorectal cancer, and to explore the indicators that can accurately assess the prognosis of patients with colorectal cancer.

METHODS

A total of 374 patients with colorectal cancer were enrolled. The clinical data, tumor-node-metastasis (TNM) stage, and Dukes stage were recorded. All patients received examinations including carcinoembryonic antigen (CEA), carbohydrate antigen 199, C-reactive protein, albumin, D-dimer, and fibrinogen as well as routine blood tests one week before surgery. The tumor location, size, depth of invasion, lymph node metastasis, and distant metastasis were recorded during surgery. The pathological tissue typing and expression of proliferating cell nuclear antigen (PCNA) and p53 were observed. All patients were followed for 3 years, and patients with endpoint events were defined as a poor prognosis group, and the remaining patients were defined as a good prognosis group. The differences in clinical data, serology, and histology were analyzed between the two groups. Multivariate COX regression was used to analyze the independent

Manuscript source: Unsolicited manuscript

Received: June 12, 2019
Peer-review started: June 18, 2019
First decision: July 31, 2019
Revised: August 5, 2019
Accepted: August 26, 2019
Article in press: August 26, 2019
Published online: December 15, 2019

P-Reviewer: Jorge AG, Orbell JH, Garcia SS
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Liu MY

influencing factors for the prognosis of colorectal cancer. The receiver operating characteristic curve was used to evaluate the predictive value of each of the independent influencing factors and their combination for the prognosis of colorectal cancer.

RESULTS

The follow-up outcomes showed that 81 patients were in the good prognosis group and 274 patients in the poor prognosis group. The TNM stage, PCNA, Glasgow prognostic score (GPS), neutrophil-lymphocyte ratio (NLR), C-reactive protein/albumin ratio (CAR), D-dimer, and CEA were independent influencing factors for the prognosis of colorectal cancer (P = 0.000). NLR had the highest predictive power for colorectal cancer prognosis [area under the receiver operating characteristic curve (AUC) = 0.925], followed by D-dimer (AUC = 0.879) and GPS (AUC = 0.872). The accuracy of the combination of all indicators in predicting the prognosis of colorectal cancer was the highest (AUC = 0.973), which was significantly higher than that of any of the indicators alone (P < 0.05). The sensitivity and specificity of the combination were 92.59% and 90.51%, respectively.

CONCLUSION

The independent influence factors for the prognosis of colorectal cancer include TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA. The combined assessment of the independent factors is the most accurate predictor of the prognosis after colorectal cancer surgery.

Key words: Colorectal cancer; Prognosis; Influencing factors; Combination assessment

Core tip: Accurate assessment of the prognosis after colorectal cancer surgery is of great importance in patients with colorectal cancer. This study systematically analyzed the influence of clinical data and serological and histological indicators on the prognosis of patients with colorectal cancer and the results revealed that the independent influence factors for the prognosis of colorectal cancer include tumor-node-metastasis stage, proliferating cell nuclear antigen, Glasgow prognostic score, neutrophil-lymphocyte ratio, C-reactive protein/albumin ratio, D-dimer, and carcinoembryonic antigen. The combined assessment of the independent factors is the most accurate predictor of the prognosis after colorectal cancer surgery.

Citation: Jin LJ, Chen WB, Zhang XY, Bai J, Zhao HC, Wang ZY. Analysis of factors potentially predicting prognosis of colorectal cancer. *World J Gastrointest Oncol* 2019; 11(12): 1206-1217
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1206.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1206

INTRODUCTION

The prognosis of colorectal cancer is poor, and its mortality rate ranks third among all malignancies in the world[1-3]. Surgery is the main method of colorectal cancer treatment. Accurate assessment of surgical prognosis plays an important role in the treatment of colorectal cancer patients. Currently, studies on the factors affecting the prognosis of colorectal cancer are very popular. Studies have shown that high Glasgow prognostic score (GPS) in colorectal cancer is more likely to predict a poor prognosis[4-6]. The results of Arfa *et al*[7] showed that tumor-node-metastasis (TNM) stage is associated with the prognosis of colorectal cancer. Studies by Kanemitsu *et al*[8] have demonstrated that histological type and degree of differentiation of tumors can affect their biological behavior and further affect clinical outcomes. In addition, studies have found that *in vivo* inflammatory response can have an impact on the occurrence and development of tumors[9]. Serum C-reactive protein[10,11], albumin[12,13], C-reactive protein/albumin ratio (CAR)[14-16], and neutrophil-lymphocyte ratio (NLR)[17,18] reveal the possibility of inflammation-related complications, and have

certain predictive value for prognosis[19]. However, there is no systematic analysis about the factors affecting the prognosis of colorectal cancer. Therefore, this study defined colorectal cancer patients as the research subjects. We statistically analyzed the influence of clinical data and serology and histology on the prognosis of patients with colorectal cancer, and assessed the accuracy of the combination of all indicators for the prognosis evaluation, aiming to find a more accurate assessment. This study is expected to provide a new method for predicting the prognosis of colorectal cancer in the early stage of clinical diagnosis and improve the prognosis of patients.

MATERIALS AND METHODS

Research subjects

A total of 374 patients with colorectal cancer who were admitted to Cangzhou Central Hospital from March 2012 to March 2015 were recruited. The inclusion criteria were: (1) Pathological diagnosis of colorectal cancer; (2) Patients who underwent radical surgery; (3) Complete clinical data, disease history, and family history data; and (4) Complete physical examination, routine blood tests, detection of tumor markers, and relevant laboratory examinations such as coagulation function within one week before surgery. The exclusion criteria were: (1) Other carcinomas combined; (2) Pre-operative infection or insufficient infection evidence but body temperature > 38 °C; (3) Patients combined with cardiovascular disease, cerebrovascular disease, and diseases of the liver, kidney, and other important organs; and (4) Undergoing radiotherapy, chemotherapy, biotherapy, or gene therapy before surgery. The study was approved by the Ethics Committee of Cangzhou Central Hospital. All patients included in the study had a detailed understanding of the research content and provided informed consent.

Research methods

Clinical data collection: After admission, complete clinical data, including age, gender, height, weight, family history (colorectal cancer in three generations of close relatives) and smoking history were collected. Body mass index (BMI) was calculated. All patients were staged by the TNM staging method and the improved Dukes staging method according to pathological findings[20].

Serological examination: All patients received carcinoembryonic antigen (CEA), carbohydrate antigen 199 (CA199), C-reactive protein, albumin, D-dimer, and fibrinogen detection as well as routine blood tests one week before surgery. CEA and CA199 were tested by chemiluminescence with a Roche kit. Routine blood tests were performed with a CC-3200 automatic blood tester and supporting kit. The numbers of neutrophil granulocytes, white blood cells, lymphocytes, and platelets were recorded, and NLR was calculated. C-reactive protein, albumin, D-dimer, and fibrinogen were detected using an Abbott C16000 automatic biochemical analyzer (Abbott Laboratories, Inc.). CAR was calculated. According to the results of C-reactive protein and albumin detection, GPS was calculated according to the following rules: (1) Increased C-reactive protein; and (2) Hypoproteinemia. Two points were recorded if both (1) and (2) were satisfied, 1 point if (1) or (2) was satisfied, and 0 points if normal C-reactive protein and no hypoproteinemia were found.

Surgery and histological examination: Under general anesthesia, colorectal cancer radical surgery was performed. Tumor location, size, depth of invasion, lymph node metastasis, and distant metastasis were recorded. The excised specimens were flattened and fixed, and pathological examination was performed. The pathological tissues were serially sectioned, fixed with 10% formalin, and embedded in paraffin, followed by hematoxylin-eosin and immunohistochemical staining. All the sections were observed by two experienced pathologists. The pathological tissue classification and expression of proliferating cell nuclear antigen (PCNA) and p53 were observed. After surgery, patients' vital signs and wound oozing were closely observed. Anti-inflammatory drugs and intravenous nutrition were given regularly.

Follow-up

A total of 374 patients who participated in the study were followed for 3 years. The first follow-up was performed 1 mo after the end of treatment, followed by every 3 mo within 2 years. After 2 years, follow-up was performed every 6 mo. The endpoint events were defined as adverse prognostic events during follow-up, including recurrence of colorectal cancer, increased stage, other organ metastases, and death from colorectal cancer and its complications. Patients' refusal to visit, halfway out, and death from other reasons unrelated to the study were defined as loss to follow-

up. According to the follow-up results, patients with endpoint events were defined as a poor prognosis group, and the remaining patients were defined as a good prognosis group. Differences in clinical data, serology, and histology were analyzed.

Statistical analysis

Analyses were performed using Statistical Product and Service Solutions software. The measurement data are expressed as the mean ± standard deviation, and comparisons were performed using an independent sample *t*-test. The count data are expressed in case (percentage), and the chi-square test was used for comparison. Survival analysis was performed by the Kaplan-Meier method. The poor prognosis rate was calculated and the survival curve was drawn. Multivariate COX regression was used to analyze independent influencing factors for the prognosis of colorectal cancer. The receiver-operating characteristic (ROC) curve was used to evaluate the predictive value of each independent influencing factor and their combination for the prognosis of colorectal cancer. The difference was considered statistically significant at $P < 0.05$.

RESULTS

Patient follow-up results

A total of 374 patients were enrolled in the study. The Kaplan-Meier survival curve showed an increase trend in the number of adverse prognosis cases over time (Figure 1). At the end of follow-up, 19 patients were lost to follow-up. There were a total of 355 patients with complete follow-up data, of whom 81 developed endpoint events. In these 81 patients, 40 had recurrence of colorectal cancer (Figure 2A), 26 had liver metastases (Figure 2B), and 15 had bone metastases (Figure 2C). The poor prognosis rate was 22.82%.

Comparison of indicators between the prognosis group and poor prognosis group

Of the 355 patients with colorectal cancer who received complete follow-up data, 186 were male and 169 were female, with an average age of 59.83 ± 13.92 years and mean tumor size of 4.32 ± 2.56 cm. In terms of tumor location, there were 52 cases in the ascending colon, 21 in the transverse colon, 35 in the descending colon, 106 in the sigmoid colon, and 141 in the rectum. In terms of TNM stage, there were 16 cases of stage I, 251 stage II, 54 stage III, and 34 stage IV. In terms of Dukes stage, there were 17 cases of grade A, 225 grade B, and 112 grade C. Among the pathological types, there were 230 cases of adenocarcinoma and 125 cases of mucinous adenocarcinoma and signet ring cell carcinoma.

According to the prognosis results, the prognosis group included 274 patients and the poor prognosis group included 81 patients. The comparison of the indicators before treatment in the two groups of patients is shown in Table 1. The age, tumor diameter, TNM stage, PCNA index, GPS, C-reactive protein, albumin, white blood cell (WBC) count, NLR, CAR, D-dimer, fibrinogen, CEA, and CA199 in the poor prognosis group were significantly higher than those in the good prognosis group ($P < 0.05$). The gender, BMI, smoking proportion, alcohol abuse ratio, family history ratio, tumor location, tumor histology type, Dukes grade, proportion of positive P53, and platelet count were similar between the two groups ($P > 0.05$).

Multivariate COX regression analysis of the prognosis in patients with colorectal cancer

Further multivariate COX regression analysis was performed on the different indicators between the two groups. The results showed that the impact of age, tumor diameter, C-reactive protein, albumin, WBC count, fibrinogen, and CA199 on the prognosis was not significant ($P > 0.05$). TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA were independent influencing factors for the prognosis of colorectal cancer ($P = 0.000$) (Table 2).

Receiver operating characteristic curve analysis of potential indicators for predicting colorectal cancer prognosis

The ROC curve was used to further analyze the predictive value of TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA for the prognosis of colorectal cancer. The results showed that each indicator can predict the prognosis of colorectal cancer (AUC > 0.6 for all). Among them, NLR had the highest accuracy with an AUC of 0.925 (95%CI: 0.860-0.966), sensitivity of 94.12%, and slightly lower specificity of 80.25%. D-dimer was the second, and its AUC was 0.879 (95%CI: 0.841-0.911), with a specificity of 100% and sensitivity of only 58.02%. GPS was the third, and its AUC was 0.872

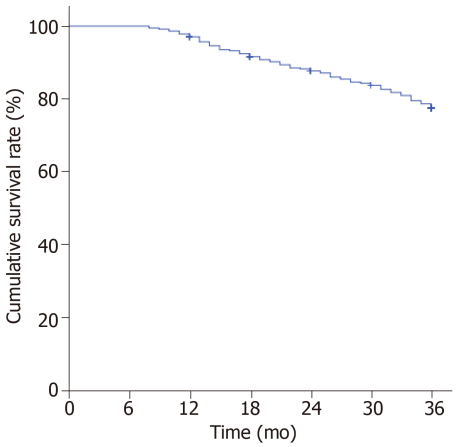

Figure 1 Survival curve analysis of patients with colorectal cancer (Kaplan-Meier). The poor prognosis rate was 22.82%.

(95%CI: 0.832-0.905), with a sensitivity and specificity of 88.89% and 85.04%, respectively (Table 3 and Figure 3).

Each indicator had some limitations in predicting the prognosis of colorectal cancer, respectively. Therefore, this study attempted to establish a combination model based on logistic regression to predict the prognosis. The result showed that the accuracy of the combination of all indicators in predicting the prognosis of colorectal cancer was the highest (AUC = 0.973, 95%CI: 0.950-0.987), which was significantly higher than that of any indicator alone ($P < 0.05$). The best diagnostic point was 0.206, with a sensitivity of 92.59% and specificity of 90.51% (Figure 4).

DISCUSSION

The detection rate of colorectal cancer has increased recently[21]. Because of the hidden symptoms in early stage, the prognosis of colorectal cancer is poor[22]. Accurate assessment of patient outcomes is critical to the choice of clinical treatments. Currently, the prognosis of colorectal cancer is predicted mainly through CEA level, pathological typing, TNM staging, *etc*.[23-25], but the accuracy of prediction cannot be guaranteed due to different individuals[26]. Recent studies have shown that GPS, C-reactive protein, albumin, CAR, NLR and other inflammatory indicators can reveal the prognosis of malignant tumors, but the accuracy of different predictive indicators for the prognosis of colorectal cancer is uncertain[27]. Therefore, this study recruited colorectal cancer patients and screened the clinical data and serology and pathology data to find the indicators that can accurately evaluate the prognosis.

Survival analysis of patients with colorectal cancer

The incidences of postoperative recurrence, metastasis, and tumor-related death are very high[28]. A 3-year follow-up analysis in the study showed that the survival curves decreased significantly at 12-18 mo and 30-36 mo, respectively. It indicated that the number of patients with a poor prognosis increased rapidly at 12-18 mo and 30-36 mo. Among the patients with a poor prognosis, the incidence of colorectal cancer recurrence was the highest, accounting for 49.4% of all patients with a poor prognosis, followed by liver metastasis (32.1%) and bone metastasis (18.5%). The overall poor prognosis rate was 22.82%, which is similar to the recent finding of Shen *et al*[29] (24.3%), and lower than the result of Lujan *et al*[30] (38.3%-45.6%). The possible reason is that with the improvement of people's health awareness, the detection rate of early colorectal cancer is increased. More patients can receive timely treatment in early stage, in addition to the popularization of endoscopic and laparoscopic treatment techniques. Hence the prognosis of colorectal cancer is improved.

Multivariate regression analysis of the prognosis after colorectal cancer surgery

With the increase of age, the immune clearance ability and postoperative recovery ability of carcinoma are significantly reduced. The possibility of poor prognosis is gradually increased[31-33]. Studies have revealed that gender, smoking history, family history, *etc*. can affect the incidence and outcome of carcinoma[34-36]. Tumor location, size, TNM stage, Dukes stage, and pathological classification can indicate the

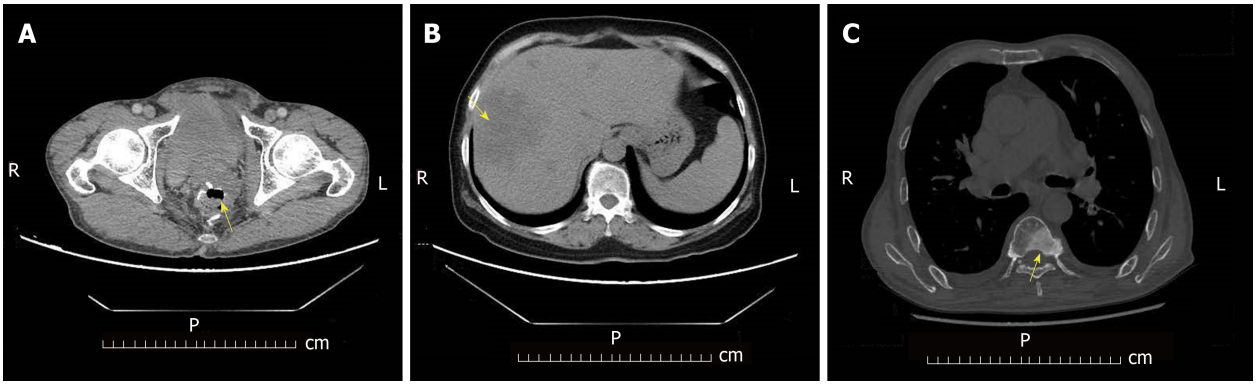

Figure 2 Endpoint events during postoperative follow-up of colorectal cancer patients. A: Colorectal cancer recurrence (arrow); B: Colorectal cancer liver metastasis (arrow); C: Colorectal cancer bone metastasis (arrow).

progression of colorectal cancer and its invasion and metastasis ability. They are often utilized to predict the prognosis of colorectal cancer[37]. PCNA and p53 are common clinical tumor-related detection indicators, and their abnormal expression levels may be associated with colorectal cancer recurrence and poor prognosis[38-42]. Serological indicators such as neutrophils, lymphocytes, albumin, and C-reactive protein are commonly used indicators for monitoring inflammation in the body. Studies have shown that inflammation can promote tumor metastasis and recurrence[43-44]. In addition, inflammatory factors can inhibit the body's immune response to tumors and stimulate tumor formation around the tumor, thereby promoting tumor growth and metastasis[45].

This study compared clinical, serological, and histological indicators of patients with different prognoses. It indicated that age, tumor diameter, TNM stage, PCNA index, GPS, C-reactive protein, albumin, WBC count, NLR, CAR, D-dimer, fibrinogen, CEA, and CA199 were possible risk factors for poor prognosis in patients with colorectal cancer. Further multivariate COX regression analysis of the above indicators showed that TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA were independent influencing factors for the prognosis of colorectal cancer. It suggested that when advanced TNM stage, high PCNA expression levels, GPS > 0, increase of NLR and CAR, or increase of D-dimer and CEA levels occurs, the possibility of poor prognosis should be guarded. In addition, early treatment, control of inflammation levels, and monitoring of D-dimer and CEA levels can prevent poor prognosis. It is worth noticing that NLR, CAR, and GPS are calculated by two serological indicators, which can simultaneously reveal changes of two indicators. Furthermore, TNM staging includes tumor diameter, lymph node metastasis, and distant metastasis. It indicated that multiple indicators are preferred when the accuracy of prognosis assessment based on single factor is poor to improve the accuracy of the evaluation results.

Potential indicators predicting the prognosis of colorectal cancer

Based on COX regression results, this study evaluated the ability of TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA to predict the prognosis of colorectal cancer. According to the evaluation results, the accuracy of NLR, D-dimer, and GPS was significantly higher than that of TNM stage, PCNA, and CAR. Among them, NLR had the highest accuracy. The possible reason may be that NLR is the ratio of neutrophils to lymphocytes. Both of them are reliable indicators for the inflammatory response *in vivo*. Neutrophils can release active factors to activate NF-κB, which is related to the formation of tumors. Also, it can inhibit the immunity of the body through the inflammatory reaction, and help tumor cells escape from immunization. In addition, it can promote tumor growth by promoting neovascularization. Hence, its increase is beneficial to tumorigenesis[43,44]. As one of the main cells of the body's immunity, lymphocytes can produce an immune response to tumor cells, and the decrease of the lymphocytes leads to a decrease in the body's ability to inhibit tumors. Therefore, increased NLR is beneficial to tumor survival and associated with a poor prognosis. The hypercoagulable state of the blood is beneficial for the metastasis of malignant tumors[46]. Studies have confirmed that preoperative D-dimer levels are associated with tumor prognosis[47-49]. In this study, D-dimer had a higher predictive ability for poor prognosis of colorectal cancer, second only to NLR. GPS is determined by the level of serum C-reactive protein and whether it has hypoproteinemia, which can simultaneously indicate the inflammatory condition and nutritional status of the

Table 1 Comparison of indicators between the good prognosis group and poor prognosis group before treatment

	Good prognosis group (*n* = 274)	Poor prognosis group (*n* = 81)	t/χ^2 value	*P* value
Age	53.64 ± 15.38	60.47 ± 8.34	3.831	0.000
Gender (Male/Female)	143/131	43/38	0.020	0.887
BMI (kg/m^2)	26.49 ± 5.34	25.39 ± 5.91	1.589	0.113
Smoking [*n* (%)]	74 (27.01%)	24 (29.63%)	0.215	0.643
History of alcohol abuse [*n* (%)]	39 (14.23%)	13 (16.05%)	0.165	0.685
Family history [*n* (%)]	46 (16.79%)	20 (24.69%)	2.580	0.108
Tumor diameter (cm)	2.08 ± 1.47	5.69 ± 2.54	16.125	0.000
Tumor location				
Ascending colon [*n* (%)]	39	13	3.139	0.535
Transverse colon [*n* (%)]	14	7		
Lower colon [*n* (%)]	25	10		
Sigmoid colon [*n* (%)]	84	22		
Rectum [*n* (%)]	112	29		
Tumor histology type				
Adenocarcinoma	177	53	0.019	0.890
Mucinous adenocarcinoma and signet ring cell carcinoma	97	28		
TNM stage				
I	10 (3.65%)	6 (7.41%)	29.292	0.000
II	213 (77.74%)	38 (46.91%)		
III	30 (10.95%)	24 (29.63%)		
IV	21 (7.66%)	13 (16.05%)		
Dukes stage				
A	17 (6.20%)	1 (1.23%)		0.062
B	177 (64.60%)	48 (59.26%)		
C	80 (29.20%)	32 (39.51%)		
PCNA				
1	98	4	114.291	0.000
2	122	11		
3	45	43		
4	9	23		
P53				
-	34	11	4.908	0.297
+	64	14		
++	61	17		
+++	78	23		
++++	37	19		
GPS				
0	233 (85.04%)	9 (11.11%)	157.579	0.000
1	24 (8.76%)	40 (49.38%)		
2	17 (6.20%)	32 (39.51%)		
C-reactive protein (mg/L)	2.73 ± 0.64	21.83 ± 16.34	19.364	0.000
Albumin (g/L)	42.43 ± 7.93	33.74 ± 6.21	9.071	0.000
WBC count (9 × 10^3/mm^3)	5.89 ± 1.98	8.53 ± 2.83	9.481	0.000
Platelet count (9 × 10^4/mm^3)	237.43 ± 103.28	247.23 ± 116.39	0.728	0.467
NLR	2.68 ± 1.73	4.53 ± 3.29	6.699	0.000
CAR	0.089 ± 0.017	0.175 ± 0.092	14.693	0.000
D-dimer (μg/mL)	0.553 ± 0.207	0.943 ± 0.375	13.117	0.000
Fibrinogen (g/L)	3.121 ± 1.542	3.524 ± 1.053	2.204	0.028
CEA (ng/mL)	3.34 ± 1.82	6.13 ± 2.36	11.281	0.000
CA199 (U/mL)	186.82 ± 139.74	635.24 ± 284.38	15.067	0.000

BMI: Body mass index; TNM: Tumor-node-metastasis; PCNA: Proliferating cell nuclear antigen; GPS: Glasgow prognostic score; WBC: White blood cell;

NLR: Neutrophil-lymphocyte ratio; CAR: C-reactive protein/albumin ratio; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 199.

body. In this study, GPS also had a high predictive ability for poor prognosis of colorectal cancer, which is similar to the study by Wind *et al*[50].

However, each indicator has a certain limitation in predicting the prognosis of colorectal cancer. Therefore, this study combined the independent risk factors to evaluate the accuracy for predicting the prognosis. It revealed that the accuracy of the combination in predicting the prognosis of colorectal cancer was significantly higher than the accuracy of individual indicators. It suggested that in the evaluation of surgical outcomes in patients with colorectal cancer, the combination of TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA has a higher accuracy.

Insufficient and prospects

This study is a single-center study and may have certain limitations. The follow-up time was short, and the indicators affecting long-term prognosis may be ignored. In future, multi-center research is considered to expand the sample size for improving the reliability of the research results. Meanwhile, the length of follow-up can be extended. The influencing factors on short-term and long-term prognosis of colorectal cancer should be analyzed respectively.

In conclusion, this study analyzed the clinical data and serological and pathological indicators that may affect the prognosis of colorectal cancer. It indicated that the independent influencing factors for the prognosis of colorectal cancer include TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA. Among them, NLR, D-dimer, and GPS have higher prediction capabilities. The combination of all independent factors can make a more accurate assessment of the prognosis of colorectal cancer.

Table 2 COX regression analysis of influence factors on the prognosis of colorectal cancer

	B	SE	Wald	*P* value	RR	95%CI	
						Lower limit	Upper limit
Age	0.241	0.223	1.328	0.154	1.273	0.822	1.971
Tumor diameter	0.160	0.315	1.142	0.328	1.173	0.633	2.175
TNM Stage	1.762	0.442	7.364	0.000	5.824	2.449	13.850
PCNA	1.534	0.382	6.338	0.000	4.635	2.192	9.800
GPS	2.238	0.448	8.927	0.000	9.377	3.897	22.564
C-reactive protein	0.454	0.355	2.773	0.058	1.574	0.785	3.156
Albumin	-0.129	0.371	2.538	0.105	0.879	0.425	1.819
WBC count	0.149	0.433	1.292	0.183	1.161	0.497	2.713
NLR	0.816	0.233	6.792	0.000	2.261	1.432	3.570
CAR	2.678	0.341	9.338	0.000	14.552	7.459	28.391
D-dimer	2.498	0.636	8.923	0.000	12.153	3.494	42.272
Fibrinogen	0.603	0.386	0.751	0.625	1.827	0.857	3.893
CEA	1.342	0.492	6.877	0.000	3.827	1.459	10.038
CA199	0.226	0.553	1.149	0.322	1.253	0.424	3.704

TNM: Tumor-node-metastasis; PCNA: Proliferating cell nuclear antigen; GPS: Glasgow prognostic score; WBC: White blood cell; NLR: Neutrophil-lymphocyte ratio; CAR: C-reactive protein/albumin ratio; CEA: Carcinoembryonic antigen; CA199: Carbohydrate antigen 199.

Table 3 Receiver-operating characteristic (ROC) curve analysis of potential indicators for predicting the prognosis of colorectal cancer

	AUC	95%CI	Best diagnostic point	Sensitivity (%)	Specificity (%)
TNM Stage	0.613	0.560-0.663	2	45.68	81.39
PCNA	0.837	0.794-0.874	2	81.48	80.29
GPS	0.872	0.832-0.905	1	88.89	85.04
NLR	0.925	0.860-0.966	0.206	94.12	80.25
CAR	0.743	0.695-0.788	4.81	45.68	94.53
D-dimer	0.879	0.841-0.911	0.795 (μg/mL)	58.02	100
CEA	0.801	0.755-0.841	3.891 (ng/mL)	76.54	64.60

TNM: Tumor-node-metastasis; PCNA: Proliferating cell nuclear antigen; GPS: Glasgow prognostic score; NLR: Neutrophil-lymphocyte ratio; CAR: C-reactive protein/albumin ratio; CEA: Carcinoembryonic antigen.

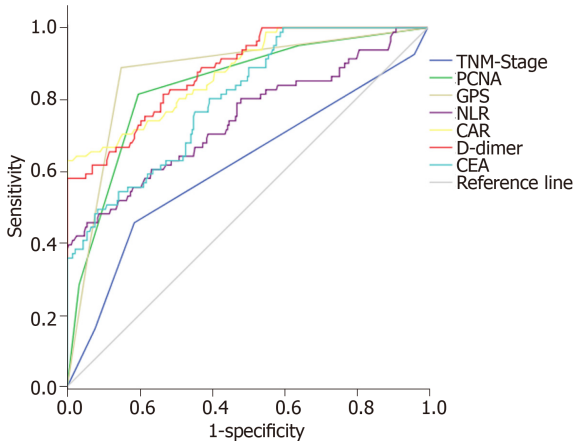

Figure 3 Receiver operating characteristic curve analysis of potential indicators for predicting the prognosis of colorectal cancer.

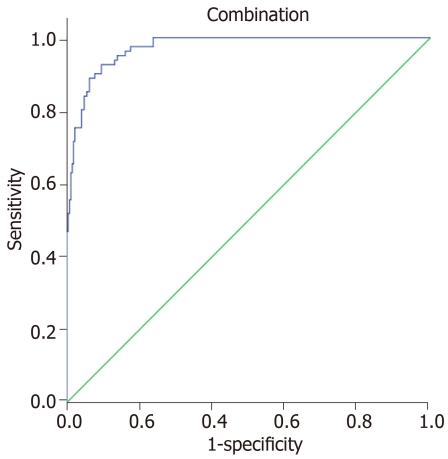

Figure 4 Receiver operating characteristic curve analysis of the combination assessment for predicting the prognosis of colorectal cancer.

ARTICLE HIGHLIGHTS

Research background

The prognosis of colorectal cancer is poor. Surgery is the main treatment for patients with colorectal cancer. Accurate assessment of surgical prognosis has an important impact on the choice of treatments for patients. Currently, there are many methods to evaluate the prognosis after colorectal cancer surgery, including tumor-node-metastasis (TNM) stage, Glasgow prognostic score (GPS) score and so on. However, the systematic analysis about the factors affecting the prognosis of colorectal cancer is still limited.

Research motivation

Currently, the prognosis of colorectal cancer is mainly predicted by carcinoembryonic antigen (CEA) level, pathological classification, and TNM stage. However, the accuracy of prediction cannot be guaranteed due to the influence of individual and environmental factors. Besides, studies have revealed that some inflammatory indicators are also related to the prognosis of cancer.

Research objectives

In this study, we analyzed the influence of clinical data, serology, and histology on the prognosis of patients with colorectal cancer, and assessed the accuracy of the combination of all indicators for the prognosis evaluation. The purpose of this study was to provide a new method for predicting the prognosis of colorectal cancer in the early stage.

Research methods

A total of 374 patients were recruited, and the patients were divided into a good prognosis group and a poor prognosis group. Relevant clinical indicators were recorded. The differences in clinical data, serology, and histology between the two groups were analyzed. Multivariate COX regression was used to analyze the independent influencing factors for the prognosis of colorectal cancer. The receiver operating characteristic curve was used to test the accuracy of different indicators and their combination for the prognostic evaluation of colorectal cancer.

Research results

The TNM stage, proliferating cell nuclear antigen (PCNA), GPS, neutrophil-lymphocyte ratio (NLR), C-reactive protein/albumin ratio (CAR), D-dimer, and CEA were independent influencing factors for the prognosis of colorectal cancer ($P = 0.000$). NLR, D-dimer, and GPS had the highest predictive power for colorectal cancer prognosis. But their accuracies were significantly lower than that of the combination of all indicators (AUC = 0.973; sensitivity, 92.59%; specificity, 90.51%).

Research conclusions

TNM stage, PCNA, GPS, NLR, CAR, D-dimer, and CEA are the independent influencing factors for the prognosis of colorectal cancer. Combined evaluation of independent factors is the most accurate method to predict the prognosis of colorectal cancer.

Research perspectives

On the purpose of avoiding the interference caused by the differences of individual and environmental factors, multi-center studies would be considered to enlarge the size of simple to improve the reliability of the research results. Besides that, long-term research is also planned to make up the ignorance of the factors affecting long-term prognosis in this study.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1218-1230

DOI: 10.4251/wjgo.v11.i12.1218

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

Deep learning with convolutional neural networks for identification of liver masses and hepatocellular carcinoma: A systematic review

Samy A Azer

ORCID number: Samy A Azer (0000-0001-5638-3256).

Author contributions: The author SAA created the idea of the review, generated the rationale and the research question, designed the project, searched the databases, analysed the findings, created the tables, wrote the manuscript, and approved the final manuscript.

Supported by the College of Medicine Research Centre, Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia.

Conflict-of-interest statement: The author declares that he has no competing interests.

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

Samy A Azer, Department of Medical Education, King Saud University College of Medicine, Riyadh 11461, Saudi Arabia

Corresponding author: Samy A Azer, FACG, Professor of Medical Education, Gastroenterologist, Department of Medical Education, King Saud University College of Medicine, P O Box 2925, Riyadh 11461, Saudi Arabia. azer2000@optusnet.com.au
Telephone: +966-11-8066393
Fax: +966-11-4699174

Abstract

BACKGROUND

Artificial intelligence, such as convolutional neural networks (CNNs), has been used in the interpretation of images and the diagnosis of hepatocellular cancer (HCC) and liver masses. CNN, a machine-learning algorithm similar to deep learning, has demonstrated its capability to recognise specific features that can detect pathological lesions.

AIM

To assess the use of CNNs in examining HCC and liver masses images in the diagnosis of cancer and evaluating the accuracy level of CNNs and their performance.

METHODS

The databases PubMed, EMBASE, and the Web of Science and research books were systematically searched using related keywords. Studies analysing pathological anatomy, cellular, and radiological images on HCC or liver masses using CNNs were identified according to the study protocol to detect cancer, differentiating cancer from other lesions, or staging the lesion. The data were extracted as per a predefined extraction. The accuracy level and performance of the CNNs in detecting cancer or early stages of cancer were analysed. The primary outcomes of the study were analysing the type of cancer or liver mass and identifying the type of images that showed optimum accuracy in cancer detection.

RESULTS

A total of 11 studies that met the selection criteria and were consistent with the aims of the study were identified. The studies demonstrated the ability to differentiate liver masses or differentiate HCC from other lesions (n = 6), HCC from cirrhosis or development of new tumours (n = 3), and HCC nuclei grading

Manuscript source: Invited manuscript

Received: March 2, 2019
Peer-review started: March 4, 2019
First decision: June 5, 2019
Revised: July 9, 2019
Accepted: October 3, 2019
Article in press: October 3, 2019
Published online: December 15, 2019

P-Reviewer: Aykan NF
S-Editor: Dou Y
L-Editor: Filipodia
E-Editor: Liu MY

or segmentation (n = 2). The CNNs showed satisfactory levels of accuracy. The studies aimed at detecting lesions (n = 4), classification (n = 5), and segmentation (n = 2). Several methods were used to assess the accuracy of CNN models used.

CONCLUSION
The role of CNNs in analysing images and as tools in early detection of HCC or liver masses has been demonstrated in these studies. While a few limitations have been identified in these studies, overall there was an optimal level of accuracy of the CNNs used in segmentation and classification of liver cancers images.

Key words: Deep learning; Convolutional neural network; Hepatocellular carcinoma; Liver masses; Liver cancer; Medical imaging; Classification; Segmentation; Artificial intelligence; Computer-aided diagnosis

Core tip: Artificial intelligence, such as convolutional neural networks (CNNs), have been used in the interpretation of images, including pathology and radiology images with potential application in the diagnosis of hepatocellular cancer (HCC) and liver masses. CNN, a machine-learning algorithm similar to deep learning, has demonstrated its capability to recognise specific features that can detect pathological lesions. The primary aim of this review is to assess the use of CNNs in examining HCC and liver masses images in the diagnosis of cancer. The second aim is to evaluate the accuracy level of CNNs and their clinical performance.

Citation: Azer SA. Deep learning with convolutional neural networks for identification of liver masses and hepatocellular carcinoma: A systematic review. *World J Gastrointest Oncol* 2019; 11(12): 1218-1230
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1218.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1218

INTRODUCTION

Significant progress has been made in image recognition primarily due to the recent revival of deep learning, particularly the convolutional neural network (CNN), a class of artificial neural networks that have been widely used in biomedical and clinical research[1]. For example, the potential use of CNNs has been shown in the detection of gastrointestinal bleeding in wireless capsule endoscopy images using handcrafted and CNN features[2], diagnosis of *Helicobacter pylori* infection based on endoscopy images[3,4], and detection of gastrointestinal polyps using endoscopy images[5,6]. There is also a surge of interest in the potential of CNNs in radiology research[1,7] and in cellular and histopathological examinations[8]. Several studies have shown the ability of the CNN algorithms in (1) Lesion detection, a prevalent task for endoscopists, radiologists, and pathologists to detect abnormalities with medical images. These include the detection of colonic polyps, the detection of lesions on radiological images, and the detection of histopathological malignant changes on biopsy images[1,5-9]. CNN algorithms are also useful for (2) Classification, the CNNs utilise target lesions depicted in medical images, and these lesions are classified into classes. One of these is classifying lesions into particular categories (lesions or normal; malignant or benign). Other examples may include classification of precancerous gastric disease using the CNNs[10] or classification of skin cancer[11]. Therefore, the task is to determine "optimal" boundaries for separating classes in the multi-dimensional feature space that is formed by input features. At least three significant techniques have been described that use CNNs for medical image classifications, including training the CNNs from scratch[12], using "off-the-shelf CNN" features as complementary information channels to existing hand-crafted image features[13], and performing unsupervised pre-training on natural or medical images and fine-tuning using deep learning models[14]. (3) Segmentation of organs or anatomical structures is a functional image processing technique for the analysis of medical images such as quantitative evaluation of clinical parameters and computer-aided diagnosis system[15]; and (4) Image reconstruction, which may include obtaining a noiseless

computed tomography (CT) image reconstructed from a subsampled sonogram[16]. With the above information in mind, this study aims at reviewing and identifying the applications and uses of CNNs in the interpretation of liver cancers, including hepatocellular carcinoma (HCC), liver metastasis (secondaries), and other liver masses.

Primary liver cancer, mainly HCC, is the fifth most common cancer in men and the seventh most common in women and is the third leading cause of cancer-related death worldwide[17]. In general, the disease is less common in females, and in most areas in the world, the male to female liver cancer rates are two- to three-fold higher, possibly due to the higher prevalence of risk factors in males and differences in sex steroid hormones, and perhaps epigenetic factors[18]. Studies showed that there is an increasing rate of HCC worldwide, which may be related partly to hepatitis B virus and hepatitis C virus infections, obesity, diabetes, metabolic syndrome, and non-alcoholic fatty infiltration of the liver[19].

However, the burden of HCC varies depending on geographical location. For example, in the Asia-Pacific region, it is a significant public health problem[20]. Because the liver is a common site of metastasis from cancers of other organs, mainly colorectal, gastric, pancreatic, breast, and lung cancers, secondaries to the liver add to the burden of liver cancer[21].

Currently and as per the guidelines of the American and European liver societies and World Gastroenterology Organisations, ultrasound is widely used in surveillance of HCCs, and CT and magnetic resonance images (MRI) are indicated to characterise a focal lesion suspected in the liver[22-24]. The diagnosis of HCC relies on either MR images or contrast-enhanced CT-scan, which enable the identification of up to 65% of small cell nodules < 2 mm in size[22-25]. However, the detection of small nodules is dependent on vascular dynamic enhancement pattern throughout the different phases of the study[26]. Also, there is inter-operator variability induced by visual qualitative assessment[27]. Therefore, the use of computer-aided diagnosis framework may enable us to resolve these limitations and enhance the diagnosing outcomes of these radiological modalities.

Based on these findings, this study aims to evaluate the use of CNN in HCC, liver metastasis (secondaries), or images of other liver masses. The rationales for the study were to assess the current status of convolutional neural networks and their applications in liver oncology images, identify gaps and deficiencies in ongoing research in this new field, particularly in relation to liver oncology images, and discuss future directions and research priorities that may maximise the applications of research in hepatic oncology. Therefore, our research questions are: (1) What is the current status of research output in the use of CNN in assessing HCC, liver metastases (secondaries), or images of other liver masses? and (2) What is the accuracy of CNN, deep learning systems, in lesion detection, classification, or segmentation of these images?

MATERIALS AND METHODS

This manuscript was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines[28].

Literature search

The databases PubMed, EMBASE, and the Web of Science were searched for studies on CNNs in liver cancer and liver masses images. Also, research books that published full papers from conferences and scientific meetings were searched. The search covered studies up to January 2019. Only studies in the English language and conducted on humans were included. Studies on animals or animal models were not included. We searched for articles with contributions of the subject headings and the following key words: “Cancer”, “Liver”, “Hepatocellular carcinoma”, “HCC”, “Liver mass”, “Metastasis”, “Hepatic”, “Secondaries in liver”, “Radiology”, “Pathology”, “Histology”, “Histopathology”, “Malignancy”, “Primary liver cancer”, “Ultrasound”, “Computed tomography”, and “Magnetic resonance images”. To maximize the yield of the search, another search was performed manually by searching the list of references of the primary articles and reviews to identify studies not found by the database search[29].

We also searched the journals listed by the Journal Citation Reports-2017 of the Web of Science under the categories Gastroenterology and Hepatology (n = 32 journals), Oncology (n = 41 Journals), Radiology (n = 6 journals), Pathology (n = 14 journals), Computer Sciences and Engineering (n = 18 journals), and Medical Informatics (n = 7).

Criteria for consideration of studies

To identify targeted studies, we created a PICOS framework (Population, Intervention, Comparison, Outcome, Studies) for the inclusion and exclusion. Table 1 summarises the PICOS framework used. The following inclusion and exclusion criteria were used in selecting studies. Studies that reported data on the use of the CNNs in liver cancers images (HCC or liver metastasis/other liver masses) were included. Full research papers of conferences and scientific meetings were also considered if they fulfil the research purpose. The search was limited to studies in the English language and conducted on humans. Studies on animals or animal models were not included. Reviews, editorials, commentaries, letters to the Editors, abstracts published in conference proceedings, were not included.

Study selection

Two researchers (the author and a research assistant) independently reviewed the titles and abstracts of all citations identified by the literature search. Relevant studies were retrieved and reviewed in detail. Any disagreement was discussed by the two evaluators. The full texts of potentially relevant articles were sought, and the selection criteria were applied. Reviewers were not blinded to authors' names or institutions. Studies were selected if they match the selection criteria.

Data extraction

Data were extracted independently by the two researchers using a predefined extraction form. The following data were abstracted in the form: (1) First author's name; (2) Year of publication; (3) Objectives/research question; (4) Method used; (5) Liver cancer/liver masses investigated; (6) Main results; (7) Accuracy, sensitivity, and specificity of method used; and (8) Institute, university, city, and country where the study was conducted. Details on reported statistical associations and comparison of the results obtained with those obtained by using other methods were also evaluated. The agreement between evaluators measured by the degree of inter-rater agreement using Cohen kappa coefficient was also carried out using SPSS software (Armonk, NY, United States)[30].

RESULTS

Literature search and selection process

Figure 1 is a flow diagram summarizing the search results and selection process of articles. One hundred and twenty-nine potentially relevant publications were identified through the search of the three databases and research books. After removal of duplicates, 78 articles remained. Of these, 42 were not relevant to the inclusion criteria. Thirty-six full-text articles were assessed for eligibility. Finally, we identified 11 articles that met our selection criteria and were consistent with the aims of the systematic review[31-41].

Characteristics of included studies

Table 2 summarises details of the 11 studies included[31-41]. The studies demonstrated the ability of CNN models in analysed images of liver cancers as follows: Classification of liver masses into five categories: category A: Classic HCC; category B: Malignant liver tumour other than HCC; category C: intermediate masses (early HCC, dysplastic nodules, or benign liver masses; category D: Haemangiomas; category E: Cysts[31], detection of small metastasis in the liver[32], discrimination between primary liver cancer (HCC) and secondaries in the liver[33], differentiation between chronic liver diseases such as cirrhosis and the presence of HCC on top of cirrhosis[34], classification of grade of HCC nuclei and segmentation of HCC nuclei on pathology images[35,36], classification of liver lesions[31,33,34,37,41], and detection of liver tumour or liver masses and identification of their types and phases[38-40]. While these studies examined liver CT images[31,32,37-40], ultrasound images[34], and 3D multi-parameter MRI scan images[33,41], other images such as cellular and histopathological images were also included[35,36].

Countries and institutes/universities involved

Geographically, these studies were performed in Japan (2), China (3), the United States (2), India (1), Greece (1), and Israel (4). Some papers were written by authors from two countries. Top universities, hospitals, and research institutes that led such research were: Department of Radiology, the University of Tokyo Hospital, Tokyo, Japan; Faculty of Engineering, Department of Biomedical Engineering Medical Image Processing Laboratory, University of Tel Aviv, Israel; Department of Informatics

Table 1 PICOS framework to identify studies for inclusion

Population	Worldwide, datasets, patients, males and females, humans
Intervention	Use of CNN-diagnostic proposals and computer models
Comparison	Normal population, use of manual detection by radiologists, hepatologists, or anatomical pathologists examining images/slides.
Outcome	Lesion detection, classification, segmentation, or image reconstruction.
Studies	Controlled, or comparison to manual (routine) assessment, or comparison to benchmark or other artificial intelligence models.

CNN: Convolutional neural network; PICOS: Population, intervention, comparison, outcome, studies.

Engineering Technology Education Institute of Crete, Greece; Thapar Institute of Engineering and Technology, Patiala, India; Manipal Hospital, Bangalore, India; Software College, Northeastern University, Shenyang, China; Electric and Computer Engineering Stevens Institute of Technology, NJ, United States; Department of Diagnostic Images, the Chaim Sheba Medical Centre; School of Computer Science and Engineering, The Hebrew University of Jerusalem, Jerusalem, Israel; Information Science and Engineering, Ritsumeikan University, Shiga, Japan; Medical School, Zhejiang University, Hangzhou, China; Department of Biomedical Engineering, Yale University, CT, United States; and Department of Electrical Engineering, Yale University, CT, United States.

Methods used

Description of reported methods: The description of the method used in these studies and the content described provided significant detail about the dataset, the generation of the CNN algorithm, the architecture used, the experiments carried out to assess system performance, system evaluation, and accuracy of automatic liver segmentation or classification[14,31-41]. However, in most studies identified no details were given regarding clinical information, such as number of patients included, sources and number of images used, and clinical procedures carried out. This imbalance in the methods described may be related to the background of the authors of these studies and the journals that published these studies. The 11 studies were published by 58 authors; of these, five were from radiology departments, one was from the pathology department, and two were possibly with medical background. The remaining 50 authors were non-medical and were from engineering, computer science, and medical image processing laboratory. Except for *Radiology*[31], and *Ultrasonic Imaging*[34], the majority of these articles were published in journals specialised in computer science and Biomedical informatics, such as *Neurocomputing*[32,36,37], *IEEE Journal of Biomedical Health Informatics*[33], *Computers in Biology and Medicine*[35], *Medical and Biological Engineering and Computing*[38], and *International Journal of Computer Assisted Radiology and Surgery*[39]. These two factors may explain the dominance of technical information regarding computer science information in the study methods.

Datasets and number of patients: The number of patients and images used in the studies varied significantly. Ben-Cohen *et al*[32] reported that their study involved 20 patients with 68 lesions in total and that they were tested on a testing set that included CT examinations for 14 patients with a total of 55 lesions[32]. Another study that used CT imaging was Frid-Adar *et al*[37], who reported a limited dataset of 182 liver lesions (53 cysts, 64 metastases, and 65 haemangiomas). The authors highlighted difficulties in obtaining datasets, and although there are medical datasets available online, they felt that they are still limited and only applicable to specific medical problems[37]. Also, the study by Todoroki *et al*[40] used 3D multi-phase contrast-enhanced liver CT images from 75 patients. The cases comprised five different lesions, namely-cysts, focal nodular hyperplasia, HCC, haemangioma, and metastases[40]. The study by Yasaka *et al*[31] is the fourth study that used contrast agent-enhanced CT images of the liver. The study was a retrospective study and used images of liver masses over three phases (non-contrast-agent enhanced, arterial, and delayed). The masses were grouped under five categories, as stated earlier[31]. On the other hand, the study by Trivizakis *et al*[33] examined upper abdominal MRI scans of 134 patients (37.7% primary liver mass and 62.3% metastatic lesion). The study by Zhang *et al*[41] also used MRI scan images of 20 patients, with HCC generating 1700 non-overlapping patches. Only one study used liver ultrasound images acquired from 94 patients. The images datasets comprised 48 normal liver, 50 chronic liver, 50 cirrhosis, and 41 HCC evolved over cirrhosis. Other datasets comprised experimental data involving 127 liver pathology images[35,36].

Methods and study design: Methods using CNN models to study HCC and liver

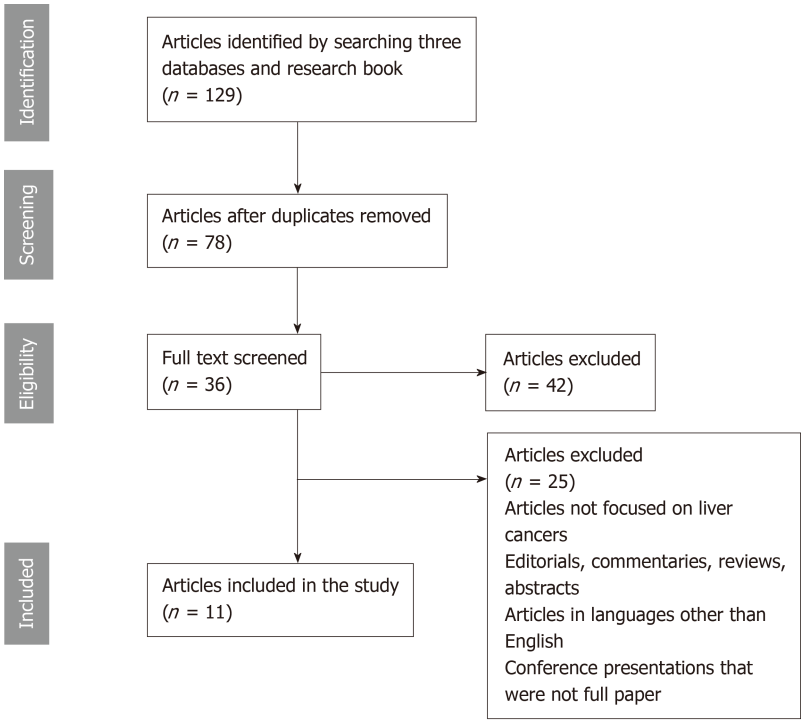

Figure 1 A PRISMA flowchart showing articles searched on use of convolutional neural networks in gastrointestinal and liver cancers images.

masses (secondaries or metastases or other liver masses) in these 11 studies varied significantly. While the study by Ben-Cohn *et al*[32] used a global context with a fully convoluted network and a local patch level analysis with superpixel sparse based classification, the study by Trivizakis *et al*[33] proposed a CNN model comprising four consecutive strided 3D convolutional layers with 3 × 3 × 3 kernel size and ReLU as activation functions. The design has a fully connected layer with 2048 neurons and a soft max layer for binary classification. For training and validation, a dataset of 130 Diffusion Weighted MR images scans was used[33]. The method used by Bharti *et al*[34] aimed at examining ultrasound images through visual interpretation of liver images.

Accuracy measures used

The accuracy measurements used in these studies varied and can be summarised as follows: (1) Assessing system performance and system evaluation[32,34]; (2) Assessing accuracy of automatic liver segmentation and lesion detection[32,34]; (3) Generating operating characteristics curves and precision recall[33]; (4) Comparing the method used outcomes with the results obtained from other CNNs[35,37,41]; (5) Measuring sensitivity, specificity, and accuracy parameters[37,38]; (6) Comparing accuracy of the deep CNN method with those measured by Bayesian model and the benchmark method[40,41]; (7) Comparing the outcomes with visual inspection by expert radiologists (precision and recall rates)[37]; and (8) Comparing the performance of the CNN-based system to non-network state-of-the-art methods for liver lesion classification[37]. Some studies did not measure sensitivity or specificity[34,40,41].

Key findings

The studies demonstrated a medium accuracy of differential diagnosis of liver masses of 0.84 for test data and the under receiver operating characteristic curve was 0.92[31]. Ben-Cohen *et al*[32] reported satisfactory results and demonstrated that on using 3-fold cross validation, experiments resulted in a true positive rate of 94.6% with 2.9 false positive result per case. The classification performance of the work conducted by Trivizakis *et al*[33] was 83% for 3D *vs* 69.6% and 65.2% for 2D, which indicates significance of tissue classification accuracy compared to two 2D CNNs. Another classification CNN system of four liver stages by Bharti *et al*[34] had an accuracy of 96.6%. Other studies showed that the proposed method had superior performance when compared with related works by other techniques[35,36,40,41]. Li *et al*[35] showed that external validation of the proposed method multiple fully connected CNN with extreme learning machine model they created by using Hep-2 cells indicates that their method can be generalised in grading HCC nuclei. The work of Vivanti *et al*[39] showed

Table 2 Studies on convolutional neural networks and liver masses and liver cancers

Ref.	Research question/Purpose	Method used	Key findings	Conclusions
Yasaka *et al*[31], 2018	Can deep learning techniques enable the classification of liver masses into categories?	A retrospective study. CNN together with dynamic contrast enhanced CT image set of liver masses were used over 3 phases (55 536 images obtained from 460 patients used in training and 100 liver mass image sets were used in testing).	The CNN model enabled classification of liver masses into 5 categories. The median area under the receiver operating characteristic curve to differentiate between categories was 0.92.	Deep learning with CNN enabled classification of liver masses and differentiate between 5 categories at dynamic CT with high accuracy.
Ben-Cohen *et al*[32], 2018	Can a proposed CNN enable the detection of liver metastasis in CT examinations of the liver?	The method involved both a global context and a fully convolutional network and a local patch level analysis with superpixel sparse based classification. A total of 20 patients with a total of 68 lesions and a testing set with CT examinations from 14 patients with overall 55 lesions were included.	The true positive rate was 94.6% with 2.9 false positive per case.	The system enhanced the detection of small metastasis in the liver and is clinically promising.
Trivizakis *et al*[33], 2019	Evaluate the use of a novel 3D CNN model in tissue classification of medical images and application for discriminating between primary and metastatic liver tumours.	The proposed model consisted of 4 consecutive strided 3D convolutional layers with 3 × 3 × 3 Kernel size and ReLU for activation functions.	The classification performance was 83% for 3D *vs* 69.6% and 65.2% for two 2D CNNs models; demonstrating significant tissue classification accuracy improvement compared to two 2D CNNs.	The proposed 3D CNN architecture can differentiate between primary and secondary liver tumours.
Bharti *et al*[34], 2018	Test a proposed model to differentiate between chronic liver and cirrhosis and presence of hepatocellular carcinoma (HCC).	The system is based on higher order features, a hierarchical organisation and multi-resolution, which enabled the characterisation of echotexture and roughness of liver surface.	The proposed CNN feature was able to differentiate four liver stages acquired from ultrasound images. The classification accuracy of the model in differentiating between the four groups was 96.6%.	The model is able to differentiate between normal liver, chronic liver disease, cirrhosis and HCC evolved on top of cirrhosis.
Li *et al*[35], 2017	Can a joint multiple fully connected CNN with extreme learning machine (MFC-CNN-ELM) architecture be used in hepatocellular carcinoma (HCC) nuclei grading?	Centre-proliferation segmentation (CPS) method was used and labels of grayscale image patch were marked under the guidance of three pathologists. A multiple fully convolutional neural network (MFC-CNN) was designed to extract the multi-form feature vectors of each input image automatically. Finally, the CNN-ELM model was used to grade HCC nuclei.	External validation using ICPR 2014 HEP-2 cell shows the good generalisation of MFC-CNN-EL architecture.	The proposed MFC-CNN-ELM has superior performance in grading HCC nuclei compared with related works.
Li *et al*[36], 2018	Can a proposed structured convolutional extreme learning machine (SC-ELM) and case-based shape template (CBST) method used in HCC nucleus segmentation?	The model SC-ELM was developed for global segmentation of pathology images and is used for coarse segmentation.	The model was used for experimentation with 12.7 liver pathology images.	The proposed model demonstrated higher performance compared with related and published work.

Frid-Adar *et al*[37], 2018	Can generated medical images be used for synthetic data augmentation and improving the performance of CNN medical image classification?	Using a limited data of CT images of 182 liver lesions (53 cysts, 64 metastasis and 65 haemangiomas), and generating synthetic medical images using recently presented deep learning Generative Adversarial Networks (GANs), the authors then used CNN to compare classification. Performance was compared with synthetic data augmentation and classic data augmentation.	The classification performance on using classic data augmentation yielded 78.6% sensitivity and 88.4% specificity. The synthetic data augmentation resulted in an increase to 85.7% for sensitivity and 92.4% for specificity.	The approach of synthetic data augmentation can be generalized to other classification applications.
Vivanti *et al*[38], 2018	Can CNN-based method enable robust automatic liver tumour delineation in longitudinal CT studies?	The inputs are baseline scans and the tumour delineation, a follow-up scan, and a liver tumour global CNN.	Results from 222 tumours from 31 patients yielded an average overlap error of 17% (SD = 11.2) and surface distance of 2.1 mm (SD = 1.8) which is far better than stand-alone segmentation.	The method shows that follow-up framework yields accurate tumour tracking with a small training dataset.
Vivanti *et al*[39], 2017	Automatic detection and segmentation of new tumours in longitudinal CT scan studies and for liver tumours burden quantification.	Inputs were the baseline and follow-up CT scans. The outputs were the new tumours segmentations in the follow-up scans, and measurement of tumour burden quantification and tumour burden change.	246 tumours of which 97 were new tumours from 37 longitudinal liver CT studies, yielded positive new tumours detection rate of 86% *vs* 72% with stand-alone detection, and a tumour burden volume overlap error of 16%.	Compared to existing methods, this method enables accurate and reliable detection of known tumours and detection of new tumours in the follow-up scans.
Todoroki *et al*[40], 2018	Can a deep CNN method detect liver masses regardless to their types and phases (stages) from CT images?	The proposed method is based on deep learning which used multi-layered CNN. The Tumour detection methods comprised two steps: (1) Segmentation of the liver from CT liver images; and (2) Calculation of probability of each pixel in the segmented liver.	3D multi-phase contrast-enhanced CT liver images of 75 cases were used in the study. The cases comprised 5 types of lesions-cysts, focal nodular hyperplasia, hepatocellular carcinoma, haemangioma, and metastases, each type was represented in 15 cases. The detection results for each type was superior to detected boundaries by doctors and outperformed other methods.	The proposed deep CNN tumour detection method demonstrated an ability to discriminate between the 5 different types, and the performance outperformed that of other convolutional methods.
Zhang *et al*[41], 2018	Can a proposed 3D multi-parameter magnetic resonance images and a novel deep CNN method classify different types of liver tissue in patients with hepatocellular carcinoma?	A novel deep CNN incorporating auto-context elements with U-net-like architecture was developed. The model uses multi-level hierarchical architecture and multi-phase training procedures.	Liver magnetic resonance images from 20 patients yielded promising results in classifying liver tissues. The method was compared with a benchmark method, multi-resolution input single-phase training and single-resolution-input, single-phase training and demonstrated higher discriminatory performance.	The multi-resolution input, the auto-context design, and the multi-phase training procedure have helped in improving the performance of the model.

CNNs: Convolutional neural networks; CT: Computed tomography.

an overlap error of 17% (SD = 11.2) and surface distance of 2.1 mm (SD = 1.8), which was far better than stand-alone segmentation. The proposed system did not require large annotated training datasets, which is an advantage compared to other systems[38]. Another study by Vivanti *et al*[39] showed that the experiments resulted in a yield of true positive new tumours detection rate of 86% *versus* 72% with stand-alone detection. The tumour burden volume overlap error was 16%, which means that the follow-up CT scans enable not only the detection of new tumours but also the estimation of tumour burden volumetric; both are required in the diagnosis and management of liver tumours[39].

The agreement between the evaluators

The inter-rater agreement between evaluators had overall κ scores in the range of 0.779-0.894.

DISCUSSION

During the last 5-6 years a significant shift in research trends in image processing has taken place, and the use of artificial intelligence in the field has moved from hand crafted algorithms to deep learning architectures[14]. In this study, our aims were to assess the use of the CNNs in deep learning of liver cancer (HCC and secondaries in the liver or liver masses) images. In this review, 11 studies were identified. Because of the heterogeneous data, gaps in the reported results, and the variability in the design of methods, it was not possible to conduct a meta-analysis. Nevertheless, certain parameters were evident from these studies. First, deep learning architectures and particularly the CNNs have been usefully implemented into medical imaging domain. The studies have shown the accuracy of the CNNs in analysing and offering the diagnosis (segmentation, and classification) of radiology images (CT scans, ultrasound, and MRI scans) and histopathology and cellular images of liver cancers. Although the number of full original articles on each cancer was small, the 11 studies were published in the years 2017 and 2018; reflecting the fact that deep learning as outlined in the CNNs and their applications in medical sciences is a recently developed discipline[42]. Second, although the technical information and the description of the test data preparation are important, the training and evaluation algorithm development are vital, and information about the sources of images, the patients involved in the study, and the clinical information collected is equally important. In this review, it was noted that the methods used varied and were not standardised. While these differences might be related to differences in the focus of the journals that published these studies and the background of the authors (medical *vs* engineering), there is a need for more studies that equally represent authors from medical, computer science, and engineering and address both aspects in the methods and results to maximise the research readability and applications of publications across disciplines involved. Third, it would be of interest to assess the performance of different research articles that were validated on the same datasets; however, most studies did not provide enough information about the exact sources of their datasets and it was not possible to trace which studies used the same testing protocol[43]. Such information is vital for comparison and assessment. We hope that journals interested in publishing such studies on deep learning, the CNNs, and artificial intelligence develop standardized guidelines that require authors to state such information, including details about the sources of datasets and protocols used in testing.

This study, however, is not free from limitations. Considering this diversity and lesions included, we must interpret the findings with caution. There could be publication bias that precluded the publication of negative studies. Studies included were only those published in the English language, and there could be good articles related to this topic that were published in other languages. However, this study presents what has been accomplished in this area in the English language literature. Studies varied in terms of patient type, study design, design of the CNN models, pathology of diseases included, and details of methods used[44].

Future research directions

This study highlights a number of future directions for research that uses CNNs to interpret liver cancer images. These can be summarised as follows: First, while the improvement in the design of the CNNs has required relatively small training sets of images, as it is the case with the models presented by Vivanti *et al*[38,39], there is a need for multi-institute and multicentre collaborations in studies including a large number of patients with cirrhosis due to different pathological causes and patients with HCC on top of cirrhosis, liver secondaries, or liver masses. Such collaborations could resolve concerns about the insufficient amount of training data in the medical image domain and enable measurement of accuracy and performance of the CNNs algorithms. Several researchers reported difficulties in obtaining images, which make the direct application of machine learning algorithms inappropriate for medical datasets and hence affect the capacity to conduct image classification or image segmentation with high accuracy[42,44]. Second, differentiation between primary and secondary (metastases in the liver) or other liver masses is difficult on the basis of radiological imaging. The studies included in this review showed that CT-based deep learning methods can enable the categorisation of liver metastases[32,33]. Although such differentiation between primary and secondary liver tumours may be clinically useful, other priorities that we need to explore further are: (1) Examination of HCC on top of liver cirrhosis to observe the characteristics of HCC on cirrhosis and non-cirrhotic liver tissue[34]; and (2) Conducting longitudinal liver CT scan studies and comparing outcomes with existing stand-alone and follow-up methods. We hypothesise that longitudinal studies could enable researchers to compare changes with the baseline scan and thus could offer a better detection of new small

tumours[38,39]. Third, we need case control studies where the use of CNN could be compared with manual assessment of images by experts, radiologists, hepatologists, and pathologists. One of the major challenges we face with the use of CNN is the difficulty in choosing discriminant features to represent the clinical characteristics and using them as the key features in the CNN algorithm in segmentation and in classification functions. Again, this goal cannot be achieved without the collaboration of medical experts, radiologists, pathologists, computer engineering programmers, and experts designing these systems. Fourth, future studies should give more attention to the assessment of accuracy and sensitivity of the CNNs in evaluating the performance of systems and calculating the positive predictive values. Ideally, a study should use two or three different methods and compare accuracy parameters for the same set of images using these different methods. Currently, we are lacking such studies in the literature, and so any comparison of accuracy is not optimum because of several variables interfering with the methods/models reported[45].

Conclusions

With increasing research applying CNNs in liver cancer images there is a demand to evaluate carefully their accuracy and define future research directions. While current studies have covered major liver cancers, the number of studies conducted so far is small and limited, and more research is needed to answer questions about the accuracy and sensitivity of the CNN algorithms. The CNNs have demonstrated abilities in segmentation, classification, and lesion detection of radiological and anatomical pathology images of common cancers. However, several deficiencies in current studies were observed. In most studies there was no balance in the content of methods among the description of patients involved, the medical component, and the technical computer related component. Furthermore, comparing the use of the CNNs with other models is needed particularly in regard to accuracy and sensitivity of each model on the same set of images. Therefore, future studies that focus on these areas should be multi-institute and the outcome of multicentre collaborations should include with a large number of patients. This is particularly important in view of the growing demand of CNNs in liver oncology.

ARTICLE HIGHLIGHTS

This study highlighted several aspects related to convolutional neural networks (CNNs). First, CNNs have potential use in identifying HCC and differentiating HCC from other liver masses with high accuracy. Second, CNNs can offer several functions concerning liver cancer, including lesion detection, classification, and segmentation. Third, the use of CNN in liver cancer is not limited to radiological images, but it is of value in pathological and cellular studies. However, the study identified several limitations in the literature in this area, mainly the smaller number of studies on the topic and the lack of studies from multi-centres as well as the lack of longitudinal liver computed tomography (CT) scan studies that can enable comparing outcomes with existing stand-alone and follow-up methods. These longitudinal studies could allow researchers to compare changes with the baseline scan and thus could offer better detection of new small liver tumours.

Research background

Recently, an increasing interest in the use of deep learning has emerged in research, particularly CNNs, a class of artificial intelligence that has been widely used in biomedical research. This study reviews the current literature on the use of CNNs in assessing hepatocellular carcinoma (HCC) and liver masses and how such advanced technology can help improve clinical diagnosis.

Research motivation

While the study focuses on an evolving field in gastroenterology and oncology with promising outcomes, several researchers reported difficulties in obtaining images, which make the direct application of machine learning algorithms inappropriate for medical datasets and hence affect the capacity to conduct image classification with high accuracy. Therefore, improvement in the design of CNNs and multi-institute and multi-centre collaborations with a large number of patients with cirrhosis due to different pathological causes and patients with HCC on top of cirrhosis or liver secondaries is needed.

Research objectives

The study aimed at assessing the use of CNNs in examining HCC and liver masses images in the diagnosis of cancer and evaluating the accuracy level of the CNNs and their performance.

Research methods

Several databases, including PubMed, EMBASE, and Web of Science, were systematically searched for studies that covered pathological anatomy, cellular, and radiological images on HCC or liver masses using the CNNs. The data were extracted as per a predefined extraction protocol, and the accuracy level and performance of the CNNs in detecting cancer or early stages

of cancer were analysed. The primary outcomes of the study were investigating the type of cancer or liver mass and identifying the type of images that showed optimum accuracy in cancer detection.

Research results

A small number of studies were identified. The studies demonstrated the ability to differentiate liver masses, differentiate HCC from other liver lesions, and differentiate HCC from cirrhosis or development of new tumours. Two studies focused on HCC nuclei grading or segmentation. In these studies, the CNNs showed satisfactory levels of accuracy. The studies aimed at detecting lesions, classification, and segmentation. Several methods were used to assess the accuracy of CNN models used.

Research conclusions

While the current studies have covered liver cancers, the number of studies conducted so far is small and limited, and more research is needed to answer questions about the accuracy and sensitivity of the CNN algorithms. The CNNs demonstrated abilities in segmentation, classification, and lesion detection in radiological and anatomical pathology images of common cancers. However, several deficiencies in current studies were observed.

Research perspectives

A large multi-centre trial is needed to evaluate carefully the use of CNNs and their clinical applications in HCC and liver masses. Differentiation between primary and secondary (metastases in the liver) or other liver masses is hard based on radiological imaging. The studies included in this review showed that CT-based deep learning methods could enable the categorisation of liver metastases from primary liver cancers. Future studies should give more attention to the assessment of accuracy and sensitivity of the CNNs in evaluating the performance of systems and calculating the positive predictive values.

ACKNOWLEDGEMENTS

The author would like to thank Dr Sarah Azer of St Vincent Hospital, University of Melbourne, for her help during writing this research article.

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World Journal of Gastrointestinal Oncology

Submit a Manuscript: https://www.f6publishing.com

World J Gastrointest Oncol 2019 December 15; 11(12): 1231-1239

DOI: 10.4251/wjgo.v11.i12.1231

ISSN 1948-5204 (online)

CASE REPORT

Inflammatory pseudotumor-like follicular dendritic cell sarcoma: A brief report of two cases

Bi-Xi Zhang, Zhi-Hong Chen, Yu Liu, Yuan-Jun Zeng, Yan-Chun Li

ORCID number: Bi-Xi Zhang (0000-0001-7443-5072); Zhi-Hong Chen (0000-0002-8994-5187); Yu Liu (0000-0001-6734-3241); Yuan-Jun Zeng (0000-0002-3898-3992); Yan-Chun Li (0000-0001-6462-5868).

Author contributions: All authors contributed to the study; Zhang BX wrote the manuscript; Chen ZH, Liu Y, Zeng YJ, and Li YC collected and analysed the data and contributed to the follow-up results; all authors read and approved the final manuscript.

Informed consent statement: All study participants, or their legal guardian, provided written informed consent prior to the study.

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

CARE Checklist (2016) statement: The manuscript was prepared and revised according to the CARE Checklist (2016).

Bi-Xi Zhang, Zhi-Hong Chen, Yu Liu, Yuan-Jun Zeng, Yan-Chun Li, Department of Pathology, People's Hospital of Hunan Province, Changsha 410005, Hunan Province, China

Corresponding author: Yan-Chun Li, PhD, Doctor, Department of Pathology, People's Hospital of Hunan Province, No. 61, Jiefang West Road, Changsha 410005, Hunan Province, China. lychglx@163.com
Telephone: +86-731-83929288

Abstract

BACKGROUND

Follicular dendritic cell (FDC) sarcoma/tumor is a rare malignant tumor of follicular dendritic cells, which is considered a low-grade sarcoma that can involve lymph nodes or extranodal sites. Conventional FDC sarcomas are negative for Epstein-Barr virus (EBV), whereas the inflammatory pseudotumor-like variant consistently shows EBV in the neoplastic cells.

CASE SUMMARY

We report two cases of inflammatory pseudotumor-like FDC sarcoma in the liver that received 3D laparoscopic right hepatectomy and open right hepatectomy separately.

CONCLUSION

EBV probe-based *in situ* hybridization and detection of immunohistochemical markers of FDC play an important role in the diagnosis and differential diagnosis of inflammatory pseudotumor-like FDC sarcoma. Complete surgical excision combined with regional lymphadenectomy may be effective in reducing the postoperative recurrence and metastasis and improving long-term survival rates.

Key words: Inflammatory pseudotumor-like follicular dendritic cell sarcoma; Epstein-Barr virus; Liver; Spleen; Case report

Core tip: There have been 48 previously reported cases of inflammatory pseudotumor-like follicular dendritic cell (FDC) sarcoma, which occurs almost exclusively in the liver and spleen. Here we report two cases of inflammatory pseudotumor-like FDC sarcoma in the liver that were treated by 3D laparoscopic right hepatectomy and open right hepatectomy separately.

Manuscript source: Unsolicited manuscript

Received: February 20, 2019
Peer-review started: February 22, 2019
First decision: June 4, 2019
Revised: September 1, 2019
Accepted: September 26, 2019
Article in press: September 26, 2019
Published online: December 15, 2019

P-Reviewer: Ebrahimifar M, Grizzi F, Pandey A, Souto Nacif L
S-Editor: Zhang L
L-Editor: Wang TQ
E-Editor: Ma YJ

Citation: Zhang BX, Chen ZH, Liu Y, Zeng YJ, Li YC. Inflammatory pseudotumor-like follicular dendritic cell sarcoma: A brief report of two cases. *World J Gastrointest Oncol* 2019; 11(12): 1231-1239
URL: https://www.wjgnet.com/1948-5204/full/v11/i12/1231.htm
DOI: https://dx.doi.org/10.4251/wjgo.v11.i12.1231

INTRODUCTION

Follicular dendritic cell (FDC) sarcoma/tumor is a rare malignant tumor of follicular dendritic cells, which are mesenchymal cells in the lymphoid follicles with antigen presenting ability. It is considered a low-grade sarcoma that can involve lymph nodes or extranodal sites[1-5]. In 1996, Shek *et al*[6] reported the first case of primary FDC sarcoma in the liver. The histology was similar to an inflammatory pseudotumor and it was related to Epstein-Barr virus (EBV)-related clonal proliferation[6]. Inflammatory pseudotumor-like FDC sarcoma was first described as a distinctive variant of FDC sarcoma and associated with EBV in 2001[7]. There have been 48 previously reported cases of inflammatory pseudotumor-like FDC sarcoma, which occurs almost exclusively in the liver and spleen (Table 1). Ancillary tests, including detection of immunohistochemical markers of FDC such as CD21, CD23, or CD35 and EBV probe-based *in situ* hybridization, are required for this diagnosis. Here we report two cases of inflammatory pseudotumor-like FDC sarcoma in the liver that were treated by 3D laparoscopic right hepatectomy and open right hepatectomy separately.

CASE PRESENTATION

Chief complaints

Case 1: A 31-year-old woman was admitted to hospital for evaluation of a four-week history of anorexia.

Case 2: A 48-year-old man stumbled across a liver mass through a routine ultrasound examination.

History of present illness

Unremarkable.

History of past illness

Case 1: Her past medical history was chronic hepatitis B for more than 10 years without antiviral treatment.

Case 2: Unremarkable.

Personal and family history

Unremarkable.

Physical examination upon admission

Case 1: Physical examination revealed mild tenderness to palpation in the right upper quadrant.

Case 2: Physical examination was unremarkable.

Laboratory examinations

Case 1: Laboratory tests showed seropositivity for HBsAg, HBeAb, and HBcAb. Furthermore, serum level of hepatitis B virus-DNA was lower than detection limit.

Case 2: Laboratory tests were unremarkable.

Imaging examinations

Case 1: Abdominal magnetic resonance imaging revealed two well-circumscribed masses in the right posterior lobe of the liver (Figure 1).

Case 2: An abdominal computed tomography examination revealed an ill-defined 10 cm mass in the right lobe of the liver accompanied with enlargement of hepatic portal lymph nodes (Figure 2).

Table 1 Review of inflammatory pseudotumor-like follicular dendritic cell tumor/sarcoma

Ref.	Sex/age	Location	Maximum diameter (cm)	Symptom	Treatment	Follow-up (mo)	Outcome
Li *et al*[13]	F/64	Spleen	7.2	Upper abdominal pain	Laparoscopic splenectomy	8	NED
	M/61	Spleen	6.2	Asymptomatic	Laparoscopic splenectomy	16	NED
	F/42	Spleen	4	Left-sided flank pain	Laparoscopic splenectomy	9	NED
	F/57	Spleen	13.3	Upper abdominal pain	Laparoscopic splenectomy	4	LWD, pulmonary metastasis
	M/52	Spleen	2 masses: 3.7, 2.9	Back pain	Laparoscopic splenectomy	5	LWD, bone metastasis
Hang *et al*[14]	M/57	Spleen	2.7	Asymptomatic	Laparoscopic partial splenectomy	9	NED
Ge *et al*[15]	F/54	Spleen	3.5	Left-sided flank pain	Splenectomy	10	NED
	M/79	Spleen	6	Asymptomatic	Splenectomy	18	NED
Pan *et al*[16]	F/78	Colon	3.9	Abdominal discomfort, bloody stool	Polypectomy	5	NED
Choe *et al*[17]	F/64	Spleen	5.5	Asymptomatic	Splenectomy	78	NED
	F/72	Spleen	7.2	Asymptomatic	Splenectomy	18	NED
	F/53	Spleen	3.2	Asymptomatic	Splenectomy	13	NED
	M/76	Spleen	3.2	Asymptomatic	Splenectomy	8	NED
	M/72	Spleen	6	Asymptomatic	Splenectomy	18	NED
	M/75	Spleen	3.5	Abdominal pain	Splenectomy	30	NED
Granados *et al*[18]	F/57	Liver	13	Abdominal pain, vomiting	Partial hepatectomy	24	NED
Cheuk *et al*[7]	F/19	Liver	12	Right upper quadrant pain, abdominal mass, weight loss	Partial hepatectomy	40	NED
	F/56	Liver	15	Abdominal discomfort	Partial hepatectomy	56	LWD, recurrence in liver
	F/40	Liver	12.5	Upper abdominal pain, weight loss	Partial hepatectomy	108	LWD, intraabdominal recurrence
	F/49	Liver	4.2	Asymptomatic	Partial hepatectomy	9	NED
	M/37	Liver	15	Abdominal mass, weight loss	Partial hepatectomy	42	NED
	F/35	Liver	20	Abdominal discomfort, fever, weight loss	Partial hepatectomy	95	DOD, disseminated in liver and peritoneum
	F/31	Liver	15	Abdominal distension, weight loss	Partial hepatectomy	60	NED
	F/58	Spleen	22	Abdominal mass	Splenectomy	4	NED
	F/39	Spleen	7.5	Weight loss, fever	Splenectomy	2	LWD, persistent fever
	F/61	Spleen	3.5	Asymptomatic	Splenectomy	NA	NA
	F/49	Peri-pancreas	15	Abdominal distension	Whipple's operation	NA	NA
Li *et al*[19]	F/49	Spleen	4.7	Asymptomatic	Splenectomy	NA	NA
	F/56	Spleen	8	Abdominal pain	Splenectomy	17	NED
	M/38	Liver	8.5	Anorexia	Partial hepatectomy	11	NED
	F/42	Liver	2 masses: 2, 1.7	Abdominal pain	Partial hepatectomy	36	NED

	M/50	Spleen and liver	Spleen: 10 Liver: 3	Abdominal bloating	Splenectomy and partial hepatectomy	17	NED
	F/39	Liver	9	Asymptomatic	Partial hepatectomy	84	NED
Chen *et al*[20]	F/28	Liver	6	Abdominal pain, fatigue, anorexia	Partial hepatectomy	48	LWD, recurrence in liver
	M/39	Spleen	7.4	Asymptomatic	Splenectomy	40	NED
	M/48	Liver	23.3	Abdominal pain, fever, fatigue	Partial hepatectomy	23	NED
	M/65	Spleen and liver	Spleen: 22.3 Liver: 5.8 (multi masses)	Abdominal pain, fever, fatigue, anorexia, weight loss	Splenectomy	2	DOD
	M/51	Spleen	8.5	Weight loss	Splenectomy	19	NED
	M/68	Spleen	2.3	Asymptomatic	Splenectomy	6	NED
	F/51	Spleen	5.3	Abdominal discomfort	Splenectomy	5	NED
	M/67	Spleen	7.5	Asymptomatic	Splenectomy	5	NED
	M/60	Liver	3	Asymptomatic	Partial hepatectomy	3	NED
	F/52	Spleen	0.9	Asymptomatic	Splenectomy	12	NED
Kitamura *et al*[21]	F/74	Spleen	3.6	Asymptomatic	Splenectomy	24	NED
Bui *et al*[22]	F/50	Spleen	6	Abdominal pain	Splenectomy	NA	NA
Vardas *et al*[23]	M/61	Spleen	10	Abdominal pain	Splenectomy	12	NED
Kim *et al*[24]	M/76	Spleen	3.2	Asymptomatic	Splenectomy	NA	NA
Horiguchi *et al*[25]	F/77	Spleen	8.5	Abdominal pain	Splenectomy	36	NED
Present case	F/31	Liver	2 masses: 3.5, 2.5	Anorexia	3D laparoscopic right hepatectomy	10	NED
	M/48	Liver and hepatoduodenal ligament lymph node	Liver: 10 Lymph node: 3.5	Asymptomatic	Open right hepatectomy, lymph node excision	2	NED

M: Male; F: Female; NED: No evidence of disease; DOD: Dead of disease; LWD: Live with disease; NA: Not available.

FINAL DIAGNOSIS

Case 1

EBV-positive inflammatory pseudotumor-like FDC sarcoma in the liver (Figure 3).

Case 2

EBV-positive inflammatory pseudotumor-like FDC sarcoma in the liver with hepatoduodenal ligament lymph node involvement (Figure 4).

TREATMENT

Case 1

3D laparoscopic right hepatectomy.

Case 2

Open right hepatectomy combined with regional lymphadenectomy.

OUTCOME AND FOLLOW-UP

Case 1

Follow-up for 10 mo showed no recurrence or metastasis.

Case 2

Follow-up for 2 mo showed no recurrence or metastasis.

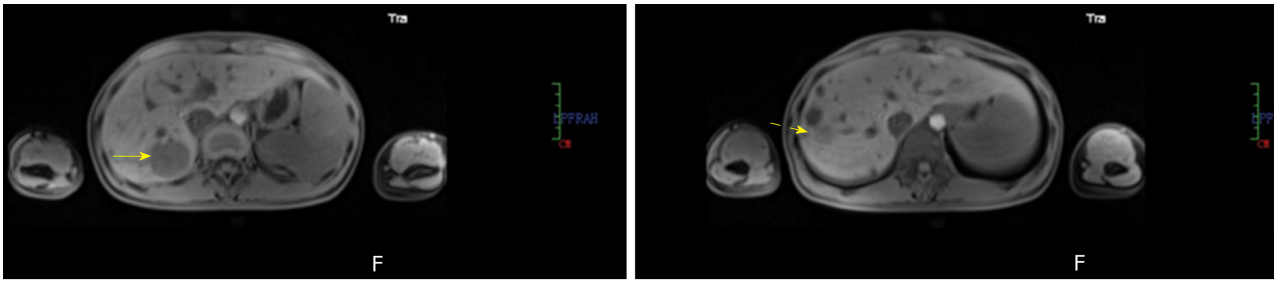

Figure 1 Magnetic resonance imaging. Two well-circumscribed lesions with long T1 and long or equal T2 signal (arrows). The multiple lesions with long T1 and long T2 signal are hepatic cysts verified by pathological examination later.

DISCUSSION

FDC sarcoma is a neoplastic proliferation of spindled to ovoid cells exhibiting morphological and immunophenotypic features of FDCs. Histologically, FDC sarcomas are classified into two types: (A) Conventional FDC sarcoma consisting of spindled to ovoid cells forming fascicles, storiform arrays, whorls, diffuse sheets, or vague nodules with an array of small lymphocytes; and (B) Inflammatory pseudotumor-like FDC sarcoma composed of neoplastic spindled cells that are dispersed within a prominent lymphoplasmacytic infiltrate[3]. To date, 48 cases of inflammatory pseudotumor-like FDC sarcoma have been reported in the English-language literature, located in the liver (16/48), spleen (32/48), colon (1/48), and peri-pancreas (1/48), respectively. These cases included 19 males and 29 females (male/female ratio of 1: 1.5), with a mean age of 55 years (range, 19-79 years). Clinical manifestations include abdominal pain, abdominal bloating, abdominal mass, weight loss, fever, fatigue, and anorexia, but most cases are asymptomatic (Table 1).

The origin of FDC sarcoma remains controversial. Phenotypic marker studies and *in vitro* experiments with fibroblast-like cell lines have developed FDCs from fibroblast-like cells[8]. The neoplastic cells are often positive for FDC markers, such as CD21, CD23, and CD35, with the staining ranging from extensive to very local. FDCs appear to be closely related to bone marrow stromal progenitors, with several myofibroblast features[9]. Two studies examining the transcriptional profile of FDC sarcoma have revealed: (A) A peculiar immunological microenvironment enriched in follicular helper T cells and Treg populations, with special relevance to the inhibitory immune receptor programmed cell death protein 1 and its ligands, programmed cell death-Ligand 1 and programmed cell death-Ligand 2; and (B) The highly specific expression of the genes encoding for FDC secreted peptide and serglycin[10-11].

Conventional FDC sarcomas are negative for EBV, whereas the inflammatory pseudotumor-like variant consistently shows EBV in the neoplastic cells[7]. EBV-encoded small RNA was detected in both of the present cases by *in situ* hybridization. EBV-encoded latent membrane protein 1, which has been found to have an oncogenic role, has been identified in 74% (26/35) cases of inflammatory pseudotumor-like FDC sarcomas by immunohistochemical staining[7,17,19-21,25]. Recently, Takeuchi *et al*[12] reported increased numbers of EBV-infected cells in IgG4-related lymphadenopathy, compared with other reactive lymphadenopathy or extranodal IgG4-related disease, which suggests that there may be a relationship between IgG4-related disease and EBV[12]. Interestingly, Choe *et al*[17] reported that significant numbers of IgG4-positive plasma cells were found in six cases of EBV-positive inflammatory pseudotumor-like FDC sarcoma of the spleen, suggesting that EBV plays a critical role in inflammatory pseudotumor-like FDC sarcoma and IgG4-related sclerosing disease[17]. Generally, the pathogenic mechanism of EBV in inflammatory pseudotumor-like FDC sarcoma remains unclear and further investigation is required.

FDC sarcoma is usually treated by complete surgical excision, with or without adjuvant radiotherapy or chemotherapy. A pooled analysis of the literature revealed local recurrence and distant metastasis rates of 28% and 27%, respectively. Large tumor size (≥ 6 cm), coagulative necrosis, high mitotic count (≥ 5 mitoses per 10 high-power fields), and significant cytological atypia are associated with a worse prognosis[2,5]. Regarding the prognosis of patients with inflammatory pseudotumor-like FDC sarcoma, based on the literature reports of inflammatory pseudotumor-like FDC sarcoma with a median follow-up period of 17 mo, 35 patients had no evidence of disease. Five patients exhibited distant metastasis and two had local recurrence, with traits similar to large tumors and multiple masses. One of the current cases presented with liver and hepatoduodenal ligament lymph node involvement,

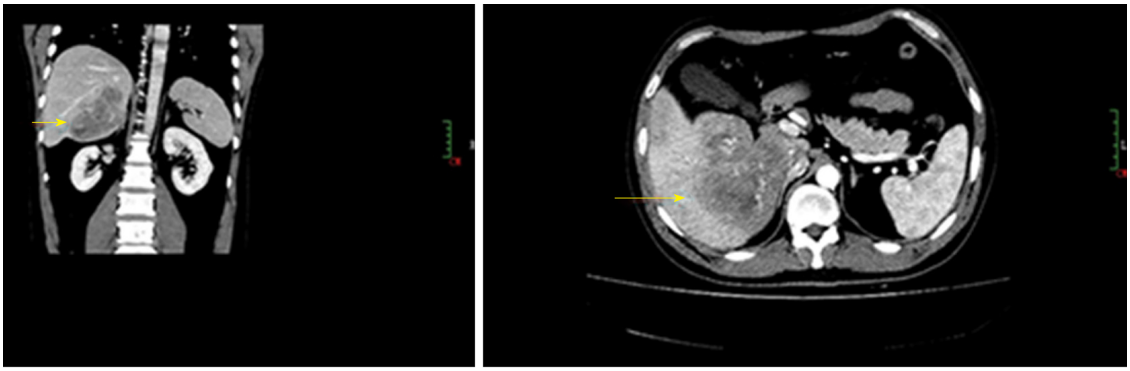

Figure 2 Abdominal computed tomography examination. The images show an ill-defined and low-density 10 cm mass (arrows) in the right lobe of the liver, accompanied with enlargement of hepatic portal lymph nodes.

suggesting that inflammatory pseudotumor-like FDC sarcoma presents an increased risk of lymph node metastasis. Complete surgical excision combined with regional lymphadenectomy may be effective in reducing the postoperative recurrence and metastasis and improving the long-term survival rates.

CONCLUSION

In conclusion, there is little specificity in the clinical manifestations of inflammatory pseudotumor-like FDC sarcoma. EBV probe-based *in situ* hybridization and detection of immunohistochemical markers of FDC play important roles in the diagnosis and differential diagnosis of inflammatory pseudotumor-like FDC sarcoma. Radical surgical resection is the main therapeutic intervention for inflammatory pseudotumor-like FDC sarcoma, especially for cases with lymph node involvement, and patients require long-term post-surgical follow-up.

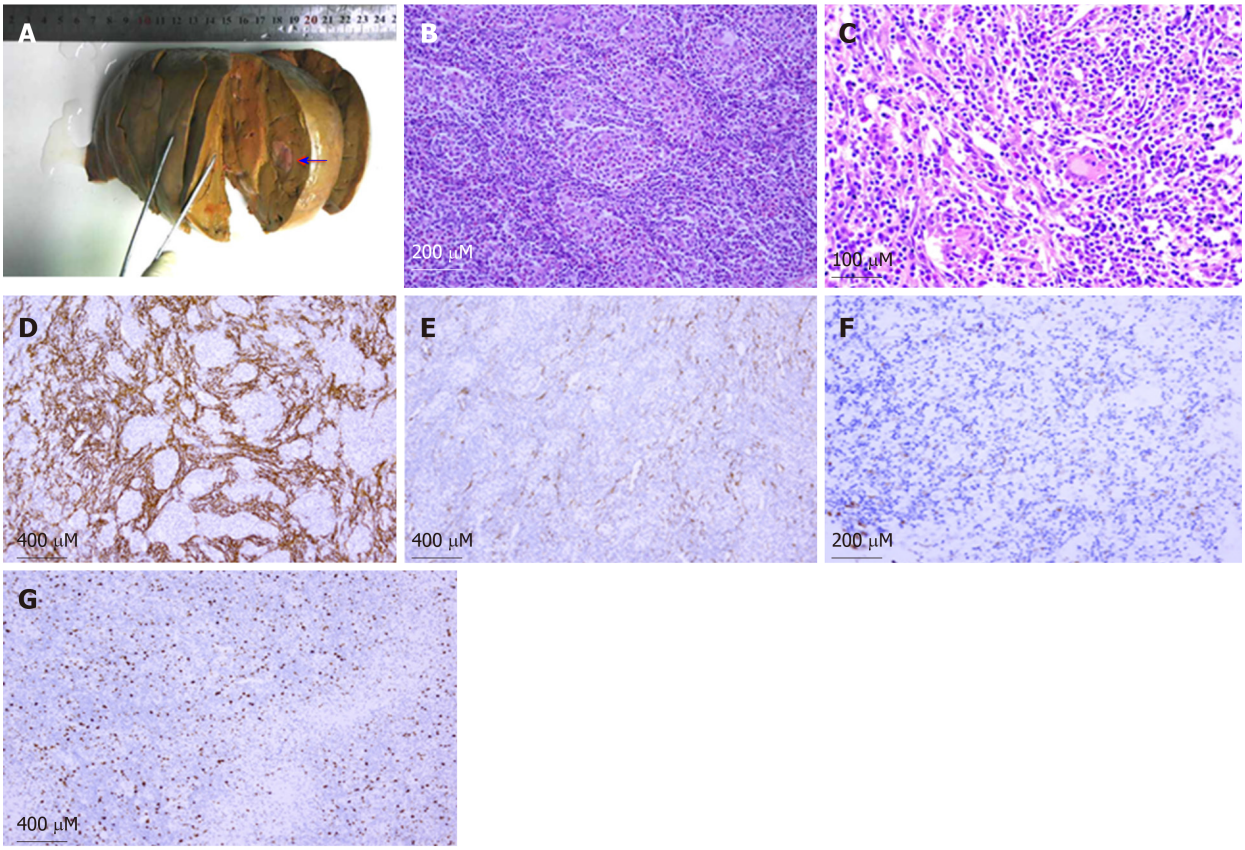

Figure 3 Epstein-Barr virus-positive inflammatory pseudotumor-like follicular dendritic cell sarcoma in the liver. A: Gross picture of an inflammatory pseudotumor-like follicular dendritic cell sarcoma of the liver. A well-circumscribed solid nodule was found in the liver. Note the grayish-white colored and soft cut surface with focal hemorrhage (arrow); B: Haematoxylin and eosin stained image showing that the tumor tissue had a meshwork-like architecture (× 200); C: On high-power field, the tumor was composed of oval to spindle cells with vesicular chromatin and distinct nucleoli. There was less degree of atypia. The background showed abundant lymphocytes and plasma cells (× 400); D: CD21 was detected on the membrane of almost all of tumor cells by immunohistochemistry (× 100); E: Smooth muscle actin was detected in the cytoplasm of a part of tumor cells by immunohistochemistry (× 100); F: Epstein-Barr virus-encoded small RNA-based *in situ* hybridization demonstrated positive nuclei of the neoplastic dendritic cells (× 200); G: Ki-67 was detected in the nuclei of almost all of tumor cells by immunohistochemistry (30%; × 100).

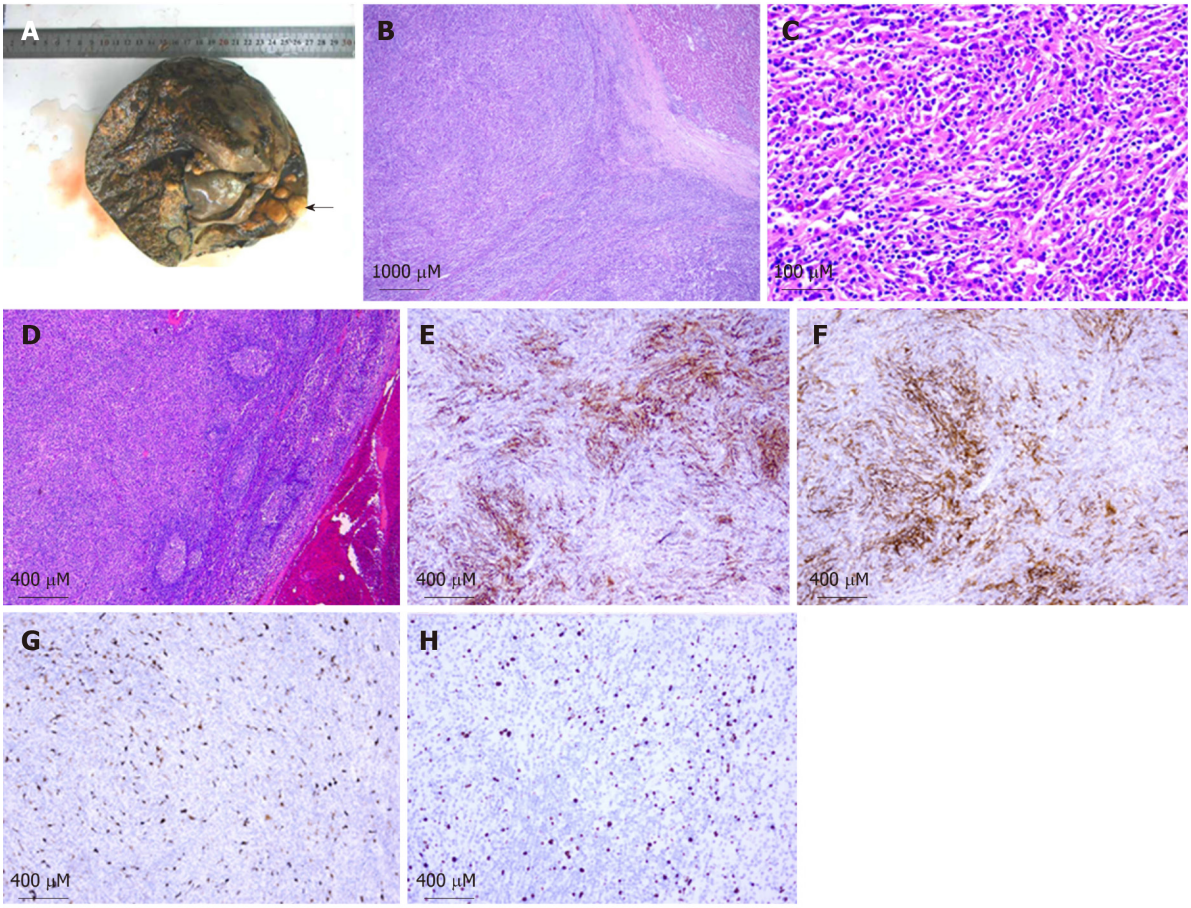

Figure 4 Epstein-Barr virus positive inflammatory pseudotumor-like follicular dendritic cell sarcoma in the liver with hepatoduodenal ligament lymph node involvement. A: Gross picture of an inflammatory pseudotumor-like follicular dendritic cell sarcoma of the liver. A large and multinodular confluent tumor was found in the liver (arrow); B: Histologic sections of follicular dendritic cell sarcoma showing an unencapsulated tumor (left) with a sharp margin from the adjacent liver parenchyma (right). The tumor tissue was arranged in whorls (× 40); C: On high-power field, the tumor was composed of oval to spindle cells with vesicular chromatin and distinct nucleoli. There was less degree of atypia. The background showed abundant lymphocytes and plasma cells (× 400); D: In the hepatoduodenal ligament lymph node, lymphoid follicles were pushed aside by tumor tissue (× 100); E: CD21 was detected on the membrane of almost all of tumor cells by immunohistochemistry (× 100); F: S100 was detected in the membrance and cytoplasm of almost all of tumor cells by immunohistochemistry (× 100); G: Epstein-Barr virus-encoded small RNA *in situ* hybridization demonstrated positive nuclei of the neoplastic dendritic cells (× 100); H: Ki-67 was detected in the nuclei of almost all of tumor cells (20%; × 100).

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