ISSN 1948-5204 (online)

# World Journal of *Gastrointestinal Oncology*

*World J Gastrointest Oncol* 2019 March 15; 11(3): 181-269

Published by Baishideng Publishing Group Inc

WJGO

World Journal of Gastrointestinal Oncology

Contents

Monthly Volume 11 Number 3 March 15, 2019

## ORIGINAL ARTICLE

### Basic Study



### Retrospective Cohort Study



### Observational Study



## SYSTEMATIC REVIEWS



## CASE REPORT

**Contents**

*World Journal of Gastrointestinal Oncology*

**Volume 11 Number 3 March 15, 2019**

## ABOUT COVER

Editorial Board Member of *World Journal of Gastrointestinal Oncology*, Marco E Allaix, MD, PhD, Assistant Professor, Department of Surgical Sciences, University of Torino, Torino 10126, Italy

## AIMS AND SCOPE

*World Journal of Gastrointestinal Oncology* (*World J Gastrointest Oncol*, *WJGO*, online ISSN 1948-5204, DOI: 10.4251) is a peer-reviewed open access academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

*WJGO* covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, *etc*. The current columns of *WJGO* include editorial, frontier, field of vision, review, original articles, case report.

We encourage authors to submit their manuscripts to *WJGO*. We will give priority to manuscripts that are supported by major national and international foundations and those that are of great clinical significance.

## INDEXING/ABSTRACTING

The *WJGO* is now indexed in Science Citation Index Expanded (also known as SciSearch®), PubMed, and PubMed Central. The 2018 edition of Journal Citation Reports® cites the 2017 impact factor for *WJGO* as 3.140 (5-year impact factor: 3.228), ranking *WJGO* as 39 among 80 journals in gastroenterology and hepatology (quartile in category Q2), and 114 among 222 journals in oncology (quartile in category Q3).

## RESPONSIBLE EDITORS FOR THIS ISSUE

**Responsible Electronic Editor:** *Han Song* **Proofing Editorial Office Director:** *Jin-Lei Wang*

**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 Murtaza Kasi

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

**EDITORIAL OFFICE**
Jin-Lei Wang, Director

**PUBLICATION DATE**
March 15, 2019



**INSTRUCTIONS TO AUTHORS**
https://www.wjgnet.com/bpg/gerinfo/204

**GUIDELINES FOR ETHICS DOCUMENTS**
https://www.wjgnet.com/bpg/GerInfo/287

**GUIDELINES FOR NON-NATIVE SPEAKERS OF ENGLISH**
https://www.wjgnet.com/bpg/gerinfo/240

**PUBLICATION MISCONDUCT**
https://www.wjgnet.com/bpg/gerinfo/208

**ARTICLE PROCESSING CHARGE**
https://www.wjgnet.com/bpg/gerinfo/242

**STEPS FOR SUBMITTING MANUSCRIPTS**
https://www.wjgnet.com/bpg/GerInfo/239

**ONLINE SUBMISSION**
https://www.f6publishing.com

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 181-194

DOI: 10.4251/wjgo.v11.i3.181

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

# Glycerophospholipids pathways and chromosomal instability in gastric cancer: Global lipidomics analysis

Cheng-Yu Hung, Ta-Sen Yeh, Cheng-Kun Tsai, Ren-Chin Wu, Ying-Chieh Lai, Meng-Han Chiang, Kuan-Ying Lu, Chia-Ni Lin, Mei-Ling Cheng, Gigin Lin

**ORCID number:** Cheng-Yu Hung (0000-0003-4158-5079); Ta-Sen Yeh (0000-0002-1830-9466); Cheng-Kun Tsai (0000-0002-9214-2801); Ren-Chin Wu (0000-0003-1439-0874); Ying-Chieh Lai (0000-0003-0148-9488); Meng-Han Chiang (0000-0002-2697-4757); Kuan-Ying Lu (0000-0002-4677-9000); Chia-Ni Lin (0000-0002-2722-0164); Mei-Ling Cheng (0000-0003-2006-133X); Gigin Lin (0000-0001-7246-1058).

**Author contributions:** Lin G conceived and designed the experiments; Yeh TS, Chiang MH, Lu KY and Hung CY performed the experiments; Hung CY and Chiang MH analyzed the data; Wu RC, Lai YC, Lin CN and Cheng ML contributed reagents, materials, and analysis tools; Hung CY and Lin G wrote the paper.

**Supported by** the funding from the Ministry of Science and Technology Taiwan grant, No. MOST 106-2314-B-182A-019-MY3; and the Chang Gung Foundation, No. CMRPG3E1321-2.

**Institutional review board statement:** All procedures in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Conflict-of-interest statement:** The authors declare that they have no

**Cheng-Yu Hung,** Molecular Medicine Research Center, Chang Gung University, Taoyuan 333, Taiwan

**Cheng-Yu Hung, Cheng-Kun Tsai, Ying-Chieh Lai, Meng-Han Chiang, Kuan-Ying Lu, Mei-Ling Cheng, Gigin Lin,** Clinical Metabolomics Core Lab, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Taoyuan 333, Taiwan

**Cheng-Yu Hung, Cheng-Kun Tsai, Ying-Chieh Lai, Meng-Han Chiang, Kuan-Ying Lu, Gigin Lin,** Department of Medical Imaging and Intervention, Imaging Core Lab, Institute for Radiological Research, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Taoyuan 333, Taiwan

**Ta-Sen Yeh,** Department of Surgery, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Taoyuan 333, Taiwan

**Ren-Chin Wu,** Department of Pathology, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Taoyuan 333, Taiwan

**Chia-Ni Lin,** Department of Laboratory Medicine, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Taoyuan 333, Taiwan

**Mei-Ling Cheng,** Department of Biomedical Science, College of Medicine, Chang Gung University, Taoyuan 333, Taiwan

**Corresponding author:** Gigin Lin, MD, PhD, Director, Department of Medical Imaging and Intervention, Imaging Core Lab, Institute for Radiological Research, Metabolomics Core Lab, Chang Gung Memorial Hospital at Linkou and Chang Gung University, Fuhsing 5, Taoyuan 333, Taiwan. giginlin@cgmh.org.tw
**Telephone:** +886-3-3281200-2575
**Fax:** +886-3-3971936

## Abstract

*BACKGROUND*

Based on the breakthrough of genomics analysis, The Cancer Genome Atlas Research Group recently proposed an integrative genomic analysis, dividing gastric cancer (GC) into four subtypes, characterized by the chromosomal instability (CIN) status. However, the CIN status of GC is still vaguely characterized and lacking the valuable easy-to-use CIN markers to diagnosis in molecular and histological detection.

conflict of interest.



**Manuscript source**: Unsolicited manuscript

**Received:** November 15, 2018
**Peer-review started:** November 15, 2018
**First decision:** December 7, 2018
**Revised:** December 17, 2018
**Accepted:** December 23, 2018
**Article in press:** December 24, 2018
**Published online:** March 15, 2019

***AIM***
To explore the associations of CIN with downstream lipidomics profiles.

***METHODS***
We collected cancerous and noncancerous tissue samples from 18 patients with GC; the samples were divided into CIN and non-CIN types based on the system of The Cancer Genome Atlas Research Group and 409 sequenced oncogenes and tumor suppressor genes. We identified the lipidomics profiles of the GC samples and samples of their adjacent noncancerous tissues by using liquid chromatography–mass spectrometry. Furthermore, we selected leading metabolites based on variable importance in projection scores of > 1.0 and $P$ < 0.05.

***RESULTS***
Twelve men and six women participated in this study; the participants had a median age of 67.5 years (range, 52–87 years) and were divided into CIN ($n$ = 9) and non-CIN ($n$ = 9) groups. The GC samples exhibited distinct profiles of lysophosphocholine, phosphocholine, phosphatidylethanolamine, phosphatidylinositol, phosphoserine, sphingomyelin, ceramide, and triglycerides compared with their adjacent noncancerous tissues. The glycerophospholipid levels (phosphocholine, phosphatidylethanolamine, and phosphatidylinositol) were 1.4- to 2.3-times higher in the CIN group compared with the non-CIN group ($P$ < 0.05). Alterations in the glycerolipid and glycerophospholipid pathways indicated progression of GC toward CIN.

***CONCLUSION***
The lipidomics profiles of GC samples were distinct from those of their adjacent noncancerous tissues. CIN status of GC is primarily associated with downstream lipidomics in the glycerophospholipid pathway.

**Key words:** Chromosomal instability; Gastric cancer; Glycerophospholipids; Metabolomics; Lipidomics profile



**Core tip:** We investigated the correlation between comprehensive lipidomic profiles of gastric cancer and the tumor's chromosomal instability (CIN) status. In this disease landscape study, which involved no pre-specified hypotheses, we combined a gene molecule classification method with a lipidomic method to discover metabolic information for accurate tumor classification. CIN-status-based lipidomics profiling demonstrated translational potential for biomarker discovery and development of novel therapeutic strategies.

**Citation:** Hung CY, Yeh TS, Tsai CK, Wu RC, Lai YC, Chiang MH, Lu KY, Lin CN, Cheng ML, Lin G. Glycerophospholipids pathways and chromosomal instability in gastric cancer: Global lipidomics analysis. *World J Gastrointest Oncol* 2019; 11(3): 181-194
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/181.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.181

## INTRODUCTION

Gastric cancer (GC) is traditionally subdivided into intestinal, diffuse, and mixed types according to Lauren classification based on histopathology[1,2]. Although widely used, the Lauren classification system does not provide precise information on treatments suitable for individual patients, and selecting a subtype-optimized therapeutic approach can be difficult[2]. Recently, The Cancer Genome Atlas (TCGA) Research Group proposed an integrative genomic analysis method, namely dividing GC into four subtypes—Epstein Barr Virus positive, microsatellite unstable, chromosomally instable, and genomically stable[3] - on the basis of gene expression profiling of exome sequences, copy-number alterations, gene expression, DNA methylation, and protein activity[2-4]. However, the chromosomal instability (CIN)

status of GC is still characterized only vaguely and lacks valuable and user-friendly markers for diagnosis in molecular and histological detection[5].

Metabolomics—the study of results of interaction between the biosystem's genome and its environment and the detection of end products of gene expression - offers opportunities to understand complex molecular mechanisms and identify the diagnostic biomarkers of human GC[4,6]. Previous metabolomics studies based on mass spectrometry (MS) and nuclear magnetic resonance systems have been limited to focusing on water-soluble compounds and volatile metabolites[7-10]. Lipid metabolites have several pivotal functions, including energy storage, modulation of cell membranes, the formation of "fat-soluble" vitamins, cellular massage, and hormonal regulation[11], and they thus warrant further research. Furthermore, increased *de novo* lipogenesis is frequently associated with the development of many cancer types[12]. For example, the lipid content of phospholipids could compromise membrane fluidity and signal transduction and in turn affect tumorigenesis and GC progression[13]. In addition, perturbation of lipid metabolism contributes to cancer progression through detection of dysregulated core enzyme activity in lipid pathways and global lipid metabolic alterations in cancer metastasis[14,15]. Global lipidomics analysis using liquid chromatography–MS (LC/MS) provides the most detailed detection and qualification of cellular lipids in systems biology. To the best of our knowledge, no prior studies have exploited the links between CIN and non-CIN status in GC and lipid alteration by using the lipidomics approach.

The present study hypothesized that lipidomic alternations reflect the CIN or non-CIN status of GC. Through global lipidomics profiling using LC/MS, we explored the correlation between lipidomic metabolites and the CIN status of GC.

## MATERIALS AND METHODS

### Patient and Histopathology

The Institutional Review Board approved this prospective study (IRB103-7448B). Informed consent to screen patient enrollment was provided by a tertiary referral center with a GC-dedicated interdisciplinary team, and tissue samples were obtained from Chang Gung Memorial Hospital in Linkou, Taiwan. We screened a continuous cohort of patients with GC from May 2015 to April 2017. The inclusion criteria were (1) histologically confirmed GC with surgical resection; and (2) age of 20–80 years. The exclusion criteria were (1) receipt of neoadjuvant therapy before surgery; (2) tumor smaller than 1 cm in computed tomography images; (3) prior gastric surgery; (4) anti-*Helicobacter pylori* eradication therapy; and (5) receipt of nonsteroidal anti-inflammatory drugs within the 1 week prior to surgery[16]. We used 18 primary GC tissue samples for genomic analysis and re-evaluated the pathological diagnoses and histological Lauren classifications of all tumors, with samples from their adjacent noncancerous tissues as controls.

### Genomic analysis

The tumor samples were divided into CIN or non-CIN by using TCGA system. We extracted genomic DNA from formalin-fixed paraffin-embedded tumor samples by using the QIAamp DNA FFPE Tissue Kit (Qiagen, Hilden, Germany) and quantified the DNA by using the Quant-iT dsDNA High-Sensitivity Assay Kit (Invitrogen, USA). In total, 409 leading oncogenes and tumor suppressor genes in GC tissue were sequenced; the protocol for TCGA analysis was detailed in our previous study[16]. The present study classified patients with GC based on high and low proportions of alteration genes.

### Lipidomic metabolite extraction

Tumor tissue samples of similar weight were extracted from the organic layer through Folch extraction and analyzed using an LC/MS system for lipidomic analysis. A modified version of Folch's method was employed[17]. In brief, we transferred approximately 50 mg of homogenized tissue into a glass tube and then added 6 mL of chloroform/methanol (2:1, v/v) solution and 1.5 mL of water. The sample was vortexed four times for 30 s each and then centrifuged at 8000 rpm for 30 min at 4 °C. The lower phase (hydrophobic phase and lipid layer) was transferred to new glass tubes and then dried using nitrogen gas. We stored the dried samples at −80 °C. Before analysis, the sample was dissolved in isopropanol/acetonitrile/water (2:1:1, V/V/V) through vortexing (four times for 30 s each) and centrifugation (12000 rpm for 20 min at 4 °C). Subsequently, the supernatant was transferred to vials for LC/MS analysis.

### Global analysis of lipidomic metabolites by LC-TOF-MS

We performed liquid chromatographic separation on an ACQUITY CSH C18 column (2.1 × 100 mm, 1.7 μm; Waters Co.) at a constant temperature of 55 °C by using the ACQUITY UltraPerformance LC system (Waters MS Technologies, UK). For metabolite profiling, mobile phase A was acetonitrile/water (60:40, v/v) and mobile phase B was isopropanol/acetonitrile (90:10, v/v); both phases were solvents containing 10 mM ammonium formate and 0.1% formic acid. The flow rate was 0.4 mL/min with a time-resolved solvent gradient[18]. We performed MS analysis by using Waters time-of-flight (TOF)–MS (SYNAPT HDMS; Waters MS Technologies, UK) operated in electrospray ionization (ESI)-positive (ESI+) and ESI-negative (ESI−) ion modes. We set the capillary and cone voltage at 2700 V (2000 V in ESI− mode) and 35 V, respectively. The desolvation gas flow rate was 800 L/h, maintained at 25 L/h. The desolvation and source temperatures were 400 °C and 100 °C, respectively. We acquired MS data in centroid mode within 20 to 990 m/z at a rate of 10 scans/s. Leucine–enkephalin served as a reference compound. The LockSpray frequency was set at 0.5 s and averaged over 10 scans for correction. We performed three technical replicates for tissue samples in both ESI+ and ESI− modes.

### Data processing and statistical analysis

We analyzed the lipidomic metabolites of the GC samples and their surrounding adjacent noncancerous tissues by using LC/TOF/MS with an untargeted metabolic approach to screen all potential biomarkers according to the application notes database (Waters, Milford, MA, USA)[19]. All MS data, namely retention times, m/z, and ion intensities, were extracted using MarkerLynx XS software (Waters) and then input to a matrix. Subsequently, the data were analyzed using orthogonal projections to latent structures discriminant analysis (OPLS-DA) run through SIMCA-P+ (version 13.0, Umetrics) with Pareto scaling. The variable importance in projection (VIP) score of each metabolite indicated a metabolite's contribution to the model. In this analysis, VIP > 1.0 and $P < 0.05$ were considered significant. In addition, we evaluated diagnostic performance by analyzing receiver operating characteristic curves with 95% confidence intervals; the areas under these curves were calculated using MetaboAnalyst 4.0[20].

### Metabolite identification

Lipids are composed of fats, oils, waxes, and sterols. As demonstrated by the LIPID MAPS classification system, lipids are broadly divided into eight categories: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, sterol lipids, prenol lipids, saccharolipids, and polyketides[21]. Significant metabolites were sought in the Human Metabolome Database (www.hmdb.ca) and confirmed using in-house data (standards based on retention times and MS spectra). Candidates for LC/MS/MS analysis were confirmed according to chemical standards, the METLIN database[22], or LIPID MAPS database[21], depending on the m/z results for daughter fragments under chromatographic conditions identical to those of the profiling experiment. The *sn*-positions of fatty acids on the glycerol backbones of lipids were not identified in this study.

## RESULTS

### Patient demographics

In total, 18 patients with GC enrolled in this study (median age, 67.5 years; range, 52–87 years) and were divided into CIN ($n$ = 9) and non-CIN ($n$ = 9) groups by using a 5% frequency of genetic variation as the demarcation point; no marked differences in demographics were observed (Table 1). In this study, 85.7% of the Lauren intestinal-type tumors (6/7) belonged to the CIN GC group and all of the Lauren diffuse-type tumors belonged to the non-CIN GC group. Lauren mixed-type tumors belonged to both the CIN (50%) and non-CIN (50%) groups. The intestinal-type tumors demonstrated a high alteration rate of 92.2% (377 genes), particularly those with copy-number changes; by contrast, the diffuse-type tumors exhibited a low alteration rate of 8.56% (35 genes).

### Lipidomic profiling of GC tumors vs adjacent non-cancerous tissues

Figure 1 shows the representative MS spectra for both ESI modes. We observed significant changes in the lysoglycerophospholipid, GP, and triglyceride (TG) regions in the ESI+ mode and in the lysoglycerophospholipid, GP, and SP regions in the ESI− mode. After calculating data matrices by using MarkerLynx XS and exporting them to SIMCA-P+ software, we obtained 1374 variables (loadings) in the ESI+ mode and 539 variables in the ESI− mode. Four significant clusters between tumors and their adjacent noncancerous tissues were detected in both modes by using OPLS-DA (R2X

**Table 1 Clinical characteristics of the study**

| Term | TCGA system | |
|---|---|---|
| | CIN | Non-CIN |
| Number | 9 | 9 |
| Age (median yr, range) | 68.1 (56-79) | 66.9 (52-87) |
| Sex (male/female) | 7/2 | 5/4 |
| Size (cm) | 4.0 (1.8-6.9) | 5.4 (2.3-11.6) |
| Lauren's classification | | |
| Intestinal type | 6 | 1 |
| Diffuse type | 0 | 5 |
| Mixed type | 3 | 3 |
| Stage | | |
| I | 1 | 0 |
| II | 2 | 2 |
| III | 5 | 6 |
| IV | 1 | 1 |

TCGA: The Cancer Genome Atlas; CIN: Chromosomal instability.

= 0.844, R2Y = 0.89, and Q2 = 0.747 in ESI+ mode; R2X = 0.815, R2Y = 0.841, and Q2 = 0.603 in ESI- mode), as illustrated in Figure 2. These clusters were divided into tumor samples with CIN status, tumor samples with non-CIN status, adjacent noncancerous tissues with CIN status, and adjacent noncancerous tissues with non-CIN status.

Loading plots of the OPLS-DA and VIP scores were used to identify potential diagnostic markers in GC tissues. Significant metabolite differences between tumors and their adjacent noncancerous tissues were identified by VIP ≥ 1.0 and $P < 0.05$ and divided into lysophosphocholine (LysoPC), phosphocholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphoserine (PS), sphingomyelin (SM), ceramide, and TG in both ESI modes (Table 2). Compared with their adjacent noncancerous tissues, the GC samples exhibited higher levels of PC and SM but lower levels of PE and TG (all $P < 0.05$). We observed no lipid species that were present in only one group. All of the metabolites observed in this study exhibited dynamic differences between tumors and their adjacent noncancerous tissues.

### *Lipidomic alterations of CIN vs non-CIN GC tumors*

The data matrices were further exported for OPLS-DA in both ESI modes to show the lipid difference between CIN and non-CIN status within the GC samples. Two significant clusters are illustrated in Figure 3 (R2X = 0.79, R2Y = 0.988, and Q2 = 0.874 in ESI+ mode; R2X = 0.71, R2Y = 0.914, and Q2 = 0.694 in ESI− mode). This pattern suggests that the divergence of the OPLS-DA distribution was dependent on the CIN status with goodness of fit. Based on the loading plots of OPLS-DA, significant differences between the CIN and non-CIN GC samples were filtered by VIP ≥ 1.0 and $P < 0.05$ and divided into PC, PE, PI, SM, and diglycerides (DG) in both ESI modes (Table 3). No lipid species were present in only one group. The levels of almost all lipid species were different in the CIN tumors and exhibited higher intensity in the CIN tumors than in the non-CIN tumors, except for DG (38:4) and SM (d18:1/18:0) (all $P < 0.05$). Compared with the non-CIN group, GP levels (PC, PE, and PI) demonstrated were 1.4- to 2.3-times higher in the CIN group ($P < 0.05$). We observed alteration of the lipid metabolism for both GC status and CIN status in the GL, GP, and SL pathways. We also observed changes in lipid species in the GL and GP pathways in the CIN analysis only; these findings are shown in Figure 4.

The predictive PLS-DA model based on the significant candidates (Table 3) demonstrated good differentiation between the CIN and non-CIN groups, with sensitivity of 0.852, specificity of 0.703, and an area under the curve of 0.906 (Figure 5).

## DISCUSSION

We found that several lipid species primarily affected the grouping of the GC samples and their adjacent noncancerous tissues; markedly higher levels of PC and SM and lower levels of PE and TG were detected in the GC samples, as shown in Figure 4.

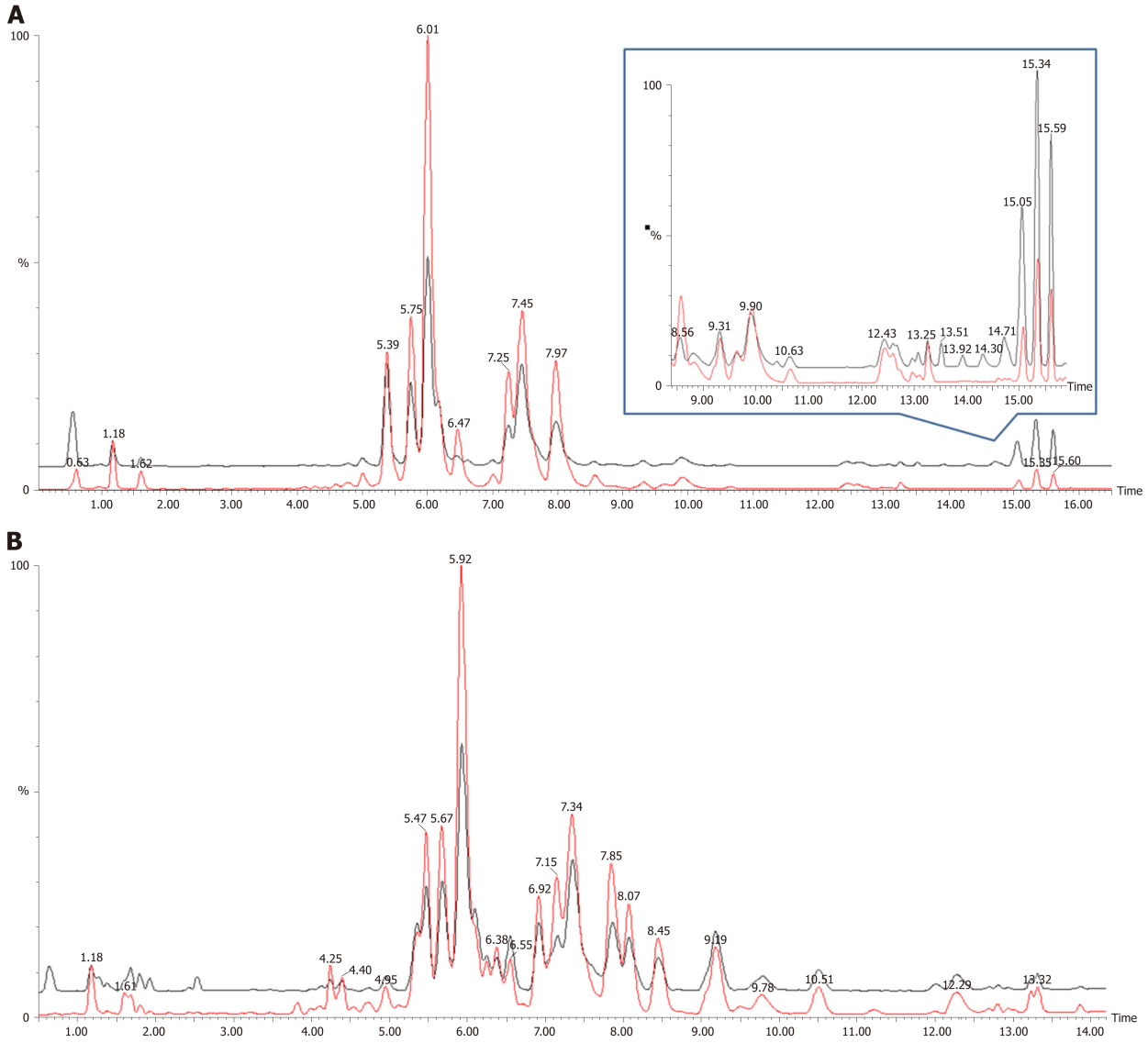


**Figure 1 Different lipidomic profiling of gastric cancer tissues based on liquid chromatography/mass spectrometry analysis.** A: Electrospray ionization (ESI) positive modes; B: ESI negative modes. Base peak chromatograms of the gastric cancer samples are shown from the different groups. Red represents for the gastric cancer tissues (NO. 38); Black represents for the adjacent non-cancerous tissues as a control.

Alterations in lipid species discovered in the GL, GP, and SL pathways of the GC samples are marked in black. Few studies have examined the differing roles of lipid metabolomics in cancerous and noncancerous samples[6,23,24]. Abbassi-Ghadi *et al*[24] reviewed several metabolites of glycolysis, the tricarboxylic acid cycle, and lipid metabolism and suggested them to be biomarkers of esophagogastric cancers. Our findings on alterations in TG are supported by the higher prevalence of an olefinic group in noncancerous gastric spheroids at 5.29 ppm, detected using 1H nuclear magnetic resonance, compared with cancerous gastric spheroids[25]. Huang *et al*[26] reported the products of SL metabolism, including SM and ceramide, which act as bioactive molecules regulating cell survival and proliferation in apoptosis. In the present study, we observed dynamic differences in several SM species between tumors and their adjacent noncancerous tissues. The elevated PC level in cancerous tissue might have been related to overexpression of lysophosphatidylcholine acyltransferase 1[13]. Moreover, the lower level of LysoPC (16:0) observed in this study resulted from conversion of LysoPC into PC due to lysophosphatidylcholine acyltransferase 1 protein activity[13].

We further identified the undisclosed correlation between lipidomic profiling of GC and CIN status. We classified lipid alterations between the CIN and non-CIN GC samples into PC, PE, PI, SM (d18:1/18:0), and DG (38:4). Significant differences in CIN status were observed in the GP (PC, PE, and PI) category alongside various fatty acyl chain lengths and the degree of saturation in the fatty acyl chain in our findings. The features of CIN status are common p53 mutation and frequent activation of genomic

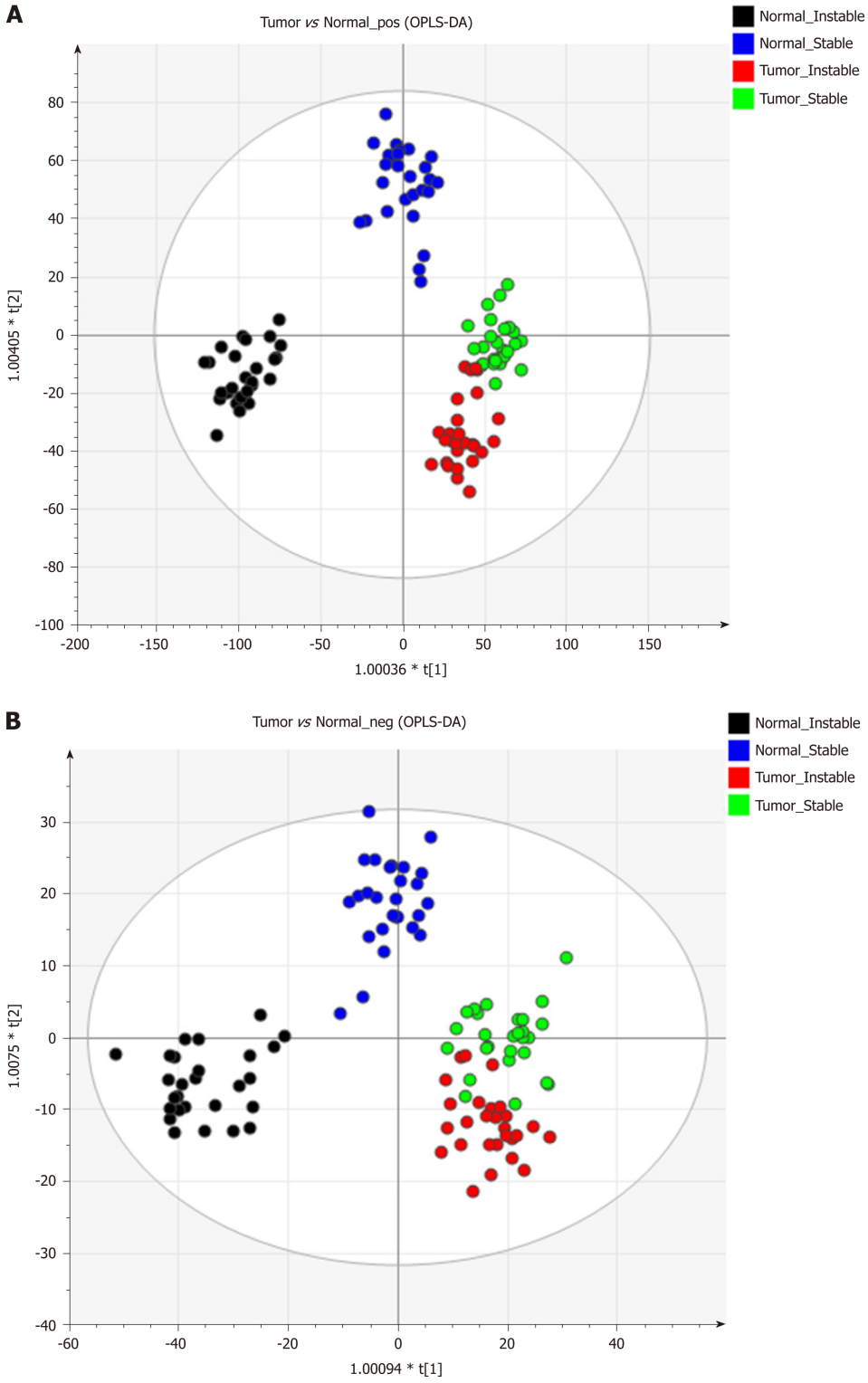


**Figure 2 Lipidomic distribution of gastric cancer tumor and the surrounding non-cancerous tissue were detected under electrospray ionization + and - mode with the orthogonal projections to latent structures discriminant analysis statistical method.** A: Electrospray ionization (ESI) +; B: ESI-. OPLS-DA: Orthogonal projections to latent structures discriminant analysis.

amplification, which encodes the receptor tyrosine kinase pathway[5]. Mitogenic signaling conducted by growth factors regulates aberrant cell growth and proliferation, which are involved in the activation of numerous lipid-metabolism-related enzymes[26]. Genetic alterations and enzyme activity in lipid perturbation accumulate over time, resulting in severe changes in lipid metabolism and ultimately leading to tumor formation in CIN tissues[27]. Dysregulation of GP metabolism has previously been described in various cancers[15,28]. Luo *et al*[15] reviewed the emerging role of lipid metabolism in cancer metastasis and revealed higher levels of PS, PI and

**Table 2 Compound list (*n* = 32) of the significant changes between tumor and normal group using electrospray ionization positive and negative modes**

| Catalog | Putative ID | RT (min) | m/z | Adduct ion | VIP | FC |
|---|---|---|---|---|---|---|
| TG | TG(54:3) | 15.6 | 902.8202 | $[M+NH_4]^+$ | 2.3 | 0.5 |
| | TG(52:4) | 15.1 | 872.7729 | $[M+NH_4]^+$ | 3.2 | 0.4 |
| | TG(52:2) | 15.6 | 876.8048 | $[M+NH_4]^+$ | 3.2 | 0.5 |
| | TG(50:3) | 15.0 | 846.7568 | $[M+NH_4]^+$ | 2.9 | 0.3 |
| | TG(50:2) | 15.3 | 848.7722 | $[M+NH_4]^+$ | 3.3 | 0.4 |
| SM | SM(d18:1/24:0) | 13.3 | 815.7015 | $[M+H]^+$ | 1.6 | 1.3 |
| | SM(d18:1/18:0) | 7.1 | 775.5980 | $[M+FA-H]^-$ | 1.7 | 0.7 |
| | SM(d18:0/22:0) | 9.9 | 811.6627 | $[M+H]^+$ | 2.1 | 1.3 |
| | SM(d18:0/16:0) | 6.0 | 705.5912 | $[M+H]^+$ | 1.0 | 1.4 |
| PS | PS(P-18:0/22:6) | 9.2 | 818.5336 | $[M-H]^-$ | 1.0 | 0.7 |
| PI | PI(22:3/16:0) | 6.1 | 887.5682 | $[M-H]^-$ | 1.4 | 1.9 |
| | PI(20:4/16:0) | 4.2 | 857.5202 | $[M-H]^-$ | 1.4 | 0.6 |
| | PI(16:0/18:2) | 4.4 | 833.520 | $[M-H]^-$ | 3.0 | 0.8 |
| PE | PE(P-18:1/20:4) | 7.1 | 748.5279 | $[M-H]^-$ | 2.5 | 0.7 |
| | PE(P-18:0/18:2) | 9.7 | 726.5437 | $[M-H]^-$ | 2.5 | 1.7 |
| | PE(P-16:0/18:2) | 7.3 | 698.5117 | $[M-H]^-$ | 4.0 | 1.9 |
| | PE(20:4/16:0) | 6.2 | 740.5237 | $[M+H]^+$ | 3.6 | 0.4 |
| | PE(18:2/18:1) | 6.6 | 740.5221 | $[M-H]^-$ | 2.3 | 0.4 |
| | PE(18:2/16:0) | 6.4 | 714.5061 | $[M-H]^-$ | 2.2 | 0.4 |
| | PE(18:1/18:1) | 8.2 | 742.5385 | $[M-H]^-$ | 2.4 | 0.6 |
| PC | PC(38:4) | 6.4 | 810.6034 | $[M+H]^+$ | 2.6 | 1.7 |
| | PC(34:3) | 4.8 | 756.5561 | $[M+H]^+$ | 14.2 | 1.5 |
| | PC(30:0) | 5.5 | 706.5394 | $[M+H]^+$ | 2.9 | 2.4 |
| | PC(18:2/16:0) | 6.0 | 758.5702 | $[M+H]^+$ | 3.0 | 0.6 |
| | PC(18:0/20:3) | 8.6 | 812.6191 | $[M+H]^+$ | 3.3 | 3.6 |
| | PC(18:0/18:1) | 9.8 | 832.6090 | $[M+FA-H]^-$ | 7.9 | 1.3 |
| | PC (16:1/16:0) | 5.6 | 776.5467 | $[M+FA-H]^-$ | 1.2 | 2.0 |
| | PC (16:0/16:0) | 7.3 | 734.5717 | $[M+H]^+$ | 1.5 | 1.4 |
| LysoPC | LysoPC (16:0) | 1.2 | 496.3395 | $[M+H]^+$ | 3.1 | 0.7 |
| Ceramide | Cer (d18:1/24:0) | 13.9 | 694.6341 | $[M+FA-H]^-$ | 1.6 | 1.6 |
| carnitine | Linoleyl carnitine (C18:2) | 1.0 | 424.3408 | $[M+H]^+$ | 1.2 | 0.6 |
| | Oleoyl carnitine (C18:1) | 1.2 | 426.3560 | $[M+H]^+$ | 1.1 | 0.8 |

All $P$ value < 0.05. The protonated $[M+H]^+$ and anionized adduct $[M+NH_4]^+$ in positive mode, and deprotonated $[M-H]^-$ and formic acid adduct $[M+FA-H]^-$ in negative ion mode. FC: Fold changes (tumor/normal); LyPC: Lysophosphocholine; PC: Phosphocholine; PE: Phosphatidylethanolamine; SM: Sphingomyelin; PI: Phosphoinositol; TG: Triglyceride; VIP: Variable importance in projection.

PC in metastatic groups than in noncancerous cells. Several core enzymes involved in the GP pathway might directly or indirectly regulate downstream biochemical alterations. Furthermore, Tsai *et al*[16] reported higher levels of PC in CIN samples after hydrophilic analysis. In our findings, CIN tumors contained significantly higher levels of PC (*i.e.*, PC-containing lipids) than did non-CIN tumors; this finding facilitated discrimination between CIN and non-CIN status in lipidomic profiling, and this supports their results. Lipidomics analysis can provide further insight into other lipid classes. We provided evidence of the difference in the DG (38:4) level of CIN status, which could be affected by the activity of phosphatidic acid phosphatase—which is encoded by a family of genes named lipins—and dephosphorylate of phosphatidic acid to form diglycerides[15].

From the perspective of molecular biology, identification of genetic and epigenetic prognostic biomarkers in various cancers contributes to identification of potential therapeutic targets by upregulating genes in cancer tissues[29]. Potential roles of lipidomics identified by TCGA classification of genomic analysis facilitate diagnosis and surveillance of GC[3,23]. Metabolic phenotypes result from a combination of genomic, transcriptomic, and proteomic conditions and their interactions with the

**Table 3 Compound list (*n* = 17) of the significant changes between chromosomal instability and non-chromosomal instability groups using electrospray ionization positive and negative modes**

| Catalog | Putative ID | RT (min) | m/z | Adduct ion | VIP | FC |
|---|---|---|---|---|---|---|
| SM | SM(d18:1/18:0) | 7.2 | 731.6077 | $[M+H]^+$ | 5.8 | 0.49 |
| PI | PI(22:6/18:0) | 5.1 | 909.554 | $[M-H]^-$ | 1.6 | 1.53 |
| | PI(22:3/16:0) | 6.1 | 887.568 | $[M-H]^-$ | 3.0 | 1.75 |
| PE | PE(P-18:0/18:2) | 9.7 | 726.544 | $[M-H]^-$ | 2.8 | 1.92 |
| | PE(P-16:0/20:5) | 5.8 | 720.499 | $[M-H]^-$ | 2.8 | 2.15 |
| | PE(37:4) | 5.8 | 754.5381 | $[M+H]^+$ | 2.9 | 1.77 |
| | PE(22:6/18:1) | 5.8 | 788.523 | $[M-H]^-$ | 1.9 | 1.43 |
| | PE(18:2/16:0) | 6.5 | 716.5233 | $[M+H]^+$ | 2.7 | 1.52 |
| PC | PC(38:6) | 6.5 | 806.571 | $[M+H]^+$ | 3.2 | 2.06 |
| | PC(38:4) | 6.7 | 810.6041 | $[M+H]^+$ | 5.9 | 2.34 |
| | PC(36:5) | 4.8 | 780.5556 | $[M+H]^+$ | 5.7 | 2.30 |
| | PC(33:2) | 5.3 | 744.5562 | $[M+H]^+$ | 3.2 | 1.73 |
| | PC(33:1) | 6.5 | 746.5715 | $[M+H]^+$ | 3.2 | 1.59 |
| | PC(30:0) | 5.5 | 706.5394 | $[M+H]^+$ | 4.6 | 1.89 |
| | PC(16:1/16:1) | 4.6 | 730.5399 | $[M+H]^+$ | 4.1 | 1.98 |
| | PC(16:1/16:0) | 5.6 | 776.547 | $[M+FA-H]^-$ | 4.3 | 1.46 |
| DG | DG(38:4) | 12.6 | 667.5287 | $[M+Na]^+$ | 1.6 | 0.66 |

All *P* value < 0.05. The protonated $[M+H]^+$ and sodium adduct $[M+Na]^+$ in positive mode, and deprotonated $[M-H]^-$ and formic acid adduct $[M+FA-H]^-$ in negative ion mode. FC: Fold changes [Chromosomal instability (CIN)/non-CIN]; PC: Phosphocholine; PE: Phosphatidylethanolamine; PI: Phosphatidylinositol; SM: Sphingomyelin; DG: Diglyceride; VIP: Variable importance in projection.

environment[30]. Our preliminary results have potential clinical implications. First, rapid lipidomics profiling could be used to identify patients at high risk of GC at various stages. We combined TCGA classification of genomic analysis with a lipidomics method to determine the distribution of lipid species for accurate diagnosis of GC and identify potential biomarkers for translational discovery and novel therapeutic strategies. Analyzing changes in GP levels (especially PC, PE, and PI) can not only provide insight into GC pathology and diagnosis but also determine novel biomarkers of CIN status in GC. Full molecular classification of GC advances the knowledge of the biology of GC, and identification of biomarkers for early diagnosis may improve effective treatment through precision medicine[8]. However, these preliminary results must be interpreted with caution until they are validated using an independent dataset because the small sample size relative to the number of features extracted may have resulted in model overfitting.

This study had some limitations. First, the sample size was small. Our objective of analyzing genomics and metabolomics data inadvertently limited the number of participants willing to contribute tissue samples in each category of this study. Therefore, more extensive research is warranted to further validate the utility of the analyzed biomarkers, and translation into clinical settings should follow. Second, the methodology of this study could be improved for development of a more comprehensive lipid extraction method for identifying more lipid species such as free fatty acids and cholesteryl ester and its derivatives. Third, potential classes were missing from this exploratory experiment. Although *Helicobacter pylori* plays a crucial role in gastric carcinogenesis, we aim to the CIN status influences on the outcome of gastric cancer, and tried to exclude the other possible factors including microbiota in gastrointestinal in this study. To further identify potential biomarkers, determining absolute concentrations in multiple biological organs is necessary. Therefore, further investigation that establishes a database of potential biomarkers - including their relative concentrations in multiple organs - for application in precision medicine is warranted.

In conclusion, CIN status of GC was primarily associated with downstream lipidomics in the GP pathway, namely PC, PE, and PI. These findings based on TCGA classification reflected regulation of the cellular signal pathway of apoptosis in CIN tumors. We employed a genomic classification method to obtain lipidomic information correlated with CIN status.

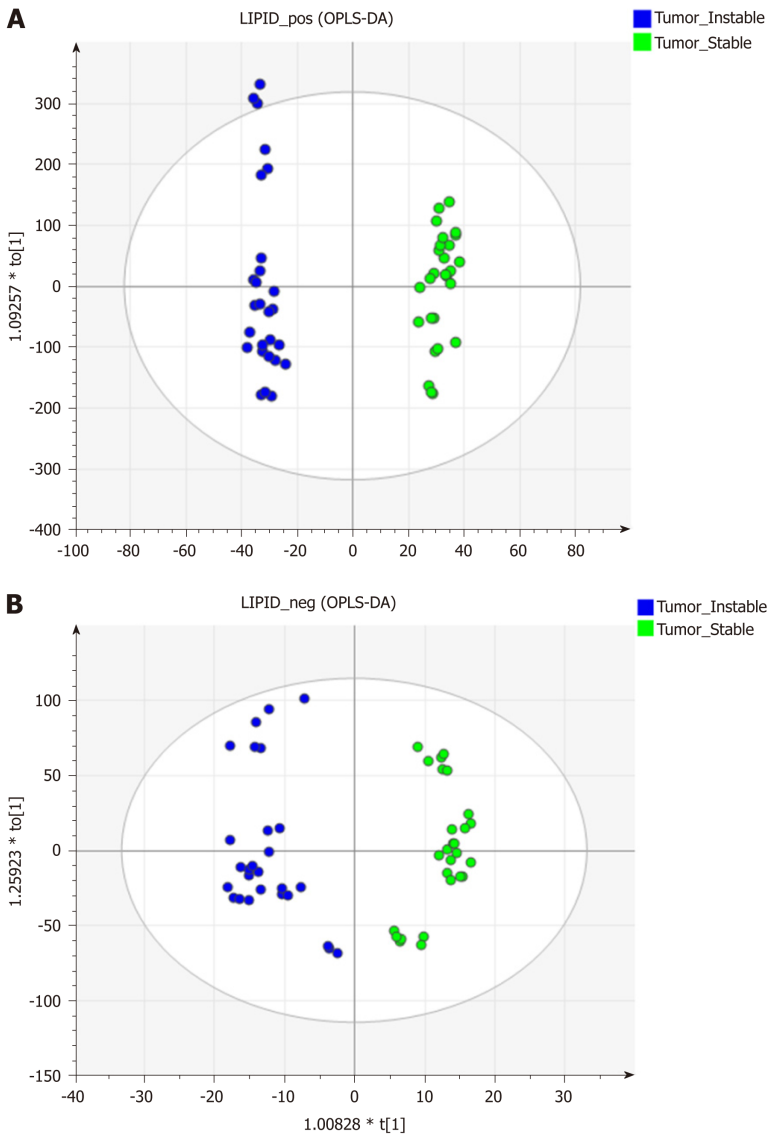


**Figure 3 Lipidomics distribution of the chromosomal instability and non-chromosomal instability type of the gastric cancer samples under electrospray ionization + and - mode using the orthogonal projections to latent structures discriminant analysis statistical method.** A: Electrospray ionization (ESI) +; B: ESI-. OPLS-DA: Orthogonal projections to latent structures discriminant analysis.

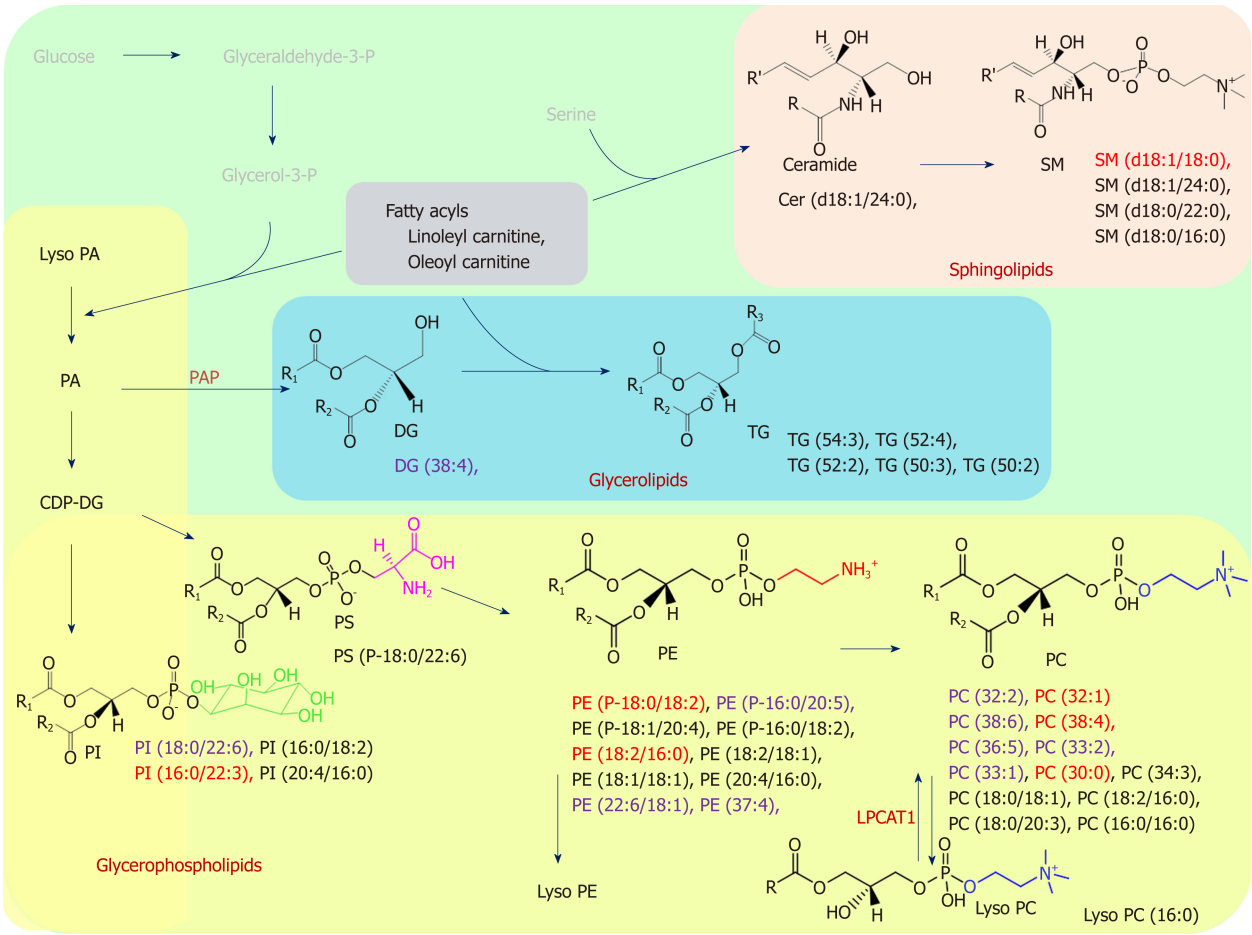


**Figure 4 Schematic overview of the lipid biosynthesis pathways in this study was summarized.** Black: Changes according to gastric cancer status; Purple: Only represent in chromosomal instability (CIN) analysis; Red: Both CIN and non-CIN status. We showed the lipid categories which involved in the significant changes of metabolites in this study. R is a carbon chain. PAP: phosphatidic acid phosphatase; LPCAT1: lysophosphatidylcholine acyltransferase 1.

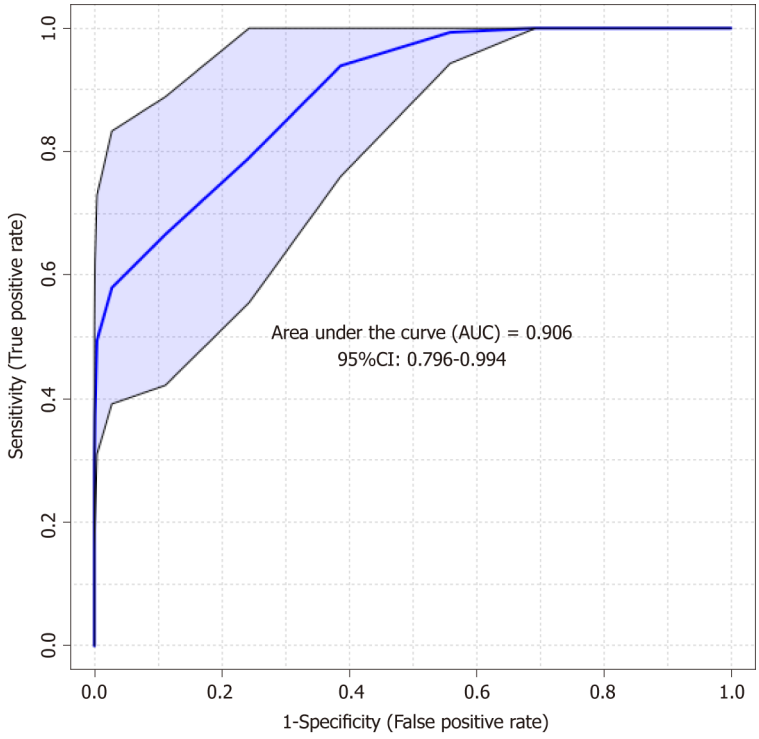


**Figure 5 The receiver operating characteristic curve analysis on the outstanding metabolites of chromosomal instability and non-chromosomal instability gastric cancer status with projections to latent structures discriminant analysis model.**

## ARTICLE HIGHLIGHTS

### Research background

Gastric cancer (GC) leads to worldwide cancer mortality, especially in developing countries. Recently, The Cancer Genome Atlas (TCGA) Research Group proposed an integrative genomic analysis, dividing gastric cancer into four subtypes—Epstein Barr Virus positive, microsatellite unstable, chromosomally instable (CIN), and genomically stable, based on gene expression profiling of the exome sequences, copy-number alterations, gene expression, DNA methylation, and protein activities. However, the CIN status of GC is still vaguely characterized and lacking the valuable easy-to-use CIN markers to diagnosis in molecular and histological detection. Metabolomics, which study the result of the interaction of the biosystem's genome with its environment and detect the end product of gene expression, offers the opportunity to understand the complex molecular mechanisms and to identify the diagnostic biomarkers of human GC. Although mass spectrometry (MS) and nuclear magnetic resonance system have been used widely to investigate metabolic changes in biological processes, most of those findings were limited to focus on water-soluble compounds, and volatile metabolites. Perturbation of lipid metabolism would also contribute to observing in the cancer progression by detecting the activity of the dysregulated core enzymes in lipid pathways and the global lipid metabolic alterations in cancer metastasis. Global lipidomics provides the most details detection and qualification of the cellular lipids in systems biology. The background, present status, and significance of the study should be described in detail.

### Research motivation

In our previous study, metabolomic profiles of GC tumors and the adjacent healthy tissue are distinct, and altered pathways involving amino acid metabolism, glyoxylate and dicarboxylate metabolism. In this study, we hypothesize that lipidomic alternations reflect the CIN or non-CIN status of GC to provide the exploration of the correlation the lipidomic metabolites of GC with its CIN status.

### Research objectives

The main objectives aimed to discover the numerous biomarkers from lipidomic studies and explore the associations of CIN with its downstream lipidomics profiles.

### Research methods

Tumor samples were categorized as CIN or non-CIN type by the TCGA system. We extracted the genomic DNA, and quantified them for genomic analysis. In total 409 leading oncogenes and tumor suppressor genes in the GC tumor tissue were sequenced. For lipidomic metabolite research, tissue extraction through Folch method and performed profiling using an LC/MS system. Data processing and statistical analysis for lipidomic analysis to discover the potential metabolites using MarkerLynx XS software, SIMCA-P+ and MetaboAnalyst 4.0.

### Research results

This study demonstrated the Lipidomic profiling of GC tumors showed distinct profiles in glycerolipid, glycerophospholipid and sphingolipid compared with adjacent non-cancerous tissues. The glycerophospholipid levels (phosphocholine, phosphatidylethanolamine, and phosphatidylinositol) demonstrated a 1.4- to 2.3-fold increase in the CIN group, compared with the non-CIN group ($P < 0.05$). Alteration of the glycerolipid and glycerophospholipid pathways involved throughout the evolutions of GC formation toward chromosomal instability.

### Research conclusions

Lipidomics profiles of GC tumors were distinct against the adjacent non-cancerous tissue. The CIN status of GC primarily associated with the downstream lipidomics in glycerophospholipid pathway.

### Research perspectives

Our study provided the genomic classification method and discovered lipidomic information to correlate with its CIN status. To validate our initial findings, more sample collections with longer follow up times will be considered.

## ACKNOWLEDGEMENTS

The authors thank all the members of the Cancer Centre, Chang Gung Memorial Hospital. LC-MS was carried out with the help from the Metabolomics Core Laboratory, Healthy Aging Research Center, Chang Gung University and Clinical Metabolomics Core Laboratory, Chang Gung Memorial Hospital.

## REFERENCES

1 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675 DOI: 10.1111/apm.1965.64.1.31]

2 **Grabsch HI**, Tan P. Gastric cancer pathology and underlying molecular mechanisms. *Dig Surg* 2013; **30**: 150-158 [PMID: 23867592 DOI: 10.1159/000350876]
3 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [PMID: 25079317 DOI: 10.1038/nature13480]
4 **Rochfort S**. Metabolomics reviewed: a new "omics" platform technology for systems biology and implications for natural products research. *J Nat Prod* 2005; **68**: 1813-1820 [PMID: 16378385 DOI: 10.1021/np050255w]
5 **Strand MS**, Lockhart AC, Fields RC. Genetics of Gastric Cancer. *Surg Clin North Am* 2017; **97**: 345-370 [PMID: 28325191 DOI: 10.1016/j.suc.2016.11.009]
6 **Jayavelu ND**, Bar NS. Metabolomic studies of human gastric cancer: review. *World J Gastroenterol* 2014; **20**: 8092-8101 [PMID: 25009381 DOI: 10.3748/wjg.v20.i25.8092]
7 **Chan AW**, Mercier P, Schiller D, Bailey R, Robbins S, Eurich DT, Sawyer MB, Broadhurst D. (1)H-NMR urinary metabolomic profiling for diagnosis of gastric cancer. *Br J Cancer* 2016; **114**: 59-62 [PMID: 26645240 DOI: 10.1038/bjc.2015.414]
8 **Liang Q**, Wang C, Li B. Metabolomic Analysis Using Liquid Chromatography/Mass Spectrometry for Gastric Cancer. *Appl Biochem Biotechnol* 2015; **176**: 2170-2184 [PMID: 26088916 DOI: 10.1007/s12010-015-1706-z]
9 **Jung J**, Jung Y, Bang EJ, Cho SI, Jang YJ, Kwak JM, Ryu DH, Park S, Hwang GS. Noninvasive diagnosis and evaluation of curative surgery for gastric cancer by using NMR-based metabolomic profiling. *Ann Surg Oncol* 2014; **21** Suppl 4: S736-S742 [PMID: 25092158 DOI: 10.1245/s10434-014-3886-0]
10 **Yu L**, Aa J, Xu J, Sun M, Qian S, Cheng L, Yang S, Shi R. Metabolomic phenotype of gastric cancer and precancerous stages based on gas chromatography time-of-flight mass spectrometry. *J Gastroenterol Hepatol* 2011; **26**: 1290-1297 [PMID: 21443661 DOI: 10.1111/j.1440-1746.2011.06724.x]
11 **Wymann MP**, Schneiter R. Lipid signalling in disease. *Nat Rev Mol Cell Biol* 2008; **9**: 162-176 [PMID: 18216772 DOI: 10.1038/nrm2335]
12 **Guo S**, Wang Y, Zhou D, Li Z. Significantly increased monounsaturated lipids relative to polyunsaturated lipids in six types of cancer microenvironment are observed by mass spectrometry imaging. *Sci Rep* 2014; **4**: 5959 [PMID: 25091112 DOI: 10.1038/srep05959]
13 **Uehara T**, Kikuchi H, Miyazaki S, Iino I, Setoguchi T, Hiramatsu Y, Ohta M, Kamiya K, Morita Y, Tanaka H, Baba S, Hayasaka T, Setou M, Konno H. Overexpression of Lysophosphatidylcholine Acyltransferase 1 and Concomitant Lipid Alterations in Gastric Cancer. *Ann Surg Oncol* 2016; **23** Suppl 2: S206-S213 [PMID: 25752890 DOI: 10.1245/s10434-015-4459-6]
14 **Lamaziere A**, Wolf C, Quinn PJ. Perturbations of lipid metabolism indexed by lipidomic biomarkers. *Metabolites* 2012; **2**: 1-18 [PMID: 24957365 DOI: 10.3390/metabo2010001]
15 **Luo X**, Cheng C, Tan Z, Li N, Tang M, Yang L, Cao Y. Emerging roles of lipid metabolism in cancer metastasis. *Mol Cancer* 2017; **16**: 76 [PMID: 28399876 DOI: 10.1186/s12943-017-0646-3]
16 **Tsai CK**, Yeh TS, Wu RC, Lai YC, Chiang MH, Lu KY, Hung CY, Ho HY, Cheng ML, Lin G. Metabolomic alterations and chromosomal instability status in gastric cancer. *World J Gastroenterol* 2018; **24**: 3760-3769 [PMID: 30197481 DOI: 10.3748/wjg.v24.i33.3760]
17 **Folch J**, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 1957; **226**: 497-509 [PMID: 13428781]
18 **Cajka T**, Fiehn O. Increasing lipidomic coverage by selecting optimal mobile-phase modifiers in LC-MS of blood plasma. *Metabolomics* 2016; **12**: 34-44 [DOI: 10.1007/s11306-015-0929-x]
19 **Want EJ**, Wilson ID, Gika H, Theodoridis G, Plumb RS, Shockcor J, Holmes E, Nicholson JK. Global metabolic profiling procedures for urine using UPLC-MS. *Nat Protoc* 2010; **5**: 1005-1018 [PMID: 20448546 DOI: 10.1038/nprot.2010.50]
20 **Chong J**, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS, Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Res* 2018; **46**: W486-W494 [PMID: 29762782 DOI: 10.1093/nar/gky310]
21 **Fahy E**, Subramaniam S, Murphy RC, Nishijima M, Raetz CR, Shimizu T, Spener F, van Meer G, Wakelam MJ, Dennis EA. Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* 2009; **50** Suppl: S9-14 [PMID: 19098281 DOI: 10.1194/jlr.R800095-JLR200]
22 **Smith CA**, O'Maille G, Want EJ, Qin C, Trauger SA, Brandon TR, Custodio DE, Abagyan R, Siuzdak G. METLIN: a metabolite mass spectral database. *Ther Drug Monit* 2005; **27**: 747-751 [PMID: 16404815 DOI: 10.1097/01.ftd.0000179845.53213.39]
23 **Chan AW**, Gill RS, Schiller D, Sawyer MB. Potential role of metabolomics in diagnosis and surveillance of gastric cancer. *World J Gastroenterol* 2014; **20**: 12874-12882 [PMID: 25278684 DOI: 10.3748/wjg.v20.i36.12874]
24 **Abbassi-Ghadi N**, Kumar S, Huang J, Goldin R, Takats Z, Hanna GB. Metabolomic profiling of oesophago-gastric cancer: a systematic review. *Eur J Cancer* 2013; **49**: 3625-3637 [PMID: 23896378 DOI: 10.1016/j.ejca.2013.07.004]
25 **Ramachandran GK**, Yong WP, Yeow CH. Identification of Gastric Cancer Biomarkers Using 1H Nuclear Magnetic Resonance Spectrometry. *PLoS One* 2016; **11**: e0162222 [PMID: 27611679 DOI: 10.1371/journal.pone.0162222]
26 **Huang C**, Freter C. Lipid metabolism, apoptosis and cancer therapy. *Int J Mol Sci* 2015; **16**: 924-949 [PMID: 25561239 DOI: 10.3390/ijms16010924]
27 **Riquelme I**, Saavedra K, Espinoza JA, Weber H, García P, Nervi B, Garrido M, Corvalán AH, Roa JC, Bizama C. Molecular classification of gastric cancer: Towards a pathway-driven targeted therapy. *Oncotarget* 2015; **6**: 24750-24779 [PMID: 26267324 DOI: 10.18632/oncotarget.4990]
28 **Perrotti F**, Rosa C, Cicalini I, Sacchetta P, Del Boccio P, Genovesi D, Pieragostino D. Advances in Lipidomics for Cancer Biomarkers Discovery. *Int J Mol Sci* 2016; **17**: E1992 [PMID: 27916803 DOI: 10.3390/ijms17121992]
29 **Shimizu D**, Kanda M, Kodera Y. Review of recent molecular landscape knowledge of gastric cancer. *Histol Histopathol* 2018; **33**: 11-26 [PMID: 28447336 DOI: 10.14670/HH-11-898]
30 **Lario S**, Ramírez-Lázaro MJ, Sanjuan-Herráez D, Brunet-Vega A, Pericay C, Gombau L, Junquera F, Quintás G, Calvet X. Plasma sample based analysis of gastric cancer progression using targeted metabolomics. *Sci Rep* 2017; **7**: 17774 [PMID: 29259332 DOI: 10.1038/s41598-017-17921-x]


**P- Reviewer:** Pellicano R
**S- Editor:** Wang JL **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 195-207

DOI: 10.4251/wjgo.v11.i3.195

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Basic Study

# Human colorectal cancer cells frequently express IgG and display unique Ig repertoire

Zi-Han Geng, Chun-Xiang Ye, Yan Huang, Hong-Peng Jiang, Ying-Jiang Ye, Shan Wang, Yuan Zhou, Zhan-Long Shen, Xiao-Yan Qiu

**ORCID number:** Zi-Han Geng (0000-0002-4367-6962); Chun-Xiang Ye (0000-0001-7015-3166); Yan Huang (0000-0001-8063-6110); Hong-Peng Jiang (0000-0003-3815-5799); Ying-Jiang Ye (0000-0002-7904-3163); Shan Wang (0000-0002-4273-4307); Yuan Zhou (0000-0001-5685-066X); Zhan-Long Shen (0000-0002-4277-7067); Xiao-Yan Qiu (0000-0002-4805-8362).

**Author contributions:** Qiu XY and Shen ZL initiated and designed the research; Geng ZH and Ye CX performed all the experiments; Huang Y and Zhou Y carried out data analyzing; Jiang HP contributed in analyzing and interpreting results; Geng ZH and Qiu XY wrote the manuscript; Ye YJ, Wang S contributed in clinical diagnosis of patients; Jiang HP and Shen ZL provided clinical specimens, and clinical and pathological information.

**Supported by** Key support projects of the National Natural Science Foundation's major research program, No. 91642206; Major international cooperation projects of the National Natural Science Foundation, No. 81320108020; Beijing Natural Science Foundation, No. 7182171; Research institute fund of NHC Key Laboratory of Medical Immunology, Peking University, No. BMU2018JDJS010; and Non-profit central research institute fund of Chinese Academy of Medical Sciences, No. 2018PT31039.

**Zi-Han Geng, Xiao-Yan Qiu,** Department of Immunology, School of Basic Medical Sciences, Peking University, Beijing 100191, China

**Zi-Han Geng, Xiao-Yan Qiu,** NHC Key Laboratory of Medical Immunology (Peking University), Beijing 100191, China

**Zi-Han Geng, Xiao-Yan Qiu,** Key Laboratory of Molecular Immunology, Chinese Academy of Medical Sciences, Beijing 100191, China

**Chun-Xiang Ye, Hong-Peng Jiang, Ying-Jiang Ye, Shan Wang, Zhan-Long Shen,** Department of Gastrointestinal Surgery, Peking University People's Hospital, Beijing 100044, China

**Chun-Xiang Ye, Hong-Peng Jiang, Ying-Jiang Ye, Shan Wang, Zhan-Long Shen,** Key Laboratory of Colorectal Cancer Diagnosis and Treatment Research, Beijing 100044, China

**Yan Huang,** Institute of Computational Medicine, School of Artificial Intelligence, Hebei University of Technology, Tianjin 300401, China

**Yuan Zhou,** Department of Biomedical Informatics, School of Basic Medical Sciences, Center for Noncoding RNA Medicine, Peking University, Beijing 100191, China

**Corresponding author:** Xiao-Yan Qiu, MD, PhD, Doctor, Department of Immunology, School of Basic Medical Sciences, Peking University Health Science Center, Xueyuan Road, Beijing 100191, China. qiuxy@bjmu.edu.cn
**Telephone:** +86-10-82805477
**Fax:** +86-10-82801149

## Abstract

### *BACKGROUND*

There is growing evidence proving that many human carcinomas, including colon cancer, can overexpress immunoglobulin (Ig); the non B cancer cell-derived Ig usually displayed unique V(D)J rearrangement pattern that are distinct from B cell-derived Ig. Especially, the cancer-derived Ig plays important roles in cancer initiation, progression, and metastasis. However, it still remains unclear if the colon cancer-derived Ig can display unique V(D)J pattern and sequencing, which can be used as novel target for colon cancer therapy.

### *AIM*

To investigate the Ig repertoire features expressed in human colon cancer cells.

**Institutional review board statement:** This work is supported by Medical Ethics Committee of Peking University People's Hospital (2018PHB 193-01).

**Conflict-of-interest statement:** The authors have no conflicts of interest to declare.

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



**Manuscript source**: Unsolicited manuscript

**Received:** October 26, 2018
**Peer-review started:** October 26, 2018
**First decision:** November 14, 2018
**Revised:** January 3, 2019
**Accepted:** January 8, 2019
**Article in press:** January 9, 2019
**Published online:** March 15, 2019

*METHODS*

Seven cancerous tissue samples of colon adenocarcinoma and corresponding noncancerous tissue samples were sorted by fluorescence-activated cell sorting using epithelial cell adhesion molecule as a marker for epithelial cells. Ig repertoire sequencing was used to analyze the expression profiles of all 5 classes of Ig heavy chains (IgH) and the Ig repertoire in colon cancer cells and corresponding normal epithelial cells.

*RESULTS*

We found that all 5 IgH classes can be expressed in both colon cancer cells and normal epithelial cells. Surprisingly, unlike the normal colonic epithelial cells that expressed 5 Ig classes, our results suggested that cancer cells most prominently express IgG. Next, we found that the usage of Ig in cancer cells caused the expression of some unique Ig repertoires compared to normal cells. Some $V_H$ segments, such as $V_H$3-7, have been used in cancer cells, and $V_H$3-74 was frequently present in normal epithelial cells. Moreover, compared to the normal cell-derived Ig, most cancer cell-derived Ig showed unique $V_HDJ_H$ patterns. Importantly, even if the same $V_HDJ_H$ pattern was seen in cancer cells and normal cells, cancer cell-derived IgH always displayed distinct hypermutation hot points.

*CONCLUSION*

We found that colon cancer cells could frequently express IgG and unique IgH repertoires, which may be involved in carcinogenesis of colon cancer. The unique IgH repertoire has the potential to be used as a novel target in immune therapy for colon cancer.

**Key words:** Immunoglobulin repertoire; Sequencing; Colorectal cancer; VDJ pattern; VJ pattern



**Core tip:** It has been found that colon cancer cells can express immunoglobulin (Ig); however, the expression profile and features of the Ig repertoire in colon cancer cells remain unclear. Here, we first sorted colon cancer cells and normal cells from 7 patients with colon cancer. Using the Ig repertoire sequencing, we analyzed the features of the Ig heavy chain (IgH) repertoire in these cells. We found that Ig in colon cancer cells had a significant tendency to choose IgG compared to the other classes of IgH, and showed unique $V_HDJ_H$ patterns and somatic hypermutation hotspots, which might be potential targets for immune therapy for colon cancer.

**Citation:** Geng ZH, Ye CX, Huang Y, Jiang HP, Ye YJ, Wang S, Zhou Y, Shen ZL, Qiu XY. Human colorectal cancer cells frequently express IgG and display unique Ig repertoire. *World J Gastrointest Oncol* 2019; 11(3): 195-207
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/195.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.195

## INTRODUCTION

Colorectal cancer is the third most common cancer worldwide and the fourth most common cause of oncological death[1]. Although using the epidermal growth factor receptor in therapies targeting colon cancer has improved the survival rate of patients, this type of cancer is still the second leading cause of deaths in men and the third in women in the United States according to cancer statistics[2-5]. Thus, more effective therapy targets need to be found.

Immunoglobulin (Ig), also classically known as an antibody, consists of two identical heavy chains (IgHs) and two identical light chains (IgLs) arranged in a roughly Y-shaped configuration[6]. Structurally, each IgH or IgL chain has its own unique viable region, which is a crucial structure that enables Igs to specifically recognize antigens, and a C-terminal conservative constant region that specifies the effector functions of the molecule[6]. The primary diversification of Ig occurs during assembly of the variable region, a process called V(D)J recombination[6]. The variable

region of a heavy chain is assembled from component variables (V), diversity (D), and joining (J) gene segments and then combined with a constant region that determines the class of Ig [IgA(α), IgD(δ), IgE(ε), IgG(γ) and IgM(μ)]. Similarly, κ and λ light chains are composed of rearranged V and J gene segments[7]. After being challenged by an antigen, the variable region of Ig undergoes somatic hypermutation (SHM) that introduces point mutations into the V region antigen-binding pocket, further boosting its affinity for a particular antigen[6] (Figure 1A). Thus, Ig can initiate specific immune responses against antigens by generating a nearly infinite diversity of antigen receptors within the constraints of a finite genome[8].

Traditionally, Ig is believed to be produced only by B lymphocytes. However, our research group and others have confirmed that non-B cells[9-11], especially epithelial cancer cells (such as human lung, breast, colon, liver, cervical and oral cancer cells), can also produce Ig, including IgG, IgM and IgA[12-17]. The non B cell-derived Igs (non B-Ig) displayed several unique features, such as a conservative V(D)J usage and mutation patterns among the same lineage. Moreover, the cancer cell-derived Ig (Cancer-Ig) showed unique glycosylation modification[18,19]. Mechanistically, cancer cell-derived Ig is involved in the proliferation of cancer cells[20,21], cancer cell invasion and metastasis[19,22-24]. These findings suggest that non-B-Ig performs a different function from B-Ig. Specifically, the Cancer-Ig acts as an oncogene in cancer development; thus, there is an increased need to get a full picture of the characteristics of Cancer-Ig sequences for both basic research and clinical application.

In this study, we used immune repertoire sequencing (IR-Seq), which avoided the depth restriction of Sanger sequencing. We completed analysis of the IgH repertoire in 7 samples of epithelial cancer cells and counterpart 7 control samples from the surgical edge of resected colon tissues (taken as normal colonic epithelial cells) in patients with colorectal cancer. Our results confirmed individually biased Ig repertoires with the presence of SHM in colon cancer, which could be recognized as an indicator of their potential as neoantigen and therapeutic targets.

## MATERIALS AND METHODS

### Patient samples

Cancer tissue and normal tissue from the surgical edge of resected colon were obtained from patients at Peking University Peoples' Hospital with written informed consent. The study was conducted according to an institutional review board-approved protocol and was approved by the Clinical Research Ethics Committee of Peking University Peoples' Hospital.

### Cell sorting

To obtain cancer cells and normal epithelial cells, tissues were first cut into small pieces (approximately 1 $mm^3$) and washed with 1 × PBS. Epithelial cells were separated from the tissue by incubating for 1 h at 37 °C with shaking in 1 × PBS supplemented with 5 mmol/L EDTA and 5 mmol/L DTT. Digested epithelial cells were then dissociated by gentleMACS Dissociator (Miltenyi Biotec, Bergisch Gladbach, Germany) and filtered through nylon mesh. Cells were then washed in 1 × PBS with 2% fetal bovine serum (FBS) (10099141, Gibco, USA) 3 times, blocked in 1 × PBS with 5% FBS for 30 min at 4 °C, and stained for 30 min at 4 °C with anti-human CD19 (11-0199-41, eBioscience, USA) and anti-human epithelial cell adhesion molecule (EpCAM) (12-9326-42, eBioscience). Fluorescence-activated cell sorting (FACS) of $EpCAM^+$ cells was then performed by FACSAria II (BD Biosciences, Franklin Lakes, NJ, USA).

### Sample preparation for IR-Seq

Total RNA of sorted epithelial cancer cells and normal colonic epithelial cells were extracted by TRIzol Reagent (15596018, Life Technology, USA). Primer sets for IgH (iRepertoire Inc.) were used to perform two rounds of polymerase chain reaction (PCR) under the reaction conditions specified by iRepertoire® (Huntsville, AL, USA). During the first round, reverse transcription was completed and nested gene-specific primers that were complementary to V and C genes were used to introduce barcodes and sequencing primers into PCR products. The second round of PCR was carried out using communal (sequencing) primers for exponential amplification. Therefore, the entire repertoire was amplified evenly and semiquantitatively, without introducing additional amplification bias (Figure 1C). The DNA concentration of eluted PCR products were measured and 100 ng of DNA was pooled for sequencing. The following sequencing using the 2 × 250 bp Illumina MiSeq platform were performed by Novogene Corporation (Beijing, China).

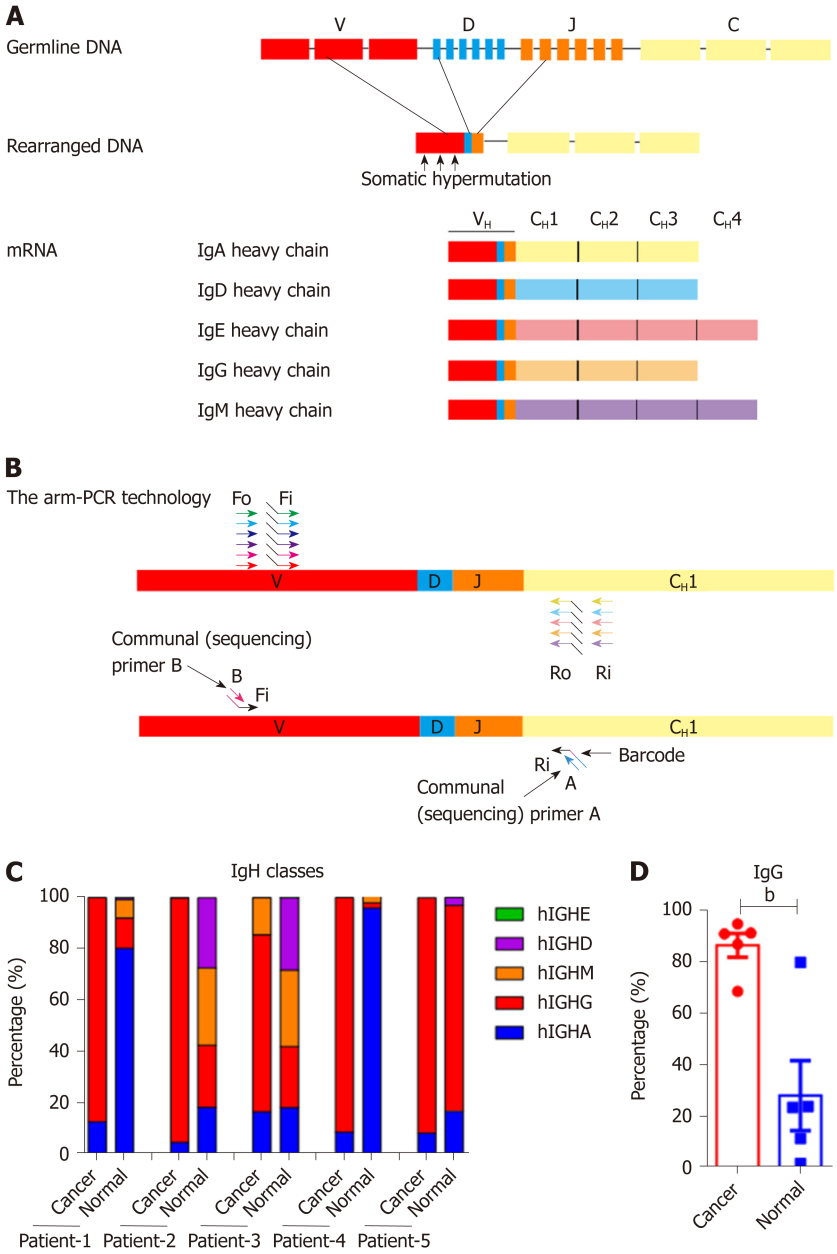


**Figure 1 Proportions of Ig classes in colon cancer cells and normal epithelial cells.** A: The process of immunoglobulin heavy chain (IgH) gene rearrangement and the structure of rearranged IgH; B: Design of the primers for the arm-polymerase chain reaction (PCR) technology used to amplify the immune repertoire. During the first round of PCR, multiple forward primers Fo (forward-out) and Fi (forward-in) were used to target V genes. The reverse primers Ro (reverse-out) and Ri (reverse-in) were targeted to the 5 classes of IgH. The Fi and Ri primers included sequencing adaptors. The second round PCR was carried out with communal primers B and A. The barcodes were in between primer A and the C gene specific primers; C: Proportions of the five IgH classes in cancer and normal cells; D: The proportion of IgG in the 5 patients. Small horizontal lines indicate the mean ± SD. Statistical significance was determined by a two-tailed unpaired Student's *t*-test. $^bP < 0.01$.

### *Data analysis*

iRepertoire® provided basic data analysis such as barcode demultiplexing and filtering, V(D)J alignment, and CDRs identification. For SHM analysis, filtered DNA sequences were uploaded to the IMGT/High V-Quest web-based analysis tool. The IMGT mutation analysis files were used to calculate mutant rates and find SHM hotspots. Data rendering and mapping was completed with GraphPad Prism5 software.

### *Statistical analysis*

All data were analyzed with GraphPad Prism software and presented as a mean ± SD or SEM. Statistical significance was determined by the two-tailed paired or unpaired Student's *t*-test, with significance level of $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.0001$, and

ns, not significant ($P > 0.05$).

## RESULTS

### *IgG can be preferentially expressed in colon cancer cells*

We obtained cell samples from 7 patients, who were diagnosed with colon adenocarcinoma (Table 1). EpCAM$^+$ epithelial cancer cells and normal epithelial cells were sorted by flow cytometry. Total RNA was extracted and reverted to cDNA. All five classes of IgH and their Ig repertoires were amplified by multiplex PCR and sequencing by IR-Seq (Figure 1A and B). We have captured the IgH expression from 5 pairs of colon cancer cells and normal cells of corresponding noncancerous tissue samples, and unpaired colon cancer cells of 2 tissue samples. We first analyzed the expression profile of IgH in the cancer cells and normal cells and found that all classes of IgH were expressed in colonic epithelial cells, but IgA and IgG appeared most frequently. Next, we compared the expression profile of IgH between 5 pairs of colon cancer cells and normal cells. We found that the normal epithelial cells could express all classes of IgH, among which IgA showed the highest frequency (5/5, mean proportion: 46.00%), followed by IgG (5/5, mean proportion: 28.35%), IgM (4/5, mean proportion: 13.77%) and IgD (4/5, mean proportion: 11.86%); however, IgE was rarely observed (3/5, mean proportion: 0.02%). Unexpectedly, the colon cancer cells mainly expressed IgG. The average percentage of IgG was significantly higher in cancer cells (86.68%) than in normal cells (28.35%) (Figure 1C and D).

### *IgH repertoire in cancer cells displayed unique features*

We compared the features of IgH repertoires between cancer cells and normal epithelial cells by analyzing the complementarity determining region 3 (CDR3) pattern of the variable region, which can represent the $V_HDJ_H$ usage of each Ig. As with our previous findings[25], all IgH repertoires from both cancerous and normal cells showed restricted $V_HDJ_H$ patterns compared with the great diversity of IgH in B cells[26] (Figure 2A). However, in each case, cancer cell-derived IgH showed different IgH repertoire profiles compared to the normal epithelial cells (Table 2). Subsequently, we genetically analyzed the distribution feature of IgH expressed in cancer and normal epithelial cells. Unlike the Ig $V_H$ segments expressed in B cells that randomly distribute in the Ig chromosome, the proximal $V_H$s, which were closer to $J_H$ segments in genomic sequence, were more frequently expressed in cancer cells than in normal cells (53.78% *vs* 38.92%) (Figure 2B), suggesting that cancer cells prefer to use these $V_H$ segments that appeared earlier in our evolution. In addition, according to the sequence characteristics, IgH can be divided into 7 families[27]. Obviously, both cancer and normal epithelial cells preferred to use $V_H3$ segments which was consistent with B cell-expressed IgH (B-IgH)[28]; however, $V_H3$-7 was usually used by cancer cells, and $V_H3$-74 was frequently used by normal epithelial cells (Figure 2C and D). We also analyzed the $J_H$ usage, and found that, unlike B cell- expressed IgH, which mostly preferred $J_H4$ and $J_H6$, both cancer and normal epithelial cells preferred to use $J_H4$ and $J_H5$ (Figure 2D). The results suggest that there are diverse mechanisms of Ig gene rearrangement between B cells and colonic epithelial cells.

### *$V_HDJ_H$ rearrangements displayed unique feature in cancer cells*

$V_HDJ_H$ rearrangement pattern represents the characteristic structure of each Ig heavy chain. We first explored $V_HDJ_H$ rearrangement patterns in each sample, and the top 10 $V_HDJ_H$ patterns of both cancer cells and normal cells in each case are listed in Table 2. Next, we investigated if there are some dominant $V_HDJ_H$ patterns shared by most cancer cells in different patient samples. Obviously, no identical $V_HDJ_H$ patterns were shared by cancer cells of different individual-derived IgH, but several $V_HDJ_H$ rearrangements, for example, $V_H1$-8/D7-27/$J_H4$ and $V_H1$-18/D4-17/$J_H4$, were found to be used by normal cells from more than one sample (Table 2). Moreover, we found that each $V_H$ of cancer-derived IgH showed unique $V_HDJ_H$ patterns in all 5 pairs of cancer tissues. For example, in patient-1, patient-2 and patient-3, the $V_H3$-23 was shared by cancer cells and normal cells but was joined by totally different Ds and $J_H$s in cancer cells compared to normal cells (Figure 3). These findings suggest that the unique $V_HDJ_H$ patterns may have a potential role, as neoantigens, in the development of future treatments for individual patients with colon cancer.

### *IgG expressed by cancer cells displays different mutation hot points than normal epithelial cells*

According to classical theory, the variable region of IgH undergoes an extremely high rate of SHM during B cell proliferation, producing a high affinity antibody[6]. Some

**Table 1 Clinical information of 7 patients with colon cancer**

| ID | Sex | Age | Clinical diagnosis | Tumor size (cm) | Differentiation | Vascular invasion | LNM | Distant metastasis | TMN | Histological type | MLH1 | MSH2 | MSH6 | PMS2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 77 | Horizontal colon cancer | 5.2 × 4.9 | Moderately and poorly differentiated | + | 0 | N/A | T4aN0M0 | Adenocarcinoma | N/A | N/A | N/A | N/A |
| 2 | F | 63 | Sigmoid colon cancer | N/A | Poorly differentiated | N/A | N/A | N/A | T3aN1bM0 | Adenocarcinoma | N/A | N/A | N/A | N/A |
| 3 | M | 77 | Right-sided colon cancer | 3 × 3 | Moderately differentiated | N/A | 0 | N/A | T4aN0M0 | Adenocarcinoma | + | + | + | - |
| 4 | F | 52 | Right-sided colon cancer | 4 × 2.5 | Well differentiated | N/A | 0 | N/A | TisN0M0 | Adenocarcinoma | + | + | + | ± |
| 5 | M | 61 | Colon cancer | 2.8 × 1.8 | Moderately differentiated | + | 1/12 | Sacrum metastasis | T4N1M1 | Adenocarcinoma | + | + | + | ± |
| 6 | M | 74 | Right-sided colon cancer | 12 × 9 × 7 | N/A | + | 15/17 | N/A | T4N3M1 | Adenocarcinoma | + | + | + | + |
| 7 | M | 89 | Right-sided colon cancer | 8.5 × 5 | Moderately differentiated | N/A | 0 | Small bowel metastasis | T4bN0M0 | Adenocarcinoma | ± | ± | ± | ± |

oncogenes are frequently mutated in cancer cells. Accordingly, we further investigated to determine if SHM also exists in IgH expressed by cancer cells. An IgH gene was defined as mutated if there were ≥ 2% mutations compared with the germline sequences. Any sequence with fewer mutations than that were considered unmutated[29]. We compared the SHM of $V_H$3-23, which was frequently used in both cancer cells and normal cells (detected in all 6/7 cases), and found that $V_H$3-23 in cancer cells showed significantly higher rates of mutation compared to normal cells (Figure 4A). Mutation hotspots of $V_H$3-23 showed a significant difference between IgG expressed in cancer cells and normal cells (Figure 4B). Similarly, $V_H$3-74/D6-19/$J_H$4 was utilized by both cancer and normal cells, but the mutant hotspots were different (Figure 4C).

## DISCUSSION

In this study, using Ig repertoire sequencing, we explored the expression profiles and Ig repertoires of Ig heavy chains in colon cancer cells compared to colonic epithelial cells from along the surgical margin. We found that cancer cells mainly express IgG, rather than all the Ig classes expressed by normal epithelial cells. Moreover, Ig repertoires in colon cancer cells displayed several unique features, such as $V_H$3-7 being preferentially used. Importantly, colon cancer cell-derived Ig always displayed unique V(D)J rearrangements or mutation hot points compared to those expressed in paired normal cells. These results provide us a better understanding for the variable region characteristics of Cancer-Ig, and open a window for further studies on the role of predominant V(D)J sequences in tumorigenesis, and which might provide new targets for colon cancer therapy.

As is already known, there are 5 classes of Ig. According to our previous findings, different classes of non-B Ig display different biological activities. Under physiological condition, IgM produced by epithelial cells displays natural antibody activity[30,31], IgA expressed by normal skin epidermal cells has potential microbial-binding activity[9]. Under pathological condition, IgG and IgA are closely related to pro-tumor activity and the maintenance of stemness of cancer cells. As early as 20 years ago, Qiu *et al*[12] found that IgG was widely expressed in many types of cancer cells; the cancer-

**Table 2 Top 10 of $V_HDJ_H$ rearrangement patterns in colon cancer and normal epithelial cells**

| | Cancer | | | | Normal | | | |
|---|---|---|---|---|---|---|---|---|
| | V | D | J | % | V | D | J | % |
| Patient-1 | hIGHV3-13 | hIGHD3-9 | hIGHJ6 | 66.84% | hIGHV1-8 | hIGHD7-27 | hIGHJ4 | 27.25% |
| | hIGHV3-30 | hIGHD6-19 | hIGHJ6 | 15.81% | hIGHV3-74 | hIGHD6-19 | hIGHJ4 | 24.71% |
| | hIGHV3-30-3 | hIGHD2-8 | hIGHJ4 | 4.90% | hIGHV3-23 | hIGHD2-21 | hIGHJ5 | 18.58% |
| | hIGHV5-51 | hIGHD3-16 | hIGHJ4 | 3.69% | hIGHV1-8 | hIGHD1-7 | hIGHJ3 | 6.31% |
| | hIGHV3-23 | hIGHD6-13 | hIGHJ4 | 3.58% | hIGHV5-51 | hIGHD3-16 | hIGHJ4 | 5.00% |
| | hIGHV3-7 | hIGHD3-9 | hIGHJ6 | 1.00% | hIGHV3-23 | hIGHD3-22 | hIGHJ6 | 2.92% |
| | hIGHV3-33 | hIGHD6-19 | hIGHJ6 | 0.79% | hIGHV3-74 | hIGHD4-17 | hIGHJ4 | 2.20% |
| | hIGHV3-13 | hIGHD6-13 | hIGHJ6 | 0.53% | hIGHV3-21 | hIGHD2-15 | hIGHJ5 | 1.19% |
| | hIGHV3-30 | hIGHD2-8 | hIGHJ4 | 0.37% | hIGHV3-74 | hIGHD2-21 | hIGHJ5 | 1.17% |
| | hIGHV3-64 | hIGHD6-19 | hIGHJ6 | 0.26% | hIGHV3-23 | hIGHD6-19 | hIGHJ4 | 1.06% |
| Patient-2 | hIGHV1-58 | hIGHD3-22 | hIGHJ4 | 43.86% | hIGHV1-18 | hIGHD4-17 | hIGHJ4 | 24.34% |
| | hIGHV1-46 | hIGHD3-9 | hIGHJ4 | 18.00% | hIGHV3-11 | hIGHD5-5 | hIGHJ2 | 10.95% |
| | hIGHV3-43 | hIGHD3-22 | hIGHJ4 | 10.20% | hIGHV3-74 | hIGHD6-13 | hIGHJ1 | 10.28% |
| | hIGHV3-23 | hIGHD3-9 | hIGHJ4 | 6.28% | hIGHV3-9 | hIGHD6-19 | hIGHJ4 | 10.02% |
| | hIGHV1-69 | hIGHD4-17 | hIGHJ3 | 5.31% | hIGHV3-23 | hIGHD3-10 | hIGHJ4 | 6.67% |
| | hIGHV1-69 | hIGHD2-15 | hIGHJ5 | 3.98% | hIGHV4-4 | hIGHD3-22 | hIGHJ4 | 5.92% |
| | hIGHV1-2 | hIGHD5-5 | hIGHJ4 | 2.77% | hIGHV3-15 | hIGHD3-10 | hIGHJ4 | 3.87% |
| | hIGHV1-58 | hIGHD5-5 | hIGHJ4 | 1.08% | hIGHV3-49 | hIGHD6-19 | hIGHJ4 | 2.88% |
| | hIGHV3-53 | hIGHD6-6 | hIGHJ4 | 1.01% | hIGHV4-59 | hIGHD3-22 | hIGHJ4 | 2.34% |
| | hIGHV1-3 | hIGHD7-27 | hIGHJ6 | 0.61% | hIGHV3-53 | hIGHD6-19 | hIGHJ4 | 2.14% |
| Patient-3 | hIGHV3-23 | hIGHD4-4 | hIGHJ5 | 11.63% | hIGHV1-18 | hIGHD4-17 | hIGHJ4 | 24.71% |
| | hIGHV7-4-1 | hIGHD1-26 | hIGHJ6 | 11.28% | hIGHV3-9 | hIGHD6-19 | hIGHJ4 | 11.82% |
| | hIGHV3-74 | hIGHD6-6 | hIGHJ5 | 11.16% | hIGHV3-11 | hIGHD5-5 | hIGHJ2 | 10.21% |
| | hIGHV3-23 | hIGHD3-16 | hIGHJ5 | 6.15% | hIGHV3-74 | hIGHD6-13 | hIGHJ1 | 9.42% |
| | hIGHV3-48 | hIGHD4-4 | hIGHJ4 | 5.29% | hIGHV3-23 | hIGHD3-10 | hIGHJ4 | 6.20% |
| | hIGHV2-5 | hIGHD5-12 | hIGHJ4 | 5.16% | hIGHV4-4 | hIGHD3-22 | hIGHJ4 | 5.93% |
| | hIGHV1-69 | hIGHD5-5 | hIGHJ4 | 4.83% | hIGHV3-15 | hIGHD3-10 | hIGHJ4 | 4.39% |
| | hIGHV3-23 | hIGHD3-22 | hIGHJ5 | 4.18% | hIGHV4-59 | hIGHD3-22 | hIGHJ4 | 2.78% |
| | hIGHV3-53 | hIGHD5-12 | hIGHJ4 | 2.14% | hIGHV3-49 | hIGHD6-19 | hIGHJ4 | 2.50% |
| | hIGHV3-11 | hIGHD2-15 | hIGHJ4 | 2.07% | hIGHV3-53 | hIGHD6-19 | hIGHJ4 | 2.06% |
| Patient-4 | hIGHV3-7 | hIGHD3-22 | hIGHJ5 | 57.78% | hIGHV3-74 | hIGHD2-8 | hIGHJ5 | 88.00% |
| | hIGHV3-48 | hIGHD3-22 | hIGHJ5 | 13.33% | hIGHV1-8 | hIGHD7-27 | hIGHJ4 | 4.00% |
| | hIGHV1-58 | hIGHD3-22 | hIGHJ4 | 11.11% | hIGHV3-74 | hIGHD6-19 | hIGHJ4 | 2.00% |
| | hIGHV3-43 | hIGHD3-22 | hIGHJ4 | 6.67% | hIGHV3-74 | hIGHD2-21 | hIGHJ5 | 2.00% |
| | hIGHV3-23 | hIGHD3-9 | hIGHJ4 | 4.44% | hIGHV3-23 | hIGHD2-21 | hIGHJ5 | 2.00% |
| | hIHGV3-74 | hIGHD2-8 | hIGHJ5 | 2.22% | hIGHV3-74 | hIGHD5-5 | hIGHJ6 | 2.00% |
| | hIGHV3-21 | hIGHD3-22 | hIGHJ5 | 2.22% | | N/A | | |
| | hIHGV1-46 | - | hIGHJ4 | 2.22% | | N/A | | |
| Patient-5 | hIHGV3-7 | hIHGD3-22 | hIHGJ5 | 65.96% | hIGHV3-7 | hIGHD3-22 | hIGHJ5 | 60.00% |
| | hIHGV3-21 | hIHGD3-22 | hIHGJ5 | 4.26% | hIGHV1-8 | hIGHD7-27 | hIGHJ4 | 12.31% |
| | hIHGV3-74 | hIHGD6-6 | hIHGJ5 | 4.26% | hIGHV1-8 | hIGHD1-7 | hIGHJ3 | 6.15% |
| | hIHGV7-4-1 | hIHGD1-26 | hIHGJ6 | 4.26% | hIGHV3-23 | hIGHD2-21 | hIGHJ5 | 4.62% |
| | hIHGV3-48 | hIHGD3-22 | hIHGJ5 | 4.26% | hIGHV3-74 | hIGHD6-19 | hIGHJ4 | 4.62% |
| | hIHGV3-48 | hIHGD4-4 | hIHGJ4 | 4.26% | hIGHV3-74 | hIGHD2-8 | hIGHJ4 | 3.08% |
| | hIHGV3-11 | hIHGD2-15 | hIHGJ4 | 2.13% | hIGHV5-51 | hIGHD3-16 | hIGHJ4 | 1.54% |
| | hIHGV3-33 | hIHGD3-22 | hIHGJ5 | 2.13% | hIGHV3-9 | hIGHD2-21 | hIGHJ5 | 1.54% |
| | hIHGV3-23 | hIHGD3-22 | hIHGJ5 | 2.13% | hIGHV3-33 | hIGHD3-22 | hIGHJ5 | 1.54% |
| | hIHGV3-72 | hIHGD1-7 | hIHGJ6 | 2.13% | hIGHV3-74 | hIGHD6-25 | hIGHJ4 | 1.54% |
| Patient-6 | hIGHV1-8 | hIGHD6-13 | hIGHJ5 | 44.32% | | N/A | | |
| | hIGHV4-61 | hIGHD3-16 | hIGHJ6 | 16.60% | | | | |
| | hIGHV1-8 | hIGHD3-10 | hIGHJ5 | 14.34% | | | | |

| | | | | | |
|---|---|---|---|---|---|
| | hIGHV3-23 | hIGHD3-22 | hIGHJ3 | 11.74% | |
| | hIGHV1-8 | hIGHD7-27 | hIGHJ4 | 6.00% | |
| | hIGHV3-23 | hIGHD2-21 | hIGHJ5 | 4.57% | |
| | hIGHV3-74 | hIGHD6-19 | hIGHJ4 | 0.96% | |
| | hIGHV4-61 | hIGHD2-21 | hIGHJ6 | 0.17% | |
| | hIGHV3-74 | hIGHD3-22 | hIGHJ3 | 0.13% | |
| | hIGHV1-8 | hIGHD3-16 | hIGHJ5 | 0.13% | |
| Patient-7 | hIGHV3-7 | hIGHD3-22 | hIGHJ5 | 80.95% | N/A |
| | hIGHV3-21 | hIGHD3-22 | hIGHJ5 | 1.28% | |
| | hIGHV3-33 | hIGHD3-22 | hIGHJ5 | 0.92% | |
| | hIGHV3-23 | hIGHD4-4 | hIGHJ5 | 0.92% | |
| | hIGHV7-4-1 | hIGHD1-26 | hIGHJ6 | 0.92% | |
| | hIGHV3-23 | hIGHD3-22 | hIGHJ5 | 0.73% | |
| | hIGHV3-48 | hIGHD3-22 | hIGHJ5 | 0.73% | |
| | hIGHV2-5 | hIGHD5-12 | hIGHJ4 | 0.73% | |
| | hIGHV3-48 | hIGHD4-4 | hIGHJ4 | 0.55% | |
| | hIGHV3-48 | hIGHD3-22 | hIGHJ5 | 0.55% | |

derived IgG could promote growth and survival of cancer cells. Recently, they found that an unique IgG, with a novel sialylated modification in Asn162 of CH1, was widely expressed in cancer stem cells of epithelial cancers, and promoted tumor progression via activating integrin-FAK signaling[23,24]. The expression of IgA by epithelial cancer cells of nasopharyngeal carcinoma and its participation in the evolution of cell cycle was confirmed by Zheng *et al*[15,32]. Meanwhile, they also found Igκ light chain expression in nasopharyngeal carcinoma cells regulated by NF-κB and Activator protein 1 (AP-1) pathways[33]. Chen *et al*[14], Liu *et al*[34] and Qiu *et al*[35] have confirmed that the IgG expressed by human prostate cancer, esophagus carcinoma and papillary thyroid cancer could promote tumor migration. In addition, Lee *et al*[36-38] developed the cancer-specific antibody RP215, which was initially produced using cell extracts of the human OC-3-VGH ovarian cancer cell line as antigen and specifically recognize almost all of the cancer cells but not normal cells. Moreover, the RP215 could specifically recognizes carbohydrate-associated epitope(s) localized in the variable region of IgG heavy chains expressed by cancer cells[36-38]. In this study, we found that unlike the paired normal colon epithelial cells which mainly expressed IgA, colon cancer cells mainly expressed IgG. These results suggest that IgG may be closely related to tumor progression of colon cancer.

We previously reported that Ig expressed in non-B cells had restricted $V_HDJ_H$ patterns, especially in some cancer cells, including colon cancer cells. We found that the colon cancer cell-derived Ig usually expressed some unique $V_HDJ_H$ patterns, such as the $V_H$5-51/D3-16/$J_H$4 and $V_H$3-15/D3-10/$J_H$4 by sanger sequencing[25]. NGS of the immune repertoire allows for the sequencing of millions of V(D)J sequences in parallel, and has a wide use in immune repertoire analyzing nowadays. In this study, using primers with IR-Seq that were different from our previous primers, we not only detected $V_H$5-51/D3-16/$J_H$4 in cancer cells of 3 samples, but more unique Ig $V_HDJ_H$ patterns were also seen in colon cancer cells. The cancer cells tended to utilize proximal $V_H$ genes such as $V_H$3-7 and $V_H$3-23, but with different Ds and $J_H$s connected to the $V_H$ segments. Several rearrangements of the sequences were predominant in cancer cells, such as $V_H$3-7/D3-22/$J_H$5, $V_H$3-23/D4-4/$J_H$5, and $V_H$3-13/D3-9/$J_H$6, but there were few common advantage $V_HDJ_H$ rearrangements shared between different patients, increasing the importance of individualized analysis and treatment plans in the future according to the characteristics of Cancer-Ig variable regions. More importantly, the SHM sites were totally different between cancer cells and normal cells in the same individual and the same $V_HDJ_H$ rearrangement, such as $V_H$3-74/D6-19/$J_H$4, suggested that this difference contributed to the growth of cancer cells.

In summary, our results confirmed that the Cancer-Ig repertoire is biased with SHM, indicating its potency as a target in individualized treatment. Sequencing the Ig repertoire opens a window for deeper understanding and new diagnostics of colon cancer, which will hopefully help the development of new molecular targets for this disease.

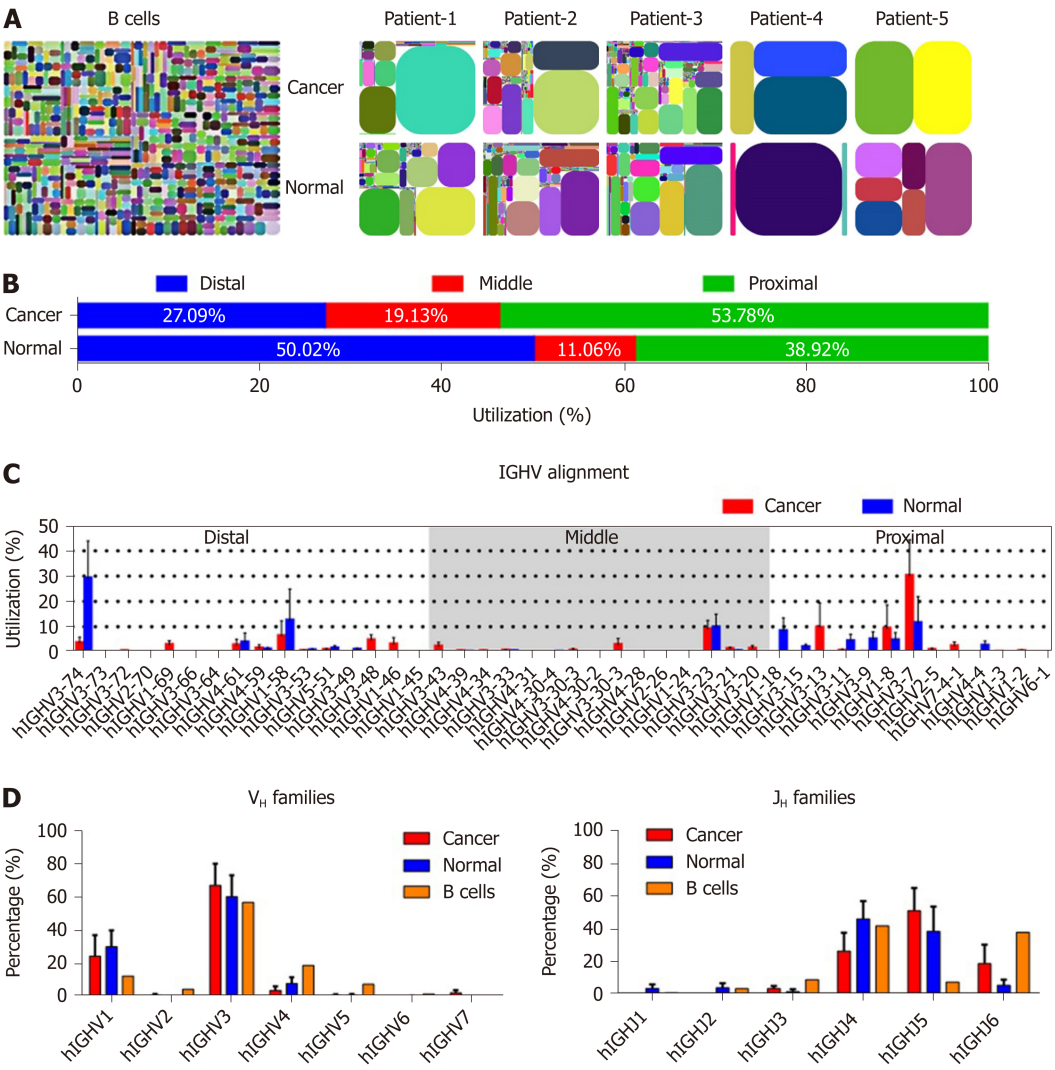


**Figure 2 The restricted VDJ patterns and distribution of immunoglobulin heavy chain in cancer and normal cells.** A: V-J-CDR3 map of immunoglobulin heavy chain (IgH) expressed in normal B cells[26], colon cancer cells and normal epithelial cells. Each rectangle represents a unique V-J-CDR3 nucleotide sequence and the size denotes its relative frequency. Colors for each rectangle are chosen randomly and, thus, do not match between plots; B: The distribution of $V_H$s expressed in cancer and normal epithelial cells; C: The utilizations of $V_H$s in cancer and normal epithelial cells. The order of $V_H$s on the X-axis corresponds to its position on a chromosome; D: Utilizations of 7 $V_H$ and 6 $J_H$ families in cancer and normal cells from patients with colon cancer (red and blue columns), and B cells from peripheral blood of a healthy donor[28] (orange columns). Small horizontal lines indicate the mean ± SEM. All data comparing cancer with normal cells were determined by the two-tailed unpaired Student's *t*-test and none of the differences were significant ($P > 0.05$). The percentage of $V_H$ and $J_H$ families in B cells (D) was derived from the data from other sources[28]; thus, statistical analysis was not performed.

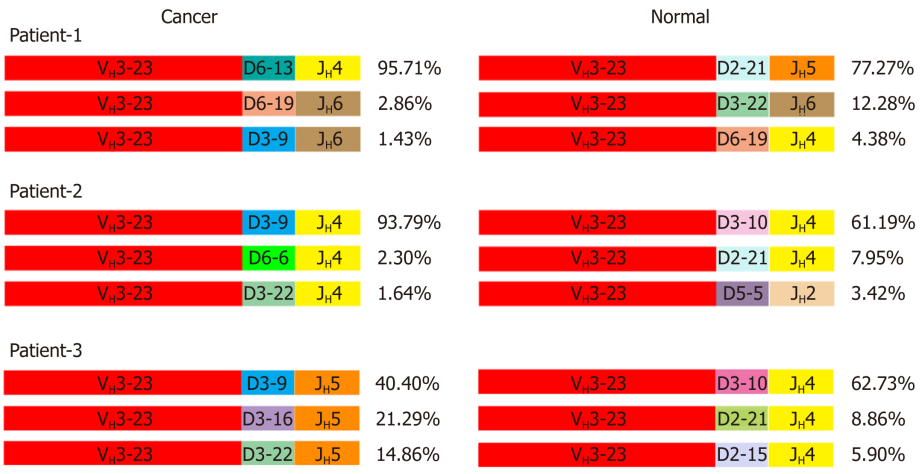


**Figure 3 Different $V_H$3-23/D/$J_H$ usages in cancer and normal cells.** The top three $V_H$3-23/D/$J_H$ and their percentage to total $V_H$3-23 rearrangements in cancer cells and normal epithelial cells of the first 3 patients. The same color represents the same V, D or J segment.

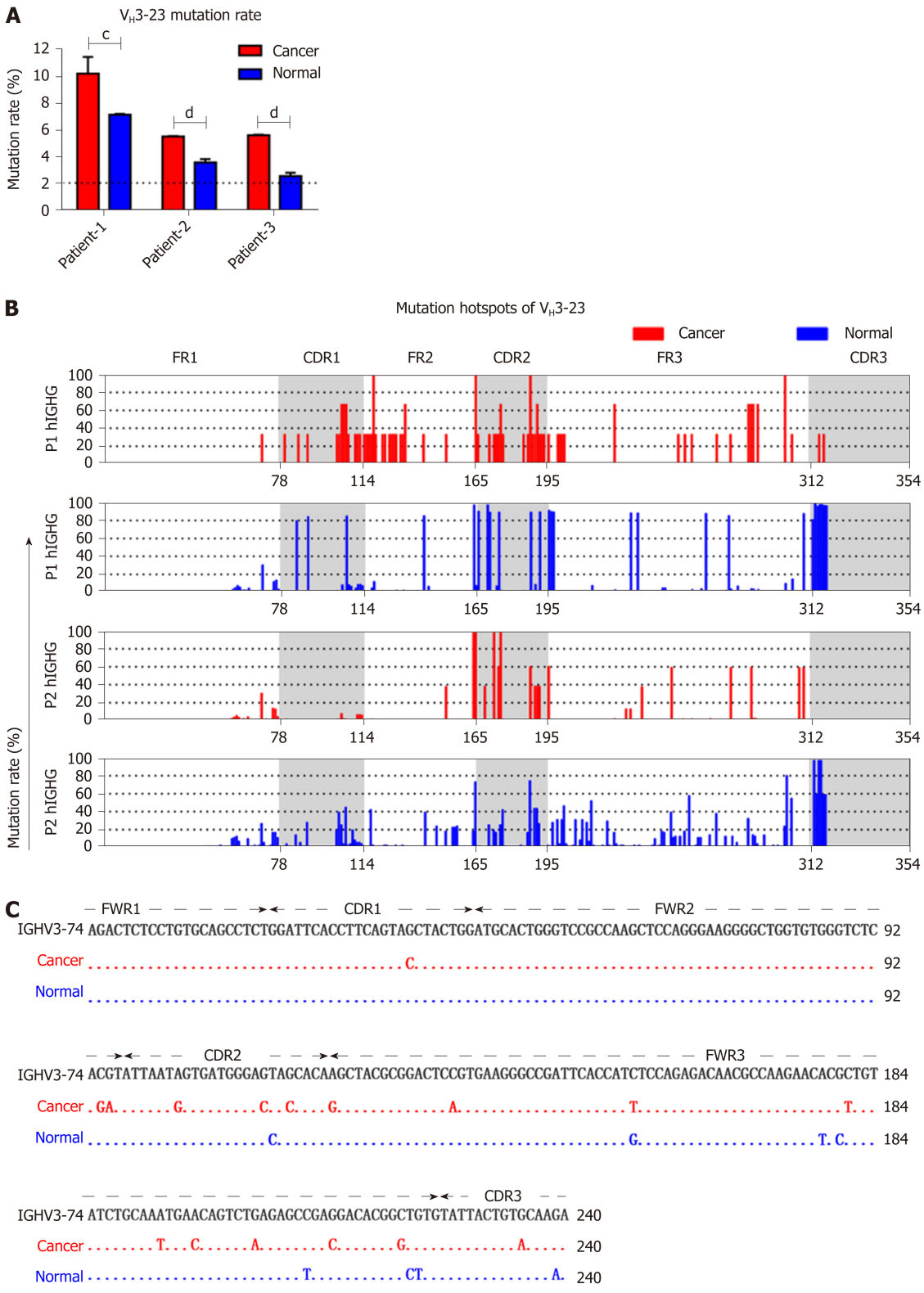


**Figure 4 Somatic hypermutation in colon cancer and normal epithelial cells.** A: Mutation rates of $V_H$3-23 in cancer and normal cells. Dotted lines represent the cut-off value 2%; B: Mutant positions and corresponding frequencies of $V_H$3-23 in different patients. P1: Patient-1. P2: Patient-2. The X-axis represents the 1st to 354th nucleotides using IMGT-numbering; C: Representative mutant positions of $V_H$3-74/D6-19/$J_H$4 in cancer and normal cells of patient-3 compared to the germline sequence of $V_H$3-74. Small horizontal lines indicate the mean ± SEM. All data comparing cancer with normal cells were determined by the two-tailed unpaired Student's *t*-test. $^cP < 0.001$. $^dP < 0.0001$.

## ARTICLE HIGHLIGHTS

### *Research background*

Traditionally, immunoglobulin (Ig) was believed to be only produced by B cells; however,

studies from our group and others have revealed that except B cells, most of non B cells, especially the non B cancer cells, including the colon cancer cells, can frequently express Ig (non B-Ig). According to our previous findings, cancer cell-derived IgG can significantly promote cancer initiation, progression and metastasis by promoting cancer stem cell behavior. IgG overexpression predicts poor prognosis of patients with cancer. Furthermore, comparing to the B cell-derived Ig repertoire, the non B cancer cell-derived Ig displays restricted and conservative V(D)J pattern rather than diversity. However, we do not know if the colon cancer cell-derived Ig is structurally different from its counterpart normal epithelial cell-derived Ig.

### Research motivation

In our previous work, we have found that colon cancer cells can overexpress the IgG compared to normal colonic epithelial cells, but it remains unclear if the colon cancer cell-derived Ig repertoire display unique feature compared to its counterpart normal cell-derived Ig, and whether the unique feature is potential for colon cancer target therapy.

### Research objectives

In this study, we used Ig repertoire sequencing (IR-Seq), which allows for the sequencing of millions of V(D)J sequences in parallel, to investigate the Ig repertoire features expressed in human colon cancer cells.

### Research methods

We first sorted $EPCAM^+$ colon cancer cells and $EPCAM^+$ normal colonic epithelial cells from corresponding noncancerous tissues as control. Then, using IR-Seq, the expression profile of Ig, $V_HDJ_H$ gene usage of Ig heavy chain (IgH) and somatic hypermutation (SHM) feature in Ig variable region were detected.

### Research results

We surprisingly found that comparing to the control normal cells, Ig expressed by colon cancer cells had a significant tendency to choose IgG among the five Ig classes. Furthermore, unlike B-Ig that can generate nearly great diversity, the non B-Ig from either colon cancer or normal epithelial cells showed restricted $V_HDJ_H$ rearrangement patterns. However, comparing to normal cell-derived $V_HDJ_H$ rearrangement patterns, cancer cell-derived $V_HDJ_H$ patterns displayed unique feature, including the usage of $V_H$, D and $J_H$ gene, and the SHM feature.

### Research conclusions

We found that colon cancer cells could frequently express IgG and unique IgH repertoires, which may be involved in carcinogenesis of colon cancer. The unique IgH repertoire has the potential to be used as a novel target in immune therapy for colon cancer.

### Research perspectives

These findings suggest that distinguishing the distinctive mutation sites of cancer cell-derived Ig from normal cell-derived Ig can help finding new target for precise treatment of patients with colon cancer.

## ACKNOWLEDGEMENTS

We would like to thank Xiu-Yuan Sun (Department of Immunology, School of Basic Medical Sciences, Peking University, Beijing, 100191, China), Hong-Yan Jin and Xiao-Hui Zhu (Center for Reproductive Medicine, Peking University Third Hospital; Biomedical Pioneering Innovation Center and Key Laboratory of Assisted Reproduction, Ministry of Education, Beijing, China) for assistant in cell sorting.

## REFERENCES

1 **Arnold M**, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut* 2017; **66**: 683-691 [PMID: 26818619 DOI: 10.1136/gutjnl-2015-310912]
2 **Knijn N**, Tol J, Punt CJ. Current issues in the targeted therapy of advanced colorectal cancer. *Discov Med* 2010; **9**: 328-336 [PMID: 20423677]
3 **Saltz LB**, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Lichinitser M, Yang TS, Rivera F, Couture F, Sirzén F, Cassidy J. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008; **26**: 2013-2019 [PMID: 18421054 DOI: 10.1200/JCO.2007.14.9930]
4 **Siegel RL**, Miller KD, Jemal A. Cancer Statistics, 2017. *CA Cancer J Clin* 2017; **67**: 7-30 [PMID: 28055103 DOI: 10.3322/caac.21387]
5 **Siegel RL**, Miller KD, Fedewa SA, Ahnen DJ, Meester RGS, Barzi A, Jemal A. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; **67**: 177-193 [PMID: 28248415 DOI: 10.3322/caac.21395]
6 **Teng G**, Papavasiliou FN. Immunoglobulin somatic hypermutation. *Annu Rev Genet* 2007; **41**: 107-120 [PMID: 17576170 DOI: 10.1146/annurev.genet.41.110306.130340]
7 **Jung D**, Giallourakis C, Mostoslavsky R, Alt FW. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu Rev Immunol* 2006; **24**: 541-570 [PMID: 16551259 DOI: 10.1146/annurev.immunol.23.021704.115830]

8 **Davis MM**, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988; **334**: 395-402 [PMID: 3043226 DOI: 10.1038/334395a0]

9 **Jiang D**, Ge J, Liao Q, Ma J, Liu Y, Huang J, Wang C, Xu W, Zheng J, Shao W, Lee G, Qiu X. IgG and IgA with potential microbial-binding activity are expressed by normal human skin epidermal cells. *Int J Mol Sci* 2015; **16**: 2574-2590 [PMID: 25625513 DOI: 10.3390/ijms16022574]

10 **Jing Z**, Deng H, Ma J, Guo Y, Liang Y, Wu R, A L, Geng Z, Qiu X, Wang Y. Expression of immunoglobulin G in human podocytes, and its role in cell viability and adhesion. *Int J Mol Med* 2018; **41**: 3296-3306 [PMID: 29512722 DOI: 10.3892/ijmm.2018.3525]

11 **Deng H**, Ma J, Jing Z, Deng Z, Liang Y, A L, Liu Y, Qiu X, Wang Y. Expression of immunoglobulin A in human mesangial cells and its effects on cell apoptosis and adhesion. *Mol Med Rep* 2018; **17**: 5272-5282 [PMID: 29393471 DOI: 10.3892/mmr.2018.8544]

12 **Qiu X**, Zhu X, Zhang L, Mao Y, Zhang J, Hao P, Li G, Lv P, Li Z, Sun X, Wu L, Zheng J, Deng Y, Hou C, Tang P, Zhang S, Zhang Y. Human epithelial cancers secrete immunoglobulin g with unidentified specificity to promote growth and survival of tumor cells. *Cancer Res* 2003; **63**: 6488-6495 [PMID: 14559841]

13 **Babbage G**, Ottensmeier CH, Blaydes J, Stevenson FK, Sahota SS. Immunoglobulin heavy chain locus events and expression of activation-induced cytidine deaminase in epithelial breast cancer cell lines. *Cancer Res* 2006; **66**: 3996-4000 [PMID: 16618718 DOI: 10.1158/0008-5472.CAN-05-3704]

14 **Chen Z**, Gu J. Immunoglobulin G expression in carcinomas and cancer cell lines. *FASEB J* 2007; **21**: 2931-2938 [PMID: 17475920 DOI: 10.1096/fj.07-8073com]

15 **Zheng H**, Li M, Ren W, Zeng L, Liu HD, Hu D, Deng X, Tang M, Shi Y, Gong J, Cao Y. Expression and secretion of immunoglobulin alpha heavy chain with diverse VDJ recombinations by human epithelial cancer cells. *Mol Immunol* 2007; **44**: 2221-2227 [PMID: 17174398 DOI: 10.1016/j.molimm.2006.11.010]

16 **Lv WQ**, Peng J, Wang HC, Chen DP, Yang Y, Zhao Y, Qiu XY, Jiang JH, Li CY. Expression of cancer cell-derived IgG and extra domain A-containing fibronectin in salivary adenoid cystic carcinoma. *Arch Oral Biol* 2017; **81**: 15-20 [PMID: 28460248 DOI: 10.1016/j.archoralbio.2017.04.010]

17 **Li M**, Feng DY, Ren W, Zheng L, Zheng H, Tang M, Cao Y. Expression of immunoglobulin kappa light chain constant region in abnormal human cervical epithelial cells. *Int J Biochem Cell Biol* 2004; **36**: 2250-2257 [PMID: 15313470 DOI: 10.1016/j.biocel.2004.03.017]

18 **Liu Y**, Liu D, Wang C, Liao Q, Huang J, Jiang D, Shao W, Yin CC, Zhang Y, Lee G, Qiu X. Binding of the monoclonal antibody RP215 to immunoglobulin G in metastatic lung adenocarcinomas is correlated with poor prognosis. *Histopathology* 2015; **67**: 645-653 [PMID: 25753759 DOI: 10.1111/his.12686]

19 **Sheng Z**, Liu Y, Qin C, Liu Z, Yuan Y, Hu F, Du Y, Yin H, Qiu X, Xu T. IgG is involved in the migration and invasion of clear cell renal cell carcinoma. *J Clin Pathol* 2016; **69**: 497-504 [PMID: 26519488 DOI: 10.1136/jclinpath-2015-202881]

20 **Li M**, Zheng H, Duan Z, Liu H, Hu D, Bode A, Dong Z, Cao Y. Promotion of cell proliferation and inhibition of ADCC by cancerous immunoglobulin expressed in cancer cell lines. *Cell Mol Immunol* 2012; **9**: 54-61 [PMID: 22036905 DOI: 10.1038/cmi.2011.40]

21 **Liang PY**, Li HY, Zhou ZY, Jin YX, Wang SX, Peng XH, Ou SJ. Overexpression of immunoglobulin G prompts cell proliferation and inhibits cell apoptosis in human urothelial carcinoma. *Tumour Biol* 2013; **34**: 1783-1791 [PMID: 23483488 DOI: 10.1007/s13277-013-0717-z]

22 **Jiang C**, Huang T, Wang Y, Huang G, Wan X, Gu J. Immunoglobulin G expression in lung cancer and its effects on metastasis. *PLoS One* 2014; **9**: e97359 [PMID: 24853685 DOI: 10.1371/journal.pone.0097359]

23 **Liao Q**, Liu W, Liu Y, Wang F, Wang C, Zhang J, Chu M, Jiang D, Xiao L, Shao W, Sheng Z, Tao X, Huo L, Yin CC, Zhang Y, Lee G, Huang J, Li Z, Qiu X. Aberrant high expression of immunoglobulin G in epithelial stem/progenitor-like cells contributes to tumor initiation and metastasis. *Oncotarget* 2015; **6**: 40081-40094 [PMID: 26472025 DOI: 10.18632/oncotarget.5542]

24 **Tang J**, Zhang J, Liu Y, Liao Q, Huang J, Geng Z, Xu W, Sheng Z, Lee G, Zhang Y, Chen J, Zhang L, Qiu X. Lung squamous cell carcinoma cells express non-canonically glycosylated IgG that activates integrin-FAK signaling. *Cancer Lett* 2018; **430**: 148-159 [PMID: 29778566 DOI: 10.1016/j.canlet.2018.05.024]

25 **Zheng J**, Huang J, Mao Y, Liu S, Sun X, Zhu X, Ma T, Zhang L, Ji J, Zhang Y, Yin CC, Qiu X. Immunoglobulin gene transcripts have distinct VHDJH recombination characteristics in human epithelial cancer cells. *J Biol Chem* 2009; **284**: 13610-13619 [PMID: 19293154 DOI: 10.1074/jbc.M809524200]

26 **Lee YN**, Frugoni F, Dobbs K, Tirosh I, Du L, Ververs FA, Ru H, Ott de Bruin L, Adeli M, Bleesing JH, Buchbinder D, Butte MJ, Cancrini C, Chen K, Choo S, Elfeky RA, Finocchi A, Fuleihan RL, Gennery AR, El-Ghoneimy DH, Henderson LA, Al-Herz W, Hossny E, Nelson RP, Pai SY, Patel NC, Reda SM, Soler-Palacin P, Somech R, Palma P, Wu H, Giliani S, Walter JE, Notarangelo LD. Characterization of T and B cell repertoire diversity in patients with RAG deficiency. *Sci Immunol* 2016; **1**: eaah6109 [PMID: 28783691 DOI: 10.1126/sciimmunol.aah6109]

27 **Matsuda F**, Ishii K, Bourvagnet P, Kuma Ki, Hayashida H, Miyata T, Honjo T. The complete nucleotide sequence of the human immunoglobulin heavy chain variable region locus. *J Exp Med* 1998; **188**: 2151-2162 [PMID: 9841928 DOI: 10.1084/jem.188.11.2151]

28 **Nzula S**, Going JJ, Stott DI. Antigen-driven clonal proliferation, somatic hypermutation, and selection of B lymphocytes infiltrating human ductal breast carcinomas. *Cancer Res* 2003; **63**: 3275-3280 [PMID: 12810659]

29 **Ghiotto F**, Marcatili P, Tenca C, Calevo MG, Yan XJ, Albesiano E, Bagnara D, Colombo M, Cutrona G, Chu CC, Morabito F, Bruno S, Ferrarini M, Tramontano A, Fais F, Chiorazzi N. Mutation pattern of paired immunoglobulin heavy and light variable domains in chronic lymphocytic leukemia B cells. *Mol Med* 2011; **17**: 1188-1195 [PMID: 21785810 DOI: 10.2119/molmed.2011.00104]

30 **Hu F**, Zhang L, Zheng J, Zhao L, Huang J, Shao W, Liao Q, Ma T, Geng L, Yin CC, Qiu X. Spontaneous production of immunoglobulin M in human epithelial cancer cells. *PLoS One* 2012; **7**: e51423 [PMID: 23251529 DOI: 10.1371/journal.pone.0051423]

31 **Shao W**, Hu F, Ma J, Zhang C, Liao Q, Zhu Z, Liu E, Qiu X. Epithelial cells are a source of natural IgM that contribute to innate immune responses. *Int J Biochem Cell Biol* 2016; **73**: 19-29 [PMID: 26820901 DOI: 10.1016/j.biocel.2016.01.017]

32 **Zheng H**, Li M, Liu H, Ren W, Hu DS, Shi Y, Tang M, Cao Y. Immunoglobulin alpha heavy chain derived from human epithelial cancer cells promotes the access of S phase and growth of cancer cells. *Cell Biol Int* 2007; **31**: 82-87 [PMID: 17074514 DOI: 10.1016/j.cellbi.2006.09.009]

33 **Liu HD**, Zheng H, Li M, Hu DS, Tang M, Cao Y. Upregulated expression of kappa light chain by Epstein-Barr virus encoded latent membrane protein 1 in nasopharyngeal carcinoma cells via NF-kappaB and AP-1

pathways. *Cell Signal* 2007; **19**: 419-427 [PMID: 16979873 DOI: 10.1016/j.cellsig.2006.07.012]
34 **Liu Y**, Chen Z, Niu N, Chang Q, Deng R, Korteweg C, Gu J. IgG gene expression and its possible significance in prostate cancers. *Prostate* 2012; **72**: 690-701 [PMID: 22430367 DOI: 10.1002/pros.21476]
35 **Qiu Y**, Korteweg C, Chen Z, Li J, Luo J, Huang G, Gu J. Immunoglobulin G expression and its colocalization with complement proteins in papillary thyroid cancer. *Mod Pathol* 2012; **25**: 36-45 [PMID: 21909078 DOI: 10.1038/modpathol.2011.139]
36 **Lee G**, Laflamme E, Chien CH, Ting HH. Molecular identity of a pan cancer marker, CA215. *Cancer Biol Ther* 2008; **7**: 2007-2014 [PMID: 19158477 DOI: 10.4161/cbt.7.12.6984]
37 **Lee G**. Cancer cell-expressed immunoglobulins: CA215 as a pan cancer marker and its diagnostic applications. *Cancer Biomark* 2009; **5**: 137-142 [PMID: 19407368 DOI: 10.3233/CBM-2009-0610]
38 **Lee G**, Ge B. Cancer cell expressions of immunoglobulin heavy chains with unique carbohydrate-associated biomarker. *Cancer Biomark* 2009; **5**: 177-188 [PMID: 19729827 DOI: 10.3233/CBM-2009-0102]

**P- Reviewer:** Ashley S, Cappuzzo F, Ishibashi H
**S- Editor:** Wang JL **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 208-226

DOI: 10.4251/wjgo.v11.i3.208

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

# Post-operative computed tomography scan – reliable tool for quality assessment of complete mesocolic excision

Cristian Livadaru, Stefan Morarasu, Tudor Cristian Frunza, Florina A Ghitun, Elena Florina Paiu-Spiridon, Florina Sava, Cristina Terinte, Dan Ferariu, Sorinel Lunca, Gabriel Mihail Dimofte

**ORCID number:** Cristian Livadaru (0000-0002-5872-4058); Stefan Morarasu (0000-0001-7767-0975); Tudor Cristian Frunza (0000-0002-4656-5360); Florina A Ghitun (0000-0003-2753-0493); Elena Florina Paiu-Spiridon (0000-0001-8916-7373); Florina Sava (0000-0001-6062-0066); Cristina Terinte (0000-0003-0434-9069); Dan Ferariu (0000-0003-2029-8652); Sorinel Lunca (0000-0002-9749-0610); Gabriel Mihail Dimofte (0000-0002-7839-9512).

**Author contributions:** Dimofte GM and Livadaru C conceived the original idea, elaborated the study design and generated the manuscript up to its final form; Livadaru C, Ghitun FA, Morarasu S, Frunza TC, Paiu-Spiridon EF, Sava F, Terinte C, Ferariu D and Lunca S, each provided significant input in documentation, bibliography research, acquisition of data and statistical analysis; Livadaru C, Paiu-Spiridon EF and Sava F performed the radiological measurements and data interpretation; Dimofte GM, Morarasu S, Frunza TC and Lunca S performed the surgeries; Terinte C and Ferariu F performed the pathological examination; Livadaru C and Ghitun FA wrote the initial manuscript, with Morarasu S, Frunza TC, Paiu-Spiridon EF, Sava F, Terinte C, Ferariu D, Lunca S and Dimofte GM providing essential intellectual content and thorough comments; Livadaru C, Morarasu S, Frunza TC, Ghitun FA, Paiu-Spiridon EF,

**Cristian Livadaru, Florina A Ghitun,** "Grigore T Popa" University of Medicine and Pharmacy, Iasi 700115, Romania

**Stefan Morarasu, Tudor Cristian Frunza, Sorinel Lunca, Gabriel Mihail Dimofte,** 2nd Clinic of Surgical Oncology, Regional Oncology Institute, "Grigore T Popa" University of Medicine and Pharmacy, Iasi 700115, Romania

**Elena Florina Paiu-Spiridon, Florina Sava,** Department of Radiology, Regional Oncology Institute, "Grigore T Popa" University of Medicine and Pharmacy, Iasi 700115, Romania

**Cristina Terinte, Dan Ferariu,** Department of Pathology, Regional Oncology Institute, "Grigore T Popa" University of Medicine and Pharmacy, Iasi 700115, Romania

**Corresponding author:** Stefan Morarasu, MD, Surgeon, 2nd Clinic of Surgical Oncology, Regional Oncology Institute, "Grigore T Popa" University of Medicine and Pharmacy, 2-4 General Henri Mathias Berthelot St., Iasi 700115, Romania. stefan.morarasu@hse.ie
**Telephone:** +35-385-8760635

## Abstract

### *BACKGROUND*

Quality control in colon cancer surgery is an ongoing debate ever since standardization proved to be highly efficient in improving survival in rectal cancer. Complete mesocolic excision (CME) is widely acclaimed as the new gold-standard in colon cancer resections, thus it is imperative to establish quality criteria of CME in order to make it easily understood and verified by surgeons worldwide. One simple and reproducible tool could be the measurement of arterial stumps postoperatively and a straightforward way to test its reliability is to test it in a comparative study between CME and non-CME surgery.

### *AIM*

To validate arterial stump measurement as a surgical quality tool by comparing CME with conventional radical colectomies.

### *METHODS*

This was a retrospective study, carried out on a prospective database. We collected data from two groups of patients, divided according to standard CME with D2 central vascular ligation (group A) and non-standardized surgery (group B). The two groups were compared with regard to the arterial stump length after right- and left-sided colectomies for colon cancer. The actual stump lengths of the

Sava F, Terinte C, Ferariu D, Lunca S and Dimofte GM provided the final critical review and approval of the manuscript.

**Institutional review board statement:** The Regional Oncology Institute's Institutional Review Board approved this study (No. 16321/20.08.2018).

**Conflict-of-interest statement:** The authors declare no conflicts of interest.

**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.



**Manuscript source**: Invited manuscript

**Received:** October 2, 2018
**Peer-review started:** October 2, 2018
**First decision:** November 15, 2018
**Revised:** December 6, 2018
**Accepted:** January 9, 2019
**Article in press:** January 10, 2019
**Published online:** March 15, 2019

ileocolic artery (ICA) and inferior mesenteric artery (IMA) were compared with their theoretical best D2 position of predicted ligation levels ($D_2$PLLs) for calculating the potential for improvement. Measurements on follow-up computed tomography scans were carried out by three observers. Pathological data were recorded (specimen length, lymph node yield) and correlated with stump length.

*RESULTS*

We analysed 58 colectomies. The stump lengths (mean ± SD) in group A were 16.97 ± 4.77 mm for ICA and 31.70 ± 15.71 mm for IMA, whereas group B had 49.93 ± 20.29 mm for ICA and 67.24 ± 28.71 mm for IMA. Shorter lengths were obtained in group A, by a mean difference of 35.66 mm ($\chi^2$ = 27.38, $P$ < 0.001), which was significant for all types of colectomies. Except for a 5.85 ± 4.71 mm difference for right colectomies, all the ligations from group A significantly reached their potential height (0.26 ± 12.18 mm from $D_2$PLL; $\chi^2$ = 0.005, $P$ = 0.944). Apart from three left colectomies, group B failed to reach $D_2$PLL, by a mean difference of 32.14 ± 26.15 mm ($\chi^2$ = 21.77, $P$ < 0.001). The calculated improvement potentials were significantly shorter in group A than in group B, by a mean of 31.88 mm ($\chi^2$= 22.13, $P$ < 0.001). The large spread of results in group B showed that there is significant variability ($P$ = 0.004) when compared to standard surgery. Significant correlations were found between stump length, specimen length and number of lymph nodes ($P$ = 0.018 and $P$ = 0.008 respectively). No statistical difference was found between observers' measurements ($P$ = 0.866).

*CONCLUSION*

Arterial stump monitoring is a significant step in defining surgical quality, as longer stumps contain residual mesocolic tissue and correlate with major prognostic factors.

**Key words:** Complete mesocolic excision; Central vascular ligation; Colon surgery; Arterial stump measurement; Computed tomography



**Core tip:** This is the first study to assess arterial stump measurement as a tool for comparative analysis between complete mesocolic excision and non-standardized colonic surgery. We demonstrated its benefit as a tool for evaluating surgical quality, mainly due to its correlation with a major prognostic factor. It is a simple and reproducible measurement on routine computed tomography scans without metallic markers. We showed that the central vascular ligation can be performed without extensive D3 lymphatic dissection.

**Citation:** Livadaru C, Morarasu S, Frunza TC, Ghitun FA, Paiu-Spiridon EF, Sava F, Terinte C, Ferariu D, Lunca S, Dimofte GM. Post-operative computed tomography scan – reliable tool for quality assessment of complete mesocolic excision. *World J Gastrointest Oncol* 2019; 11(3): 208-226
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/208.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.208

## INTRODUCTION

The surgical treatment of colon cancer is the only treatment with a curable visa. Complete mesocolic excision (CME), as Hohenberger *et al*[1] initially presented it in 2009, is based on three principles. First and foremost, the mobilization of the colon needs to strictly respect the avascular plane of dissection; this ensures removal of the colonic tumour together with a sealed, embryologically-defined mesocolon, which contains all lymphovascular potential routes of tumour dissemination. The second principle refers to the central vascular ligation (CVL), which ensures vertical removal of the entire mesocolic root and apical lymph nodes (LNs). The third principle involves clearing the LNs in the pericolic station, with the advised longitudinal resection margin being up to 5 cm proximally and 10 cm distally from the tumour,

depending on the feeding vessels[1,2].

CME has proved to be a reliable technique for standardizing colon surgery; however, the debate on defining the recommended point of CVL has not reached unanimity[2-5]. Studies that comparatively analysed CME with conventional surgery showed more surgical complications in CME. Although no randomized trials exist to support this, it seems that attempting a true central ligation causes extra risks, unlike the fascial plane dissection[6-8]. Complete mesenterectomy at the root of the superior mesenteric artery (SMA) poses danger for splanchnic nerves laceration, which will cause refractory diarrhoea[6]. Significantly more injuries to the spleen and the superior mesenteric vein (SMV) have been correlated to central ligation[7], and the high variability of venous collaterals giving rise to the gastrocolic trunk of Henle has been shown to add further haemorrhagic risk[8,9]. On the left side, ligation of the inferior mesenteric artery (IMA) in proximity to the aorta can ensue permanent urological disorders and faecal incontinence in some patients, while sexual dysfunction persists in most patients[2,10,11].

Recently, the theory of tailoring central ligation to the crossing variations between the ileocolic artery (ICA) and SMV has been raised[12,13]. Additionally, the recommendation of a 10 mm safe margin from the arterial origin has been put forward in the CME consensus[2]; although, this stump length could also be obtained when performing a D2 lymphadenectomy, considering that the ICA runs in front of the SMV[13]. Further insight was given by Japanese research groups, who stated that the lymphatic drainage of the right colon ends in front of the SMV, making dissection beyond it unwarranted. They advocate for a full clearance alongside the anterior aspect of the SMV, without reaching beyond it[2].

Regardless of the continuing debate, some surgical societies have understood the value of standardization and implemented the central ligation as a guideline procedure; these include the French Society of Digestive Surgery[14] and the American Society of Colon and Rectal Surgeons[15,16]. Furthermore, the Japanese recommend at least D2 resections for cT2 colon cancer[17]. The Norwegian Health Directive expects a standard CME with complete D2 dissection to be performed on all interventions with curative intent and advises additional D3 lymphadenectomy whenever possible[18], by following the evidence on increased 5-year overall survival from 82.1% to 89.1% as reported by Hohenberger *et al*[1].

Nevertheless, if the surgery does not closely respect the embryologic cleavage plan, contamination with malign cytology through the disrupted mesocolon could render null any potential benefit of the central ligation[1]. Consequently, analysing surgical specimens is crucial for evaluating the quality of CME. In this way, the integrity of the mesocolon and the number of LNs can be accurately measured for stratifying the survival outcomes, as the difference between mesocolic plane and muscularis propria planes of resection translates to a 27% increase in 5-year overall survival for stage III disease[19]. Another indicator, proposed by West *et al*[20,21], is measurement of the distance from the CVL to the bowel margin; however, this does not directly reflect the residual stump length and accompanying LNs because the main pedicle's length is variable and thus the arterial stump may be longer than the pathologist suggests[22,23].

As a shorter arterial stump would imply fewer residual nodes, Hohenberger *et al*[24] applied angiography to patients with local recurrence of colorectal cancer to visualize the residual IMA. Thus, they appreciated that long stumps illustrated an incomplete primary lymphadenectomy which had caused the local cancer recurrence[24]. Recent studies, however, proposed a simpler technique to evaluate the point of CVL—by measuring the arterial stumps on post-operative computed tomography (CT) scans[22,25,26]. Their results showed promise in evaluating the quality of colon resections, thus calling for further studies to be made for validation of the technique.

### Aim

Our aim was to analyse the existing variation in the technique of radical colectomy and to demonstrate the utility of the arterial stump as a tool for evaluating surgical quality. Our first objective was to compare the arterial stump lengths from our own CME series without local recurrences[27], with a different series of conventional radical colectomies. Our second objective was to calculate the improvement potential in each group, by comparing the actual lengths with their theoretical best position of D2 and D3 predicted ligation levels ($D_2$PLL and $D_3$PLL respectively).

## MATERIALS AND METHODS

### Design and setting

This study was designed as a retrospective analysis and conducted on a prospectively

maintained database of patients treated at the Regional Oncology Institute (IRO) in Iasi, Romania.

### Patients

The study was approved by the Institutional Ethics Committee of the IRO, and patients had given consent for inclusion in the research database.

### Inclusion criteria

Two groups of adult patients (groups A and B, see below) were included in consecutive order. All underwent surgery with curative intent for colon cancer (stages I-III UICC 7$^{th}$ edition) and had at least one post-operative good quality contrast-enhanced CT scan that was available for re-evaluation.

Group A patients were operated on at the 2$^{nd}$ Surgery Department of IRO between the dates of March 2012 and February 2018; all surgeries were performed by one surgical team. Group B patients were operated on in other surgical units between the dates of July 2007 and February 2018, and are currently being followed up at the Medical Oncology Outpatient Clinic in our institution.

Exclusion criteria for both groups were stage IV colonic tumours, palliative colectomies, and tumours in the transverse colon.

### Surgical technique

Colectomies in group A patients were performed according to CME principles with the following adjustments to the high vascular ligation site: (1) Ligation of the ICA at the right side of the SMV for right colectomies (D2 resection); (2) Ligation of the IMA at roughly 1 cm from its origin in left colectomies; or (3) Low ligation of the IMA in segmental sigmoidectomy, distal to the branching point of the left colic artery (LCA) but with subsequent LN dissection along the IMA trunk, up to its origin from the aorta (D3 lymphadenectomy).

Group B colectomies were non-CME surgical procedures, but were performed with intent of oncological radicality. Surgeons involved in this group represent a heterogenous group working in district hospitals without a major interest in colonic cancer surgery. There was no additional selection, except for availability of the post-operative CT scan and curative intent of the surgical procedure.

### Data collection

The individual data for group A patients were gathered from a prospectively maintained electronic database, containing all the patients who had undergone colectomies performed by the same surgical team. The individual data for group B patients were obtained from the registries of the Outpatient Clinic of Medical Oncology at IRO. All these patients were cross-referenced in the hospital's electronic registry, for confirmation of data. Only patients with an available post-operative abdominal CT scan were included in the study and the images were retrieved from the Picture Archiving and Communication System (known as "PACS") for analysis.

### Radiological examination protocol

The majority of patients ($n$ = 51) had undergone spiral CT scans in our radiology department, by means of a BrightSpeed 16SL scanner with 16 detectors (GE Healthcare, Waukesha, WI, United States). The protocol included 16 mm x 1.25 mm increment, pitch of 1.75, table speed of 35 mm/rot, 120 kV and 260 mA, with slight variations according to patient particularities. Contrast media was injected (816.5 mg/mL of Iomeron 400®; Bracco Imaging, Milano, Italy) with a power injector at a rate of 2.5 mL/s. Scans were obtained in the arterial and venous phases, with an acquisition delay of 15 sec and 50 sec after the bolus threshold (150 HU). Datasets were reconstructed with a 1.25–3 mm section thickness, and the reconstruction interval varied between 1.3 mm and 5 mm. A minority of CTs ($n$ = 7) originated in private radiology departments, but biases were minimised as the acquisition protocols and reconstruction intervals were similar and only the reconstructed thickness varied from 1.25 mm to 5 mm, accepted as standard for colorectal follow-up.

### Measurement technique

No metal clips were used on the arterial stumps. We examined the CT scans from both groups using a dedicated radiology imaging software (RadiAnt DICOM Viewer v. 4.2.1; Medixant, Poznan, Poland) with 3D reconstructions in multiple planes and a maximum intensity projection of 10 mm. Lengths were measured with the software's dedicated ruler[28].

On the right side, we only chose the ICA because it is the most constant collateral of the SMA[9]. It was defined as the last vessel arising from the SMA to the right side, at the level of the 5$^{th}$ and 6$^{th}$ jejunal artery emergence, and its trajectory is anterior,

inferior and to the right, thus always making an acute angle with the SMA stem[29]. The IMA was defined as originating at the level of the 3rd lumbar vertebral body and descending on a discrete left trajectory[30]. Arterial lumen continuity could be verified by applying negative contrast in different oblique planes, and the stumps were frequently identified through the presence of a granuloma on their tip[26]. Additionally, the anatomical crossing of the ICA (anteriorly or posteriorly) in relation to the SMV was registered.

We measured the presumed and actual arterial stump lengths on all the colectomies included in the study. In right colectomies, the actual arterial length was measured as the length from the origin of the ICA to the ligation point. The presumed arterial length was measured as the length from the origin of ICA to the right-hand side of the SMV, as this is the standard length for D2 ligation (Figure 1). In left-sided colectomies, the ligation point was analysed in relation to the IMA and the LCA. The actual stump length was measured from the origin of the IMA to the ligation point, while the presumed stump length was measured from the IMA origin to immediately distal to the LCA origin, as is done for standard D2 ligation with preservation of the LCA. The D2 improvement potential ($D_2$IP) was defined as the difference between actual stump lengths and the $D_2$PLL (Figure 1). When we compared this with the D3 central ligation, a value of 10 mm was considered as the presumed stump length in left colectomies[2]. The D3 improvement potential ($D_3$IP) was calculated only for the IMA and represents the difference between actual stump lengths and the 10 mm safe length ($D_3$PLL) recommended by consensus[2] (Figure 1).

Measurements were performed by the main author (Livadaru C) and validated independently by two radiologists (Paiu-Spiridon EF and Sava F) from the Radiology Department of IRO, in order to access inter-observer variations. The quantitative parameter (arterial length) was expressed as the average between the three observers.

### Statistical analysis

Statistical analysis was carried out with SPSS v20.0 (IBM Corp, Armonk, NY, United States). Descriptive statistics were performed on all collected data. For verifying the normal distribution of all the data, we used the Shapiro-Wilk test. Only homogenous data were averaged. The inter-observer agreement between the three sets of measurements was confirmed using the Kruskal-Wallis test. We applied the independent Student's *t*-test for comparing the mean arterial length between groups A and B. We used the paired *t*-test to verify whether or not the actual residual lengths of the arteries differed from the D2 and D3 presumed lengths. Furthermore, by using the paired *t*-test we calculated a specific value that quantified the potential for improvement of the ligation height (defined as the statistically significant difference between the mean actual and presumed stump length). By using the independent *t*-test, we also compared the potentials for improvement found in each group. Levene's test was used to compare the actual stump length variation between groups. Whenever the data showed a non-normal distribution, non-parametric tests such as the Kruskal-Wallis were used. A *P*-value of $< 0.05$ was considered statistically significant for all tests.

## RESULTS

From the 124 consecutively registered patients in group A, 29 colectomies were selected after applying the exclusion criteria. Similarly, for group B, the search in the Oncology Outpatient Clinic registries provided 69 consecutive cases, from which 29 colectomies were selected after applying the inclusion criteria (Figure 2). When stratified for type of colectomy, the stump measurements were normally distributed (Shapiro-Wilk's test), but overall, when all measurements were considered for each group, they were non-normally distributed - a fact explained by the differences in category between the ICA and IMA. The median time from surgery to post-operative CT scan was 13.5 mo [inter-quartile range (IQR): 6.0-27.0]. The two groups had similar mean time intervals from surgery to the CT scan (18 mo *vs* 27 mo; $t = -1.23$, $P = 0.227$). Patient demographics are listed in Table 1.

Overall, there were a total of 22 ICA stumps, with the $D_2$PLL median length of 9.97 mm (IQR: 7.85-12.70), and a total of 36 IMA stumps, with the $D_2$PLL mean ± SD length of 37.87 ± 8.22 (95%CI: 35.09-40.65). The level of inter-observer agreement assessed for the three observers with the Kruskal-Wallis test revealed no significant difference between the sets (Table 2, $P = 0.999$) (Figure 3).

### Comparison between groups A and B

Overall, the groups differed significantly (Table 2, $P < 0.001$) in actual stump length (-35.66 ± 30.83 mm; $P < 0.001$) and in D2 improvement potential (-31.88 ± 26.52 mm; $P <$

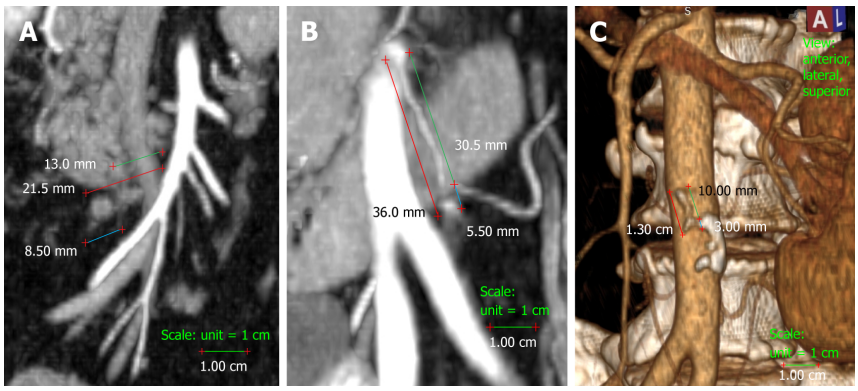


**Figure 1 Representative cases of stump length measurement.** A: Actual ICA stump after a group A right colectomy. The lengths measured on contrast-enhanced CT were 21.5 mm – actual length with granuloma (red line), 13.0 mm – D2 predicted ligation level (green line), and 8.5 mm – D2 improvement potential (blue line); B: Actual IMA stump after a group A sigmoidectomy. The lengths measured on contrast-enhanced CT were 36.0 mm – actual length with granuloma (red line), 30.5 mm – D2 predicted ligation level (green line), and 5.50 mm – D2 improvement potential (blue line); C: Reconstruction in 3D form, showing the actual inferior mesenteric artery stump after a group A high ligation. The measurements on contrast-enhanced CT were 13.0 mm – actual length (red line), 10.0 mm – D3 predicted ligation level (green line), and 3.0 mm – D3 improvement potential (blue line). The scale bar illustrates the size of 1 cm. CT: Computed tomography; ICA: Ileocolic artery; IMA: Inferior mesenteric artery.

0.001). The $D_2$PLL was similar between the two groups (-3.79 ± 13.98 mm; $P$ = 0.353), both overall (Table 3, Figure 4) and when stratified for type of colectomy (Tables 4, 5 and 6). Levene's test showed that the variances of actual stump lengths between the groups (204.5 *vs* 732.2) reached the level of statistical significance ($P$ = 0.04).

### *Right colectomies*

When we compared the actual ICA stumps between the two groups, the *t*-test showed that group A had significantly shorter stumps than group B, the difference being -32.96 ± 6.56 mm (mean ± SE) (Table 4, $P$ = 0.001). Furthermore, the $D_2$IP was still significantly longer in group B, by almost 4 cm (Table 4, $P$ < 0.001) (Figure 5).

### *Sigmoidectomies*

In Table 5, the comparison of IMA stumps between the two groups shows that, apart from $D_2$PLL, all the parameters from group A were significantly shorter, as shown by the independent *t*-test. The actual difference (mean ± SE) was -34.50 ± 9.28 mm (Table 5, $P$ < 0.001), and the $D_2$IP difference was -29.57 ± 8.01 mm (Table 5, $P$ = 0.001) while the $D_3$IP difference was -34.50 ± 9.28 mm (Table 5, $P$ < 0.001) (Figure 6).

### *Left colectomies*

This subgroup followed the same trend of significance, with shorter actual stumps in group A (by 33.33 ± 8.37 mm) (Table 6, $P$ = 0.011). The $D_2$IP appeared similar between the groups ($P$ = 0.056), but group A significantly surpassed the D2 level and achieved D3 as intended, with a $D_3$IP difference of -33.33 ± 8.37 mm (Table 6, $P$ = 0.011) (Figure 7).

Table 7 presents a summary of all the IMA stumps from this study, by taking sigmoidectomies and left colectomies together. Except for the $D_2$PLL, all the parameters in group A are significantly shorter then group B (Table 7, $P$ < 0.001) (Figure 8).

### *Comparison between actual arterial stumps and predicted ligation levels in group A*

For the 29 actual stumps in group A, the median length was 22.63 mm and the Kruskal-Wallis test showed no statistically significant D2 improvement potential (mean ± SD: 0.26 ± 12.18 mm; $\chi^2$ = 0.005, $P$ = 0.944) (Table 3).

In right colectomies, the actual ICA length (mean ± SD) was 16.97 ± 4.77 mm. The mean difference between actual stump lengths and $D_2$PLL was statistically significant, as determined by the paired *t*-test, revealing a D2 improvement potential of 5.85 ± 4.71 mm (Table 4, $P$ = 0.001).

On the left side, there were a total of 17 residual IMAs examined; the mean length was 31.70 ± 15.71 mm for which the ligation had been done correctly at the D2 level in all colectomies, without any $D_2$IP remaining (-3.68 ± 14.30 mm; $P$ < 0.304), as shown in Table 7.

When stratified for type of colectomy, the paired *t*-test revealed no $D_2$IP for sigmoidectomies (0.27 ± 12.90 mm) (Table 5, $P$ = 0.942) and left colectomies (-16.52 ±

**Table 1 Patient demographics**

| Characteristic | Group A | Group B |
|---|---|---|
| Age (yr) | | |
| Mean | 64 | 59 |
| Range | 49-80 | 40-76 |
| Sex | | |
| Male | 14 | 16 |
| Female | 15 | 13 |
| Type of colectomy | | |
| Right colectomy | 12 | 10 |
| Left-sided colectomies | 17 | 19 |
| Left colectomy | 4 | 16 |
| Sigmoidectomy | 13 | 3 |
| pT stage | | |
| pTis | 1 | 3 |
| pT2 | 3 | 17 |
| pT3 | 19 | 9 |
| pT4 | 6 | |
| pN stage | | |
| pNx | 1 | |
| pN0 | 13 | 14 |
| pN1 | 11 | 9 |
| pN2 | 4 | 6 |
| M stage | | |
| M0 | 29 | 29 |
| M1 | 0 | 0 |

11.71 mm) (Table 6, $P$ = 0.067). When we tested for the D3 ligation, only left colectomies reached this level (9.60 ± 9.21 mm) (Table 6, $P$ = 0.129), whereas sigmoidectomies fell short of this objective (by 25.42 ± 15.62 mm) (Table 5, $P$ = 0.001).

### *Comparison between actual arterial stumps and predicted ligation levels in group B*

For the 29 actual stumps in group B, the median length was 61.27 mm, while the mean potential for improvement of 32.14 ± 26.15 mm was statistically significant ($\chi^2$ = 21.77, $P$ < 0.001) (Table 3).

In right colectomies, the actual ICA length (mean ± SD) was 49.93 ± 20.29 mm. The mean difference between actual stump lengths and $D_2$PLL was statistically significant by the paired $t$-test, revealing a $D_2$IP of 42.13 ± 21.50 mm (Table 4, $P$ < 0.001).

The mean IMA stump length from 19 left-sided colectomies (sigmoidectomies and left colectomies) was 67.24 ± 28.71 mm. The paired $t$-test showed a statistically significant difference between actual and presumed lengths, showing that in group B the ligation had been made lower than the D2 level, with a mean $D_2$IP of 27.14 ± 27.74 mm (Table 7, $P$ < 0.01).

When stratified for type of colectomy, the 16 sigmoidectomies had mean IMA stumps of 69.92 ± 30.29 mm and statistically significant $D_2$IP (29.84 ± 28.67 mm) (Table 5, $P$ < 0.01) and $D_3$IP (59.92 ± 30.29 mm) (Table 5, $P$ < 0.01). The subgroup of left colectomies was the only one to output no statistically significant $D_2$IP ($P$ = 0.38), but significant $D_3$IP (42.93 ± 13.15 mm) (Table 6, $P$ = 0.03).

### *Correlations between arterial stump length and pathology*

In group A, the mean ± SD number of LNs harvested with the CME technique was 34.83 ± 16.75 and Spearman's test showed moderate ($rs$ = -0.40) inverse correlation with the stump lengths (Table 8, $P$ = 0.032). Similarly, the significance persisted for the specimen length with moderate correlation ($rs$ = -0.44); as such, the greater the specimen length was, the shorter the stump length (Table 8, $P$ = 0.016).

In group B, LNs were available in the Oncology outpatient registries only for 18 out of the 29 patients. The median number of LNs was 20 (IQR: 10.75-26.50). Spearman's correlation with the stump length was negligible ($rs$ = -0.112) and without significance ($P$ = 0.659).

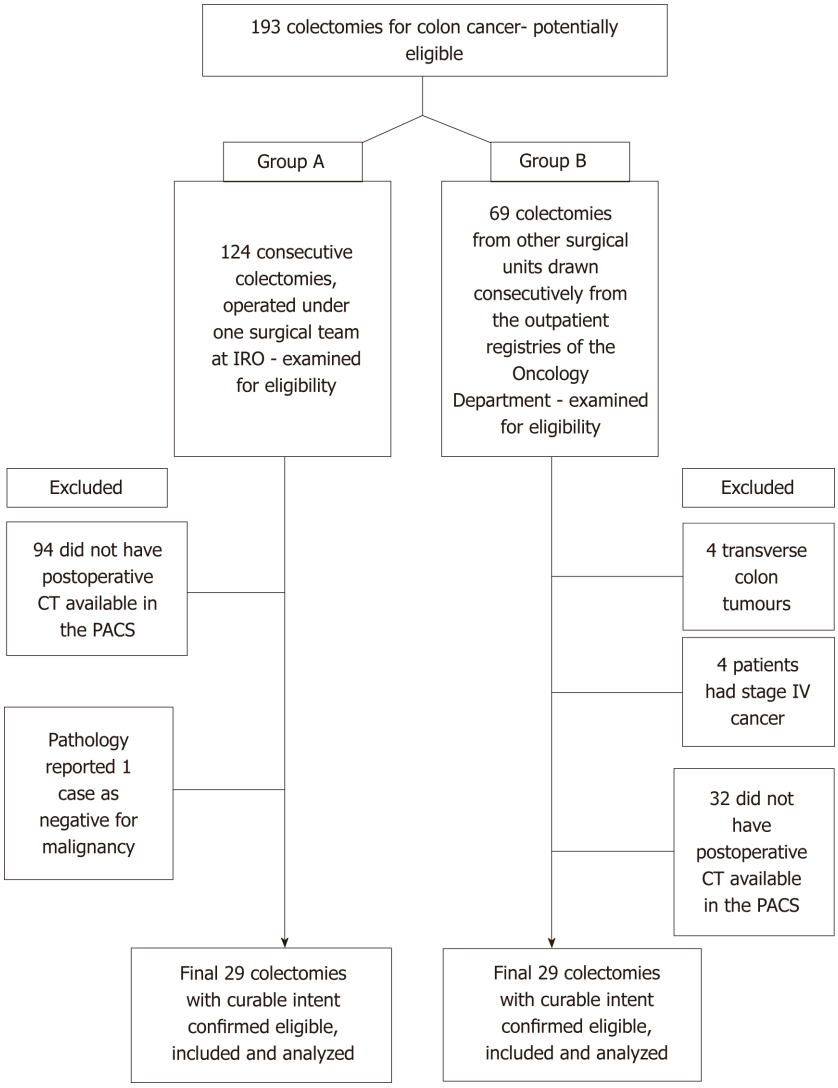


**Figure 2 Flow diagram of eligibility.** CT: Computed tomography; PACS: Picture Archiving and communication system.

Out of the 22 ileocolic stumps, the SMV was crossed posteriorly by the ICA in 68% of cases. In group A, surgeons preserved the LCA in 77% of sigmoidectomies, demonstrating a D2 ligation intent, whereas none of the left colectomies had a preserved LCA, showing D3 ligation intent. In group B, the LCA was present in 81% of sigmoidectomies and in all left colectomies, suggesting a D2 intent for sigmoidectomies and a questionable D2 intent for left colectomies.

## DISCUSSION

As far as our research into the literature has shown, this is the first study to radiologically compare post-resectional stumps between two groups, of which one provides standard resection quality and the second study, after that of Munkedal *et al*[22], assessed both ileocolic and IMA stumps after CME. The retrospective setting of our study ensured that the surgical team operated based on their regular standard, without making efforts for achieving shorter stumps. In this sense, there is the advantage of reflecting our surgical practice in a more trustworthy manner.

The tight distribution of arterial stump lengths from our series (group A) is proof of a uniform and disciplined surgical procedure (Figures 4, 5 and 8). The argument behind this statement is the 0% locoregional recurrence at a minimum of 2 years for the 88 colectomies from which group A was extracted[27]. By constantly performing the D2 ligation, rigorous harvesting of LNs with their corresponding mesocolons was achieved and, thus, contributed to a zero local recurrence rate in the larger cohort[27]. The fact that group B did not use a standardized technique was also revealed by the

**Table 2 Comparison of arterial stump length between the three observers**

| Observer | *n* | Median length, mm | IQR, mm | $\chi^2$ | [1]*P*-value |
|---|---|---|---|---|---|
| Livadaru C | 58 | 35.70 | 21.38-60.30 | 0.01 | 0.999 |
| 1st Radiologist | 58 | 34.00 | 21.50-61.63 | | |
| 2nd Radiologist | 58 | 35.00 | 20.98-62.50 | | |

[1]Reference to RESULTS text. IQR: Interquartile range.

significantly larger variation in stump size, as compared to group A ($P$ = 0.04). This probably reflects an incomplete mesocolic excision because, theoretically, the mesocolic fascia leads up to the origin of the primary feeding vessels and would result in a short stump if completely excised. This presumption will be verified in the future, but a 60-mm stump is certainly representative of an incomplete resection. Other similar studies[20,26] showed that the wider the variation in stump length, the larger the variation in fascial plane of dissection. Hence, when we have the prerequisites for a large variance in technique (*i.e*. non-standardized surgery), there is a higher risk of having low quality surgery, which impacts correct staging, local recurrence, and overall survival[19,31-34].

Although our results are comparable with the null loco-regional recurrence from the prospectively performed CME surgeries of Galizia *et al*[35] and with the 3.6% from Hohenberger and colleague's[1] series of 1329 CME colectomies with D3 CVL, the clear extra benefit of D3 *vs* D2 ligation is controversial when compared to the benefit of pristine embryologic plane dissection[3,4,36,37]. We believe that the foremost important factor for minimizing local recurrence is respecting the mesocolic plane, in order to prevent spillage of cancerous cells. Performing the central ligation in right colectomy at the D3 rather than the D2 level is not supported by any randomized trial yet[5], increasing peri-operative risks without major benefits for the general population.

All the arterial stumps were shorter in group A than group B, not only overall but also when stratified for type of colectomy. We wanted to assess if this was caused by the variability in the ICA-SMV crossing lengths, on the right side, or the LCA emerging lower down from the IMA, on the left side. Hence, we also measured the personalized presumed lengths for true D2 ligation, in each group. By subtracting the actual stumps from the presumed stumps, the D2 potential for improvement was calculated as a personal quality measurement for the surgical team. These were significantly shorter in group A, proving that across group B colectomies, the standardized D2 level of ligation was systematically not reached (Tables 4, 5, 6 and 7). The only exception was the subgroup of left colectomies that showed statistical similarity at the D2 level, even though this did not reflect similar ligation intents. Group A surgeons intended and significantly reached D3 ($P$ = 0.129), whereas in group B, the D3 potential was not reached ($P$ = 0.03) (Table 6).

The right colectomy subgroup from group A had slightly longer stumps than expected for D2 (by 5.80 mm), but they were still the shortest CT-evaluated post-colectomy stumps in the literature[22,25,26] (Table 9). Moreover, by calculating the potentials for improvement for left-sided colectomies performed in our department (0.27 mm), we determined that they are correctly realised at the D2 level ($P$ = 0.94). In contrast to other similar studies[21], our experience showed no excess IMA stumps compared to pre-determined vascular and anatomical boundaries for the D2 ligation (Table 10). The left colectomy subgroup was the only one in which the D3 level had been achieved in all cases (9.60 mm; $P$ = 0.129).

The LN yield is considered to be a very important independent prognostic factor for colon cancer survival[32-34,38]. Using our D2 CME technique, we harvested a mean number of 34.8 LNs, which proved to be superior to the number of LNs reported through the employment of CME with D3 CVL from Erlangen: a median of 32 LNs reported in the first series[1] and of 30 LNs reported in a subsequent series[20]. The length between the origin of the IMA and the departure of the LCA was 37.87 ± 8.22 mm (mean ± SD) in our study, and this segment of artery alone was previously shown to harbour up to 10 LNs[39]. Rosi *et al*[40] demonstrated that up to 22% of patients with sigmoid tumours had positive LNs at a distance of 10 to 30 mm from the origin of the IMA, thus showing that the ligation height impacted survival. Since we showed that LNs inversely correlate to the feeding stump length, their prognostic value could be transferred to some extent onto the stump length. These results aid the arguments for considering the measurement of post-colectomy arterial stumps as an additional marker for surgical quality evaluation, with the potential to impact survival.

One similar study[22] that reported longer stumps found no correlations between

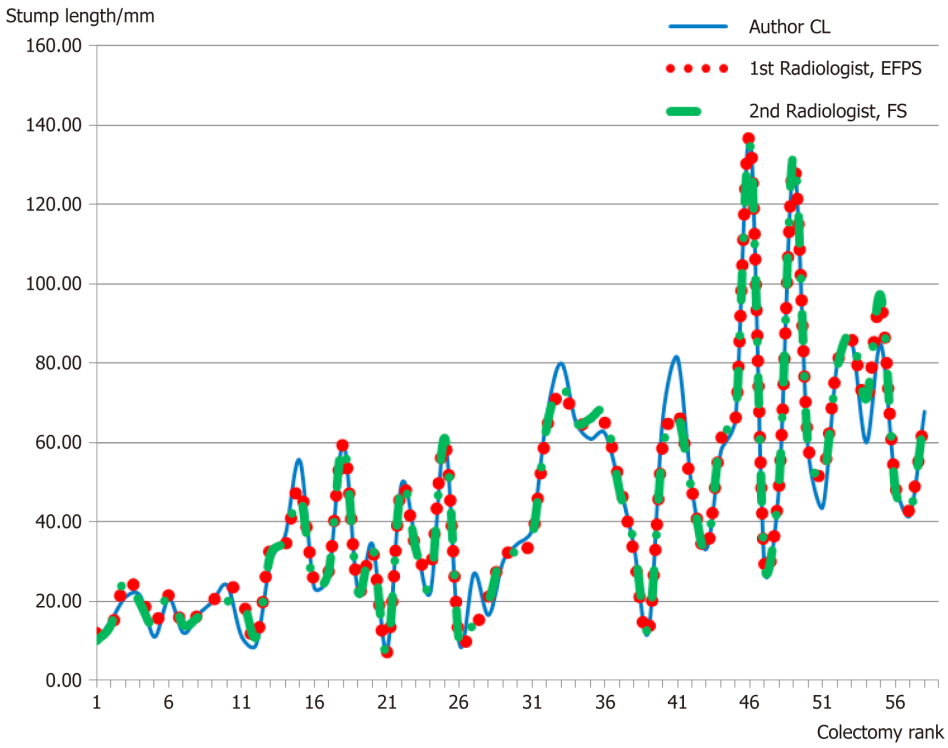


**Figure 3 Comparison of arterial stump lengths between the three observers.** CL: Cristian livadaru; EFPS: Elena florina paiu-spiridon; FS: Florina sava.

node count and stump length. In that sense it supports the argument that, a more central ligation provides a more realistic correlation with harvested LNs, assuming that a dedicated pathology department counted the LNs[27]. This impacts a more accurate staging[41] and could theoretically transfer an under-staged patient from stage II to III, in order to provide benefit not just from the adjuvant chemotherapy but also from the additional prognostic factor. Additionally, the advantage of a surgical technique that comprehensively retrieves the mesocolon ensures that significantly more of the newly described tumour deposits are being included in the specimen, when comparing CME specimens to classic colectomy specimens[35]. The prognostic capability of tumour deposits in the mesocolon is analogous to that of positive LNs, with node-negative patients in stages I or II requiring adjuvant chemotherapy[38].

A limit of our study could be the small population size, but we used strict inclusion and exclusion criteria and analysed a single surgical team with unchanging practice of surgical technique to determine the accuracy of stump measurement. Seven scans originated in different radiology departments; hence, for these, the acquisition protocols varied slightly in terms of slice reconstruction interval, but we do not believe this produced a sizeable difference. The presence of metal clips on the stump would have accelerated the identification process, but from our radiologists' viewpoint, they tend to add measuring artefacts. The first author (Livadaru C), who measured and interpreted the quantitative and qualitative parameters, is not a radiologist, but all the data were validated by two independent radiologists (Paiu-Spiridon EF and Sava F) and the inter-observer agreement test showed no statistically significant difference between the measurements of the actual stump lengths. This strong correlation between observers indicates that there is high feasibility of performing the arterial stump evaluation on follow-up contrast CTs by non-radiological observers, regardless of metal clips. Pre-operative CT scans paired with the follow-up scans would have aided the stump identification but we do not consider pre-operative scans essential, as a member of the surgical team would be able to clearly identify the desired artery based on familiarity with anatomical landmarks from the operating field.

Regarding the time interval from surgery to the post-operative CT, other authors have already shown that the stumps are clearly visible more than 2 years after surgery[26]. Even if the residual arteries could be affected by post-resectional retraction over this time, the two groups in our study had statistically similar time intervals from operation to CT scan ($P$ = 0.227), precluding it from having a major influence on our comparison.

The CME consensus study group advises a 10 mm safety stump when performing the CVL[2]. However, a recent systematic review and meta-analysis on mesenteric

**Table 3 All colectomies: Comparison between groups A and B**

| | Group A, median (mean) in mm, (IQR) | Group B, median (mean) in mm, (IQR) | A – B difference, mean in mm ± SD, (95%CI) | Statistics for A – B difference[1] | *P*-value |
|---|---|---|---|---|---|
| All colectomies, *n* | 29 | 29 | - | - | - |
| Actual stump | 22.63 (25.60), (20.16-31.04) | 61.80 (61.27), (45.07-69.87) | -35.66 ±30.83, (21.24-62.26) | $\chi^2$ = 27.38, $\eta^2$ = 0.47 | < 0.001[2] |
| $D_2$PLL | 26.00 (25.34), (20.15-30.53) | 34.00 (29.13), (11.00-41.10) | -3.79 ±13.98, (-11.90–4.32) | $t$ = -0.936, df = 56 | 0.353 |
| $D_2$IP, mean ± SD | 0.26 ± 12.18 | 32.14 ± 26.15 | -31.88 ± 26.52, (-41.96-21.79) | $\chi^2$ = 22.13, $\eta^2$ = 0.38 | < 0.001[2] |
| Statistics for $D_2$IP, $\chi^2$ (df), *P* | 0.005 (1), 0.944 | 21.77 (1), < 0.001 | - | - | - |

[1]$\chi^2$ = Kruskal-Wallis test; $\eta^2$ = effect size estimate; $t$ = *t*-test.
[2]Reference to RESULTS text. $D_2$IP: D2 improvement potential, calculated as difference of actual stump; $D_2$PLL: D2 predicted ligation level; IQR: Interquartile range.

vessels variations determined the mean distance from the origin of the ICA to the right side of the SMV to be 15.2 mm[9]; our study also showed a median of 9.97 mm. Although these values correspond to a D2 ligation, they are comparable with the safety margin of 10 mm proposed for the central ligation. Thus, it would not be wrong to state that a CME with D2 ligation could be equivalent to a CME with CVL in the cases when the crossing distance is less or equal to 10 mm, thus sparing the patient from unnecessary haemorrhagic risks that dissection under the SMV would entail. The original work of Spasojevic *et al*[13] shows a benefit of five to six LNs when the D2 ligation properly includes the vertical compartment situated lateral to the SMV. Furthermore, as they showed the LN distribution is dependent to anterior or posterior ICA crossing patterns, they emphasised tailoring the central ligation with respect to these anatomical variants. Hence, a correct D3 lymphadenectomy could be performed without dissecting behind the SMV in 42.6% of the reported cases, where the ICA ran in front of the SMV (calculated from a recent pooled analysis of 6090 ileocolic arteries[9]) and the retrieved nodal compartment would additionally yield a mean of three LNs[13]. Whether these three LNs would make a substantial difference in the outcomes remains unknown.

Most importantly, we showed both significantly longer stumps and greater spread of results upon comparing non–standardized colectomies with CME. We believe that measuring the arterial stump after colectomies is a simple and reproducible tool for controlling surgical quality and does not require a specialist radiologist. The fact that it quantifies the potential for improving the ligation allows for the surgical technique to be monitored and improved accordingly. In order to ensure the best survival outcomes for each patient, this instrument should be associated with the pathologist's evaluation of fascial plane stratification[19]. With these two instruments of standardization, standardized interventions can be promoted amongst surgeons and the learning curve for D2 CME could grow in non-tertiary surgical centres.

## Conclusion

We put forward now the feasibility of performing accurate arterial stump evaluation by non-radiologist interpreters. The tight distribution of arterial stumps reflects the constancy and quality of a disciplined surgery. The retrospective setting offers insurance that stumps had not been ligated shorter for the sake of comparison, as their length reflects only strong adherence to the oncological principles of CME. As the mesocolic root inserts high on the mesenteric vessels, longer stumps would inherently be surrounded by residual mesocolic tissue which allows risk of local recurrence. Measuring arterial stumps could be an additional tool for a surgical team's personal quality measurement. As we showed it to significantly correlate with a major prognostic factor (LN yield), this straightforward and reproducible tool may similar potential to predict outcomes. Considering that no serious risks are added when attempting a D2 vascular ligation, it is recommendable that all surgeons apply D2-CME as standardized technique for colon cancer, in order to maximize benefits for their patients.

**Table 4 Right colectomies: Ileocolic artery stump comparison between groups A and B**

| | Group A, mean in mm ± SD, (95%CI) | Group B, mean in mm ± SD, (95%CI) | A – B difference, mean in mm ± SE, (95%CI) | Statistics for A – B difference, *t* (df)[1] | *P*-value |
|---|---|---|---|---|---|
| Right colectomies, *n* | 12 | 10 | - | - | - |
| Actual stump | 16.97 ± 4.77, (13.94-19.96) | 49.93 ± 20.29, (35.40-64.44) | -32.96 ± 6.53, [-47.62-(-18.30)] | -5.02 (9.83) | 0.001[2] |
| $D_2$PLL | 11.12 ± 2.97, (9.23-13.00) | 7.80 ± 6.53, (3.13-12.47) | 3.32 ± 2.10, (-1.06-7.69) | 1.58 (20) | 0.130 |
| $D_2$IP | 5.85 ± 4.71, (2.85-8.84) | 42.13 ± 21.50, (26.75-57.50) | -35.78 ± 6.74, [-50.83-(-20.72)] | -5.31 (9.77) | < 0.001[2] |
| Statistics for $D_2$IP, *t* (df), *P* | 4.30 (11), 0.001[2] | 6.20 (9), < 0.001[2] | - | - | - |

[1]*t*-test.
[2]Reference to RESULTS text. $D_2$IP: D2 improvement potential, calculated as difference of actual stump; $D_2$PLL: D2 predicted ligation level.

**Table 5 Sigmoidectomies: Inferior mesenteric artery stump comparison between groups A and B**

| | Group A, mean in mm ± SD, (95%CI) | Group B, mean in mm ± SD, (95%CI) | A – B difference, mean in mm ± SE, (95%CI) | Statistics for A – B difference, *t* (df) | *P*-value |
|---|---|---|---|---|---|
| Sigmoidectomies, *n* | 13 | 16 | - | - | - |
| Actual stump | 35.42 ± 1 5.62, (25.98-44.86) | 69.92 ± 30.29, (53.78-86.06) | -34.50 ± 9.28, [-53.55-(-15.45)] | -3.72 (27) | < 0.001[1] |
| $D_2$PLL | 35.15 ± 8.85, (29.80-40.50) | 40.08 ± 8.36, (35.62-44.53) | -4.93 ± 3.20, (-11.50-1.65) | -1.54 (27) | 0.136 |
| $D_2$IP | 0.27 ± 12.90, [-7.53-(-8.06)] | 29.84 ± 29.67, (14.56-45.12) | -29.57 ± 8.01, [-46.20-(-12.94)] | -3.69 (21.72) | 0.001[1] |
| $D_3$IP | 25.42 ± 15.62, (15.98-34.86) | 59.92 ± 30.29, (43.78-76.06) | -34.50 ± 9.28, [-53.55-(-15.45)] | -3.72 (27) | < 0.001[1] |
| Statistics for $D_2$IP, *t* (df), *P* | 0.07 (12), 0.942[1] | 4.16 (15), < 0.01[1] | - | - | - |
| Statistics for $D_3$IP, *t* (df), *P* | 5.87 (12), 0.001[1] | 7.91 (15), < 0.01[1] | - | - | - |

[1]Reference to RESULTS text. $D_2$IP: D2 improvement potential, calculated as difference of actual stump - $D_2$PLL; $D_3$IP improvement potential, calculated as difference of actual stump – 10 mm (D3 level). $D_2$PLL: D2 predicted ligation level.

**Table 6 Left colectomies: Inferior mesenteric artery stump comparison between groups A and B**

| | Group A, mean in mm ± SD, (95%CI) | Group B, mean in mm ± SD, (95%CI) | A – B difference, mean in mm ± SE, (95%CI) | Statistics for A – B difference, *t* (df) | *P*-value |
|---|---|---|---|---|---|
| Left colectomy, n | 4 | 3 | - | - | - |
| Actual stump | 19.60 ± 9.22, (4.94-34.27) | 52.93 ± 13.15, (20.27-85.60) | -33.33 ± 8.37, [-54.85-(-11.81)] | -3.98 (5) | 0.011[1] |
| $D_2$PLL | 36.12 ± 3.52, (30.52-41.73) | 40.17 ± 8.25, (19.67-60.66) | -4.04 ±4.50, (-15.60-7.52) | -0.90 (5) | 0.410 |
| $D_2$IP | -16.52 ± 11.71, (-35.16-2.12) | 12.77 ± 19.79, [-36.40-(-61.93)] | -29.29 ± 11.8, [-59.64-(-1.06)] | -2.48 (5) | 0.056 |
| $D_3$IP | 9.60 ± 9.21, (-5.06-24.27) | 42.93 ± 13.15, (10.27-75.60) | -33.33 ± 8.37, [-54.85-(-11.81)] | -3.98 (5) | 0.011[1] |
| Statistics for $D_2$IP, *t* (df), *P* | -2.82 (3), 0.067[1] | 1.12 (2), 0.38 | - | - | - |
| Statistics for $D_3$IP, *t* (df), *P* | 2.08 (3), 0.129[1] | 5.66 (2), 0.03[1] | - | - | - |

[1]Reference to RESULTS text. $D_2$IP: D2 improvement potential, calculated as difference of actual stump; $D_3$IP improvement potential, calculated as difference of actual stump – 10 mm (D3 level). $D_2$PLL: D2 predicted ligation level.

**Table 7 Left colectomies and sigmoidectomies: Overview of all the inferior mesenteric artery stumps between groups A and B**

| | Group A, mean in mm ± SD, (95%CI) | Group B, mean in mm ± SD, (95%CI) | A – B difference, mean in mm ± SE, (95%CI) | Statistics for A – B difference, *t* (df) | *P*-value |
|---|---|---|---|---|---|
| Left colectomy, *n* | 17 | 19 | - | - | - |
| Actual stump | 31.70 ± 15.71, (23.62-39.77) | 67.24 ± 28.71, (53.40-81.07) | -35.54 ± 7.85, [-51.48-(-19.59)] | -4.53 (34) | < 0.001 |
| $D_2$PLL | 35.38 ± 7.83, (31.36-39.41) | 40.09 ± 8.11, (36.18-44.00) | -4.71 ± 2.66, (-10.13-0.70) | -1.77 (34) | 0.086 |
| $D_2$IP | -3.68 ± 14.30, (-11.04-3.67) | 27.14 ± 27.74, (13.77-40.51) | -30.83 ± 7.25, [-45.68-(-15.97)] | -4.25 (27.5) | < 0.001 |
| $D_3$IP | 21.70 ± 15.71, (13.62-29.78) | 57.24 ± 28.71, (43.40-71.07) | -35.54 ± 7.85, [-51.48-(-19.59)] | -4.53 (34) | < 0.001 |
| Statistics for $D_2$IP, *t* (df), *P* | -1.06 (16), [1]0.304 | 4.26 (18), < 0.01[1] | - | - | - |
| Statistics for $D_3$IP, *t* (df), *P* | 5.69 (16), < 0.001 | 8.68 (18), < 0.001 | - | - | - |

[1]Reference to RESULTS text. $D_2$IP: D2 improvement potential, calculated as difference of actual stump; $D_3$IP improvement potential, calculated as difference of actual stump – 10 mm (D3 level). $D_2$PLL: D2 predicted ligation level.

**Table 8 Group A: Correlation of lymph node count and specimen length with stump length**

| | *n* | Mean (median) | SD | IQR | *rs* | *P*-value |
|---|---|---|---|---|---|---|
| LN count | 29 | 34.83 (36.00) | 16.75 | (20.50-43.00) | -0.40 | 0.032[1] |
| Specimen length in cm | 29 | 26.26 (23.00) | 9.90 | (18.00-34.00) | -0.44 | 0.016[1] |

[1]Reference to RESULTS text. IQR: Interquartile range; LN: Lymph node.

**Table 9 Computed tomography evaluated ileocolic artery stumps in the literature**

| Study, year | *n* | Actual ileocolic stump in mm | Presumed D2 ileocolic stump in mm |
|---|---|---|---|
| Group A - our study, 2018, mean ± SD (95%CI) | 12 | 17.69 ± 4.77 (13.94-19.96) | 11.12 ± 2.97 (9.23-13.00) |
| Munkedal *et al*[22] 2017, mean (95%CI) | 32 | 31.0 (25-37) | 10.0[1] |
| Kaye *et al*[26] 2015, mean, (range) | 128 | 28.1 (2.5–74.3) | 14.4 (6.4) |
| Spasojevic *et al*[25] 2011, mean ± SD | 11 | 28.0 ± 9.3 | 18.1 ± 4.0 |

[1]Chosen as reference value from the complete mesocolic excision guideline[2,21].

**Table 10 Computed tomography evaluated inferior mesenteric artery stumps in the literature**

| Study, year | *n* | Actual IMA stump in mm | Presumed IMA stump in mm |
|---|---|---|---|
| Group A from our study, mean ± SD (95%CI) | 17 | 31.70 ± 15.6 (26.0-44.9) | 35.38 ± 7.83 (31.36-39.41) |
| Munkedal *et al*[22] 2017, mean (95%CI) | 20 | 49.0 (40-57) | 35.0 |
| Prevot *et al*[42] 2013, mean (range) | 26 | 30.3 (0.3–60) | - |

IMA: Inferior mesenteric artery.

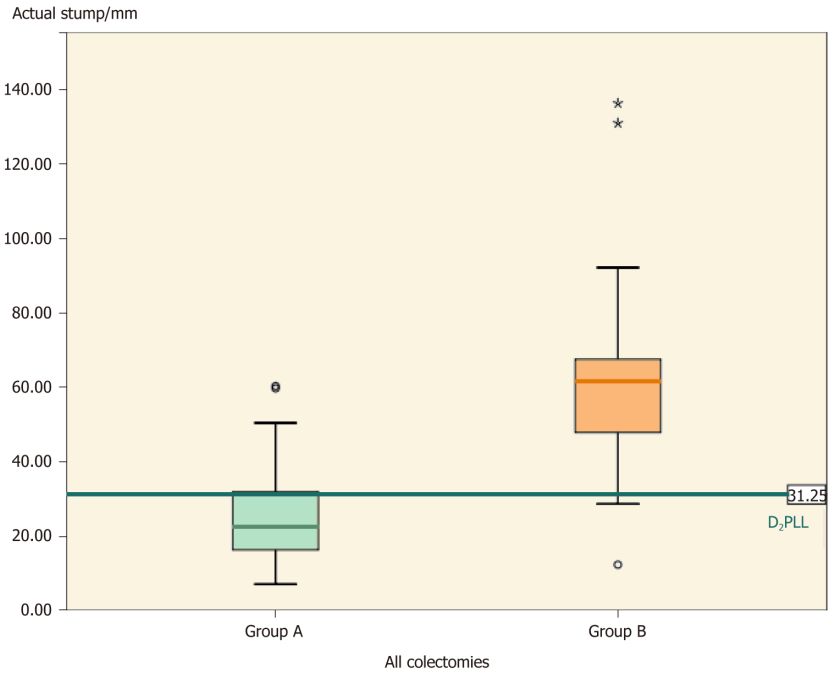


**Figure 4 Comparison of all the actual stumps between groups with horizontal reference to D2 predicted ligation levels.** $D_2$PLL: D2 predicted ligation level.

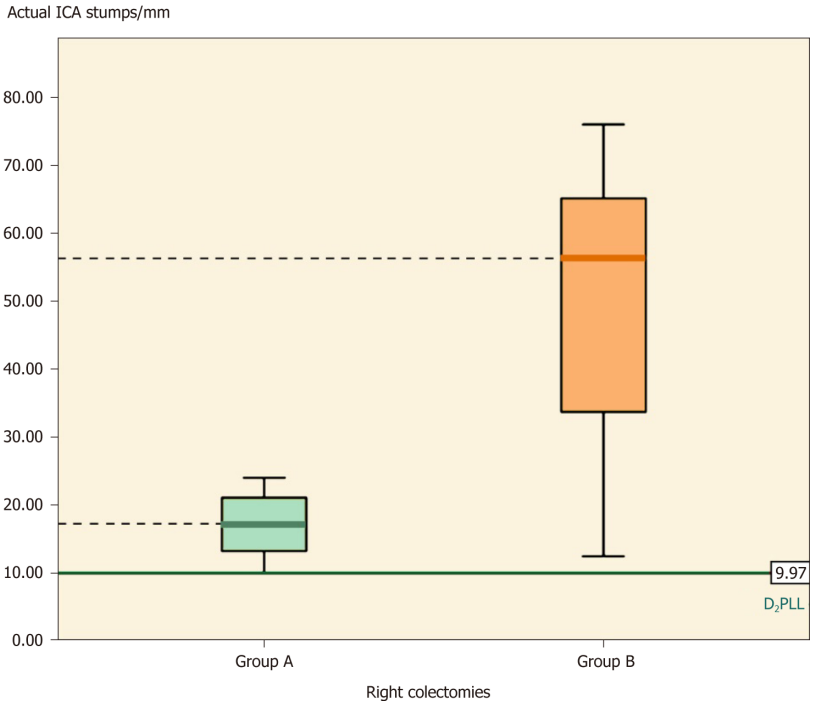


**Figure 5 Comparison of ileocolic artery actual stump from right colectomies between groups, with horizontal reference to D2 predicted ligation levels.** $D_2$PLL: D2 predicted ligation level; ICA: Ileocolic artery.

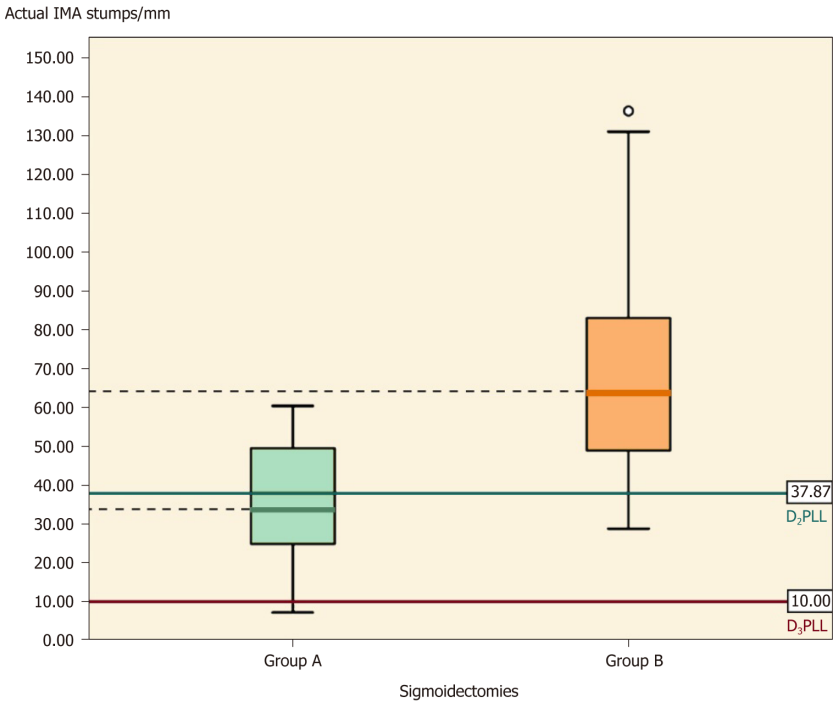


**Figure 6 Comparison of inferior mesenteric artery actual stumps from sigmoidectomies between groups, with horizontal reference to D2 predicted ligation levels and D2 predicted ligation levels.** $D_2$PLL: D2 predicted ligation level; $D_3$PLL: D3 predicted ligation level; IMA: Inferior mesenteric artery.

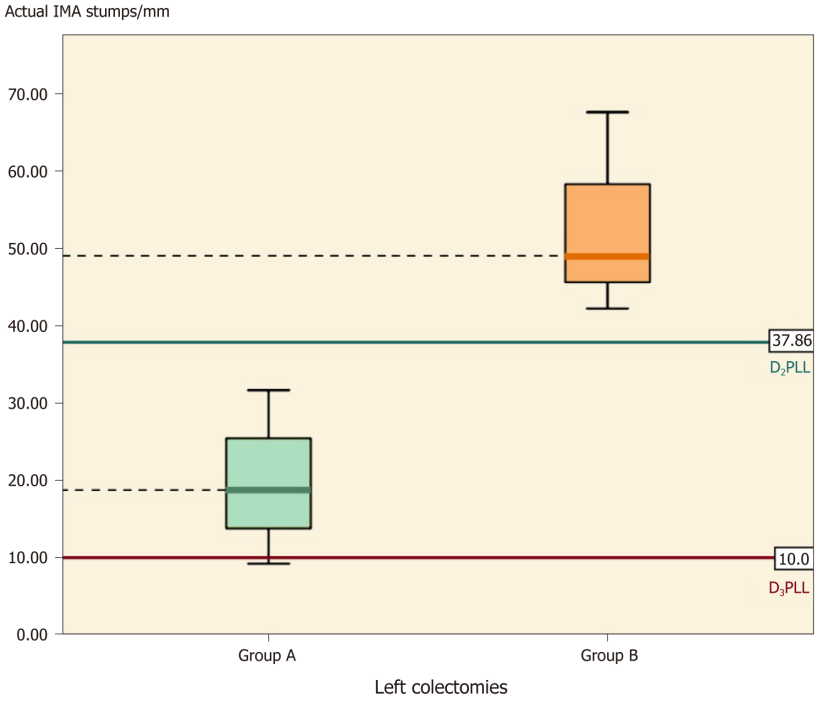


**Figure 7 Comparison of inferior mesenteric artery actual stumps from left colectomies between groups, with horizontal reference to D2 predicted ligation levels and D3 predicted ligation levels.** $D_2$PLL: D2 predicted ligation level; $D_3$PLL: D3 predicted ligation level; IMA: Inferior mesenteric artery.

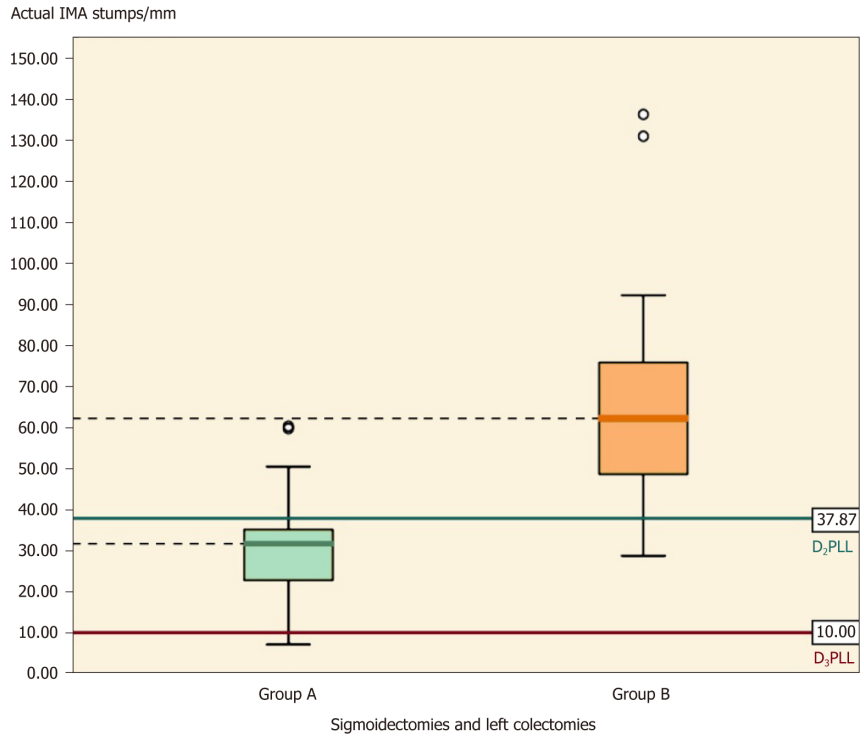


**Figure 8 Comparison of inferior mesenteric artery actual stumps from sigmoidectomies and left colectomies between groups, with horizontal reference to D2 predicted ligation levels and D3 predicted ligation levels.** $D_2$PLL: D2 predicted ligation level; $D_3$PLL: D3 predicted ligation level; IMA: Inferior mesenteric artery.

## ARTICLE HIGHLIGHTS

### *Research background*

Surgery has witnessed a paradigm shift in colon cancer management ever since prominent researchers have proposed complete mesocolic excision (CME) as the optimal surgical technique. CME specimens have been demonstrated as superior to standard resections, and patient outcomes have significantly improved. Despite this, adoption among surgeons is still not clear because quality criteria are not well defined. On this basis, researchers have proposed various quality markers - lymph node yield, mesocolon area, distance from central vascular ligation (CVL) to colon margin, *etc*.

### *Research motivation*

Because quality criteria in colon surgery are not yet defined, CME adoption is still behind total mesorectal excision. A consensus is needed to group already-proven pathological quality markers with newly-advocated radiological markers and to establish standards in colon surgery. The value of measuring arterial stumps on post-operative computed tomography (CT) scans has been previously analysed, but never in a comparative study between CME and standard specimens.

### *Research objectives*

In our advent to better define quality criteria for colon resections, we sought to analyse the value of measuring arterial stumps on post-operative CT scans in a comparative setting between CME and non-CME specimens, for the first time. By testing our hypothesis that arterial stumps are shorter in the CME group and are correlated with prognosis, we aimed to establish arterial stumps as tools to assess CME surgery.

### *Research methods*

This study was designed as a retrospective analysis and conducted on a prospectively maintained database. Two groups of adult patients were included in consecutive order. All underwent surgery with curative intent for colon cancer (stages I-III UICC 7$^{th}$ edition) and had at least one post-operative good quality contrast-enhanced CT scan that was available for re-evaluation. Group A were operated based on standard CME principles whereas group B underwent conventional colectomy. Measurements of arterial stumps were done by three observers. Shapiro-Wilk test was used to verify normal distribution of data. Kruskal-Wallis test confirmed inter-observer correlation. Stump measurements were analysed comparatively using Student's *t*-test. Paired and independent *t*-test was used to quantify potential for improvement of the ligation height and to compare potentials for improvement between the two groups. Non-normal distribution and non-parametric data was analysed using Kruskal-Wallis test.

### *Research results*

From 193 consecutive patients, 58 patients were selected after applying the inclusion and exclusion criteria (29 in CME group, 29 in non-CME group). After comparatively analyzing stump length in both groups, Shorter lengths were obtained in group A, by a mean difference of 35.66 mm ($\chi^2$= 27.38, $P$ < 0.001), which was significant for all types of colectomies. Ligations from group A significantly reached their potential height (0.26 ± 12.18 mm from $D_2$PLL; $\chi^2$= 0.005, $P$ = 0.944) in comparison with group B were the overwhelming majority failed to reach $D_2$PLL, by a mean difference of 32.14 ± 26.15 mm ($\chi^2$ = 21.77, $P$ < 0.001). Moreover, improvement potentials were far shorter in group A than group B ($\chi^2$= 22.13, $P$ < 0.001). Significant more variability was found in resections of group B ($P$ = 0.004). No significant difference was found when measurements of three different observers were analysed ($P$ = 0.866). Stump length was statistically correlated with specimen length and lymph node yield ($P$ = 0.018 and $P$ = 0.008 respectively).

### Research conclusions

Measuring arterial stumps is a simple and standard tool for defining surgical quality of colon resections. It may be, as well, a straightforward prognostic factor given its correlation with lymph node yield.

### Research perspectives

Our study is a step forward in refining quality criteria for colon surgery. Further research is needed on larger cohorts to compare the value of stump measurement to specimen measurements such as CVL distance or mesocolon surface area. The threshold for CVL should be further analysed, as D3 dissection may not aid in significantly better surgical specimens and outcomes.

## ACKNOWLEDGEMENTS

We would like to express our gratitude to Mrs. Oana Frunza for illustrating the statistical data into graphical forms.

## REFERENCES

1 **Hohenberger W**, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome. *Colorectal Dis* 2009; **11**: 354-364; discussion 364-5 [PMID: 19016817 DOI: 10.1111/j.1463-1318.2008.01735.x]

2 **Søndenaa K**, Quirke P, Hohenberger W, Sugihara K, Kobayashi H, Kessler H, Brown G, Tudyka V, D'Hoore A, Kennedy RH, West NP, Kim SH, Heald R, Storli KE, Nesbakken A, Moran B. The rationale behind complete mesocolic excision (CME) and a central vascular ligation for colon cancer in open and laparoscopic surgery: proceedings of a consensus conference. *Int J Colorectal Dis* 2014; **29**: 419-428 [PMID: 24477788 DOI: 10.1007/s00384-013-1818-2]

3 **Quirke P**, West N. Quality of surgery: has the time come for colon cancer? *Lancet Oncol* 2015; **16**: 121-122 [PMID: 25555422 DOI: 10.1016/S1470-2045(14)71223-9]

4 **Madoff RD**. Defining quality in colon cancer surgery. *J Clin Oncol* 2012; **30**: 1738-1740 [PMID: 22473171 DOI: 10.1200/JCO.2011.40.9615]

5 **Ong ML**, Schofield JB. Assessment of lymph node involvement in colorectal cancer. *World J Gastrointest Surg* 2016; **8**: 179-192 [PMID: 27022445 DOI: 10.4240/wjgs.v8.i3.179]

6 **Bertelsen CA**. Complete mesocolic excision an assessment of feasibility and outcome. *Dan Med J* 2017; **64** [PMID: 28157065]

7 **Bertelsen CA**, Neuenschwander AU, Jansen JE, Kirkegaard-Klitbo A, Tenma JR, Wilhelmsen M, Rasmussen LA, Jepsen LV, Kristensen B, Gögenur I; Copenhagen Complete Mesocolic Excision Study (COMES); Danish Colorectal Cancer Group (DCCG). Short-term outcomes after complete mesocolic excision compared with 'conventional' colonic cancer surgery. *Br J Surg* 2016; **103**: 581-589 [PMID: 26780563 DOI: 10.1002/bjs.10083]

8 **Wang C**, Gao Z, Shen K, Shen Z, Jiang K, Liang B, Yin M, Yang X, Wang S, Ye Y. Safety, quality and effect of complete mesocolic excision vs non-complete mesocolic excision in patients with colon cancer: a systemic review and meta-analysis. *Colorectal Dis* 2017; **19**: 962-972 [PMID: 28949060 DOI: 10.1111/codi.13900]

9 **Negoi I**, Beuran M, Hostiuc S, Negoi RI, Inoue Y. Surgical Anatomy of the Superior Mesenteric Vessels Related to Colon and Pancreatic Surgery: A Systematic Review and Meta-Analysis. *Sci Rep* 2018; **8**: 4184 [PMID: 29520096 DOI: 10.1038/s41598-018-22641-x]

10 **Dobrowolski S**, Hać S, Kobiela J, Sledziński Z. Should we preserve the inferior mesenteric artery during sigmoid colectomy? *Neurogastroenterol Motil* 2009; **21**: 1288-e123 [PMID: 19508489 DOI: 10.1111/j.1365-2982.2009.01331.x]

11 **Liang JT**, Huang KC, Lai HS, Lee PH, Sun CT. Oncologic results of laparoscopic D3 lymphadenectomy for male sigmoid and upper rectal cancer with clinically positive lymph nodes. *Ann Surg Oncol* 2007; **14**: 1980-1990 [PMID: 17458586 DOI: 10.1245/s10434-007-9368-x]

12 **Ignjatovic D**, Sund S, Stimec B, Bergamaschi R. Vascular relationships in right colectomy for cancer: clinical implications. *Tech Coloproctol* 2007; **11**: 247-250 [PMID: 17676266 DOI: 10.1007/s10151-007-0359-5]

13 **Spasojevic M**, Stimec BV, Dyrbekk AP, Tepavcevic Z, Edwin B, Bakka A, Ignjatovic D. Lymph node distribution in the d3 area of the right mesocolon: implications for an anatomically correct cancer resection. A postmortem study. *Dis Colon Rectum* 2013; **56**: 1381-1387 [PMID: 24201392 DOI: 10.1097/01.dcr.0000436279.18577.d3]

14 **Slim K**, Blay JY, Brouquet A, Chatelain D, Comy M, Delpero JR, Denet C, Elias D, Fléjou JF, Fourquier

P, Fuks D, Glehen O, Karoui M, Kohneh-Shahri N, Lesurtel M, Mariette C, Mauvais F, Nicolet J, Perniceni T, Piessen G, Regimbeau JM, Rouanet P, sauvanet A, Schmitt G, Vons C, Lasser P, Belghiti J, Berdah S, Champault G, Chiche L, Chipponi J, Chollet P, De Baère T, Déchelotte P, Garcier JM, Gayet B, Gouillat C, Kianmanesh R, Laurent C, Meyer C, Millat B, Msika S, Nordlinger B, Paraf F, Partensky C, Peschaud F, Pocard M, Sastre B, Scoazec JY, Scotté M, Triboulet JP, Trillaud H, Valleur P. [Digestive oncology: surgical practices]. *J Chir (Paris)* 2009; **146** Suppl 2: S11-S80 [PMID: 19435621 DOI: 10.1016/S0021-7697(09)72398-1]

15 **Otchy D**, Hyman NH, Simmang C, Anthony T, Buie WD, Cataldo P, Church J, Cohen J, Dentsman F, Ellis CN, Kilkenny JW, Ko C, Moore R, Orsay C, Place R, Rafferty J, Rakinic J, Savoca P, Tjandra J, Whiteford M; Standards Practice Task Force; American Society of Colon and Rectal Surgeons. Practice parameters for colon cancer. *Dis Colon Rectum* 2004; **47**: 1269-1284 [PMID: 15484340 DOI: 10.1007/s10350-004-0598-8]

16 **Chang GJ**, Kaiser AM, Mills S, Rafferty JF, Buie WD; Standards Practice Task Force of the American Society of Colon and Rectal Surgeons. Practice parameters for the management of colon cancer. *Dis Colon Rectum* 2012; **55**: 831-843 [PMID: 22810468 DOI: 10.1097/DCR.0b013e3182567e13]

17 **Watanabe T**, Muro K, Ajioka Y, Hashiguchi Y, Ito Y, Saito Y, Hamaguchi T, Ishida H, Ishiguro M, Ishihara S, Kanemitsu Y, Kawano H, Kinugasa Y, Kokudo N, Murofushi K, Nakajima T, Oka S, Sakai Y, Tsuji A, Uehara K, Ueno H, Yamazaki K, Yoshida M, Yoshino T, Boku N, Fujimori T, Itabashi M, Koinuma N, Morita T, Nishimura G, Sakata Y, Shimada Y, Takahashi K, Tanaka S, Tsuruta O, Yamaguchi T, Yamaguchi N, Tanaka T, Kotake K, Sugihara K; Japanese Society for Cancer of the Colon and Rectum. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2016 for the treatment of colorectal cancer. *Int J Clin Oncol* 2018; **23**: 1-34 [PMID: 28349281 DOI: 10.1007/s10147-017-1101-6]

18 **Helsedirektoratet**. *National action plan with guidelines for diagnosis, treatment and follow up of cancer in the colon and rectum*. Oslo: Helsedirektoratet 2017;

19 **West NP**, Morris EJ, Rotimi O, Cairns A, Finan PJ, Quirke P. Pathology grading of colon cancer surgical resection and its association with survival: a retrospective observational study. *Lancet Oncol* 2008; **9**: 857-865 [PMID: 18667357 DOI: 10.1016/S1470-2045(08)70181-5]

20 **West NP**, Hohenberger W, Weber K, Perrakis A, Finan PJ, Quirke P. Complete mesocolic excision with central vascular ligation produces an oncologically superior specimen compared with standard surgery for carcinoma of the colon. *J Clin Oncol* 2010; **28**: 272-278 [PMID: 19949013 DOI: 10.1200/JCO.2009.24.1448]

21 **West NP**, Kobayashi H, Takahashi K, Perrakis A, Weber K, Hohenberger W, Sugihara K, Quirke P. Understanding optimal colonic cancer surgery: comparison of Japanese D3 resection and European complete mesocolic excision with central vascular ligation. *J Clin Oncol* 2012; **30**: 1763-1769 [PMID: 22473170 DOI: 10.1200/JCO.2011.38.3992]

22 **Munkedal DLE**, Rosenkilde M, Nielsen DT, Sommer T, West NP, Laurberg S. Radiological and pathological evaluation of the level of arterial division after colon cancer surgery. *Colorectal Dis* 2017; **19**: O238-O245 [PMID: 28590033 DOI: 10.1111/codi.13756]

23 **Solon JG**, Cahalane A, Burke JP, Gibbons D, McCann JW, Martin ST, Sheahan K, Winter DC. A radiological and pathological assessment of ileocolic pedicle length as a predictor of lymph node retrieval following right hemicolectomy for caecal cancer. *Tech Coloproctol* 2016; **20**: 545-550 [PMID: 27231119 DOI: 10.1007/s10151-016-1483-x]

24 **Hohenberger P**, Schlag P, Kretzschmar U, Herfarth C. Regional mesenteric recurrence of colorectal cancer after anterior resection or left hemicolectomy: inadequate primary resection demonstrated by angiography of the remaining arterial supply. *Int J Colorectal Dis* 1991; **6**: 17-23 [PMID: 2033347 DOI: 10.1007/BF00703955]

25 **Spasojevic M**, Stimec BV, Gronvold LB, Nesgaard JM, Edwin B, Ignjatovic D. The anatomical and surgical consequences of right colectomy for cancer. *Dis Colon Rectum* 2011; **54**: 1503-1509 [PMID: 22067178 DOI: 10.1097/DCR.0b013e318232116b]

26 **Kaye TL**, West NP, Jayne DG, Tolan DJ. CT assessment of right colonic arterial anatomy pre and post cancer resection - a potential marker for quality and extent of surgery? *Acta Radiol* 2016; **57**: 394-400 [PMID: 25940063 DOI: 10.1177/0284185115583033]

27 **Morarasu S**, Frunza T, Bilavschi K, Patrascu AM, Lunca S, Dimofte G. Histopathology report on colon cancer specimens; measuring surgical quality, an increasing stress for surgeons. *J Mind Med Sci* 2018; **5**: 75-81 [DOI: 10.22543/7674.51.P7581]

28 **Spasojevic M**, Stimec BV, Fasel JF, Terraz S, Ignjatovic D. 3D relations between right colon arteries and the superior mesenteric vein: a preliminary study with multidetector computed tomography. *Surg Endosc* 2011; **25**: 1883-1886 [PMID: 21136104 DOI: 10.1007/s00464-010-1480-5]

29 **Vandamme JP**, Van der Schuren G. Re-evaluation of the colic irrigation from the superior mesenteric artery. *Acta Anat (Basel)* 1976; **95**: 578-588 [PMID: 970095 DOI: 10.1159/000145493]

30 **Panagouli E**, Lolis E, Venieratos D. A morphometric study concerning the branching points of the main arteries in humans: relationships and correlations. *Ann Anat* 2011; **193**: 86-99 [PMID: 21169000 DOI: 10.1016/j.aanat.2010.10.009]

31 **Bertelsen CA**, Neuenschwander AU, Jansen JE, Wilhelmsen M, Kirkegaard-Klitbo A, Tenma JR, Bols B, Ingeholm P, Rasmussen LA, Jepsen LV, Iversen ER, Kristensen B, Gögenur I; Danish Colorectal Cancer Group. Disease-free survival after complete mesocolic excision compared with conventional colon cancer surgery: a retrospective, population-based study. *Lancet Oncol* 2015; **16**: 161-168 [PMID: 25555421 DOI: 10.1016/S1470-2045(14)71168-4]

32 **Le Voyer TE**, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, Haller DG. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol* 2003; **21**: 2912-2919 [PMID: 12885809 DOI: 10.1200/JCO.2003.05.062]

33 **Johnson PM**, Porter GA, Ricciardi R, Baxter NN. Increasing negative lymph node count is independently associated with improved long-term survival in stage IIIB and IIIC colon cancer. *J Clin Oncol* 2006; **24**: 3570-3575 [PMID: 16877723 DOI: 10.1200/JCO.2006.06.8866]

34 **Chen SL**, Bilchik AJ. More extensive nodal dissection improves survival for stages I to III of colon cancer: a population-based study. *Ann Surg* 2006; **244**: 602-610 [PMID: 16998369 DOI: 10.1097/01.sla.0000237655.11717.50]

35 **Galizia G**, Lieto E, De Vita F, Ferraraccio F, Zamboli A, Mabilia A, Auricchio A, Castellano P, Napolitano V, Orditura M. Is complete mesocolic excision with central vascular ligation safe and effective

in the surgical treatment of right-sided colon cancers? A prospective study. *Int J Colorectal Dis* 2014; **29**: 89-97 [PMID: 23982425 DOI: 10.1007/s00384-013-1766-x]

36 **Hogan AM**, Winter DC. Complete mesocolic excision--a marker of surgical quality? *J Gastrointest Surg* 2009; **13**: 1889-1891 [PMID: 19655207 DOI: 10.1007/s11605-009-0976-6]

37 **Dimitriou N**, Griniatsos J. Complete mesocolic excision: Techniques and outcomes. *World J Gastrointest Oncol* 2015; **7**: 383-388 [PMID: 26689921 DOI: 10.4251/wjgo.v7.i12.383]

38 **Jessup JM**, Goldberg RM, Asare EA, Benson III AB, Brierley JD, Chang GJ, Chen V, Compton CC, De Nardi P, Goodman KA, Gress D, Guinney J, Gunderson LL, Hamilton SR, Hanna NN, Kakar S, Kosinski LA, Negoita S, Ogino S, Overman MJ, Quirke P, Rohren E, Sargent DJ, Schumacher-Penberthy LT, Shibata D, Sinicrope FA, Steele SR, Stojadinovic A, Tejpar S, Weiser MR, Welton ML, Washington MK, Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE. Colon and Rectum. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE. *AJCC Cancer Staging Manual*. New York: Springer 2017; 251-274

39 **Grinnell RS**. Results Of Ligation Of Inferior Mesenteric Artery At The Aorta In Resections Of Carcinoma Of The Descending And Sigmoid Colon And Rectum. *Surg Gynecol Obstet* 1965; **120**: 1031-1036 [PMID: 14269834 DOI: 10.1177/0954409713508109]

40 **Rosi PA**, Cahill WJ, Carey J. A ten year study of hemicolectomy in the treatment of carcinoma of the left half of the colon. *Surg Gynecol Obstet* 1962; **114**: 15-24 [PMID: 14494101]

41 **Titu LV**, Tweedle E, Rooney PS. High tie of the inferior mesenteric artery in curative surgery for left colonic and rectal cancers: a systematic review. *Dig Surg* 2008; **25**: 148-157 [PMID: 18446037 DOI: 10.1159/000128172]

42 **Prevot F**, Sabbagh C, Deguines JB, Potier A, Cosse C, Yzet T, Regimbeau JM. Are there any surgical and radiological correlations to the level of ligation of the inferior mesenteric artery after sigmoidectomy for cancer? *Ann Anat* 2013; **195**: 467-474 [PMID: 23735577 DOI: 10.1016/j.aanat.2013.03.008]

**P- Reviewer:** Cao ZF, Chen XZ, Hori T, Jeong KY
**S- Editor:** Ma YJ **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 227-237

DOI: 10.4251/wjgo.v11.i3.227

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Retrospective Cohort Study

# Hepatic resection *vs* percutaneous radiofrequency ablation of hepatocellular carcinoma abutting right diaphragm

Kyoung Doo Song, Hyo Keun Lim, Hyunchul Rhim, Min Woo Lee, Tae Wook Kang, Yong Han Paik, Jong Man Kim, Jae-Won Joh

**ORCID number:** Kyoung Doo Song (0000-0002-2767-3622); Hyo Keun Lim (0000-0003-3269-7503); Hyunchul Rhim (0000-0002-9737-0248); Min Woo Lee (0000-0001-9048-9011); Tae Wook Kang (0000-0002-8991-6407); Yong Han Paik (0000-0002-3076-2327); Jong Man Kim (0000-0002-1903-8354); Jae-Won Joh (0000-0003-1732-6210).

**Author contributions:** Song KD and Lim HK designed the study, analyzed data and wrote the manuscript; Lim HK, Rhim H, Lee MW, Paik YH, Joh JW and Kim JM were all responsible for the provision of study materials or patients; Kang TW and Kim JM contributed to collection and assembly of data; all authors provided critical review of the manuscript and approved the final version of the manuscript.

**Institutional review board statement:** This study was reviewed and approved by the Institutional Review Board of the Samsung Medical Center.

**Informed consent statement:** Informed consent was waived by the Institutional Review Board of the Samsung Medical Center because this study was a retrospective study.

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



**Kyoung Doo Song, Hyunchul Rhim, Min Woo Lee, Tae Wook Kang,** Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, South Korea

**Hyo Keun Lim,** Department of Radiology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Health Sciences and Technology, SAIHST, Sungkyunkwan University, Seoul 06351, South Korea

**Yong Han Paik,** Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, South Korea

**Jong Man Kim, Jae-Won Joh,** Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, South Korea

**Corresponding author:** Hyo Keun Lim, MD, PhD, Professor, Department of Radiology and Department of Health Sciences and Technology, Samsung Medical Center, SAIHST, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea. hyokeun.lim@samsung.com
**Telephone:** +82-2-34102505
**Fax:** +82-2-34102559

## Abstract

### *BACKGROUND*

It is usually difficult to adequately conduct percutaneous ultrasound-guided radiofrequency (RF) ablation for hepatocellular carcinomas (HCCs) abutting the diaphragm. Our hypothesis was that the subphrenic location of HCC could have an effect on the long-term therapeutic outcomes after hepatic resection and RF ablation.

### *AIM*

To compare the long-term therapeutic outcomes of hepatic resection and percutaneous RF ablation for HCCs abutting the diaphragm.

### *METHODS*

A total of 143 Child-Pugh class A patients who had undergone hepatic resection ($n$ = 80) or percutaneous ultrasound-guided RF ablation ($n$ = 63) for an HCC (≤ 3 cm) abutting the right diaphragm were included. Cumulative local tumor progression (LTP), cumulative intrahepatic distant recurrence (IDR), disease-free survival (DFS), and overall survival (OS) rates were estimated. Prognostic factors

**Manuscript source**: Invited manuscript

**Received:** October 23, 2018
**Peer-review started:** October 23, 2018
**First decision:** November 29, 2018
**Revised:** December 12, 2018
**Accepted:** January 5, 2019
**Article in press:** January 6, 2019
**Published online:** March 15, 2019

for DFS and OS were analyzed. Complications were evaluated.

***RESULTS***

The cumulative IDR rate, DFS rate, and OS rate for the hepatic resection group and RF ablation group at 5 years were "35.9% *vs* 65.8%", "64.1% *vs* 18.3%", and "88.4% *vs* 68.7%", respectively. Hepatic resection was an independent prognostic factor for DFS ($P \leq 0.001$; hazard ratio, 0.352; 95%CI: 0.205, 0.605; with RF ablation as the reference category); however, treatment modality was not an independent prognostic factor for OS. The LTP rate was 46.6% at 5 years for the RF ablation group. The major complication rate was not significantly different between the groups ($P = 0.630$). The rate of occurrence of peritoneal seeding was higher in the RF ablation group (1.3% *vs* 9.5%, $P = 0.044$).

***CONCLUSION***

Although OS was not significantly different between patients who had gone hepatic resection or percutaneous RF ablation for HCCs abutting the diaphragm, DFS was better in the hepatic resection group.

**Key words:** Hepatic resection; Radiofrequency ablation; Hepatocellular carcinoma; Diaphragm; Treatment outcome



**Core tip:** The aim of this study was to compare the long-term therapeutic outcomes of hepatic resection and percutaneous radiofrequency (RF) ablation for hepatocellular carcinomas abutting the diaphragm. The disease-free survival (DFS) rate was 64.1% and 18.3% for the hepatic resection group and the RF ablation group, and overall survival (OS) rate was 88.4% and 68.7% for the hepatic resection group and the RF ablation group at 5 years. The local tumor progression rate was as high as 46.6% for the RF ablation group. Although OS was not significantly different between two groups, DFS was better in the hepatic resection group.

**Citation:** Song KD, Lim HK, Rhim H, Lee MW, Kang TW, Paik YH, Kim JM, Joh JW. Hepatic resection *vs* percutaneous radiofrequency ablation of hepatocellular carcinoma abutting right diaphragm. *World J Gastrointest Oncol* 2019; 11(3): 227-237
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/227.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.227

## INTRODUCTION

Both hepatic resection and radiofrequency (RF) ablation are considered curative procedures for very early or early-stage hepatocellular carcinoma (HCC)[1]. Many studies have revealed that RF ablation is comparable to hepatic resection in terms of long-term survival for patients with early-stage HCC[2-4]. However, most studies have not taken into account the location of HCCs. Tumor location is an important factor affecting local tumor control especially for RF ablation due to its technical complexity[5].

When an HCC is located in the liver abutting the right diaphragm, an adequate accomplishment of percutaneous ultrasound (US)-guided RF ablation is difficult due to the poor sonic window resulting from lung shadowing and the potential risk of collateral thermal injury to the diaphragm. According to a preliminary study, local tumor progression (LTP) after percutaneous RF ablation was more frequent in patients with subphrenic HCCs (29%) than in nonsubphrenic HCCs (6%)[6]. To overcome this inherent limitation, many investigators have used the infusion of artificial ascites or pleural effusion. Several studies have reported that percutaneous RF ablation with infusion of artificial ascites or pleural effusion was safe and effective[7-10]. However, the LTP rate after RF ablation for subphrenic HCCs remained high even with the application of these special techniques[9]. The effect of the specific location of HCC on the long-term therapeutic outcomes after hepatic resection and RF ablation has not yet been investigated. Thus, the aim of this study was to compare the long-term therapeutic outcomes of hepatic resection *vs* percutaneous RF ablation for

the curative treatment of HCCs abutting the diaphragm.

## MATERIALS AND METHODS

Our Institutional Review Board approved this retrospective study, and informed consent was waived.

### Patients

Between January 2006 and October 2010, 5981 patients were diagnosed with HCC at our institution. This study included patients from the same population as in a previous study that was conducted at our institution; however, the study design and result analysis methods are different[11]. Inclusion criteria for our study were as follows: (1) patients who had undergone percutaneous US-guided RF ablation or hepatic resection for HCC as a first-line treatment; (2) patients who had a single HCC ≤ 3 cm; (3) patients with HCC abutting the right diaphragm (subphrenic HCC); and (4) patients with Child-Pugh class A. A subphrenic HCC in our study was defined as a tumor that abutted the right diaphragm on axial or coronal images of computed tomography (CT) or magnetic resonance imaging. We excluded tumors that abutted the left diaphragm because they are different from the tumors abutting the right diaphragm in many ways, in terms of treatment. Most tumors abutting the left diaphragm are located under the heart and are, hence, considered more technically difficult to treat compared to those close to the right diaphragm. In addition, the use of artificial ascites or pleural effusion is usually ineffective for tumors abutting the left diaphragm. Instead, hepatic resection of tumors abutting the left diaphragm (especially in the left lateral segment) is easily performed either after laparotomy or with a laparoscopic approach. Finally, our study included 63 patients (49 men, 14 women; mean age, 60.3 years; range, 41–78 years) who had undergone percutaneous RF ablation and 80 patients (62 men, 18 women; mean age, 53.5 years; range, 30–78 years) who had been treated with hepatic resection. The patient inclusion flowchart is shown in Figure 1.

In 3 (4.7%) patients in the RF ablation group, HCC was confirmed histologically via percutaneous US-guided biopsy. In the remainder of the patients in the RF ablation group, HCC was diagnosed based on one of two clinical guidelines from the American Association for the Study of Liver Diseases at the time of RF ablation[1,12]. For all patients in the hepatic resection group, HCC was diagnosed histologically after hepatic resection.

### Treatment of HCC and follow-up

The general inclusion criteria for hepatic resection at our institution were as follows: (1) a single tumor or oligonodular tumors within a monosegment of the liver; (2) an indocyanine green retention rate less than 20% at 15 min; (3) serum total bilirubin level less than 1.5 mg/dL; (4) no severe portal hypertension; and (5) no gross ascites. The inclusion criteria for percutaneous RF ablation at our institution were as follows: (1) a single tumor (≤ 5 cm in the greatest dimension) or multiple nodular tumors (three or fewer, each ≤ 3 cm in the greatest dimension); (2) Child-Pugh class A or B disease; (3) no evidence of portal vein thrombosis or extrahepatic metastasis; and (4) prothrombin time ratio > 50%, and platelet count > 50000/mm$^3$ (50 × 10$^9$/L). Treatment modality was decided based on age, liver function reserve, tumor location, surgical risk, and patient preference by a multidisciplinary tumor board composed of hepatologists, radiologists, surgeons, and medical and radiation oncologists.

Hepatic resection was performed by one of two surgeons (JHK and JWJ) with more than 10 years of experience in hepatobiliary surgery by the end of the study. The types of hepatic resection were as follows: subsegmentectomy in 58 patients, bisegmentectomy in five patients, posterior sectionectomy in 12 patients, right hemihepatectomy in two patients, anterior sectionectomy in one patient, central hepatectomy in one patient, and extended left hemihepatectomy in one patient. As a result, anatomical resection was performed in 17 (21.3%) patients and non-anatomical resection was performed in 63 (78.8%) patients[13]. Hepatic resection was performed after laparotomy in 78 (97.5%) patients and with laparoscopy in two (2.5%) patients. RF ablation was performed by one of five interventional radiologists (MWL, DC, HR, HKL, and YK) with more than 6 years of experience in RF ablation by the end of the study. The process and method of RF ablation were the same as those described in a previous study[14]. In brief, RF ablation was performed percutaneously under the guidance of real-time US. We used internally cooled electrode systems with generators (Cool-tip RF System, Covidien, Mansfield, MA, United States; or VIVA RFA System, STARmed, Goyang, South Korea). Sedation was performed via an intravenous injection of pethidine hydrochloride (Samsung Pharmaceuticals, Seoul,

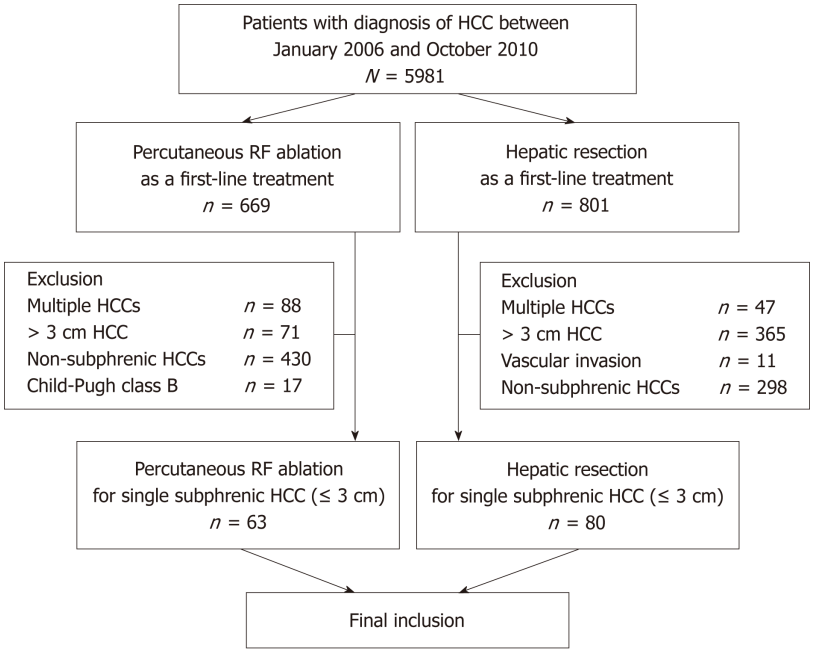


**Figure 1 Flowchart of patient inclusion.** HCC: Hepatocellular carcinoma; RF: Radiofrequency.

South Korea) and fentanyl citrate (GUJU Pharma, Seoul, South Korea). To improve the sonic window and avoid thermal injury to the diaphragm, artificial ascites (5% dextrose in a water solution) was infused into the perihepatic space using a 5F angiosheath in 39 (61.9%) patients.

After RF ablation, immediate follow-up contrast agent-enhanced CT was performed to evaluate the therapeutic response and possible complications. Contrast agent-enhanced CT was performed at the 1 mo follow-up, every 3 mo during the first 2 years, followed by every 4-6 mo according to the risk of recurrence for both the hepatic resection group and RF ablation group.

### Data acquisition

Baseline characteristics of patients and HCCs were obtained through review of their electronic medical record from our institution. To compare the therapeutic outcomes between the two groups, intrahepatic distant recurrence (IDR), disease-free survival (DFS), and overall survival (OS) were calculated. IDR was defined as a new tumor appearing in the liver separate from the treated area. DFS was defined as the time interval from the date of treatment to one of the following events: intrahepatic recurrence, extrahepatic recurrence, or death. OS was defined as the time interval from the date of treatment to death. If the patients had undergone liver transplantation, they were considered to have been censored at the time of liver transplantation. Complications were stratified according to the Clavien classification of postoperative complications, and complications of grade II or higher were considered major complications[15]. Local tumor progression (LTP) was evaluated for the RF ablation group. LTP was defined as the appearance of new tumor foci at the margin of the ablation zone after at least one contrast-enhanced follow-up study had demonstrated an absence of viable tumors[16].

### Statistical analysis

Continuous data were compared using two-sample *t* tests, and categorical variables were compared using chi-squared tests between the two groups. Cumulative LTP, cumulative IDR, DFS, and OS rates were estimated using the Kaplan-Meier method. Prognostic factors for DFS and OS were assessed using Cox regression models. Proportional hazard (PH) assumption for the Cox proportional hazard model was tested using Schoenfeld's method. For the variables with violation of PH assumption, the time-dependent Cox regression was applied. When the time dependence was not significant, the Cox proportional hazard model was applied. Possible risk factors with *P* values of 0.1 or less at univariate analyses were entered into the multivariate Cox proportional hazard models. Subgroup analysis for patients with ≤ 2 cm HCCs was performed with Cox proportional hazard models. All statistical analyses were performed using a software (PASW statistical software, version 18.0; SPSS, Chicago, IL). For all tests, a *P* value < 0.05 was defined as a significant difference.

## RESULTS

Baseline characteristics of patients and HCCs are shown in Table 1. The median follow-up period was 74.9 mo (range, 10.3-117.8 mo) in the hepatic resection group and 65.3 mo (range, 4.1-113.9 mo) in the RF ablation group. The RF ablation group was significantly older, and they exhibited a lower α-fetoprotein level, platelet count, and serum albumin level, and a higher prothrombin time. In the RF ablation group, the proportion of patients with liver cirrhosis and hepatitis C virus was higher and the proportion of patients with hepatitis B virus was lower compared to that in the hepatic resection group. The mean size of HCCs was not significantly different between the two groups.

### *Therapeutic outcomes*

The cumulative IDR rates at 1-, 3-, and 5-years were 15.0%, 29.1%, and 35.9%, respectively, for the hepatic resection group and 13.1%, 54.5%, and 65.8%, respectively, for the RF ablation group (Figure 2A). The estimated DFS rates at 1-, 3-, and 5-years were 85.0%, 70.9%, and 64.1%, respectively, for the hepatic resection group and 69.5%, 27.5%, and 18.3%, respectively, for the RF ablation group (Figure 2B). The estimated OS rates at 1-, 3-, and 5-years were 97.5%, 92.3%, and 88.4%, respectively, for the hepatic resection group and 100%, 81.4%, and 68.7%, respectively, for the RF ablation group (Figure 2C). For the RF ablation group, the cumulative LTP rates were 22.5%, 37.8%, and 46.6% at 1-, 3-, and 5-years, respectively (Figure 3).

### *Analysis of risk factors*

Based on multivariate analysis, there was no independent prognostic factor for OS. Hepatic resection [$P \leq 0.001$; hazard ratio (HR), 0.352; 95% confidence interval (CI): 0.205, 0.605; with RFA as the reference category], alanine aminotransferase level ($P = 0.006$; HR, 1.011; 95%CI: 1.003, 1.020), and serum albumin level ($P = 0.014$; HR, 0.481; 95%CI: 0.269, 0.860) were independent prognostic factors for DFS (Tables 2 and 3).

### *Subgroup analysis for patients with ≤ 2 cm HCC*

Thirty-seven patients in the hepatic resection group and 27 patients in the RF ablation group had ≤ 2 cm HCC. The cumulative IDR rates at 1-, 3-, and 5-years were 13.5%, 27.3%, and 33.1%, respectively, for the hepatic resection group and 15.3%, 60.3%, and 70.2%, respectively, for the RF ablation group. The estimated DFS rates at 1-, 3-, and 5-years were 86.5%, 72.7%, and 66.9%, respectively, for the hepatic resection group and 81.0%, 27.4%, and 18.3%, respectively, for the RF ablation group. The estimated OS rates at 1-, 3-, and 5-years were 100%, 94.5%, and 91.7%, respectively, for the hepatic resection group and 100%, 83.8%, and 65.4%, respectively, for the RF ablation group. In multivariate analysis, hepatic resection was an independent prognostic factor for DFS ($P = 0.018$; HR, 0.365; CI: 0.158-0.844), but was not an independent prognostic factor for OS.

### *Complications and treatment for recurrent HCC*

There was no treatment-related mortality in either group. Major complications occurred in three patients (3.8%) in the hepatic resection group: Grade II, pneumonia ($n = 1$) and intraperitoneal hemorrhage ($n = 1$); and Grade III, wound infection requiring surgery ($n = 1$). In the RF ablation group, a major complication occurred in one patient (1.6%): Grade III, pleural effusion requiring drainage. The major complication rate was not significantly different between the two groups ($P = 0.060$). The posttreatment hospital stay was significantly longer in the hepatic resection group (median, 9 d; range, 5-23 d) than in the RF ablation group (median, 1.0 d; range, 1-4 d; $P < 0.001$).

During the follow-up period, peritoneal seeding occurred in one patient (1.3%) in the hepatic resection group and six patients (9.5%) in the RF ablation group, and the rate of peritoneal seeding was significantly different ($P = 0.044$).

During the follow-up period, LTP occurred in 29 (46.0%) of the 63 patients in the RF ablation group. The initial treatment modalities for LTP were as follows: transarterial chemoembolization (TACE) ($n = 14$), RF ablation ($n = 12$), hepatic resection ($n = 1$), combined TACE and RF ablation ($n = 1$), and combined TACE and radiation therapy ($n = 1$). In 26 of 29 patients, LTP was controlled with additional treatments, and the number of additional treatments was as follows: One ($n = 17$), two ($n = 3$), three ($n = 4$), and six ($n = 2$). For the remaining three patients, LTP was not controlled even though they received repeated treatments with TACE or RF ablation. In addition, multiple intra- and extrahepatic metastases occurred. Finally, sorafenib treatment was administered. IDR occurred in 31 (38.8%) of the 80 patients in the hepatic resection group, and treatment modalities were as follows: TACE ($n = 18$), RF ablation ($n = 11$), cryoablation ($n = 1$), and hepatic resection ($n = 1$). IDR occurred in 42 (66.7%) of the 63

**Table 1 Baseline patient characteristics**

| Variable | Hepatic resection (*n* = 80) | RF ablation (*n* = 63) | *P* value |
|---|---|---|---|
| Mean age (yr) | 53.5 ± 9.0 | 60.3 ± 8.7 | < 0.001 |
| Male sex | 62 (78) | 49 (78) | 0.968 |
| Etiology | | | 0.042 |
| HBV | 68 (85) | 43 (68) | |
| HCV | 6 (8) | 13 (20) | |
| NBNC | 6 (8) | 7 (11) | |
| Liver cirrhosis | 50 (63) | 50 (79) | 0.029 |
| Tumor size | 2.1 ± 0.6 | 2.1 ± 0.5 | 0.906 |
| α-fetoprotein (ng/mL) | 200 ± 489 | 72 ± 165 | 0.031 |
| Platelet count (× 103/mm$^3$) | 148 ± 49 | 107 ± 49 | < 0.001 |
| Alanine aminotransferase (IU/L) | 41 ± 21 | 40 ± 30 | 0.849 |
| Total bilirubin (mg/dL) | 0.7 ± 0.3 | 0.7 ± 0.4 | 0.99 |
| Albumin (g/dL) | 4.2 ± 0.4 | 3.9 ± 0.4 | < 0.001 |
| Prothrombin time (INR) | 1.1 ± 0.1 | 1.2 ± 0.1 | < 0.001 |

Continuous data were evaluated using two-sample *t* tests and categorical variables were analyzed using Chi-square tests. Data represent the number of patients with percentage in parentheses or the mean ± SD. RF: Radiofrequency; HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNC: Non-B non-C; INR: International normalized ratio.

patients in the RF ablation group, and treatment modalities were as follows: TACE (*n* = 16), RF ablation (*n* = 20), combined TACE and RF ablation (*n* = 3), hepatic resection (*n* = 1), liver transplantation (*n* = 1), and sorafenib treatment (*n* = 1).

## DISCUSSION

In our study, we compared long-term therapeutic outcomes for treatments using hepatic resection and percutaneous RF ablation for HCCs (≤ 3 cm) abutting the right diaphragm; we found that the treatment modality was a significant prognostic factor for DFS, but was not an independent prognostic factor for OS. For the RF ablation group, the LTP rate was as high as 46.6% at 5 years. The location of tumors can affect the technical difficulty in local control of tumors, especially for RF ablation. Although there have been many studies that compared therapeutic outcomes between hepatic resection and RF ablation for HCC, most of them did not consider the location of tumors. In this way, the results of our study, which compares hepatic resection and percutaneous RF ablation for HCCs with consideration of the location of tumors, can provide important data for the proper management of HCCs abutting the diaphragm.

In our study, the LTP rate was 46.6% at 5 years for the RF ablation group. The LTP rate was much higher than rates reported in previous studies that included all HCCs located in the liver[11,14,17-19]. Percutaneous RF ablation for subphrenic HCCs is difficult to adequately perform for several reasons. First, the poor sonic window resulting from the lung shadow makes it difficult to accurately target tumors with the electrodes. Second, all tumors were subcapsular HCCs in our study. In general, subcapsular HCCs are considered to be more difficult to treat with percutaneous HCC than nonsubcapsular HCCs because of the difficulty of placing an electrode and not being able to obtain enough ablative margin along the hepatic capsule.

In this study, patients who had undergone hepatic resection exhibited longer DFS compared to those who had undergone RF ablation. This result is in line with previous studies that compared DFS outcomes for hepatic resection and RF ablation for HCC[20,21]. In our study, the estimated DFS rates at 1-, 3-, and 5-years were 85.0%, 70.9%, and 64.1%, respectively, for the hepatic resection group and 69.5%, 27.5%, and 18.3%, respectively, for the RF ablation group. In the previous study at our institution that compared RF ablation with hepatic resection for single HCC ≤ 3 cm located in the liver, the estimated DFS rate at 5 years was 61.1% for the hepatic resection group and 31.7% for the RF ablation group[11]. The DFS rate for the hepatic resection group of this study was similar to our previous result. However, the DFS rate for the RF ablation group of this study was lower than our previous result. This difference can most likely be explained by the high LTP rate for the RF ablation group in this study.

According to previous studies, RF ablation was comparable to hepatic resection for

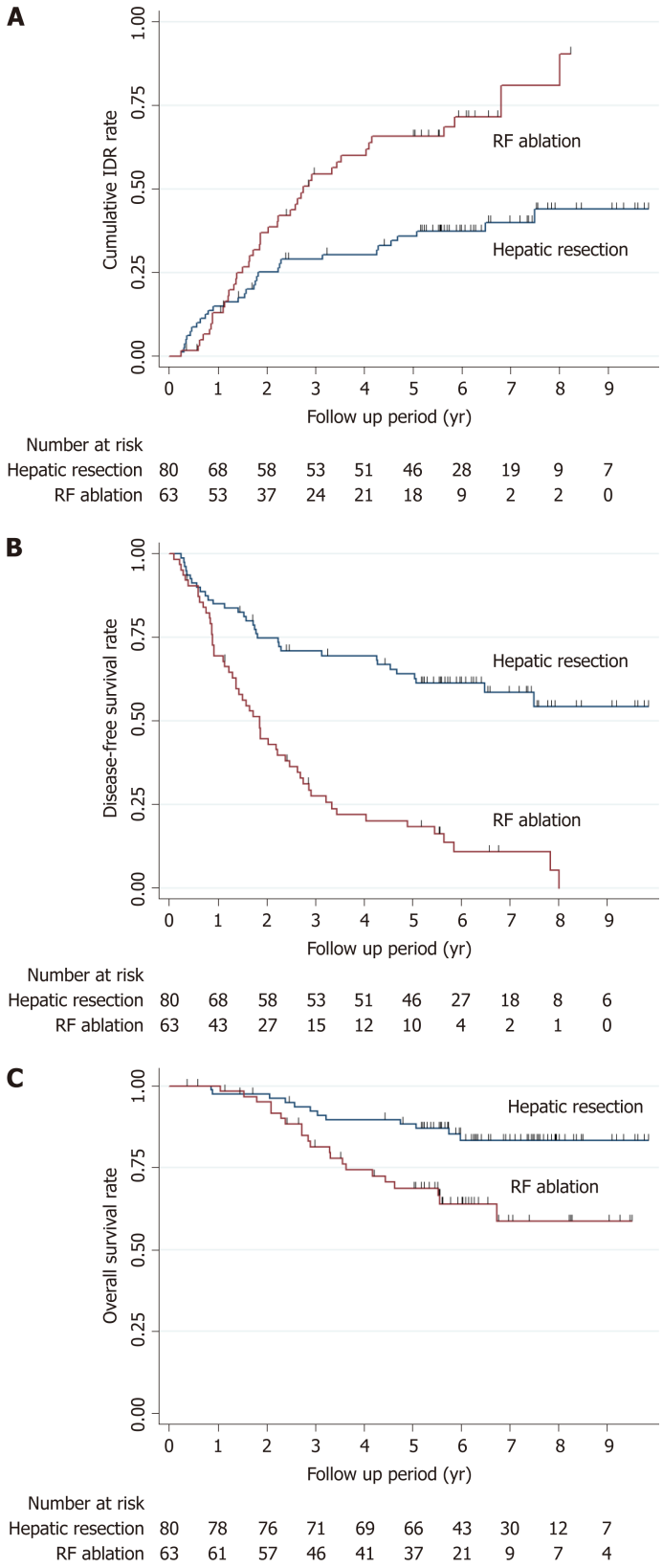


**Figure 2 Cumulative intrahepatic distant recurrence rates (A), disease-free survival rates (B), and overall survival rates (C).** IDR: Intrahepatic distant recurrence; RF: Radiofrequency.

very early and early-stage HCCs in terms of OS[22-24]. In our study, estimated OS rates for the hepatic resection group (97.5%, 92.3%, and 88.4% at 1-, 3-, and 5-years, respectively) appeared to be better than those for the RF ablation group (100%, 81.4%, and 68.7% at 1-, 3-, and 5-years). However, similar to previous studies, treatment modality was not an independent prognostic factor for OS according to multivariate

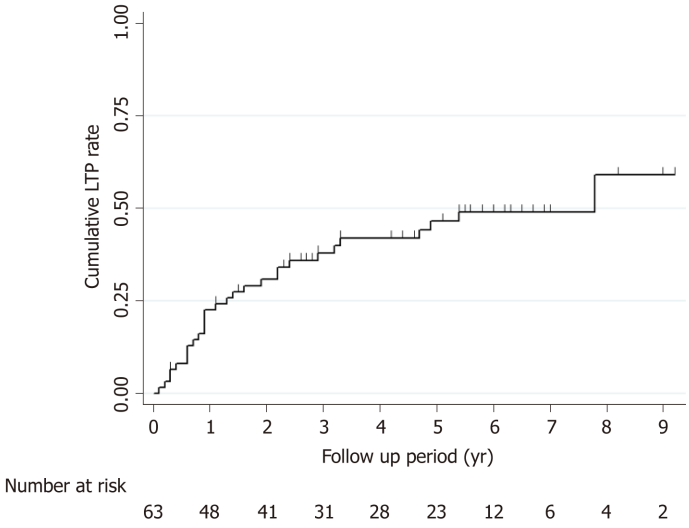


**Figure 3 Local tumor progression rate in the radiofrequency ablation group.** LTP: Local tumor progression.

analyses in our study.

Previous studies have reported comparable outcomes between RF ablation and hepatic resection in terms of long-term survival for patients with early-stage HCC. Based on these results, both hepatic resection and RF ablation are considered as curative treatment options for early stage HCC. Although treatment modality was not an independent prognostic factor for OS in patients with subphrenic HCCs, there were some differences in treatment outcomes between patients with subphrenic HCCs and nonsubphrenic HCCs that need to be considered when treatment modality is determined. First, the LTP rate after RF ablation was much higher for patients with subphrenic HCCs. Second, recurrent LTP was common in patients with subphrenic HCCs. In 12 (41%) of 29 patients who had LTP, multiple treatments were performed to control the LTP. Third, the peritoneal seeding rate for subphrenic HCCs was as high as 9.5% in the RF ablation group. Considering these unfavorable outcomes of RF ablation for subphrenic HCCs, it may be reasonable to preferentially consider hepatic resection as the first-line treatment for subphrenic HCCs rather than percutaneous RF ablation. Otherwise, laparoscopic RF ablation or combined TACE and RF ablation should be considered because these modalities can be more effective than percutaneous RF ablation alone in terms of local tumor control[25-27]. However, this issue needs to be investigated further.

Our study has some limitations. First, because this is a retrospective study, the treatment groups were not randomized, and we could not exclude the possibility of selection bias. However, we analyzed the effect of treatment modality (hepatic resection *vs* percutaneous RF ablation) after controlling for potential compounding factors. Second, HCC was diagnosed based on clinical guidelines in most patients in the RF ablation group. Therefore, there was a possibility of false-positive diagnosis, which could affect the outcomes. Third, this is a single-center study. In general, the outcomes of both hepatic resection and RF ablation greatly depend on the expertise and experience of the operators. In addition, we only used the single straight type of RF electrode and US as a guiding modality. Using other types of RF electrodes or guiding modalities may result in different therapeutic outcomes. Therefore, care should be taken when generalizing our results to that from other institutions.

In conclusion, although OS was not significantly different between patients who had undergone hepatic resection or percutaneous RF ablation for HCCs abutting the diaphragm, DFS was better in the hepatic resection group, and LTP was as high as 46.6% at 5 years in the RF ablation group. Therefore, it may be reasonable that hepatic resection should be preferentially considered over percutaneous US-guided RF ablation as a first-line treatment for HCCs abutting the diaphragm.

**Table 2 Univariate and multivariate analysis of prognostic factors for overall survival**

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR | *P* value | HR | *P* value |
| Group (RF ablation) | 0.364 (0.179, 0.742) | 0.005 | 0.676 (0.309, 1.482) | 0.329 |
| Age | 1.025 (0.988, 1.063) | 0.187 | | |
| Sex (female) | 0.975 (0.439, 2.165) | 0.95 | | |
| Etiology (hepatitis B virus)[1] | | 0.028 | | 0.176 |
| Hepatitis C virus | 3.053 (1.240, 7.516) | 0.01 | 2.180 (0.854, 5.566) | 0.124 |
| NBNC | 2.292 (0.671, 7.831) | 0.26 | 2.433 (0.686, 8.636) | 0.232 |
| Liver cirrhosis (absence) | 1.834 (0.756, 4.446) | 0.18 | | |
| Tumor size | 1.505 (0.782, 2.899) | 0.221 | | |
| α-fetoprotein | 1.000 (1.000, 1.001) | 0.345 | | |
| Platelet count | 0.989 (0.982, 0.996) | 0.003 | 0.991 (0.982, 1.000) | 0.051 |
| Alanine aminotransferase | 1.006 (0.993, 1.019) | 0.36 | | |
| Total bilirubin | 1.031 (0.393, 2.704) | 0.951 | | |
| Albumin | 0.327 (0.148, 0.727) | 0.006 | 0.485 (0.189, 1.244) | 0.132 |
| Prothrombin time (INR) | 19.351 (1.354, 1798.539) | 0.034 | 0.325 (0.002, 46.731) | 0.657 |

The Cox proportional hazards model was used for univariate and multivariate analysis. The reference category for each categorical variable is provided in the square brackets in the first column.
[1]Bonferroni correction was used owing to multiple comparisons. Numbers in parentheses represent the 95%CI. HR: Hazard ratio; RF: Radiofrequency; NBNC: Non-B non-C; INR: International normalized ratio.

**Table 3 Univariate and multivariate analysis of prognostic factors for disease-free survival**

| Variable | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | HR | *P* value | HR | *P* value |
| Group (RF ablation) | 0.272 (0.174, 0.427) | < 0.001 | 0.352 (0.205, 0.605) | < 0.001 |
| Age | 1.036 (1.012, 1.060) | 0.003 | 1.015 (0.987, 1.043) | 0.306 |
| Sex (female) | 1.096 (0.650, 1.847) | 0.732 | | |
| Etiology (hepatitis B virus) 1 | | 0.726 | | |
| Hepatitis C virus | 1.507 (0.789, 2.878) | 0.31 | | |
| NBNC | 1.121 (0.483, 2.601) | 1 | | |
| Liver cirrhosis (absence) | 1.526 (0.916, 2.544) | 0.105 | | |
| Tumor size | 0.976 (0.658, 1.448) | 0.904 | | |
| α-fetoprotein | 1.000 (0.999, 1.000) | 0.391 | | |
| Platelet count | 0.991 (0.987, 0.995) | < 0.001 | 0.998 (0.993, 1.004) | 0.542 |
| Alanine aminotransferase | 1.008 (1.000, 1.017) | 0.045 | 1.011 (1.003, 1.020) | 0.006 |
| Total bilirubin | 0.995 (0.540, 1.833) | 0.988 | | |
| Albumin | 0.281 (0.168, 0.470) | < 0.001 | 0.481 (0.269, 0.860) | 0.014 |
| Prothrombin time (INR) | 147.887 (13.992, 1563.115) | < 0.001 | 4.212 (0.195, 90.886) | 0.359 |

The Cox proportional hazards model was used for univariate and multivariate analysis. The reference category for each categorical variable is in the square brackets in first column. [1]Bonferroni correction was used owing to multiple comparisons. Numbers in parentheses represent the 95%CI. HR: Hazard ratio; RF: Radiofrequency; NBNC: Non-B non-C; INR: International normalized ratio.

## ARTICLE HIGHLIGHTS

### *Research background*

Many studies have revealed that radiofrequency (RF) ablation is comparable to hepatic resection in terms of long-term survival for patients with early stage hepatocellular carcinoma (HCC). However, most studies have not taken into account the location of HCCs.

### *Research motivation*

Our study attempted to analyze the effect of the subphrenic location of HCC on the long-term

therapeutic outcomes after hepatic resection and RF ablation.

### Research objectives
To compare the long-term therapeutic outcomes between hepatic resection *vs* percutaneous RF ablation for HCCs abutting the diaphragm.

### Research methods
A total of 143 Child-Pugh class A patients who had undergone hepatic resection ($n$ = 80) or percutaneous RF ablation ($n$ = 63) for an HCC (≤ 3 cm) abutting the right diaphragm were included. Therapeutic outcomes were compared.

### Research results
Hepatic resection was an independent prognostic factor for disease-free survival (DFS) ($P \leq 0.001$; hazard ratio, 0.352; 95%CI: 0.205, 0.605; with RF ablation as the reference category); however, treatment modality was not an independent prognostic factor for overall survival (OS). The local tumor progression rate was 46.6% at 5 years for the RF ablation group.

### Research conclusions
Although OS was not significantly different between patients who had undergone hepatic resection or percutaneous RF ablation for HCCs abutting the diaphragm, DFS was better in the hepatic resection group.

### Research perspectives
Further studies with large sample size and multicenter prospective studies are needed to confirm the conclusion of this study.

## REFERENCES


1 **Bruix J**, Sherman M; American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
2 **Cho YK**, Kim JK, Kim WT, Chung JW. Hepatic resection versus radiofrequency ablation for very early stage hepatocellular carcinoma: a Markov model analysis. *Hepatology* 2010; **51**: 1284-1290 [PMID: 20099299 DOI: 10.1002/hep.23466]
3 **Wang JH**, Wang CC, Hung CH, Chen CL, Lu SN. Survival comparison between surgical resection and radiofrequency ablation for patients in BCLC very early/early stage hepatocellular carcinoma. *J Hepatol* 2012; **56**: 412-418 [PMID: 21756858 DOI: 10.1016/j.jhep.2011.05.020]
4 **Molinari M**, Helton S. Hepatic resection versus radiofrequency ablation for hepatocellular carcinoma in cirrhotic individuals not candidates for liver transplantation: a Markov model decision analysis. *Am J Surg* 2009; **198**: 396-406 [PMID: 19520354 DOI: 10.1016/j.amjsurg.2009.01.016]
5 **Chen J**, Peng K, Hu D, Shen J, Zhou Z, Xu L, Chen J, Pan Y, Wang J, Zhang Y, Chen M. Tumor Location Influences Oncologic Outcomes of Hepatocellular Carcinoma Patients Undergoing Radiofrequency Ablation. *Cancers (Basel)* 2018; 10 [PMID: 30309001 DOI: 10.3390/cancers10100378]
6 **Kang TW**, Rhim H, Kim EY, Kim YS, Choi D, Lee WJ, Lim HK. Percutaneous radiofrequency ablation for the hepatocellular carcinoma abutting the diaphragm: assessment of safety and therapeutic efficacy. *Korean J Radiol* 2009; **10**: 34-42 [PMID: 19182501 DOI: 10.3348/kjr.2009.10.1.34]
7 **Rhim H**, Lim HK, Kim YS, Choi D. Percutaneous radiofrequency ablation with artificial ascites for hepatocellular carcinoma in the hepatic dome: initial experience. *AJR Am J Roentgenol* 2008; **190**: 91-98 [PMID: 18094298 DOI: 10.2214/AJR.07.2384]
8 **Koda M**, Ueki M, Maeda Y, Mimura K, Okamoto K, Matsunaga Y, Kawakami M, Hosho K, Murawaki Y. Percutaneous sonographically guided radiofrequency ablation with artificial pleural effusion for hepatocellular carcinoma located under the diaphragm. *AJR Am J Roentgenol* 2004; **183**: 583-588 [PMID: 15333339 DOI: 10.2214/ajr.183.3.1830583]
9 **Kang TW**, Rhim H, Lee MW, Kim YS, Choi D, Lee WJ, Lim HK. Radiofrequency ablation for hepatocellular carcinoma abutting the diaphragm: comparison of effects of thermal protection and therapeutic efficacy. *AJR Am J Roentgenol* 2011; **196**: 907-913 [PMID: 21427344 DOI: 10.2214/AJR.10.4584]
10 **Kondo Y**, Yoshida H, Tateishi R, Shiina S, Kawabe T, Omata M. Percutaneous radiofrequency ablation of liver cancer in the hepatic dome using the intrapleural fluid infusion technique. *Br J Surg* 2008; **95**: 996-1004 [PMID: 18581421 DOI: 10.1002/bjs.6058]
11 **Kang TW**, Kim JM, Rhim H, Lee MW, Kim YS, Lim HK, Choi D, Song KD, Kwon CH, Joh JW, Paik SW, Paik YH, Ahn JH. Small Hepatocellular Carcinoma: Radiofrequency Ablation versus Nonanatomic Resection--Propensity Score Analyses of Long-term Outcomes. *Radiology* 2015; **275**: 908-919 [PMID: 25688888 DOI: 10.1148/radiol.15141483]
12 **Bruix J**, Sherman M; Practice Guidelines Committee, American Association for the Study of Liver Diseases. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]
13 **Cucchetti A**, Qiao GL, Cescon M, Li J, Xia Y, Ercolani G, Shen F, Pinna AD. Anatomic versus nonanatomic resection in cirrhotic patients with early hepatocellular carcinoma. *Surgery* 2014; **155**: 512-521 [PMID: 24439747 DOI: 10.1016/j.surg.2013.10.009]
14 **Kim YS**, Lim HK, Rhim H, Lee MW, Choi D, Lee WJ, Paik SW, Koh KC, Lee JH, Choi MS, Gwak GY, Yoo BC. Ten-year outcomes of percutaneous radiofrequency ablation as first-line therapy of early hepatocellular carcinoma: analysis of prognostic factors. *J Hepatol* 2013; **58**: 89-97 [PMID: 23023009 DOI: 10.1016/j.jhep.2012.09.020]
15 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID:

15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]
16 **Ahmed M**, Solbiati L, Brace CL, Breen DJ, Callstrom MR, Charboneau JW, Chen MH, Choi BI, de Baère T, Dodd GD, Dupuy DE, Gervais DA, Gianfelice D, Gillams AR, Lee FT, Leen E, Lencioni R, Littrup PJ, Livraghi T, Lu DS, McGahan JP, Meloni MF, Nikolic B, Pereira PL, Liang P, Rhim H, Rose SC, Salem R, Sofocleous CT, Solomon SB, Soulen MC, Tanaka M, Vogl TJ, Wood BJ, Goldberg SN; International Working Group on Image-guided Tumor Ablation; Interventional Oncology Sans Frontières Expert Panel; Technology Assessment Committee of the Society of Interventional Radiology,; Standard of Practice Committee of the Cardiovascular and Interventional Radiological Society of Europe. Image-guided tumor ablation: standardization of terminology and reporting criteria--a 10-year update. *Radiology* 2014; **273**: 241-260 [PMID: 24927329 DOI: 10.1148/radiol.14132958]
17 **Kang TW**, Lim HK, Lee MW, Kim YS, Rhim H, Lee WJ, Paik YH, Kim MJ, Ahn JH. Long-term Therapeutic Outcomes of Radiofrequency Ablation for Subcapsular versus Nonsubcapsular Hepatocellular Carcinoma: A Propensity Score Matched Study. *Radiology* 2016; **280**: 300-312 [PMID: 26824711 DOI: 10.1148/radiol.2016151243]
18 **Shiina S**, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; **107**: 569-577; quiz 578 [PMID: 22158026 DOI: 10.1038/ajg.2011.425]
19 **Lee DH**, Lee JM, Lee JY, Kim SH, Yoon JH, Kim YJ, Han JK, Choi BI. Radiofrequency ablation of hepatocellular carcinoma as first-line treatment: long-term results and prognostic factors in 162 patients with cirrhosis. *Radiology* 2014; **270**: 900-909 [PMID: 24475823 DOI: 10.1148/radiol.13130940]
20 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]
21 **Vivarelli M**, Guglielmi A, Ruzzenente A, Cucchetti A, Bellusci R, Cordiano C, Cavallari A. Surgical resection versus percutaneous radiofrequency ablation in the treatment of hepatocellular carcinoma on cirrhotic liver. *Ann Surg* 2004; **240**: 102-107 [PMID: 15213625 DOI: 10.1097/01.sla.0000129672.51886.44]
22 **Nishikawa H**, Inuzuka T, Takeda H, Nakajima J, Matsuda F, Sakamoto A, Henmi S, Hatamaru K, Ishikawa T, Saito S, Nasu A, Kita R, Kimura T, Arimoto A, Osaki Y. Comparison of percutaneous radiofrequency thermal ablation and surgical resection for small hepatocellular carcinoma. *BMC Gastroenterol* 2011; **11**: 143 [PMID: 22204311 DOI: 10.1186/1471-230X-11-143]
23 **Hong SN**, Lee SY, Choi MS, Lee JH, Koh KC, Paik SW, Yoo BC, Rhee JC, Choi D, Lim HK, Lee KW, Joh JW. Comparing the outcomes of radiofrequency ablation and surgery in patients with a single small hepatocellular carcinoma and well-preserved hepatic function. *J Clin Gastroenterol* 2005; **39**: 247-252 [PMID: 15718869 DOI: 10.1097/01.mcg.0000152746.72149.31]
24 **Hasegawa K**, Makuuchi M, Takayama T, Kokudo N, Arii S, Okazaki M, Okita K, Omata M, Kudo M, Kojiro M, Nakanuma Y, Takayasu K, Monden M, Matsuyama Y, Ikai I. Surgical resection vs. percutaneous ablation for hepatocellular carcinoma: a preliminary report of the Japanese nationwide survey. *J Hepatol* 2008; **49**: 589-594 [PMID: 18620773 DOI: 10.1016/j.jhep.2008.05.018]
25 **Shibata T**, Isoda H, Hirokawa Y, Arizono S, Shimada K, Togashi K. Small hepatocellular carcinoma: is radiofrequency ablation combined with transcatheter arterial chemoembolization more effective than radiofrequency ablation alone for treatment? *Radiology* 2009; **252**: 905-913 [PMID: 19567647 DOI: 10.1148/radiol.2523081676]
26 **Peng ZW**, Zhang YJ, Chen MS, Xu L, Liang HH, Lin XJ, Guo RP, Zhang YQ, Lau WY. Radiofrequency ablation with or without transcatheter arterial chemoembolization in the treatment of hepatocellular carcinoma: a prospective randomized trial. *J Clin Oncol* 2013; **31**: 426-432 [PMID: 23269991 DOI: 10.1200/JCO.2012.42.9936]
27 **Hirooka M**, Kisaka Y, Uehara T, Ishida K, Kumagi T, Watanabe Y, Abe M, Matsuura B, Hiasa Y, Onji M. Efficacy of laparoscopic radiofrequency ablation for hepatocellular carcinoma compared to percutaneous radiofrequency ablation with artificial ascites. *Dig Endosc* 2009; **21**: 82-86 [PMID: 19691779 DOI: 10.1111/j.1443-1661.2009.00836.x]

**P- Reviewer:** Aykan NF, He J, Huang C, Lambrecht NW
**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 238-249

DOI: 10.4251/wjgo.v11.i3.238

ISSN 1948-5204 (online)

ORIGINAL ARTICLE

Observational Study

# Risk of cholangiocarcinoma in patients undergoing therapeutic endoscopic retrograde cholangiopancre-atography or cholecystectomy: A population based study

Chi-Chih Wang, Ming-Chang Tsai, Wen-Wei Sung, Tzu-Wei Yang, Hsuan-Yi Chen, Yao-Tung Wang, Chang-Cheng Su, Ming-Hseng Tseng, Chun-Che Lin

**ORCID number:** Chi-Chih Wang (0000-0002-8222-0503); Ming-Chang Tsai (0000-0002-5770-9358); Wen-Wei Sung (0000-0002-2375-0029); Tzu-Wei Yang (0000-0002-1522-8177); Hsuan-Yi Chen (0000-0003-1001-7968); Yao-Tung Wang (0000-0002-0300-0896); Chang-Cheng Su (0000-0002-7211-4192); Ming-Hseng Tseng (0000-0001-8868-1610); Chun-Che Lin (0000-0002-2474-6734).

**Author contributions:** Tseng MH and Lin CC contributed equally to this manuscript; Wang CC, Tseng MH and Sung WW contributed to conception and design; Tseng MH contributed to acquisition of data; Tsai MC, Wang CC, Wang YT and Chen HY contributed to analysis and interpretation of data; Wang CC, Yang TW and Chen HY contributed to drafting of the manuscript; Yang TW, Sung WW and Lin CC contributed to critical revision of the manuscript; Tsai MC, Sung WW and Su CC contributed to statistical analysis; Tseng MH and Lin CC contributed to supervision.

**Supported by** Chung Shan Medical University Hospital research program, Taichung, Taiwan, No. CSH- 2013-C-032.

**Institutional review board statement:** This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital,

**Chi-Chih Wang, Ming-Chang Tsai, Wen-Wei Sung, Yao-Tung Wang, Chun-Che Lin,** Institute of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan

**Chi-Chih Wang, Ming-Chang Tsai, Wen-Wei Sung, Tzu-Wei Yang, Hsuan-Yi Chen, Yao-Tung Wang, Chun-Che Lin,** School of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan

**Chi-Chih Wang, Ming-Chang Tsai, Tzu-Wei Yang, Hsuan-Yi Chen, Chang-Cheng Su, Chun-Che Lin,** Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

**Wen-Wei Sung,** Department of Urology, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

**Tzu-Wei Yang,** Institute and Department of Biological Science and Technology, National Chiao Tung University, Hsinchu 30010, Taiwan

**Yao-Tung Wang,** Division of Pulmonary Medicine, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

**Ming-Hseng Tseng,** Department of Medical Informatics, Chung Shan Medical University, Taichung 40201, Taiwan

**Ming-Hseng Tseng,** Information Technology Office, Chung Shan Medical University Hospital, Taichung 40201, Taiwan

**Corresponding author:** Chun-Che Lin, MD, PhD, Attending Doctor, Chief Doctor, Doctor, Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Chung Shan Medical University Hospital, No.110, Sec.1, Jianguo N.Rd., Taichung 40201, Taiwan. cshy333@csh.org.tw
**Telephone:** +886-424730022-11603
**Fax:** +886-423248130

## Abstract

*BACKGROUND*

Cholangiocarcinoma is a highly lethal disease that had been underestimated in the past two decades. Many risk factors are well documented for in cholangiocarcinoma, but the impacts of advanced biliary interventions, like endoscopic sphincterotomy (ES), endoscopic papillary balloon dilatation (EPBD),

Taiwan.

**Informed consent statement:** The Institutional Review Board waved the need of informed consent in this study as it is a retrospective study based on the National Health Insurance Research Database.

**Conflict-of-interest statement:** None.

**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.



**Manuscript source**: Invited manuscript

**Received:** October 25, 2018
**Peer-review started:** October 25, 2018
**First decision:** December 10, 2018
**Revised:** January 16, 2019
**Accepted:** January 29, 2019
**Article in press:** January 30, 2019
**Published online:** March 15, 2019

and cholecystectomy, are inconsistent in the previous literature.

*AIM*
To clarify the risks of cholangiocarcinoma after ES/EPBD, cholecystectomy or no intervention for cholelithiasis using the National Health Insurance Research Database (NHIRD).

*METHODS*
From data of NHIRD 2004-2011 in Taiwan, we selected 7938 cholelithiasis cases as well as 23814 control group cases (matched by sex and age in a 1:3 ratio). We compared the previous risk factors of cholangiocarcinoma and cholangiocarcinoma rate in the cholelithiasis and control groups. The incidences of total and subsequent cholangiocarcinoma were calculated in ES/EPBD patients, cholecystectomy patients, cholelithiasis patients without intervention, and groups from the normal population.

*RESULTS*
In total, 537 cases underwent ES/EPBD, 1743 cases underwent cholecystectomy, and 5658 cholelithiasis cases had no intervention. Eleven (2.05%), 37 (0.65%), and 7 (0.40%) subsequent cholangiocarcinoma cases were diagnosed in the ES/EPBD, no intervention, and cholecystectomy groups, respectively, and the odds ratio for subsequent cholangiocarcinoma was 3.13 in the ES/EPBD group and 0.61 in the cholecystectomy group when compared with the no intervention group.

*CONCLUSION*
In conclusion, symptomatic cholelithiasis patients who undergo cholecystectomy can reduce the incidence of subsequent cholangiocarcinoma, while cholelithiasis patients who undergo ES/EPBD are at a great risk of subsequent cholangiocarcinoma according to our findings.

**Key words:** Cholangiocarcinoma; Endoscopic sphincterotomy; Endoscopic papillary balloon dilatation; Cholecystectomy



**Core tip:** There are many risk factors well demonstrated in cholangiocarcinoma, but the impacts of advanced biliary interventions, like endoscopic sphincterotomy (ES), endoscopic papillary balloon dilatation (EPBD) and cholecystectomy, are inconsistence in previous literature. We tried to evaluate the subsequent cholangiocarcinoma risk in cholelithiasis patients who underwent ES, EPBD and cholecystectomy. Cholecystectomy can reduce the incidence of subsequent cholangiocarcinoma, while cholelithiasis patients underwent ES/EPBD are in a huge risk of subsequent cholangiocarcinoma in our database study.

**Citation:** Wang CC, Tsai MC, Sung WW, Yang TW, Chen HY, Wang YT, Su CC, Tseng MH, Lin CC. Risk of cholangiocarcinoma in patients undergoing therapeutic endoscopic retrograde cholangiopancre-atography or cholecystectomy: A population based study. *World J Gastrointest Oncol* 2019; 11(3): 238-249
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/238.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.238

## INTRODUCTION

Cholangiocarcinoma, which arises from the epithelial cells of the intrahepatic or extrahepatic bile ducts, is a highly lethal disease that has been underestimated in the past two decades. Unlike the decline in mortality due to primary liver cancer, the mortality of intra-hepatic cholangiocarcinoma (ICC) has increased in both sexes in Europe[1]. At the same time, previous studies have shown that the incidence of ICC has been rising, while the incidence of extra-hepatic cholangiocarcinoma (ECC) has declined internationally[2-5] in the past thirty years, except in Denmark[6]. Unfortunately, the global incidence data may be inaccurate because of ICC registered as

part of primary liver cancer and ECC mixed with gallbladder cancers in the databases of many countries.

The previous literature has listed many well known risk factors for cholangiocarcinoma, such as primary sclerosing cholangitis[7-9], choledochal cyst disease[10,11], specific parasite infection[12], cholelithiasis[13,14], chronic hepatitis B and C (CHB and CHC) infection[15,16], diabetes mellitus (DM)[17,18] and *Helicobacter* infection (HP)[19,20]. However, the true etiology of cholangiocarcinoma is still a mystery, although several hypotheses have been proposed, including destruction of the integrity of the bile duct through procedures like therapeutic endoscopic retrograde cholangiopancreatography (ERCP) or cholecystectomy. The major indications for ERCP are choledocholithiasis, rather than biliary or pancreatic neoplasms, or the need to manage postoperative biliary complications[21-23]. Therapeutic ERCP, including endoscopic sphincterotomy (ES) and endoscopic papillary balloon dilatation (EPBD), has been considered to have increased future cholangiocarcinoma incidence for over a decade[24-26]. Because cholelithiasis itself is one of the risk factors of cholangiocarcinoma, the impact of the incidence of a subsequent cholangiocarcinoma for advanced bile duct management is hard to evaluate.

ES had been shown to increase biliary epithelial atypia[27], and previous data have indicated that therapeutic ERCP can increase the subsequent cholangiocarcinoma rate[28]. At the same time, many recent larger population-based studies have demonstrated that ES does not increase the incidence of cholangiocarcinoma[29-31]. Even some evidence has suggested that ES does not increase the subsequent cholangiocarcinoma rate over that seen with EPBD[29]. At the same time, cholelithiasis and cholecystectomy had been of concern due to the increase in ICC[32] and ECC[33], but some studies have shown that cholecystectomy decreases the subsequent cholangiocarcinoma rate in cholelithiasis patients[34].

The inconsistency of the previous evidence led us to conduct this study using the National Health Insurance Research Database (NHIRD) 2004-2011 in Taiwan. Our goal was to re-confirm the old risk factors in modern society and to clarify the risk of cholangiocarcinoma in the medium time period following therapeutic ERCP or cholecystectomy in cholelithiasis patients.

## MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Chung Shan Medical University Hospital, Taiwan. The IRB waved the need for informed consent in this study as it is a retrospective study based on the NHIRD. All authors declare no any conflicts of interest.

### *Study design*

This study is a population-based retrospective cohort study based on Taiwan's NHIRD, which covers more than 99% of the Taiwanese population[35]. The study methods of NHIRD have been described in detail in previous studies[36,37]. Symptomatic cholelithiasis cases with above 18 years of age were included from one million random samples of NHIRD data obtained between January 2005 and December 2007 using Codes of International Statistical Classification of Diseases and Related Health Problems-9th Edition (ICD-9), which were registered once in admission or three times in outpatient clinics to avoid bias from possible classification errors. After study group selection, we built the control group with propensity score matching by sex and age in a 1:3 ratio. The control group cases were defined as individuals who had neither been diagnosed with cholelithiasis nor undergone a related medical procedure, such as cholecystectomy or ERCP, in the previous year. Cholelithiasis patients who had undergone ES, EPBD, or cholecystectomy in the previous year or who were diagnosed after cholangiocarcinoma were excluded from further analysis. We then excluded patients, who diagnosed with cholangiocarcinoma from January to December 2004 in both the control and study groups. The cholangiocarcinoma patients in Taiwan have catastrophic illness cards that waive their medical expenses by ICD-9 registration; therefore, we considered that a one year time period for exclusion was adequate. The variables such as economic status, place of residence, follow-up time, and cholangiocarcinoma rate, as well as the historical common risk factors, such as CHB, CHC, HP, DM, end-stage renal disease (ESRD) on dialysis, congenital cystic disease of liver (CCDL), Clonorchis Opisthorchis (CO), and inflammatory bowel disease (IBD), were compared in cholelithiasis and control group.

The cases of cholelithiasis were divided into three groups of patients who underwent ES or EPBD, patients who underwent cholecystectomy, and patients without any therapeutic intervention between January 2005 to December 2011. The

patients who underwent both ES/EPBD and cholecystectomy were registered in the ES/EPBD group in our settings. The details of study design are shown in Figure 1. The ICD-9 codes for the listed diseases and procedure codes are listed in Supplementary Table 1. The stratification of age, gender, economic status, place of residence, follow-up time, cholangiocarcinoma rate, and historical common risk factors were compared in each group. Patients who experienced cholangiocarcinoma in the first 6 mo after ES, EPBD, or cholecystectomy were excluded from further analysis, because these cases should be considered as misdiagnoses or concurrent malignancies rather than subsequent cholangiocarcinoma. The time cumulative risk of cholangiocarcinoma in the different groups was calculated.

### Data processing and statistical analysis

The NHIRD, which includes one a representative population of one million persons residing in Taiwan between 2004 and 2011 was managed using Microsoft SQL Server 2008 R2 (Microsoft Corporation, Redmond, WA, United States) and the SQL programming language for the data query and data processing jobs. Statistical analysis was done using OpenEpi: Open source epidemiologic statistics for public health, version 3.01[38]. Kaplan-Meier survival analyses were conducted using SPSS version 19. Person time analyses were done using OpenEpi version 3.01.

Data obtained from the study were compared with the use of the $\chi^2$ test for categorical variables, the *t*-test, or one-way ANOVA (Analysis of Variance) for continuous variables, and the Log Rank (Mantel-Cox) test for survival curves. A two-tailed *P*-value of 0.05 was considered statistically significant in this study.

## RESULTS

Because we used age and sex to find three times as many normal population subjects without cholelithiasis to be our control group, we could not evaluate age and sex as risk stratification in our comparisons of the cholelithiasis and control groups.

### Cholelithiasis cases and their matched controls

In total, 7938 adult cholelithiasis cases were selected from one million random samples of NHIRD data obtained between January 2005 and December 2007. The control group consisted of 23814 cases without cholelithiasis and matched by age and sex. The mean age of both groups was 59.15 ± 16.53 and the proportion of female patients was 52.15% in both groups. The mean follow up time was 57.96 ± 21.48 mo in cholelithiasis group and 63.12 ± 15.6 mo in the normal population in our analysis. Demographic data revealed that the cholelithiasis patients had a minimum basic salary (49.92%) and residence in a lived in remote villages (1.65%) and the differences were statistically significant when compared to the control group. The proportion of historical risk factors for cholangiocarcinoma, like CHB, CHC, HP, DM, ESRD, CCDL, and IBD, were 9.50% *vs* 2.80%, 6.83% *vs* 1.99%, 1.61% *vs* 0.55%, 29.21% *vs* 18.17%, 2.34% *vs* 1.50%, 0.64% *vs* 0.03% and 1.5% *vs* 0.77% in the cholelithiasis group versus the normal population, respectively. All the proportions of comorbidity were significantly high ($P < 0.001$) in the cholelithiasis group, except for CO infection because neither group showed CO infection. In total,147 cholelithiasis cases and 39 normal population cases experienced cholangiocarcinoma during the follow-up period. After exclusion of cases with cholangiocarcinoma in the initial 6 mo in both groups, 55 cholelithiasis cases and 35 normal population cases developed cholangiocarcinoma, with a mean follow up of 36.73 ± 20.57 mo and 35.27 ± 19.94 mo, respectively. The subsequent cholangiocarcinoma rate was higher in the cholelithiasis group than in the control group (0.69% *vs* 0.15%, $P < 0.001$). The detailed information is shown in Table 1.

### Cholelithiasis cases that underwent ES/EPBD, cholecystectomy, and no intervention

There were 537 cases that underwent ES/EPBD, 1743 cases that underwent cholecystectomy, and 5658 cases that received no intervention, and we observed no significant difference in the mean age. However, the mean age after age stratification of patients above 70 years old was higher in the ES/EPBD group (79.11 ± 5.13), followed by the no intervention group (78.78 ± 6.08) and the cholecystectomy group (78.01 ± 5.54). Other demographic data in our analysis showed some differences: Follow-up time, place of residence, proportion of CHB, proportion of CHC, and proportion of CCDL. The details are shown in Table 2.

In total, 27 patients (5.03%) were diagnosed with cholangiocarcinoma in the ES/EPBD group, while 105 (1.86%) were diagnosed with cholangiocarcinoma in the no intervention group, and 15 (0.86%) were diagnosed in the cholecystectomy group

**Table 1 Demographic data of study and normal population**

| | Cholelithiasis group *n* = 7938 | | Control group *n* = 23814 | | *P* value |
|---|---|---|---|---|---|
| | *N* | SD, % | *N* | SD, % | |
| Age, mean (SD) | 59.15 | 16.53 | 59.15 | 16.53 | 1 |
| Age, yr | | | | | |
| 18-49 | 38.89 | 7.38 | 38.94 | 7.38 | |
| 50-69 | 59.13 | 5.52 | 59.13 | 5.52 | |
| > 70 | 78.67 | 5.95 | 78.67 | 5.95 | |
| Gender | | | | | 1 |
| Male | 3798 | 47.85 | 11394 | 47.85 | |
| Female | 4140 | 52.15 | 12420 | 52.15 | |
| Follow up time (mo), mean (SD) | 57.96 | 21.48 | 63.12 | 15.6 | < 0.001 |
| Economic status | | | | | < 0.001 |
| MBS | 3963 | 49.92 | 11216 | 47.1 | |
| 1-3 times MBS | 3136 | 39.51 | 10217 | 42.9 | |
| Above 3 times MBS | 825 | 10.39 | 2336 | 9.81 | |
| Place of residence | | | | | 0.007 |
| City | 5046 | 63.57 | 15078 | 63.32 | |
| Countryside | 2747 | 34.61 | 8403 | 35.29 | |
| Remote village | 131 | 1.65 | 287 | 1.21 | |
| Comorbidity | | | | | |
| CHB | 754 | 9.5 | 667 | 2.8 | < 0.001 |
| CHC | 542 | 6.83 | 474 | 1.99 | < 0.001 |
| HP | 128 | 1.61 | 131 | 0.55 | < 0.001 |
| DM | 2319 | 29.21 | 4327 | 18.17 | < 0.001 |
| ESRD | 186 | 2.34 | 357 | 1.5 | < 0.001 |
| CCDL | 51 | 0.64 | 7 | 0.03 | < 0.001 |
| CO | 0 | 0 | 0 | 0 | NA |
| IBD | 119 | 1.5 | 184 | 0.77 | < 0.001 |
| Cholangiocarcinoma | | | | | |
| Number (rate) | 147 | 1.85 | 39 | 0.16 | < 0.001 |
| Follow up time (mo), mean (SD) | 13.92 | 21.96 | 31.8 | 21.48 | < 0.001 |
| Cholangiocarcinoma after first 6 mo | | | | | |
| Number (rate) | 55 | 0.69 | 35 | 0.15 | < 0.001 |
| Follow up time (mo), mean (SD) | 36.73 | 20.57 | 35.27 | 19.94 | 0.86 |

SD: Standard deviation; MBS: Minimum basic salary; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; HP: *Helicobacter* infection; DM: Diabetes mellitus; ESRD: End-stage renal disease; CCDL: Congenital cystic disease of liver; CO: Clonorchis Opisthorchis; IBD: Inflammatory bowel disease.

during the follow-up period. After exclusion of possible misdiagnoses and concurrent cholangiocarcinoma, by excluding cholangiocarcinoma diagnosed within 6 mo after the procedure, 11 (2.05%), 37 (0.65%), and 7 (0.40%) cholangiocarcinoma cases were diagnosed in the ES/EPBD, no intervention, and cholecystectomy groups, respectively. The time to diagnosis for subsequent cholangiocarcinoma was 41.17 ± 22.51 mo in the ES/EPBD group, 35.46 ± 19.08 mo in the no intervention group, and 33.70 ± 23.35 mo in the cholecystectomy group. The odds ratio for subsequent cholangiocarcinoma was 3.13 in the ES/EPBD group and 0.61 in cholecystectomy group when compared with the no intervention group. The results were similar if we excluded the cholangiocarcinoma cases within one year after the procedure or the diagnosis of cholelithiasis. The cumulative cholangiocarcinoma rates in the three groups in the 7-year follow-up period are demonstrated in Figure 2.

### *The incidence of cholangiocarcinoma*

The incidence of cholangiocarcinoma after the initial 6 mo was compared using incidence rate/1000 person-years. In the ES/EPBD group, the incidence of

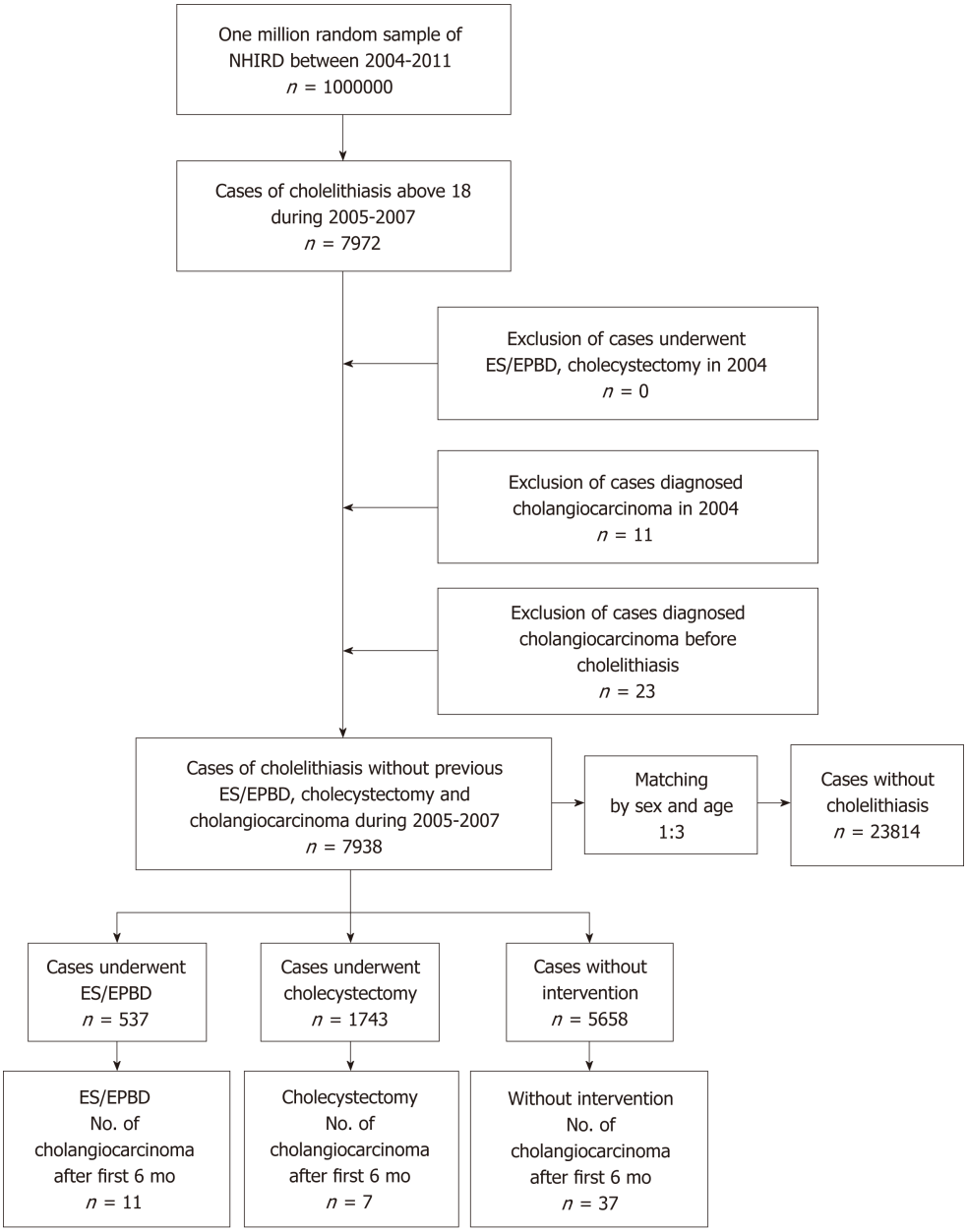


**Figure 1 Case selection flow chart of one million nationwide representative data base.** ES/EPBD: Endoscopic sphincterotomy/endoscopic papillary balloon dilatation; NHIRD: National Health Insurance Research Database.

cholangiocarcinoma was 4.37 (2.30-7.59) per 1000 person-years, which is more than 15 times of the incidence of the normal population. The incidence of cholangiocarcinoma in ES/EPBD was especially high in females (6.31/1000 person-years) and patients older than 70 years (7.53/1000 person-years).

In the cholecystectomy group, the incidence of cholangiocarcinoma was 0.79 (0.34–1.55) per 1000 person-years, which is still higher than the cholangiocarcinoma incidence in the normal population. The highest incidence of cholangiocarcinoma was found in patients older than 70 years (2.15/1000 person-years).

The cholelithiasis patients without advanced intervention had an incidence of cholangiocarcinoma of 1.38 (0.99–1.88) per 1000 person-years. The highest incidence of cholangiocarcinoma in this subgroup was observed in men (1.72/1000 person-years) and in elderly patients (2.80/1000 person-years). The incidence comparisons are shown in Table 3. For the recurrent biliary events, the comparisons between cholangiocarcinoma patients and non-cholangiocarcinoma patients in ES/EPBD group were listed in the Supplementary Table 2.

## DISCUSSION

**Table 2 The comparisons of cholelithiasis patients underwent therapeutic endoscopic retrograde cholangiopancreatography, cholecystectomy or no intervention**

| | ES/EPBD *n* = 537 | | Cholecystectomy *n* = 1743 | | Without intervention *n* = 5658 | | *P* value |
|---|---|---|---|---|---|---|---|
| | *N* | SD, % | *N* | SD, % | *N* | SD, % | |
| Age, mean (SD) | 64.33 | 16.33 | 56.95 | 16.53 | 59.34 | 16.43 | 0.941 |
| Age, yr | | | | | | | |
| 18-49 | 39.29 | 7.59 | 38.26 | 7.6 | 39.09 | 7.27 | 0.391 |
| 50-69 | 60 | 5.2 | 59.3 | 5.56 | 58.99 | 5.53 | 0.559 |
| > 70 | 79.11 | 5.73 | 78.01 | 5.54 | 78.78 | 6.08 | 0.002 |
| Gender | | | | | | | 0.692 |
| Male | 264 | 49.16 | 843 | 48.36 | 2691 | 47.56 | |
| Female | 273 | 50.84 | 900 | 51.64 | 2967 | 52.44 | |
| Follow up time (mo), mean (SD) | 56.3 | 22.24 | 61.38 | 18.4 | 56.88 | 22.27 | < 0.001 |
| Economic status | | | | | | | 0.16 |
| MBS | 319 | 59.4 | 1137 | 65.23 | 3590 | 63.45 | |
| 1-3 times MBS | 209 | 38.92 | 574 | 32.93 | 1964 | 34.71 | |
| Above 3 times MBS | 9 | 1.68 | 28 | 1.61 | 94 | 1.66 | |
| Place of residence | | | | | | | 0.009 |
| City | 296 | 55.12 | 854 | 49 | 2813 | 49.72 | |
| Countryside | 205 | 38.18 | 681 | 39.07 | 2250 | 39.77 | |
| Remote village | 36 | 6.7 | 204 | 11.7 | 585 | 10.34 | |
| Comorbidity | | | | | | | |
| CHB | 50 | 9.31 | 137 | 7.86 | 567 | 10.02 | 0.026 |
| CHC | 22 | 4.1 | 73 | 4.19 | 447 | 7.9 | < 0.001 |
| HP | 11 | 2.05 | 23 | 1.32 | 94 | 1.66 | 0.433 |
| DM | 167 | 31.1 | 478 | 27.42 | 1674 | 29.59 | 0.135 |
| ESRD | 12 | 2.23 | 37 | 2.12 | 137 | 2.42 | 0.76 |
| CCDL | 13 | 2.42 | 12 | 0.69 | 26 | 0.46 | < 0.001 |
| CO | 0 | 0 | 0 | 0 | 0 | 0 | NA |
| IBD | 6 | 1.12 | 23 | 1.32 | 90 | 1.59 | 0.54 |
| Cholangiocarcinoma | | | | | | | |
| Number of cholangiocarcinoma | 27 | 5.03 | 15 | 0.86 | 105 | 1.86 | < 0.001 |
| Number of cholangiocarcinoma after first 6 mo | 11 | 2.05 | 7 | 0.4 | 37 | 0.65 | < 0.001 |
| Odds ratio | 3.13 | | 0.61 | | 1 | | |
| Number of cholangiocarcinoma after first 12 mo | 10 | 1.86 | 6 | 0.34 | 35 | 0.62 | < 0.001 |
| Odds ratio | 3.01 | | 0.56 | | 1 | | |
| Time to diagnosis of cholangiocarcinoma (excluding case in initial 6 mo), month | 41.17 | 22.51 | 33.7 | 23.35 | 35.46 | 19.08 | 0.698 |

SD: Standard deviation; MBS: Minimum basic salary; CHB: Chronic hepatitis B; CHC: Chronic hepatitis C; HP: *Helicobacter* infection; DM: Diabetes mellitus; ESRD: End-stage renal disease; CCDL: Congenital cystic disease of liver; CO: Clonorchis Opisthorchis; IBD: Inflammatory bowel disease.

In our study, the intervention rate was higher than that reported previously[39], because this was a hospital-based cohort database, which meant that nearly all cholelithiasis cases were regarded as symptomatic patients. We found a higher incidence of cholelithiasis in people with a minimum basic salary and the highest economic status. The former seems connected with a poor health environment, as shown in previous literature[40], while the latter can be explained by diets high in cholesterol, saturated fat, and excess carbohydrates[41]. The same conditions explained the higher portion of cholelithiasis patients among residents of remote villages than in the normal population. Because primary sclerosing cholangitis[7-9], CCDL[10,11], CO[12], cholelithiasis[13,14], CHB and CHC[15,16], DM[17,18] and HP infection[19,20] are important risk factors for cholangiocarcinoma, we subjected these factors to further evaluation to compare cholelithiasis patients and a normal population. In our analysis, CHB, CHC, DM, HP infection, ESRD, CCDL, and IBD were more common in cholelithiasis patients and some of these factors logically increased the rate of cholangiocarcinoma

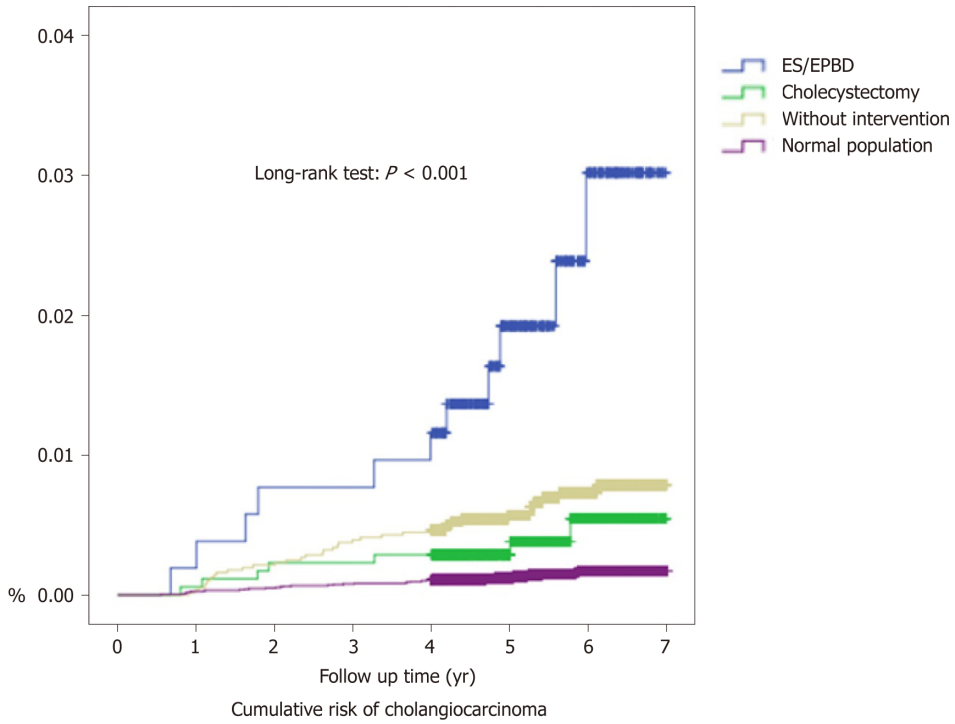


**Figure 2 Cumulative risk of cholangiocarcinoma.** The cases of cholangiocarcinoma within 6 mo after the therapeutic procedure or the diagnosis of cholelithiasis were excluded. ES/EPBD: Endoscopic sphincterotomy/endoscopic papillary balloon dilatation.

by increasing the incidence of cholelithiasis[42]. Because CO infection is extremely rare in modern Taiwanese society, no CO-infected patient was found in our study in either group. The cholangiocarcinoma rate was higher in cholelithiasis patients than in the normal population (0.69% *vs* 0.15%), thereby confirming the previous concept of cholelithiasis as an important risk factor for cholangiocarcinoma.

The rate of total cholangiocarcinoma and subsequent cholangiocarcinoma (diagnosed 6 mo after procedure) are highest in ES/EPBD patients, followed by cholelithiasis patients without intervention, and the lowest cholangiocarcinoma rate was found in cholecystectomy patients. The odds ratio of ES/EPBD patients for cholangiocarcinoma was 3.13 when compared with no intervention, indicating that the subsequent cholangiocarcinoma rate was high after ES/EPBD in cholelithiasis patients. Cholecystectomy decreased the cholangiocarcinoma rate in cholelithiasis patients in our study and this effect was compatible with previous literature reports[34].

Another interesting finding of our study was the high incidence of cholangiocarcinoma in the medium time period for cholelithiasis patients who had undergone ES/EPBD, especially in women and in patients older than 70 years. However, current guidelines do not suggest close follow-up in these patients.

This study has two major limitations. First, this is a retrospective database cohort study that showed an increase in the further incidence of cholangiocarcinoma after EST/EPBD in cholelithiasis patients, but the true consequence of cholangiocarcinoma and ES/EPBD is unclear. Second, even though this is a one million representative database, the incidence of cholangiocarcinoma is so low that we only found 11 cases, 7 cases, and 37 cases in the ES/EPBD, cholecystectomy, and without intervention group, respectively, but the power of our results is still credible. We will try to initiate a prospective hospital-based cohort study in cholelithiasis patients, who underwent therapeutic intervention to clarify the consequence of cholangiocarcinoma in ES/EPBD and cholecystectomy patients.

In conclusion, symptomatic cholelithiasis did increase the cholangiocarcinoma rate in our analysis, and patients with cholelithiasis who underwent cholecystectomy could reduce the incidence of subsequent cholangiocarcinoma, but the incidence is still significantly higher than the incidence in the normal population. Meanwhile, the patients with cholelithiasis who undergo ES/EPBD are at high risk of subsequent cholangiocarcinoma.

**Table 3 Incidence of cholangiocarcinoma amount patient with cholelithiasis underwent therapeutic endoscopic retrograde cholangiopancreatography, cholecystectomy or no intervention compared with normal population (excluding cholangiocarcinoma in the initial 6 mo)**

| Variables | Person-years at risk in study cohort | Person-years at risk in control cohort | No. of observed cases of cholangiocarcino-ma in study cohort | No. of observed cases of cholangiocarcino-ma in control cohort | Incidence rate/1000 person-years (95%CI) in study cohort | Incidence rate/1000 person-years (95%CI) in control cohort |
|---|---|---|---|---|---|---|
| ES/EPBD | | | | | | |
| Total | 2519.33 | 125339.21 | 11 | 35 | 4.37 (2.30-7.59) | 0.28 (0.20-0.38) |
| Gender | | | | | | |
| Male | 1252.12 | 59176.6 | 3 | 20 | 2.40 (0.61-6.52) | 0.34 (0.21-0.51) |
| Female | 1267.21 | 66162.61 | 8 | 15 | 6.31 (2.93-11.99) | 0.23 (0.13-0.37) |
| Age, yr | | | | | | |
| 18-49 | 561.74 | 37789.95 | 1 | 5 | 1.78 (0.09-8.78) | 0.13 (0.05-0.29) |
| 50-69 | 895.32 | 48272.57 | 2 | 14 | 2.23 (0.38-7.38) | 0.29 (0.17-0.48) |
| > 70 | 1062.27 | 39276.69 | 8 | 16 | 7.53 (3.50-14.30) | 0.41 (0.24-0.65) |
| Cholecystectomy | | | | | | |
| Total | 8911.32 | 125339.21 | 7 | 35 | 0.79 (0.34-1.55) | 0.28 (0.20-0.38) |
| Gender | | | | | | |
| Male | 4187.56 | 59176.6 | 3 | 20 | 0.72 (0.18-1.95) | 0.34 (0.21-0.51) |
| Female | 4723.76 | 66162.61 | 4 | 15 | 0.85 (0.27-2.04) | 0.23 (0.13-0.37) |
| Age, yr | | | | | | |
| 18-49 | 3173.23 | 37789.95 | 1 | 5 | 0.32 (0.02-1.55) | 0.13 (0.05-0.29) |
| 50-69 | 3413.76 | 48272.57 | 1 | 14 | 0.29 (0.01-1.45) | 0.29 (0.17-0.48) |
| > 70 | 2324.33 | 39276.69 | 5 | 16 | 2.15 (0.79-4.77) | 0.41 (0.24-0.65) |
| Cholelithiasis without intervention | | | | | | |
| Total | 26820.41 | 125339.21 | 37 | 35 | 1.38 (0.99-1.88) | 0.28 (0.20-0.38) |
| Gender | | | | | | |
| Male | 12201.3 | 59176.6 | 21 | 20 | 1.72 (1.09-2.59) | 0.34 (0.21-0.51) |
| Female | 14619.11 | 66162.61 | 16 | 15 | 1.09 (0.65-1.74) | 0.23 (0.13-0.37) |
| Age, yr | | | | | | |
| 18-49 | 8423.77 | 37789.95 | 4 | 5 | 0.48 (0.15-1.15) | 0.13 (0.05-0.29) |
| 50-69 | 10889.04 | 48272.57 | 12 | 14 | 1.10 (0.60-1.87) | 0.29 (0.17-0.48) |
| > 70 | 7507.6 | 39276.69 | 21 | 16 | 2.80 (1.78-4.20) | 0.41 (0.24-0.65) |

ES/EPBD: Endoscopic sphincterotomy/endoscopic papillary balloon dilatation.

## ARTICLE HIGHLIGHTS

### *Research background*

Cholangiocarcinoma is a highly lethal disease. There are many well known risk factors of cholangiocarcinoma, most of them result from chronic biliary system inflammation, such as primary sclerosing cholangitis, choledochal cyst disease, specific parasite infection, cholelithiasis, chronic hepatitis B and C infection, diabetes mellitus and Helicobacter infection, but the impacts of advanced biliary interventions, like endoscopic sphincterotomy (ES), endoscopic papillary balloon dilatation (EPBD) and cholecystectomy, are inconsistence in previous literature. It is important to understand the major hypothesis result in cholangiocarcinoma.

### *Research motivation*

We focused on the most common disease, cholelithiasis, which can result in cholangiocarcinoma. We conducted this study using the National Health Insurance Research Database to clarify the risks of cholangiocarcinoma after ES/EPBD, cholecystectomy or no intervention for cholelithiasis.

### *Research objectives*

We try to evaluate hospital base cholelithiasis retrospective cohort and analyzed further

cholangiocarcinoma risk in patients underwent ES/EPBD, cholecystectomy or no intervention for cholelithiasis. Further studies, to clarify whether the inflammation location or the different methods of therapeutic managements affect the incidence of cholangiocarcinoma, are needed in this field.

### Research methods

Because of cholangiocarcinoma is still a disease with very low incidence in normal population, we collect data of NHIRD 2004-2011 in Taiwan using one million random samples. We selected 7938 cholelithiasis cases as well as 23814 control group cases (matched by sex and age in 1:3 ratio). The incidences of total and subsequent cholangiocarcinoma were calculated in ES/EPBD patients, cholecystectomy patients, cholelithiasis patients without intervention and normal population. This topic is hard to be analyzed because subsequent cholangiocarcinoma incidence is low and both cholelithiasis and the managements for cholelithiasis maybe influence the cholangiocarcinoma rate.

### Research results

There are 537 cases underwent ES/EPBD, 1743 cases underwent cholecystectomy and 5658 cases without intervention in our cholelithiasis cohort. Eleven (2.05%), 37 (0.65%) and 7 (0.40%) subsequent cholangiocarcinoma cases diagnosed in ES/EPBD, no intervention and cholecystectomy group respectively and the odds ratio for subsequent cholangiocarcinoma is 3.13 in ES/EPBD group and 0.61 in cholecystectomy group comparing with no intervention group.

### Research conclusions

Symptomatic cholelithiasis patients underwent cholecystectomy had the lowest incidence of subsequent cholangiocarcinoma, but the incidence is still higher than normal population. Patients underwent ES/EPBD are in a high risk of subsequent cholangiocarcinoma and a follow-up plane should be needed in these kinds of patients. The hypotheses of these results can be explained by both inflammation at bile ducts increases incidence of cholangiocarcinoma than inflammation at gallbladder, or cholecystectomy reduce recurrent biliary events in cholelithiasis patients and decrease future cholangiocarcinoma rates. We need a series studies to clarify this mystery we left today.

### Research perspectives

The future direction of research is to evaluate choledocholithiasis patients, who underwent therapeutic endoscopic retrograde cholangiopancreatography with or without further cholecystectomy, and their subsequent cholangiocarcinoma incidence. Because we think the procedure related cholangiocarcinoma need longer time period to take place, the influences of subsequent cholangiocarcinoma between ES and EPBD may be clarified in whole population based cohort study.

## REFERENCES


1 **Bertuccio P**, Bosetti C, Levi F, Decarli A, Negri E, La Vecchia C. A comparison of trends in mortality from primary liver cancer and intrahepatic cholangiocarcinoma in Europe. *Ann Oncol* 2013; **24**: 1667-1674 [PMID: 23378539 DOI: 10.1093/annonc/mds652]

2 **Welzel TM**, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 2006; **98**: 873-875 [PMID: 16788161 DOI: 10.1093/jnci/djj234]

3 **Patel T**. Worldwide trends in mortality from biliary tract malignancies. *BMC Cancer* 2002; **2**: 10 [PMID: 11991810 DOI: 10.1186/1471-2407-2-10]

4 **West J**, Wood H, Logan RF, Quinn M, Aithal GP. Trends in the incidence of primary liver and biliary tract cancers in England and Wales 1971-2001. *Br J Cancer* 2006; **94**: 1751-1758 [PMID: 16736026 DOI: 10.1038/sj.bjc.6603127]

5 **Patel T**. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001; **33**: 1353-1357 [PMID: 11391522 DOI: 10.1053/jhep.2001.25087]

6 **Jepsen P**, Vilstrup H, Tarone RE, Friis S, Sørensen HT. Incidence rates of intra- and extrahepatic cholangiocarcinomas in Denmark from 1978 through 2002. *J Natl Cancer Inst* 2007; **99**: 895-897 [PMID: 17551150 DOI: 10.1093/jnci/djk201]

7 **Bergquist A**, Ekbom A, Olsson R, Kornfeldt D, Lööf L, Danielsson A, Hultcrantz R, Lindgren S, Prytz H, Sandberg-Gertzén H, Almer S, Granath F, Broomé U. Hepatic and extrahepatic malignancies in primary sclerosing cholangitis. *J Hepatol* 2002; **36**: 321-327 [PMID: 11867174 DOI: 10.1016/s0168-8278(01)00288-4]

8 **Burak K**, Angulo P, Pasha TM, Egan K, Petz J, Lindor KD. Incidence and risk factors for cholangiocarcinoma in primary sclerosing cholangitis. *Am J Gastroenterol* 2004; **99**: 523-526 [PMID: 15056096 DOI: 10.1111/j.1572-0241.2004.04067.x]

9 **Chapman MH**, Webster GJ, Bannoo S, Johnson GJ, Wittmann J, Pereira SP. Cholangiocarcinoma and dominant strictures in patients with primary sclerosing cholangitis: a 25-year single-centre experience. *Eur J Gastroenterol Hepatol* 2012; **24**: 1051-1058 [PMID: 22653260 DOI: 10.1097/MEG.0b013e3283554bbf]

10 **Scott J**, Shousha S, Thomas HC, Sherlock S. Bile duct carcinoma: a late complication of congenital hepatic fibrosis. Case report and review of literature. *Am J Gastroenterol* 1980; **73**: 113-119 [PMID: 6249119]

11 **Lipsett PA**, Pitt HA, Colombani PM, Boitnott JK, Cameron JL. Choledochal cyst disease. A changing pattern of presentation. *Ann Surg* 1994; **220**: 644-652 [PMID: 7979612 DOI: 10.1097/00000658-199411000-00007]

12 **Watanapa P**, Watanapa WB. Liver fluke-associated cholangiocarcinoma. *Br J Surg* 2002; **89**: 962-970

[PMID: 12153620 DOI: 10.1046/j.1365-2168.2002.02143.x]
13 **Welzel TM**, Mellemkjaer L, Gloria G, Sakoda LC, Hsing AW, El Ghormli L, Olsen JH, McGlynn KA. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case-control study. *Int J Cancer* 2007; **120**: 638-641 [PMID: 17109384 DOI: 10.1002/ijc.22283]
14 **Hsing AW**, Gao YT, Han TQ, Rashid A, Sakoda LC, Wang BS, Shen MC, Zhang BH, Niwa S, Chen J, Fraumeni JF. Gallstones and the risk of biliary tract cancer: a population-based study in China. *Br J Cancer* 2007; **97**: 1577-1582 [PMID: 18000509 DOI: 10.1038/sj.bjc.6604047]
15 **Shin HR**, Lee CU, Park HJ, Seol SY, Chung JM, Choi HC, Ahn YO, Shigemastu T. Hepatitis B and C virus, Clonorchis sinensis for the risk of liver cancer: a case-control study in Pusan, Korea. *Int J Epidemiol* 1996; **25**: 933-940 [PMID: 8921477 DOI: 10.1093/ije/25.5.933]
16 **Shaib YH**, El-Serag HB, Davila JA, Morgan R, McGlynn KA. Risk factors of intrahepatic cholangiocarcinoma in the United States: a case-control study. *Gastroenterology* 2005; **128**: 620-626 [PMID: 15765398 DOI: 10.1053/j.gastro.2004.12.048]
17 **Jing W**, Jin G, Zhou X, Zhou Y, Zhang Y, Shao C, Liu R, Hu X. Diabetes mellitus and increased risk of cholangiocarcinoma: a meta-analysis. *Eur J Cancer Prev* 2012; **21**: 24-31 [PMID: 21857525 DOI: 10.1097/CEJ.0b013e3283481d89]
18 **Zhang LF**, Zhao HX. Diabetes mellitus and increased risk of extrahepatic cholangiocarcinoma: a meta-analysis. *Hepatogastroenterology* 2013; **60**: 684-687 [PMID: 23321031]
19 **Chang JS**, Tsai CR, Chen LT. Medical risk factors associated with cholangiocarcinoma in Taiwan: a population-based case-control study. *PLoS One* 2013; **8**: e69981 [PMID: 23894567 DOI: 10.1371/journal.pone.0069981]
20 **Murphy G**, Michel A, Taylor PR, Albanes D, Weinstein SJ, Virtamo J, Parisi D, Snyder K, Butt J, McGlynn KA, Koshiol J, Pawlita M, Lai GY, Abnet CC, Dawsey SM, Freedman ND. Association of seropositivity to Helicobacter species and biliary tract cancer in the ATBC study. *Hepatology* 2014; **60**: 1963-1971 [PMID: 24797247 DOI: 10.1002/hep.27193]
21 **ASGE Standards of Practice Committee**; Maple JT, Ben-Menachem T, Anderson MA, Appalaneni V, Banerjee S, Cash BD, Fisher L, Harrison ME, Fanelli RD, Fukami N, Ikenberry SO, Jain R, Khan K, Krinsky ML, Strohmeyer L, Dominitz JA. The role of endoscopy in the evaluation of suspected choledocholithiasis. *Gastrointest Endosc* 2010; **71**: 1-9 [PMID: 20105473 DOI: 10.1016/j.gie.2009.09.041]
22 **Baron TH**, Mallery JS, Hirota WK, Goldstein JL, Jacobson BC, Leighton JA, Waring JP, Faigel DO. The role of endoscopy in the evaluation and treatment of patients with pancreaticobiliary malignancy. *Gastrointest Endosc* 2003; **58**: 643-649 [PMID: 14595292 DOI: 10.1016/S0016-5107(03)01994-1]
23 **Costamagna G**, Shah SK, Tringali A. Current management of postoperative complications and benign biliary strictures. *Gastrointest Endosc Clin N Am* 2003; **13**: 635-648, ix [PMID: 14986791 DOI: 10.1016/S1052-5157(03)00103-X]
24 **Sheth SG**, Howell DA. What are really the true late complications of endoscopic biliary sphincterotomy? *Am J Gastroenterol* 2002; **97**: 2699-2701 [PMID: 12425534 DOI: 10.1111/j.1572-0241.2002.07051.x]
25 **Bergman JJ**, van Berkel AM, Groen AK, Schoeman MN, Offerhaus J, Tytgat GN, Huibregtse K. Biliary manometry, bacterial characteristics, bile composition, and histologic changes fifteen to seventeen years after endoscopic sphincterotomy. *Gastrointest Endosc* 1997; **45**: 400-405 [PMID: 9165322 DOI: 10.1016/S0016-5107(97)70151-2]
26 **Kurumado K**, Nagai T, Kondo Y, Abe H. Long-term observations on morphological changes of choledochal epithelium after choledochoenterostomy in rats. *Dig Dis Sci* 1994; **39**: 809-820 [PMID: 8149847 DOI: 10.1007/BF02087428]
27 **Kalaitzis J**, Vezakis A, Fragulidis G, Anagnostopoulou I, Rizos S, Papalambros E, Polydorou A. Effects of endoscopic sphincterotomy on biliary epithelium: a case-control study. *World J Gastroenterol* 2012; **18**: 794-799 [PMID: 22371639 DOI: 10.3748/wjg.v18.i8.794]
28 **Oliveira-Cunha M**, Dennison AR, Garcea G. Late Complications After Endoscopic Sphincterotomy. *Surg Laparosc Endosc Percutan Tech* 2016; **26**: 1-5 [PMID: 26679684 DOI: 10.1097/SLE.0000000000000226]
29 **Peng YC**, Lin CL, Hsu WY, Chow WK, Lee SW, Yeh HZ, Chang CS, Kao CH. Association of Endoscopic Sphincterotomy or Papillary Balloon Dilatation and Biliary Cancer: A Population-Based Cohort Study. *Medicine (Baltimore)* 2015; **94**: e926 [PMID: 26061315 DOI: 10.1097/MD.0000000000000926]
30 **Langerth A**, Sandblom G, Karlson BM. Long-term risk for acute pancreatitis, cholangitis, and malignancy more than 15 years after endoscopic sphincterotomy: a population-based study. *Endoscopy* 2015; **47**: 1132-1136 [PMID: 26165737 DOI: 10.1055/s-0034-1392482]
31 **Strömberg C**, Böckelman C, Song H, Ye W, Pukkala E, Haglund C, Nilsson M. Endoscopic sphincterotomy and risk of cholangiocarcinoma: a population-based cohort study in Finland and Sweden. *Endosc Int Open* 2016; **4**: E1096-E1100 [PMID: 27747285 DOI: 10.1055/s-0042-114982]
32 **Guo L**, Mao J, Li Y, Jiao Z, Guo J, Zhang J, Zhao J. Cholelithiasis, cholecystectomy and risk of hepatocellular carcinoma: a meta-analysis. *J Cancer Res Ther* 2014; **10**: 834-838 [PMID: 25579515 DOI: 10.4103/0973-1482.135992]
33 **Tao LY**, He XD, Qu Q, Cai L, Liu W, Zhou L, Zhang SM. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a case-control study in China. *Liver Int* 2010; **30**: 215-221 [PMID: 19840244 DOI: 10.1111/j.1478-3231.2009.02149.x]
34 **Nordenstedt H**, Mattsson F, El-Serag H, Lagergren J. Gallstones and cholecystectomy in relation to risk of intra- and extrahepatic cholangiocarcinoma. *Br J Cancer* 2012; **106**: 1011-1015 [PMID: 22240785 DOI: 10.1038/bjc.2011.607]
35 **Cheng TM**. Taiwan's new national health insurance program: genesis and experience so far. *Health Aff (Millwood)* 2003; **22**: 61-76 [PMID: 12757273 DOI: 10.1377/hlthaff.22.3.61]
36 **Wu CY**, Kuo KN, Wu MS, Chen YJ, Wang CB, Lin JT. Early Helicobacter pylori eradication decreases risk of gastric cancer in patients with peptic ulcer disease. *Gastroenterology* 2009; **137**: 1641-8.e1-2 [PMID: 19664631 DOI: 10.1053/j.gastro.2009.07.060]
37 **Wu CY**, Chan FK, Wu MS, Kuo KN, Wang CB, Tsao CR, Lin JT. Histamine2-receptor antagonists are an alternative to proton pump inhibitor in patients receiving clopidogrel. *Gastroenterology* 2010; **139**: 1165-1171 [PMID: 20600012 DOI: 10.1053/j.gastro.2010.06.067]
38 **Dean AG**, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version 3.01, updated Apr 6, 2013, accessed Jan 6, 2018. Available from: URL: http://www.openepi.com/Menu/OE_Menu.htm
39 **Lirussi F**, Nassuato G, Passera D, Toso S, Zalunardo B, Monica F, Virgilio C, Frasson F, Okolicsanyi L.

Gallstone disease in an elderly population: the Silea study. *Eur J Gastroenterol Hepatol* 1999; **11**: 485-491 [PMID: 10755250 DOI: 10.1097/00042737-199905000-00004]

40 **Naeem M**, Rahimnajjad NA, Rahimnajjad MK, Khurshid M, Ahmed QJ, Shahid SM, Khawar F, Najjar MM. Assessment of characteristics of patients with cholelithiasis from economically deprived rural Karachi, Pakistan. *BMC Res Notes* 2012; **5**: 334 [PMID: 22741543 DOI: 10.1186/1756-0500-5-334]

41 **Gaby AR**. Nutritional approaches to prevention and treatment of gallstones. *Altern Med Rev* 2009; **14**: 258-267 [PMID: 19803550 DOI: 10.1136/aim.2009.001172]

42 **Acalovschi M**, Buzas C, Radu C, Grigorescu M. Hepatitis C virus infection is a risk factor for gallstone disease: a prospective hospital-based study of patients with chronic viral C hepatitis. *J Viral Hepat* 2009; **16**: 860-866 [PMID: 19486279 DOI: 10.1111/j.1365-2893.2009.01141.x]

**P- Reviewer:** Lan C

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 250-263

DOI: 10.4251/wjgo.v11.i3.250

ISSN 1948-5204 (online)

SYSTEMATIC REVIEWS

# Near-infrared fluorescence guided esophageal reconstructive surgery: A systematic review

Elke Van Daele, Yves Van Nieuwenhove, Wim Ceelen, Christian Vanhove, Bart P Braeckman, Anne Hoorens, Jurgen Van Limmen, Oswald Varin, Dirk Van de Putte, Wouter Willaert, Piet Pattyn

**ORCID number:** Elke Van Daele (0000-0003-0336-4179); Yves Van Nieuwenhove (0000-0003-3064-5638); Wim Ceelen (0000-0001-7692-4419); Christian Vanhove (0000-0002-3988-5980); Bart P Braeckman (0000-0002-0085-8264); Anne Hoorens (0000-0002-0736-2034); Jurgen Van Limmen (0000-0001-5105-4154); Oswald Varin (0000-0001-9749-0568); Dirk Van de Putte (0000-0001-7068-9518); Wouter Willaert (0000-0002-0885-6749); Piet Pattyn (0000-0002-1139-3394).

**Author contributions:** Van Daele E, Pattyn P, Van Nieuwenhove Y and Ceelen W designed the research; Van Daele E and Pattyn P performed the research and analysed the data; Van Daele E, Van Nieuwenhove Y and Ceelen W wrote the paper; Van Daele E and Ceelen W performed the statistical analysis; Vanhove C, Braeckman BP, Hoorens A, Van Limmen J, Varin O, Willaert W and Van de Putte D contributed to the manuscript reviewing and editing;

**Supported by** " Kom op tegen Kanker" (Stand up to Cancer), the Flemish cancer society which has no role in the design of the study, nor in the collection, analysis or interpretation of the data or in the manuscripts' writing.

**Conflict-of-interest statement:** The authors declare no conflicting interests related to this article.

**PRISMA 2009 Checklist statement:**

**Elke Van Daele, Yves Van Nieuwenhove, Wim Ceelen, Oswald Varin, Dirk Van de Putte, Wouter Willaert, Piet Pattyn,** Department of Gastrointestinal Surgery, Ghent University Hospital/Ghent University, Ghent B-9000, Belgium

**Christian Vanhove,** Department of Electronics and information systems, Ghent University, Ghent B-9000, Belgium

**Bart P Braeckman,** Department of Biology, Ghent University, Ghent B-9000, Belgium

**Anne Hoorens,** Department of Pathology, Ghent University Hospital/Ghent University, Ghent B-9000, Belgium

**Jurgen Van Limmen,** Department of Anaesthesiology, Ghent University Hospital/ Ghent University, Ghent B-9000, Belgium

**Corresponding author:** Elke Van Daele, MD, Surgeon, Department of Gastrointestinal Surgery, Ghent University Hospital/Ghent University, University Hospital 2K12 IC, C. Heymanslaan 10, Ghent B-9000, Belgium. elke.vandaele@uzgent.be
**Telephone:** +32-9-3320829
**Fax:** +32-9-3323891

## Abstract

### *BACKGROUND*

After an esophagectomy, the stomach is most commonly used to restore continuity of the upper gastrointestinal tract. These esophago-gastric anastomoses are prone to serious complications such as leakage associated with high morbidity and mortality. Graft perfusion is considered to be an important predictor for anastomotic integrity. Based on the current literature we believe Indocyanine green fluorescence angiography (ICGA) is an easy assessment tool for gastric tube (GT) perfusion, and it might predict anastomotic leakage (AL).

### *AIM*

To evaluate feasibility and effectiveness of ICGA in GT perfusion assessment and as a predictor of AL.

### *METHODS*

This study was designed according to the PRISMA guidelines and registered in the PROSPERO database. PubMed and EMBASE were independently searched by 2 reviewers for studies presenting data on intraoperative ICGA GT perfusion

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



**Manuscript source**: Invited manuscript

**Received:** November 27, 2018
**Peer-review started:** November 27, 2018
**First decision:** January 4, 2019
**Revised:** January 21, 2019
**Accepted:** January 29, 2019
**Article in press:** January 30, 2019
**Published online:** March 15, 2019

assessment during esophago-gastric reconstruction after esophagectomy. Relevant outcomes such as feasibility, complications, intraoperative surgical changes based on ICGA findings, quantification attempts, anatomical data and the impact of ICGA on postoperative anastomotic complications, were collected by 2 independent researchers. The quality of the included articles was assessed based on the Methodological Index for Non-Randomized Studies. The 19 included studies presented data on 1192 esophagectomy patients, in 758 patients ICGA was used perioperative to guide esophageal reconstruction.

***RESULTS***

The 19 included studies for qualitative analyses all described ICGA as a safe and easy method to evaluate gastric graft perfusion. AL occurred in 13.8% of the entire cohort, 10% in the ICG guided group and 20.6% in the control group ($P < 0.001$). When poorly perfused cases are excluded from the analyses, the difference in AL was even larger (AL well-perfused group 6.3% *vs* control group 20.5%, $P < 0.001$). The AL rate in the group with an altered surgical plan based on the ICG image was 6.5%, similar to the well perfused group (6.3%) and significantly less than the poorly perfused group (47.8%) ($P < 0.001$), suggesting that the technique is able to identify and alter a potential bad outcome.

***CONCLUSION***

ICGA is a safe, feasible and promising method for perfusion assessment. The lower AL rate in the well perfused group suggest that a good fluorescent signal predicts a good outcome.

**Key words:** Indocyanine green; Angiography; Fluorescence; Esophagectomy; Anastomotic leak; Near-infrared spectroscopy; Esophageal neoplasms; Esophageal cancer



**Core tip:** Esophagectomy is a surgery known for its complexity and potentially high morbidity associated with postoperative anastomotic leakage (AL). This review evaluates Indocyanine green fluorescence angiography (ICGA) as a safe, feasible and promising method to assess graft perfusion during esophageal reconstructive surgery. We discuss the safety, the methodology and the effectiveness of ICGA and its potential to reduce AL rate.

**Citation:** Van Daele E, Van Nieuwenhove Y, Ceelen W, Vanhove C, Braeckman BP, Hoorens A, Van Limmen J, Varin O, Van de Putte D, Willaert W, Pattyn P. Near-infrared fluorescence guided esophageal reconstructive surgery: A systematic review. *World J Gastrointest Oncol* 2019; 11(3): 250-263
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/250.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.250

## INTRODUCTION

The incidence of esophageal adenocarcinoma has rapidly increased over the past two decades[1]. For locally and locally advanced esophageal cancer (EC) patients, surgical resection remains the cornerstone of their treatment. The stomach is most commonly used to restore continuity of the upper gastrointestinal tract after an oesophagus resection. However, these esophago-gastric anastomotic sites (AS) are fragile and prone to complications as leakage, fistulas, bleeding, and stricture. Anastomotic leakage (AL) remains the main cause of postoperative morbidity and mortality in digestive reconstructive surgery. After an esophagectomy leakage incidence ranges from 5% to 20%[2-6]. Literature reports leak-associated mortality rates from 18% to 40% compared to an overall in-hospital mortality of 4% to 6%[2,7,8]. Age, male gender, smoking, alcohol abuse, American Society of Anaesthesiologists score, obesity, emergency surgery, prolonged operative time, intraoperative blood loss, diabetes, renal failure, use of corticosteroids and cardiovascular disease are identified as risk factors for AL, potentially through impaired perfusion of the gastric graft[2,4-6,8-11].

Graft perfusion is considered to be an important predictor for anastomotic

integrity. Currently, tissue perfusion is assessed using subjective parameters such as tissue colour and vessel pulsations. However, these parameters are known to be of limited predictive value, emphasizing the clear need for a safe, reproducible, and non-invasive method to objectively assess tissue viability and graft perfusion.

Several methods have been tested for intraoperative evaluation of graft perfusion, such as laser speckle contrast imaging, gastric tonometry, Doppler flowmetry, spectroscopy and angiography[12-15]. None of them have been widely accepted due to limited feasibility, reproducibility issues or costs. Near infrared fluorescent (NIRF) imaging requires a fluorescence-imaging agent that can be excited in the tissue at near infrared (NIR) wavelengths by penetrating NIR light. A 2D image of tissue deposition of the NIRF imaging agent can be created based on the fluorescence generated by the excited NIRF agent and captured by adapted cameras. Indocyanine green (ICG) is a clinically approved NIRF agent to determine cardiac output, hepatic function, and ophthalmic angiography. ICG is known for its absorption maximum around 760-780 nm, its immediate binding with plasma proteins resulting in a confinement to the vascular compartment, its low toxicity and its rapid and exclusive biliary excretion. Its excellent safety record has added to the rapid food and drug administration approval for clinical use in 1956[16]. Recent developments of new hardware have made it possible to perform Indocyanine green fluorescence angiography (ICGA) not only in open, but also during minimally invasive surgery, resulting in a general interest of the surgical community in this technique as a method to visualize anastomotic perfusion and its potential to reduce AL risk. At present ICGA is not a standard guideline during esophageal surgery because of the lack of prospective randomized studies, but many expert centers already incorporate perioperative ICGA as a routine step during esophageal reconstruction.

This study aimed to systematically review the literature on feasibility and effectiveness of ICGA use as a method to evaluate graft perfusion and as a predictor of AL after esophageal reconstructive surgery. Additionally, current methods to quantify ICGA in esophageal reconstructive surgery were reviewed.

## MATERIALS AND METHODS

This study was designed according to the PRISMA guidelines and registered in the PROSPERO 2018 database (www.crd.york.ac.uk). The registration identification number is CRD42018100503.

### *Eligibility criteria*

Only English written studies on human subjects were included in the study. Publications were excluded if they reported on a primary intervention other than ICGA or when they did not present original patient data. Conference abstracts, case reports, technical notes, letters, comments, duplications and animal studies were also excluded. Study selection was based on the population, intervention, comparison, outcome principle. The population was defined as patients undergoing esophagectomy for EC with gastric graft reconstruction. The intervention is ICGA for the intraoperative assessment of gastric graft perfusion. Comparison was done with non ICGA guided esophagectomy patients if a control group was present. The primary objective was feasibility of ICGA as a method to evaluate graft perfusion. The secondary objective was the effect of ICGA on alterations of the surgical plan and its ability to predict and avoid AL. The final objective was to evaluate current attempts to quantify ICGA in esophageal reconstructive surgery.

### *Search strategy*

PubMed and EMBASE were independently searched by 2 reviewers for studies presenting data on intraoperative ICGA gastric graft perfusion assessment during esophago-gastric reconstruction after esophagectomy. The search was last updated in October 2018. The following MeSH terms were used in the PubMed database searches: "Esophagectomy"[MeSH] OR "Esophagus surgery" OR "Esophagus cancer" OR "Esophagus" OR "Esophag*" AND "Indocyanine green"[MeSH] OR "angiography"[MeSH] OR "ICG" OR "graft perfusion" OR "near infrared imaging" OR "near infrared fluorescence". Same Emtree terms were used in the EMBASE database. Titles and abstracts were screened for eligibility. After reading full text articles, duplicates were extracted, and references were screened to identify additional articles. Consensus was reached by the 2 reviewers after re-review in case of discordances in study selection.

### *Methodological quality appraisal*

The quality of the included articles was assessed based on the Methodological Index

for Non-Randomized Studies (MINORS), a validated quality assessment system with 8 items for non-comparative and 12 items for comparative studies. The maximum score for non-comparative studies is 16 and 24 for the comparative studies[17].

### Data extraction, management and synthesis

Study design, patients' demographic data, intervention related data and outcomes were extracted from the included studies. Study data were collected by 2 independent researchers in a standard Excel™ data collection sheet which was used to calculate descriptive statistics for all measured parameters. Continuous and categorical variables were summarized as means with standard deviation, and/or median with IQR or ranges. Data were analyzed using SPSS version 22 for windows (SPSS Inc., Chicago, IL). Statistical analysis using a fisher exact- test was performed to compare AL rates between the ICGA guided and non-guided group. A $P$-value < 0.05 was considered statistically significant.

## RESULTS

### Search results

The initial database searches resulted in 251 eligible abstracts and citations and 3 additional records were found through reference checking. After adjusting for duplicates and screening of the abstracts, 23 full text articles remained and were reviewed in detail, resulting in the exclusion of another 4[18-40]. Finally, 19 papers met the inclusion criteria for qualitative analysis[18-23,25-28,30,33-40]. Six articles could be used for separate quantitative analyses as they contained data of a comparison group[25,26,35-38]. The PRISMA flowchart of the search is shown in Figure 1.

### Study and population characteristics

Study characteristics of the 19 included studies and demographic data of the included patients are described in Table 1. Ten studies were feasibility studies, 6 studies compared the ICGA study cohort to a historical non ICGA cohort, and 3 studies evaluated outcome of patients with adequately versus poorly ICGA perfused AS. In total, 1192 (82% male) esophagectomy patients were analyzed. In 758 patients, ICGA was used perioperative to guide esophageal reconstruction compared to 434 patients was the anastomotic site was determined based on clinical judgment and/or Doppler ultrasound.

### Methodological quality of the studies

All included studies were feasibility-or non-randomized controlled studies and the risk of bias within the studies was, therefore, analyzed according to the MINORS methodological index[17]. The score varied from 4 to 13 (median 6) for non-comparative studies (max score 16) and from 6 to 18 (median 16) for the comparative studies (max score 24). Most studies had a clear aim and appropriate endpoints, however the follow-up period and loss to follow-up were rarely mentioned. None had unbiased endpoint assessment or power calculations.

### ICGA feasibility and safety analysis

All studies reported ICGA to be feasible and easy. Many different imaging systems were described both for open and minimally invasive use, and different dosages of ICG were used (Table 1). Nevertheless, all authors were able to obtain good intraoperative NIRF images and no equipment malfunction was mentioned. The timeframe during which GT perfusion was visualized varied from a few seconds to a few minutes after injection of the dye. Shimada *et al*[40] described good visualization of the GT vasculature at the greater curvature after 1 min, the mucosal arterial network was nicely visible after 2 min. Kumagai *et al*[39] reported a median time of 27.9 s. (14.6-141.7) from initial fluorescence of the root of the right gastroepiploic artery to the tip of the GT in his first cohort and 35.3 s (13-204) in the second cohort, comparable to Sarkari's median 37.5 s (20-105)[21,25,39]. Karampiris *et al*[36] reported a mean total duration of the ICGA of 173 s (± 74), leading him to conclude that ICGA did not significantly increase operating time ($P$ = 0.8). All authors reported ICGA to be uneventful and well tolerated. None of the authors reported adverse reactions[18-23,25-28,30,33-40]. Murawa *et al*[19] was the only author who reported a transient but uncomplicated drop in oxygen saturation to 90% immediately after administration of the dye in his study group.

### Quantification and validation

Five studies reported an attempt to objective quantification of the ICGA signal based on time and/or intensity of the fluorescence[21,27,30,37,39]. Kumagai *et al*[21] first reported a

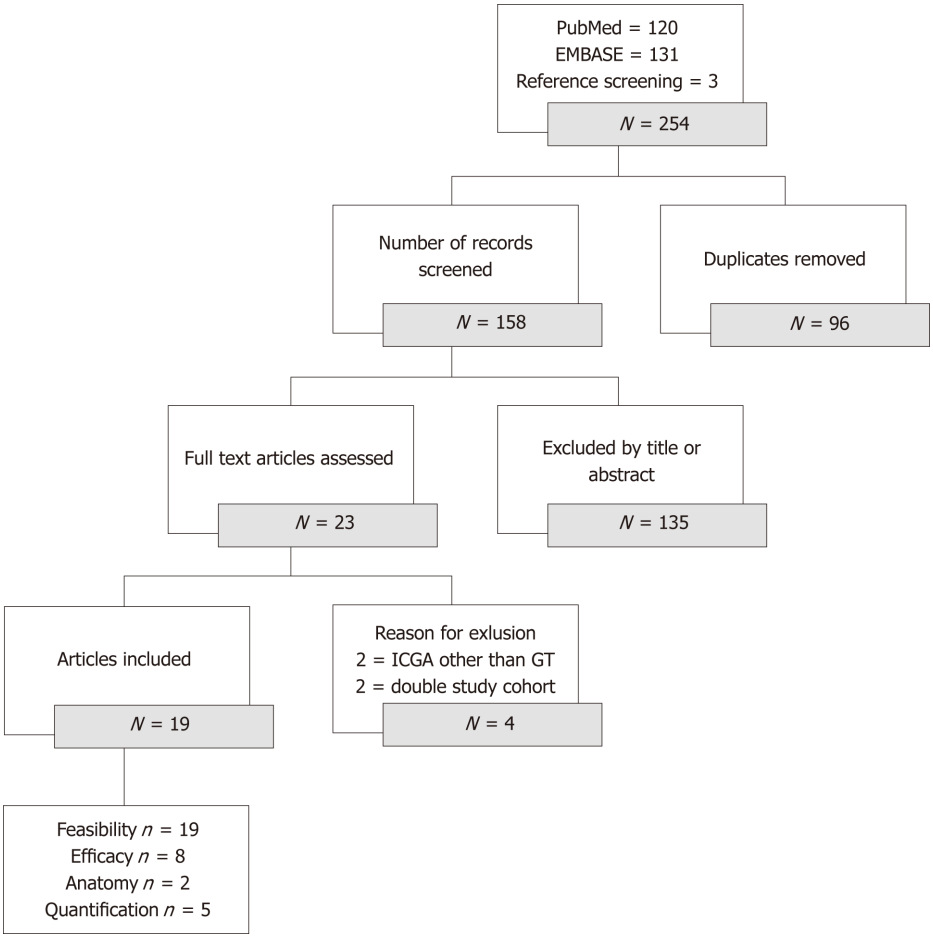


**Figure 1 PRISMA flowchart of the search.** ICGA: Indocyanine green fluorescence angiography; GT: Gastric tube.

significantly longer time registration from initial fluorescence at the root of right gastroepiploic artery until perfusion of the tip in both AL patients. Yukaya's study objectively assessed the gastric conduit vascularization based on inflow and outflow delays in perfusion detected on 2 points of the gastric conduit. Patterns of NIRF were categorized into three types of blood flow: Normal flow, delayed inflow and delayed outflow. The study could not show significant differences in AL, but the authors did provide a quantitative evaluation method of the NIRF image based on time intensity curves[27]. Koyanagi *et al*[30] quantified flow speed of NIRF in the gastric wall. Based on the length of the GT, anatomical connection between the right and left gastroepiploic artery and the speed of the fluorescence, 2 groups of patients were identified. A group with simultaneous NIRF in the gastric conduit wall and in the greater curvature vessels beyond the watershed region and a delayed group with obviously slower NIRF in the gastric conduit wall compared to the greater curvature vessels. Risk assessment identified flow speed of the NIRF as a predictor of AL ($P$ = 0.002). A receiver operating characteristics curve determined a cut-off value of 1.76 cm/s or less as a significant independent AL predictor ($P$ = 0.004)[30]. Noma *et al*[37] and Kumagai *et al*[39] published a more simplified quantification method based on time of GT perfusion. Noma's 30 s rule included an ICGA 20 s-and 30 s demarcation line on the GT. After pulling up the stomach all cervical anastomoses were made proximal from the 30 s border[37]. Recently, Kumagai *et al*[39] published the 90 s rule where all anastomoses were made in areas where less than 90 s were needed for NIRF enhancement.

### *Influence of intraoperative ICGA on the surgical plan*

The intraoperative consequences of gastric tube (GT) perfusion assessment are summarized in Table 2. Within the entire study cohort, perioperative ICGA resulted in a change of the surgical plan in 93 patients (12.4% of the study group). Different surgical approaches were described to secure sufficient perfusion to the anastomotic site for patients with an impaired perfusion on ICGA. Four authors mentioned relocation of the anastomosis with resection of the poorly perfused tip if the GT was sufficient in length[33,36,38,39]. In three studies, authors chose to switch from an end-to-

**Table 1 Demographic data and study characteristics**

| Ref. | Enrollment | Study design | Non ICG group | No. of patients (M/F) | Median age (range) | Neoadjuvant therapy | Operative technique | Dye and dose | Timing of ICGA | Imaging system | Change of procedure | MINOR score |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shimada *et al*[40] | 2008-2011 (Japan) | Prospective cohort<br>Feasibility | No | 36 GT (29/7) | 67 (49-81) | 8 CT<br>1 RT<br>1 CRT | Open surgery<br>Cervical AS | 2.5 mg Diagnogreen | After GT creation | PDE | Superdrainage (5) | 6[a] |
| Kubota *et al*[18] | 2010-2011 (Japan) | Prospective cohort<br>Feasibility | No | 4 GT (3/1) | 69 (64-71) | 4 CT | Open surgery<br>Cervical AS | 0.5 g/kg | After GT creation | HEMS | No | 4[a] |
| Murawa *et al*[19] | 2009-2010 (Poland) | Prospective cohort<br>Feasibility | No | 15 (13/2) | Mean 56 (54-74) | 0 | Not reported<br>Cervical AS<br>Transhiatal | 25 mg | After GT creation | ICView | End-to-end (4) | 6[a] |
| Pacheco *et al*[20] | 2010-2011 (United States) | Retrospective cohort<br>Feasibility | No | 11 (NR)<br>- 10 ICGA good AS<br>- 1 ICGA poor AS | Mean 56 (SD ± 9) | 7 CRT | Open surgery<br>Cervical AS<br>Transhiatal | Not reported | After GT creation | SPY | No | 4[a] |
| Kumagai *et al*[21] | 2013 (Japan) | Prospective cohort<br>Feasibility | No | 20 (16/4) | Mean 68 (50-79) | 0 | Open surgery<br>Cervical AS | 2.5 mg Diagnogreen | After GT creation | PDE | No | 13[a] |
| Rino *et al*[22] | 2009-2013 (Japan) | Prospective cohort<br>Feasibility | No | 33 (29/4) | Mean 68 (NR) | Not reported | Not reported<br>Thoracic AS<br>Ivor Lewis | 2.5 mg Diagnogreen | After GT creation | PDE | No | 4[a] |
| Sarkaria *et al*[23] | 2012-2013 (United States) | Prospective cohort<br>Feasibility | No | 30 (22/8) | 59 (37-76) | 24 CRT | RAMIE<br>Cervical + thoracic AS<br>Ivor Lewis + McKeown | 10 mg | Before GT creation | FireFly | No | 6[a] |
| Hodari *et al*[25] | 2011-2014 (United States) | Retrospective cohort<br>Historical case control | Yes | 54 (44/10)<br>- 39 ICGA<br>- 15 non ICGA | 65 (45-81) | 38 CRT | RAMIE<br>Thoracic AS<br>Ivor Lewis | Not reported | Before anastomosis during anastomosis | FireFly | Guided AS (39) | 6[b] |
| Campbell *et al*[26] | 2007-2013 (United States) | Retrospective cohort<br>Historical case control | Yes | 90 (74/16)<br>- 21 ICGA<br>- 60 non ICGA<br>- 9 Doppler | 62 (22-81) | 6 CT<br>54 CRT | MIE + Hybrid + Open<br>Thoracic AS<br>Ivor Lewis | 5 mg | After GT creation | SPY | Guided AS (21) | 15[b] |
| Yukaya *et al*[27] | 2013-2014 (Japan) | Prospective cohort<br>Feasibility | No | 27 (26/1) | 67 (40-86) | Not reported | Not reported<br>Cervical AS | 0,1 mg/kg Diagnogreen | After GT creation | HEMS | No | 11[a] |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Zehetner *et al*[28] | 2008-2011 (United States) | Prospective cohort<br>Case controlled | No | 150 (125/25)<br>- 6 delayed AS = excluded<br>- 95 ICGA good AS<br>- 49 ICGA poor AS | 67 (IQR 57-74) | 67 CRT | MIE + Open Cervical AS<br>Transhiatal + McKeown | 2.5 mg | After GT creation | SPY | Guided AS (95) | 13[b] |
| Koyonagi *et al*[30] | 2014-2015 (Japan) | Prospective cohort<br>Case controlled | No | 40 (34/6)<br>- 25 ICGA good AS<br>- 15 ICGA poor AS | 68 (26-82) | 11 CT<br>4 CRT | Not reported<br>Cervical AS | 2.5 or 1.25 mg Diagnogreen | After GT creation | PDE | No | 17[b] |
| Schlottmann *et al*[33] | NR (United States) | Prospective cohort<br>Feasibility | No | 5 (3/2) | 59 (56-70) | 1 CRT | Hybrid<br>Thoracic AS<br>Ivor Lewis | 5 mg<br>ICG-pulsion | Before anastomosis | IMAGE1 | Guided AS (5)<br>- Resection (2) | 8[a] |
| Kitagawa *et al*[29,34] | 2011-2017 (Japan) | Retrospective cohort<br>Case controlled | No | 72 (57/15)<br>- 26 ICGA<br>- 46 ICGA-LMM (line marking method) | Mean 66 (SD ±7) | 60 CT<br>2 CRT | MIE + Hybrid<br>Cervical AS<br>McKeown | 5 mg | Before GT creation<br>After GT creation | HEMS | Guided AS (26)<br>Guided GT (46)<br>- End-to-end (4)<br>- Superdrainage (1) | 18[b] |
| Ohi *et al*[32,35] | 2000-2015 (Japan) | Retrospective cohort<br>Historical case control | Yes | 120 (101/19)<br>- 59 ICGA<br>- 61 non ICGA | 68 (IQR 63-74) | 56 Not reported | Open + Hybrid<br>Cervical AS | 2.5 mg Diagnogreen | After GT creation | PDE | Guided AS 60 s (59)<br>- End-to-end (3)<br>- Supercharge/drain (1)<br>- Manubriotomy (5) | 15[b] |
| Karampiris *et al*[36] | 2010-2016 (Germany) | Retrospective cohort<br>Historical case control | Yes | 90 (65/23 + 2 NR)<br>- 35 ICGA<br>- 33 ICGA good AS<br>- 2 ICGA poor AS<br>- 55 non ICGA | Mean 62 (SD ± 9) | 69 CT<br>25 RT | Open + Hybrid<br>cervical + thoracic AS<br>Thoracophrenico + McKeown | 7.5 mg | After GT creation | PinPoint | Guided AS (33)<br>- Resection (26) | 16[b] |
| Noma *et al*[37] | 2010-2016 (Japan) | Retrospective cohort<br>Historical case control<br>Case matched | Yes | 285 (244/41)<br>- 71 ICGA<br>- 214 non ICGA | Mean 65 (SD ± 8) | 129 CT<br>21 CRT | Open+ Hybrid + MIE<br>Cervical AS<br>McKeown | 12.5 mg Diagnogreen | After GT creation | PDE | Guided AS 30 s (71)<br>- extended mobilization<br>- Supercharge/drain (1) | 17[b] |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dalton *et al*[38] | 2014-2016 (United States) | Retrospective cohort<br>Historical case control | Yes | 40 (32/8)<br>- 20 ICGA<br>- 20 non ICGA | Mean 64 (SD ± 10) | 36 Not reported | MIE<br>Thoracic AS<br>Ivor Lewis | 7.5 mg | After GT creation | PinPoint | Guided AS (20)<br>- Resection (6) | 17[b] |
| Kumagai *et al*[39] | 2014-2017 (Japan) | Prospective cohort<br>Feasibility | No | 70 (59/11) | 71 (46-82) | Not reported | Not reported<br>Cervical AS | 2.5 mg Diagnogreen | After GT creation | PDE | Guided AS 90 s (70)<br>- Resection (35) | 8[a] |

Neoadjuvant treatment: CT: Chemotherapy, RT: Radiotherapy, CRT: Chemo radiation; Fluorescent imaging system: PDE: Photodynamic Eye; Hamamatsu Photonics K.K, Hamamatsu, Japan; HEMS: Hyper Eye Medical System; Mizuho Ikakogyo Co., Tokyo, Japan; ICView: Pulsion Medical Systems, Munich, Germany; SPY: SPY Imaging System, Novadaq Industries Inc., Toronto, Canada; FireFly: Firefly Fluorescence Imaging Scope, Intuitive Surgical, Sunnyvale, Canada; IMAGE1: IMAGE1 STORZ professional image enhancement system; PinPoint: PinPoint system, Novadaq, Ontario, Canada; ICGA: Indocyanine green fluorescence angiography; AL: Anastomotic leakage; GT: Gastric tube; MINORS: Methodological Index for Non-Randomized Studies; AS: Anastomotic sites; RAMIE: Robotic assisted minimally invasive esophagectomy.

side anastomosis to an end-to-end anastomosis to reduce the necessary GT length needed for a cervical anastomosis[19,34,35]. Finally, four authors made additional arterial or venous anastomosis between the GT vasculature and carotid artery or jugular vein to increase arterial perfusion or venous drainage of the cervical anastomosis, a technique also known as supercharge or super drainage[34,35,37,40]. Both Ohi *et al*[35] and Noma *et al*[37] described a step-up surgical protocol for a well perfused anastomotic site. Changing the surgical plan seemed very efficient, as AL after an altered surgical plan occurred in 6.5% of patients, comparable to the 6.3% AL rate in the well perfused cohort and significantly less common than the 20.5% AL rate in the non ICG cohort ($P < 0.001$).

### *The effect of ICGA guided surgery on AL*

In 1186 patients, a primary esophagogastric anastomosis was made. The 6 patients, in whom Zehetner *et al*[28] decided to perform an ischemic conditioning with delayed anastomosis were excluded from further analyses. In the entire cohort, 13.8% of patients suffered from AL, 9.9% in the ICGA guided group and 20.5% in the control group ($P < 0.001$). Within the group of ICGA guided esophagogastric anastomosis, 592 had a good ICGA perfusion, but still resulted in 6.3% AL rate. Ninety-three patients had a low perfusion at the tip of the GT, for which different types of corrections were performed resulting in an adequate tip perfusion and an AL rate of 6.5%, comparable to the AL rate of the well perfused cohort and significantly lower than the 47.8% AL rate in the poorly perfused group ($P < 0.001$) (Figure 2). The difference in AL rate is even clearer considering only the cohort with a control group ($P < 0.001$) (Figure 3).

## DISCUSSION

Surgeons lack the ability to predict AL based simply on gross inspection of tissue viability, emphasizing the need for a safe, reproducible, and non-invasive method for perfusion assessment, especially in esophageal reconstructive surgery. We aimed to review the literature on the feasibility and effectiveness of ICGA as a method to evaluate graft perfusion and as a predictor of AL after esophagectomy. In addition, we evaluated published attempts to quantify ICGA based imaging data in esophageal surgery.

Based on the 1192 included patients, we conclude that ICGA is an easy, safe and feasible method for GT perfusion assessment during esophageal reconstructive surgery. There are no reported technical failures of the different ICGA platforms, nor reports of adverse events, except for an uneventful and transient drop in oxygen saturation immediately after admission of the ICG, suggesting that this is a ready to use method with very limited risk and learning curve. The transient drop in percutaneous oxygen saturation measurement via pulse oximetry is a misreading due to the interference of the dye's absorption spectra used by optical-technology-based monitors who interfere with the oximetry readings.

The time required for assessment of GT perfusion varied from a few seconds to a few minutes after injection of the dye, which does not significantly affect operating

**Table 2 Influence of Indocyanine green fluorescence angiography on intraoperative decisions and on anastomotic leakage**

| Ref. | Entire cohort *n* = 1186 | ICGA study group *n* = 752 | | | Non ICGA *n* = 434 |
|---|---|---|---|---|---|
| | | ICGA good perfused AS, *n* = 592 | ICGA good perfused altered AS, *n* = 93 | ICGA poor perfused AS, *n* = 67 | |
| Shimada *et al*[40] | 3/36 | 2/31 | 1/5 Superdrainage | - | - |
| Kubota *et al*[18] | 0/4 | 0/4 | - | - | - |
| Murawa *et al*[19] | 1/15 | 1/11 | 0/4 End-to-end | - | - |
| Pacheco *et al*[20] | 2/11 | 1/10 | - | 1/1 | - |
| Kumagai *et al*[21] | 2/20 | 2/20 | - | - | - |
| Rino *et al*[22] | 5/33 | 5/33 | - | - | - |
| Sarkaria *et al*[23] | 2/30 | 2/30 | - | - | - |
| Hodari *et al*[25] | 3/54 | 0/39 | - | - | 3/15 |
| Campbell *et al*[26] | 12/90 | 0/21 | - | - | 12/69 |
| Yukaya *et al*[27] | 9/27 | 9/27 | - | - | - |
| Zehetner *et al*[28] | 24/144 | 2/95 | - | 22/49 | - |
| Koyonagi *et al*[30] | 7/40 | 0/25 | - | 7/15 | - |
| Schlottmann *et al*[33] | 0/5 | 0/3 | 0/2 resection | - | - |
| Kitagawa *et al*[29,34] | 7/72 | 3/41 ICG-LMM<br>4/26 ICG | 0/5 ICG-LMM<br>- 0/4 End-to-end<br>- 0/1 Superdrainage | - | - |
| Ohi *et al*[32,35] | 10/120 | 0/50 | 1/9<br>- 0/3 End-to-end<br>- 1/1 Supercharge/drain<br>- 0/5 Manubriotomy | - | 9/61 |
| Karampiris *et al*[36] | 13/90 | 0/7 | 1/26 Resection | 2/2 | 10/55 |
| Noma *et al*[37] | 60/285 | 6/70 | 0/1 Supercharge/drain | - | 54/214 |
| Dalton *et al*[38] | 3/40 | 0/14 | 2/6 Resection | - | 1/20 |
| Kumagai *et al*[39] | 1/70 | 0/35 | 1/35 Resection | - | - |

ICGA: Indocyanine green fluorescence angiography.

time. Different teams used different dosages of ICG varying from 2.5 mg to 25 mg, but none reported serious adverse events, and all described good visualization. Diana[41-43] performed an optimal dosage study in a pig model resulting in a dose of 0.5 mg/kg as optimal dose for a reliable fluorescence that could be converted into a time intensity curve with their VR RENDER perfusion software. At present optimal doses of 0.2-0.5 mg/kg are mentioned in literature, mainly based on the study of Diana, nevertheless many of the included studies have shown that GT perfusion could be visualized using a much lower (and potentially less toxic) dose. A recent practical guide for ICGA guided abdominal surgery also concluded on a bolus dose of 2.5 mg as optimal dose for simple visualization of both esophagogastric and colorectal anastomotic perfusion[44].

Only a few authors have attempted to quantify the method, usually in colorectal surgery, but comparison with a golden standard method is rarely reported. The only quantitative attempt to validate ICGA as a reliable perfusion assessment tool was performed by Diana *et al*[41-43], who showed that the time intensity curves of the NIRF images correlate with hypo-perfusion and tissue ischemia in an experimental pig model of small bowel ischemia[41-43]. Only five studies reported an attempt to quantify the ICGA signal during esophageal surgery, based on the speed of fluorescent coloring of the GT after injection of the dye[21,27,30,37,39]. Koyanagi *et al*[30] added the length of the GT and anatomical connection between the right and left gastroepiploic artery. A ROC curve analysis determined a cut-off value of 1.76 cm/s or less as a significant independent AL predictor ($P$ = 0.004)[30]. Yukaya's study assessed perfusion based on inflow and outflow delays detected on 2 points of the gastric conduit. That study could not show significant differences in AL between flow types, but the authors did provide the first and only quantitative evaluation method based on time intensity curves in esophageal surgery[27].

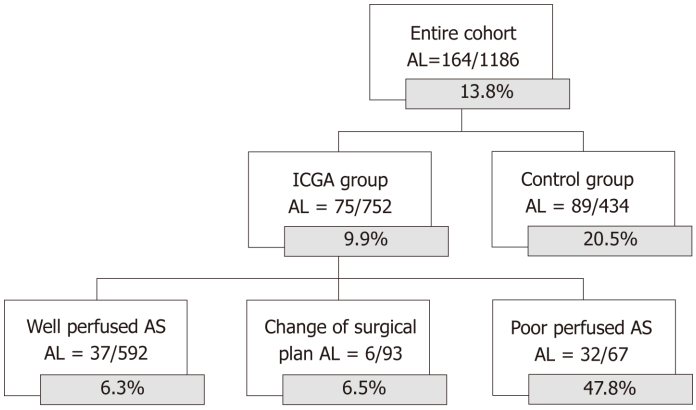


**Figure 2 Anastomotic leakage rates of the entire cohort.** ICGA: Indocyanine green fluorescence angiography; AL: Anastomotic leakage; AS: Anastomotic sites.

AL occurred in 13.8% of the entire cohort, which is in line within the normal range according to the literature. At present, there are no randomized trials to support the alteration of the surgical plan based on the ICGA images. Nevertheless, the most promising aspect of ICGA perfusion assessment is probably its ability to change the surgical plan. In this review population of 1186 patients, the surgeon chose to change the surgical plan to increase the blood flow to the anastomotic site, in 93 cases. The chosen technique varied with surgeon preference and location of the anastomosis. The AL rate in this altered anastomotic site group was 6.5%, similar to the well perfused group (6.3%) and significantly less than the poorly perfused group (47.8%) ($P < 0.001$). These results suggest that the technique is able to predict and remedy a potentially worse outcome, but randomized trials are needed to confirm these retrospective results.

This review is based on 19 studies with considerable inter-study heterogeneity, making comparison between studies difficult. Methods of ICGA interpretation and surgical consequences varied among the different study cohorts. Moreover, the included manuscripts are mainly prospective feasibility studies or retrospective analyses of low-grade evidence and with relatively low MINOR scores. The studies with a control group were all retrospective comparisons to historical control groups. Most studies had incomplete or unmentioned follow-up periods and lacked prospective sample size calculations.

The present review suggests that ICGA is a safe, feasible and promising method to assess graft perfusion that might help reducing AL. At present optimal doses of 0.2-0.5 mg/kg are mentioned in literature but based on this review a bolus dose of 2.5 mg is a sufficient optimal dose for visualization of esophagogastric anastomotic perfusion.

A few authors have attempted to quantify the method, but rarely in oesophageal surgery and without comparison with a golden standard method, stressing the need for objective quantification of the ICGA with validated cutoff levels for sufficient graft perfusion in esophageal surgery. No studies mentioned validation of the method. Objective, in depth assessment of tissue perfusion using NIRF imaging during creation of the esophagogastric anastomosis in EC surgery is lacking. Therefore, we propose a clinical study that uses NIRF dynamic images to calculate physiologically relevant parameters (blood flow, blood volume, vascular leakage) and generate pseudocolor coded parametric maps using advanced curve analysis and compartmental modelling (adiabatic approximation to tissue homogeneity model, AATH)[45,46]. In addition, this study would be the first to validate imaging-based perfusion assessment of the stomach graft using tissue, serum, and cellular hallmarks of hypo-perfusion and hypoxia during esophagectomy[47]. The study is registered in Clinicaltrials.gov as NCT03587532 and is currently recruiting.

The differences in AL rate between the well perfused and poor perfused AS clearly suggest that a good fluorescent signal is a predictor of good outcome. The AL rate in the group with an altered surgical plan based on the ICG image was similar to the well perfused group and significantly less than the poorly perfused group, suggesting that the technique is able to predict and remedy a potentially worse outcome. Reducing AL after oesophageal reconstruction is an ongoing goal of the oesophageal surgical community, this easy and safe new technique of perioperative perfusion assessment has the potential to reduce AL rate and its associated mortality, but randomized trials are needed to confirm these retrospective results. However, a

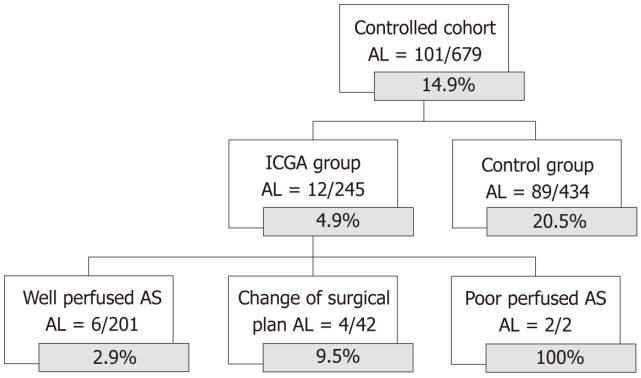


**Figure 3 Anastomotic leakage rates of the cohort with a historical control group.** ICGA: Indocyanine green fluorescence angiography; AL: Anastomotic leakage; AS: Anastomotic sites.

prospective randomized study can only compare ICGA guided surgery versus non ICGA guided surgery and that implies a large sample size demanding a multicentre study. Comparing in a randomized fashion ICGA good perfused anastomoses versus ICGA poor perfused anastomosis would be scientifically perfect but ethically impossible. At present, there are no ongoing randomized trials listed on clinical trial.gov.

## ARTICLE HIGHLIGHTS

### *Research background*

After an esophagectomy, the stomach is most commonly used to restore continuity of the upper gastrointestinal tract. These esophago-gastric anastomoses are prone to serious complications such as leakage associated with high morbidity and mortality. The main cause of anastomotic leakage (AL) is tissue hypoxia, which results from impaired perfusion of the pedicled gastric tube (GT). Clinical judgment is unreliable in determining GT perfusion.

### *Research motivation*

An objective, validated, and reproducible method to evaluate tissue perfusion at the anastomotic site is urgently needed. Based on the current literature we believe Indocyanine green fluorescence angiography (ICGA) is an easy and cheap assessment tool for GT perfusion, that might predict and therefore alter a potentially bad outcome.

### *Research objectives*

This study aimed to systematically review the literature on feasibility and effectiveness of ICGA use as a method to evaluate graft perfusion and as a predictor of AL after esophageal reconstructive surgery. Additionally, current methods to quantify ICGA in esophageal reconstructive surgery were reviewed.

### *Research methods*

This study was designed according to the PRISMA guidelines and registered in the PROSPERO database. Pubmed and Embase were independently searched by 2 reviewers for studies presenting data on intraoperative ICGA GT perfusion assessment during esophago-gastric reconstruction after esophagectomy. Relevant outcomes such as feasibility, complications, intraoperative surgical changes based on ICGA findings, quantification attempts, anatomical data and the impact of ICGA on postoperative anastomotic complications, were collected by 2 independent researchers. The quality of the included articles was assessed based on the MINORS criteria. The 19 included studies presented data on 1192 esophagectomy patients, in 758 patients ICGA was used perioperative to guide esophageal reconstruction.

### *Research results*

The 19 included studies for qualitative analyses all described ICGA as a safe and easy. AL occurred in 13.8% of the entire cohort, 10% in the ICG guided group and 20.6% in the control group ($P < 0.001$). When poorly perfused cases are excluded from the analyses, the difference in AL was even larger (AL well-perfused group 6.3% *vs* control group 20.5%, $P < 0.001$). The AL rate in the group with an altered surgical plan based on the ICG image was 6.5%, similar to the well perfused group (6.3%) and significantly less than the poorly perfused group (47.8%) ($P < 0.001$), suggesting that the technique is able to identify and alter a potential bad outcome.

### *Research conclusions*

ICGA is a safe, feasible and promising method for perfusion assessment. Based on this review a bolus dose of 2.5 mg is a sufficient optimal dose for visualization of esophagogastric anastomotic perfusion. The differences in AL rate between the well perfused and poor perfused AS clearly

suggest that a good fluorescent signal is a predictor of good outcome. The AL rate in the group with an altered surgical plan based on the ICG image was similar to the well perfused group and significantly less than the poorly perfused group, suggesting that the technique is able to predict and remedy a potentially worse outcome.

### *Research perspectives*

A few authors have attempted to quantify the method, but rarely in oesophageal surgery and without comparison with a golden standard method, stressing the need for objective quantification of the ICGA with validated cutoff levels for sufficient graft perfusion in esophageal surgery. No studies mentioned validation of the method. Therefore, we propose a clinical study that validates imaging-based perfusion assessment of the stomach graft using tissue, serum, and cellular hallmarks of hypo-perfusion and hypoxia during esophagectomy. The study is registered in Clinicaltrials.gov as NCT03587532 and is currently recruiting. This easy and safe new technique of perioperative perfusion assessment has the potential to reduce AL rate and its associated mortality, but randomized trials are needed to confirm these retrospective results. At present, there are no ongoing randomized trials listed on clinical trial.gov. Potentially because a prospective randomized study comparing ICGA guided surgery versus non ICGA guided surgery implies a large sample size demanding a large multicentre study.

## REFERENCES


1 **International Agency for Research on Cancer, WHO**. Cancer Statistics, GLOBOCAN 2012: Estimated Cancer Incidence, Mortality and Prevalence Worldwide in 2012. Available from: URL: http://gco.iarc.fr/today/data/factsheets/cancers/6-Oesophagus-fact-sheet.pdf

2 **Kassis ES**, Kosinski AS, Ross P, Koppes KE, Donahue JM, Daniel VC. Predictors of anastomotic leak after esophagectomy: an analysis of the society of thoracic surgeons general thoracic database. *Ann Thorac Surg* 2013; **96**: 1919-1926 [PMID: 24075499 DOI: 10.1016/j.athoracsur.2013.07.119]

3 **Biere SS**, Maas KW, Cuesta MA, van der Peet DL. Cervical or thoracic anastomosis after esophagectomy for cancer: a systematic review and meta-analysis. *Dig Surg* 2011; **28**: 29-35 [PMID: 21293129 DOI: 10.1159/000322014]

4 **Sauvanet A**, Mariette C, Thomas P, Lozac'h P, Segol P, Tiret E, Delpero JR, Collet D, Leborgne J, Pradère B, Bourgeon A, Triboulet JP. Mortality and morbidity after resection for adenocarcinoma of the gastroesophageal junction: predictive factors. *J Am Coll Surg* 2005; **201**: 253-262 [PMID: 16038824 DOI: 10.1016/j.jamcollsurg.2005.02.002]

5 **Sunpaweravong S**, Ruangsin S, Laohawiriyakamol S, Mahattanobon S, Geater A. Prediction of major postoperative complications and survival for locally advanced esophageal carcinoma patients. *Asian J Surg* 2012; **35**: 104-109 [PMID: 22884266 DOI: 10.1016/j.asjsur.2012.04.029]

6 **Haga Y**, Wada Y, Takeuchi H, Ikejiri K, Ikenaga M. Prediction of anastomotic leak and its prognosis in digestive surgery. *World J Surg* 2011; **35**: 716-722 [PMID: 21184072 DOI: 10.1007/s00268-010-0922-5]

7 **Rutegård M**, Lagergren P, Rouvelas I, Lagergren J. Intrathoracic anastomotic leakage and mortality after esophageal cancer resection: a population-based study. *Ann Surg Oncol* 2012; **19**: 99-103 [PMID: 21769467 DOI: 10.1245/s10434-011-1926-6]

8 **Van Daele E**, Van de Putte D, Ceelen W, Van Nieuwenhove Y, Pattyn P. Risk factors and consequences of anastomotic leakage after Ivor Lewis oesophagectomy†. *Interact Cardiovasc Thorac Surg* 2016; **22**: 32-37 [PMID: 26433973 DOI: 10.1093/icvts/ivv276]

9 **Wright CD**, Kucharczuk JC, O'Brien SM, Grab JD, Allen MS; Society of Thoracic Surgeons General Thoracic Surgery Database. Predictors of major morbidity and mortality after esophagectomy for esophageal cancer: a Society of Thoracic Surgeons General Thoracic Surgery Database risk adjustment model. *J Thorac Cardiovasc Surg* 2009; **137**: 587-595; discussion 596 [PMID: 19258071 DOI: 10.1016/j.jtcvs.2008.11.042]

10 **Junemann-Ramirez M**, Awan MY, Khan ZM, Rahamim JS. Anastomotic leakage post-esophagogastrectomy for esophageal carcinoma: retrospective analysis of predictive factors, management and influence on longterm survival in a high volume centre. *Eur J Cardiothorac Surg* 2005; **27**: 3-7 [PMID: 15621463 DOI: 10.1016/j.ejcts.2004.09.018]

11 **Markar SR**, Arya S, Karthikesalingam A, Hanna GB. Technical factors that affect anastomotic integrity following esophagectomy: systematic review and meta-analysis. *Ann Surg Oncol* 2013; **20**: 4274-4281 [PMID: 23943033 DOI: 10.1245/s10434-013-3189-x]

12 **Milstein DM**, Ince C, Gisbertz SS, Boateng KB, Geerts BF, Hollmann MW, van Berge Henegouwen MI, Veelo DP. Laser speckle contrast imaging identifies ischemic areas on gastric tube reconstructions following esophagectomy. *Medicine (Baltimore)* 2016; **95**: e3875 [PMID: 27336874 DOI: 10.1097/MD.0000000000003875]

13 **Linder G**, Hedberg J, Björck M, Sundbom M. Perfusion of the gastric conduit during esophagectomy. *Dis Esophagus* 2017; **30**: 143-149 [PMID: 27766735 DOI: 10.1111/dote.12537]

14 **Pham TH**, Perry KA, Enestvedt CK, Gareau D, Dolan JP, Sheppard BC, Jacques SL, Hunter JG. Decreased conduit perfusion measured by spectroscopy is associated with anastomotic complications. *Ann Thorac Surg* 2011; **91**: 380-385 [PMID: 21256274 DOI: 10.1016/j.athoracsur.2010.10.006]

15 **Tsekov C**, Belyaev O, Tcholakov O, Tcherveniakov A. Intraoperative Doppler assessment of gastric tube perfusion in esophagogastroplasty. *J Surg Res* 2006; **132**: 98-103 [PMID: 16154594 DOI: 10.1016/j.jss.2005.07.037]

16 **Alander JT**, Kaartinen I, Laakso A, Pätilä T, Spillmann T, Tuchin VV, Venermo M, Välisuo P. A review of indocyanine green fluorescent imaging in surgery. *Int J Biomed Imaging* 2012; **2012**: 940585 [PMID: 22577366 DOI: 10.1155/2012/940585]

17 **Slim K**, Nini E, Forestier D, Kwiatkowski F, Panis Y, Chipponi J. Methodological index for non-randomized studies (minors): development and validation of a new instrument. *ANZ J Surg* 2003; **73**: 712-716 [PMID: 12956787 DOI: 10.1046/j.1445-2197.2003.02748.x]

18 **Kubota K**, Yoshida M, Kuroda J, Okada A, Ohta K, Kitajima M. Application of the HyperEye Medical System for esophageal cancer surgery: a preliminary report. *Surg Today* 2013; **43**: 215-220 [PMID: 22782594 DOI: 10.1007/s00595-012-0251-4]

19 **Murawa D**, Hünerbein M, Spychała A, Nowaczyk P, Połom K, Murawa P. Indocyanine green angiography for evaluation of gastric conduit perfusion during esophagectomy--first experience. *Acta Chir Belg* 2012; **112**: 275-280 [PMID: 23008991 DOI: 10.1080/00015458.2012.11680838]

20 **Pacheco PE**, Hill SM, Henriques SM, Paulsen JK, Anderson RC. The novel use of intraoperative laser-induced fluorescence of indocyanine green tissue angiography for evaluation of the gastric conduit in esophageal reconstructive surgery. *Am J Surg* 2013; **205**: 349-352; discussion 352-3 [PMID: 23414958 DOI: 10.1016/j.amjsurg.2012.11.005]

21 **Kumagai Y**, Ishiguro T, Haga N, Kuwabara K, Kawano T, Ishida H. Hemodynamics of the reconstructed gastric tube during esophagectomy: assessment of outcomes with indocyanine green fluorescence. *World J Surg* 2014; **38**: 138-143 [PMID: 24196170 DOI: 10.1007/s00268-013-2237-9]

22 **Rino Y**, Yukawa N, Sato T, Yamamoto N, Tamagawa H, Hasegawa S, Oshima T, Yoshikawa T, Masuda M, Imada T. Visualization of blood supply route to the reconstructed stomach by indocyanine green fluorescence imaging during esophagectomy. *BMC Med Imaging* 2014; **14**: 18 [PMID: 24885891 DOI: 10.1186/1471-2342-14-18]

23 **Sarkaria IS**, Bains MS, Finley DJ, Adusumilli PS, Huang J, Rusch VW, Jones DR, Rizk NP. Intraoperative near-infrared fluorescence imaging as an adjunct to robotic-assisted minimally invasive esophagectomy. *Innovations (Phila)* 2014; **9**: 391-393 [PMID: 25238427 DOI: 10.1097/IMI.0000000000000091]

24 **Kamiya K**, Unno N, Miyazaki S, Sano M, Kikuchi H, Hiramatsu Y, Ohta M, Yamatodani T, Mineta H, Konno H. Quantitative assessment of the free jejunal graft perfusion. *J Surg Res* 2015; **194**: 394-399 [PMID: 25472574 DOI: 10.1016/j.jss.2014.10.049]

25 **Hodari A**, Park KU, Lace B, Tsiouris A, Hammoud Z. Robot-Assisted Minimally Invasive Ivor Lewis Esophagectomy With Real-Time Perfusion Assessment. *Ann Thorac Surg* 2015; **100**: 947-952 [PMID: 26116484 DOI: 10.1016/j.athoracsur.2015.03.084]

26 **Campbell C**, Reames MK, Robinson M, Symanowski J, Salo JC. Conduit Vascular Evaluation is Associated with Reduction in Anastomotic Leak After Esophagectomy. *J Gastrointest Surg* 2015; **19**: 806-812 [PMID: 25791907 DOI: 10.1007/s11605-015-2794-3]

27 **Yukaya T**, Saeki H, Kasagi Y, Nakashima Y, Ando K, Imamura Y, Ohgaki K, Oki E, Morita M, Maehara Y. Indocyanine Green Fluorescence Angiography for Quantitative Evaluation of Gastric Tube Perfusion in Patients Undergoing Esophagectomy. *J Am Coll Surg* 2015; **221**: e37-e42 [PMID: 26206660 DOI: 10.1016/j.jamcollsurg.2015.04.022]

28 **Zehetner J**, DeMeester SR, Alicuben ET, Oh DS, Lipham JC, Hagen JA, DeMeester TR. Intraoperative Assessment of Perfusion of the Gastric Graft and Correlation With Anastomotic Leaks After Esophagectomy. *Ann Surg* 2015; **262**: 74-78 [PMID: 25029436 DOI: 10.1097/SLA.0000000000000811]

29 **Kitagawa H**, Namikawa T, Munekage M, Akimori T, Kobayashi M, Hanazaki K. Visualization of the Stomach's Arterial Networks During Esophageal Surgery Using the HyperEye Medical System. *Anticancer Res* 2015; **35**: 6201-6205 [PMID: 26504051]

30 **Koyanagi K**, Ozawa S, Oguma J, Kazuno A, Yamazaki Y, Ninomiya Y, Ochiai H, Tachimori Y. Blood flow speed of the gastric conduit assessed by indocyanine green fluorescence: New predictive evaluation of anastomotic leakage after esophagectomy. *Medicine (Baltimore)* 2016; **95**: e4386 [PMID: 27472732 DOI: 10.1097/MD.0000000000004386]

31 **Nakashima Y**, Saeki H, Yukaya T, Tsutsumi S, Nakanishi R, Sugiyama M, Ohgaki K, Sonoda H, Oki E, Maehara Y. Blood Flow Assessment with Indocyanine Green Fluorescence Angiography for Pedicled Omental Flap on Cervical Esophagogastric Anastomosis after Esophagectomy. *J Am Coll Surg* 2016; **222**: e67-e69 [PMID: 27113525 DOI: 10.1016/j.jamcollsurg.2016.01.048]

32 **Ohi M**, Saigusa S, Toiyama Y, Ichikawa T, Shimura T, Yasuda H, Okita Y, Yoshiyama S, Kobayashi M, Araki T, Inoue Y, Mohri Y, Kusunoki M. Evaluation of Blood Flow with Indocyanine Green-Guided Imaging to Determine Optimal Site for Gastric Conduit Anastomosis to Prevent Anastomotic Leak after Esophagectomy. *Am Surg* 2017; **83**: e197-e199 [PMID: 28637544]

33 **Schlottmann F**, Patti MG. Evaluation of Gastric Conduit Perfusion During Esophagectomy with Indocyanine Green Fluorescence Imaging. *J Laparoendosc Adv Surg Tech A* 2017; **27**: 1305-1308 [PMID: 28817358 DOI: 10.1089/lap.2017.0359]

34 **Kitagawa H**, Namikawa T, Iwabu J, Fujisawa K, Uemura S, Tsuda S, Hanazaki K. Assessment of the blood supply using the indocyanine green fluorescence method and postoperative endoscopic evaluation of anastomosis of the gastric tube during esophagectomy. *Surg Endosc* 2018; **32**: 1749-1754 [PMID: 28916846 DOI: 10.1007/s00464-017-5857-6]

35 **Ohi M**, Toiyama Y, Mohri Y, Saigusa S, Ichikawa T, Shimura T, Yasuda H, Okita Y, Yoshiyama S, Kobayashi M, Araki T, Inoue Y, Kusunoki M. Prevalence of anastomotic leak and the impact of indocyanine green fluorescein imaging for evaluating blood flow in the gastric conduit following esophageal cancer surgery. *Esophagus* 2017; **14**: 351-359 [PMID: 28983231 DOI: 10.1007/s10388-017-0585-5]

36 **Karampinis I**, Ronellenfitsch U, Mertens C, Gerken A, Hetjens S, Post S, Kienle P, Nowak K. Indocyanine green tissue angiography affects anastomotic leakage after esophagectomy. A retrospective, case-control study. *Int J Surg* 2017; **48**: 210-214 [PMID: 29146267 DOI: 10.1016/j.ijsu.2017.11.001]

37 **Noma K**, Shirakawa Y, Kanaya N, Okada T, Maeda N, Ninomiya T, Tanabe S, Sakurama K, Fujiwara T. Visualized Evaluation of Blood Flow to the Gastric Conduit and Complications in Esophageal Reconstruction. *J Am Coll Surg* 2018; **226**: 241-251 [PMID: 29174858 DOI: 10.1016/j.jamcollsurg.2017.11.007]

38 **Dalton BGA**, Ali AA, Crandall M, Awad ZT. Near infrared perfusion assessment of gastric conduit during minimally invasive Ivor Lewis esophagectomy. *Am J Surg* 2018; **216**: 524-527 [PMID: 29203037 DOI: 10.1016/j.amjsurg.2017.11.026]

39 **Kumagai Y**, Hatano S, Sobajima J, Ishiguro T, Fukuchi M, Ishibashi KI, Mochiki E, Nakajima Y, Ishida H. Indocyanine green fluorescence angiography of the reconstructed gastric tube during esophagectomy: efficacy of the 90-second rule. *Dis Esophagus* 2018; **31** [PMID: 29897432 DOI: 10.1093/dote/doy052]

40 **Shimada Y**, Okumura T, Nagata T, Sawada S, Matsui K, Hori R, Yoshioka I, Yoshida T, Osada R, Tsukada K. Usefulness of blood supply visualization by indocyanine green fluorescence for reconstruction during esophagectomy. *Esophagus* 2011; **8**: 259-266 [PMID: 22557942 DOI: 10.1007/s10388-011-0291-7]

41 **Diana M**, Noll E, Diemunsch P, Dallemagne B, Benahmed MA, Agnus V, Soler L, Barry B, Namer IJ, Demartines N, Charles AL, Geny B, Marescaux J. Enhanced-reality video fluorescence: a real-time assessment of intestinal viability. *Ann Surg* 2014; **259**: 700-707 [PMID: 23532109 DOI:

10.1097/SLA.0b013e31828d4ab3]

42 **Diana M**, Halvax P, Dallemagne B, Nagao Y, Diemunsch P, Charles AL, Agnus V, Soler L, Demartines N, Lindner V, Geny B, Marescaux J. Real-time navigation by fluorescence-based enhanced reality for precise estimation of future anastomotic site in digestive surgery. *Surg Endosc* 2014; **28**: 3108-3118 [PMID: 24912446 DOI: 10.1007/s00464-014-3592-9]

43 **Diana M**, Agnus V, Halvax P, Liu YY, Dallemagne B, Schlagowski AI, Geny B, Diemunsch P, Lindner V, Marescaux J. Intraoperative fluorescence-based enhanced reality laparoscopic real-time imaging to assess bowel perfusion at the anastomotic site in an experimental model. *Br J Surg* 2015; **102**: e169-e176 [PMID: 25627131 DOI: 10.1002/bjs.9725]

44 **van Manen L**, Handgraaf HJM, Diana M, Dijkstra J, Ishizawa T, Vahrmeijer AL, Mieog JSD. A practical guide for the use of indocyanine green and methylene blue in fluorescence-guided abdominal surgery. *J Surg Oncol* 2018; **118**: 283-300 [PMID: 29938401 DOI: 10.1002/jso.25105]

45 **St Lawrence K**, Verdecchia K, Elliott J, Tichauer K, Diop M, Hoffman L, Lee TY. Kinetic model optimization for characterizing tumour physiology by dynamic contrast-enhanced near-infrared spectroscopy. *Phys Med Biol* 2013; **58**: 1591-1604 [PMID: 23417099 DOI: 10.1088/0031-9155/58/5/1591]

46 **Milej D**, Abdalmalak A, Desjardins L, Ahmed H, Lee TY, Diop M, Lawrence KS. Quantification of blood-brain barrier permeability by dynamic contrast-enhanced NIRS. *Sci Rep* 2017; **7**: 1702 [PMID: 28490806 DOI: 10.1038/s41598-017-01922-x]

47 **Van Daele E**, Van Nieuwenhove Y, Ceelen W, Vanhove C, Braeckman BP, Hoorens A, Van Limmen J, Varin O, Van de Putte D, Willaert W, Pattyn P. Assessment of graft perfusion and oxygenation for improved outcome in esophageal cancer surgery: Protocol for a single-center prospective observational study. *Medicine (Baltimore)* 2018; **97**: e12073 [PMID: 30235661 DOI: 10.1097/MD.0000000000012073]

**P- Reviewer:** Mastoraki A, Senchukova MA

**S- Editor:** Ji FF **L- Editor:** A **E- Editor:** Song H

World Journal of Gastrointestinal Oncology

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

*World J Gastrointest Oncol* 2019 March 15; 11(3): 264-269

DOI: 10.4251/wjgo.v11.i3.264

ISSN 1948-5204 (online)

CASE REPORT

# Stent placement followed by preoperative chemotherapy and elective surgery for acute malignant colorectal obstruction: Six cases of report

Jun-Jie Liu, Teng-Hui Ma, Qi-Yuan Qin, Lei Wang

**ORCID number:** Jun-Jie Liu (0000-0002-0005-8849); Teng-Hui Ma (0000-0003-4547-865X); Qi-Yuan Qin (0000-0002-0745-7180); Lei Wang (0000-0003-3724-7392).

**Author contributions:** Wang L and Ma TH designed the study; Liu JJ and Qin QY collected the patients' clinical data; Liu JJ wrote the paper; Wang L, Ma TH and Qin QY revised the paper.

**Institutional review board statement:** The study was reviewed and approved by the Ethics Committee of the Sixth Affiliated Hospital of Sun Yat-sen University.

**Informed consent statement:** All study participants, or their legal guardian, provided informed written consent prior to study.

**Conflict-of-interest statement:** The authors declare that they have no conflicts of interest.

**CARE Checklist (2016) statement:** The guidelines of the CARE Checklist (2016) have been adopted.



**Jun-Jie Liu, Teng-Hui Ma, Qi-Yuan Qin, Lei Wang,** Department of Colorectal Surgery, the Sixth Affiliated Hospital of Sun Yat-sen University, Guangzhou 510655, Guangdong Province, China

**Corresponding author:** Lei Wang, MD, PhD, Doctor, Professor, Surgeon, Department of Colorectal Surgery, the Sixth Affiliated Hospital of Sun Yat-sen University, 26 Yuancun Erheng Road, Guangzhou 510655, Guangdong Province, China. wangl9@mail.sysu.edu.cn
**Telephone:** +86-020-38254052
**Fax:** +86-020-38254000

## Abstract

*BACKGROUND*

The self-expandable metal stent is used as a bridge to surgery in the treatment of acute malignant colorectal obstruction (AMCO). However, recent studies have shown inferior long-term outcomes and increased risk of tumor dissemination after stent placement. In addition, the optimal interval between stent placement and surgery is not clear. The aim of the current study was to present a new strategy for AMCO: stent placement followed by preoperative chemotherapy and elective surgery.

*CASE SUMMARY*

Six patients were diagnosed as acute obstruction. There was one patient with descending cancer, four with sigmoid cancers and one with rectal cancer. The obstructive symptoms of these six patients were relieved within 3 d after stent placement. After receiving two cycles of preoperative chemotherapy, consisting of modified infusional fluorouracil, leucovorin and oxaliplatin [modified FOLFOX6 (mFOLFOX6)], they underwent elective surgery of primary tumor resection. None of the 6 patients received colostomy or colonic lavage during surgery. There was no complication of anastomotic leak, ileus or surgical site infection after surgery. In addition, the patients had low operation time and blood loss, adequate lymph nodes harvest and fast postoperative recovery.

*CONCLUSION*

The two-cycle mFOLFOX6 preoperative chemotherapy and elective surgery after stent placement is a safe and feasible strategy in the management of AMCO.

**Key words:** Stent; Colorectal cancer; Obstruction; Preoperative chemotherapy; Case report

**Manuscript source**: Unsolicited manuscript

**Received:** October 13, 2018
**Peer-review started:** October 15, 2018
**First decision:** October 25, 2018
**Revised:** December 18, 2018
**Accepted:** January 8, 2019
**Article in press:** January 9, 2019
**Published online:** March 15, 2019



**Core tip:** The favorable short-term outcomes of stent placement, as a bridge to surgery for acute malignant colorectal obstruction, have been well described comparing with emergency surgery. However, the risk of tumor dissemination after stent placement is still a topic of concern. In addition, the current interval between stent placement and surgery is inadequate for the patient's recovery and primary anastomosis. This study is to present the strategy of arranging two cycles of preoperative chemotherapy between stent placement and elective surgery for obstructive colorectal cancers. The six cases of patients showed no stoma creations and no adverse events.

**Citation:** Liu JJ, Ma TH, Qin QY, Wang L. Stent placement followed by preoperative chemotherapy and elective surgery for acute malignant colorectal obstruction: Six cases of report. *World J Gastrointest Oncol* 2019; 11(3): 264-269
**URL**: https://www.wjgnet.com/1948-5204/full/v11/i3/264.htm
**DOI**: https://dx.doi.org/10.4251/wjgo.v11.i3.264

## INTRODUCTION

Acute malignant colorectal obstruction (AMCO) occurs in approximately 8.3% of colorectal cancers[1]. Management of AMCO requires thorough assessment of patients, comprehensive understanding of the pathology and careful choice of treatment options. Since first introduced in 1994[2], stent placement has been widely used as a bridge to surgery for AMCO. Many randomized controlled trials and systematic reviews have demonstrated that stent placement followed by elective surgery is a safe and effective approach that has a lower stoma rate and reduced postoperative complications when compared with emergency surgery[3-9].

However, there are some concerns about the oncological safety of stent placement for AMCO. Recent studies have shown that stent use in AMCO resulted in higher local recurrence rate or inferior survival outcomes[10,11]. In clinical practice, surgery of tumor resection was arranged about one week after stent placement. However, there are still some patients who cannot recovery well or obtain primary anastomosis without stoma creation in this short time. A recent meta-analysis indicated that the postoperative complication rate of using a stent as a bridge to surgery was up to 37.84%, and that the stoma rate was 28.8%[8]. Adding preoperative chemotherapy into the prolonged interval between stent placement and elective surgery may reduce the risk of tumor dissemination and improve the general status of patients. In the current study, we presented six patients treated with two cycles of preoperative chemotherapy and elective surgery after stent placement and evaluated their outcomes.

## CASES PRESENTATION

### *Chief complaints*

From March 2016 to October 2017, six patients were hospitalized with the chief complaints of abdominal distention, abdominal pain and stop of flatus and defecation.

### *History of present illness*

They had the chronic symptoms of colorectal cancers for at least two week, such as abdominal pain, change of the bowel habit and change of the stool character. The acute obstruction was occurred within 48 h before hospital admission. The signs of bowel perforation and strangulated obstruction were not found in these patients.

### *History of past illness*

The six patients had Eastern Cooperative Oncology Group (ECOG) performance status ≥ 2 and adequate hematologic, liver and renal function. They had no other cancers or clinically significant cardiovascular diseases.

### *Physical examination*

Abdominal distention and hyperactive bowel sounds were found. Peritoneal irritation signs were not found.

### *Laboratory testing*

The blood routine index, biochemical function and tumor biomarker were tested after hospitalization. The initial testing results of hemoglobin, albumin and creatinine were shown in Table 1.

### *Imaging examination*

Bowel distention with gas and effusion was shown in abdominal X-ray. Computed tomography (CT) showed that the obstructing tumor was located on left-sided colon or upper rectum (more than 10 cm from the anal verge). Obvious distention of proximal bowel was also found in CT scan. The tumor stage was examined through enhanced CT scanning.

## FINAL DIAGNOSIS

The basic characteristics of the six patients are shown in Table 1. Six patients were diagnosed with AMCO. There was one patient with descending cancer, four with sigmoid cancers and one with rectal cancer. Two patients had synchronous hepatic metastasis. One patient had two metastastic sites in the left lobe of liver (maximum diameter: 1.6 cm and 5.8 cm), and the other patient had one metastatic site in S8 (maximum diameter: 2.2 cm).

## TREATMENT

### *Stent placement*

Before stent placement, an endoscopic biopsy was carried out to confirm the malignancy diagnosis. An endoscope was used to cross the stricture caused by the tumor. When the stricture could not be crossed, a guide wire in a catheter was used. Contrast agent was injected through the catheter to estimate the length and width of the stricture. An appropriately sized of self-expandable metal stent (SEMS) was chosen according to the estimation of the stricture. The stent delivery system was pushed into the area of the stricture with radio-endoscopic guidance. The stent was deployed to crossover the distal and proximal end of the stricture. The passing of fecal material through the stent indicated successful placement. If complications from the stent occurred, including re-obstruction, migration of the stent or bowel perforation, patients were sent to emergency surgery.

### *Preoperative chemotherapy*

After stent placement, the six patients were scheduled for preoperative chemotherapy, which consisted of two cycles of modified infusional fluorouracil, leucovorin and oxaliplatin [modified FOLFOX6 (mFOLFOX6)], instead of direct surgery. The timing of preoperative chemotherapy after stent placement was determined by the time when the obstructive symptoms disappeared. Each cycle consisted of oxaliplatin 85 mg/m$^2$, leucovorin 400 mg/m$^2$ and fluorouracil 400 mg/m$^2$ given intravenously on day 1 and fluorouracil 2400 mg/m$^2$ given over 48-h of continuously intravenous infusion on days 1-2. Acute adverse events associated with chemotherapy were graded according to the Common Terminology Criteria Adverse Events Version 4.03.

### *Elective surgery*

Surgery of primary tumor resection was performed two to three weeks after the end of chemotherapy. Mechanical bowel preparation was used preoperatively. Laparoscopic or open surgery was performed by senior surgeons. Complete mesocolic or mesorectal excision with central vascular ligation was adopted as the standard technique. Stoma creation or colonic lavage was selectively performed in the cases with obvious bowel edema or distention, or doubtful anastomosis.

### *Postoperative treatment*

The patient with one metastatic site in S8 underwent microwave ablation. The patient with two hepatic metastatic tumors in left liver lobe underwent partial hepatectomy, but he refused postoperative chemotherapy. The other five patients received postoperative chemotherapy for three to six months.

## OUTCOME AND FOLLOW UP

The obstructive symptoms of these six patients were disappeared within 3 d after

**Table 1 Patient characteristics**

| Characteristics | Data ( *n* = 6) |
|---|---|
| Sex | |
| Male | 4 (66.7%) |
| Female | 2 (33.3%) |
| Age, yr | 65 (38-76) |
| BMI*, kg/m$^2$ | 20.1 (17.1-23.6) |
| Hemoglobin*, g/L | 123.5 (88-143) |
| Albumin*, g/L | 38.5 (34.1-43.1) |
| Creatinine*, μmoI/L | 66 (42.3-94.7) |
| Tumor site, clinical stage# | |
| Descending colon, III | 1 (16.7%) |
| Sigmoid colon, III | 1 (16.7%) |
| Sigmoid colon, III | 2 (33.3%) |
| Sigmoid colon, IV | 1 (16.7%) |
| Rectum, II | 1 (16.7%) |

Data are median (range) or *n* (%). *The initial testing during the first hospitalization. #Evaluated before chemotherapy.

stent placement. No patients had complications caused by stents. The median time between stent placement and preoperative chemotherapy was 9 d (range, 3-34). None of the grade 3/4 toxicities were found. The median time between stent placement and elective surgery was 38 d (range, 33-43). All of the six patients underwent primary tumor resections with primary anastomosis. No patient had stoma creation, colonic lavage or postoperative complication. Five patients (83.3%) received laparoscopic surgery. The median operation time was 162.5 min (range, 112-270). The median blood loss was 50 mL (range, 30-100). The median lymph node harvest was 26 (range, 13-45). The median time to first flatus was 2 d (range, 2-4). The median postoperative stay was 11.5 d (range, 9-17). The perioperative outcomes are shown in Table 2.

Eighteen months after surgery of primary tumor resection was set for the follow-up period. Two patients were found with peritoneal metastasis at the end of follow-up period (both of them received postoperative chemotherapy).

## DISCUSSION

The use of SEMS as a bridge to surgery for AMCO has been an increasingly common practice in qualified medical centers. In the present study, we introduced the clinical use of preoperative chemotherapy and elective resection after stent placement for AMCO. The case series showed that this new approach provided no stoma creation and no morbidity.

The original intention of adding preoperative chemotherapy after stent placement was to lighten the potential adverse effect of stent placement. Maruthachalam *et al*[12] found that insertion of a stent resulted in increased levels of CK20 mRNA in the peripheral blood of patients with colorectal cancer, which was considered to promote the distribution of occult tumor cells. Fryer *et al*[13] analyzed the histopathological changes induced by stent placement in 72 patients and concluded that the changes of the tumor included tumor necrosis (100%) and flat ulceration (77.8%). Colonic perforation caused by the stent may potentially result in the peritoneal seeding[14]. In a retrospective study of Korea, a higher rate of perineural invasion was observed in patients of the stent group compared to surgery group (76% *vs* 51.4%, respectively)[15]. Sabbagh *et al*[11] first reported significantly lower overall survival for the patients with stent placement after conducting a propensity score analysis. Although receiving preoperative and postoperative chemotherapy, two patients in our study were found with peritoneal metastasis at the end of follow-up period. It demonstrates that the evidence about the inferior oncological outcomes of stent placement may be underestimated in our clinical practice.

Most obstructing colorectal cancers are in the advanced stage. For the metastatic colorectal cancers that are suitable for curative resection, it is appropriate to carry out preoperative chemotherapy in order to reduce the tumor load. In terms of tumors

**Table 2 Perioperative outcomes**

| Variables | Data (*n* = 6) |
|---|---|
| Grade 3/4 toxicities; Surgical approach | 0 |
| Laparoscopic | 5 (83.3%) |
| Open | 1 (16.7%) |
| Conversion | 0 |
| Operation time, min | 162.5 (112-270) |
| Blood loss, mL | 50 (30-100) |
| Lymph nodes harvest | 26 (13-45) |
| Stoma creation | 0 |
| Colonic lavage | 0 |
| Complication | 0 |
| Anastomotic leakage | 0 |
| Surgical site infection | 0 |
| Ileus | 0 |
| Time to first flatus, d | 2 (2-4) |
| Postoperative stay, d | 11.5 (9-17) |

Data are median (range) or *n* (%).

without macroscopic metastasis, preoperative chemotherapy was considered to eradicate micrometastasis and to reduce the risk of incomplete excision. Preoperative chemotherapy in locally advanced rectal cancer has been recommended by clinical guidelines. For locally advanced and operable colon cancers, preoperative chemotherapy is feasible with encouraging pathological responses, although long-term outcomes are unknown[16].

The optimal time interval between stent placement and subsequent surgery has not been clearly mentioned by previous studies. Five to ten days was recommended by the guidelines of the European Society of Digestive Endoscopy[17]. A meta-analysis showed a median interval of ten days from stent placement to surgery[9]. A retrospective study from Turkey concluded that time intervals of seven to nine days after stent placement is sufficient for safe surgery[18]. However, extending the time interval will lead to better recovery of bowel and nutritional status. The six cases showed that primary anastomosis without stoma creation was obtained after the interval of four weeks. In our experiences, a time interval of ten days is not enough for the bowel to restore. We considered that the better bowel environment could be obtained at least four weeks after stent placement. However, a prolonged interval may increase the technical difficulty of surgery because of more local tumor infiltration and fibrosis in patients with stents. In addition, more cycles of chemotherapy during stent placement might induce more complications such as bowel reobstruction, stent migration and bowel perforation. In our study, the two cycles of preoperative chemotherapy have excellent clinical outcomes and patient compliance. However, it needs further studies to confirm.

## CONCLUSION

The study demonstrates that the placement of SEMS followed by two-cycle preoperative chemotherapy and elective surgery is a safe and feasible strategy for the treatment of AMCO. This new strategy brings no stoma creation and low postoperative morbidity. Surgeons can apply this method to selected patients after thorough evaluations and multidisciplinary collaborations. However, the oncological improvement of this strategy and the optimal cycles of preoperative chemotherapy need confirmation in further studies, in particular clinical trials.

## REFERENCES

1 **Cheynel N**, Cortet M, Lepage C, Benoit L, Faivre J, Bouvier AM. Trends in frequency and management of obstructing colorectal cancers in a well-defined population. *Dis Colon Rectum* 2007; **50**: 1568-1575 [PMID: 17687610 DOI: 10.1007/s10350-007-9007-4]

2 **Tejero E**, Mainar A, Fernández L, Tobío R, De Gregorio MA. New procedure for the treatment of colorectal neoplastic obstructions. *Dis Colon Rectum* 1994; **37**: 1158-1159 [PMID: 7956588 DOI: 10.1007/BF02049822]

3 **Arezzo A**, Balague C, Targarona E, Borghi F, Giraudo G, Ghezzo L, Arroyo A, Sola-Vera J, De Paolis P, Bossotti M, Bannone E, Forcignanò E, Bonino MA, Passera R, Morino M. Colonic stenting as a bridge to surgery versus emergency surgery for malignant colonic obstruction: results of a multicentre randomised controlled trial (ESCO trial). *Surg Endosc* 2017; **31**: 3297-3305 [PMID: 27924392 DOI: 10.1007/s00464-016-5362-3]

4 **Pirlet IA**, Slim K, Kwiatkowski F, Michot F, Millat BL. Emergency preoperative stenting versus surgery for acute left-sided malignant colonic obstruction: a multicenter randomized controlled trial. *Surg Endosc* 2011; **25**: 1814-1821 [PMID: 21170659 DOI: 10.1007/s00464-010-1471-6]

5 **van Hooft JE**, Bemelman WA, Oldenburg B, Marinelli AW, Lutke Holzik MF, Grubben MJ, Sprangers MA, Dijkgraaf MG, Fockens P; collaborative Dutch Stent-In study group. Colonic stenting versus emergency surgery for acute left-sided malignant colonic obstruction: a multicentre randomised trial. *Lancet Oncol* 2011; **12**: 344-352 [PMID: 21398178 DOI: 10.1016/S1470-2045(11)70035-3]

6 **Ho KS**, Quah HM, Lim JF, Tang CL, Eu KW. Endoscopic stenting and elective surgery versus emergency surgery for left-sided malignant colonic obstruction: a prospective randomized trial. *Int J Colorectal Dis* 2012; **27**: 355-362 [PMID: 22033810 DOI: 10.1007/s00384-011-1331-4]

7 **Huang X**, Lv B, Zhang S, Meng L. Preoperative colonic stents versus emergency surgery for acute left-sided malignant colonic obstruction: a meta-analysis. *J Gastrointest Surg* 2014; **18**: 584-591 [PMID: 24170606 DOI: 10.1007/s11605-013-2344-9]

8 **Allievi N**, Ceresoli M, Fugazzola P, Montori G, Coccolini F, Ansaloni L. Endoscopic Stenting as Bridge to Surgery versus Emergency Resection for Left-Sided Malignant Colorectal Obstruction: An Updated Meta-Analysis. *Int J Surg Oncol* 2017; **2017**: 2863272 [PMID: 28761765 DOI: 10.1155/2017/2863272]

9 **De Ceglie A**, Filiberti R, Baron TH, Ceppi M, Conio M. A meta-analysis of endoscopic stenting as bridge to surgery versus emergency surgery for left-sided colorectal cancer obstruction. *Crit Rev Oncol Hematol* 2013; **88**: 387-403 [PMID: 23845505 DOI: 10.1016/j.critrevonc.2013.06.006]

10 **Gorissen KJ**, Tuynman JB, Fryer E, Wang L, Uberoi R, Jones OM, Cunningham C, Lindsey I. Local recurrence after stenting for obstructing left-sided colonic cancer. *Br J Surg* 2013; **100**: 1805-1809 [PMID: 24227368 DOI: 10.1002/bjs.9297]

11 **Sabbagh C**, Browet F, Diouf M, Cosse C, Brehant O, Bartoli E, Mauvais F, Chauffert B, Dupas JL, Nguyen-Khac E, Regimbeau JM. Is stenting as "a bridge to surgery" an oncologically safe strategy for the management of acute, left-sided, malignant, colonic obstruction? A comparative study with a propensity score analysis. *Ann Surg* 2013; **258**: 107-115 [PMID: 23324856 DOI: 10.1097/SLA.0b013e31827e30ce]

12 **Maruthachalam K**, Lash GE, Shenton BK, Horgan AF. Tumour cell dissemination following endoscopic stent insertion. *Br J Surg* 2007; **94**: 1151-1154 [PMID: 17541987 DOI: 10.1002/bjs.5790]

13 **Fryer E**, Gorissen KJ, Wang LM, Guy R, Chetty R. Spectrum of histopathological changes encountered in stented colorectal carcinomas. *Histopathology* 2015; **66**: 480-484 [PMID: 24889189 DOI: 10.1111/his.12467]

14 **Kim SJ**, Kim HW, Park SB, Kang DH, Choi CW, Song BJ, Hong JB, Kim DJ, Park BS, Son GM. Colonic perforation either during or after stent insertion as a bridge to surgery for malignant colorectal obstruction increases the risk of peritoneal seeding. *Surg Endosc* 2015; **29**: 3499-3506 [PMID: 25676202 DOI: 10.1007/s00464-015-4100-6]

15 **Kim HJ**, Choi GS, Park JS, Park SY, Jun SH. Higher rate of perineural invasion in stent-laparoscopic approach in comparison to emergent open resection for obstructing left-sided colon cancer. *Int J Colorectal Dis* 2013; **28**: 407-414 [PMID: 22885839 DOI: 10.1007/s00384-012-1556-x]

16 **Foxtrot Collaborative Group**. Feasibility of preoperative chemotherapy for locally advanced, operable colon cancer: the pilot phase of a randomised controlled trial. *Lancet Oncol* 2012; **13**: 1152-1160 [PMID: 23017669 DOI: 10.1016/S1470-2045(12)70348-0]

17 **van Hooft JE**, van Halsema EE, Vanbiervliet G, Beets-Tan RG, DeWitt JM, Donnellan F, Dumonceau JM, Glynne-Jones RG, Hassan C, Jiménez-Perez J, Meisner S, Muthusamy VR, Parker MC, Regimbeau JM, Sabbagh C, Sagar J, Tanis PJ, Vandervoort J, Webster GJ, Manes G, Barthet MA, Repici A; European Society of Gastrointestinal Endoscopy. Self-expandable metal stents for obstructing colonic and extracolonic cancer: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2014; **46**: 990-1053 [PMID: 25325682 DOI: 10.1055/s-0034-1390700]

18 **Abdussamet Bozkurt M**, Gonenc M, Kapan S, Kocatasş A, Temizgönül B, Alis H. Colonic stent as bridge to surgery in patients with obstructive left-sided colon cancer. *JSLS* 2014; 18 [PMID: 25408602 DOI: 10.4293/jsls.2014.00161]


**P- Reviewer:** Sawaki A, Yarema R, Lin Q
**S- Editor:** Dou Y **L- Editor:** A **E- Editor:** Song H


Published By Baishideng Publishing Group Inc
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA
Telephone: +1-925-2238242
Fax: +1-925-2238243
E-mail: bpgoffice@wjgnet.com
Help Desk:https://www.f6publishing.com/helpdesk
https://www.wjgnet.com