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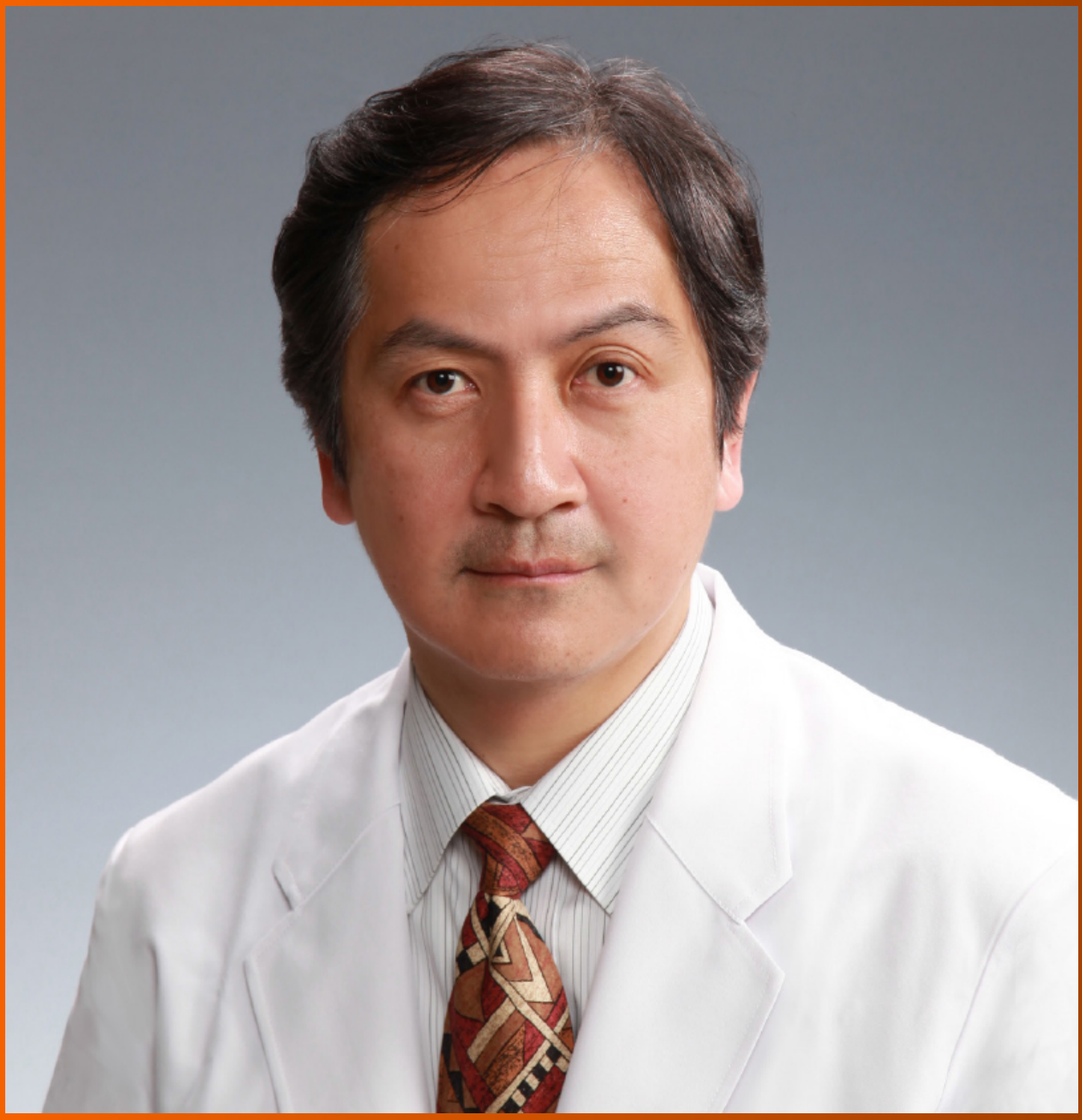

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*REVIEW*

# Endoscopic diagnosis and treatment of gastric dysplasia and early cancer: Current evidence and what the future may hold

Edward Young, Hamish Philpott, Rajvinder Singh

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## Abstract

Gastric cancer accounts for a significant proportion of worldwide cancer-related morbidity and mortality. The well documented precancerous cascade provides an opportunity for clinicians to detect and treat gastric cancers at an endoscopically curable stage. In high prevalence regions such as Japan and Korea, this has led to the implementation of population screening programs. However, guidelines remain ambiguous in lower prevalence regions. In recent years, there have been many advances in the endoscopic diagnosis and treatment of early gastric cancer and precancerous lesions. More advanced endoscopic imaging has led to improved detection and characterization of gastric lesions as well as superior accuracy for delineation of margins prior to resection. In addition, promising early data on artificial intelligence in gastroscopy suggests a future role for this technology in maximizing the yield of advanced endoscopic imaging. Data on endoscopic resection (ER) are particularly robust in Japan and Korea, with high rates of curative ER and markedly reduced procedural morbidity. However, there is a shortage of data in other regions to support the applicability of protocols from these high prevalence countries. Future advances in endoscopic therapeutics will likely lead to further expansion of the current indications for ER, as both technology and proceduralist expertise continue to grow.

**Key Words:** Gastric cancer; Endoscopy; Endoscopic imaging; Endoscopic mucosal resection; Endoscopic submucosal dissection

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**Core Tip:** There have been numerous recent advances in the endoscopic detection, characterization and treatment of precancerous gastric lesions and early gastric cancers. Accumulating evidence suggests that endoscopic submucosal dissection results in at least equivalent disease-related outcomes with a marked reduction in morbidity compared to gastrectomy. Supportive data are robust in regions with high gastric cancer prevalence, however a paucity of western data results in inconsistencies in clinical practice in these regions. This article serves to review existing evidence regarding endoscopic imaging and therapeutics in gastric cancer, as well as identify future areas for research and development.

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## INTRODUCTION

Gastric cancer remains a leading cause of cancer-related morbidity and mortality, accounting for 780000 deaths and more than 1 million new diagnoses worldwide in 2020 alone[1,2]. Conventional surgical management with gastrectomy is associated with significant morbidity and mortality[3]. Countries with a high prevalence of gastric cancer have implemented systematic screening programs and demonstrated the benefit of early detection and endoscopic resection (ER) of precancerous gastric lesions and early gastric cancers (EGCs), offering curative treatment with considerably less morbidity[4,5]. Future challenges and opportunities remain, including the judicious surveillance of gastric metaplasia in lower prevalence communities, utilising improvements in endoscopic imaging and further refining existing ER techniques, while at all times accumulating high-quality collaborative data sets to inform and shape future management algorithms.

## ENDOSCOPIC DIAGNOSIS OF GASTRIC NEOPLASIA

### *Endoscopic gastric cancer screening*

Rates of gastric cancer in Eastern Asia (particularly Japan and Korea) are markedly higher than Western regions, which has resulted in implementation of population screening programs as an evidence based, cost effective measure for preventing gastric cancer-related mortality and morbidity[4-6]. Screening programs facilitate the detection of precancerous lesions and EGCs at an endoscopically resectable stage. Japanese guidelines suggest biennial or triennial endoscopic screening for those over the age of 50. This approach could prevent up to 63% of gastric cancer related mortality, resulting in 27.2 quality-adjusted life years gained *per* 1000 individuals[4]. In Korea, guidelines similarly suggest biennial screening but commencing at age 40. This is supported by data showing a 38% reduction in age-standardised mortality rate in the screening group at large, at a cost of less than the average gross expenditure product *per* capita[5].

In Western regions including North America and Australasia, the age-standardised incidence of gastric cancer is 6.5-8.8 *per* 100000 population, four-times lower than East Asia[6]. The lower incidence may be attributable to a combination of reduced *Helicobacter pylori* prevalence as well as environmental, lifestyle (diet, smoking) and genetic factors[6]. Given this comparatively low gastric cancer incidence, Western countries are yet to adopt population screening. Instead, guidelines in these regions are varied. The American Gastroenterology Association does not recommend population screening, only surveillance for patients with gastric intestinal metaplasia (GIM), while the British Society of Gastroenterology (BSG) recommends endoscopic screening in patients with multiple risk factors for gastric cancer (male, smoker, pernicious anaemia, family history in first degree relative), citing low-grade evidence[7,8]. Unsurprisingly, low disease prevalence combined with variable guidelines leads to

inconsistencies in clinical practice, as demonstrated in a 2016 survey of American endoscopists[9]. An additional challenge in these low prevalence regions is the management of individuals who have migrated from areas where gastric cancer incidence is high, as data show persistently elevated gastric cancer risk for at least two generations, yet the infrastructure and guidelines in terms of screening are not established[10,11]. These factors would undoubtedly contribute to the discrepancy in gastric cancer survival between Western and East Asian regions, highlighting the need for additional research in these populations[12].

### Progression of precancerous gastric lesions

Gastric cancer develops through a well-established precancerous cascade: From atrophic gastritis (AG) to GIM, low-grade dysplasia (LGD), high-grade dysplasia (HGD) and eventually carcinoma, with the likelihood of progression increasing as this cascade advances[13]. Although eradication of *Helicobacter Pylori* has been clearly demonstrated to reduce rates of development of this precancerous cascade, data have been more variable regarding prevention of progression from GIM, implying a 'point of no return' in the gastric cancer cascade[14-16]. These observations underpin the utility of screening and surveillance endoscopy, providing the opportunity to identify and treat curable lesions. In order to maximise yield, guidelines also suggest routine 'mapping' biopsies according to the updated Sydney protocol (including two biopsies taken from the antrum and one from each of the incisura, lesser curve and greater curve) in addition to biopsies of any suspicious areas during surveillance endoscopy, which results in improved AG and GIM detection compared to non-systematic biopsies[8,17].

Japanese data suggests an overall 5-year cumulative gastric cancer incidence of 1.9%-10% in AG and 5.3%-9.8% in GIM[18]. The Operative Link on Gastritis/Intestinal Metaplasia Assessment (OLGIM) scoring system further assists in differentiating between degrees of AG and GIM with regard to gastric cancer risk[19]. Those with high-risk lesions meeting OLGIM stage III/IV have an odds ratio for gastric cancer of 2.41 and 3.99 respectively[20]. Accordingly, guidelines suggest that patients with OLGIM stage III/IV AG or with GIM have 3-yearly surveillance endoscopy or 1- to 2-yearly endoscopy in the presence of a family history of gastric cancer[21]. Although these scoring systems are consistently reported in high-prevalence regions, the limited experience in lower prevalence regions again results in inconsistencies, as pathologists rarely report according to OLGIM staging and thus endoscopist adherence to GIM surveillance guidelines is variable[9].

In the context of biopsy-detected LGD and HGD, the risks of progression to gastric cancer are reportedly between 2.8%-11.5% and 10%-68.8% respectively; therefore guidelines suggest endoscopic removal of any defined lesion with dysplasia[22-26]. Importantly, lesions are also frequently upgraded histologically once endoscopically resected. In a 2015 study, 25% of lesions initially diagnosed as LGD were upgraded after ER; 16.7% to HGD and 6.9% to carcinoma[27]. Accordingly, gastric lesions with any degree of biopsy proven dysplasia should be regarded as potentially housing adenocarcinoma.

### Endoscopic imaging techniques

The endoscopic detection and characterisation of EGCs and precancerous lesions is therefore critical in establishing cancer risk and directing appropriate treatment and surveillance. Consensus guidelines from the BSG and the Japanese Gastroenterological Endoscopy Society (JGES) define endoscopic standards to maximise detection of gastric mucosal lesions, including a minimum 7 min gastric examination time, as well as adequate mucosal visualisation using sufficient air insufflation, mucosal cleaning techniques (mucolytic and defoaming agents) and consideration of peristalsis inhibiting medications if views are obstructed by movement[28,29]. While a consensus has not yet been reached regarding the role of advanced endoscopic imaging techniques, a number of recent advances in endoscopic imaging have demonstrated higher detection rates and more accurate characterisation of mucosal lesions.

**White light endoscopy:** White light endoscopy (WLE) is the conventional endoscopic imaging modality. It is sufficient for detecting some features of carcinomatous transformation of gastric lesions, including red discolouration of the mucosal surface, depressed-type lesions and mucosal ulceration[30,31]. However, the sensitivity for WLE in detecting GIM, LGD/HGD and EGC has been reported to be as low as 29%-59.1%, 51%-74% and 48%-72% respectively[32-36]. As a result, more advanced endoscopic imaging techniques are now used for lesion detection and characterisation.

**Chromoendoscopy:** Traditional chromoendoscopy requires the topical application of stains or dyes (usually methylene blue, indigo carmine and/or acetic acid) in an effort to enhance tissue characterisation[37,38]. Studies have demonstrated the superiority of chromoendoscopy in the detection of EGCs and precancerous gastric lesions, with a 2016 meta-analysis demonstrating pooled sensitivity of 90% and specificity of 82% [38]. However, the requirement for local dye application has limited its use in the context of the wide field required for gastric lesion detection, particularly following the development of virtual chromoendoscopy.

**Narrow-band imaging:** Narrow-band imaging (NBI) is the most commonly used form of virtual chromoendoscopy; utilising a rotating optical interference filter to restrict incident light into two narrow bands of different wavelengths (Figure 1). This enhances the definition of the surface mucosa, while emphasising the contrast of the vascular network[39]. NBI is superior to WLE in detection of early precancerous gastric lesions, with pooled sensitivity of 69% and specificity of 91% for GIM[40]. Prospective trials have demonstrated a 40% increase in detection of all focal gastric lesions and more than twice the detection rates for GIM using NBI compared to WLE [32,41].

**High-magnification NBI:** High-magnification endoscopy (Figure 2) uses a movable lens in the tip of the endoscope to allow up to 150 times optical zoom without any degradation of image quality[42]. This is usually combined with the use of a translucent cap to stabilize the focal length between the lens and the target tissue, as well as near-focus imaging that allows the endoscope to be positioned closer to the mucosal surface[42]. High-magnification endoscopy has been combined with NBI (ME-NBI) to facilitate detailed assessment of the mucosa and enhance lesion detection and characterisation.

Uedo *et al*[42] first described the 'light blue crest' seen on ME-NBI (Figure 2) which has been demonstrated to detect GIM with a sensitivity of 80%-89% and a specificity of 93%-96%[36,43,44]. In regard to characterisation of dysplasia, a prospective multicentre study in 2016 showed an improvement in sensitivity from 74% to 92% with the use of ME-NBI over WLE[36]. Detailed assessment of surface microvascular patterns on polypoid lesions using ME-NBI results in sensitivity of up to 86.2% and specificity of up to 97% for the presence of dysplasia[45].

In the assessment of EGCs, there have been a number of classification systems using ME-NBI. Yao *et al*[45] first described the 'VS' system in 2008, which has a sensitivity of 86%-97% for EGCs[46-49]. This system requires detailed assessment of the microvascular and surface mucosal pattern to ascertain features of irregularity, which in the presence of a demarcation line is suggestive of carcinoma[46]. The VS classification was further simplified by Yamada *et al*[49] in 2014, who demonstrated that the presence of a demarcation line with an irregular microvascular pattern was 95% sensitive and 96% specific for EGC[49].

The accuracy of ME-NBE was confirmed in a 2015 meta-analysis of a combined 2171 patients, demonstrating 86% sensitivity and 96% specificity for the diagnosis of EGC [50,51]. Direct comparator studies of ME-NBI *vs* WLE have also shown up to twice the rate of EGC detection with ME-NBI[52-54].

In addition, ME-NBI is valuable in determining tumour margins at the time of ER, resulting in 97.4%-98.1% accuracy of endoscopic markings[55-57]. Comparator studies have demonstrated the superior precision of ME-NBI *vs* chromoendoscopy in this context, with 17%-20% higher rates of accurate endoscopic markings[55,57]. The use of ME-NBI is also beneficial when lesion margins are unclear on chromoendoscopy, as demonstrated by Nagahama *et al*[57] who found that 72.6% of lesions with an unclear margin on chromoendoscopy were able to be clearly delineated using ME-NBI[57].

**Blue laser imaging:** Blue laser imaging (BLI) uses laser to create a highly narrowed blue band, enhancing vascular structures without using a filter as is required for NBI, thereby resulting in a higher light intensity[58,59]. There is a paucity of evidence regarding BLI in comparison to other endoscopic imaging, however recent studies have demonstrated superior sensitivity (93%-94%) in detection of EGCs compared to WLE (46%-50%)[60,61]. While direct comparator studies are limited, Kaneko *et al*[61] compared BLI and NBI in 39 patients with gastrointestinal neoplasia and found that BLI maintained sufficient brightness and contrast up to 40 mm while NBI deteriorated beyond 20 mm. This could be of relevance in detection of EGCs where endoscopists may benefit from increased field of view, however further studies in this area are required.

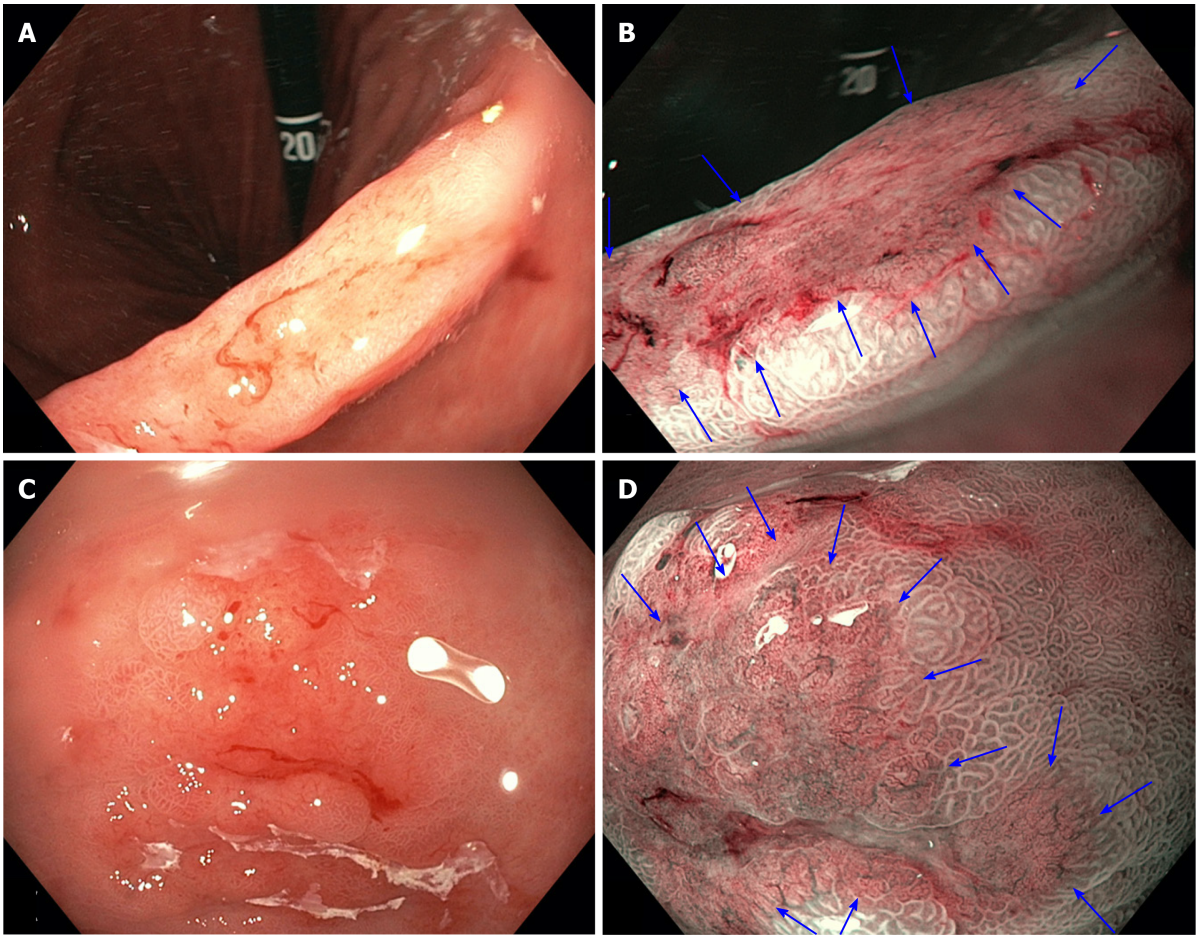


**Figure 1 White light endoscopy compared to narrow-band imaging in gastric lesions, demonstrating clear demarcation lines and irregular microvascular/microsurface patterns on narrow-band imaging.** A: Early gastric cancer at the incisura seen on white light endoscopy (WLE); B: The same lesion seen on narrow-band imaging (NBI) (blue arrows); C: Early gastric cancer in the antrum seen on WLE; D: The same lesion seen on NBI (blue arrows).

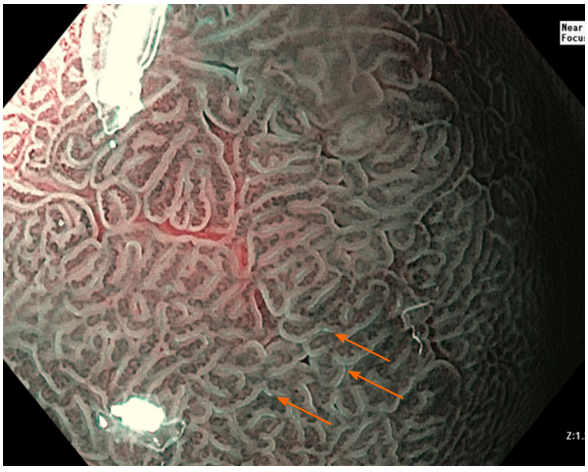

**Figure 2 Narrow-band imaging demonstrating the 'light blue crest' (orange arrows) consistent with intestinal metaplasia.**

**Confocal laser endomicroscopy:** Confocal laser endomicroscopy (CLE) uses a low-power laser to illuminate tissue, detecting reflected fluorescent light and allowing high-resolution endoscopic histological assessment[62,63]. CLE was first assessed in precancerous gastric lesions by Guo *et al*[63] who reported sensitivity and specificity of 98% and 95% for the detection of GIM[63]. A more recent meta-analysis demonstrated the accuracy of CLE for the diagnosis of all stages of precancerous gastric lesions and

EGCs, with pooled sensitivity of 92% for GIM, 81% for LGD/HGD and 91% for EGC [64,65]. However, its use is limited by the equipment required (either a dedicated endoscopic system or a probe-based system inserted *via* the therapeutic channel), the time required for image acquisition, as well as the learning curve of image interpretation[63].

### Future prospects

Artificial intelligence (AI) has demonstrated efficacy in detection and classification of multiple gastrointestinal lesions[66]. Real-time AI gastric lesion detection has not yet been studied, however convolutional neural networks have been generated from endoscopic images with a sensitivity of 92% using WLE and 97% using ME-NBI[67-69]. This could be of particular benefit for improving lesion detection and characterisation for endoscopists without expertise in advanced imaging interpretation.

Texture and colour enhancement imaging (TXI) is a recently developed technology aiming to improve lesion detection by enhancing texture, brightness and colour tone (Figure 3) using stacked images, while maintaining a similar colour spectrum to WLE [70]. By improving lesion detection within WLE, TXI may facilitate superior detection rates during endoscopic screening, although studies are required to investigate this.

Whilst WLE remains the mainstay for the majority of endoscopists, accumulating evidence as presented above points to a significant additive benefit in repeat interrogation of suspicious areas with chromoendoscopy. The trade-off between time, cost, availability and feasibility means that most proceduralists employ virtual chromoendoscopy with ME-NBI.

### Assessment of invasion depth and nodal metastases

**Endoscopic ultrasound:** While computed tomography (CT) and positron emission tomography/CT are endorsed by guidelines for the assessment of distant metastases and locally advanced gastric cancers, endoscopic ultrasound (EUS) has been recommended for EGCs, in particular for distinction of T1 and T2 lesions[71]. However, data on the utility of EUS in assessment of EGC depth of invasion have been varied. Han *et al*[72] reported under-staging in 16.7% of T2 gastric cancers with EUS, and Choi *et al*[71] reported no advantage of EUS over conventional endoscopic assessment using surface nodularity and fold convergence[71]. In comparison, Mouri *et al*[73] reported that lesions classified by EUS as being intramucosal or submucosal border lesions were histologically either intramucosal or invading < 500 μm into the submucosal space (SM1) in 99% and 87% of cases[73]. A 2015 Cochrane review also demonstrated 87% sensitivity for submucosal invasion[74,75]. Regarding specific EUS characteristics, Kim *et al*[76] reported that an arch-shaped submucosal deformity on EUS was associated with a negative predictive value for SM2 (> 500 μm submucosal invasion) or greater of 94.4%, with a sensitivity and specificity of 84% and 83%[76]. More recently, EUS miniature probes have been demonstrated to have higher resolution but less penetration compared to conventional EUS[77]. Miniature probes have demonstrated superiority for assessment of submucosal invasion, with an overall accuracy of 84.5% *vs* 61.6% using conventional EUS when assessing lesions less than 2 cm in diameter without endoscopic features of submucosal invasion[78]. While the role of conventional EUS in the confirmation of suitability for ER remains unclear, miniature probe EUS may have a role in < 2 cm lesions which are indeterminate endoscopically.

Concurrent to these improvements in EUS, the addition of technologies such as CT with multiplanar reformations and virtual gastroscopy has led to overall accuracy as high as 94% for the diagnosis of EGC[79]. In addition, high-speed magnetic resonance imaging (MRI) and diffusion-weighted imaging have also addressed many of the limitations of MRI for the assessment of T-stage, though this remains infrequently used in the assessment of EGCs[80,81]. While a detailed comparison of radiological methods for staging gastric cancers is beyond the scope of this review, improvements in these technologies may facilitate accurate non-invasive assessment of EGCs in the future.

With respect to exclusion of nodal disease prior to ER for EGC, the use of EUS has again been associated with variable accuracy. In a 2008 meta-analysis, the pooled sensitivity of EUS for N1 disease was as low as 58.2%[82]. More recent data have shown an improved sensitivity and specificity of 74%-83% and 67%-70% in detecting nodal positivity[75,83,84]. However, this improved accuracy still remains inadequate to guide consideration of endoscopic management of disease with metastatic potential. Despite technological advances, CT also remains inaccurate for the detection of nodal metastases, with sensitivity between 62.5%-91.9%[85]. Accordingly, gastric cancers

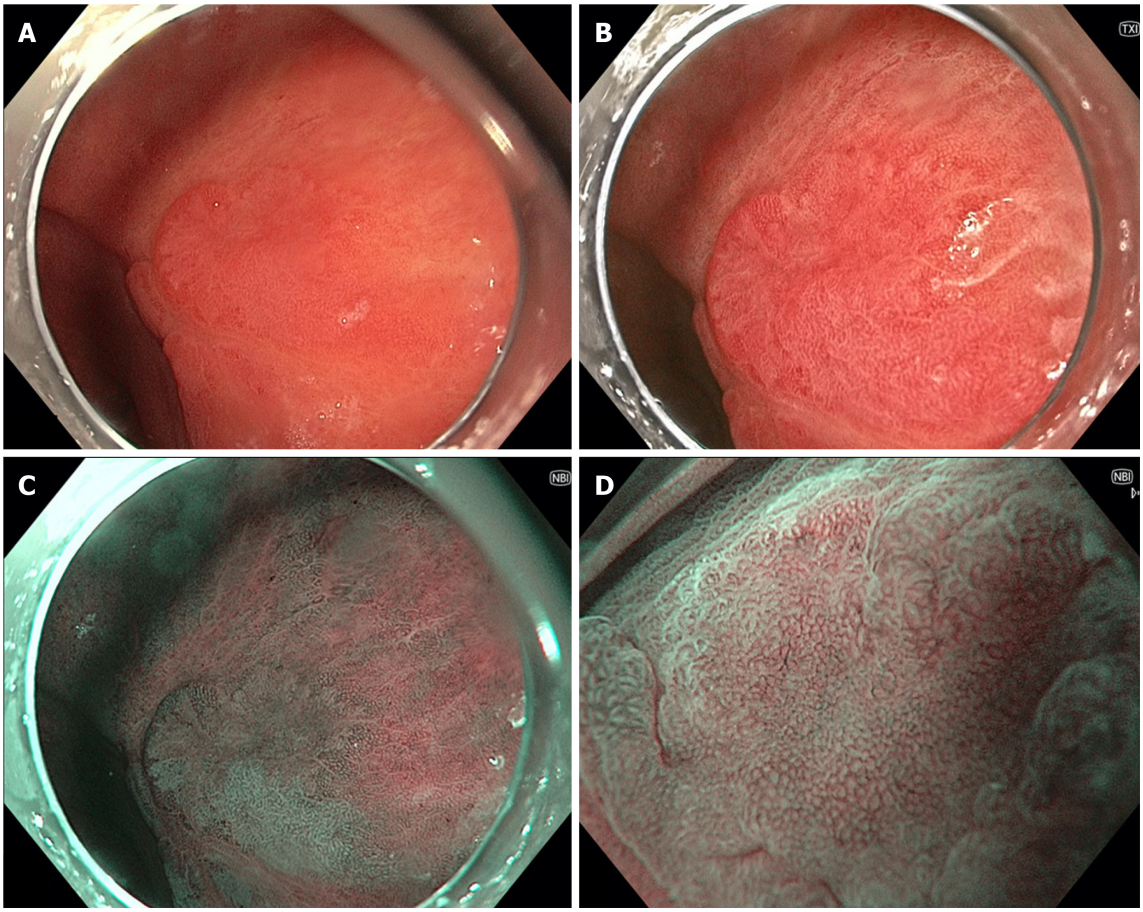


**Figure 3 Gastric body lesion with low-grade dysplasia seen on multiple forms of endoscopic imaging.** A: White light endoscopy; B: Texture and colour enhancement imaging; C: Narrow-band imaging (NBI); D: high-magnification NBI.

with a significant potential for lymph node metastasis based on lesion characteristics are generally managed surgically with lymphadenectomy to evaluate for lymphatic involvement, as reliable non-invasive exclusion of lymph node metastases (LNM) remains elusive.

## ENDOSCOPIC TREATMENT OF GASTRIC NEOPLASIA

ER is indicated for all discrete gastric lesions with histological evidence of dysplasia given the risk of concurrent or future EGC. In addition, data supports ER for lesions histologically indefinite for dysplasia, as the rate of true dysplasia or EGC in resected specimens is as high as 90.8%, particularly in males; lesions > 5 mm in diameter; or lesions with erosions[86]. For gastric mucosal lesions < 1 cm in diameter with LGD, either endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD) can be performed, however ESD is preferred in larger lesions and those demonstrating HGD/EGC[87].

### *Indications for ESD*

In the context of histologically confirmed carcinoma, ESD was initially indicated only for the resection of macroscopically intramucosal (T1a) differentiated gastric carcinomas < 2 cm in diameter without ulcer or scar[87]. These have subsequently been labelled 'absolute criteria', as data now support the efficacy of ESD for the resection of a broader range of EGCs termed 'expanded criteria'. In the 2015 JGES guidelines, expanded criteria lesions include all differentiated T1a EGCs > 2 cm without ulcer/scar, differentiated T1a lesions < 3 cm with ulcer/scar, and undifferentiated EGCs < 2 cm in diameter, while the 2015 European Society of Gastrointestinal Endoscopy (ESGE) guidelines also include differentiated lesions < 3 cm with superficial submucosal invasion (SM1 ≤ 500 μm)[87,88].

Another evolving potential indication for ESD is for the resection of submucosal gastric tumours. He *et al*[88] first demonstrated the efficacy and safety of ESD for gastrointestinal stromal tumours (GIST) in 2013, when they reported successful ESD in 25 large gastric GISTs with a mean diameter of 2.7 cm[88]. More recently, An *et al*[89] published data on 168 cases of ESD for gastric GISTs, with complete and en bloc resection in 100% of lesions[89]. Delayed bleeding occurred in only 1.2% and there were no other significant complications. While additional data are required before recommending ESD as a standard of care option for GIST management, early data support this as a potential future indication.

### *Efficacy of ESD*

Data on the efficacy of ESD (Table 1) are particularly robust in East Asian countries where the incidence of gastric cancer is significantly higher than it is in Western countries. Studies in Japan and South Korea have demonstrated en bloc resection in 95.3%-99.2%, complete resection in 87.7%-95.5% and curative resection in 81.7%-84.1% [90-94]. In Western countries however, data are more limited and results are variable. En bloc resection rates are between 92%-97.8%, with complete resection in 75.6%-89% and curative resection in 72.2%-79.2%[95-100].

One contributing factor to this discrepancy is the differing incidence of gastric cancer, resulting in comparatively limited experience in Western centres. This was supported by a 2020 Italian survey where only 41% of included interventionalists had performed more than 80 ESDs and 31% had performed < 40[98]. This study also demonstrated that rates of en bloc resection were higher and rates of perforation lower in more experienced proceduralists. However, there are multiple other contributing factors. Firstly, there are differences in lesion classification, as the Japanese classification of gastric carcinoma includes EGCs which would by European guidelines be classified as HGD[101]. The non-aggressive and non-invasive nature of HGD would result in bias in favour of Japanese outcomes. Further to this, there appear to be differences in disease behaviour in East Asian compared to Western populations. Studies in Korea reviewing rates of LNM in surgical specimens at gastrectomy have reported LNM in 0.3% of absolute criteria lesions and 0.4% in expanded criteria, while marginally higher at 3.2% in undifferentiated carcinomas[102-104]. In contrast, American studies report LNM in 7.5%-13.6% of expanded criteria lesions at gastrectomy[105,106]. Guidelines developed based on data from East Asian populations should therefore be used with caution in Western populations until further evidence is able to clarify this.

**EMR *vs* ESD:** ESD is associated with longer procedure times and an almost three times higher perforation rate compared to EMR, however multiple meta-analyses have established the superiority of ESD over EMR in regard to en bloc resection rates (OR 9-10 for ESD), complete resection rates (OR 5.7-8.4) and curative resection rates (OR 2.9 [38,107,108]). Tao *et al*[107] also reported a reduction in local recurrence (OR 0.18), likely resulting from the aforementioned superior rates of en bloc, complete and curative resection[107].

**ESD *vs* surgery:** Increasing evidence suggests that ESD is associated with equivalent overall survival compared to gastrectomy, while reducing procedural complications. A 2015 retrospective Chinese study used propensity score matching to compare outcomes for surgery and ESD in 176 patients with a median follow up of 77 mo[109]. There was no significant difference in overall survival, and while local recurrence occurred in 1.7% of ESD, all but one of the recurrent lesions were treated endoscopically. There was no difference in early complication rates, but late complications were significantly more common in the surgical group (6.8% *vs* 0%). A similar 2017 Korean study found no difference in 5-year survival between groups, with a complication rate of 15% in gastrectomy *vs* 5.1% in ESD[110]. In regard to expanded criteria lesions, data have also reported equivalent survival with a reduction in complications using ESD, with a 2017 study actually demonstrating improved 5-year survival after propensity score matching in the ESD group (97.1% *vs* 85.8%)[109,111,112]. Unsurprisingly, a recent meta-analysis reported higher rates of local recurrence and metachronous cancer in the ESD group, resulting in lower disease-free survival (HR 4.58)[113]. However, the majority of recurrence is able to be treated with repeat ESD and thus lower disease-free survival is not reflected in any difference in disease-specific survival [109,113]. Further to this, the same meta-analysis reported a mean 128 min shorter operation time, 7 d shorter hospital stay, lower procedure-related death and fewer complications[113].

**Table 1 Studies reporting *en bloc*, complete and curative resection rates using endoscopic submucosal dissection**

| Ref. | Location | Total lesions | *En bloc* resection | Complete resection | Curative resection |
|---|---|---|---|---|---|
| Suzuki *et al*[90], 2019 | Japan | 10821 | 99.2% | 91.6% | 81.7% |
| Ryu *et al*[91], 2018 | Korea | 1541 | 97.3% | 95.5% | N/A |
| Chung *et al*[92], 2009 | Korea | 1000 | 95.3% | 87.7% | N/A |
| Watanabe *et al*[93], 2017 | Japan | 511 | 97.4% | 92.9% | 84.1% |
| Ngamruengphong *et al*[94], 2020 | North America | 347 | 92% | 82% | N/A |
| Manta *et al*[95], 2020 | Italy | 299 | 97.6% | 89% | 72.5% |
| Abdelrahim *et al*[96], 2019 | Europe | 175 | 92.5% | 83.4% | N/A |
| Tate *et al*[99], 2019 | Australia | 135 | 94.8% | 86.7% | 79.2% |
| Pagano *et al*[98], 2019 | Italy | 41 | 97.8% | 75.6% | 72.2% |

N/A: No application.

In regard to complications of surgical management, gastrectomy is associated with significant post-operative complication rates of 9.6%-18.1% for laparoscopic and 17.4%-29.3% for open gastrectomy, as well as a 30-d mortality rate of 2%-4.1%[3,114-116]. In addition, Yu *et al*[116] assessed quality of life scores after gastrectomy and found that fatigue, diarrhoea, reflux, dysphagia and eating restriction scores all remained persistently worse at 5-year follow-up after surgery[116].

A 2015 study demonstrated the lower overall medical costs of ESD compared to surgery for EGC, with ESD costing a median $2374 USD compared to $4954 USD for surgery ($P < 0.001$)[117,118]. This was confirmed by Shin *et al*[109] who reported approximate total hospital stay costs averaging $1871 USD for ESD *vs* $5925 USD for subtotal gastrectomy and $6476 for total gastrectomy[109].

**Long-term outcomes:** Long-term data on ESD for EGCs has been extensively reported in Japan and South Korea, where studies including up to 5-9 years of follow-up have documented local recurrence rates of 0%-1.8% in absolute criteria lesions and 0.6%-7.0% in expanded criteria lesions[119-123]. In these same cohorts there were no cases of LNM in absolute criteria lesions, and LNM rates were between 0%-0.48% in expanded criteria lesions. Min *et al*[123] reported long-term outcomes (48 mo follow-up) for only lesions meeting criteria for curative resection, in whom 0.3% of absolute and no expanded criteria lesions had local recurrence[123]. In a study by Suzuki *et al*[124] in 2015 (again reporting only on curative resections), despite overall 5-year overall survival being as low as 92.2% due to other comorbidities, the 5-year disease-specific survival was 99.9% in both absolute and expanded criteria lesions[124]. In Western populations, long-term data are limited, but studies have reported local recurrence rates of 4.8%-7% for expanded criteria lesions[125-127].

**ESD in 'outside of criteria' lesions:** ESD is often also employed for the treatment of lesions outside of even the expanded criteria when patients are unsuitable for major surgery. Abe *et al*[127] followed 14 patients who had ESD for undifferentiated non-curatively resected lesions who were not suitable for or had declined further surgery [127]. Over a median of 76.4 mo follow-up, there was only one case of local recurrence which was managed with repeat ESD. A 2013 Japanese study reviewed 104 patients who undergone ESD despite being outside of criteria in regard to lesion characteristics, who had declined or been deemed unsuitable for surgical management[128,129]. Patients were followed-up for a median of 47 mo after resection, with 5-year overall survival of 70% but disease-specific survival of 91.5%. In patients with comorbidities precluding them from major surgery, ESD may therefore be beneficial even in lesions outside of the expanded criteria.

### *Approach to incomplete and non-curative resection*

**Incomplete resection:** The limited available evidence supports repeat ESD where possible for lesions with positive horizontal margins after initial resection. Jung *et al*[129] reported 28 patients with a positive horizontal margin after initial ESD, in whom the curative resection rate of repeat ESD was 89.3%[129]. In a 2018 study, Jeon *et al*[130] also reported improved disease-free survival following repeat ESD for non-

curative resections due to positive lateral margins (89.2% *vs* 69.1%)[130].

**Non-curative resection:** The management of lesions determined to be beyond the expanded criteria histologically after ER (non-curative resection) remains controversial. A large 2019 meta-analysis demonstrated improved 5-year overall and disease-specific survival (OR 3.5 and 3.99 respectively) following additional surgery after non-curative resection[131,132]. However, these data are retrospective and are therefore susceptible to bias associated with the selection of less systemically unwell and comorbid patients in the additional surgery group. Other studies have reported rates of LNM or residual tumour as low as 5.1%, with differences in overall survival but no difference in disease-specific survival between those treated with additional gastrectomy and those managed with surveillance[133,134]. These studies have hypothesised that further data may delineate a group in whom surveillance after non-curative resection is associated with equivalent long-term outcomes.

Multiple studies have addressed factors to stratify the risk of LNM or residual tumour in patients who have had non-curative ESD. In regard to LNM, lymphovascular invasion (LVI) and depth of submucosal invasion histologically are reported to be the most consistent predictors[135,136]. Goto *et al*[134] reported that the presence of depth of invasion > SM1 or LVI was 100% sensitive and 86% specific for the presence of LNM[135]. For predicting the presence of residual tumour, a 2020 meta-analysis reported significant risk factors to be tumour size > 30 mm and the presence of positive horizontal margins[137]. Although additional data are required to guide decision making, patients who have non-curative resections may not warrant additional surgery provided the invasion depth is ≤ SM1 without evidence of LVI, while patients with tumour size > 30 mm or positive horizontal margin may be appropriate for repeat ESD.

This concept led to a 2017 study by Hatta *et al*[137], who developed the eCura scoring system for predicting the risk of LNM using data from 1101 patients who underwent radical surgery after non-curative ESD[137]. The presence of a positive vertical margin, submucosal invasion > SM1, tumour size > 30 mm and vascular invasion were each assigned 1 point, while lymphatic invasion was assigned 3 points. Patients scoring 0-1 points were classified as low-risk (2.5% risk of LNM), 2-4 points intermediate-risk (6.7% risk of LNM) and 5-7 points high-risk (22.7% risk of LNM). The scoring system was then validated on 905 patients with non-curative ESD who declined surgery. 5-year cancer-specific survival was 99.6% in the low-risk group (60.4% of patients), 96% in the intermediate risk group (27.6% of patients) and 90% in the high-risk group (11.9% of patients), suggesting that ESD without additional surgical management may be sufficient in low-risk patients according to the eCura system[138].

These risk stratification tools are of particular importance when considering patients with radiologically enlarged lymph nodes that may or may not signify the presence of LNM. Lee *et al*[138] reported 47 cases of ESD for expanded criteria lesions, where patients had at least one enlarged lymph node on CT[138]. 12 of 47 patients had surgical resection, with no evidence of metastatic disease, while the remaining 35 patients had serial CT monitoring over a median 56 mo. Of these 35 patients, 21 patients had reduction in size or complete resolution of lymphadenopathy, while 13 patients had no change. Only one case had progressive lymph node enlargement but declined biopsy due to age. Assessment of the risk of LNM may therefore help direct decision making regarding the need for additional surgery in the context of radiologically enlarged lymph nodes.

In patients at risk of LNM, future studies may explore the role of ER with additional laparoscopic lymph node resection. A 2011 study by Cho *et al*[139] described 9 patients who had endoscopic full thickness resection (FTR) with laparoscopic regional lymph node resection[139]. Despite 4 lesions having histological LVI, there were no cases of LNM. There may also be a future role for targeted lymph node resection using sentinel node detection strategies. While data have been inconsistent regarding the accuracy of gastric sentinel node detection, these strategies have been most successful in T1 EGCs and thus may be of use following higher-risk ESD[140,141]. Studies using dye have reported a sensitivity of only 75%, however in the context of dual tracer with radiolabelled tin colloid and blue dye, the sensitivity for LNM is up to 93%, with an overall accuracy of 99%[142]. Further studies are therefore required to assess the adequacy of sentinel node guided laparoscopic lymph node resection as a treatment strategy after non-curative ESD.

### *Complications of ESD*

While ESD offers the promise of reduced morbidity, both acute (bleeding, perforation)

and chronic (stenosis, recurrence, metachronous lesions) complications can occur[107,108,113].

**Bleeding:** The risk of delayed bleeding with ESD is equivalent to that in EMR, with most studies reporting rates between 4% and 6%[143-146]. Nam *et al*[145] retrospectively reviewed 1,864 cases of ESD, in which post-procedural bleeding occurred in 4.1% of patients, with the majority occurring within 24 h of ESD[145]. In their multivariate analysis, predictors of delayed bleeding were patient age ≤ 65 years, resection size > 30 mm, procedure time > 20 min, lesions located in the lower third of the stomach and the presence of erosions. In regard to treatment, delayed bleeding after ESD is generally amenable to routine endoscopic management[146].

Studies have addressed prevention strategies for post-ESD bleeding, with data supporting post-operative proton-pump inhibitor (PPI) use after ESD with an OR of 0.4-0.49 for delayed bleeding[147,148]. A 2016 meta-analysis on the use of PPI prior to ESD found a reduction in gastric pH at the time of procedure, but no pooled difference in delayed bleeding[149]. Previously, routine re-look endoscopies were performed in many centres after ESD in an attempt to prevent rebleeding; a method extrapolated from data supporting second endoscopy after treatment of bleeding peptic ulcers [150]. However, Goto *et al*[144] reported no difference in rates of bleeding before or after routine follow-up endoscopy within one week of initial ESD, suggesting limited benefit from this strategy[144].

**Perforation:** Perforation occurs more frequently in ESD than in EMR[107,108]. Rates of perforation are generally reported between 1.5% and 9.6%, with a large meta-analysis by Arezzo *et al*[150] demonstrating a pooled perforation rate of 4.9%[95,100,151-156]. Risk factors for perforation include lesions located in the upper third of the stomach, the presence of submucosal invasion or fibrosis and longer procedure times[152,153,157]. Longer procedure times may reflect either more complicated resections or less proceduralist experience, while perforations in upper third gastric lesions likely reflect thinner proximal gastric wall in comparison to the antrum[158].

When macroscopic perforations occur during ESD, more than 97% of cases are able to be treated endoscopically with clip closure[155,156]. Micro-perforations, or suspected perforations that are not endoscopically visible, are usually able to be conservatively managed with fasting and intravenous antibiotics[155].

Delayed perforation, when no intraprocedural perforation occurs but symptoms of peritonism and radiological evidence of perforation develop post-procedure, is a phenomenon that appears to be specific to ESD. It is most common in the thin upper third of the stomach and the majority of cases occur within 24 h[159,160]. Yamamoto *et al*[158] reported 5 cases of delayed perforation in a 2017 case series, of which 4 out of 5 were able to be managed endoscopically[158].

**Stenosis:** Post-ESD stenosis occurs most commonly following resection of gastric antral (occurring in up to 7% of cases) or cardiac lesions, presenting with either oesophageal or gastric outlet obstruction[161,162]. Sumiyoshi *et al*[160] analysed predisposing factors, with the only risk factor after multivariate analysis being a > 75% circumferential post-resection defect[160]. In regard to treatment, while data is limited, most patients have improvement in their symptoms following either balloon dilation if severe, or conservative management if mild-moderate symptoms[162,163].

**Local recurrence and metachronous gastric lesions:** Guidelines recommend close ongoing surveillance after ESD for EGC, with ESGE and JGES guidelines suggesting 3- to 6-monthly and 6- to 12-monthly surveillance endoscopy respectively[87,88]. The aim of surveillance after ESD is for early detection of not only local recurrence but also metachronous precancerous gastric lesions. These lesions occur in up to 6.9%-13% of patients after ESD, with a 2019 study reporting a cumulative incidence of metachronous gastric cancer of 33.2 cases *per* 1000 patient-years[95,164,165]. In regard to treatment of metachronous lesions, Kim *et al*[165] reported 117 cases of metachronous gastric neoplasms, of which 77% were able to be retreated with curative ESD[165].

## FUTURE DIRECTIONS FOR THE ENDOSCOPIC TREATMENT OF GASTRIC NEOPLASIA

### *Methods for generating counter-traction*

Generating counter-traction during ESD is of benefit in reducing procedural time and

complications[166,167]. Depending on the position of the lesion being resected, gravity may not provide sufficient access to submucosal tissue planes. There have been a number of recent developments with regard to endoscopic equipment and techniques to generate counter-traction.

**Magnetic anchor guided ESD**: Magnetic anchor guided ESD uses a large external electromagnet combined with an internal magnet attached to the lesion *via* a clip (Figure 4). No comparative studies have assessed the advantages of this technique over conventional ESD, however studies have shown feasibility as well as subjective benefit from the point of view of endoscopists[168,169]. The main limitations of this technique are the equipment required and the coupling strength of the magnets relative to the abdominal wall thickness[170].

**Dual channel endoscope**: This technique requires a dedicated dual working channel endoscope, often employed for the 'grasp-and-snare' technique in EMR[171]. Hua *et al* [171] compared conventional ESD (24 patients) with dual channel ESD (22 patients), using one channel for dissection while grasping the lesion *via* the second channel[171]. Mean procedure times were significantly shorter with dual channel ESD (20.5 min *vs* 49.1 min) with no other differences in outcomes or complications. There are two main limitations of dual channel endoscopy: The requirement for a dedicated endoscope and the limited distance between working channels inhibiting angulation of traction.

**Additional working channel**: The use of an attachable additional working channel (Figure 5) aims to overcome some of these limitations of dual channel endoscopy by providing greater distance between working channels as well as allowing the use of a conventional therapeutic endoscope. The additional working channel consists of a flexible attachment, a long shaft and an adaptor for fixation at the endoscope handle [172,173]. This technique has been compared with conventional ESD in porcine stomachs, where it resulted in a reduction in procedure time (24.5 min *vs* 32.5 min) and lower rates of muscularis damage (3.13% *vs* 18.75%)[173]. However, minimal data exist in humans apart from feasibility studies reporting successful resection of 8 lesions by ESD and 6 lesions by EMR[174,175]. The main limitation of this method is the inability to generate significant angulation on the grasping forceps, preventing the most effective direction of counter-traction.

**Double endoscopes**: Using two separate endoscopes allows maximal angulation for the grasping forceps, optimising counter-traction. Generally, the first endoscope is inserted and a circumferential incision is made. Following this, a second smaller calibre endoscope is inserted to grasp and lift the edge of the lesion while the proceduralist proceeds with dissection. In 2017, Ogata *et al*[175] demonstrated the efficacy of this method in 122 patients, with a 97.5% en bloc resection rate and 86.9% curative resection rate, with perforation and delayed bleeding in 3.3% and 2.5% respectively [175]. While there are no direct comparator trials with conventional ESD, Çolak *et al* [176] employed this method in 6 patients where positioning made conventional ESD difficult[176]. They reported no difference in resection times compared to more straightforward ESDs. There are a number of obvious limitations to this method, including the requirement for two endoscopists, endoscopes and light sources, as well as space limitations in both the oral cavity and within the lumen where one endoscope can obstruct the view of the other[176]. Other groups have reported various methods to overcome some of these limitations. Higuchi *et al*[177] reported double endoscope ESD using a single light source, where the light source from the first endoscope is removed and attached to the second endoscope for insertion until the lesion is grasped [177]. Following this, the light source is reattached to the initial main endoscope. This technique resulted in improved accuracy of dissection compared to historical controls, without any serious intraprocedural complications. Alternatively, Ahn *et al*[178] employed a trans-nasal endoscope for applying traction, maximising space within the oral cavity and within the gastrointestinal tract due to the smaller calibre endoscope [178].

**Endo-lifter**: The 'endo-lifter' (Figure 6) consists of a transparent hood with grasping forceps, mounted on the tip of an endoscope, with the forceps running externally to the endoscope to allow dissection *via* the working channel[179]. The attachment of the forceps to the hood produces an arc that aims to provide superior angulation for countertraction. Minimal data exist using the endo-lifter in animal models, with variable results. Schölvinck *et al*[180] reported shortened procedure times when used by ESD-experienced but not inexperienced endoscopists[180]. Teo *et al*[179] reported increased visualisation of the submucosa and a reduction in subjective procedural

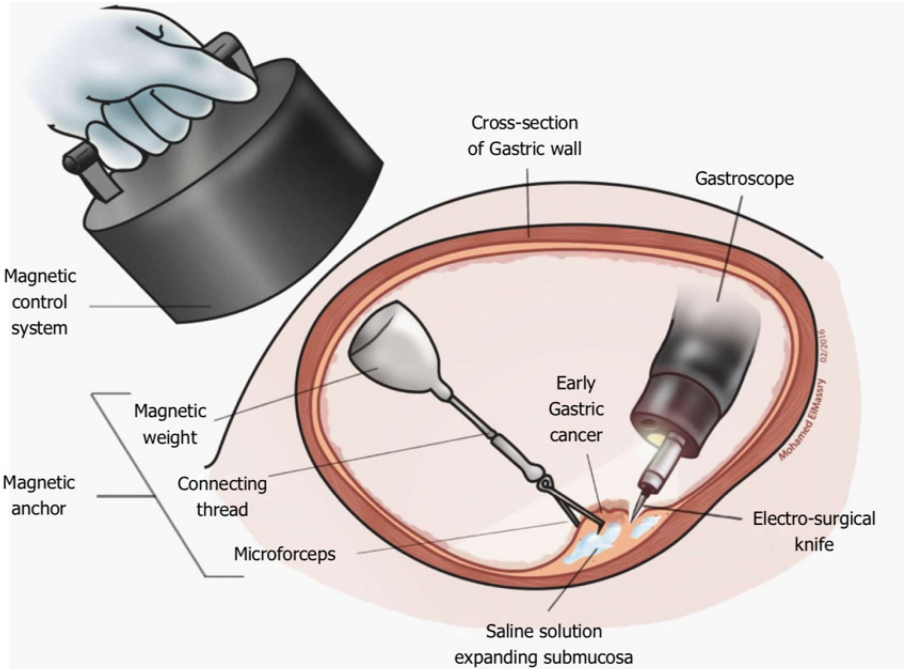


**Figure 4 Magnetic anchor-guided endoscopic submucosal dissection[169].** Citation: Mortagy M, Mehta N, Parsi MA, Abe S, Stevens T, Vargo JJ, Saito Y, Bhatt A. Magnetic anchor guidance for endoscopic submucosal dissection and other endoscopic procedures. *World J Gastroenterol* 2017; 23: 2883-2890. ©The Author(s) 2017. Published by Baishideng Publishing Group Inc.

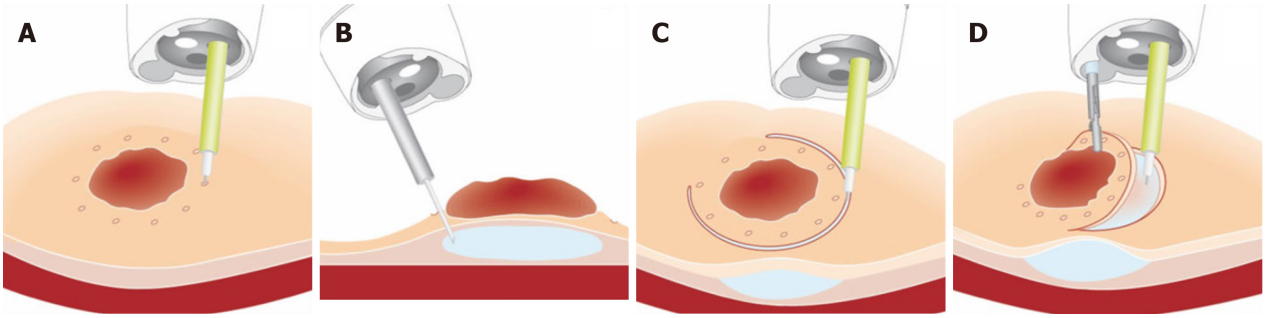


**Figure 5 Endoscopic submucosal dissection using an additional working channel[172].** A: Lesion marked *via* usual working channel; B: Submucosal injection; C: Near-circumferential incision made; D: Lesion grasped for countertraction *via* additional working channel while dissection underway. Citation: Knoop RF, Wedi E, Petzold G, Bremer SCB, Amanzada A, Ellenrieder V, Neesse A, Kunsch S. Endoscopic submucosal dissection with an additional working channel (ESD+): a novel technique to improve procedure time and safety of ESD. *Surg Endosc* 2021; 35: 3506-3512. ©The Author(s) 2021. Published by Springer Open Access Article.

difficulty rating from endoscopists[179].

**Spring and loop clip traction (using an S-O clip)**: This method uses the S-O clip (Figure 7) which is attached to a spring and then a loop of nylon[181,182]. One clip is attached to the edge of the lesion, while the other clip is attached to an area of opposing gastric wall to provide traction. Nagata[181] reported data from 140 patients of which 51 had spring and loop clip traction, with shorter procedure times and no difference in complication rates compared to conventional ESD[181]. The main limitations of this technique are the requirement for specific equipment (*i.e.*, the S-O clip) as well as only allowing one direction of traction once the clips are deployed.

**Other clip methods**: Various methods have been described employing readily available endoscopic equipment with clips to minimise cost. Yoshida *et al*[182] reported the efficacy of the 'dental floss clip' (DFC) traction device using dental floss tied to the proximal end of a clip before attaching the clip to the edge of the lesion, with the dental floss running externally to the endoscope[182]. There was no difference in procedure time overall, however in upper and middle greater curvature lesions DFC traction reduced procedure time by approximately 50%. In addition, the

**Figure 6 'Endo-lifter' (Olympus-Tokyo, Japan)[192].** Citation: Harlow C, Sivananthan A, Ayaru L, Patel K, Darzi A, Patel N. Endoscopic submucosal dissection: an update on tools and accessories. *Ther Adv Gastrointest Endosc* 2020; 13: 2631774520957220. ©The Author(s) 2020. Published by Open Access Article.

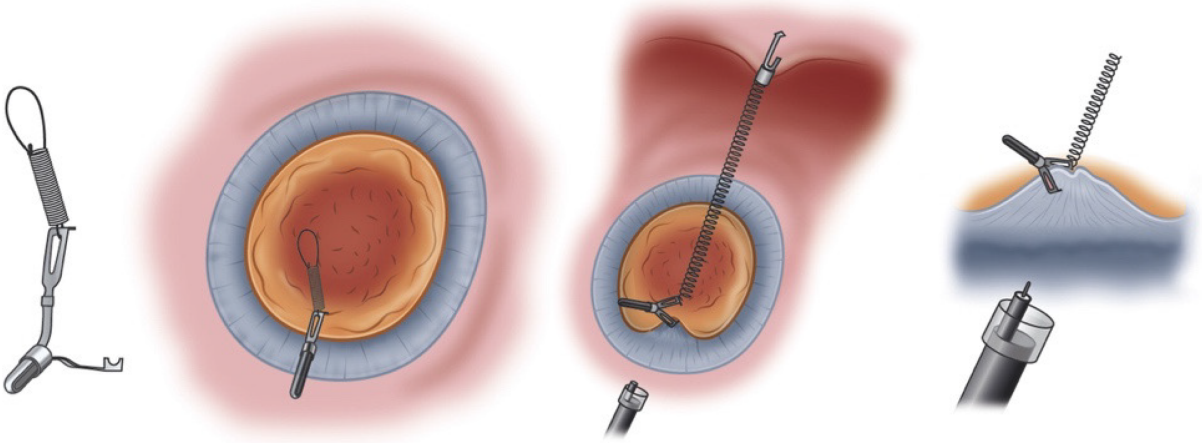

**Figure 7 Spring and loop clip traction[193].** Citation: Nagata M, Fujikawa T, Munakata H. Comparing a conventional and a spring-and-loop with clip traction method of endoscopic submucosal dissection for superficial gastric neoplasms: a randomized controlled trial (with videos). *Gastrointest Endosc* 2021; 93: 1097-1109. ©The Author(s) 2021. Published by Open Access Article.

use of DFC traction reduced perforation rates compared to conventional ESD (0.3% *vs* 2.2%)[183]. Noda *et al*[183] added a polypectomy snare sheath external to the endoscope through which the thread (dental floss) was passed, which was then inserted along with the endoscope. The use of the sheath allows the thread to be moved without interference from the endoscope, as well as allowing both 'pull' and 'push' motions by advancing the entire sheath. In an 88 patient study comparing polypectomy snare sheath ESD with conventional ESD, this technique resulted in a reduction in procedure time and lower rates of significant bleeding[183]. Yoshida *et al*[184] also reported a reduction in procedure time by simplifying this technique to their 'clip and snare' technique: A snare with its sheath external to the endoscope is attached to the proximal end of a clip, then inserted and attached to the lesion[184]. Zhang *et al*[185] then modified the clip and snare technique (Figure 8), by using additional clips to attach the snare to the circumference of the lesion, allowing multi-focal lifting of the lesion, as well as placing clips attached to the snare on opposing gastric wall mucosa to alter the direction of traction[185].

### *Endoscopic full-thickness resection*

FTR can either be performed free-hand, attempting to maintain the serosa although often resulting in macroscopic perforation, or device assisted (Figure 9) using an 'over-the-scope' clip[186,187]. While some data exist regarding the use of FTR for gastric GIST where successful resection rates are as high as 96.8%, there is no evidence

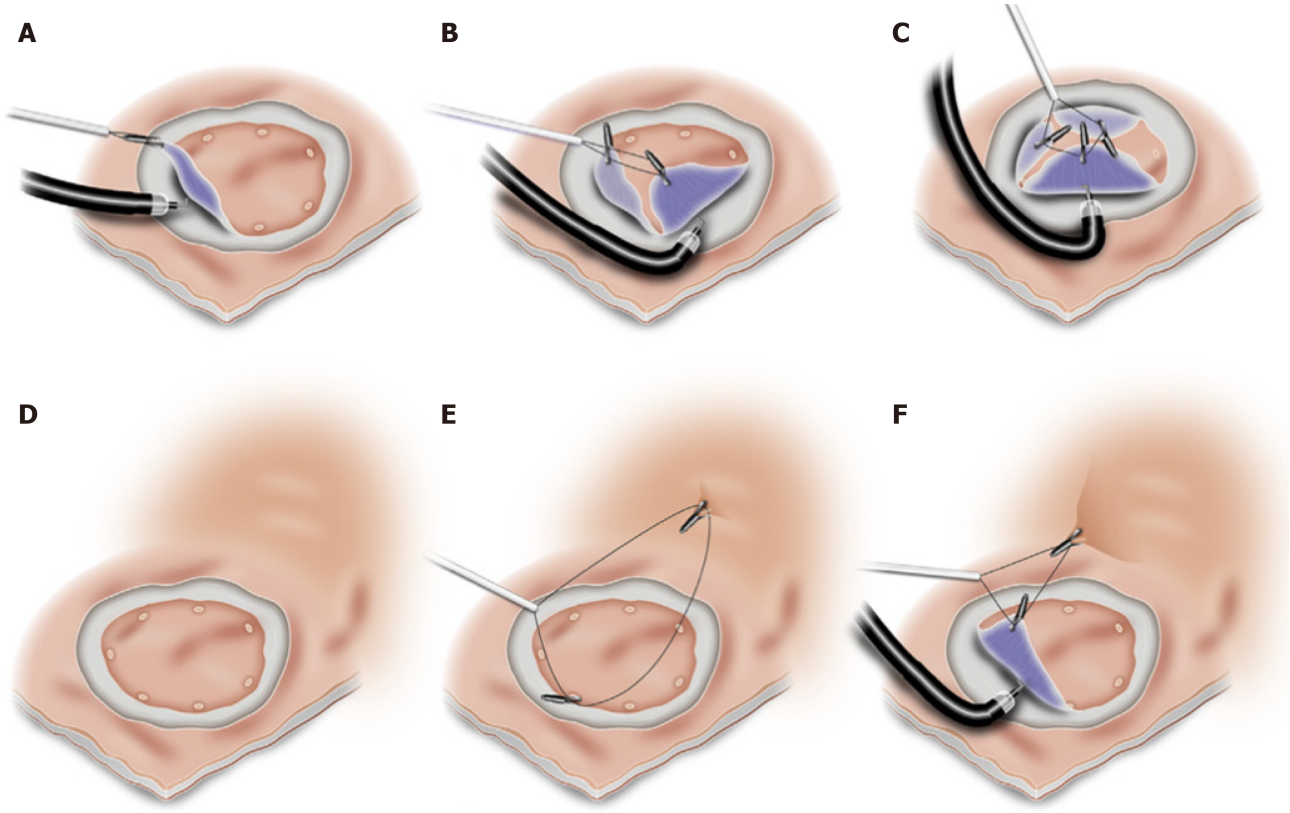


**Figure 8 Modified endo-clip and snare[185].** A-C: Multiple clips used to provide multifocal traction; D-F: Clip applied to opposing gastric wall for countertraction. Citation: Zhang Q, Yao X, Wang Z. A modified method of endoclip-and-snare to assist in endoscopic submucosal dissection with mucosal traction in the upper GI tract. *VideoGIE* 2018; 3: 137-141. ©The Author(s) 2018. Published by Open Access Article.

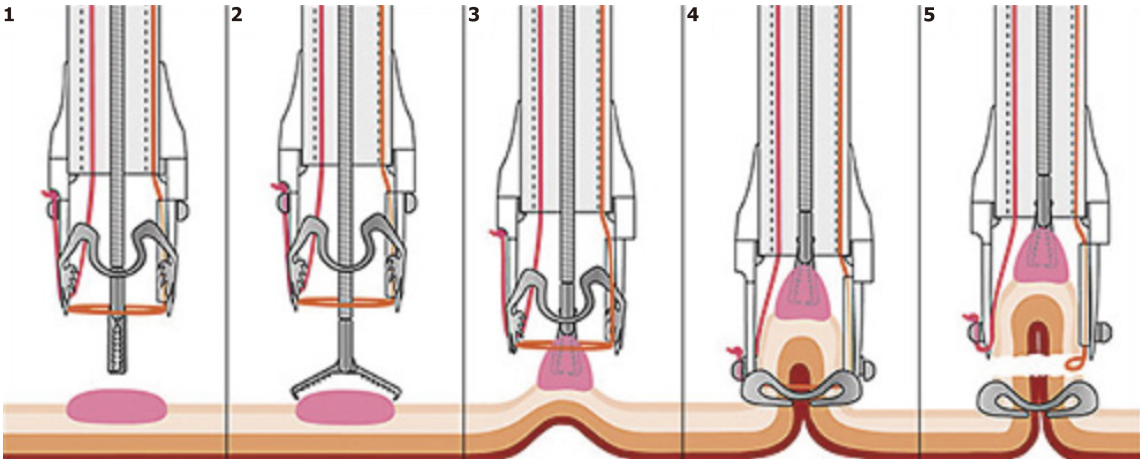


**Figure 9 'Over-the-scope clip' (Ovesco, Germany) full-thickness resection[194].** Citation: Mão de-Ferro S, Castela J, Pereira D, Chaves P, Dias Pereira A. Endoscopic Full-Thickness Resection of Colorectal Lesions with the New FTRD System: Single-Center Experience. *GE Port J Gastroenterol* 2019; 26: 235-241. ©The Author(s) 2019. Published by Open Access Article.

regarding the use of FTR in EGCs[188]. Theoretical roles for gastric FTR could include treatment of recurrent lesions within EMR/ESD scar limiting repeat resection, or using FTR in combination with laparoscopic nodal resection in non-candidates for gastrectomy with lesions outside of expanded criteria for ESD. Chae *et al*[188] reported a successful case of FTR for an EGC with a positive horizontal margin after initial ESD which had resulted in severe fibrosis[188]. Cho *et al*[138] reported a series of 9 patients treated with FTR and laparoscopic lymph node resection who had lesions outside of expanded criteria, in whom lymph nodes were positive in only 1 case[138].

### *Robot-assisted ESD*

The 'Master And Slave Transluminal Endoscopic Robot' (MASTER) was developed in an attempt to mitigate the technical difficulties commonly encountered in interventional endoscopy[189-191]. Initial feasibility studies were performed in 2010 in ex-vivo and in-vivo porcine stomachs, where 20 gastric lesions were successfully resected using MASTER assisted ESD with no difference in procedure time compared to conventional ESD[190]. Subsequently, a small case series was reported in 2012, using MASTER assisted ESD in 5 patients, demonstrating clear resection margins in all cases with no major complications[192]. More studies are required to further explore the role of robot-assisted ESD, however this technique will clearly be limited by the cost and availability of equipment, while proceduralists already successfully perform more conventional ESD techniques at a fraction of the cost.

## CONCLUSION

Advances in endoscopic imaging and therapeutics have enhanced the detection, characterisation and treatment of precancerous gastric lesions and EGC, allowing for pre-emptive minimally invasive intervention at an early stage. Endoscopic treatment of precancerous lesions and EGCs not only reduces rates of advanced carcinoma, but also avoids the requirement for high-risk surgical interventions with associated short- and long-term complications. The significant disparity in gastric cancer outcomes between East Asian and Western regions reflects extensive endoscopist experience, exhaustive research and clear guidelines in East Asia. Accordingly, there is a requirement for high-quality collaborative data from Western populations to aid in the development of clear management algorithms. Globally there are a multitude of opportunities for further research in both endoscopic imaging and resection techniques to optimise lesion detection and outcomes of ER, as well as potentially establish new indications for ER into the future.

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REVIEW

# *Helicobacter pylori* eradication: Exploring its impacts on the gastric mucosa

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## Abstract

*Helicobacter pylori (H. pylori)* infects approximately 50% of all humans globally. Persistent *H. pylori* infection causes multiple gastric and extragastric diseases, indicating the importance of early diagnosis and timely treatment. *H. pylori* eradication produces dramatic changes in the gastric mucosa, resulting in restored function. Consequently, to better understand the importance of *H. pylori* eradication and clarify the subsequent recovery of gastric mucosal functions after eradication, we summarize histological, endoscopic, and gastric microbiota changes to assess the therapeutic effects on the gastric mucosa.

**Key Words:** *Helicobacter pylori*; Gastric mucosa; Histology; Endoscopic findings; Gastrointestinal microbiota; Eradication therapy

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**Core Tip:** Eradication of *Helicobacter pylori* (*H. pylori*) is important. Multiple gastrointestinal diseases and extragastric diseases would emerge if *H. pylori* infection persists, whereas they would improve after *H. pylori* eradication. Thus, *H. pylori* eradication produces dramatic changes in the gastric mucosa. This review highlights the most recent literature and presents a comprehensive evaluation about the impact of *H. pylori* eradication on the gastric mucosa.

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## INTRODUCTION

*Helicobacter pylori* (*H. pylori*) represents a type of Gram-negative microaerophilic bacterium with a helical shape, generally infecting humans in early childhood[1,2]. O'Connor *et al*[3] have generated a table with some of the latest epidemiological findings about *H. pylori* infection, whose rate remains high, especially in certain parts of China as well as some Eastern European and South American countries. *H. pylori* infects ~50% of the global population[4]. Some researchers have reported that *H. pylori* infection rate is associated with socioeconomic status, including educational resources and living conditions, indicating that elevated *H. pylori* prevalence is more likely to happen in underdeveloped countries[5,6]. *H. pylori* is transmitted *via* iatrogenic, fecal–oral, and oral–oral routes[7].

Gastrointestinal diseases develop if *H. pylori* infection persists, including acute and chronic gastritis, gastric and duodenal ulcers[8], gastric mucosa-associated lymphoid tissue lymphoma (MALToma)[9], and autoimmune gastritis (AIG)[10]. Several studies have reported that *H. pylori* infection plays a role in extragastric diseases, including immune thrombocytopenia, unexplained iron-deficiency anemia, and Alzheimer's disease[11-15]. Moreover, the World Health Organization has included *H. pylori* among group 1 carcinogens for its critical role in gastric cancer (GC) etiology[16,17].

Besides curing gastritis, complete eradication of *H. pylori* can permanently cure peptic ulcers[18] and induce MALToma regression[19]. Additional evidence also suggests that *H. pylori* eradication treatment decreases precancerous lesions[20] and successfully prevents GC development[21,22], even after resection of early GC[23]. There is an urgent need to clearly assess the importance and necessity of *H. pylori* eradication. Therefore, the purpose of this review is to examine the impact on the gastric mucosa of *H. pylori* eradication to better understand the importance of *H. pylori* eradication.

## GASTRIC MUCOSAL CHANGES AFTER *H. PYLORI* ERADICATION

The gastric mucosa, the innermost layer of the stomach, consists of the epithelium, lamina propria, and muscularis mucosae, constituting three protective mucosal barriers. The most important barrier is called epithelial–bicarbonate barrier, the first line of defense of the gastric mucosa[24]. On the one hand, long-term *H. pylori* infection induces a sequence of histopathological changes, from gastritis (acute, chronic, and atrophic), intestinal metaplasia (IM), dysplasia, and ultimately to neoplasia according to the classical Correa sequence[25,26]. On the other hand, after anti-*H. pylori* therapy using antibiotics and proton pump inhibitors (PPIs)[27], the gastric mucosa undergoes various changes.

## HISTOLOGICAL CHANGES UPON *H. PYLORI* ERADICATION

With *H. pylori* infection, the histological changes in the gastric mucosa, such as gastritis, are among the important and obvious manifestations. Evaluation of the extent of gastritis was proposed and revised based on the Sydney System[28] and/or the Updated Sydney System[29], comprising endoscopic and pathological findings. However, the *H. pylori* eradication efficiency can be also evaluated by histological indicators of activity (neutrophil polymorph density), inflammation (lymphocyte and plasma cell elevations), atrophy, and IM.

### *Changes of inflammation and activity*

Regarding changes in histological indicators of gastric mucosal activity and inflammation, comparable trends of improvement have been reported[30-35]. Activity was improved in all studies. In addition, several studies have reported neutrophil

disappearance early after *H. pylori* eradication; consequently, activity score is considered a highly sensitive index for assessing *H. pylori* presence. Meanwhile, the inflammatory index PGII declines rapidly within 1–2 mo after successful *H. pylori* eradication[36,37]. Furthermore, inflammation is cleared at a significantly reduced rate, but with overt improvement[38].

### Changes in atrophy and IM

Atrophic gastritis (AG) and IM are premalignant conditions for GC. It remains controversial whether *H. pylori* eradication reverses AG and IM.

Various parts of the stomach exhibit different histological recoverability. With a 1-year follow-up, Sung *et al*[34] carried out a study in 2000, screening 587 *H. pylori*-positive subjects, randomizing them to the omeprazole, amoxicillin and clarithromycin ($n$ = 295) or placebo ($n$ = 292), and indicating that GA and IM in the antrum and corpus could be alleviated by *H. pylori* eradication, as did other studies by Annibale *et al*[39] and Ohkusa *et al*[40]. However, a recent study performed by Sung *et al*[35] in 2020 corrected the above results, demonstrating that GA is improved significantly with radical treatment of *H. pylori* in the antrum and corpus, while IM did not follow the same trend. With 3 years of follow-up, our team previously assessed 197 *H. pylori*-infected patients, including 92 receiving *H. pylori* eradication therapy and 87 control patients, and found markedly decreased atrophy in individuals with successful *H. pylori* eradication[33]. However, Kang *et al*[41] found that AG was improved in the corpus but not in the antrum. Furthermore, Kodama *et al*[42] showed that atrophy was markedly reduced after *H. pylori* eradication, both in the antrum and corpus after 5–13 years of follow-up. At the same time, IM was significantly decreased in the corpus but not in the antrum, with no differences observed in the untreated group. With 10 years of follow-up, Kodama *et al*[43] evaluated the gastric mucosa at five points based on the Updated Sydney System, revealing that atrophy at every site in the stomach and IM in the lesser curvature of the corpus showed continuous and significant decreases. In addition, Hwang *et al*[44] prospectively assessed patients with a 10-year follow-up, demonstrating that AG and IM in the antrum and corpus were gradually alleviated and reached a point at which they were comparable to those of *H. pylori*-negative individuals. There are three meta-analyses[45-47] concerning improvements of AG and IM. The first[45] assessed the long-term impact of *H. pylori* eradication on histological features in the stomach, and demonstrated that eradicating *H. pylori* improved atrophy but not IM, a finding similar to that of another meta-analysis[46]. However, Kong *et al*[47] reported that IM improvement only occurred in the gastric antrum and not in the corpus.

The discrepant responses of AG and IM to *H. pylori* eradication may have several reasons. On the one hand, the methods of histological assessment of biopsy specimens, sample sizes, and amounts of biopsy specimens are different across studies. On the other hand, progression from AG to serious AG, IM, and GC takes decades, indicating that a longer follow-up period could better mimic the actual situation[48,49]. Moreover, different risk factors for AG and IM, such as bile reflux, other bacterial infections, age, and dietary structure, could also influence the final results[33,50].

Different follow-up times result in different recoverability degrees of AG and IM. Since follow-up is tightly associated with improvement data in the majority of studies, follow-up times were divided into three groups for further assessment of AG and IM, including short (< 3 years), medium (3–10 years), and long (≥ 10 years) terms (Table 1). Activity and inflammation improvements following *H. pylori* eradication were consistent. However, whether AG and IM can be completely cured upon *H. pylori* eradication remains debatable. It is worth noting that a research team in Colombia conducted a large trial with long-term follow-up in the 1990s. After 6 years[51], 12 years[32], 16 years[52], and 20 years[53], the results indicated that *H. pylori* infection increased histological progression, and anti-*H. pylori* treatment significantly induced histological improvement and disease regression, and reduced progression of precancerous lesions of GC. Therefore, AG could be reversed, and even IM, with prolonged follow-up.

The above findings suggest that *H. pylori* eradication improves AG and IM, and anti-*H. pylori* treatment confers long-term benefits in decreasing the progression of precancerous lesions. The earlier the *H. pylori* eradication, the greater the benefits.

## CHANGES IN ENDOSCOPIC FINDINGS AFTER *H. PYLORI* ERADICATION

Endoscopy is an important gastrointestinal examination method. The Kyoto Classi-

**Table 1 Major features of the eight trials examining for histological parameters**

| Ref. | Study arm, *n* | | Follow-up, yr | Medication | Methods | Histologic parameter | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Eradicated | Not eradicated | | | 1 = OS | AG | | | | | | IM | | | | | |
| | | | | | 2 = RCT | Antrum | | | Corpus | | | Antrum | | | Corpus | | |
| | | | | | | Before | After | *P* value | Before | After | *P* value | Before | After | *P* value | Before | After | *P* value |
| Sung *et al* [34] | 226 | 245 | 1 | OAC | 2 | 0.64 ± 0.78 | 0.70 ± 0.82 | *P* = 0.627 | 0.06 ± 0.31 | 0.02 ± 0.18 | *P* = 0.682 | 0.78 ± 0.98 | 0.61 ± 0.94 | *P* = 0.014 | 0.04 ± 0.32 | 0.06 ± 0.30 | *P* = 0.391 |
| Annibale *et al*[39] | 25 | 7 | 0.5 | BAM | 1 | 0.56 ± 0.24 | 0.5 ± 0.2 | NS | 1.64 ± 0.11 | 1.36 ± 0.18 | NS | 0.58 ± 0.25 | 0.53 ± 0.23 | NS | 0.52 ± 0.13 | 0.76 ± 0.16 | NS |
| Ohkusa *et al* [40] | 115 | 48 | 1-1.25 | PPI/A/C | 1 | 0.8 ± 1 | At 1–3 mo: 0.8 ± 1 | *P* > 0.2 | 0.5 ± 0 | At 1–3 mo: 0.3 ± 0 | *P* = 0.020 | 0.7 ± 0 | At 1–3 mo: 0.6 ± 0 | *P* = 0.14 | 0.0 ± 0.0 | At 1–3 mo: 0.2 ± 0 | *P* = 0.022 |
| | | | | | | | At 12–15 mo: 0.9 ± 1 | *P* = 0.15 | | At 12–15 mo: 0.2 ± 0 | *P* = 0.001 | | At 12–15 mo: 0.4 ± 0 | *P* < 0.001 | | At 12–15 mo: 0.1 ± 0 | *P* > 0.2 |
| Lu *et al*[33] | 92 | 62 | 3 | O/LAC | 1 | 1.25 ± 0.44 | 0.97 ± 0.83 | *P* < 0.01 | NA | NA | NA | 0.64 ± 0.76 | 0.73 ± 0.77 | NS | NA | NA | NA |
| Kang *et al* [41] | 210 | 16 | 3 | PPI/A/C | 1 | 0.85 ± 0.06 | 1 yr: 0.83 ± 0.06 | NS | 0.70 ± 0.07 | 1 yr: 0.42 ± 0.06 | *P* < 0.001 | 0.91 ± 0.07 | 1 yr: 0.83 ± 0.06 | NS | 0.60 ± 0.07 | 1 yr: 0.54 ± 0.06 | NS |
| | 54 | 16 | 3 | PPI/A/C | 1 | 0.96 ± 0.14 | 3 yr: 1.32 ± 0.20 | NS | 0.91 ± 0.20 | 3 yr: 0.45 ± 0.15 | *P* = 0.033 | 1.02 ± 0.14 | 3 yr: 1.29 ± 0.14 | NS | 0.68 ± 0.15 | 3 yr: 0.83 ± 0.14 | NS |
| Kodama *et al* [42] | 118 | 21 | 8.6 | PPI/A/C | 1 | 1.60 ± 0.09 | 1.02 ± 0.08 | *P* < 0.001 | 0.71 ± 0.10 | 0.02 ± 0.02 | *P* < 0.001 | 0.60 ± 0.11 | 0.43 ± 0.09 | NS | 0.17 ± 0.12 | 0.00 ± 0.00 | *P* < 0.05 |
| Kodama *et al* [43] | 176 | 21 | 10 | PPI/A/C | 1 | AI: 1.39 ± 0.07 | 6 yr: 0.90 ± 0.09 | *P* < 0.05 | B1: 1.08 ± 0.08 | 1 yr: 0.78 ± 0.11 | *P* < 0.05 | A1: 1.14 ± 0.10 | NA | NS | B1: 0.97 ± 0.09 | 6y: 0.42 ± 0.17 | *P* < 0.05 |
| | | | | | | A2: 1.39 ± 0.06 | 1 yr: 1.06 ± 0.08 | *P* < 0.01 | B2: 0.52 ± 0.06 | 0.6 mo: 0.29 ± 0.07 | *P* < 0.05 | A2: 0.50 ± 0.07 | NA | NS | B2: 0.13 ± 0.04 | NA | NS |
| | | | | | | IA: 1.51 ± 0.08 | 1 yr: 1.24 ± 0.09 | *P* < 0.05 | | | | A1: 1.04 ± 0.09 | NA | NS | | | |
| Hwang *et al* [44] | 442 | 91 | 10 | PPI/A/C E/A/C/M | 1 | *n* = 178 | *n* = 89 (50.0) | *P* = 0.002 | *n* = 105 | *n* = 72 (68.8) | *P* = 0.01 | *n* = 221 | *n* = 45 (20.4) | *P* = 0.002 | *n* = 142 | *n* = 31 (21.8) | *P* = 0.01 |

A: Amoxicillin; A1: Lesser curvature of the antrum; A2: Greater curvature of the antrum; B1: Lesser curvature of the corpus; B2: Greater curvature of the corpus; B: Bismuth subcitrate; C: Clarithromycin; E: Esomeprazole; GA: Gastric atrophy; IA: Lesser curvature of the angulus; IM: Intestinal metaplasia; L: lansoprazole; M: Metronidazole; NA: Not applied; NS: Not significant; O: Omeprazole; OS: Observational study; PPI: Proton pump inhibitor; RCT: Randomized controlled trial; data are presented as *n* (%) or the median ± standard deviation or mean ± SE/median.

fication of Gastritis, categorizing *H. pylori* infection into three phases (non-gastritis, active gastritis, and inactive gastritis[54]), was proposed to better assess the status of *H. pylori* infection and GC risk by endoscopy[55] (Figure 1). In a healthy stomach, an easily detectable feature, non-gastritis, was the regular arrangement of collecting venules (RAC), featured as small red spots on the mucosal surface[56,57]. However, after being infected with *H. pylori*, the stomach was characterized as irregular arrangement or absence of the so-called collecting venules[58]. AG after infection by *H. pylori* presents with diffuse redness, spotty redness, mucosal edema, and enlarged folds. This phenomenon can decrease and disappear after *H. pylori* eradication[59-61]. In addition, with *H. pylori* eradication, nodular gastritis (NG), whose endoscopic character is "goose flesh" in the antrum, can also disappear with the passage of time [62,63].

After a period of *H. pylori* infection, AG turns into inactive gastritis upon eradication therapy or spontaneously disappears because of advanced atrophy, featuring map-like redness, and flat or depressed erythematous tumors, which is the characteristic change of AG after *H. pylori* eradication, *i.e.*, nonatrophied areas dissipate the inflammation, and the atrophied areas are relatively red compared to the nonatrophied areas. Using white-light imaging (WLI) and linked color imaging (LCI), Majima *et al*[64] found that map-like redness is closely associated with GC occurrence upon effective *H. pylori* eradication. Another study also revealed map-like redness upon *H. pylori* eradication as the sole predictive factor for metachronous cancer[65]. With *H. pylori* infection, atrophic change expands from the antrum to the fundus, and is improved after eradication[66,67]. Another characteristic was described as mottled patchy erythema (MPE) after *H. pylori* infection, showing many flat/slightly depressed erythematous lesions detected by white light endoscopy, and highly predicting the impact of *H. pylori* eradication.

The typical endoscopic finding of IM is mixed patchy pink and pale mucosal areas surrounding grayish slightly elevated plaques generating an irregular, uneven surface. Moreover, villus-like structures, whitish mucosa, and rough mucosal surface can help diagnose IM by endoscopy[68,69]. In addition, endoscopic IM contributes to recognition of current and past *H. pylori* infections, similar to endoscopic atrophy[70]. *H. pylori* eradication reduces the development of hyperplastic polyps (HPPs); either sessile or pedunculated polyps result from *H. pylori* infection[71]. Gastric xanthoma (GX) is a typical endoscopic manifestation of *H. pylori* infection that persists upon *H. pylori* eradication, showing one or more yellowish well-delineated nodules or plaques of 1–10 mm in diameter[72]. However, GX may be a precancerous lesion of GC[72,73]. After treatment with PPI, the endoscopic phenomena of multiple white elevated lesions and cobblestone-like mucosa became more evident in comparison with PPI nonusers[74].

Overall, endoscopic features represent additional indexes for evaluating *H. pylori* therapy for efficacy. Atrophy, IM, HPPs, and fundic gland polyps are detected in active and inactive gastritis. In addition, atrophy boundaries are unclear with map-like redness observed upon *H. pylori* eradication[75]. However, endoscopic atrophy and IM may show no rapid improvement[76,77], and prolonged follow-up is required for detecting gastric mucosal changes endoscopically following *H. pylori* eradication[78].

## EFFECT OF *H. PYLORI* ERADICATION THERAPY ON GASTRIC MICROBIOTA

There are many microorganisms in the human stomach, constituting alongside *H. pylori* the so-called gastric microbiota[79], whose balance and stability are indispensable for normal gastric mucosal digestion and metabolism. With more advanced techniques, such as culture-free molecular methods (*e.g.*, 16S rDNA sequencing), the human stomach is currently known to host multiple resident microbes. Based on such techniques, many reports have shown that *H. pylori*-negative individuals have a greatly diverse gastric microbiome with four dominating phyla, including Proteobacteria (including *H. pylori*), Firmicutes, Bacteroidetes, and Actinobacteria; the commonest genera are *Streptococcus*, *Lactobacillus*, and *Propionibacterium*[80-85].

Upon *H. pylori* infection, changes in gastric microorganisms arise, including gastric microbial diversity, composition, and predictive pathways[86], leading to various diseases[87-89]. Generally, colonization by *H. pylori* is associated with significantly reduced alpha and beta diversities (representing inter-sample and in-sample diversities, respectively)[90-92]. Additionally, several studies have revealed that *H.*

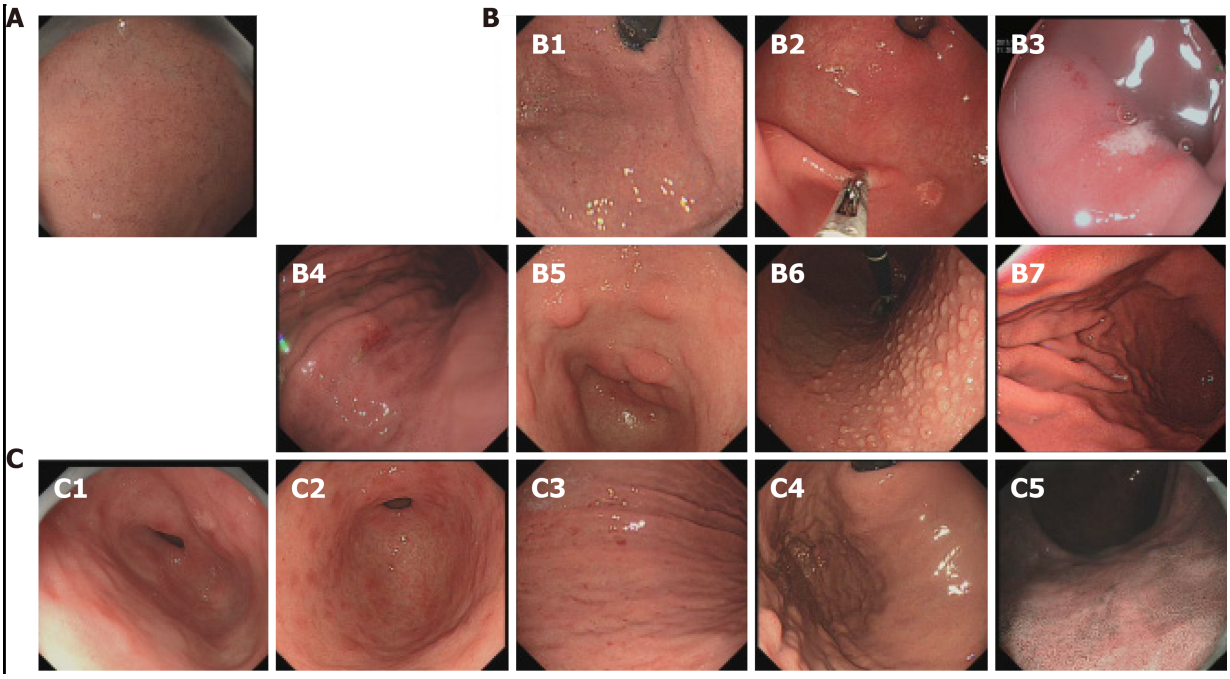


**Figure 1 Endoscopic features for *Helicobacter pylori* infection.** A: Normal gastric mucosa. Regular arrangement of collecting venules is seen; B: Infected gastric mucosa. B1: Spotty redness; B2: Gastric xanthoma; B3: Erosion; B4: Multiple redness and erosion; B5: Hyperplastic polyp; B6: Nodular gastritis; B7: Intestinal metaplasia; C: Gastric mucosa after eradication. C1: Patchy redness; C2: Map-like redness; C3: Redness; C4: Atrophy; C5: Intestinal metaplasia.

*pylori*-infected individuals have different community structures in comparison with their *H. pylori*-negative counterparts[93-96]. Compositionally, Proteobacteria often dominate the gastric mucosa upon *H. pylori* infection, becoming the single most abundant bacteria and almost reaching 90% abundance at the phylum level, while other phyla (Actinobacteria, Bacteroidetes, Firmicutes, and Fusobacteria) show reduced numbers[82,90,92-94,96,97].

After anti-*H pylori* treatment, the gastric microbiome undergoes major reshaping (Table 2). Mounting evidence indicates that gastric microbial diversity markedly increases upon effective *H. pylori* eradication but does not improve if treatment fails [35,82,86,93,98,99]. Recovery may take some time as microbial diversity increases gradually from week 0 to weeks 6 and 26[86]. Additionally, alpha diversity can regain the level of uninfected individuals following effective eradication[98]. Although the community structure can also be partly restored upon *H. pylori* eradication, whether in post-eradication groups it can be restored to that of healthy control groups appeared to be age related. Specifically, several studies indicated that the adult specimens from 6 mo after successful treatment still showed altered community structure *vs* the negative control group[98], while others recruiting children reported the close community structures between the eradication and *H. pylori*-negative groups at 4 wk post-therapy [99] and the restored gastric microbiota composition in individuals administered with anti-*H. pylori* therapy at 2 mo post-treatment[82]. We believe that in adult patients, further research is needed to see whether the recovery in microbial composition can be observed over a longer observation period.

Compositionally, the relative abundance of *H. pylori* starkly decreases post-treatment, although it remains the dominant bacterium[86,93]. Meanwhile, Actinobacteria, Firmicutes, Bacteroidetes, and Fusobacteria are significantly enriched after successful eradication[82,90,93,98]. At the genus level, the probiotics *Lactobacillus* and *Bifidobacterium* are markedly increased post-therapy[86]. Functional analysis was performed in multiple studies[82,86,98]. The activities of disease-associated categories in *H. pylori* infection (lipopolysaccharide biosynthesis, bacterial motility proteins, *etc*.) were more pronounced[82,98]. In addition, the metabolic pathways (protein digestion and absorption, gastric acid secretion, and carbohydrate digestion and absorption) in the presence of *H. pylori* were downregulated[100]. After eradication therapy, these functions might be partly restored[86].

*H. pylori* infection is associated with reduced bacterial diversity and causes a shift in bacterial structure. Clearance of *H. pylori* significantly increases bacterial diversity. The relative abundance of *Helicobacter* decreases after therapy, while other phyla are increased, partly restoring bacterial structure and improving microbiota functions,

**Table 2 Major features of five meta-analyses**

| Ref. | Year | Total No. of study | Eradication group | | | Control group | | | OR/RR | 95%CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Total No. | Total events | Incidence rate | Total No. | Total events | Incidence rate | | |
| Sugimoto *et al*[118] | 2020 | 4RCTs | 2731 | 73 | 2.7% | 2733 | 49 | 1.80% | 0.67 | 0.47–0.96 |
| Sugano *et al*[119] | 2019 | 32 | 16301 | 316 | 1.90% | 14805 | 535 | 3.60% | 0.46 | 0.39-0.55 |
| Doorakkers *et al* [120] | 2016 | 8 Cohort | 12899 | 119 | 0.90% | 18654 | 208 | 1.10% | 0.46 | 0.32-0.66 |
| Chen *et al*[121] | 2016 | 8RCTs | 3992 | 74 | 1.90% | 3962 | 116 | 2.90% | 0.64 | 0.48-0.85 |
| Ford *et al*[104] | 2014 | 6RCTs | 3294 | 51 | 1.60% | 3203 | 76 | 2.40% | 0.66 | 0.46-0.95 |

RR: Risk ratio; OR: Odds ratio; 95% C: 95% confidence interval.

such as metabolism.

## CHANGES IN GC AFTER *H. PYLORI* ERADICATION

Many studies have confirmed that *H. pylori* infection is the main etiological agent of GC[101,102], whose risk can be reduced by *H. pylori* eradication[103-108].

To explore this, Wong *et al*[109] performed a study demonstrating that GC incidence rates were comparable in the treatment and placebo groups (7 cases in either group), which may have been due to a underpowered design despite the 7.5-year follow-up of 1630 participants. However, with the follow-up time gradually extended, the incidence rates of GC in both groups gradually showed differences. Another study demonstrated significantly decreased GC incidence after 6 years of follow-up after *H. pylori* eradication, and the standardized incidence ratio (SIR) was 1.62 in the initial 5 years but was reduced thereafter to reach 0.14[21]. A Swedish cohort study found significantly decreased risks of gastric adenocarcinoma and non-cardia gastric adenocarcinoma upon cure of *H. pylori* infection (SIRs were 8.65 in 1–3 years, 2.02 in 3–5 years, and 0.31 in 5–7.5 years)[22,109]. After *H. pylori* treatment, the risk was 39% lower over an extended follow-up of 15 years and 52% over an extended follow-up of 22 years among individuals with *H. pylori* eradication compared with those showing persistent infection, whereas there was no difference during the initial 7.3-year follow-up[20,110,111]. Having a first-degree relative with diagnosed GC doubles or triples GC risk[112]. In *H. pylori*-infected individuals with a first-degree relative diagnosed with GC, eradication of *H. pylori* also reduces GC risk[106,113]. A South Korean study utilized a prospective randomized design (832 and 844 in the cure and placebo groups, respectively, of first-degree relatives of GC cases). GC risk was reduced by 55% after *H. pylori* eradication *vs* the placebo group, with an average follow-up of 9.2 years. Of note, GC risk was 73% lower upon *H. pylori* eradication compared with the placebo group.

GC, as the end point of gastric disease, is also inextricably linked to *H. pylori*. Choi and collaborators[114] found that *H. pylori* eradication had no significant relationship with metachronous GC (MGC) incidence within an average follow-up of 3 years, whereas *H. pylori* eradication markedly reduced MGC incidence with a median follow-up duration of 71.6 mo[115]. A recent randomized trial involving early GC cases (a population that usually has severe atrophic alterations in the gastric mucosa) demonstrated that treating *H. pylori* infection reduced MGC risk by half[106]. A similar effect was also reported in another Chinese trial[116]. Successful eradication therapy cannot completely eliminate the development of GC. Take *et al*[117] performed a retrospective cohort trial in Japan, including 2737 patients treated for *H. pylori* infection with yearly endoscopic follow-up for 21.4 years. The degree of atrophy was related to a high yearly risk of GC. They also found an elevated risk of diffuse-type GC in individuals with mild to moderate gastric atrophy at baseline. The above findings suggest that endoscopic monitoring for GC should continue beyond 10 years post-*H. pylori* eradication regardless of the degree of gastric mucosal atrophy at the time of eradication treatment[117].

Several meta-analyses have demonstrated that the risk of GC is correlated with *H. pylori* eradication (Table 3)[104,118-121]. One meta-analysis including six randomized studies involving healthy, asymptomatic participants with *H. pylori* infection showed that GC risk was about 34% less after treatment compared with the control group [104]. Another meta-analysis also showed a reduced incidence of GC upon eradication therapy compared with control patients (pooled incidence rate ratio = 0.54)[122]. Sugano *et al*[119] and Doorakkers *et al*[120] reported that the lower odds ratio/relative risk was 0.46. A further meta-analysis demonstrated that no matter how varied the countries, conditions at baseline, and follow-up periods among studies, *H. pylori* eradication effectively reduces GC incidence. Consistent with the prediction, long-term (≥ 5 years) follow-up showed greater effects in reducing GC upon *H. pylori* eradication compared with shorter follow-up periods (< 5 years)[119]. This was consistent with other meta-analyses[104,118,120-122]. Thus, the above meta-analyses provided further robust evidence of the effect of eradication treatment.

Precancerous lesions are closely associated with GC. Consequently, whether and when *H. pylori* eradication reverses precancerous tumors has attracted increasing attention. Kiriyama *et al*[123] and Wong *et al*[109] have reported that eradicating *H. pylori* did not reverse mucosal injury in IM to yield a normal gastric mucosa or prevent GC development, indicating a histological point of no return. In agreement, others have indicated that GC progression continues following *H. pylori* eradication[109,124]. However, the Taipei global consensus and Matsu Islands consensus proposed that eradicating *H. pylori* reduces GC risk[107,108], which may be due to treatment effects before a certain point for preventing GC.

In general, GC risk in *H. pylori*-infected patients is increasing. A large number of studies have shown that *H. pylori* eradication can reduce the incidence of not only GC, but also MGC. In both small and large studies (community, region, or country) examining young and old individuals, and even first-degree relatives of patients, eradication of *H. pylori* results in long-term benefits.

## DISCUSSION

*H. pylori* infection induces a sequence of histological changes, especially AG and IM. A histological classification system (Figure 2A) was proposed by an international group of gastroenterologists and pathologists, to grade gastritis into stages with corresponding cancer risk in individual patients, termed the Operative Link on Gastritis Assessment (OLGA) scale[125]. However, disease severity and extent in OLGA are primary parameters, which leads to low interobserver agreement. Therefore, a staging system based on IM (Operative Link on Gastric Intestinal Metaplasia Assessment, OLGIM; Figure 2B) was proposed to assess the degree of IM and GC risk in 2010[126]. However, some individuals potentially at high risk of GC may be overlooked[127]. Therefore, the combination of OLGA and OLGIM more accurately predicts GC risk. Meanwhile, the AI system using deep learning (especially convolutional neural networks; CNNs) has been applied in gastroenterology[128-131]. For example, studies have reported the usefulness of CNN-based AI systems for diagnosing *H. pylori* infection and timely detecting gastric neoplasms[129,131,132].

Previous studies have found that only a small number of patients with *H. pylori* infection develop GC eventually, but *H. pylori* is one of the main causes of GC. The high risk of GC emphasizes the need for early detection and proper treatment of *H. pylori* infection. Along with standard endoscopy, new endoscopic techniques, such as magnifying endoscopy[133], endocytoscopy[134,135], magnifying narrow-band imaging (M-NBI)[136], I-Scan[137], endomicroscopy[138], and LCI[139-141], can be used to detect *H. pylori* infection. Magnifying endoscopy allows the structure of the mucosa and the subepithelial capillary network around the gastric fovea to be observed in detail. As a novel ultra-high magnification technology, endocytoscopy can recognize gastric mucosal minimal changes[134,135]. Moreover, the NBI system and I-Scan are also the recent developments in computed virtual chromoendoscopy imaging [137]. The diagnostic accuracy of M-NBI endoscopy for gastritis and magnifying I-Scan for *H. pylori* infection was 96.1% and 94.0%, respectively[136,137]. Currently, a novel imaging mode under blue laser endoscopy, LCI, plays an important role in endoscopic diagnosis of active *H. pylori* infection or distal gastric disease, through its enhanced slight differences in mucosal color[139-141]. With the assistance of computer-aided diagnosis (CAD) systems, LCI-CAD can effectively assess the gastric mucosal status of uninfected, currently infected, and post-*H. pylori* eradication patients[142-144].

**Table 3 Studies on gastric microbiota alteration after eradication**

| Ref. | Year | Total subjects | Follow-up time | Age | Regimen | Study group | | Main outcomes |
|---|---|---|---|---|---|---|---|---|
| | | | | 1 = Adults<br>2 = Children | 1 = TT for 7-14 d<br>2 = QT for 10-14 d | HEG | *H. pylori* (-) | |
| Li *et al*[93] | 2017 | 33 | Day 0 and week 9 | 1 | 1 | 17 | 16 | Bacterial diversity increased and the relative abundance of *Helicobacter* decreased, while the relative abundance of other phyla increased |
| Serrano *et al*[99] | 2019 | 16 | Day 0 and month 2 | 2 | 1 | 11 | 5 | Bacterial diversity increased and the structures of the uninfected group were restored |
| Guo *et al* [98] | 2020 | 164 | Day 0 and month 6 | 1 | 2 | 115 | 49 | Bacterial diversity returned to the level of the control group. The structure of the bacteria was different after treatment compared to the control group. Microbiota functional capacities were changed |
| He *et al* [86] | 2019 | 17 | Weeks 0, 6, and 26 | 1 | 2 | 10 | NA | Bacterial diversity increased and structure and microbiota functional capacities were changed |
| Miao *et al* [82] | 2020 | 55 | Day 0 and week 4 | 2 | 1, 2 and STP | 11 | 8 | Diversity was similar compared to the control group. The bacterial structure became close to controls |
| Sung *et al* [35] | 2020 | 102 | Day 0 and 1 year | 1 | 1 | 102 | NA | Bacterial diversity increased and structure was changed |

HEG: *Helicobacter pylori* eradication group; *H. pylori*: *Helicobacter pylori*; NA: Not applicable; QT: Quadruple therapy; TT: Triple therapy; STP: Sequential therapy with proton pump inhibitor and amoxicillin.

In this review, we describe the changes in gastric histology, endoscopic appearances, gastric microbiota, and decreased risk of GC and MGC[108]. Dyspeptic symptoms, AIG, and recurrence of peptic ulcer disease significantly declined after eradication of *H. pylori*. The risk of synchronous GC after endoscopic resection of early GC was also reduced. Many extragastric disorders, such as iron deficiency anemia, MALToma, and idiopathic thrombocytopenic purpura, were also associated with the presence of *H. pylori* and they were improved after eradication of *H. pylori*[8,9,10,145,146]. Therefore, consensus reports recommend eradication of *H. pylori* in infected patients, decreasing the risk of these diseases[38,147].

However, there are some potential concerns for *H. pylori* therapy due to the significantly increased antibiotic (particularly metronidazole and clarithromycin) resistance rates for *H. pylori*[148,149], and development of novel and alternative antimicrobial agents specific for *H. pylori* is urgent. These approaches are broadly divided into two main categories: (1) Novel synthetic treatment, which includes new classes of antimicrobial peptides (AMPs) and small molecule inhibitors; and (2) natural treatment options, which include the use of probiotics and phytotherapy to treat *H. pylori* infection. First, AMPs play a pivotal role in the innate immune responses to *H. pylori* in humans. AMPs can be roughly divided into nine categories: Pexiganan, tilapia piscidins, epinecidin-1, cathelicidins, defensins, bicarinalin, odorranain-HP, PGLa-AM1, and bacteriocins[150]. Among them, cathelicidins and defensins, both secreted by epithelial cells of many tissues, exhibit the key therapeutic potential[151]. SQ109, a typical representative of small molecule inhibitors for treating *H. pylori* infection, displays robust thermal and pH stability, induces low/no spontaneous drug resistance, and shows anti-*H. pylori* superiority over metronidazole and amoxicillin [152]. Second, adjuvant probiotics and phytotherapy therapy are designed to increase the eradication rate of *H. pylori* and reduce the adverse effects of treatment[153,154]. Phytotherapy, including herbs and spices, cruciferous vegetables, Korean red ginseng and green tea, and extracts of oils, resveratrol, and beta-carotene, is another naturopathic therapy. Specifically, herbal-based therapies, one of the most popular forms of phytotherapy, can act as anti-inflammatory agents to treat *H. pylori* infection[155]. Nevertheless, the active component for the majority of agents and the molecular mechanism of inhibition against *H. pylori* remain unknown. After the eradication of *H. pylori*, the risk of gastroesophageal reflux disease is increased due to the restoration of gastric acid secretion[156,157]. Alterations in gut microbiota might decrease the secretion levels of insulin, and fasting glucose, total cholesterol, and triglyceride were reduced after *H. pylori* eradication[148,158]. However, the findings remain contro-

**A**

| Atrophy score | | Corpus | | | |
|---|---|---|---|---|---|
| | | No atroph (score 0) | Mild atrophy (score 1) | Moderate atrophy (score 2) | Severe atrophy (score 3) |
| Antrum (Including incisura angularis) | No atroph (score 0) | Stage 0 | Stage I | Stage II | Stage II |
| | Mild atrophy (score 1) | Stage I | Stage I | Stage II | Stage III |
| | Moderate atrophy (score 2) | Stage II | Stage II | Stage III | Stage IV |
| | Severe atrophy (score 3) | Stage III | Stage III | Stage IV | Stage IV |

**B**

| IM score | | Corpus | | | |
|---|---|---|---|---|---|
| | | No IM (score 0) | Mild IM (score 1) | Moderate IM (score 2) | Severe IM (score 3) |
| Antrum (Including incisura angularis) | No IM (score 0) | Stage 0 | Stage I | Stage II | Stage II |
| | Mild IM (score 1) | Stage I | Stage I | Stage II | Stage III |
| | Moderate IM (score 2) | Stage II | Stage II | Stage III | Stage IV |
| | Severe IM (score 3) | Stage III | Stage III | Stage IV | Stage IV |

**Figure 2 Operative link on gastritis assessment staging system (A) and operative link on gastric intestinal metaplasia assessment (B) staging system.** IM: Intestinal metaplasia; OLGA: Operative link on gastritis assessment staging system; OLGIM: Operative link on gastric intestinal metaplasia assessment.

versial and further well-designed randomized trials are warranted to clarify the impact of *H. pylori* eradication on metabolic parameters.

More significantly, the relative immutability of IM is of concern, as the condition carries a high GC risk not only in the presence of *H. pylori* infection, but also after *H. pylori* eradication. In other words, GC can still develop even after successful eradication in the presence of IM[159]. Previous studies have indicated that the detection of map-like erythema, a histological indicator of IM, is correlated with a high risk of GC development after *H. pylori* eradication[65]. Even worse, the eradication therapy can cause some characteristics, such as a gastritis-like appearance, resulting in a difficult diagnosis of GC[160-162]. This is why post-eradication status should be distinguished from *H. pylori* negativity.

## CONCLUSION

Whether *H. pylori* eradication confers long-term benefits has been debated for a long time. Obviously, eradication of *H. pylori* is more important, because the disadvantages can be avoided based on clinical experience and continuous technological development. More importantly, *H. pylori* eradication offers lifelong benefits, and the earlier it is eradicated, the better. In addition, more sensitive and accurate tools can be developed to detect *H. pylori* infection in the early and post-eradication stages. This could be a promising area of research.

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*MINIREVIEWS*

# Molecular advances in pancreatic cancer: A genomic, proteomic and metabolomic approach

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## Abstract

Pancreatic ductal adenocarcinoma (PDAC) represents a challenging pathology with very poor outcomes and is increasing in incidence within the general population. The majority of patients are diagnosed incidentally with insidious symptoms and hence present late in the disease process. This significantly affects patient outcomes: the only cure is surgical resection but only up to 20% of patients present with resectable disease at the time of clinical presentation. The use of "omic" technology is expanding rapidly in the field of personalised medicine - using genomic, proteomic and metabolomic approaches allows researchers and clinicians to delve deep into the core molecular processes of this difficult disease. This review gives an overview of the current findings in PDAC using these "omic" approaches and summarises useful markers in aiding clinicians treating PDAC. Future strategies incorporating these findings and potential application of these methods are presented in this review article.

**Key Words:** Pancreatic ductal adenocarcinoma, Pancreatic adenocarcinoma; Genomic; Proteomic; Metabolomic

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**Core Tip:** Treatment for pancreatic ductal adenocarcinoma is limited by the severity of the pathology, limited biomarkers and late presentation of patients. Utilising genomic, proteomic and metabolomic research into pancreatic ductal adenocarcinoma has provided insight into understanding the disease process as well as providing suitable markers of diagnosis and treatment to improve clinical outcomes.

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## INTRODUCTION

Pancreatic adenocarcinoma or pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy with a 1-year and 5-year survival rate of 24% and 9% respectively and make up the majority of pancreatic cancers (PC) (85%) with others arising from the endocrine tissue of the pancreas[1]. According to the GLOBOCAN statistics in 2020, PC were the 12$^{th}$ most commonly diagnosed cancer in the world with the incidence increasing with the Human Development Index; the highest cumulative risks being in North America and Europe[2]. Unfortunately, developments in treatment have not progressed as rapidly as other cancers and PC are projected to become the second leading cause of cancer related death in the United States by 2030[3]. Majority of PDAC cases are diagnosed at a late stage owing to the lack of clinical features early on in its course. Currently the only curative option is through surgical resection, but only roughly 20% of all cases are operable at the time of diagnosis, with limited chemotherapy and radiotherapy options at present[4]. However, with the emergence of "omics" research, new advances have been made in improving the treatment of PC.

## GENOMICS, PROTEOMICS AND METABOLOMICS

The field of "omics" research refers to the use of high throughput technologies to globally analyse a biological system at the molecular level. This takes place on multiple "levels" depending on the nature of the molecules being studied i.e. genetic material, proteins and metabolites[5].

The first of these fields to emerge was genomics, with the goal of characterising the genome and the variations of its structure and expression leading to pathogenesis[5]. Cancer being essentially a disease of the genome, occurring through the accumulation of genetic mutations, the insights gleaned from such analysis is invaluable for cancer research[6].

With the advent of next-generation sequencing [whole-genome sequencing (WGS), whole-exome sequencing (WES) and RNA sequencing], this can now be done quicker and more accurately than traditional methods[7]. WES, which sequences all protein coding exons, is more abundantly utilised as it is more accurate and relatively less expensive compared to WGS, which also has the issue of complex data analysis and interpretation[6].

At the protein level, proteomic studies identify and quantify the proteome of a biological system, their interactions and post-translational modifications[5]. Proteomics technologies are largely based in protein separation (gel-based techniques and chromatography) and mass spectrometry (MS) for high throughput analysis of proteins in tissues and fluids[8,9]. Quantification methods include isobaric tags for relative and absolute quantification, isotope coded affinity tag and differential image gel electrophoresis, with tandem MS[8,9]. Modifications can also be detected by MS through the corresponding change in mass brought on by the modifying process[5].

Metabolomics involves the study of all the low molecular weight metabolites within a sample that gives a comprehensive reflection of the sample's phenotype at a given time[10]. Identification and quantification of these metabolites can indicate the metabolic processes occurring and changes in these can then be associated with disease processes. Like proteomics, metabolomics is largely driven by MS and chromatography, however there is added complexity due to the variation of the physical properties of these metabolites. Due to this, the metabolites need to be stratified along these properties followed by the application of analytic methods optimised for each type of metabolite[10].

## MOLECULAR SUBTYPES FOR PERSONALISED MEDICINE

### *Genomic subtypes*

Waddell *et al*[11] utilised WGS to map the genome of 100 PDAC specimens. Their findings reinforced the known main drivers of PDAC (KRAS, TP53, CDKN2A and

SMAD4) and also found multiple other mutations at much lower prevalence. The authors described four subtypes of PDAC based on the quantity of variations in chromosome structure: (1) Stable (20%; < 50 structural variations and widespread aneuploidy); (2) Locally rearranged (30%; significant events on 1-2 chromosomes); (3) Scattered (36%; < 200 structural variations and moderate chromosomal damage); and (4) Unstable (14%; large number of structural variation and defects in DNA damage repair). The unstable subtype was associated with a high BReast CAncer gene (BRCA) mutational signature, suggesting defects in DNA damage repair, possibly sensitising these to platinum based chemotherapy or poly (ADP-ribose) polymerase inhibitors (PARPi). Indeed, from their sample, 4 out of 5 of these patients treated with platinum based chemotherapy showed response to treatment[11].

Singhi *et al*[12] utilised real time targeted sequencing of exons and introns of 3594 PDAC specimens during the course of clinical care and reported that 17% of these may be susceptible to current therapies based on this genomic data. They found genetic alterations in receptor tyrosine kinase (RTK)/Ras/mitogen-activated protein kinase (MAPK) activation, DNA damage repair, cell cycle control, TGF-B signalling, histone modification, SWI/SNF protein complex, PI3L/mTOR signalling, WNT/B-catenin pathway, RNA splicing, NOTCH pathway, angiogenesis and Hedgehog signalling. Interestingly, the investigators also found that 14% of their sample exhibited mutations in DNA damage repair genes (BRCA-FANC family). Further to this, they identified genetic alterations in receptor tyrosine kinases as potential targets on a background of wild-type KRAS PDAC (12% of the sample)[12].

Aguirre *et al*[13] performed deep WES of PDAC primaries and metastases for 73 patients having clinically indicated biopsies. Average time for the results of WES to return to the clinicians was 39 d, longer than that of research only biopsies (28 d) due to the need for histological diagnosis prior to sequencing. Analysis of these findings resulted in three mutational signatures: SigA (homologous recombination deficiency), SigB (aging) and SigC (unknown aetiology). Around 40% of these patients had potentially targetable genomic findings, when excluding KRAS or CDKN2A, 48% were eligible for clinical trials or off-label use of other therapeutic agents and 24% of patients were indeed enrolled onto a clinical trial or treated with an off-label agent. This shows the feasibility of implementing WES clinically to guide treatment choice. Clinically relevant findings included DNA damage repair (DDR) mutations and BRAF mutations in KRAS wild-type PDAC which may confer sensitivity to platinum-based chemotherapy/PARPi and MAPK inhibition respectively. The authors also presented two such case studies with significant responses to treatment[13].

### *Proteomic subtypes*

Using 56 PDAC liver metastases specimens, Law *et al*[14] performed liquid chromatography-mass spectrometry (LC-MS/MS) to identify 30811 peptides that mapped to 916 proteins comprising of at least 5 peptides in 80% of the sample. Functional analysis of these proteins showed that they play a role in, "extracellular matrix organization, protein processing and transport, translation, glycolytic processes, NADPH metabolism, cell migration, immune response, fibronectin binding, and cell homeostasis"[14].

These proteins were analysed to categorise four PDAC subtypes and three protein clusters. The matched subtypes and clusters are: "Inflammatory" (cluster 3 - pentose phosphate pathway, adaptive immune response, complement activation, IL8 production and extracellular fibril organisation), "proliferative" (cluster 2 - translation, cell proliferation and telomere maintenance), "progenitor-like" (cluster 1 - ethanol oxidation pathways, mitochondrial fatty acid B-oxidation and retinoic acid signaling pathways) and "metabolic" (cluster 1). Both the "progenitor-like" and "metabolic" subtypes exhibit cluster 1 proteins however, the latter shows higher expression of these proteins. The authors were also able to map these subtypes onto previously defined transcriptomic subtypes, making this the first proteomic study with data robust enough to make such a correlation[14].

Clinically, the "proliferative" subtype was associated with a history of alcohol use and the "metabolic" subtype was associated with tobacco use. The "metabolic" and "progenitor-like" subtypes had a decreased risk of death when treated with FOLFIRINOX + gemcitabine compared to the other two subtypes. Further analysis also showed that there was a significant increase in survival when "progenitor-like" subtypes are treated with gemcitabine and that the "metabolic" subtype had a negative correlation between survival probability and abraxane/paclitaxel treatment. These analyses support the use of proteomics derived subtypes as a method for selecting targeted therapeutics however more robust trials are needed to validate these findings [14].

Another interesting finding from this study is the role of serine hydro- xymethyltransferase (SHMT1) (involved in the folic acid cycle) in gemcitabine resistance. Comparison of untreated samples and samples treated with only gemcitabine showed that SHMT1 was significantly down-regulated in the treatment group. The investigators further displayed increased EC50 of gemcitabine in cell lines with SHMT1 knockdown compared to the control group, showing that SHMT1 is a potential mediator of gemcitabine resistance. Expression of SHMT1 was higher in "metabolic" and "progenitor-like" subtypes compared to the other two subtypes regardless of gemcitabine treatment. Expression of this protein may potentially guide the choice of gemcitabine as treatment or monitor those on gemcitabine for resistance to treatment [14].

Humphrey *et al*[15] used liquid chromatography-mass spectrometry (LC-MS/MS) to stratify two cohorts of PDAC cell lines American Type Culture Collection (ATCC) and The Kinghorn Cancer Centre (TKCC), along tyrosine phosphorylation (pTyr) sites. The authors produced a list of 1622 pTyr sites from 797 proteins. Of these, 144 had significant subtype specificity. ATCC subtype 1 showed hypophosphorylation of 65 pTyr sites and the enriched proteins were involved in formation and regulation cell-cell adheren junctions and tight junctions. ATCC subtype 2 contained 54 up-regulated pTyr sites (specifically increased relative phosphorylation rather than increased protein levels) with enrichment of proteins involved in mRNA processing and spliceosome pathways. ATCC subtype 3 showed significantly increased phosphorylation (in both relative phosphorylation and protein expression) in 15 pTyr sites of RTKs including EGFR, MET, RON, EPHA4, EPHB2/3/4 and DDR2[15].

When this methodology was applied to the TKCC cohorts, 1220 pTyr sites were identified, of which 383 were subtype specific. TKCC subtypes 1 and 2 showed 101 and 73 down-regulated pTyr sites, while TKCC subtype 3 showed up-regulation of 209 pTyr sites and was enriched for Ephrin and EGFR signalling. Targeting RTK pTyr sites in the TKCC cohort showed increased phosphorylation of RTKs in subtype 3 including sites on EGFR, EPHA2, DDR1, FGFR1, INSR, MERTK, MET, and RON[15].

Of the subtype specific pTyr sites identified, 8 were identified as "common classifier sites", able to predict the subtype in the ATCC cohort. Subtypes 1, 2 and 3 were identified to exhibit low medium and high phosphorylation of these sites respectively. As both cohorts had subtypes that were "RTK-enriched", the investigators tested the cell lines in this cohort against erlotinib, an EGFR kinase inhibitor. Indeed, the cell lines in this subtype showed increased sensitivity to erlotinib. The authors recognised that the pTyr signature of these RTKs are what conferred sensitivity to RTK blockade rather than expression levels and so stratifying patients with such a signature can potentially allow targeted therapeutic regimes to be studied[15].

### Metabolomic subtypes

Metabolomics profiling by Daemen *et al*[16] using LC-MS/MS and gas chromatography-MS represents the only metabolomic study to stratify PDAC. The investigators examined 38 "PDAC-derived" cell lines to quantify 256 metabolites. Analysis of these metabolites revealed three subtypes of PDAC, described as: (1) Slow proliferating (34%); (2) Glycolytic (27%); and (3) Lipogenic (39%). The slow proliferating subtype was low in amino acids and carbohydrates, and the cells had a significantly longer doubling time than the other two subtypes. The glycolytic subtype showed increased levels of metabolites of the glycolytic and serine pathways (phosphoenolpyruvate, glyceraldehyde-3-phosphate, lactate, and serine) and decreased redox balance metabolites (NAD, NADH, NADP, NADPH, GSSG, GSH and flavin adenine dinucleotide). The lipogenic pathway showed increased levels of lipid metabolites (palmitic acid, oleic acid, palmitloeic acid and myristic acid), oxidative phosphorylation (OXPHOS) metabolites (coenzymes Q9 and Q10) and aspartate-malate shuttle metabolites (aspartate and glutamate)[16].

Moreover, the authors predicted and demonstrated in vitro, sensitivity of the glycolytic subtypes to aerobic glycolysis inhibitors (oxamate and the LDHA inhibitor GNE-140), glutaminolysis inhibitor (BPTES) and inhibitors of gamma-glutamylcysteine synthetase (BSO) and cystine transporter [(S)-4-CPG], and also demonstrated sensitivity of the lipogenic subtype to lipid synthesis inhibitors (FASN inhibitor GSK1195010, SCD inhibitor, cerulenin, and orlistat). They also performed *in vivo* confirmatory tests, inducing 68% tumour growth inhibition of the glycolytic cell line with LDHA inhibition and 52% tumour growth inhibition in the lipogenic cell line with SCD inhibition[16]. Table 1 summarises these studies and the clinically significant findings.

**Table 1 Summary of molecular subtyping studies**

| Ref. | Subtypes | Clinical significance |
|---|---|---|
| | **Genomic** | |
| Waddell *et al* [11] | Stable, locally re-arranged, scattered and unstable | High BRCA mutational signature in the unstable subtype, sensitizing to PARPi and PBC. |
| Singhi *et al*[12] | - | Real time genetic sequencing. 17% of specimens found to have sensitivities to available treatments. Potential therapeutic targets. |
| Aguirre *et al* [13] | SigA, SigB and SigC | Potential targets in 40% of patients. 48% eligible for trials/off-label use. Of 24% enrolled onto a clinical trial. |
| | Proteomic | |
| Law *et al*[14] | Inflammatory, proliferative, progenitor-like and metabolic | ↓Risk of death in metabolic and progenitor-like subtypes treated with FOLFIRINOX+Gemcitabine.<br>↑Survival in progenitor-like subtype treated with gemcitabine. SHMT1 a potential mediator of gemcitabine resistance. |
| Humphrey *et al*[15] | TKCC subtypes 1, 2 and 3<br>ATCC subtypes 1, 2 and 3 | Subtypes 3 in both cohorts showed increased sensitivity to erlotinib, potentially mediated by tyrosine phosphorylation of RTK sites. |
| | Metabolomic | |
| Daemen *et al* [16] | Slow proliferating, glycolytic and lipogenic | Glycolytic subtype sensitive to inhibitors of aerobic glycolysis, glutaminolysis, γ-glutamylcysteine and Xct. Lipogenic subtype sensitive to lipid synthesis inhibitors. |

BRCA; Breast cancer gene; RTK: Receptor tyrosine kinase; PBC: Platinum-based chemotherapy; PARPi: Poly (ADP-ribose) polymerase inhibitors; xCT: Cystine transporter; SHMT1: Serine hydroxymethyltransferase 1.

## BIOMARKER DISCOVERY

Currently, the only biomarker for PDAC is carbohydrate antigen 19-9 (CA19-9), approved by the Food and Drug Administration for use in clinical practice[17]. Unfortunately, the median sensitivity and specificity of CA19-9 is 79% and 82% respectively, making it unsuitable for use as a diagnostic marker, with it being raised in other gastrointestinal pathology[18]. Further complicating this is the fact that roughly 10% of the population with a Lewis-negative genotype do not express CA19-9 (a sialyl-Lewis A tetrasaccheride) at all. Its use currently is limited to monitoring CA19-9 positive PDAC for progression or recurrence after resection[17]. There is an obvious need for further investigation for potential biomarkers for early diagnosis, prognosis and sensitivity/resistance to therapeutics. "Omics" techniques have the capability to produce large amounts of data which can be correlated with specific states of the biological system and so there is great potential for this data to be used for biomarker discovery.

As mentioned earlier, one of the main factors that influence the outcomes in PDAC is that the majority are diagnosed at an advanced stage. With surgical resection currently being the only curative option, improving the proportion of patients eligible for surgery would drastically improve outcomes. The use of "omics" technologies to screen for potential diagnostic markers that can accurately differentiate PDAC from other pathologies or normal healthy tissue can lead the way to targeted diagnostic panels that can be implemented clinically.

An emerging area of research is the use of "liquid biopsy", which is the sampling of tumour material which spills into the circulation[19]. The main components of such a biopsy include circulating tumour cells (CTC), cell-free DNA (cfDNA) and circulating exosomes. cfDNA is genetic material released into the circulation from benign and malignant cells during cell death, circulating tumour DNA (ctDNA) being the subset of this derived from malignant cells. Exosomes are extracellular vesicles released from cells into various bodily fluids and can contain proteins and genetic material for analysis. They have a longer half-life and are constantly being produced by cells making them more readily available for isolation than cfDNA[19].

Zhu *et al*[20] conducted a systematic review and meta-analysis of 19 studies utilising ctDNA, CTCs and exosomes to diagnose "pancreatic adenocarcinoma", "pancreatic ductal adenocarcinoma" or "pancreatic cancer". They found that the overall sensitivity, specificity and area under the curve (AUC) for liquid biopsy was 0.8 [95% confidence interval (CI): 0.77-0.82], 0.89 (95%CI: 0.87-0.91) and 0.93 respectively, demonstrating the feasibility of liquid biopsy as a diagnostic tool. Of the three components,

exosomes were found to have the highest accuracy with a sensitivity, specificity and AUC of 0.93 (95%CI: 0.90-0.95), 0.92 (95%CI: 0.88-0.95), and 0.9819 respectively. The authors suggested that the high sensitivity of the exosomes was due to the exocrine function of the pancreases and the high specificity due to the methods used to analyse the exosomes in these studies [polymerase chain reaction (PCR) and flow cytometry] [20].

Overall sensitivity, specificity and AUC of the CTC studies was 0.74 (95%CI: 0.68-0.79), 0.83 (95%CI: 0.78-0.88), and 0.8166[20]. This was thought to be due to hepatic trapping of CTCs and due to reduced blood flow in pancreatic malignancies compared to normal tissue (decreasing the chances of cell shedding into the circulation)[20]. Indeed, it was found that for PC, the levels of CTC was the lowest compared to other types of cancer[21].

ctDNA's overall sensitivity, specificity and AUC was 0.64 (95%CI: 0.58-0.70), 0.92 (95%CI: 0.88-0.95), and 0.9478[20]. All of the ctDNA studies utilised PCR of KRAS mutations to distinguish PC from either healthy controls, pancreatitis or benign lesions. As mentioned before, KRAS is the most common genetic driver of PDAC and so utilising this as the marker for detection may be the reason for the high specificity. The relatively low sensitivity however may have been due to the abundance of ctDNA in the circulation[20]. It has been found that the abundance of ctDNA has a positive correlation with tumour load, supported by the physiology of ctDNA mentioned above: It is released through cell death[22]. Due to this, ctDNA may not be an ideal candidate for use in early detection of PDAC but may have a role as a prognostic biomarker or a marker of response to treatment, especially in those that are CA19-9 negative.

In terms of protein biomarkers, many studies have been done to characterise the differences in the proteomes of PDAC and normal control (NC) specimens from various sources including tissue samples, cell lines, serum/plasma and pancreatic juice[8]. While quite a few studies have been done, only a few of the biomarkers described overlap between studies. Further to this, none of these biomarkers have been put to use clinically, mainly due to the lack of validation in clinical trials, and standardised, reproducible and cost-effective analytical methods.

MS data combined with the results of a literature review by Capello *et al*[23] yielded 17 plasma protein biomarkers to distinguish early stage PDAC from benign pancreatic disease and NC. They tested these biomarkers using ELISA, first in a triage set which narrowed these down to 7 biomarkers, followed by validation of these 7 biomarkers in three independent plasma sample sets. Statistical analysis of the performance of these 7 biomarkers led to the development of a 3 biomarker panel of metalloproteinase inhibitor 1, leucine rich alpha-2-glycoprotein 1 and CA 19-9 which was able to differentiate PDAC cases from healthy controls with an AUC of 0.887 (95%CI: 0.817-0.957) in a blinded test set, which was a statistically significant improvement compared to CA 19-9 alone. The sensitivities at a fixed 95% and 99% specificity were 0.667 and 0.410 respectively, compared to the sensitivities of 0.538 and 0.462 respectively for CA 19-9 [23].

In terms of prognostic biomarkers, de Oliveira *et al*[24] recently conducted a meta-analysis of MS data from two systematic reviews of PDAC secretome and proteome. No protein was found to be present in all the studies and so the authors selected those that were presented in at least 2 studies, generating a list of 39 secreted proteins. Further gene expression analysis of 4747 tumours (of 10 types of cancers) and 2737 corresponding normal tissues for these proteins revealed that 31 of these were unregulated in PDAC, and when analysed for 10 cancer types showed that all 39 genes were enriched in PDAC *vs* the other types, with an opposite expression profile in acute myeloid leukaemia. Further to this, the authors displayed a correlation between the gene expression for these proteins and significantly shorter survival (hazard ratio = 5.36) in PDAC patients, which was validated in three independent data sets[24].

Peng *et al*[25] developed a protein signature to predict response to chemotherapy through LC-MS/MS of prepared serum samples from 16 stage IV PDAC patients. The three protein biomarker candidates vitamin-K dependent protein Z, sex hormone-binding globulin and von Willebrand factor, combined with CA 19-9 were used to make a biomarker panel, with biomarker positive patients having significantly shorter median survival in both stage III and stage IV patients [8.7 mo (95%CI: 6-11.7) for biomarker negtive *vs* 19.2 mo (95%CI: 11.4-22.1) for biomarker positive].

A meta-analysis of metabolomic biomarkers for PC by Mehta *et al*[26] yielded 21 deregulated blood based biomarkers that appeared in at least 2 studies. The authors developed a 10 metabolite diagnostic panel from these biomarkers which was tested on plasma samples of 192 patients from four diagnostic groups: PC ($n$ = 59), NC ($n$ = 48), colorectal cancer (CRC, $n$ = 66) and type 2 diabetes mellitus (T2DM, $n$ = 19). The

**Table 2 Summary of biomarker discovery studies**

| Ref. | Biomarkers | Sensitivity | Specificity | AUC |
|---|---|---|---|---|
| Zhu *et al*[20] | ctDNA | 0.64 | 0.92 | 0.9478 |
| | CTC | 0.74 | 0.83 | 0.8166 |
| | Exosome | 0.93 | 0.92 | 0.9819 |
| Capello *et al*[23] | TIMP1, LRG1 and CA19-9 | 0.667/0.410 | 0.95/0.99 | 0.887 |
| Mehta *et al*[26] | Panel of: Lactate, LysoPC (18:2), Alanine, Choline, Threonine, Asparagine, Tyrosine, Lysine, Palmitate and 3-hydroxybutyrate | - | - | 0.992 *vs* NC |
| | | | | 0.957 *vs* T2DM |
| | | | | 0.653 *vs* CRC |
| | | Biomarker + ve median survival | | Biomarker - ve median survival |
| Peng *et al*[25] | Panel of: PZ, SHBG, VWF and CA19-9 | 19.2 mo | | 8.7 mo |
| | | | *AUC* | |
| Martín-Blázquez *et al*[27] | Panel of: PS (12:0/15:1), TG (22:2/15:0/18:3), 4-oxo-Retinoic acid, Androsterone sulfate, LysoPE (18:2), Phenylalanylphenylalanine, all-trans-Decaprenyldiphosphate, LysoPC (18:2) and Dehydroepiandrosterone sulfate | | 0.992 (CI: 0.972-1.000) | |

ctDNA: Circulating tumour DNA; CTC: Circulating tumour cells; TIMP1: Metalloproteinase inhibitor 1; LRG1: Leucine rich alpha-2-glycoprotein 1; CA19-9: Carbohydrate antigen 19-9; CI: Confidence interval; NC: Normal control; T2DM: Type 2 Diabetes mellitus; CRC: Colorectal cancer.

AUC of PC, T2DM and CRC *vs* NC were 0.992 (95%CI: 0.977-1), 0.957 (95%CI: 0.868-1) and 0.986 (95%CI: 0.967-1) respectively. The AUC of PC *vs* CRC was only 0.653 (95%CI: 0.543-0.757), suggesting a lack of specificity of the panel between these two groups. An index of the 10 metabolite panel showed that higher index values correlated with increased risk of malignancy, with a value of ≥ 12.5 representing a 100% risk of PC [26]. While the authors used the term "pancreatic cancer", in their study, the authors manually curated the literature to ensure the comparison groups were PDAC patients and controls.

More recently, Martín-Blázquez *et al*[27] analysed serum from unresectable PDAC patients and NC using reverse-phase liquid chromatography and high-resolution MS to identify 86 significant metabolites. With these, the researchers proposed a model of 9 markers that discriminated PDAC from healthy controls with an AUC of 0.992 (95%CI: 0.972-1.000). Table 2 summarises these molecular subtyping studies while Figure 1 demonstrates subtypes and biomarkers by "Omics" level[28].

## CONCLUSION

### *Closing thoughts and future considerations*

"Omics" technologies have allowed mining of massive amounts of data, giving new insights into the complex, heterogeneous nature of PDAC. As described above, many studies have been done to describe molecular classifications and potential biomarkers, but none of these have yet been translated into clinical practice[28]. Several trials, as reviewed by Du *et al*[29], faced difficulties in sample procurement, low quality of samples and waiting time for sample analysis leading to patient deterioration or withdrawal. The authors suggest a multi-disciplinary approach with specialist input in sample acquisition in designated centres with high levels of experience. Issues regarding the technology should improve as these become more accessible to the clinical setting. Indeed, these difficulties were echoed by lessons learnt from the Individualized Molecular Pancreatic Cancer Therapy trial, a study that aimed to match patients with recurrent or metastatic PDAC to treatment based on genomic data[30]. Unfortunately, no patients were able to be enrolled into the study largely due to the quick progression of PDAC and due to inadequate numbers meeting the eligibility criteria. The investigators described four criteria that would need to be met for such a study to take place: (1) Screening of a sufficient number of patients; (2) Timely acquisition of tumour material for analysis; (3) Quick turnaround time of usable data; and (4)

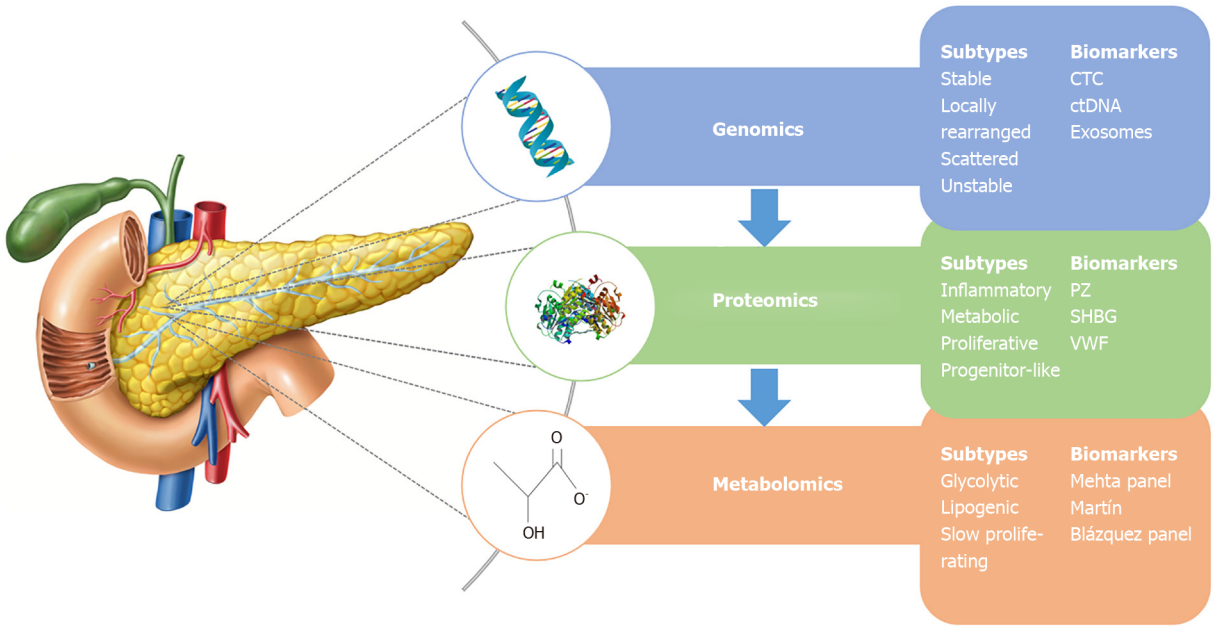


**Figure 1 Genomic, proteomic and metabolomic subtypes and biomarkers of pancreatic cancer.** Double stranded DNA image[28]. CTC: Circulating tumour cells; ctDNA: Circulating tumour DNA; PZ: Vitamin-K dependent protein Z; SHBG: Sex hormone-binding globulin; VWF: Von Willebrand Factor.

Effective treatments or clinical trials for enrollment[30]. Currently, there is an exciting initiative by PrecisionPanc in the United Kingdom, where through a "Master Protocol", patients suspected or diagnosed with PDAC will undergo prospective molecular profiling to guide enrollment into one of their five PRIMUS trials[31].

Additionally, many of these studies are largely comparative, most of them describing the difference in data between disease and normal states. Few studies have correlated their findings with upstream/downstream "omics" data and so there is an obvious need for integrated analysis of multiple "omics" levels. An example of integrated analysis is seen in the study by The Cancer Genome Atlas Research Network, who performed genomic, transcriptomic and proteomic analysis of 150 PDAC specimens using whole-exon sequencing, DNA methylation assays, RNA sequencing and reverse phase protein arrays[32]. The authors described molecular profiles along each of these "omic" levels, potential subtypes along transcriptomic and proteomic lines, potential therapeutic targets and through integrated analysis were able to describe some of the interplay between these levels, giving further insight into the complexity of PDAC. Another example is the study by Follia *et al*[33] who used genomic and transcriptomic data to describe four metabolic subtypes of PDAC (2 glycolytic and 2 non-glycolytic) which they correlated with the subtypes described by Daemen *et al*[16].

Finally, with such high throughput technologies being used, cancer research is moving into the realm of "big data". "Wide" data sets (where the number of variables exceed the number of subjects) such as those produced by "omics" technologies are better analysed through machine learning/artificial intelligence than traditional statistical analysis[34]. Over the past few years, many studies have been done using machine learning methods for molecular subtyping and biomarker discovery in other types of malignancies, simultaneously using data from multiple "omics" levels and there is great potential for machine learning and artificial intelligence applications in PDAC research[35].

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*MINIREVIEWS*

# Screening and prevention of hepatitis C virus reactivation during chemotherapy

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**Author contributions:** Li YR developed the theory and performed the computations; Chen WC and Hsu PI verified the analytical methods; Hu TH, Chen HC and Hsu PI supervised the findings of this work; all authors discussed the results and contributed to the final manuscript.

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## Abstract

Hepatitis C virus (HCV) reactivation occurs in 23% of HCV-infected cancer patients receiving chemotherapy. Forty-three percent of the patients with reactivation of HCV during chemotherapy develop a hepatitis flare. Most of the cancer patients with HCV reactivation have an unremarkable clinical course following an HCV-related hepatitis flare during chemotherapy. However, 26%–57% of the cancer patients developing an acute flare of chronic hepatitis C during chemotherapy require unanticipated discontinuation or dose reduction of chemotherapy, which results in deleterious changes in the cancer treatment plan. Although an optimal strategy for HCV screening in cancer patients receiving chemotherapy has not been established, universal pre-chemotherapy HCV testing for patients with hematological malignancies is recommended by current guidelines. All the currently approved direct-acting antivirals (DAAs) can be used in cancer patients, but the use of DAAs during chemotherapy should avoid drug–drug interactions between chemotherapy and antiviral agents. If there are no contraindications or anticipated drug–drug interactions, DAAs treatment can be administered before, during, or after chemotherapy. In conclusion, HCV reactivation occurs in approximately one-fourth of HCV-infected cancer patients receiving chemotherapy. An HCV-related hepatitis flare during chemotherapy may lead to the discontinuation of potentially life-saving chemotherapy.

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Currently, universal HCV screening is recommended in hematological malignancy patients before chemotherapy, but there is no evidence-based guideline for other cancer patients. DAAs treatment can cure HCV infection and prevent HCV reactivation during chemotherapy.

**Key Words:** Hepatitis C virus; Chemotherapy; Screening; Reactivation; Hepatitis flare

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**Core Tip:** Hepatitis C virus (HCV) reactivation occurs in approximately one-fourth of HCV-infected cancer patients receiving chemotherapy. An HCV-related hepatitis flare during chemotherapy may lead to the discontinuation of potentially life-saving chemotherapy. Currently, universal HCV screening is recommended in hematological malignancy patients before chemotherapy, but there is no evidence-based guideline for other cancer patients. direct-acting antivirals treatment can cure HCV infection and prevent HCV reactivation during chemotherapy.

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## INTRODUCTION

Hepatitis C virus (HCV) infection affects millions of people worldwide and is a significant burden for cancer patients[1,2]. The prevalence of chronic HCV infection among cancer patients in the United States ranges from 1.5% to 10.6%[3]. A recent study with universal pre-chemotherapy screening of HCV infection by testing for anti-HCV antibody showed that the prevalence of HCV infection in cancer patients receiving chemotherapy was 6.0% (337/5601) in Taiwan[4]. Chemotherapy can lead to immunosuppression and reactivate quiescent HCV infection in cancer patients[5-13]. The acute flare of HCV infection may result in deleterious changes in the cancer treatment plan and has a negative impact on the treatment outcome of cancer patients. Currently, evidence-based guidelines for HCV screening and treatment in cancer patients undergoing chemotherapy have not been established because data on the efficacy of pre-chemotherapy HCV testing and treatment are very limited. In this article, we review the incidence of HCV reactivation during chemotherapy and the outcome of HCV-related hepatitis flare in cancer patients receiving chemotherapy. Additionally, the recommendations for the pre-chemotherapy HCV screening and the prevention of HCV reactivation during chemotherapy are also reviewed.

## DEFINITION OF QUIESCENT HCV INFECTION, HCV REACTIVATION AND HCV-RELATED HEPATITIS FLARE IN CANCER PATIENTS RECEIVING CHEMOTHERAPY

This article summarizes current evidences dealing with HCV reactivation and HCV-related hepatitis flare in cancer patients receiving chemotherapy. It is worthy to note that the definitions of HCV reactivation and hepatitis flare during chemotherapy varied in previous studies[4,14-22]. In this review article, we list the definitions of HCV reactivation and HCV-related hepatitis flare in each study quoted in the Tables. The definitions of HCV reactivation and HCV-related hepatitis flare recommended by authors are summarized in Table 1. Recommended HCV reactivation during chemotherapy is increase in HCV-RNA level of ≥ 1 $Log_{10}$ IU/mL over baseline[17], and recommended definition of HCV-related hepatitis flare is unexplained increase in alanine aminotransferase (ALT) to 3 times the upper limit of normal during chemotherapy and increase in HCV-RNA level of ≥ 1 $Log_{10}$ IU/mL over baseline[18]. Most retrospective works lacked the data of HCV viral load before chemotherapy. The

**Table 1 Recommended definitions of quiescent hepatitis C virus infection, hepatitis C virus reactivation and hepatitis C virus-related hepatitis flare in cancer patients receiving chemotherapy**

| Term | Definition | Ref. |
|---|---|---|
| Quiescent HCV infection | An HCV infection with a positive serum HCV-RNA and normal serum levels of liver enzymes | IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 59[30] |
| HCV reactivation | Increase in HCV-RNA level during chemotherapy of ≥ 1 $\log_{10}$ IU/mL over baseline | Talima *et al*[17] |
| HCV-related hepatitis flare | Unexplained increase in ALT to 3 times the upper limit of normal during chemotherapy and increase in HCV-RNA level of ≥ 1 $\log_{10}$ IU/mL over baseline | Torres *et al*[18] |

IARC: International Agency for Research on Cancer; HCV: Hepatitis C virus.

scientific strengths of these retrospective evidences were therefore not robust.

## INCIDENCE OF HCV REACTIVATION IN CANCER PATIENTS RECEIVING CHEMOTHERAPY

In a retrospective observation study by Lee *et al*[16], enhanced replication of HCV (increase in HCV-RNA level of ≥ 1 $\text{Log}_{10}$ IU/mL over baseline) was noted in 9 (27%) of 33 HCV-infected cancer patients who underwent chemotherapy. Another retrospective study by Talima *et al*[17] demonstrated that the incidence of HCV reactivation (increase in HCV-RNA level of ≥ 1 $\text{Log}_{10}$ IU/mL over baseline) in 34 HCV-infected breast cancer patients receiving chemotherapy was 6% (2/34). In a prospective observation study by Torres *et al*[18], reactivation of HCV infection (increase in HCV-RNA level of ≥ 1 $\text{Log}_{10}$ IU/mL over baseline) occurred in 23 (23%) of 100 cancer patients undergoing chemotherapy. Among these cancer patients, those with hematological malignancies had a higher incidence of HCV reactivation than those with solid tumors (36% *vs* 10%).

## INCIDENCE OF HCV-RELATED HEPATITIS FLARE IN CANCER PATIENTS RECEIVING CHEMOTHERAPY

HCV-related hepatitis flare is a significant burden for cancer patients undergoing chemotherapy[6,19-20]. Table 2 displays the incidence of HCV-related hepatitis flare in cancer patients receiving chemotherapy. In a retrospective observation study by Li *et al*[4], universal pre-chemotherapy screening of HCV infection by testing for anti-HCV antibody was conducted in 5601 cancer patients undergoing chemotherapy. HCV-infected cancer patients had a higher incidence of severe acute liver injury (serum ALT increases beyond 10 times the upper limit of normal during chemotherapy or 6 mo following chemotherapy) than those without HCV infection (2.3% *vs* 0.7%). Among the HCV-infected patients who did not have chronic HBV infection, the incidences of severe liver injury in those with hematological malignancy, hepatocellular carcinoma (HCC) and non-HCC solid tumors were 9.4% (3/32), 1.9% (2/105), and 1.2% (2/169), respectively. In this study, the incidence of severe acute liver injury in HCV-infected hematological cancer patients was higher than that in those with HCC patients and non-HCC solid tumor patients (9.4% *vs* 1.9 % and 1.1%, respectively). Rituximab-containing chemotherapy and hematological malignancy were identified risk factors related to severe acute exacerbation of HCV infection in cancer patients undergoing chemotherapy. In another retrospective study by Mahale *et al*[5], the incidence of acute exacerbation of HCV infection (3-fold or greater increase in serum ALT level) during chemotherapy was 23% in 104 patients with hematological malignancy and 4% in 204 patients with solid tumors. The former also had a higher incidence of HCV-related acute exacerbation than the latter. In a prospective observation study at MD Anderson Cancer Center[18], reactivation of HCV infection occurred in 23% of 100 cancer patients receiving chemotherapy. Among those with HCV reactivation, 10 patients (43%) developed a hepatitis flare (unexplained increase in ALT to 3 times the upper limit of normal). Overall, the incidence of HCV-related hepatitis flare in cancer patients receiving chemotherapy was 10% in this prospective study.

**Table 2 Incidence of hepatitis C virus-related hepatitis flare in cancer patients receiving chemotherapy**

| Ref. | Study type | Cancer type | Results |
|---|---|---|---|
| Torres *et al* [18] | Prospective observation study | Hematological tumor ($n$ = 50) | A hepatitis flare occurred in 10% of HCV-infected cancer patients receiving chemotherapy |
| | | Non-HCC solid tumor ($n$ = 50) | Definition of HCV-related hepatitis flare: unexplained increase in ALT to 3 times the upper limit of normal and increase in HCV-RNA level of ≥ 1 $\log_{10}$ IU/mL over baseline |
| Li *et al*[4] | Retrospective observation study | Hematological tumor ($n$ = 569); HCC ($n$ = 256); Non-HCC solid tumor ($n$ = 3900) | The incidence of severe acute liver injury in HCV-infected hematological cancer patients was higher than that in those with HCC patients and non-HCC solid tumor patients (9.4% *vs* 1.9 % and 1.1%, respectively). Definition of severe acute liver injury: ALT increased beyond 10 times the upper limit of normal during chemotherapy or 6 months following chemotherapy |
| Tomizawa *et al*[28] | Retrospective observation study | Colorectal cancer ($n$ = 24) | The incidence of severe acute exacerbation rate in HCV-infected patients was 8%. Definition of severe acute exacerbation: ALT increased beyond 5 times the upper limit of normal |
| Hsu *et al* [22] | Retrospective observation study | Hematological tumor ($n$ = 104); Solid tumor ($n$ = 204) | The incidence of HCV acute exacerbation was 11% (hematological tumor: 23%; solid tumor: 4.4%). Definition of HCV acute exacerbation: 3-fold or greater increase in serum ALT level |

HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma; ALT: Alanine aminotransferase.

## CLINICAL OUTCOME OF HCV-RELATED HEPATITIS FLARE DURING CHEMOTHERAPY

Most of the cancer patients with HCV reactivation have an unremarkable clinical course following HCV flare during chemotherapy. In a prospective observation study by Fujii *et al*[6], 6 (28.6%) of 21 Leukemia patients with HCV-related hepatitis flare during chemotherapy developed hepatic decompensation (Table 3). Another prospective observation study by Torres *et al*[18] showed that none of 23 cancer patients (hematological malignancy: $n$ = 18; solid tumor: $n$ = 5) with HCV-related hepatitis flare during chemotherapy developed liver decompensation or mortality. In a retrospective study including 33 hematological cancer patients with HCV-related hepatitis flare, the incidence of hepatic decompensation was also 0%[7]. Although most of the cancer patients with HCV reactivation or hepatitis flare during chemotherapy have a benign clinical course, a significant number of patients with severe flare of HCV infection have to discontinue potentially life-saving chemotherapy. Currently, there are no randomized controlled trials comparing the outcomes of the patients with chemotherapy-related HCV reactivation who stop chemotherapy and who go on cancer treatment. Therefore, whether chemotherapy should be stopped in cancer patients with HCV reactivation remains unclear. Nonetheless, physicians often discontinue chemotherapy in HCV-infected cancer patients who develop severe liver dysfunction during cancer treatment because it is a life-threatening condition and can be induced by either chemotherapeutic drugs or viral reactivation.

In a retrospective study by Li *et al*[4], four of seven patients (57.1%) with HCV-related severe acute liver injury discontinued chemotherapy due to hepatitis flare. In a prospective study by Torress *et al*[18], 6 of 23 HCV-infected patients (26%) with hepatitis flare required unanticipated discontinuation or dose reduction of chemotherapy. Since interruption of chemotherapy in cancer patients would result in deleterious changes in the cancer treatment plan and has a negative impact on patient outcome, the aforementioned findings support the identification and treatment of chronic HCV infection to prevent HCV reactivation and hepatitis flare.

## PRE-CHEMOTHERAPY HCV SCREENING IN CANCER PATIENTS

A standard strategy for HCV screening in cancer patients before chemotherapy has not been established. However, a retrospective study by Hosry *et al*[21] demonstrated that early diagnosis of HCV infection with virological cure improved the outcomes of cancer and survival of HCV-infected patients who developed non-Hodgkin lymphoma. To improve pre-chemotherapy HBV and HCV testing, Hsu *et al*[22] developed a computerized order entry-based therapeutic control system (e-CONTROL) to notify healthcare providers in a medical center in Taiwan for pre-

**Table 3 Clinical outcomes of hepatitis C virus-related hepatitis flare during chemotherapy**

| Ref. | Study type | Cancer type | Liver decompensation | Interruption of chemotherapy | Mortality due to HCV reactivation |
|---|---|---|---|---|---|
| Torres *et al*[18] | Prospective observation study | Hematological tumor ($n$ = 18)<br>Solid tumor ($n$ = 5) | Total: 0% | Total: 26% | Total: 0% |
| Fujii *et al*[6] | Prospective study | Leukemia ($n$ = 21) | Total: 28.6% | – | – |
| Li *et al*[4] | Retrospective observation study | Hematological tumor ($n$ = 3)<br>HCC ($n$ = 2)<br>Non-HCC solid tumor ( $n$ = 2) | Total: 0%<br>(Hematological tumor: 0%;<br>HCC: 0%;<br>Non-HCC solid tumor: 0%) | Total: 57%<br>(Hematological tumor: 67%;<br>HCC: 50%;<br>solid tumor: 50%) | Total: 0%<br>(Hematological tumor: 0%;<br>HCC: 0%;<br>Non-HCC solid tumor: 0%) |
| Lee *et al*[16] | Retrospective study | Hematological tumor ($n$ = 14)<br>Solid tumor ($n$ = 11) | Total: 0% | Total: 32% | Total: 0% |
| Mahale *et al*[5] | Retrospective study | Hematological tumor ($n$ = 24)<br>Solid tumor ($n$ = 9) | Total: 0% | Total: 45% | Total: 0% |
| Zuckerman *et al*[7] | Retrospective study | Hematological tumor ($n$ = 33) | Total: 0% | – | Total: 0% |

HCV: Hepatitis C virus; HCC: Hepatocellular carcinoma.

chemotherapy HBV and HCV testing. The e-CONTROL system achieved a pre-chemotherapy HCV screening rate of 97.7% (5601/5735). The HCV screening rate in cancer patients receiving chemotherapy is low in the United States. According to an observation study at MD Anderson Cancer Center, the HCV screening rate in cancer patients receiving chemotherapy was only 13.9%[23]. Currently, an optimal strategy for HCV screening in cancer patients receiving chemotherapy has not been established. Figure 1 illustrates current recommendations for testing and treating HCV infection in cancer patients receiving chemotherapy[24,25,29]. The 5th European Conference on Infections in Leukemia recommended that all patients with hematological malignancy be screened for hepatotropic viruses (HBV and HCV) before cancer treatment[24]. The European Association for the Study of the Liver (ESAL) recommends that all patients with malignancy should be screened for HCV infection before cancer treatment due to HCV reactivation possibility after treatment[25].

Anti-HCV antibody is a recommended tool for initial screening of HCV infection in cancer patients receiving chemotherapy because of its cheap and cost-effective advantages. However, the disadvantage of screening HCV infection by serum anti-HCV antibody is a positive result indicating either current or past HCV infection. Therefore, cancer patients with a positive result of anti-HCV antibody should be further tested for serum HCV RNA to confirm current infection status of HCV (Figure 1). In clinical practice, serum HCV RNA is not recommended as a routine screening tool for HCV infection in cancer patients because it is an expensive diagnostic method.

## TREATMENT OF HCV INFECTION IN CANCER PATIENTS RECEIVING CHEMOTHERAPY

All the currently approved direct-acting antiviral (DAA) agents can be applied in cancer patients, but the use of DAA treatment during chemotherapy should avoid drug–drug interactions between chemotherapy agents and antivirals (Figure 1). The initiation of DAA treatment in HCV-infected cancer patients undergoing chemotherapy should be individualized and determined by the cancer treatment plan. Contraindications to DAA treatment in HCV-infected cancer patients include (1)

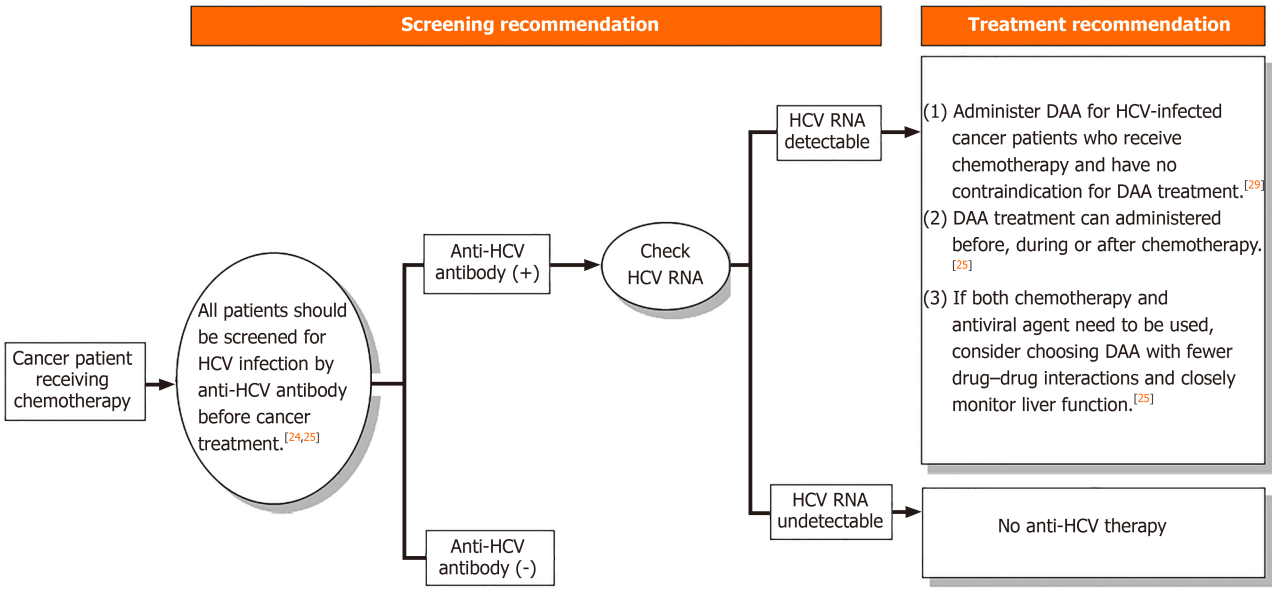


**Figure 1 Current recommendations for testing and treating hepatitis C virus infection in cancer patients receiving chemotherapy.** HCV: Hepatitis C virus; DAA: Direct-acting antiviral.

Pregnancy; (2) Uncontrolled cancer; (3) Patients with a life expectancy of < 12 mo that cannot be remediated by cancer treatment; (4) Hypersensitivity or intolerance to DAAs; and (5) Anticipated major drug–drug interactions with cancer treatment. If there are no contraindications or anticipated drug–drug interactions, DAA treatment can be administered before, during or after chemotherapy.

The use of DAAs for HCV infection in infected cancer patients receiving chemotherapy may increase the risk of drug–drug interactions. Physicians can identify potentially significant interactions between DAAs and chemotherapy agents based on information obtained from current databases (http://hepdruginteractions.org) and then choose adequate DAAs for HCV treatment or alter the regimen of chemotherapy to avoid drug-drug interactions. In general, treatment for HCV infection in cancer patients receiving chemotherapy by sofosbuvir/Ledipasvir or glecaprevir/pibrentasvir is safe and effective[26]. A prospective observation study from MD Anderson Cancer Center showed that the cure rate of HCV infection by sofosbuvir-based therapy in HCV-infected cancer patients was 91%[27].

## CONCLUSION

In conclusion, chemotherapy can lead to immunosuppression and reactivate quiescent HCV infection. Most of the cancer patients with HCV reactivation have an unremarkable clinical course following HCV hepatitis flare during chemotherapy. However, 26%–57% of the cancer patients developing acute exacerbation of chronic hepatitis C during chemotherapy require unanticipated discontinuation or dose reduction of chemotherapy. Currently, the optimal strategy for HCV screening in cancer patients receiving chemotherapy has not been established. Nonetheless, the ESAL recommends that all patients with malignancy should be screened for HCV before cancer treatment due to HCV reactivation possibility after treatment. Currently, universal HCV screening is recommended in hematological malignancy patients before chemotherapy, but there is no evidence-based guideline for other cancer patients. Administration of DAAs can cure HCV infection and prevent HCV reactivation during chemotherapy.

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*MINIREVIEWS*

# Role of near-infrared fluorescence in colorectal surgery

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## Abstract

Near-infrared fluorescence (NIRF) is a technique of augmented reality that, when applied in the operating theatre, allows the colorectal surgeon to visualize and assess bowel vascularization, to identify lymph nodes draining a cancer site and to identify ureters. Herein, we review the literature regarding NIRF in colorectal surgery.

**Key Words:** Fluorescence; Enhanced reality; Anastomotic leak; Ureter; Anastomosis

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**Core Tip:** Near-infrared fluorescence appears to be a useful tool that assists surgeons performing colorectal surgery by identifying poorly vascularized areas of the bowel and therefore decreasing the incidence of anastomotic leak, visualizing lymphatic drainage and identifying the ureters during difficult surgery.

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## INTRODUCTION

Augmented reality (AR) is increasingly used in the operating room to help surgeons in

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their work. AR is created by overlaying digital information on an image being viewed through a device in real time, allowing us to extend our perception of reality.

Fluorescence is the property of absorbing light of short wavelengths and emitting light of longer wavelengths. In near-infrared (NIR) fluorescence (NIRF) imaging, a fluorescent dye, namely a fluorochrome emitting in the NIR spectrum, is injected intravenously or directly into the tissue. The AR acquisition system functions as a laparoscope but uses two cameras at the same time: A white light camera acquiring real-world images and a second infrared camera for acquiring the fluorescence images emitted by the dye. On the fluorescent images, the rays emitted by the fluorochromes are visualized by white pixels on a black background. The AR image is then constructed by superposition of the real-world image and the recoloured NIR image.

Radiation, like light, penetrates biological tissue while becoming increasingly depleted in energy up to the depth at which all energy transported has been absorbed. Biological tissues absorb and scatter shorter wavelength light, and NIR light can penetrate biological tissues (such as skin and blood) more efficiently than visible light. Therefore, it creates a "transparency" effect of the tissues and allows the acquisition of fluorescent images through several millimetres of depth.

Indocyanine green (ICG) is the most commonly used fluorophore in NIRF imaging and has been approved by the Food and Drug Administration for clinical and research use in humans since 1956. Its fluorescent capabilities have been used increasingly since the 2000s in various specialties, such as neurosurgery, plastic surgery, gynaecology or general surgery. ICG is an iodine dye[1] that can be injected intravenously. The compound is 98% bound to plasma proteins and has a hepatic clearance rate of 18% to 24% *per* minute into the bile. This leads to a half-life of generally 3 to 4 min depending on the vascularization of the organ of interest, with no known metabolites. Its quick clearance rate allows the dye to be used for multiple injections during a procedure. ICG is excited between 750 and 800 nm, and fluorescence is viewed at approximately 830 nm[2]. This fluorochrome is well-tolerated in patients. However, there have been few described cases of hypotension, tachycardia, dyspnoea, urticarial and anaphylactic shock[3]. In colorectal surgery, NIRF imaging uses not only ICG but also other fluorochromes and is employed for three main functions: Estimation of the blood supply, oncological applications and highlighting of anatomical elements such as the ureters.

## PREVENTION OF ANASTOMOTIC LEAKS

In colorectal surgery involving intestinal resection, the most feared complication is anastomotic leak (AL), which occurs in 8.1% of procedures according to the European Society of Coloproctology snapshot audit for right hemicolectomies[4] and in up to 17.5% of low colorectal anastomoses. AL causes devastating morbidity and mortality [5,6]. Complete anastomosis healing requires adequate perfusion; therefore, one of the most important risk factors for AL is poor perfusion of the anastomosis[7,8]. Intraoperatively, the selection of an optimal site for anastomosis depends on subjective clinical indicators of good perfusion, such as the colour of the bowel wall, palpable pulsations of the mesenteric arteries, or bleeding of the resection margins[9,10]. Nonetheless, two studies[11,12] showed that the surgeon's subjective AL prediction based on perfusion underestimated the risks of AL. Numerous objective quantitative techniques of intraoperative bowel viability assessment exist, such as measurement of tissue oxygen levels, laser Doppler flowmetry, pulse oximetry, pH measurement and NIR fluorescence angiography[13], but only a few are applicable in colorectal surgery, especially in laparoscopic surgery.

NIR fluorescence angiography using ICG as a fluorochrome is an ever-increasing technique used in the operating room. The first clinical study reporting this method in colorectal surgery was performed by Kudszus *et al*[14] in 2010. ICG was injected intravenously to highlight the microvasculature and enabled surgeons to detect poorly perfused areas more precisely[3]. Indeed, ischaemic demarcation of the intestine is visible with normal light more than 10 min after vessel division, whereas NIRF nearly immediately distinguishes vascularized from ischaemic tissue areas[15]. Fluorescence is visible within 30 to 60 s after fluorochrome injection[16,17], which is performed after the division of the mesenteric vessels when the operator has chosen the resection margins and/or after the anastomosis in order to verify good perfusion of the surrounding tissues.

Thereafter, in a meta-analysis of 10 studies from 2010 to 2017 (894 patients), van den Bos *et al*[18] reported a change in the initial surgical plan in 10.8% of patients. In most articles, the resection was extended to better perfused tissues or the anastomosis was

redone if the perfusion was unsatisfactory[16]. The use of ICG also helped to invalidate the clinical impression of poor perfusion and thus indicated that the resection margins did not need to be extended further. For instance, Kudszus *et al*[14] described a non-extended resection in 2.5% of patients (5/201) despite a clinical impression of poor perfusion. In the study by Kim *et al*[17], the use of ICG determined competent perfusion of the bowel at the anastomosis in 13 patients (10.6%) at risk of ischaemia due to restrictive mesocolon, malrotation, marginal vessel deficiency, accidental left colic artery excision, and oedema due to irradiation.

The effect of NIRF angiography on intraoperative decision-making seems to allow a reduction in the risk of AL (Table 1). However, due to the small sample sizes, only 4 out of 11 studies reported a statistically significant ($P < 0.05$) reduction in AL[16-20]. In other investigations, subgroup analysis showed significant reductions in AL for colorectal cancer (CRC) surgery[14,21], rectal surgery[22], elective resection and hand-sewn anastomosis[14]. However, in two larger studies[23,24], the use of fluorescence angiography did change the decision-making, but there was no discernible change in AL outcome.

The first randomized controlled trials in the field were released in 2019 and 2020 by De Nardi *et al*[25] and Jafari *et al*[26], respectively. In these prospective, single-blinded trials, the authors did not find a significant difference in the AL rate between the NIRF group and the control group (De Nardi *et al*[25]: Incidence of AL = 5% in the NIR group *versus* 9% in the control group; $P = 0.2$; Jafari *et al*[26]: Incidence of AL = 9.0% in the NIR group *vs* 9.6% in the control group; $P = 0.37$). However, these studies might be underpowered. Of note, Jafari *et al*[26] stopped the trial before including the minimum number of 450 patients, as mentioned in their research protocol. Nevertheless, Alekseev *et al*[27] randomized controlled trial including 380 patients showed significant results, with an incidence of AL of 9.1% in the NIR group *vs* 16.3% in the control group ($P = 0.04$). This might be explained by the marked incidence of AL in the included population. Indeed, the authors pro-actively searched for leaks *via* contrast exams performed on every patient 30 d after surgery if the post-operative period was entirely uneventful.

Based on these randomized controlled trials, it can be concluded that the effect of fluorescence probably exists but that this effect is most likely modest and more pronounced in patients with a high risk for AL. New randomized studies are ongoing and will allow us to reach a conclusion on the subject (notably: Armstrong *et al*[28], NCT03602677, NCT03390517).

Regarding economic aspects, AL leads to extended hospitalization. Therefore, the use of NIRs may reduce the length of hospital stay[14] and reduce costs by preventing the occurrence of AL. The initial outlay for the material of the NIR fluorescence system was 70.000€ for Kim *et al*[17], then 13€ *per* patient for the dye; the corresponding cost was 110.000€ (purchase and 5-year maintenance) for Ris *et al*[16], and then 130€ *per* patient for 3 injections. AL increases the treatment costs by €12600[29] to €21500[30]. Therefore, the NIRF technique seems to be cost effective[31], and the acquisition costs of a NIRF-ICG system could be covered in a year if it prevents two ALs *per* year. All the studies do not share this point of view, as Jafari *et al*[32] reported an initial cost of $167500 to $223750 and a cost *per* case of $999 to $1099. Consequently, a randomized controlled trial assessing the effect of NIRF on AL, including a cost-benefit analysis, is needed.

In addition to being used to reduce the rate of AL in elective surgery, NIRF can be used during emergency surgery, *e.g.*, in acute ischaemic disease, to identify bowel segments to be resected. However, only a few studies have applied NIRF to emergency surgery, and these studies included only small numbers of patients ($n = 4$ to 56)[33-35].

In the Liot *et al*[34]'s study, NIR fluorescence led to a change of plan in 32% of the cases: 67% were slated for a less aggressive approach, while only 33% were scheduled for larger resection. None of those patients underwent reoperation for ischaemia. Nevertheless, another study reported two false positive cases where the ICG injection showed good perfusion, while the pathological report finally revealed signs of necrosis.

## IDENTIFICATION OF METASTATIC LYMPH NODES

CRC is the third most common cancer worldwide, accounting for approximately 10% of all new cases, and is the fourth most common cause of death from cancer[36]. The 5-year survival of CRC is determined by the stage of the disease at diagnosis and ranges from 90% in early-stage localized tumours to 14% in distant metastatic disease[37].

**Table 1 Retrospective cohort studies**

| Ref. | Indication for resection | Sample size | ICG injection time | Change of plan (%) | AL rate (%) |
|---|---|---|---|---|---|
| Kudszus *et al* [14], 2010 | Colorectal cancer | ICG: 201. Control: 201 | Before anastomosis | 16.4 | ICG: 3.5. Control: 7.5 |
| Kin *et al*[23], 2015 | Colorectal cancer, Diverticulitis, IBD, other | ICG: 173. Control: 173 | Before anastomosis | 4.6 | ICG: 7.5. Control: 6.4 |
| Jafari *et al*[3], 2013 | Rectal cancer | ICG: 16. Control: 22 | Before anastomosis | 19 | ICG: 6. Control: 18 |
| Kim *et al*[17], 2016 | Rectal cancer | ICG: 123. Control: 313 | Before ± after anastomosis | 0[2] | ICG: 0.8[a]. Control: 5.4 |
| Boni *et al*[15], 2015 | Colorectal cancer | ICG: 42. Control: 38 | Before + after Anastomosis | 4.7 | ICG: 0. Control: 5.2 |
| Wada *et al*[87], 2019 | Rectal cancer[1] | ICG: 34. Control: 34 | Before anastomosis | 27.1[1] | ICG: 8.8. Control: 14.7 |
| Dinallo *et al*[24], 2019 | ND | ICG: 234. Control: 320 | Before anastomosis | 5.6[a] | ICG: 1.3. Control: 1.3 |
| Mizrahi *et al*[88], 2018 | Rectal cancer | ICG: 30. Control: 30 | Before + after anastomosis | 13.3 | ICG: 0. Control: 6.7 |
| Kim *et al*[19], 2017 | Rectal cancer | ICG: 310. Control: 347 | Before anastomosis | ND | ICG: 0.6[a]. Control: 5.2 |
| Ris *et al*[16], 2018 | Colorectal cancer (65.5%), diverticular disease (18.8%), Crohn's disease, ulcerative colitis, other | ICG: 504 Control: 1173 | Before + after anastomosis | 5.8 | ICG: 2.4[a]. Control: 5.8 |
| Watanabe *et al* [20], 2020 | Rectal cancer | ICG: 211. Control: 211 | Before anastomosis | ND | ICG: 4.7[a]. Control: 10.4 |

[1]149 (48:101).

[2]Fluorescent imaging correctly determined competent perfusion of the bowel adjacent to the anastomosis in 10.6% who were possibly susceptible to anastomotic site ischemia.

[a]$P < 0.05$. ND: No data available; IBD: Inflammatory bowel disease; AL: Anastomotic leak; ICG: Indocyanine green.

Lymph node status is a prognostic factor and a determinant for adjuvant therapeutic intervention. The sentinel lymph node (SLN) concept is based upon the observation that tumour cells migrating from a primary tumour metastasize to one or a few lymph nodes before involving other lymph nodes. This concept was first described in 1960[38], and its application contributed to the identification of lymph nodes with the highest probability of malignant infiltration for the staging of a tumour.

There are various methods for intraoperative mapping of SLNs. Currently, surgeons use direct visualization identification after the injection of dyes such as isosulfan blue [39], patent blue[40], methylene blue[41], or scintigraphy after the injection of radiocolloids around the tumour[42], and for over ten years, the use of NIRF after the injection of ICG.

The capability of ICG NIRF to identify metastatic LNs in various types of malignancies was extensively investigated. The sensitivity of ICG NIRF varied according to the site of the primary malignancy: 50% to 100% for endometrial and cervical carcinoma[43], 50%-100% for gastric cancer[44], and up to 95% to 100% for breast cancer[44]. Lymph nodes and lymph vessels draining the injection site and thus containing ICG can be visualized by NIR without ionizing radiation or radioactivity involvement. In the meta-analysis performed by Emile *et al*[45] in 2017 regarding SLN in CRC, the pooled sensitivity and specificity rates were 71 and 84.6, respectively. On the other hand, when the proportion of patients with early-stage tumours was > 50% of the sample size[46-48], the median sensitivity and specificity increased to 100%. This difference has also been found in the meta-analysis of Burghgraef *et al*[49], where the T1-T2 group had a 1.25 higher accuracy rate than the T3-T4 group (CI: 1.05-1.47). The hypothesis was that the flow through nodes with obvious nodal metastases could be obstructed by the tumour, leading to lymph drainage through alternative pathways (aberrant drainage concept). According to these results, sentinel node mapping with ICG is feasible for early-stage cancer/radiologically localized colorectal neoplasia[50]. Additionally, preoperative T staging should be performed before SLN mapping[46].

The ability of ICG NIRF to detect metastatic LN was compared with patent blue dye in two studies[51,52], which respectively examined both techniques in 20 and in 50 patients with colon cancer and reported that both methods had the same detection rates (95%, 99.5%) for SLN, but higher sensitivity in favour of ICG was observed by Liberale *et al*[51], especially in patients with higher BMIs (> 25 kg/m$^2$).

Regional lymphadenectomy directly influences the risk of distant failure, provides prognostic information and guides postoperative management, such as chemotherapy administration. The number of nodes retrieved from the surgical specimen improves the accuracy of prognosis and influences survival outcomes in patients with colon cancer. NIR lymph node mapping can modify the surgical procedure when ICG shows additional lymph nodes outside of the proposed resection margins to achieve radical lymph resection for curative surgery[48,53-55]. For that purpose, ICG injection is usually performed between 1 and 3 d before surgery[55]. This technique may serve to optimize and individualize the excision of patient specificities rather than theoretical anatomic generalization.

Another way to use ICG in metastatic lymph node mapping is as an intravenous injection. In tumour tissue, neoangiogenesis is responsible for the presence of immature and permeable vessels allowing ICG-bound molecules to pass into the extravascular space. As the half-life of ICG in blood circulation is approximately 5 min, ICG is rapidly cleared from the vascular space, but extravascular ICG accumulation will be responsible for the observed hyperfluorescence of tumour tissue in contrast to surrounding normal tissue[56]. This phenomenon is known as the enhanced permeability and retention effect. In one study (Liberale *et al*[57], 15 min after intraoperative intravenous injection of ICG, no lymph nodes other than those containing cancer cells were fluorescent, suggesting that fluorescence was directly related to the presence of tumoural tissue inside LNs .

This method also enables us to highlight peritoneal metastasis (PM). The first application of ICG-based fluorescence imaging in patients with PM of colorectal origin was demonstrated in 2016[58]. According to the last study by Lieto *et al*[59], the diagnostic performance of ICG NIRF was significantly better than preoperative and intraoperative conventional procedures. In this study, intraoperative ICG identified additional hyperfluorescent metastatic nodules and then confirmed them by histopathology (16/17). The sensitivity increased from 76.9% to 96.9% with NIRF.

## PREVENTION OF IATROGENIC URETERAL INJURY

Iatrogenic ureteral injury is a complication occurring in 0.15% to 1.9%[60,61] of patients undergoing colorectal surgery. These lesions include ureteral sections, ligations, crushing, coagulation or indirect injuries, such as burn and ischaemic lesions, and may require secondary/subsequent surgery in a later stage. Even if it is a rare event, the consequences of such injuries are important and lead to increased postoperative mortality, morbidity, hospital stay and health costs[62].

Preoperative ureter stent placement is a technique used in surgery to help identify the ureters. These stents can be palpated during open surgery, but in laparoscopic surgery, in which tactile feedback is limited, this technique loses its interest[63]. In an effort to enhance visualization of ureters throughout laparoscopic dissection, lighted ureteral stents were devised[64,65]. However, preoperative ureteral stent placement is an invasive procedure that harbours increased risks of complications, such as ureteral perforation and urinary tract infection[66]. New techniques have been or are being developed for ureter visualization with AR assistance (Table 2).

Because of the hepatic clearance of ICG, its use in ureter visualization requires the placement of a ureteral catheter for retrograde injection of the dye directly into the lumen of the ureter. This technique allows identification of the entire course of the ureters from the surrounding tissue in laparoscopic and open surgery and enables the location of a lesion made during the surgery in real time by leakage of the dye in the intraperitoneal space[67]. A small cohort study including 10 patients showed 100% visualization of ureters directly after injection[68]. The importance of this method was confirmed by two case studies[69,70]. Considering the potential complications of ureteral catheters, new dyes were investigated to offer non-invasive alternatives to improve preoperative ureteral identification.

Methylene blue is a dye that has long been used in humans with a good safety profile. Its NIR fluorescent properties were discovered at micromolar concentrations only ten years ago[71]. When used at the typical clinical concentration of 1% (*i.e.*, 31.3 mmol/L), methylene blue is bright blue but has virtually no NIRF because of

**Table 2 Ureteral visualization studies using intravenous dye**

| Ref. | Model | Surgery | Dye | *n* (%) | Visible ureters | Visible time after injection | Visibility duration |
|---|---|---|---|---|---|---|---|
| Matsui *et al*[71], 2010 | Pig | Open[1] | Methylene blue | 20 | 100% (40/40) | 10 min | 65 min |
| | | Laparoscopy[1] | | 2 | 50% (2/4) | 10 min | 20 min |
| Verbeek *et al*[89], 2013 | Human | Open[1] | Methylene blue | 12 | 100% (20/20) | 10 min | ≥ 50 min |
| Al-Taher *et al*[90], 2016 | Human | Laparoscopy | Methylene blue | 10 | 62,5% (5/8) | ND | ND |
| Barnes *et al*[72], 2018 | Human | Laparoscopy | Methylene blue | 34 | 93.6% (59/63) | 0 min | 2 h |
| | | Open | | 6 | 66.6% (4/6) | | |
| Tanaka *et al*[67], 2007 | Rat | Open | IRDye 800CW | 12 | 100% | 3-5 min | ND |
| | Pig | Open | IRDye 800CW | 6 | 100% | 10 min | > 20 min |
| Schols *et al*[74], 2014 | Pig | Laparoscopy | IRDye 800cw | 2 | 50% (1/2) | 10 min | ND |
| Korb *et al*[75], 2015 | Pig | Laparoscopy | IRDye 800CW | 6 | 100% | 10 min | ≥ 50 min |
| Van den Bos *et al*[18], 2018 | Pig | Laparoscopy | IRDye 800 BK | 1 | 100% (2/2) | 35 min | 3 h |
| | | | IRDye 800NOS | 1 | 100% (2/2) | 45 min | ND |
| | | | IRDye 800CW | 1 | 100% (2/2) | 10 min | ≥ 15 min |
| Al-Taher *et al*[77], 2018 | Pig | Laparoscopy | IRDye 800BK | 3 | 100% (6/6) | 1-20 min | ≥ 100 min |

[1]With dissection to highlight ureters.
ND: No data available.

quenching. However, when diluted to 20 mmol/L (quenching threshold) or below, methylene blue displays moderate NIRF[71], with absorption occurring at 668 nm and excitation emission at 688 nm[72]. When the emission wavelength is shorter than that of ICG, the fluorescence of methylene blue is visible less deeply. Because a major route of excretion of methylene blue is through the kidneys, the urinary excretion rate amounts to 28.6% (± 3.0/24 h)[73], and urine becomes NIR fluorescent after a single intravenous dose, which permits real-time identification of the ureters. As the urine flow in the ureter is not continuous but pulsatile and the fluorescence signal is related to urine flow, the fluorescence signal in ureters varies over time. However, a preclinical study using this technique showed that a diuretic, which increased the flux of urine through the ureters, did not increase NIRF[71].

Barnes *et al*[72] showed that 93% of the ureters were seen with NIR techniques. The ureteric fluorescence was bright enough to assure the surgeon that the ureter was positively identified; therefore, no additional dissection was required to identify the ureter under white light. Moreover, 20% of all ureters were visible only under fluorescence and not seen with white light. In laparoscopy, fluorescence was deemed useful; in 10 cases, fluorescence revealed the ureter to be in a place different from the one that the surgeon predicted, and in 2 cases, the surgical plan was subsequently modified. Fluorescence was not deemed useful in open surgery, as tactile feedback is sufficient to detect ureters. The ureters can be seen directly after the injection of methylene blue for more than two hours. According to various studies, the fluorescence time and the visualization quality seemed to depend on the dose and concentration of dye administered.

There were no adverse events observed after the administration of the dye in these studies. However, methylene blue is known to cause severe adverse reactions, such as anaphylaxis, methemoglobinemia or severe haemolysis, in subjects with known glucose-6-phosphate dehydrogenase deficiency[71]. It is important to note that intravenous injection of methylene blue causes an artificial drop in oxygen saturation measured *via* pulse oximetry that lasts up to several minutes. This phenomenon is caused by the principle of pulse oximetry, which is based on the red and infrared light absorption characteristics of oxygenated and deoxygenated haemoglobin.

Recently, experimental dyes have been studied for intraoperative visualisation of ureters using NIRF imaging in laparoscopic surgery. First, IRDye 800CW (LI-COR Biosciences, Lincoln, Nebraska, United States) is a tetrasulfonated heptamethine indocyanine dye that, after intravenous injection, is cleared by the kidneys and excreted into urine. Its maximum absorption occurs at 775 nm, and its maximum

excitation emission occurs at 796 nm, which enables a visualization depth equivalent to ICG. Animal studies[67,74,75] have successfully demonstrated the potential of this dye for the identification of ureters. However, a major disadvantage of IRDye 800CW is its cost, which is almost tenfold that of ICG. In 2018, a new preclinical dye, IRDye 800BK (LI-COR Biosciences), with a maximum absorption of 774 nm and a maximum emission of 790 nm, was developed for NIRF visualization, and its price should be similar to that of ICG. Because of its hydrophilic nature, IRDye 800BK is primarily cleared by the kidneys and enables visualization of the ureters in pigs[76,77].

A comparison of IRDye 800BK *vs* 800CW by Van den Bos *et al*[76] showed that IRDye 800BK successfully identified the course of the ureter in living pig models but also resulted in the highest contrast between the ureter and background. This new dye would allow ureter visualization from 20 min to ≥ 120 min post intravenous injection [77], and the duration and quality of the visualization would be influenced by the concentration of dye. The first study in humans is currently underway (NCT03387410 Thomas Barnes, Oxford University Hospitals NHS Trust).

## DISCUSSION

This review of the literature presents the role of NIRF imaging in colorectal surgery. The literature in the field suggests that NIRF imaging is safe and feasible. Furthermore, NIRF imaging seems to be useful for helping the surgeon make decisions during surgical procedures.

NIRF imaging is suitable for all types of surgery but is of particular interest in laparoscopy, where surgeons do not have tactile feedback. Indeed, this modality seems helpful for the detection of ureters to reduce the risk of iatrogenic ureteral lesions. Various dyes have been tested, and a new fluorochrome is under investigation to improve NIR brightness compared to that of methylene blue.

Even if several meta-analyses have been performed for the estimation of intestinal vascularization, very few randomized trials have validated the technique in its different indications. Despite the increase in the number of clinical studies, the interpretation of fluorescence is based on the subjective evaluation of the surgeon, and only a few published studies[19,78] have attempted to determine an objective threshold of fluorescence quantification to normalize the technique on a larger scale. Although some notions of the costs of the methods can be found in the articles read, these data come from heterogeneous studies with small sample sizes and are therefore not comparable. Thus, cost-effectiveness studies would be interesting in the future.

In oncology, the NIRF imaging system is currently used in operating rooms using non-specific fluorescent probes, such as ICG, to visualize SLNs. In recent years, many studies have focused on developing targeted fluorescent probes. Targeted fluorochromes are composed of a carrier molecule that specifically binds to a certain target ( *i.e.*, monoclonal antibodies, peptides, small molecules), conjugated to a fluorescent dye. Studies should focus on tumour markers that could provide surgeons real-time feedback about the location and extent of tumours. Seven potential targets for imaging CRC have been identified: CXCR4, EpCAM, EGFR, CEA, Muc1, MMP, and VEGF-A [79], and some have been investigated in other types of cancers. To date, in CRC, two phase I feasibility studies have been performed in humans[80,81], and more molecules have been tested in mice with human CRC xenografts[82-84].

Cancer detection is not the only indication of these new targeted dyes. Neumann *et al*[85] described using an enzymatic marker made by a dye becoming fluorescent only in response to MMP for early identification of AL by targeted fluorescence endoscopy during the healing process. Hingorani *et al*[86] demonstrated in ex vivo human nerves and in vivo mouse models that the fluorescent FAM-HNP401 peptide, when injected intravenously, localized to the connective tissue surrounding peripheral nerves and highlighted these nerves.

## CONCLUSION

NIRF imaging appears to be a useful tool to assist surgeons in colorectal surgery. This technique has been tested for three main indications: (1) In the estimation of intestinal vascularization to detect areas of poor perfusion and therefore prevent AL, or in the assessment of the extent of ischaemic segments during acute intestinal ischaemia; (2) In the visualization of lymphatic drainage and (especially in oncological contexts) SLNs and lymphatic and peritoneal metastases in order to prevent recurrence and

adapt further adjuvant treatment; and (3) In the detection of ureters in order to reduce the risk of iatrogenic ureteral lesions, particularly during laparoscopic surgery. Randomized controlled trials and prospective studies with larger sample sizes are needed to validate the methods. Additional studies are underway for the first use of new fluorescent molecules in humans.

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*MINIREVIEWS*

# Dysphagia, reflux and related sequelae due to altered physiology in scleroderma

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## Abstract

Systemic sclerosis is a connective tissue disease that presents with significant gastrointestinal involvement, commonly in the esophagus. Dysphagia is a common clinical manifestation of systemic sclerosis and is strongly related to esophageal dysmotility. However, there are multiple other contributing factors in each step in the physiology of swallowing that may contribute to development of severe dysphagia. The oral phase of swallowing may be disrupted by poor mastication due to microstomia and poor dentition, as well as by xerostomia. In the pharyngeal phase of swallowing, pharyngeal muscle weakness due to concurrent myositis or cricopharyngeal muscle tightening due to acid reflux can cause disturbance. The esophageal phase of swallowing is most commonly disturbed by decreased peristalsis and esophageal dysmotility. However, it can also be affected by obstruction from chronic reflux changes, pill-induced esophagitis, or Candida esophagitis. Other contributing factors to dysphagia include difficulties in food preparation and gastroparesis. Understanding the anatomy and physiology of swallowing and evaluating systemic sclerosis patients presenting with dysphagia for disturbances in each step can allow for development of better treatment plans to improve dysphagia and overall quality of life.

**Key Words:** Systemic sclerosis; Esophageal motility disorders; Deglutition; Deglutition disorders; Gastroesophageal reflux; Esophagitis

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**Core Tip:** Systemic sclerosis presents with significant gastrointestinal involvement, with dysphagia being a common clinical symptom. Normal swallowing physiology is broken down into the oral phase, pharyngeal phase, and esophageal phase of swallowing; systemic sclerosis can cause disease processes that affect and disrupt each stage of swallowing. We describe the disruptions to swallowing that occur in each phase and potential therapeutic options to alleviate symptoms.

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## INTRODUCTION

Scleroderma is a connective tissue disease that may affect any organ system, creating variegated patient-to-patient manifestations. Scleroderma can occur as localized scleroderma or systemic sclerosis (SSc). Localized Scleroderma involves the skin and subcutaneous tissue, but does not manifest with Raynaud phenomenon, digital ischemia, or visceral organ involvement[1]. SSc can be further divided into the limited form, formerly known as CREST syndrome, or the diffuse form. Limited SSc symptomology includes calcinosis cutis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia[1], while diffuse SSc involves widespread cutaneous involvement and earlier, more progressive systemic involvement[1]. The breakdown of types of scleroderma and features of each are shown in Figure 1.

In SSc, gastrointestinal (GI) involvement, specifically esophageal disease, is very common in both limited and diffuse forms, and may be the first manifestation of disease[2]. A previous study evaluating quality of life has shown significant correlation between GI symptoms and lower quality of life scores[3]. Dysphagia, specifically, is a common presentation of GI involvement in SSc[4]. A recent study using screening questionnaires to evaluate physical, functional, and emotional outcomes due to dysphagia shows mild disability in 74% of patients and moderate to severe disability in the remaining 26%[5]. While many studies report that dysphagia is a result of esophageal dysfunction, oral and pharyngeal involvement can also lead to oropharyngeal causes of dysphagia in SSc[5]. Given the significant impact of dysphagia on quality of life in SSc patients, swallowing dysfunction should be thoroughly worked up so it may be treated appropriately[5].

The aim of this study is to review pathophysiology of SSc as well as the physiology of swallowing and reflux to understand the various disruptions that result in dysphagia in SSc. Understanding this will allow for development of more effective therapeutic plans for SSc patients suffering from dysphagia.

## PATHOPHYSIOLOGY OF SSC

The pathophysiology of SSc is complex and has not been fully elucidated. SSc is defined by vascularity changes, autoimmunity, and tissue fibrosis[1]. Vascular insult, which can be due to autoantibody effects, infectious or inflammatory causes, environmental exposure, or any number of catalysts, results in activation of endothelial cells. This leads to activation of platelets, which release various factors, such as thromboxane A2 (TXA2), ADP, and transforming growth factor beta (TGF-beta), that promote platelet aggregation and activation of the coagulation cascade. Activated endothelial cells release endothelin 1 (ET-1), a potent vasoconstrictor that also causes leukocyte adhesion, proliferation of vascular smooth muscle cells, and activation of fibroblasts[1]. Under the influence of ET-1 and TGF-beta, endothelial cells undergo trans-differentiation into mesenchymal cells that have a blunted response to vasodilators such as nitrous oxide[1,6]. This milieu contributes to tissue hypoxia and

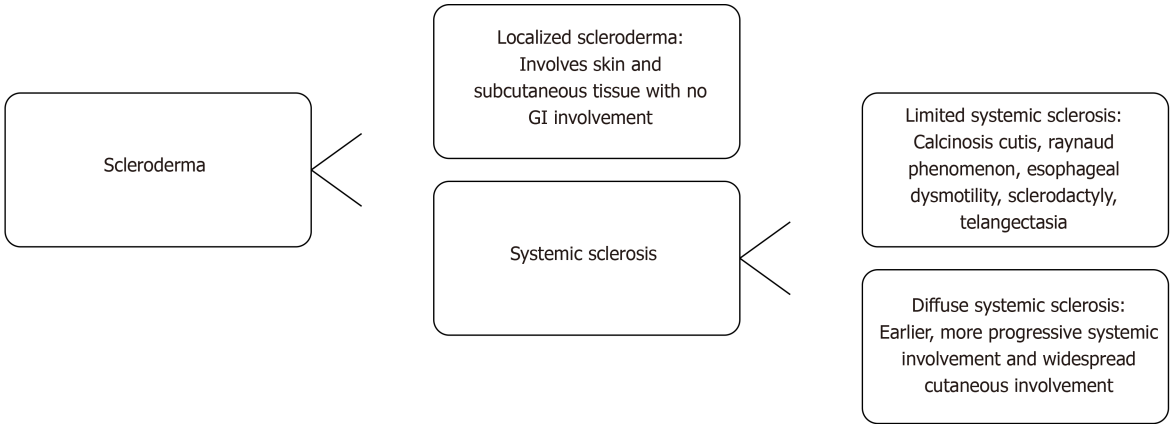


**Figure 1 The types of scleroderma and features of each disease.** Scleroderma can be broken down into localized scleroderma or systemic sclerosis. Systemic sclerosis can be further broken down into limited and diffuse types. GI: Gastrointestinal.

oxidative stress, exacerbated by diminished angiogenesis secondary to the impaired functioning of endothelial progenitor cells in SSc[1]. Furthermore, SSc may be associated with vascular endothelial growth factor isoforms that are antiangiogenic[6].

Immune system involvement also plays a role in SSc pathogenesis, with dysregulation of both innate and humoral immunity. SSc presents an imbalance of T helper cells defined by a predominance of type 2 T helper (Th2) cells[1]. The cytokines produced by these overexpressed Th2 cells, including IL-4 and IL-13, are pro-fibrotic [7]. Th2 cells also induce immunoglobulin production by B cells; these upregulated autoantibodies contribute to inflammation. Limited SSc is associated with anti-centromere antibodies while the diffuse form is associated with anti-Scl-70 antibodies or anti-DNA topoisomerase I antibodies, as well as anti-RNA polymerase III antibodies. There is an association between anti-RNPC3 antibodies and severity of SSc GI disease, with the presence of these antibodies predictive of moderate or severe GI disease. The subunit of RNA polymerase III that is targeted by autoantibodies can further stratify GI severity in SSc patients[8]. Activation of the immune system in this way leads to fibrosis. The excess collagen produced by myofibroblasts contributes to this process of structural damage[7]. The basic pathophysiology of SSc is outlined in Figure 2.

Autonomic dysfunction may also contribute to SSc symptomology and severity. Vascular damage to the vasa nervorum is important in the development of this dysfunction[9]. Autoantibodies against nicotinic acetylcholine receptor at autonomic ganglia (gAChR) may also play a role in GI autonomic dysfunction, as anti-gAChRα3 autoantibodies were significantly higher in patients with SSc GI disease than in SSc patients without GI manifestations[10]. Anti-muscarinic-3 receptor autoantibodies may also contribute by inhibiting contraction of smooth muscle cells[11].

### *SSc esophageal involvement*

GI involvement, specifically esophageal involvement, is the most common internal organ complication of SSc, affecting over 90% of patients[8]. While patients with the diffuse form have greater skin and muscular involvement, there is no difference in GI involvement between the diffuse and limited forms of SSc[12]. Since smooth muscle is targeted in the pathophysiology of SSc, the lower two-thirds of the esophagus and lower esophageal sphincter (LES) are preferentially affected, as the upper third of the esophagus is primarily composed of skeletal muscle. Esophageal symptomatology typically includes dysphagia, pseudo-obstruction, regurgitation, heartburn, nausea, or vomiting, all of which can lead to poor eating and severe weight loss[7]. Esophageal dysfunction permits gastric reflux, which can lead to erosive esophagitis, bleeding, and ulceration, as well as eventual stricture and fistulae formation and an achalasia-like syndrome. Barrett's esophagus and adenocarcinoma may result from long-term disease. Micro-aspiration due to esophageal dysmotility is also associated with interstitial lung disease (ILD)[7].

Diagnostic delay may occur in SSc GI disease since 30% of patients with esophageal involvement may be asymptomatic, especially early in the disease course[7]. Various diagnostic studies can be used in detection of esophageal disease including manometry, pH monitoring with or without impedance, and esophagogastroduodenoscopy (EGD)[4]. Radiographic imaging may also be used: Videofluorography

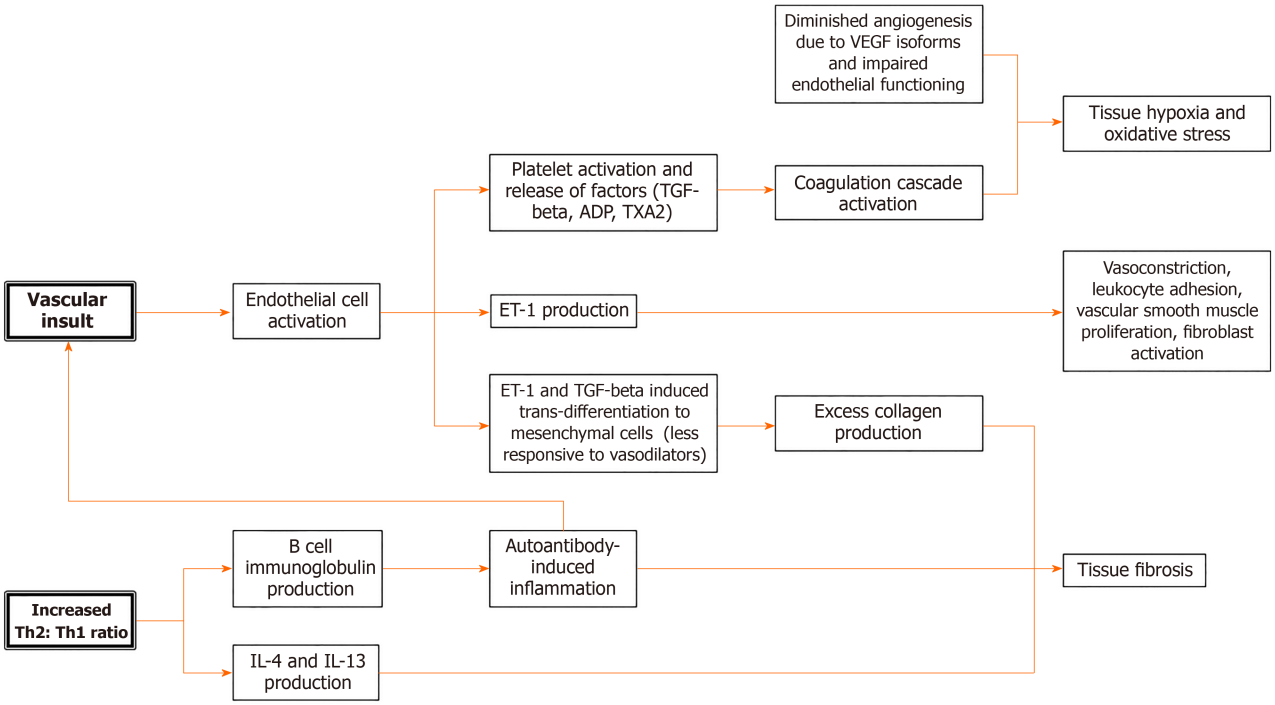


**Figure 2 Simplified pathophysiology of systemic sclerosis.** SSc: Systemic sclerosis; Th2: Type 2 T helper; Th1: Type 1 T helper; TGF-beta: Transforming growth factor beta; TXA2: Thromboxane A2; ET-1: Endothelin 1; VEGF: Vascular endothelial growth factor; IL: Interleukin.

swallow study of the esophagus can also show altered peristalsis and a standard chest computed tomography (CT) may reveal a dilated esophagus[13]. The diagnostic studies are outlined in Table 1. When patients are symptomatic, associated GI symptoms can be scored in various ways according to symptomology. UCLA Scleroderma Clinical Trial Consortium GIT 2.0 (UCLA SCTC GIT 2.0) is a scoring system used to assess GI tract symptom severity as well as health-related quality of life [14]. The scoring system has 34 questions assessing 7 different scales, which include reflux, distention/bloating, diarrhea, fecal soilage, constipation, emotional well-being, and social functioning, as outlined in Table 2[14]. The score is based on a questionnaire that asks about severity and frequency of symptoms, ranging from "no gut symptoms" to "very severe symptoms"[14]. This scoring system is found to be reliable and feasibly applicable as it takes only 6-8 min to complete[14]. The UCLA STCT GIT 2.0 is very useful in SSc patients not only for evaluating severity of GI disease but also for following improvement in symptoms with various treatments[14].

SSc patients, especially those with diffuse SSc in which complications typically occur more rapidly, report adverse effects of the disease on their quality of life. Patients describe fatigue, manual activity limitations, distress related to manifestations and unpredictability of their disease, sleep difficulties, and low self-esteem; patients do not feel fully equipped to handle their disease mentally or physically[15]. Anxiety related to the disease is prominent in the SSc population, further dampening quality of life[15]. The prevalence of GI disease in SSc, as well as the harsh symptomology that can result from GI forms of the disease, likely makes GI involvement a contributor to poor quality of life in these patients.

## PHYSIOLOGY OF SWALLOWING

Dysphagia is common in SSc and may be related to disease affecting the smooth muscle of the distal two-thirds of the esophagus and the LES as well as other disease processes in SSc[1,16]. It is therefore necessary to first review the normal swallowing physiology to understand how various manifestations of SSc can cause disruption to each step of this process. Physicians can then apply this knowledge clinically by recommending treatment plans targeting these factors through lifestyle changes, medications, and procedures to improve dysphagia symptoms and quality of life.

**Table 1 Features of various diagnostic studies in systemic sclerosis diagnosis**

| Diagnostic study | Role in SSc |
|---|---|
| Esophagogastroduodenoscopy | Evaluates for esophageal causes of dysphagia[4] |
| | Shows reflux-related complications: erosive esophagitis, strictures, Barrett's esophagus, esophageal adenocarcinoma[4] |
| | Reveals esophageal findings in asymptomatic patients[4] |
| Esophageal manometry | Detects esophageal dysmotility, even in early stages of SSc[4] |
| | Shows decreased lower esophageal sphincter pressure and absent peristalsis in distal two-thirds of esophagus[4] |
| Pharyngeal manometry | Evaluates for oropharyngeal dysphagia by assessing upper esophageal sphincter relaxation and pharyngeal propulsion[38] |
| Esophageal pH monitoring (with or without impedance) | Gold standard for gastroesophageal reflux detection[4] |
| | Used for patients with resistant reflux[4] |
| Videofluorography swallow study of esophagus | Shows esophageal dysmotility with decreased peristalsis in distal 2/3 of esophagus[13] |
| | Shows decrease of lower esophageal sphincter pressure[13] |
| | Shows dilated lumen of esophagus[13] |
| CT chest | Shows esophageal dilation[13] |

SSc: Systemic sclerosis; CT: Computed tomography.

**Table 2 Scoring system of gastrointestinal symptoms based on UCLA Scleroderma Clinical Trial Consortium GIT 2.0[14]**

| Scales | None-to-mild symptoms | Moderate symptoms | Severe-to-very severe symptoms |
|---|---|---|---|
| Reflux | | | |
| Distension/bloating | | | |
| Diarrhea | | | |
| Constipation | | | |
| Fecal soilage | | | |
| Emotional well being | | | |
| Social functioning | | | |
| Total GIT score | | | |

Swallowing is a coordinated, physiological process that is important for consumption of food and fluid. It is essential for sustenance and any disruption in it affects perception of quality of life[17]. Difficult or disordered swallowing can cause further medical complications such as malnutrition, dehydration, and aspiration pneumonia[17,18].

The physiological process of swallowing is broken down into three stages: the oral stage, the pharyngeal stage, and the esophageal stage[17]. The oral stage is the voluntary first step, and involves the lips, teeth, muscles of mastication, and tongue [17]. In the oral preparatory phase of the oral stage, mastication allows the breakdown of food as it is moved around the oral cavity and lubricated with saliva[17,19]. During the propulsive stage of the oral phase, the food bolus is first positioned on the superior surface of the tongue[17]. Tongue flexion beginning anteriorly and moving posteriorly pushes the bolus towards the pharynx, to begin the second stage of swallowing[17]. In the pharyngeal phase of swallowing, the larynx and soft palate move upwards to block off the airway and nasopharynx[19]. Several muscles of the anterior portion of the pharynx contract to cause forward displacement of the larynx and pharynx[19]. This is followed by relaxation of the cricopharyngeal muscle and relaxation and opening of the upper esophageal sphincter (UES)[19]. As the UES opens, the bolus will pass through to the esophagus, beginning the esophageal phase of swallowing[20]. Once the bolus enters the esophageal lumen, it is transported by coordinated,

sequential muscular contractions and relaxations known as peristalsis[19]. This peristalsis is triggered by distension of esophagus and allows for the bolus to travel towards the stomach[19]. This primary peristalsis may be followed by a secondary wave. Secondary peristalsis is limited to the smooth muscle portion in the bottom 2/3 of the esophagus, and functions to clear remnants of the bolus leftover from the primary wave and to remove refluxed gastric contents[19]. The final step in the esophageal phase of swallowing is relaxation of the LES and movement of the bolus into the stomach[19]. Relaxation occurs in a coordinated manner with the preceding peristalsis[19]. The LES remains tonically contracted at rest and is supported by the crural diaphragm[19].

Dysphagia, defined as difficulty in swallowing that results in delay in passage of food or liquid bolus[21], is a common symptom in SSc patients due to various disturbances in the normal swallowing process[1]. In these patients, each stage of swallowing can be disrupted in various ways, each leading to a different classification of dysphagia[21]. Good history-taking followed by necessary diagnostic testing in SSc patients can define the type of dysphagia so an appropriate therapeutic plan may be recommended. The oral phase of swallowing in SSc may be disrupted by loss of teeth and microstomia limiting mastication. Impaired saliva production may further complicate the oral phase. Pharyngeal dysphagia commonly occurs due to uncoordinated contractions, muscular weakness causing decreased contraction, or anatomic anomalies causing obstruction[21]. In SSc, there may be weakening of pharyngeal muscles due to concurrent myositis or cricopharyngeal muscle tightening secondary to reflux. Esophageal dysphagia is commonly due to mechanical obstructions or motility disorders[21]. Dysmotility characterized by decreased peristalsis or obstruction secondary to chronic reflux changes may result in esophageal dysphagia in SSc. The various disease processes affecting each stage of swallowing in SSc are outlined in Figure 3. The potential treatment options for these processes are outlined in Table 3.

## CAUSES OF ORAL DYSPHAGIA IN SSC PATIENTS

### *Pre-oral phase food preparation*

Hand function impairment in SSc can commonly lead to difficulty in food preparation and presentation. Hand dysfunction in SSc is widely recognized and is typically due to skin thickening and contracture of the hands. Tests such as the Hand Mobility in Scleroderma (HAMIS) test are frequently used to assess finger and wrist mobility[22]. In a study of 30 patients with SSc, it was found that hand dexterity was reduced to 68%-80%, and grip force was reduced to 46%-65% compared to a healthy person. Finger flexion and extension were the most impaired in hand mobility[23]. Ischemic digital ulceration in SSc also contributes to impairment of hand function as well as increased pain[24]. These impairments lead to difficulty in preparing or ingesting food in patients with SSc. These quality-of-life changes affecting eating are addressed in the Scleroderma Health Assessment Questionnaire, which asks questions such as "Are you able to cut your meat?" and "Are you able to lift a full glass to your mouth?"[25]. Those who do endorse difficulty cutting their food may have further swallowing symptoms due to ingestion of larger pieces of food. Inability to lift a glass can also limit water intake, that would have facilitated swallowing.

### *Disruption in mastication and food bolus preparation*

The oral voluntary phase of swallowing may be also severely limited in SSc due to various factors that can subsequently impact the following swallowing phases. This adds to the burden of diminished esophageal motility function and dysphagia in SSc patients. Studies show that SSc patients may suffer from microstomia due to sclerofibrosis of perioral tissue and malfunctioning of the temporomandibular joint [26]. This limits SSc patients' ability to open their mouths beyond 40 mm, and results in limited mandibular movements[27]. This causes disturbances in mastication and food bolus preparation, affecting the oral preparatory stage of the oral phase of swallowing and contributing to dysphagia in SSc patients.

Other disturbances in SSc lead to poor dental hygiene in this patient population. Teeth enamel can be eroded due to gastric acid reflux[2] and xerostomia[28]. In addition, SSc patients may have sclerodactyly and hand dysfunction, which can limit their ability to brush and care for their teeth[26]. This results in dental cavities, decay, and ultimately loss of teeth, and causes disruption in chewing of the food bolus for mastication in the oral phase of swallowing[26]. Other findings in SSc patients include

**Table 3 Treatment options for the various disease processes that contribute to the development of dysphagia in systemic sclerosis**

| Disease process | Therapeutic plan |
|---|---|
| Xerostomia | Drinking water more frequently[2] |
| | Using artificial saliva as needed[2] |
| | Using special toothpastes and mouthwashes[2] |
| | Avoiding medications that exacerbate dry mouth[2] |
| Microstomia | Performing exercises and massages to stretch the mouth[2] |
| Dental decay | Planning regular follow-up with experienced dentist[2] |
| Concurrent myositis | Frequent screening for myositis in patients with SSc and suggestive symptoms[37] |
| | Treating concurrent myositis with immunomodulatory therapy and interventional procedures[37] |
| Esophageal Dysmotility | Lifestyle management (taking smaller bites, chewing food thoroughly, drinking adequate water with food)[16] |
| GERD | Medications (PPIs, H2RAs)[2,52] |
| | Dietary modifications (avoiding acidic foods)[52] |
| | Lifestyle modifications (avoiding meals before bedtime, elevating the head of the bed while sleeping)[2,57] |
| Candida esophagitis | Screening for fungal esophagitis in patients with SSc and suggestive symptoms[73] |
| | Prompt antifungal treatment[73] |
| Pill esophagitis | Avoiding medications at high risk of causing esophagitis[79] |
| | Screening for esophagitis in SSc patients taking culprit medications with suggestive symptoms[79] |
| Gastroparesis | Medications (prokinetic agents)[2] |
| | Dietary modifications of small frequent meals with fiber[2] |

SSc: Systemic sclerosis; PPIs: Proton pump inhibitors; H2RAs: H2-receptor antagonists; GERD: Gastroesophageal reflux disease.

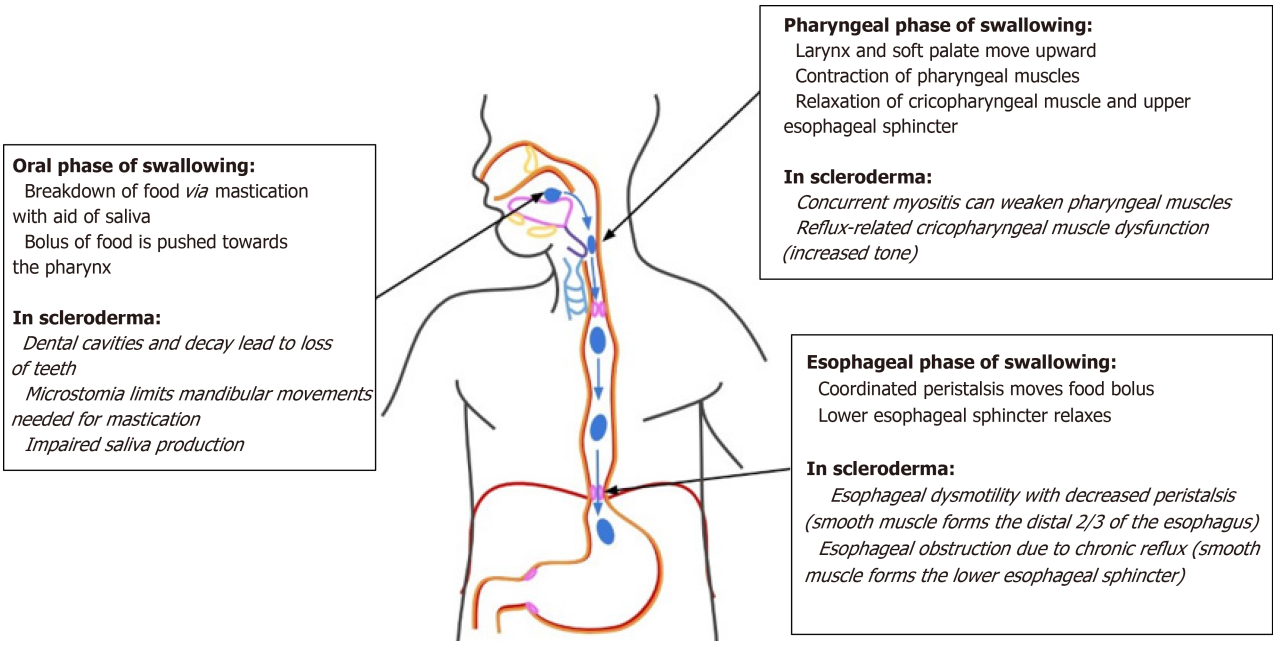


**Figure 3 Disruptions of normal swallowing physiology due to disease processes of scleroderma.**

widening of the periodontal ligament around the premolar and molar teeth[29], mandibular bone resorption[26], and gingival recession[26].

There are very few treatments to ameliorate the issue of poor dentition in SSc. Typical treatments for poor dentition include dentures and implants, which are not feasible in SSc. Dentures are difficult to apply due to impaired hand function as well as

oral cavity narrowing and tongue rigidity in SSc patients[30]. Once applied, salivary hypofunction may also reduce denture retention[30]. Implants are also difficult due to low bone mineral density in SSc patients[31]. Mandibular bone resorption, commonly seen in SSc patients, is thought to be secondary to pressure ischemia from sclerosed skin and muscle atrophy[32]. Proper dental care and hygiene is essential in preventing and treating dysphagia in SSc patients. Patients may require assistance in brushing their teeth and should be encouraged through routine checks with experienced dentists.

### *Impaired saliva production and concomitant Sjogren's syndrome*

Patients with SSc have a higher prevalence of concomitant Sjogren's Syndrome, an autoimmune disease characterized by inflammation of exocrine glands resulting in dryness of mucosal surfaces. Specifically, there is a loss of salivary glands that leads to xerostomia[33]. Sjogren's disease is seen in 17%-29% patients with SSc[33]. Saliva is necessary for mastication, digestion, oral cleansing, and speech articulation[34]. It contains water, minerals, electrolytes, buffers, and proteins that maintain neutral pH and aid in enzymatic digestion. Loss of the bicarbonate buffer contained within saliva disrupts one defense mechanism against acid reflux, and may result in more severe gastroesophageal reflux disease (GERD) symptoms[19]. Salivary dysfunction also impairs the oral preparatory stage of the oral phase of swallowing, as saliva is needed for lubrication of food and mastication[17,35]. This disturbance may contribute to the overall prevalence of dysphagia in SSc patients.

## CAUSES OF PHARYNGEAL DYSPHAGIA IN SSC PATIENTS

### *Concomitant myositis*

Patients with SSc are more prone to suffering from concurrent myopathies that may contribute to the pathophysiology of dysphagia. Prior studies note anywhere between 5%-81% of SSc patients suffer from myopathies, depending on the definition of muscle involvement. Myositis in patients with SSc is termed as Scleroderma Polymyositis Overlap. Scleroderma Polymyositis Overlap is characterized by a more active fibrotic disease and is associated with an increased rate of systemic complications as well as a higher mortality rate[36]. Polymyositis is a myopathy characterized by muscle impairment and systemic symptoms secondary to muscle inflammation of striated skeletal muscles[37]. Because coordinated contraction of the striated skeletal muscles of the oropharynx and upper esophagus is essential to the swallowing process, inflammation and dysfunction of these muscles, as seen in polymyositis, can cause pharyngeal dysphagia[37]. Dysphagia is reported in up to 36% of patients with myositis[37]. On pharyngeal high-resolution impedance manometry, there may be globally weak pressure generation, with absent hypo-pharyngeal constrictor activity as well as abnormal UES contractility[38]. These findings correlate with increased post-swallow residue and aspiration risk[38]. Given the risk of concomitant myositis in SSc patients, pharyngeal dysphagia due to striated muscle weakness should be considered a potential cause of dysphagia in SSc patients. The possibility of additional disruption in the pharyngeal phase of swallowing may result in a more severe dysphagia in SSc patients.

### *Cricopharyngeal muscle disorder*

Cricopharyngeal muscle disorders can commonly develop in SSc patients due to changes from chronic reflux. A previous study found that 51% of patients with gastroesophageal reflux have pharyngoesophageal dysphagia due to cricopharyngeal muscle dysfunction[39]. Another study in which HCl was infused proximal to the gastro-esophageal sphincter revealed an increase in UES tone 1 min after infusion, revealing the reactivity of the cricopharyngeal muscle to acid reflux[40]. Cricopharyngeal muscle dysfunction resulting in increased tone may prevent passage of food bolus from pharynx through UES into the esophagus. Given the high prevalence of reflux in SSc patients, cricopharyngeal muscle dysfunction may be an important complication to consider. Several case reports have also shown SSc patients specifically presenting with UES stenosis[41,42]. This upper esophageal involvement in SSc is an important contributor to dysphagia during the pharyngeal phase of swallowing.

## CAUSES OF ESOPHAGEAL DYSPHAGIA IN SSC PATIENTS

### *Esophageal dysmotility*

SSc commonly presents with GI involvement, manifested primarily in the esophagus as dysmotility[16]. The distal two-thirds of the esophagus is affected due to its smooth muscle makeup[2]; this area of smooth muscle is likely commonly cited as the first affected location of the GI tract because esophageal changes are readily sensed by patients due to the daily frequency of swallowing. While loss of peristalsis in other areas of the GI tract may become noticeable to patients with time, the fact that swallowing is under partial voluntary control and performed multiple times per day presumably highlights any associated discomfort. Further, perhaps the two-layered muscularis of the esophagus is more quickly affected than the thicker three-layered muscularis of the stomach, for example[43].

Prospective studies show esophageal dysmotility confirmed *via* manometry in about 70%-75% of SSc patients[16]. Esophageal involvement results in decreased amplitude of esophageal peristalsis as well as decreased tone of the LES. Esophageal manometry can confirm scleroderma as the cause of esophageal dysmotility by showing absent peristalsis in the distal two-thirds of the esophagus and a hypotensive LES[44], as seen in Figure 4. Clinically, patients most commonly present with dysphagia to both solids and liquids[16]. The lack of peristalsis in scleroderma results in esophageal dysphagia due to a motility issue, as lack of coordinated contractions/relaxations in the esophagus prevent food from being efficiently transported to the stomach[21]. Other clinical symptoms related to esophageal dysmotility in scleroderma include globus sensation, reflux, heartburn, or chest discomfort with swallowing[16]. Complications related to esophageal dysmotility include gastroesophageal reflux, stasis of foods in the esophagus, and aspiration into the lungs[16]. While there are no medications that effectively treat esophageal dysmotility, symptoms can be alleviated by lifestyle management. This includes taking smaller bites, chewing food thoroughly, and hydrating with water when having any solid, dry foods[16]. While esophageal dysmotility is commonly thought to contribute to dysphagia in SSC[1], the development and progression of further complications can cause more severe disruption to the normal swallowing physiology and may worsen dysphagia symptoms.

### *Gastroesophageal reflux*

In individuals with a normal esophagus, throughout the day, only small amounts of gastric contents reflux into the esophagus due to presence of multiple defensive barriers[19]. These include inherent LES tone with surrounding muscles, like the crural diaphragm, augmenting tone, secondary peristalsis to rapidly clear refluxed materials, bicarbonate in swallowed saliva to neutralize acid, local bicarbonate and mucus secretion, and resistance of the epithelium to degradation[19]. In patients with GERD, gastric contents with acid or pepsin reflux into the esophagus and cause inflammation or esophageal injury, possibly due to defective defensive mechanisms[19]. If the LES is impaired by disease or inflammation, it may allow contents to reflux. Defective peristalsis may limit clearance of contents, causing further injury and inflammation with longer contact times[19]. In addition, reduced salivation will decrease neutralization of refluxed contents[19]. Certain anatomic irregularities can also worsen reflux frequency and severity. For example, hiatal hernias decrease the overlap between the crural diaphragm and LES, thereby decreasing LES tone as it is no longer supported by the diaphragm[19,45]. In addition, transient LES relaxation (TLESR) has a major role in GERD pathology[19,45]. Physiologically, TLESRs occur after meals as they are triggered by gastric distension to allow the release of gas from the stomach as belching [45]. In patients with GERD, TLESR can allow acid in addition to gas to be refluxed into the esophagus[45]. Studies have shown that most episodes of acid reflux occur during TLESRs[46]. In patients with GERD, TLESRs occur more frequently, acid refluxes into the esophagus more often, and refluxed contents reach more proximal levels in the esophagus than in patients without GERD[46].

GERD is a very common diagnosis in SSc patients due to various disruptions of reflux defense mechanisms, as outlined in Figure 5. Based on large retrospective studies, the prevalence of esophageal reflux in SSc patients is 34.8%[2,47]. Clinically, patients report classic heartburn and reflux symptoms as well as dysphagia, odynophagia, laryngitis, chronic cough, hoarseness, or asthma[16]. In SSc, esophageal dysmotility prevents secondary peristalsis from efficiently clearing acidic refluxed materials[16], highly predisposing SSc patients to developing severe GERD[2]. The loss of tone in the LES also contributes to the development of GERD and complications from reflux[2]. GERD-related complications such as reflux esophagitis, strictures,

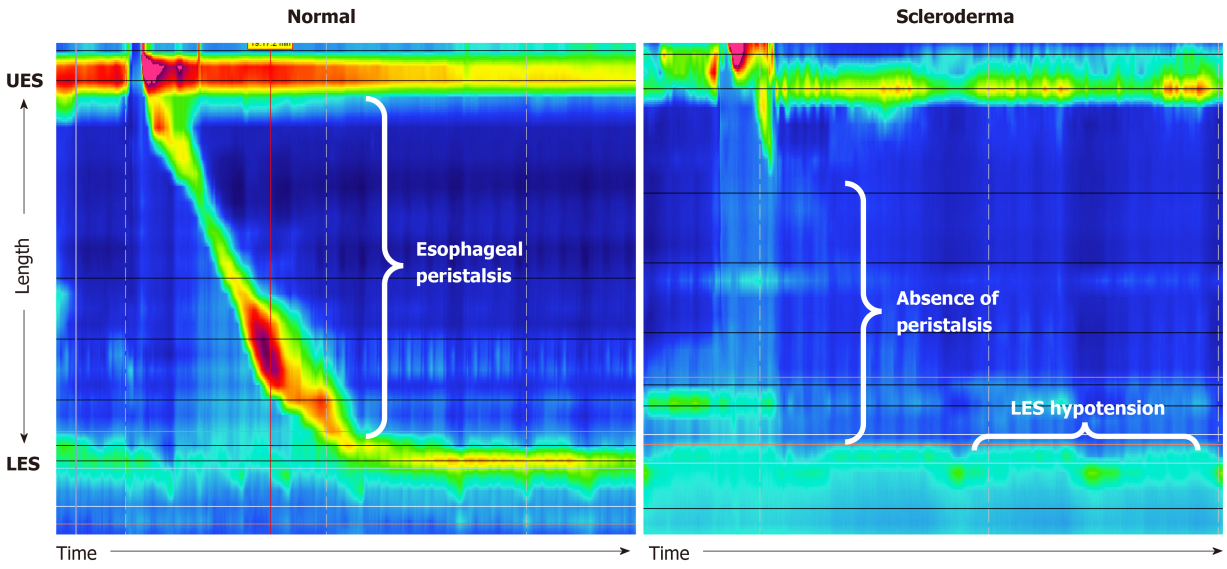


**Figure 4 Normal manometry findings showing peristalsis during swallowing and lower esophageal sphincter tone compared to manometry findings in scleroderma showing an absence of peristalsis and lower esophageal sphincter hypotension.** LES: Lower esophageal sphincter; UES: Upper esophageal sphincter.

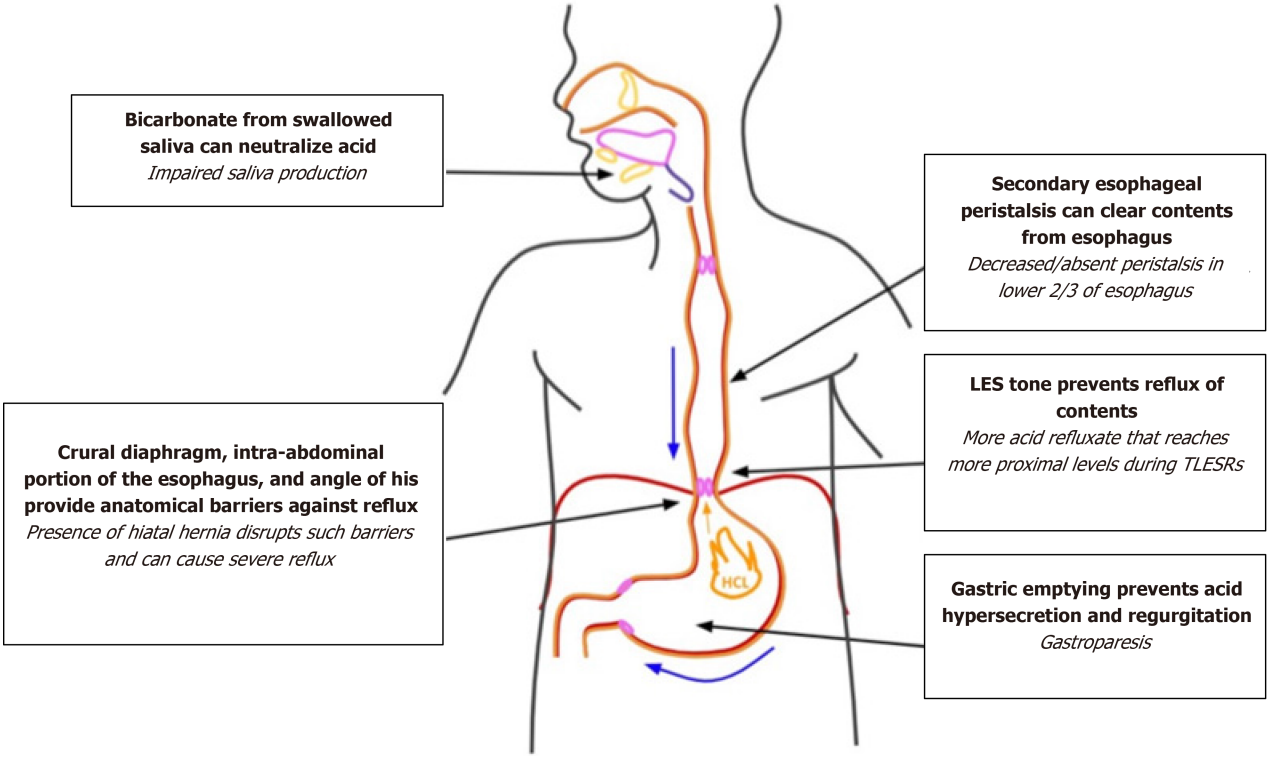


**Figure 5 Disturbances of defense mechanisms against reflux in scleroderma.** LES: Lower esophageal sphincter.

Barrett's esophagus, and adenocarcinoma may cause further dysphagia symptoms[2]. Gastroparesis (Gp) and small bowel dysmotility are also common symptoms in scleroderma and may worsen GERD symptoms[48]. SSc is also associated with impaired saliva production, which impedes the ability to neutralize the refluxed contents[48].

The presence of hiatal hernia in SSc patients may exacerbate GERD symptoms as well. One study comparing 25 SSc patients to 25 control patients found presence of hiatal hernia in 16 out of 25 SSc patients and only 3 out of 25 control patients[49]. Another cross-sectional study of SSc patients found that those with gastroesophageal symptoms had a significantly higher frequency of hiatal hernia than asymptomatic

patients[50]. Concurrent hiatal hernia likely contributes to the occurrence and severity of GERD symptoms in SSc patients. Loss of multiple defense mechanisms against reflux through the various disease processes of SSc may explain why SSc-related GERD is associated with more severe symptoms and more complications[51].

In SSc patients that present with GERD, treatment is imperative to prevent development of acid-induced complications. Medical management *via* proton pump inhibitors (PPIs) is effective in relieving GI symptoms[16]. H2-receptor antagonists (H2RAs) can also be used, though less effective than PPIs[16,52]. This medication therapy should be coupled with lifestyle measures. This includes avoiding acidic foods, abstaining from drinking alcohol and smoking, losing weight, eating smaller meals, and elevating the head of the bed[52].

### *TLESR*

TLESRs are important in development of GERD[45], as studies indicate that TLESRs may be more important than intervals of persistently low LES tone for reflux episodes [45,46]. Given that TLESRs are triggered by gastric distension[45], they may occur more frequently in SSc patients who suffer from Gp-related distension. In patients with GERD, reflux during TLESRs was found to be more acidic and reach more proximal levels of the esophagus than in normal patients[46]. This was thought to be related to decreased esophageal contraction in response to acid reflux[46]. Loss of esophageal motility in scleroderma patients may predispose patients to more acid reflux than can reach more proximal levels of the esophagus during TLESRs. The increase in frequency of TLESRs with refluxate that can reach more proximal levels of the esophagus likely increases severity of GERD symptoms in SSc patients. There has been some evidence suggesting that baclofen may decrease incidence of TLESRs, thereby reducing the number and length of reflux episodes in patients with GERD[16, 53].

### *Delayed esophageal clearance*

Despite the significant overlap between the clinical presentations of SSc esophagus and GERD, there are additional mechanisms in SSc pathophysiology to consider. While GERD is primarily due to reflux of acidic gastric contents up into the esophagus, SSc esophagus has reflux coupled with the stasis of acidic contents due to delayed esophageal clearance, exacerbating symptoms[19,54]. Consequences of delayed esophageal clearance secondary to dysmotility include both severe GERD symptoms from esophageal stasis-related acidity and a dilated distal esophagus[54].

It is important to recognize patients who present with refractory GERD symptoms despite adequate treatments and suspect SSc as a possible diagnosis. In these patients, an EGD will show a dilated esophagus with retained secretions and saliva suggestive of esophageal stasis, and CT may confirm dilated esophagus[54]. Twenty-four-hour ambulatory pH monitor with impedance monitoring and manometry may demonstrate ineffective esophageal motility with subsequent stasis of acidic contents in the esophagus, by documenting prolonged esophageal acidity compared to acid exposure in the stomach[54]. Findings indicative of esophageal stasis on 24-h impedance pH monitoring may be seen in Figure 6. If delayed esophageal clearance is noted through diagnostic testing, it should be addressed promptly, as it is likely a major exacerbator of esophageal acidity. Esophageal stasis of acidic contents may contribute to the severity of GERD symptoms and high rate of complications in SSc patients[51], and should therefore be addressed in therapeutic plans. It is also important to note that esophageal involvement of SSc is linked to development of ILD, a major cause of morbidity and mortality in SSc[2]. ILD severity has been shown to be associated with more active reflux disease, but development may be possible even in SSc patients with esophageal dilation but no clinical esophageal symptoms[2,55]. A retrospective cross-sectional study has shown that the diameter of the esophagus in SSc patients correlates with progression of ILD, as measured by diffusion capacity of the lung for carbon monoxide[2,56]. Given that delayed esophageal clearance can result in both increased acid damage to the esophagus and development of ILD, developing an appropriate treatment plan is essential. Possible lifestyle modifications to consider include eating smaller meals, remaining in an upright position after eating, elevating the head of the bed while sleeping, and avoiding eating in the three hours before sleeping[57,54]. These changes utilize the effect of gravity to promote esophageal clearance of acidic contents[54]. Lifestyle changes can be supplemented with medical therapy targeting esophageal acidity[57].

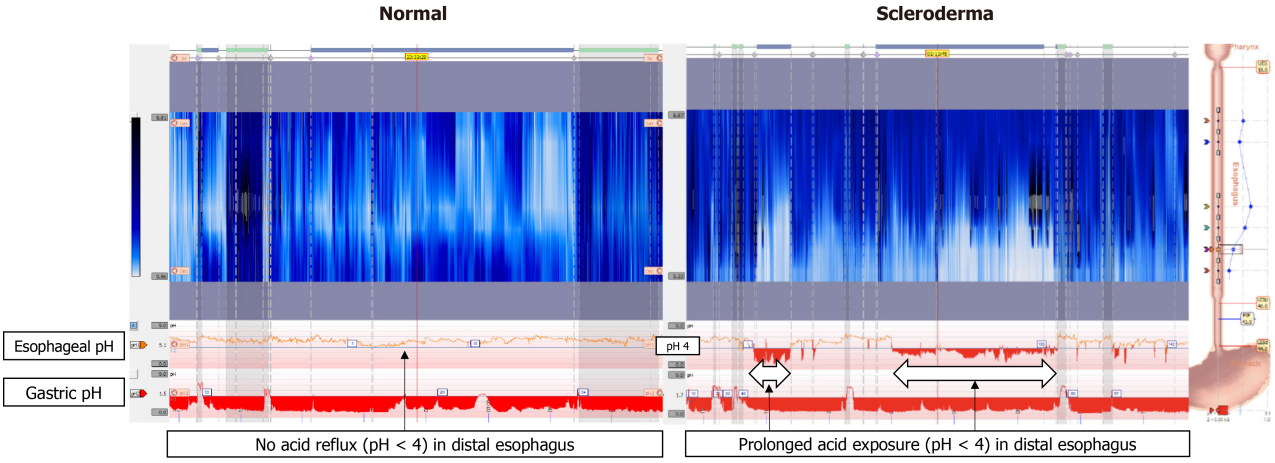


**Figure 6 Twenty-four hour impedance pH monitoring normal findings compared to findings in scleroderma.** In normal esophagus, there are no episodes of acid reflux, as indicated by no drops of pH below 4 in the esophagus. In scleroderma esophagus, there is prolonged acid exposure in the distal esophagus.

### *Erosive esophagitis*

SSc patients are more prone to developing erosive esophagitis as a complication of severe GERD, which can further contribute to their dysphagia. A collection of studies report that 30%-60% of SSc patients develop erosive esophagitis[58-60]. One study found that 24.2% of SSc patients had mild to moderate erosive esophagitis while 3.6% of patients had severe erosive esophagitis[61]. Mechanisms for development of this pathology all pertain to impaired acid clearance in the esophagus and increased acid exposure, such as impaired peristalsis, impaired LES tone, and pathologic nighttime gastroesophageal reflux[58,59]. Symptoms of dysphagia were also found to correlate with erosive esophagitis[58]. This is likely related to both the development of the erosions in esophagitis as well as their sequela, such as strictures.

### *Strictures and mechanical obstruction*

Strictures are a large contributor to dysphagia in SSc and are found in 17%-29% of SSc patients[62]. A diagnosis of SSc increases the odds ratio of developing strictures to 12 [62]. Strictures result from excess deposition of collagen during healing of erosive esophagitis and are often due to chronic gastroesophageal reflux[57]. The presence of these strictures leads to an obstructive dysphagia as they narrow the esophageal lumen and reduce esophageal distensibility[63]. This leads to further impairment of acid clearance from the esophagus, resulting in more esophageal insult and potential for dysphagia. Schatzki rings have also been found in SSc patients, with one study finding 12.5% of SSc patients with the lower esophageal rings. Though they may be more amenable to treatment *via* dilation, these rings also contribute to dysphagia[64]. However, obstructive dysphagia is not always due to strictures and Schatzki rings in SSc — though rare, adenocarcinoma may also cause obstructive esophageal dysphagia.

### *Barrett's esophagus and esophageal adenocarcinoma*

Reflux-related disorders such as SSc are naturally conducive to the development of Barrett's esophagus. Pathophysiology of Barrett's esophagus is tied to increased acidic insult of esophageal epithelium. Clinically, patients with Barrett's esophagus tend to have longer durations of dysphagia and relatively impaired LES tone[65]. It is unclear whether this association is causation or correlation, as risk factors for Barrett's esophagus may predispose patients to other causes of dysphagia as well[65]. Barrett's esophagus has been found in up to 37% of patients with SSc[62], and is more likely to develop in patients with limited SSc[65]. Patients with SSc that have Barrett's esophagus have an increased risk of developing further complications, such as strictures and adenocarcinoma[66]. Esophageal adenocarcinoma specifically may develop as a result of metaplasia and dysplasia found in Barrett's esophagus. Development is more common in SSc patients, as esophageal adenocarcinoma has an incidence of 0.7% per year in patients with SSc and Barrett's esophagus, while incidence is only 0.45% per year in patients with Barrett's esophagus without SSc[67]. Though other complications of SSc may have higher incidence, esophageal adenocarcinoma should still be considered and ruled out in patients presenting with dysphagia.

Esophageal cancer most commonly presents with mechanical obstruction leading to esophageal dysphagia, so it must be considered as a potential contributor to dysphagia symptoms in SSc patients[66].

### Sequelae of SSc treatment

*Candida*, an opportunistic fungus, is normally a symbiont of the esophagus that rarely gives rise to severe disease indications. Yet *Candida* infection remains the most common cause of infectious esophagitis, with an incidence of up to 88%[68,69]. In the setting of an impaired host-defense system, uncontrolled proliferation of *Candida* in the esophageal mucosa can occur, leading to the formation of characteristic adhesive plaques, which are visualized by endoscopy. Studies have reported that Candida esophagitis (CE) can be a result of poor emptying of the esophagus, acid suppression, and immunosuppressive therapy[62]. Connective tissue diseases, such as SSc, can promote esophageal stasis and can, therefore, lead to fungal colonization of the esophagus. In addition, acid suppression *via* PPIs and H2RAs are commonly used in SSc patients with complaints of GERD[2]. For other various manifestations of SSc such as ILD, immunosuppression therapy is commonly prescribed[70]. As a result, CE and secondary stricture formation is likely to occur with SSc, further complicating dysphagia and esophageal dysfunction in these patients[71]. Patients with CE respond well to anti-fungal treatment, limiting the progression of disease to stricture formation and necrotizing CE[72,73]. Therefore, although CE is rare, early detection and treatment of this condition in SSc esophagus is very important to avoid development of esophagus-related complications that have high mortality rates. Pill-induced esophagitis, which is characterized as direct injury to the esophageal mucosa due to use of certain culprit medications[74], is another important consideration in SSc patients. Interestingly, patient-related risk factors for pill-induced esophagitis are commonly associated with extended transit time of culprit medications in the esophagus. Studies suggest that altered anatomy, as found in SSc, can lead to delayed esophageal transit time and stasis, therefore increasing the risk for pill-induced esophagitis[74]. Other patient-related risk factors leading to increased transit time of medications include position of patient while taking pill and size of pill. Reduced water intake while ingesting pill, potentially due to impaired hand function in SSc also increases risk. Decreased saliva production secondary to Sjogren's syndrome, a condition known to have a higher prevalence in SSc patients, may also lead to dysphagia and impaired swallowing of the pill[74].

Notable culprit medications leading to pill-induced esophagitis include non-steroidal anti-inflammatory drugs, aspirin, bisphosphonates, potassium chloride, antibiotics (namely tetracycline and clindamycin), and iron. Prolonged contact of the pill with the esophageal mucosa can lead to direct irritation of the mucosa[75]. Several pathophysiologic mechanisms have been implicated in this condition including disruption of the cytoprotective prostaglandin barrier of the mucosa, caustic injury, and vascular injury resulting from hyperosmotic properties of the medication[74,76-78]. Pill-induced esophagitis often occurs at the site of esophageal narrowing which, in SSc patients, can result from stricture formation, decreased LES tone, or decreased esophageal peristalsis. This condition is typically self-limiting, but if left untreated, can lead to strictures, ulcerations, and even perforations, which can complicate dysphagia in SSc esophagus[79]. Ultimately, pill-induced esophagitis should be considered in SSc patients presenting with heartburn, dysphagia, or odynophagia and culprit medications should be avoided in this patient subset whenever possible. Special attention should be given to SSc patients that develop iron deficiency anemia, which often occurs secondary to microhemorrhages from telangiectasias in the GI mucosa and severe malabsorption along the GI tract in advanced disease[80]. Treatment with iron supplementation may be indicated, but usage of oral iron pills should be closely followed, as studies have shown that this can cause direct mucosal injury to the esophagus, leading to pill-induced esophagitis and dysphagia[81].

## OTHER CONTRIBUTING FACTORS TO DYSPHAGIA

### Gp

Gp is the delay of gastric emptying without mechanical obstruction, resulting in symptoms of bloating, nausea, and upper abdominal pain[82]. In SSc, Gp may develop due to fibrotic infiltration, resulting in subsequent dysfunction of autonomic nerves, smooth muscle, and enteric neurons[82]. Previous studies in SSc patients exhibiting GI symptoms have shown the prevalence of Gp to be 50%-67%[83]. GERD is commonly

associated with Gp, with about 27% of patients with Gp suffering from concomitant moderate to severe GERD[84]. The pathophysiology of this may be related to increased volume of gastric contents resulting in stomach distension and decreased LES pressure with more frequent TLESRs, causing regurgitation of contents into the esophagus[84]. This likely explains why GERD symptoms are found to be most severe in patients with Gp and other pre-existing conditions, such as SSc[3]. The complex interplay between Gp and severe GERD may be the reason why patients with SSc are found to have significantly greater reflux symptoms and complications than those with idiopathic reflux[51]. Correspondingly, treating GERD in patients with SSc may therefore involve treating the underlying Gp.

## CONCLUSION

While it may be intuitive to blame esophageal dysmotility and reflux as the culprit for dysphagia in SSc patients, understanding the physiology of swallowing and anti-reflux defense mechanisms can provide better insight on causative and contributive factors in patient symptomatology. Understanding the range of potential sources for dysphagia, from oral to esophageal, can help develop more effective therapeutic plans that can improve symptoms and result in a better quality of life. Xerostomia may be managed through lifestyle changes, including drinking water frequently, using artificial saliva as needed, utilizing special toothpastes and mouthwashes, and avoiding medications that may exacerbate symptoms[2,85]. SSc patients should regularly perform exercises and massages to stretch their mouth and prevent debilitation from microstomia[2]. Regular follow-up with an experienced dentist can improve dental hygiene and oral health[2]. SSc patients presenting with dysphagia should also be screened for concurrent myositis, and treated accordingly with appropriate immunomodulatory therapy and other interventional procedures[37]. While studies show limited efficacy of treatment options targeting esophageal dysmotility, appropriate GERD management is essential in limiting the development of dysphagia[2]. GERD management includes dietary and lifestyle modifications as well as medication therapy to limit progression of reflux and reflux-related complications[2,57]. Delayed esophageal clearance may also be best managed through lifestyle changes such as avoiding meals before bedtime and elevating the head of the bed while sleeping[54]. Given that Candida infection and certain culprit medications can also cause esophagitis, providers should carefully screen for these in SSc patients. Signs of fungal infection should be treated promptly to prevent complications and culprit medications should be generally avoided[73,79].Underlying Gp may also contribute to symptoms and should be managed with prokinetic agents and dietary modification[2,57]. Being cognizant of the various contributing factors that result in dysphagia can allow physicians to develop better, more well-rounded therapeutic plans. This may allow better control of the various disease processes in SSc and improved symptoms and quality of life for SSc patients.

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ORIGINAL ARTICLE

Basic Study

# Planning the hepatitis C virus elimination in Cyprus: A modeling study

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## Abstract

***BACKGROUND***

Hepatitis C virus (HCV) infection is a major global public health problem. In the Republic of Cyprus, the estimated prevalence of chronic hepatitis C (CHC) among the general population is 0.6%, while the CHC prevalence among people who inject drugs (PWID) is estimated at 46%. Direct-acting antivirals that can eliminate HCV are not yet widely available in the Republic of Cyprus. However, when direct-acting antivirals become available, a long-term strategic plan to guide elimination efforts will be needed to maximize the effect of treatment.

***AIM***

To determine the programmatic targets to eliminate HCV in the Republic of Cyprus.

***METHODS***

A dynamic, stochastic, individual-based model of HCV transmission, disease progression, and cascade of care was calibrated to data from Cyprus. The model

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stratifies the population into the infected general population and the PWID population. A variety of test, prevention, and treatment strategies concerning the general population, PWID, or both were examined. The time horizon of the analysis was until 2034.

*RESULTS*

Under the status quo scenario, the model predicted that 75 (95% confidence interval (CI): 60, 91) and 575 (95%CI: 535, 615) liver-related deaths and new infections would occur by 2034, respectively. Launching an expanded treatment program, without screening interventions, would cause modest outcomes regarding CHC prevalence (16.6% reduction in 2034 compared to 2020) and liver-related deaths (10 deaths would be prevented compared to the status quo scenario by 2034). Implementing a test and treat strategy among the general population but without any intervention in the PWID population would suffice to meet the mortality target but not the incidence target. To achieve HCV elimination in Cyprus, 3080 (95%CI: 3000, 3200) HCV patients need to be diagnosed and treated by 2034 (2680 from the general population and 400 from PWID), and harm reduction coverage among PWID should be increased by 3% per year (from 25% in 2020 to 67% in 2034).

*CONCLUSION*

Elimination of HCV is a demanding public health strategy, which requires significant interventions both among the general population and high-risk groups.

**Key Words:** Mathematical modelling; Projections; Hepatitis C virus elimination; Direct-acting antivirals; Screening campaigns

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**Core Tip:** Direct-acting antivirals (DAAs) that can eliminate hepatitis C virus are not yet available in the Republic of Cyprus. However, when DAAs become available, a long-term strategic plan to guide elimination efforts will be needed to maximize the effect of treatment. To achieve the elimination goals, 3080 patients need to be diagnosed and treated by 2034 (2680 from the general population and 400 from people who inject drugs), and harm reduction coverage among people who inject drugs should be increased by 3% per year.

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## INTRODUCTION

Hepatitis C virus (HCV) is a major public health problem that affects 1% of the world population[1]. The advent of highly effective direct-acting antivirals (DAAs) has significantly improved the management of the infection and brought great optimism that HCV could be eliminated in the near future[2,3]. Driven by the major clinical achievements, the World Health Organization (WHO) released the Global Health Sector Strategy on viral hepatitis targeting elimination by 2030[4]. The ambitious elimination targets include a 80% reduction in HCV incidence and a 65% reduction in HCV-related mortality in 2030 compared to 2015[4].

In the Republic of Cyprus (government-controlled area), the prevalence of chronic hepatitis C (CHC) among the general population is estimated at 0.6%, while the estimated CHC prevalence among people who inject drugs (PWID) is much higher at 43%[5]. Similar to other western countries, HCV transmission in the Republic of Cyprus is mostly limited to the PWID group[6].

Although DAAs are capable to eliminate HCV, they are not yet available in Cyprus. However, when DAAs become available, an appropriate long-term strategic plan to guide elimination efforts will be necessary to maximize the benefits of treatment. For example, to eliminate HCV is vital to implement screening campaigns among the general population to support treatment scale-up[7,8] and prevention/harm reduction (HR) measures among PWID to halt ongoing transmission[9-11].

Several studies from different countries have shown that the elimination of HCV is attainable using integrated strategies[7,8,12]. However, the WHO recommends countries develop country-specific targets within their national plans that align with their epidemiological situation. The optimal combination of the required intervention to achieve HCV elimination targets in Cyprus has not been studied yet. The aim of this study is to simulate the implementation of an integrated HCV strategy in the Republic of Cyprus to determine the programmatic targets to eliminate HCV.

## MATERIALS AND METHODS

### Setting

The HCV prevalence among adults in Cyprus ranges between 0.5%-1.9% with a central estimate of 0.6%[5,13]. Furthermore, according to the Cyprus National Addictions Authority, the estimated number of PWID in 2017 in Cyprus was between 499 and 909 (central estimate 700), of which, according to estimations, between 279 and 509 were HCV positive (central estimate 400) (Table 1).

The burden of HCV-related liver deaths is low since only 13 HCV-related deaths were recorded from 2005 to 2017 (2 HCV-related deaths in 2017)[5]. Regarding the fibrosis stage, only 15.7% of people living with HCV are estimated to have progressed to advanced disease (*i.e.* F3 Liver fibrosis stage and beyond) [5.2% and 10.5% are in F3 and ≥ F4, respectively (Supplementary Table 1)]. The main reason for that is that several patients with advanced disease had access to DAA treatment in the previous years individually either through the Cyprus' Committee of High-Cost Drugs or by ordering generic drugs from abroad.

Diagnosis efforts comprise a vital component of the elimination strategy of HCV. In the Republic of Cyprus, the diagnosis rate is moderate with 21 and 36 new diagnoses of CHC in 2017 and 2018, respectively[5]. However, 90 patients are currently on a waiting list to be approved for treatment with DAAs. Notably, DAAs will be available with no restrictions based on the fibrosis score.

HR strategies such as high-coverage needle and syringe programs and opioid substitution treatment have been often used to prevent the spread of HCV among PWID[14]. Several empirical studies have shown that these interventions can substantially reduce the risk of HCV acquisition among PWID[15]. In the Republic of Cyprus, the coverage of needle and syringe exchange programs is low, while the coverage of opiate substitution therapy is suboptimal because only about a quarter of PWID participate in any of them[16].

### Description of the epidemiological model

A dynamic, discrete-time, stochastic, individual-based model of HCV transmission, disease progression, and cascade of care was fitted to epidemiological and clinical data from the Republic of Cyprus. The model stratified the population into two groups: infected general population (*e.g.*, HCV+ but not PWID) and the PWID population ( Supplementary Figure 1).

The PWID population was stratified based on the following criteria: infection status (susceptible, infected), engagement in the HCV cascade care (undiagnosed, diagnosed, on DAAs), HR status (whether the PWID takes part in HR programs), and sharing status (sharer or non-sharer). PWID could transit from sharers to non-sharers and vice versa. However, the transition from sharers to non-sharers was balanced, so that the proportion of PWID in the high-risk group remained constant over time. Initially, new injectors do not participate in HR programs. Sexual transmission was not considered in the model.

The infected general population (*i.e.* infected patients without the risk to further transmit the disease) was divided by the fibrosis stage and the engagement in the HCV cascade of care (undiagnosed, diagnosed, on DAAs).

Each year, infected PWID exit the pool of injectors due to cessation of injection and transit to the infected general population. Individuals exit various states through HCV related death or background death (Supplementary Figure 1). PWID also experience additional drug-related mortality (Table 1).

**Table 1 Model parameters and references**

| Parameter | Estimate | Ref. |
|---|---|---|
| Total chronic hepatitis C population size in the Republic of Cyprus | 2900 | [13] |
| Chronic hepatitis C population size in the Republic of Cyprus among the general population | 2600 | [13] |
| Chronic hepatitis C population size in the Republic of Cyprus among PWID | 300 | [16] |
| Proportion who are acutely infected and spontaneously clear infection | 26% | [28] |
| PWID population | 700 | [16] |
| Duration of injecting carrier among PWID (yr) | 13.5 | [16] |
| Proportion of sharers | 43% | [16] |
| Overall PWID mortality | 2% | [29] |
| New diagnoses | 30 | [5] |
| Proportion participating in harm reduction programs (OST or high coverage HCNSP) | 25% | [16] |
| Relative risk for HCV infection while in a harm reduction program | 0.5 | [14] |
| SVR IFN-free DAAs | 95% | [17-19] |
| HCV Progression rates per yr | | |
| F0→F1 | 0.176 | [30] |
| F1→F2 | 0.082 | [30] |
| F2→F3 | 0.100 | [30] |
| F3→F4 | 0.161 | [30] |
| F4→Decompensated cirrhosis | 0.04 | [31,32] |
| F4→Hepatocellular carcinoma | 0.021 | [31,32] |
| Decompensated cirrhosis→Death related to HCV | 0.306 | [31,32] |
| Hepatocellular carcinoma→Death related to HCV | 0.433 | [31,32] |
| Decompensated cirrhosis→Hepatocellular carcinoma | 0.021 | [31,32] |

DAAs: Direct-acting antivirals; HCNSP: High-coverage needle and syringe programs; HCV: Hepatitis C virus; IFN: Interferon; OST: Opioid substitution treatment; PWID: People who inject drugs; SVR: Sustained virologic response.

We assume a 95% sustainable virologic response (SVR) rate for those treated with DAAs[17-19]. Individuals who have achieved SVR are considered cured. Patients who fail treatment could be retreated. If PWID achieve SVR, then they become susceptible again and are at risk of reinfection (assuming the risk of reinfection equals the initial infection rate and no behavior changes after successful treatment). Reinfected PWID could be retreated.

### *Infection probability*

The force of infection for susceptible PWID depends on HCV prevalence and on whether they are high-risk, participating in HR programs, or both. More specifically, the force of infection for susceptible PWID who participate in an HR program is multiplied by a factor Z ($Z < 1$) indicating that PWID in HR programs have lower probability of getting infected compared to PWID not in HR programs. Low-risk PWID could not be infected (*e.g.*, PWID who do not share their injection equipment are not at risk of HCV infection).

### *Model calibration*

The model was run until it achieved a steady-state (the level of prevalence in the population of PWID in 2020 without use of treatment) by varying the infection rate. After reaching a steady-state, the model was seeded with a cohort that represents the infected patients from the general population (size of the infected population, fibrosis stage, share of diagnosed, and mean age of the infected patients).

Intervention scenarios involving the expansion of testing and/or scaling-up treatment coverage and/or increased proportions of PWID in HR programs were examined. For each scenario, 1000 simulations (runs) were performed (Supplementary Figure 2). The results were summarized using the median of all simulations. To include the appropriate uncertainty (stochastic variability), credible intervals (*i.e.* 2.5 and 97.5 percentiles of simulations) are also shown. The time horizon of our analysis was 15 years (2020–2034). Further details about the description of the model are available in the appendix.

### Scenarios analysis

Modified elimination targets: The WHO recommends each country develops country-specific targets aligned with its epidemiological situation. Several studies have highlighted the issue that for some countries the proposed WHO's elimination targets may be impractical[20,21]. For example, in countries like the Republic of Cyprus with low baseline mortality (approximately 2 per year), the 65% reduction may not be feasible.

The mortality target for the Republic of Cyprus was modified as follows: to prevent the cumulative number of deaths by 2034 from surpassing the limit of 5 per 100000 people (*e.g.*, 45 deaths) and to reduce CHC prevalence among the general population by over 80% in 2034 compared to 2020. Regarding the incidence goal, we kept the incidence target of the WHO (80% reduction in incidence in 2034 compared to 2020).

### Examined scenarios

To examine the impact of different strategies on incidence, chronic prevalence, and HCV-related mortality, five different scenarios were considered.

Scenario A: A status quo scenario was used to generate predictions regarding the current management of HCV (Supplementary Figure 3). This provides a reference scenario of no regular scale-up of treatment (about 3 patients from the general population are individually treated per year). The additional four scenarios explored the impact of increased DAA uptake, HCV testing, and HR coverage.

Scenario B investigates the impact of increasing DAA coverage exclusively among the general population but without implementing awareness and screening programs (Supplementary Figure 4).

Scenario C is scenario B plus the implementation of awareness and screening programs among the general population (Supplementary Figure 5). This scenario explores the impact of an elimination strategy that exclusively focuses on the general population without any interventions among the high-risk population (*e.g.*, PWID population).

Scenario D is scenario C with increased HR coverage among PWID but without the simultaneous use of DAA therapy (Supplementary Figure 6). This scenario evaluates the impact of the expansion of primary prevention strategies among PWID without using DAA treatment. We assumed that PWID who participate in HR programs are diagnosed if they are infected and undiagnosed.

Scenario E is scenario D with the addition that DAA treatments would also be available to the PWID population (Supplementary Figures 7 and 8). This scenario assesses an integrated strategy that includes interventions both regarding the general population and the high-risk group.

### Sensitivity analysis

To examine the impact of the uncertainty around epidemiological parameters or model assumptions in the required treatments to achieve elimination, a series of univariate sensitivity analyses under scenario E were implemented. More specifically the impact of higher/lower general population (1950 or 3250 *vs* 2600), higher/lower PWID population (525 or 875 *vs* 700), the impact of adherence to treatment on SVR (85% *vs* 95%), influence of shorter/longer average duration of injecting carrier (10 or 15 *vs* 13.5 years), and the effect of changes in risk behavior after successful treatment (50% lower or higher probability of re-infection *vs* no change in risk behavior).

## RESULTS

### Status quo scenario

Under the status quo scenario, the model predicted a 4.5% (95% confidence interval (CI): 3.2%, 5.7%) increase in the number of viremic cases in 2034 compared to 2020

(Figure 1A). Concerning liver-related deaths, the model projects that 75 (95%CI: 60, 91) liver-related deaths would occur between 2020 and 2034 under the status quo scenario (Figure 2).

Regarding HCV incident cases, it was estimated that 38 PWID (95%CI: 23, 51) are newly infected every year [annual incidence per 100 person-years: 9.6 (95%CI: 5.5, 13.2)] (Figure 3). Without any PWID-specific interventions, HCV incidence is expected to remain constant through our study. The cumulative HCV incident cases, under the status quo scenario, would be 575 (95%CI: 535, 615) during 2020-2034 (Supplementary Figure 10). Finally, the total number of PWID with CHC that would stop injecting and be considered part of the general population, under the status quo scenario, would be 340 (95%CI: 305, 360) during 2020-2034.

### Scenario B

Under scenario B, 90 DAAs per year would be available for the infected population. However, without the implementation of awareness and screening interventions, the elimination program would quickly run out of patients after the first years due to the insufficient number of diagnosed patients (Figure 4B).

Scenario B would cause marginal declines in CHC prevalence and liver-related deaths. More specifically, the total number of infected people was projected to be lower by 16.6% (95%CI: 15.2%, 18.5%) in 2034 compared to 2020 (Figure 1A). Regarding deaths, scenario B would prevent 10 (95%CI: -12, 29) liver-related deaths compared to the status quo scenario by 2034. However, this decline would be insufficient to keep the number of deaths closer to the desired target (45 cumulative deaths) (Figure 3).

Finally, because in this scenario no antiviral treatments or expansion of HR coverage is provided to PWID, HCV incidence would remain constant (Figure 2).

### Scenario C

Under scenario C, the modified targets of mortality could be achieved. More specifically, using a gradually increasing program, if we treat 2715 (95%CI: 2670, 2760) patients from the general population between 2020 and 2034, the prevalence of CHC in the general population will decrease by 82.7% (95%CI: 81.5%, 84.1%) in 2034 compared to 2020. Liver-related deaths will not exceed the limit of the 5 per 100000 deaths (Figures 1B, 3, and 4).

Scenario C would prevent 46 (95%CI: 27, 63) liver-related deaths compared to the status quo scenario (Figure 3). Nevertheless, no declines in incidence would be observed under this scenario (Figure 2).

### Scenario D

Scenario D, in addition to scenario C, would prevent 59 (95%CI: 4, 120) new infections compared to the status quo scenario by 2034. However, without using DAAs for the PWID population, the estimated reduction in the incidence of HCV would be well below the WHO elimination goals (22.5% *vs* 80.0%) (Figure 2).

Furthermore, scenario D, compared to the previous scenarios, would reduce the transition of infected PWID, who stopped drug injection, to the general population. More specifically, expansion of HR would prevent 32 (95%CI: 9, 52) infections from passing from PWID to the general population.

### Scenario E

The integrated strategy E can eliminate HCV in the Republic of Cyprus. To eliminate HCV in the country, HR coverage should be increased by 3% per year (from 25% in 2020 to 67% in 2034), and 3080 (95%CI: 3000, 3200) patients (2680 from the general population and 400 from PWID) should be diagnosed and treated with DAAs by 2034 (Figure 4, Supplementary Figure 9). To create the required DAA demand for the general HCV+ population, significant awareness and screening programs should be implemented. On the contrary, regarding the PWID population, because most of the PWID would be diagnosed through the expansion of HR coverage, there is no need for a significant screening program to identify the undiagnosed.

Under scenario E, the total number of viremic cases would decrease by 87.6% (95%CI: 86.4%, 88.7%) in 2034 compared to the number of viremic cases in 2020 (Figure 1A). Additionally, 45 (95%CI: 21, 68) and 175 (95%CI: 115, 220) liver-related deaths and new infections, respectively, would be averted compared to the status quo scenario by 2034 (Supplementary Figure 10).

The total number of chronic infected PWID that would cease injecting and move to the general population under scenario E would be 185 (95%CI: 160, 220) during 2020-

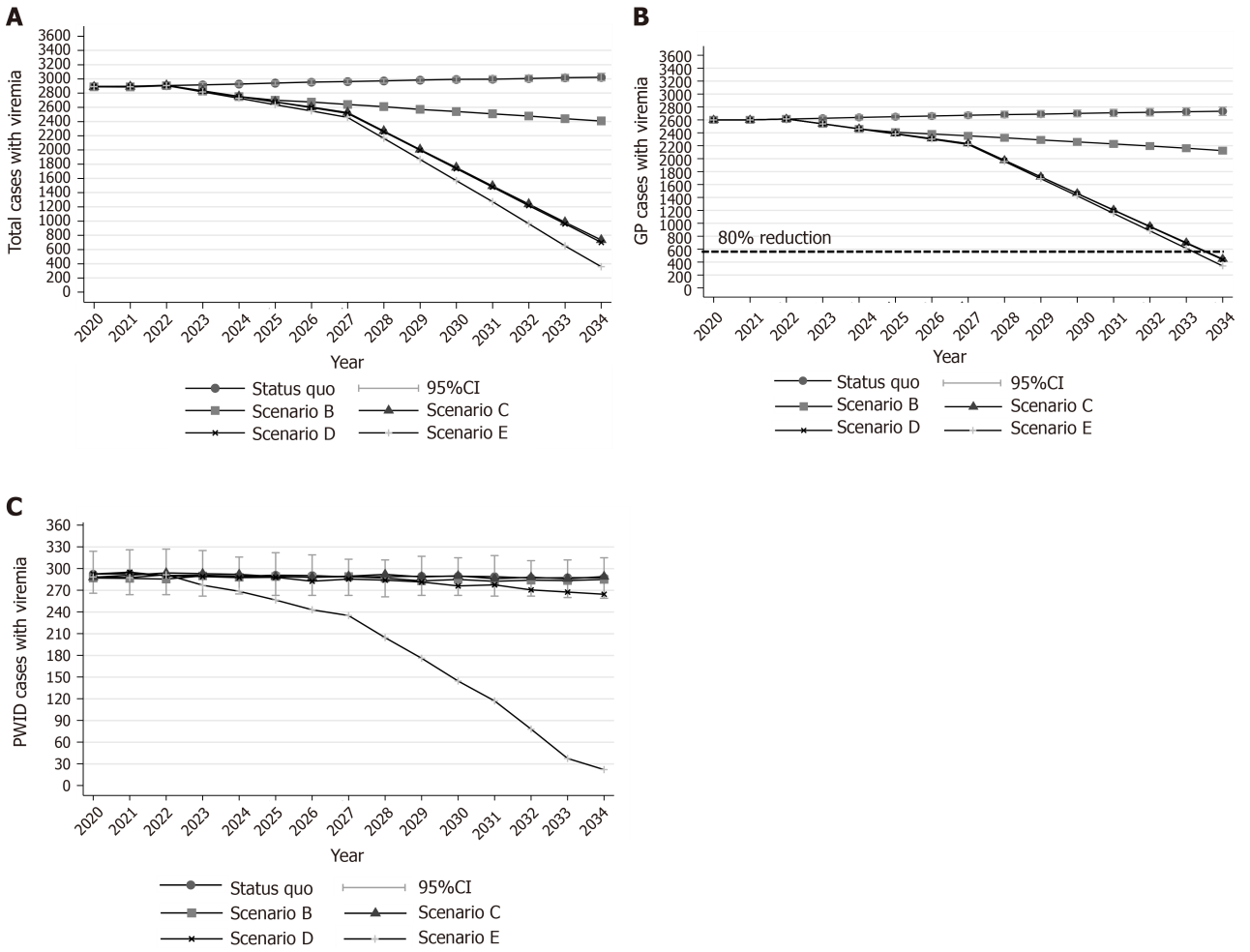


**Figure 1 Model projections of future prevalence of chronic hepatitis C virus in the Republic of Cyprus under different scenarios for the total infected population, the general population (*i.e.* total infected population minus infected people who inject drugs), and the infected people who inject drugs.** A: Total population; B: General population (GP); C: People who inject drugs (PWID). CI: Confidence interval.

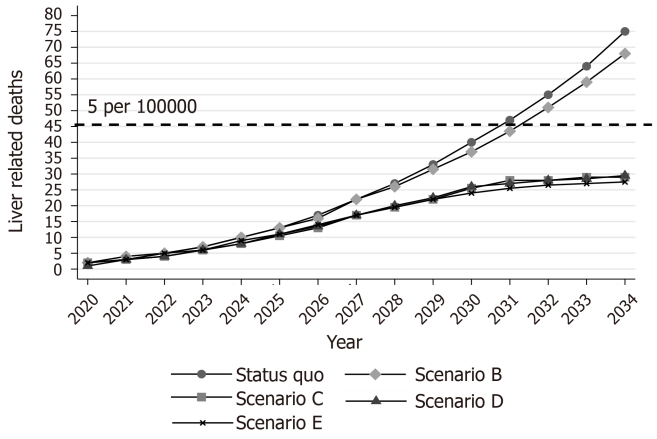


**Figure 2 Model predictions for cumulative hepatitis C virus related deaths in the Republic of Cyprus under different scenarios.**

2034.

Finally, under the elimination scenario, some reinfections among PWID would appear. Because there is no natural immunity following successful treatment, PWID with ongoing risk activities remain vulnerable to reinfection. It was estimated that 5 (95%CI: 1, 15) reinfections would occur during the horizon of the elimination strategy. (Supplementary Table 2).

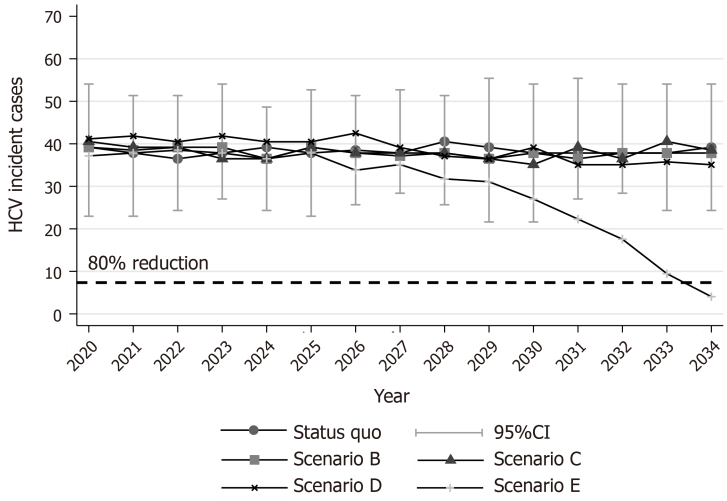


**Figure 3 Model predictions for hepatitis C virus new cases (incidence) among people who inject drugs in the Republic of Cyprus under different scenarios.** HCV: Hepatitis C virus.

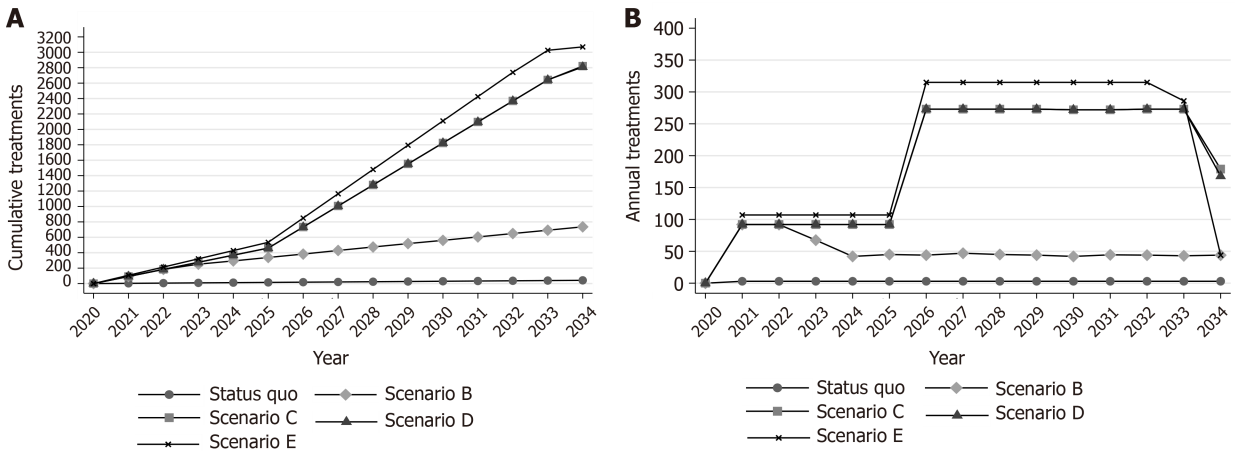


**Figure 4 Model estimations concerning the cumulative and annual number of antiviral treatments of each scenario.** A: Cumulative treatments; B: Annual treatments.

### *Sensitivity analysis*

The sensitivity analysis showed that the variation in the size of the HCV+ general population is the primary determinant of the estimated required number of treatments in order to achieve HCV elimination in the Republic of Cyprus. Specifically, under a smaller population (*e.g.*, 1950 instead of 2600), the required treatments decreased by 19.3% compared to the base case. On the contrary, if the size of the HCV+ general population is higher than the base case (*e.g.*, 3250 instead of 2600), the needed treatment increased by 20.1%, compared to the base case (Supplementary Figure 11).

The second most important factor that affects the required number of treatments to achieve elimination is the variation in the average duration of drug use injection. For a longer injecting duration (15.0 years instead of 13.5 years), the needed treatments would increase by 3.6%. On the other hand, for a shorter injecting duration (10.0 years instead of 13.5 years) the required treatments would decrease by 3.4%. Lower SVR is expected to cause a 3.7% increase in the required number of treatments to achieve elimination.

Although most of the variables had a marginal impact on the projections regarding the elimination at the country level, they have a significant impact on the micro-elimination of CHC in PWID (Supplementary Figure 11). For example, potential changes in risk behavior of PWID after successful treatment is a vital factor regarding HCV elimination within the PWID population. If the risk of reinfection following treatment is 50% higher than the primary risk of infection (due to potential complacency caused by improvements in antiviral therapy), then the required number of treatments to reduce the incidence of HCV would 10% higher.

## DISCUSSION

Our study is the first analysis that estimated the required interventions to achieve HCV elimination in the Republic of Cyprus. According to our model, HCV elimination in the Republic of Cyprus is feasible but necessitates significant improvements in the cascade of care, expansion in HR coverage, and scale-up of antiviral treatment. In this analysis, a mild gradual increase in DAA coverage was analyzed, as it is more realistic in settings where the majority of the infected population are undiagnosed and unlinked to care.

Because the elimination of HCV requires sustained and targeted efforts, well-defined targets are critical to guide action. Well-defined targets would help ensure enhanced HCV screening and committed funding to scaling-up treatment. The sustainable funding of the national action plan and the political will to achieve HCV elimination has been highlighted as key success factors for eliminating HCV as a public health threat[12].

Implementation of screening and linkage-to-care programs is a significant component of the elimination strategy[7,8,12]. To achieve the elimination targets, awareness activities should create enough demand for treatment. As scenario B illustrates, if awareness activities fail to diagnose enough patients, WHO elimination targets are unlikely to be reached because the elimination program would quickly run out of patients to treat (Figure 4B). This highlights the importance of taking a holistic approach to HCV elimination with coordinated screening, linkage-to-care, and treatment efforts. An HCV management without effective screening campaigns would be an ineffective health policy strategy[7,8].

Injection drug use remains the major risk factor for new HCV infections in the Republic of Cyprus. The efforts to eliminate HCV among PWID are a critical component of the country's elimination strategy. Elimination of HCV among PWID not only reduces new infections but also eliminates the number of infected ex-PWID who will enter the general population. For example, under the status quo scenario, the number of PWID who would acquire HCV and stop injecting is high: 340 (95%CI: 305, 360) infected ex-PWID by 2034. As most of those individuals are in an early phase of the disease and mostly without symptoms, it would be more difficult to be diagnosed and linked to care when they have been moved back to the general population. This suggests that if case finding interventions failed to identify infected PWID during their injecting carrier, intensifying screening campaigns in the general population to prevent HCV-related deaths attributed to the ex-PWID population should be implemented.

HR interventions have a vital and multifaceted role in reaching the incidence elimination target[9,22]. First, HR programs reduce both initial infections and reinfection after successful treatment and thus the required number of treatments to eliminate HCV. Second, HR services could play a potential case-finding role and serve as an access point to HCV education and counseling. It is important that if we expand HR coverage, the need for additional screening campaigns among PWID would be minimal (Supplementary Figure 8). Nevertheless, HR strategies must be provided for the long term, without any future discontinuation. Potential removal would allow the rebound of HCV infections, even after the elimination goals have been achieved[9,23]. Additionally, as scenario D highlights, if HR strategies are not coupled with antiviral therapy, the expected decreases in CHC prevalence and incidence would be limited.

Currently, HCV mortality is not considered a significant public health problem in Cyprus as there are few patients with advanced disease in the country. Nonetheless, our model highlights that complacency or inaction will cause a substantial number of preventable HCV-related deaths and HCV consequences (compensated cirrhosis, decompensated cirrhosis, and hepatocellular carcinoma) in the following years as a result of the aging of the HCV cohort.

Increases in reinfections may be a barrier to implementing treatment as prevention as an intervention to end transmission[24,25]. Reinfected cases are expected to exist during the elimination strategy because the susceptible population would increase without significant decreases in the infected population. Nevertheless, the existence of reinfections is an indirect indicator that we treat active PWID[9]. Sustained HCV treatment strategies will reduce the infected pool leading to the eventual reduction in the rate of HCV reinfection. In the Republic of Cyprus, reinfections are expected to be relatively low under scenario E due to the significant expansion of the HR coverage during the elimination strategy.

The implementation of the national elimination strategy is a dynamic procedure. Monitoring and evaluation of the progress of an elimination plan are essential to understand the effectiveness of the applied interventions and to determine the level of

improvement if needed. Although the strategy presented in our results could achieve the elimination of HCV, our mathematical model should be continuously rerun and fed with the most up-to-date data, so as to evaluate the achieved impact and keep the programs on track to achieve the elimination targets.

### Added value of the study

Our study contributes to the discussion regarding the feasibility of HCV elimination. First, the analysis underlined that HCV elimination in Cyprus is achievable and computed the required interventions.

Second, due to the very low baseline HCV-related mortality (~2 per year), we have used both an absolute (*i.e.* preventing the cumulative number of deaths by 2034 from surpassing the limit of 5 per 100000 people) and a relative target (*i.e.* reducing CHC prevalence among the general population by over 80% in 2034 compared to 2020) regarding the mortality part of the elimination. Although the relative targets have been extensively used since their introduction, the absolute HCV elimination targets have recently been put on the agenda[20,21].

### Comparisons with other studies

Our results are consistent with previous modeling studies showing that in order to meet the elimination targets, interventions to prevent transmission and increase testing simultaneously with the increase of treatment coverage should be implemented [11,22,26]. Furthermore, the outputs of our model are in line with other models showing that the impact of scaling-up treatment is significantly larger when combined with prevention programs[9-11,23]. Finally, our findings that the mortality target requires more treatment than needed to achieve the incidence target is consistent with previous studies[23,27].

### Limitations

As with any modelling study, there are several limitations to our approach. First, the model ignores the impact of social networks on HCV transmission and assumes that the population is totally mixed, *i.e.* injectors have equal contact with all other injectors in the population. Second, we assumed that the proportion of sharers and non-sharers PWID remained constant over time after 2016. Third, the model did not take into account the impact of the coronavirus disease 2019 pandemic. Fourth, we assumed that the fibrosis distribution of the patients awaiting DAA therapy was similar to the whole HCV-infected population in the Republic of Cyprus. Finally, the model did not consider the additional mortality or potential increase of HCV progression rates due to HCV/human immunodeficiency virus coinfection.

## CONCLUSION

The elimination of HCV is a demanding public health goal, which requires significant reforms. Our results show that the elimination of HCV cannot be achieved without implementing awareness and screening programs among the general population and prevention interventions among high-risk groups. Our model estimates that around 3000 patients need to be diagnosed and treated by 2034.

## ARTICLE HIGHLIGHTS

### Research background

Hepatitis C virus (HCV) infection is a major global public health problem. Although direct-acting antivirals are capable to eliminate HCV, they are not yet widely available in Cyprus. However, when direct-acting antivirals become available, an appropriate long-term strategic plan to guide elimination efforts will be necessary to maximize the benefits of treatment.

### Research motivation

An appropriate long-term elimination plan will maximize the benefits of treatment.

### Research objectives

This study aims to simulate the implementation of an integrated HCV strategy in the

Republic of Cyprus to determine the programmatic targets to eliminate HCV.

### Research methods

A dynamic, discrete-time, stochastic, individual-based model of HCV transmission, disease progression, and a cascade of care was fitted to epidemiological and clinical data from the Republic of Cyprus. The model stratifies the population into two groups: the infected general population [*e.g.*, HCV+ but not people who inject drugs (PWID)] and the PWID population. The model was run until it achieved a steady-state (the level of prevalence in PWID population in 2020 without the use of a treatment) by varying the infection rate. After reaching a steady-state, the model was seeded with a cohort that represents the infected patients from the general population (size of the infected population, fibrosis stage, share of diagnosed, and mean age of the infected patients).

### Research results

The analysis showed that under the status quo scenario 75 (95% confidence interval: 60, 91) and 575 (95% confidence interval: 535, 615) liver-related deaths and new infections would occur by 2034, respectively. Without screening interventions, launching an expanded treatment program would cause modest outcomes regarding chronic hepatitis C prevalence (16.6% reduction in 2034 compared to 2020) and liver-related deaths (10 deaths would be prevented compared to the status quo scenario by 2034). Implementing a test and treat strategy among the general population but without any intervention in the PWID population would suffice to meet the mortality target but not the incidence target. To achieve HCV elimination in Cyprus, 3080 (95% confidence interval: 3000, 3200) patients need to be diagnosed and treated by 2034 (2680 from the general population and 400 from PWID), and harm reduction coverage among PWID should be increased by 3% per year (from 25% in 2020 to 67% in 2034).

### Research conclusions

Our study highlighted that without the implementation of large awareness or screening programs, HCV elimination cannot be achieved, due to suboptimal treatment coverage. Elimination of HCV is a demanding public health strategy that requires significant public health reforms (*e.g.*, enhancing harm reduction programs, implementing case-finding, linkage to care interventions).

### Research perspectives

Elimination of HCV is a demanding public health intervention, which poses significant challenges in any health care system. Nevertheless, our analysis highlighted that HCV elimination is an achievable target.

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ORIGINAL ARTICLE

Clinical and Translational Research

# Establishment and validation of a computer-assisted colonic polyp localization system based on deep learning

Sheng-Bing Zhao, Wei Yang, Shu-Ling Wang, Peng Pan, Run-Dong Wang, Xin Chang, Zhong-Qian Sun, Xing-Hui Fu, Hong Shang, Jian-Rong Wu, Li-Zhu Chen, Jia Chang, Pu Song, Ying-Lei Miao, Shui-Xiang He, Lin Miao, Hui-Qing Jiang, Wen Wang, Xia Yang, Yuan-Hang Dong, Han Lin, Yan Chen, Jie Gao, Qian-Qian Meng, Zhen-Dong Jin, Zhao-Shen Li, Yu Bai

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## Abstract

***BACKGROUND***

Artificial intelligence in colonoscopy is an emerging field, and its application may help colonoscopists improve inspection quality and reduce the rate of missed polyps and adenomas. Several deep learning-based computer-assisted detection (CADe) techniques were established from small single-center datasets, and unrepresentative learning materials might confine their application and generalization in wide practice. Although CADes have been reported to identify polyps in colonoscopic images and videos in real time, their diagnostic performance deserves to be further validated in clinical practice.

***AIM***

To train and test a CADe based on multicenter high-quality images of polyps and preliminarily validate it in clinical colonoscopies.

***METHODS***

With high-quality screening and labeling from 55 qualified colonoscopists, a dataset consisting of over 71000 images from 20 centers was used to train and test a deep learning-based CADe. In addition, the real-time diagnostic performance of CADe was tested frame by frame in 47 unaltered full-ranged videos that contained 86 histologically confirmed polyps. Finally, we conducted a self-controlled observational study to validate the diagnostic performance of CADe in real-world colonoscopy with the main outcome measure of polyps per colonoscopy in Changhai Hospital.

***RESULTS***

The CADe was able to identify polyps in the test dataset with 95.0% sensitivity and 99.1% specificity. For colonoscopy videos, all 86 polyps were detected with 92.2% sensitivity and 93.6% specificity in frame-by-frame analysis. In the prospective validation, the sensitivity of CAD in identifying polyps was 98.4% (185/188). Folds, reflections of light and fecal fluid were the main causes of false positives in both the test dataset and clinical colonoscopies. Colonoscopists can detect more polyps (0.90 *vs* 0.82, $P < 0.001$) and adenomas (0.32 *vs* 0.30, $P = 0.045$) with the aid of CADe, particularly polyps < 5 mm and flat polyps (0.65 *vs* 0.57, $P < 0.001$; 0.74 *vs* 0.67, $P = 0.001$, respectively). However, high efficacy is not realized in colonoscopies with inadequate bowel preparation and withdrawal time ($P = 0.32$; $P = 0.16$, respectively).

***CONCLUSION***

CADe is feasible in the clinical setting and might help endoscopists detect more polyps and adenomas, and further confirmation is warranted.


**Key Words:** Computer-assisted detection; Artificial intelligence; Deep learning; Colonoscopy; Clinical validation; Colorectal polyp

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**Core Tip:** Our study indicated that the deep learning-based computer-assisted detection system trained from the dataset consisting of the largest number of polyps achieved high diagnostic performance on the test dataset of images, colonoscopy videos and clinical validation. This system might aid colonoscopists in finding more polyps and adenomas and deserves to be further validated in multicenter randomized trials.

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## INTRODUCTION

Colorectal cancers (CRC) are the third most prevalent cancer and the second highest cause of cancer deaths worldwide[1]. Colonoscopy is considered to be the gold standard for CRC screening[2] but cannot detect all colonic neoplasms[3,4]. Colonoscopy has been reported to miss 17%-48% of adenomas, which are considered to represent the causes of 50%-60% of interval cancers[5-7].

To detect more polyps, a wealth of auxiliary devices and technology have been invented to improve adenoma detection rates (ADRs) and reduce adenoma miss rates (AMRs)[8,9]. However, these devices cannot overcome the limitations of colonoscopists themselves, who could overlook the polyps flashing across the video screen [10]. The ability of colonoscopists to detect adenoma is also influenced by alertness, fatigue and monitoring and varies greatly among individual colonoscopists[11,12]. In addition, even colonoscopists with a high ADR may still miss adenomas because they may become less vigilant about inspecting the remaining colon after detecting one adenoma (one and done effect)[13].

Over the last two decades, computer-assisted polyp detection has been increasingly explored to improve inspection quality and reduce AMR[4,14]. Recently, artificial intelligence has made remarkable breakthroughs in medical fields with deep learning and convolutional neural networks (CNNs)[15,16]. Deep learning automatically makes use of CNNs, which logically imitate the structure and activity of brain neurons, to learn detailed features of medical images[17]. With sufficient learning materials, CNNs can reach even greater real-time detection accuracy than human experts, which suggests that computer-assisted detection systems (CADes) might serve as real-time "experts" to improve the quality of colonoscopies[15,18-20]. However, despite promising findings, most current CADes were established using a limited number of colonoscopic images from a single center, which might limit the robustness and generalizability of findings in wide practice[18,21]. Therefore, we trained and tested the CNN-based CADe using colonoscopic images of multicenter datasets as well as tested and validated its diagnostic performance in real-time colonoscopy videos and real-world colonoscopy.

## MATERIALS AND METHODS

### *Overview of study design*

The colonoscopic records were retrospectively collected from 20 centers (Supplementary Table 1). Figure 1A illustrates the workflow of preprocessing images. Fifty-five qualified colonoscopists screened and labeled the images in a specific labeling system (Supplementary Video 1), and the labeled images were randomly divided into training and test datasets for the construction and testing of the CADe. The detailed preprocessing is illustrated in the Appendix A. To evaluate comprehensively ADe's real-time diagnostic performance, 47 unaltered full-range colonoscopy videos of routine practice, including 86 histologically confirmed polyps, were collected (Supplementary Table 2, Appendix B), and a self-controlled observational study was conducted for clinical validation in the Changhai Endoscopy Center.

### *Construction of CADe*

The CADe was built based on the You Only Look Once v2 deep learning framework. The training dataset was used to build the model, and the testing dataset was used to test the performances with the details illustrated in the Appendix C.

### *Validation of the CADe in clinical colonoscopy*

Figure 1B shows the process of enrolling patients. Consecutive outpatients aged 18-75 years who were scheduled for screening, surveillance and diagnostic colonoscopies were invited to participate in the validation between November 1, 2018 and December 10, 2018. Exclusion criteria included declined consent, age < 18 or > 75 years, poor

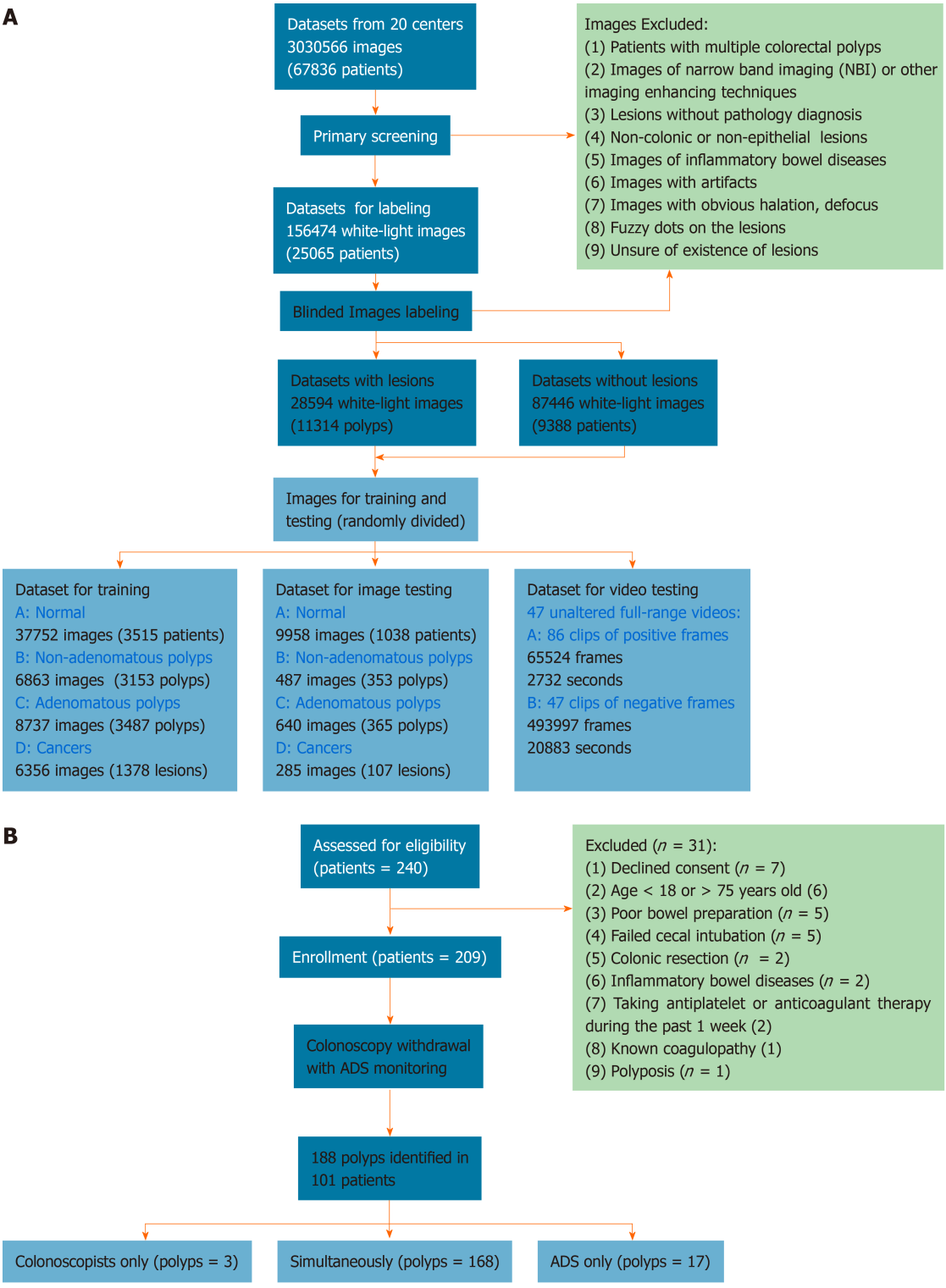


**Figure 1 Flowchart of image preprocessing and validation in clinical trials.** A: Image preprocessing; B: Validation in clinical trials.

bowel preparation quality, failed cecal intubation, history of colonic resection, inflammatory bowel disease, antiplatelet or anticoagulant therapy during the past 1 wk, known coagulopathy and polyposis. Patients received 3 L polyethylene glycol as a split-dose bowel preparation and were either sedated with propofol or without. Twenty colonoscopists, including two trainees, with experience ranging from 100 to over 20000 procedures performed colonoscopies, and an additional colonoscopist served as the full-time observer. The ADRs of the included colonoscopists ranged from 14%-33% in a mixed-indications population. Water-based colonoscopy, antispasmodics, distal attachments, optical-enhanced imaging and image-guided devices were not used in the validation.

CADe was automatically initiated when the ileocecal valve was identified. When identifying any potential polyp, the CADe presented an alert rectangle surrounding polyps on a second monitor and sounded alert, which was audible to colonoscopists and the observer. However, colonoscopists only observed the regular monitor of colonoscopy and were unable to see directly the alert rectangle since the second monitor (showing CADe's findings) was placed on their rear-right side. To view the findings identified by CADe, the colonoscopists had to turn back toward their right. When conducting withdrawal, the colonoscopists were required to offer immediately a verbal signal once they detected a potential polyp. The observer observed the two monitors, received alerts of CADe and signals from colonoscopists and finally determined the detection priority according to the following instructions.

Polyps identified in the study were finally confirmed by careful inspection of colonoscopists, biopsy and histologic examination, and the potential causes of false detection were determined by the discussion of colonoscopists and observers. Under the guidance of the observer, once hearing the alerts of the CADe, colonoscopists were required to recheck immediately all findings of the CADe, including observing the second monitor and carefully inspecting highlighted areas by the CADe. The duration of defining polyp-detection priority was confined to continuous withdrawal duration, and any process of observing the second monitor, seeking or unfolding polyps for rechecking, or performing biopsy and polypectomy was excluded. If the polyp was only identified by the CADe and colonoscopists could not localize the polyp before observing the second monitor, it was defined as type A (CADe only, missed by colonoscopists). If the polyp was reported by the colonoscopists and missed by the CADe before an unfolding or close view, it was defined as type B (colonoscopists only, missed by CADe). If the polyp was reported by both the colonoscopists and CADe during continuous withdrawal, it was defined as type C (colonoscopists and CADe). Therefore, types A + B + C included findings of the colonoscopists + CADe, whereas types B + C were classified as findings of the colonoscopists alone.

### Outcome measures

For clinical validation, the main outcome measures were the mean polyps per colonoscopy and the sensitivity of CADe[22]. The mean adenomas per colonoscopy, false positives and negatives of CADe and potential causes were also analyzed. For the test dataset, the diagnostic performance was assessed based on the accuracy, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) with definitions illustrated in the Appendix D.

### Sample size and statistical analysis

The sample estimation was based on the number of polyps per colonoscopy using the comparison of paired quantitative data. In the pilot trial, we found that the CADe could help increase the identification of 0.1 polyps per colonoscopy with a standard derivation of 0.37 for the improvement and assumed a power of 0.9 with an α-error of 0.01 to determine the sample size. Therefore, 203 patients were at least required for clinical validation. Continuous data are presented as the means and standard deviations. The number of polyps identified by each detection method was compared using a Wilcoxon signed ranks test. A 2-sided McNemar test with a significance level of 0.05 was performed to compare the polyp detection rate (PDR), ADR and sensitivity between colonoscopists and colonoscopists + CADe. A 2-sided $P$ value < 0.05 was considered statistically significant, whereas $P$ values for multiple groups were compared with relevant levels after Bonferroni correction. All statistical analyses were performed using SPSS Statistics v21 (IBM, Armonk, NY, United States).

### Ethics and study registration

This study received approval from the ethics committee of Shanghai Changhai Hospital and registered at ClinicalTrials.gov (identifier, NCT03761771) and was performed in accordance with the Declaration of Helsinki. The use of retrospective data (images and historical reports) and the study protocol was approved or exempted by the ethics committee of every hospital. Informed and written consent was obtained from all patients during clinical validation, and patients had the right to withdraw at any time. All authors had access to the study data and approved the final manuscript.

## RESULTS

### *Polyp identification in the test dataset*

Supplementary Table 3 shows the characteristics of the polyps in the test dataset. There were 285, 640 and 487 images of adenocarcinomas, adenomatous polyps and nonadenomatous polyps in the test datasets, respectively (Supplementary Table 3). Of the 285 adenocarcinomas, 17.2% (49/285) could not be classified by the Paris classification because advanced stages were suspected. A total of 99.2% (483/487) of the nonadenomatous polyps and 96.6% (618/640) of the adenomatous polyps were classified as sessile or flat type (Supplementary Table 3).

The mean time for polyp identification of CADe was 0.03 ± 0.01 s. The receiver operating characteristic curve (ROC) is presented in Figure 2. Based on the ROC curve, the optimal cutoff value of probability was determined to be 50%, which is indicated by the red dot in Figure 2. The CADe shows excellent diagnostic performance in identifying polyps with an area under the curve of 0.984. Overall, the CADe identified colorectal lesions with 95.0% sensitivity, 99.1% specificity, 93.7% PPV and 99.3% NPV (Table 1), and the highest sensitivity was noted for adenocarcinomas (97.2%, Table 1). Moreover, CADe exhibited the highest sensitivity for large polyps (96.7% for ≥ 10 mm) and the lowest sensitivity for diminutive polyps (94.1% for ≤ 5 mm, Table 1). Fecal fluid/bubble (24%), reflection of light (21%), difficult angle (19%) and shadow (11%) were considered to be the four most common causes of false negatives (Table 2, Supplementary Figure 1), whereas folds (19%), reflections of light (18%), fecal fluid/bubble (17%) and colonic/ileocecal valves (19%) were the most important causes of false positives (Table 2, Supplementary Figure 2).

### *Polyp identification in colonoscopy videos*

The video dataset consisted of 56 adenomas and 30 nonneoplastic polyps, and 53, 27 and 6 polyps exhibited diameters of ≤ 5 mm, 6-9 mm and ≥ 10 mm, respectively ( Supplementary Table 2). Most of the included polyps (71/86) exhibited flat or sessile morphology, and a small portion of polyps (13/86) were considered challenging to detect in routine practice (Supplementary Table 2).

For frame-based tests, a dataset of 86 video clips of positive frames (65524 frames and 2732 s in total) and 47 clips of negative frames (493997 frames and 20883 s) were constructed for test. Although CADe identified all 86 polyps, regarding frame-based analysis, there was an overall sensitivity of 92.2% and specificity of 93.6% for overall polyps and a sensitivity of 66.2% and specificity of 97.9% for "challenging" polyps ( Supplementary Table 4, Supplementary Video 2).

### *Validation of the CADe in clinical colonoscopy*

In total, 240 patients were recruited for validation, and 31 were excluded due to refusal of informed consent (7), age (6), poor bowel preparation (5), failed cecal intubation (5) and others (8) (Figure 1B). The baseline characteristics are shown in Supplementary Table 3, and 188 polyps were identified in 101 patients (Figure 1B), resulting in a 48.3% PDR. The withdrawal time of 107 procedures (52.2%) was shorter than 6 min (Supplementary Table 5).

The number of polyps identified by colonoscopists only, CADe only and both were three, 17 and 168, respectively, revealing a sensitivity of 98.4% (185/188) for CADe ( Supplementary Table 6). When assisted by the CADe, the number of identified polyps and adenomas significantly increased (0.82 *vs* 0.90, $P < 0.001$; 0.30 *vs* 0.32, $P < 0.05$, respectively), although the PDR and ADR did not increase ($P = 0.06$ and $P = 0.13$, respectively, Tables 3 and 4). The diagnostic sensitivity of the CADe was significantly greater than that of colonoscopists [98.4% (185/188) *vs* 91.0% (171/188), $P = 0.03$, Supplementary Table 6]. A total of 468 false positives occurred in 209 colonoscopy withdrawals (2.2 false positives per colonoscopy withdrawal); folds (59.0%), reflections of light (19.0%), fecal fluid/bubble (9.2%) and normal structures (9.8%) were considered to be the common causes of false positives (Table 2).

The diagnostic value of the CADe in polyps was observed in both age groups ($P = 0.03$ and $P = 0.002$, respectively), both sexes ($P = 0.005$ and $P = 0.01$, respectively), both hemi-colons ($P = 0.01$ and $P = 0.003$, respectively), all indications (all $P = 0.03$), inexperienced colonoscopists (< 1000 procedures) or general colonoscopists (1000-3000 procedures) ($P = 0.003$ and $P = 0.03$, respectively), and new generations of colonoscope (CF-290 and CF-260, $P < 0.001$ and $P = 0.03$, respectively), especially for diminutive (0.57 *vs* 0.65, $P < 0.001$) and flat polyps (0.67 *vs* 0.74, $P = 0.001$) (Table 3). Similarly, CADe also assisted colonoscopists in identifying more diminutive and flat adenomas ( $P = 0.025$ and $P = 0.045$, respectively) in elderly (≥ 50 years, 0.43 *vs* 0.46, $P = 0.045$) and

**Table 1 Performance of lesion detection and localization**

| | Lesions | Sensitivity, % | Specificity, % | PPV, % | NPV, % |
|---|---|---|---|---|---|
| Overall | 1412 | 95.0 (93.7-96.0) | 99.1 (98.9-99.3) | 93.7 (92.3-94.9) | 99.3 (99.1-99.4) |
| Pathology | | | | | |
| Non-adenomatous polyps | 487 | 93.4 (90.8-95.4) | 99.1 (98.9-99.3) | 83.5 (80.0-86.5) | 99.7 (99.5-99.8) |
| Adenomatous polyps | 640 | 95.2 (93.1-96.6) | 99.1 (98.9-99.3) | 87.1 (84.4-89.5) | 99.7 (99.5-99.8) |
| Adenocarcinomas | 285 | 97.2 (94.3-98.7) | 99.1 (98.9-99.3) | 75.5 (70.7-79.7) | 99.9 (99.8-100.0) |
| Size | | | | | |
| ≤ 5 mm | 713 | 94.1 (92.1-95.7) | 99.1 (98.9-99.3) | 88.2 (85.6-95.3) | 99.6 (99.4-99.7) |
| 6-9 mm | 396 | 95.2 (92.5-97.0) | 99.1 (98.9-99.3) | 80.7 (76.8-84.1) | 99.8 (99.7-99.9) |
| ≥ 10 mm | 303 | 96.7 (93.8-98.3) | 99.1 (98.9-99.3) | 76.5 (71.9-80.6) | 99.9 (99.8-99.9) |
| Paris classification | | | | | |
| Ip | 180 | 96.0 (91.8-98.3) | 99.1 (98.9-99.3) | 65.8 (59.7-71.4) | 99.9 (99.8-1.00) |
| Is | 581 | 96.1 (94.0-97.4) | 99.1 (98.9-99.3) | 86.1 (83.2-88.6) | 99.8 (99.6-99.8) |
| IIa | 525 | 92.6 (89.9-94.6) | 99.1 (98.9-99.3) | 84.4 (81.1-87.2) | 99.6 (99.5-99.7) |
| IIb | 30 | 100.0 (85.9-100.0) | 99.1 (98.9-99.3) | 25.0 (17.7-33.9) | 100.0 (99.9-100.0) |
| IIc | 2 | 100.0 (19.8-100.0) | 99.1 (98.9-99.3) | 2.2 (0.4-8.4) | 100.0 (99.9-100.0) |
| III | 32 | 96.9 (82.0-99.8) | 99.1 (98.9-99.3) | 25.6 (18.3-34.5) | 100.0 (99.9-100.0) |
| Unclassified | 62 | 96.8 (87.8-99.4) | 99.1 (98.9-99.3) | 40.0 (32.2-48.3) | 100.0 (99.9-100.0) |

PPV: Positive predictive value; NPV: Negative predictive value.

male patients (0.38 *vs* 0.41, $P < 0.001$), with the aid of a new-generation colonoscope (CF-290, $P = 0.025$) (Table 4). Notably, patients with adequate bowel preparation quality and withdrawal time showed a significantly increased number of polyps ($P < 0.001$ and $P = 0.001$) and adenomas ($P = 0.025$ and $P = 0.045$), whereas patients with < 6 Boston bowel preparation score and a < 6 min withdrawal time did not show any increase (Tables 3 and 4).

## DISCUSSION

This CADe was developed and tested using over 70000 well-labeled colonoscopic images from 20 endoscopy centers, representing the dataset with the largest number of polyps. Notably, CADe did not merely perform well on the image tests of multicenter datasets and greater than 550 thousand frames of colonoscopy videos but also aided colonoscopists in identifying more polyps and adenomas in colonoscopy practice.

One of the first CADes systems validated in real-time colonoscopy was reported to identify polyps with 96% accuracy in the tests of labeled images[19], and consistently, the current CADe also reached a sensitivity of 95.0% for localizing polyps within a 30 ms constraint[23]. Notably, our findings further demonstrated that CADe assisted colonoscopists in detecting more adenomas in clinical practice but not merely in selected colonoscopy videos[19]. Compared with Yamada's CADe[24], the current CADe also realized a high sensitivity in the dataset mainly consisting of nonpolypoid lesions in contrast to Yamada's dataset, which was mainly based on polypoid lesions. However, the performance of the CADe seemed to decline in video tests (92.2% sensitivity and 93.6% specificity), which is consistent with previously reported CADes [20,24]. Further improvements in terms of process capability and colonoscopy video materials are warranted for CADes given that a great abundance and variety of random artifacts from the quick movement of videos with higher running speeds are indispensable to optimize further CADes in avoiding false positives and reducing time delays[19].

For clinical validation, several CADe systems in colonoscopy have been validated and are mainly focused on adenoma identification and quality control[25-31]. Wang *et*

**Table 2 Potential causes of false negatives and false positives**

| Causes of false negatives | No. of images, *n* (%) | Causes of false positives[1] | No. of images, *n* (%) |
|---|---|---|---|
| Test dataset | | | |
| Fecal fluid/bubble | 17 (24) | Folds | 18 (19) |
| Reflections of light | 15 (21) | Reflections of light | 17 (18) |
| Difficult angle | 13 (19) | Fecal fluid/bubble | 16 (17) |
| Shadow | 8 (11) | Colonic valve | 11 (12) |
| Fuzzy image | 6 (9) | Ileocecal valve | 7 (7) |
| Color of background | 2 (3) | Fuzzy image | 5 (5) |
| Far distance | 2 (3) | Others[2] | 11 (12) |
| Unclassified | 7 (10) | Unclassified | 10 (11) |
| Validation in clinical colonoscopy | | | |
| Shadow | 1 (33.3) | Folds[3] | 276 (59.0) |
| Difficult angle | 1 (33.3) | Reflections of light | 89 (19.0) |
| Depressed lesion | 1 (33.3) | Fecal fluid/bubble | 43 (9.2) |
| | | Normal structures[4] | 46 (9.8) |
| | | Other lesions[5] | 14 (3.0) |

[1]Twelve lesions were considered to be missed by senior colonoscopists on colonoscopy.
[2]Others included color change (3), anus (3), lesions under the mucous (2), melanosis coli (1), and bloodstain (1).
[3]Sixteen folds were caused by suction.
[4]Normal structures included vessels (20), ileocecal valve (12), anus (8), and lymphoid follicle (6).
[5]Other lesions included ulcers (7), cysts under the mucous (4), and diverticulum (3).

*al*[25,26,31] first conducted randomized controlled trials (RCTs) in an open-label or double-blind setting, most of which shared a large number of patients (both ~1000), rigorously illustrating that CADe effectively increased the ADR and adenomas per colonoscopy by identifying additional diminutive adenomas, including easy-to-miss adenomas for experienced colonoscopists and significantly reduced AMR[31]. Despite the demonstrated improvement of ADR and lower AMR in well-designed RCTs[25-31], our preliminary validation limited by the smaller sample size and observational design could not detect the difference in PDR or ADR and might calm our excessive excitement for AI. However, the observational and pragmatic design might reflect the realistic situation of colonoscopy practice and be beneficial for the real-world application of CADe. For example, in contrast to Wang's studies, which filtered out alerts of CADe when the lumen was not inflated, the colonoscopists in our validation directly received all alerts from CADe and could better evaluate the real-world feasibility of CADe and the level of colonoscopists' acceptance. We also analyzed all false positives rather than filtering out flashing-alert false positives given that the majority of the flashing alerts [folds (59.0%) and reflections of light (19.0%)] were clinically relevant to remind colonoscopists to inflate the lumen and carefully inspect the location of possibly missed polyps. Given that the exploration and application of CADe in colonoscopy is an emerging area and most studies were conducted in a single center, it is indispensable to explore prudently CADe's feasibility and endoscopists' acceptance in colonoscopy practice[32]. Presumably, the CADe should be further developed to identify different false positives to assess the bowel preparation quality and inspecting techniques as well as to remind the colonoscopist in real time to deal with the folds and residual fecal fluid, which may finally help to optimize high-quality colonoscopy.

Consistent with current work, Klare *et al*[29] also conducted an observational validation of their CADe with 55 clinical colonoscopies, indicating that their low-delay CADe might be feasible for real-time colonoscopy. However, their CADe detected no polyps before colonoscopists with a relatively greater false-positive frequency. Presumably, the construction of non-CNN algorithms and the design in which colonoscopists finally determine the criterion standard for polyp detection without rechecking the false positives of the CADe might lead to limitations[29]. Notably, our

**Table 3 Polyp detection between colonoscopists and colonoscopists + computer-assisted detection**

| | Colonoscopists | Colonoscopists + CADe | *P* value |
|---|---|---|---|
| PDR, % | 45.9 | 48.3 | 0.06 |
| SSR, % | 3.83 | 4.78 | 0.50 |
| CRSR, % | 9.09 | 11.5 | 0.06 |
| Number of polyps, mean ± SD | 0.82 ± 1.20 | 0.90 ± 1.25 | < 0.001 |
| Pathology | | | |
| Non-adenomatous | 0.52 ± 0.94 | 0.58 ± 1.00 | 0.01 |
| Adenomatous | 0.30 ± 0.62 | 0.32 ± 0.64 | 0.025 |
| Age | | | |
| < 50 | 0.45 ± 0.70 | 0.51 ± 0.77 | 0.03 |
| ≥ 50 | 1.12 ± 1.42 | 1.22 ± 1.47 | 0.002 |
| Sex | | | |
| Male | 0.96 ± 1.28 | 1.05 ± 1.36 | 0.005 |
| Female | 0.66 ± 1.07 | 0.72 ± 1.11 | 0.01 |
| Location | | | |
| Proximal | 0.35 ± 0.69 | 0.39 ± 0.76 | 0.01 |
| Distal | 0.46 ± 0.89 | 0.51 ± 0.90 | 0.003 |
| Size | | | |
| ≥ 10 mm | 0.06 ± 0.28 | 0.06 ± 0.28 | 1 |
| 6-9 mm | 0.18 ± 0.52 | 0.19 ± 0.52 | 0.32 |
| ≤ 5 mm | 0.57 ± 0.95 | 0.65 ± 1.00 | < 0.001 |
| Morphology | | | |
| Flat | 0.67 ± 1.03 | 0.74 ± 1.08 | 0.001 |
| Subpedunculated | 0.11 ± 0.42 | 0.12 ± 0.42 | 0.16 |
| Pedunculated | 0.03 ± 0.21 | 0.03 ± 0.21 | 1 |
| Indications | | | |
| Screening | 0.76 ± 1.15 | 0.85 ± 1.21 | 0.03 |
| Surveillance | 1.19 ± 1.42 | 1.33 ± 1.53 | 0.03 |
| Diagnosis | 0.70 ± 1.10 | 0.75 ± 1.13 | 0.03 |
| Colonoscopes | | | |
| CF-Q290 | 0.78 ± 1.09 | 0.87 ± 1.17 | < 0.001 |
| CF-Q260 | 1.04 ± 1.58 | 1.15 ± 1.56 | 0.08 |
| CF-Q240 | 0.83 ± 1.64 | 0.83 ± 1.64 | 1 |
| Experience | | | |
| > 3000 | 1.24 ± 1.48 | 1.24 ± 1.48 | 1 |
| 1000-3000 | 0.79 ± 1.16 | 0.91 ± 1.26 | 0.003 |
| < 1000 | 0.54 ± 0.92 | 0.62 ± 0.99 | 0.03 |
| BBPS | | | |
| < 6 | 0.59 ± 1.19 | 0.62 ± 1.19 | 0.32 |
| ≥ 6 | 0.87 ± 1.19 | 0.96 ± 1.26 | < 0.001 |
| Withdrawal time | | | |
| < 6 min | 0.52 ± 0.97 | 0.54 ± 0.98 | 0.16 |

| | | | |
|---|---|---|---|
| ≥ 6 min | 1.13 ± 1.33 | 1.27 ± 1.39 | 0.001 |

CADe: Computer-assisted detection; BBPS: Boston bowel preparation score; PDR: Polyp detection rate; SSR: Sessile serrated adenoma/polyp detection rate; CRSR: Clinical serrated polyp detection rate.

findings indicated that the diagnostic capability of CADe tended to be susceptible to inadequate withdrawal time or inadequate bowel preparation quality ($P$ = 0.32 and $P$ = 0.16) given that CADe could only detect polyps appearing in view during the colonoscopy but was unable to identify polyps covered by fecal fluid or hidden between the folds. Therefore, the bowel preparation and withdrawal time should also be considered even if the CADe is established to monitor colonoscopy withdrawal. The combination of an AI-assisted quality control system (monitoring withdrawal speed and bowel preparation quality) and CADe could provide more remarkable benefits in adenoma detection, even for large adenomas[28]. However, the additional detection of most CADes, including our current system, is confined to nonadenomatous polyps and diminutive adenomas, of which clinical significance was undetermined for interval cancers and cost-effectiveness analysis. Although promising preliminary findings seemed to appear, the CADes should be further validated in multicenter settings and community practices as well as optimized for satisfactory integration into clinical practice.

We defined a true positive with intersection over union (IoU) > 0.3, which was also adopted in previous reports[33]. In addition, Horie *et al*[34] considered the detection of CADe correct when the CADe could recognize merely a part of the esophageal cancer and false when identifying wide noncancerous areas occupying greater than 80% of the frame, whose standard was significantly lower than the level of 0.3 in IoU. In addition, Paul *et al*[33] proposed to use > 0.3 IoU in training and > 0.1 IoU to distinguish pulmonary nodules with the explanation of respecting the clinical need for coarse localization. However, there was no uniform standard for IoU, and more studies are warranted to discuss the optimal IoU or define other potential indicators to ensure the quality and comparability between CADes. Given that colonoscopists in our study were aware that their examinations were being monitored and reminded by sound of the CADe, the result might be vulnerable to the Hawthorne effect, which may explain our study's significantly higher PDR (45.9%) compared to our previous report (PDR: 32.0-39.8%). Presumably, a fair proportion of polyps first detected by CADe or detected simultaneously by CADe and colonoscopists, which might have been ignored in real clinical practice, were additionally identified by colonoscopists in the current study. Although this weakness in our design may have prevented the benefit of CADe from being completely realized, more polyps and adenomas, especially those that were diminutive (< 5 mm) and flat, were still identified with the aid of CADe in the current study.

Compared with previous studies, our study has several strengths. First, our study retrospectively collected the datasets of 20 endoscopy centers, carefully screened patients with one polyp as well as the corresponding histological results, and deliberately labeled the images with blinding and repetition, finally constructing a large and high-quality dataset of colonoscopic images for training and testing. As a result, the dataset could represent a wider population and be ensured of the higher reliability of the original material, which is beneficial to reduce the risk of overfitting. Second, through pilot validation in clinical colonoscopy, our study not only indicated that CADe could help colonoscopists identify more polyps and adenomas but also preliminarily indicated the significance of quality control of colonoscopy (withdrawal time and bowel preparation quality) in CADe. Third, we analyzed the performance of CADe in various clinical scenarios and identified the most important causes of false negatives and positives, which might provide directions to optimize further the clinical application of the CADe.

Our study had several limitations. First, the CADe was completely established on selected retrospective white-light images without making use of the temporal coherence of videos, which contain many unclear and unfocused frames of images that could be not or mistakenly detected by CADe. As a result, the performance of CADe might be suboptimal with lower sensitivity or specificity in colonoscopy videos. Consistent with the concerns, the CADe's performance was influenced by the unclear frames (too distant, unfocused and partially appeared) of seven challenging videos, which led to a testing sensitivity of less than 80%. Further efforts should be made to train the CADe with more consecutive videos to take advantage of the temporal coherence and improve diagnostic accuracy in clinical colonoscopy. Second, we did

**Table 4 Adenoma detection between colonoscopists and colonoscopists + computer-assisted detection**

| | Colonoscopists | Colonoscopists + CADe | *P* value |
|---|---|---|---|
| ADR, % | 22.0 | 23.9 | 0.13 |
| Number of adenomas, mean ± SD | | | |
| | 0.30 ± 0.62 | 0.32 ± 0.64 | 0.025 |
| Age | | | |
| < 50 | 0.14 ± 0.40 | 0.15 ± 0.41 | 0.32 |
| ≥ 50 | 0.43 ± 0.67 | 0.46 ± 0.69 | 0.045 |
| Sex | | | |
| Male | 0.38 ± 0.70 | 0.41 ± 0.73 | 0.045 |
| Female | 0.21 ± 0.48 | 0.22 ± 0.48 | 0.32 |
| Location | | | |
| Proximal | 0.12 ± 0.38 | 0.13 ± 0.39 | 0.16 |
| Distal | 0.17 ± 0.46 | 0.19 ± 0.47 | 0.08 |
| Size | | | |
| ≥ 10 mm | 0.06 ± 0.28 | 0.06 ± 0.28 | 1 |
| 6-9 mm | 0.11 ± 0.36 | 0.11 ± 0.36 | 1 |
| ≤ 5 mm | 0.12 ± 0.38 | 0.15 ± 0.42 | 0.025 |
| Morphology | | | |
| Flat | 0.20 ± 0.47 | 0.21 ± 0.50 | 0.045 |
| Subpedunculated | 0.07 ± 0.27 | 0.07 ± 0.28 | 0.32 |
| Pedunculated | 0.03 ± 0.21 | 0.03 ± 0.21 | 1 |
| Indications | | | |
| Screening | 0.29 ± 0.65 | 0.33 ± 0.66 | 0.08 |
| Surveillance | 0.43 ± 0.67 | 0.45 ± 0.67 | 0.32 |
| Diagnosis | 0.25 ± 0.56 | 0.26 ± 0.59 | 0.32 |
| Colonoscopes | | | |
| CF-Q290 | 0.31 ± 0.63 | 0.34 ± 0.65 | 0.025 |
| CF-Q260 | 0.23 ± 0.51 | 0.23 ± 0.51 | 1 |
| CF-Q240 | 0.25 ± 0.62 | 0.25 ± 0.62 | 1 |
| Experience | | | |
| > 3000 | 0.5 ± 0.72 | 0.5 ± 0.72 | 1 |
| 1000-3000 | 0.24 ± 0.59 | 0.27 ± 0.60 | 0.08 |
| < 1000 | 0.23 ± 0.53 | 0.26 ± 0.60 | 0.16 |
| BBPS | | | |
| < 6 | 0.19 ± 0.46 | 0.19 ± 0.46 | 1 |
| ≥ 6 | 0.32 ± 0.64 | 0.35 ± 0.66 | 0.025 |
| Withdrawal time | | | |
| < 6 min | 0.22 ± 0.57 | 0.22 ± 0.60 | 0.32 |
| ≥ 6 min | 0.38 ± 0.65 | 0.42 ± 0.65 | 0.045 |

CADe: Computer-assisted detection; BBPS: Boston bowel preparation score; ADR: Adenoma detection rate.

not identify a significant difference in PDR and ADR due to a limited sample size

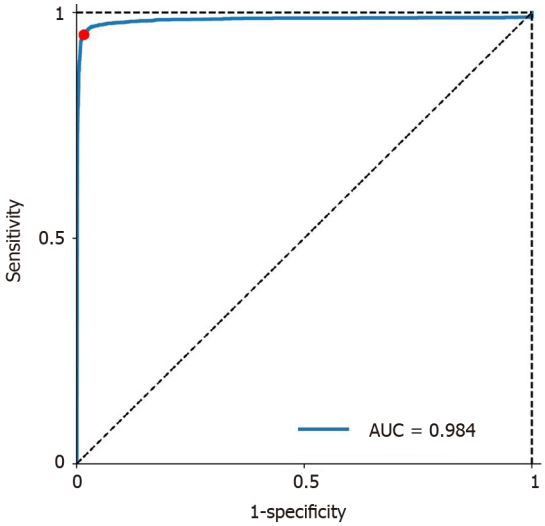


**Figure 2 Receiver operating characteristic curve for localization of polyps.** AUC: Area under the curve.

despite the fact that the polyps and adenomas identified per colonoscopy significantly increased. This information might provide a potential remedy to missed adenomas and the one-and-done effect of colonoscopists, which was demonstrated in recent tandem RCTs[31]. Finally, we artificially defined and classified the detection of polyps into three types. The validity of this classification was uncertain and might be influenced by the investigator's bias (*i.e.* the delay between the recording time and actual time of detection on the part of the colonoscopists) despite the independent determination of observer using the objective measurement of time. As a result, the current study might not be completely adequate to demonstrate the clinical implementation of CADe due to the single-center setting with a limited sample size, and future large RCTs are warranted.

## CONCLUSION

In summary, our study indicated that this deep learning-based CADe had been trained on the dataset consisting of the largest number of polyps from 20 centers and reached high diagnostic performance on the test dataset, colonoscopy videos and clinical validation with an acceptable frequency of false positives. This system might help colonoscopists identify more polyps and adenomas and deserves to be further validated in multicenter randomized trials.

## ARTICLE HIGHLIGHTS

### *Research background*

Artificial intelligence is an emerging area in the research and applications of digestive endoscopy. Current deep learning-based computer-assisted detection (CADe) for colorectal polyps is mainly constructed from single-center and limited-sample learning images.

### *Research motivation*

Although several studies reported that CADes identified polyps in colonoscopic images, videos and exams in real time, the lack of large-sample and representative learning materials might limit the diagnostic performance in wide colonoscopy practice.

### *Research objectives*

We aimed to train and test a CADe based on a multicenter high-quality dataset of polyps and preliminarily validated it in clinical colonoscopies.

### Research methods

After retrospective collection and systematic screening and labeling, over 71000 images from 20 centers and 47 unaltered full-ranged videos were used to train and test a deep learning-based CADe. We also validated its diagnostic performance in real-world colonoscopy using a single-arm self-controlled observational study.

### Research results

Our CADe identified polyps of images with 95.0% sensitivity and 99.1% specificity. For colonoscopy videos, all 86 polyps were detected with 92.2% sensitivity and 93.6% specificity for frame-by-frame analysis. The sensitivity of CAD in identifying polyps was 98.4% (185/188) in the prospective validation. Colonoscopists could detect more polyps (0.90 *vs* 0.82, $P < 0.001$) and adenomas (0.32 *vs* 0.30, $P = 0.045$) with the aid of CADe, particularly polyps < 5 mm and flat polyps/adenomas.

### Research conclusions

A CADe based on multicenter high-quality colonoscopic images was constructed and might help endoscopists detect more polyps and adenomas.

### Research perspectives

CADe is feasible in the clinical setting, and further confirmation is warranted.

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ORIGINAL ARTICLE

Case Control Study

# Tailored eradication strategy *vs* concomitant therapy for *Helicobacter pylori* eradication treatment in Korean patients

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**Author contributions:** Chung JW contributed to the study concept and design, coordinated all of the study processes as the corresponding authors; Choi YI and Seo JY analyzed the data; Choi YI interpreted the data; Choi YI and Chung JW drafted the manuscript; Park DK, Kim KO, Kwon KA, Kim JH, and Kim YJ critically revised the manuscript for important intellectual content; all the authors approved the submitted draft.

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## Abstract

### *BACKGROUND*

Antibiotic resistance to *Helicobacter pylori (H. pylori)* infection, which ultimately results in eradication failure, has been an emerging issue in the clinical field. Recently, to overcome this problem, an antibiotic sensitivity-based tailored therapy (TT) for *H. pylori* infection has received attention.

### *AIM*

To investigate the efficacy and safety profiles of TT for *H. pylori* infection treatment compared to a non-bismuth quadruple therapy, concomitant therapy (CT) regimen.

### *METHODS*

We included patients (> 18 years) with an *H. pylori* infection and without a history of *Helicobacter* eradication who visited the Gil Medical Center between March 2016 and October 2020. After being randomly assigned to either the TT or CT treatment group in 1 to 1 manner, patient compliance, eradication success rate (ESR), and patient-reported side effects profiles were assessed and compared between the two groups. *H. pylori* infection was diagnosed using a rapid urease test, Giemsa stain, or dual priming oligonucleotide polymerase chain reaction (DPO-PCR). Tailored eradication strategy based through the presence of a 23S ribosomal RNA point mutation. For the TT group, a DPO-PCR test, which detected A2142G

study protocol was approved by the ethics committee of the GMC.

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

**Conflict-of-interest statement:** All the Authors have no conflict of interest related to the manuscript.

**Data sharing statement:** The data used to support the findings of this study are available from the corresponding author upon request at ( junwonchung@gilhospital.com)

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and/or A2143G point mutations, and a clarithromycin resistance test were performed. Patients in the clarithromycin-resistant group were treated with a bismuth-containing quadruple combination therapy, while those with sensitive results were treated with the standard triple regimen.

*RESULTS*

Of the 217 patients with a treatment naive *H. pylori* infection, 110 patients [mean age: 58.66 ± 13.03, men, $n$ = 55 (50%)] were treated with TT, and 107 patients [mean age: 56.67 ± 10.88, men, $n$ = 52 (48.60%)] were treated with CT. The compliance (TT *vs* CT, 100% *vs* 98.13%, $P$ = 0.30), and follow-up loss rates (8.18% *vs* 9.35%, $P$ = 0.95) were not significantly different between the groups. The ESR after treatment was also not statistically different between the groups (TT *vs* CT, 82.73% *vs* 82.24%, $P$ = 0.95). However, the treatment-related and patient-reported side effects were significantly lower in the TT group than in the CT group (22.77% *vs* 50.52%, $P$ < 0.001).

*CONCLUSION*

The DPO-based TT regimen shows promising results in efficacy and safety profiles as a first-line *Helicobacter* eradication regimen in Korea, especially when physicians are confronted with increased antibiotic resistance rates.

**Key Words:** *Helicobacter pylori*; Eradication; Tailored therapy; Conco-mitant therapy regimen

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**Core Tip:** We investigated the efficacy and safety profiles of a tailored therapy (TT) as a first line *Helicobacter pylori* (*H. pylori*) eradication treatment compared to a concomitant therapy (CT) regimen in Korea, where clarithromycin resistance rates are high. Of 217 treatment-naïve *H. pylori* infection patients, 107 patients were treated with CT and 101 patients with TT. Although the eradication success rate was not statistically different between the groups, the treatment-related side effect rate was significantly lower in the TT group. Therefore, the TT regimen might be a promising solution to overcoming the problem of increased antibiotic resistance rates for *Helicobacter* eradication.

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## INTRODUCTION

*Helicobacter pylori (H. pylori)* eradication is associated with a reduced risk of stomach cancer, including adenocarcinoma and mucosa-associated lymphoid tissue lymphoma, thus the rising global rates of antibiotic resistant *H. pylori* infections is concerning[1-4]. Currently, the Maastricht V/Florence Consensus guidelines, and Korean *H. pylori* treatment guidelines recommend proton pump inhibitor-based standard triple therapy [5-7]. However, the treatment success rate of standard tailored therapy (TT) has been declining worldwide over the past few decades as *H. pylori* drug resistance has increased year-over-year[4,7-9]. In Korea, a recent studying assessing the use of the standard triple therapy regimen for *H. pylori* eradication showed a treatment success rate of less than 70%[8-11]. Given that the ideal eradication success rate (ESR) should be over 80% in intention-to-treatment (ITT) analyses, and 90% in per-protocol (PP) analyses according to the 1997 Asia-Pacific Agreement Report for *H. pylori* treatment, conventional standard triple therapy with an empirically chosen policy has lost its role in *H. pylori* eradication practice[12].

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Recently, to overcome the aforementioned problems of empirically chosen eradication policies, the concept of 'TT' has been introduced in the eradication policy for *H. pylori* infection[12-18]. TT for *H. pylori* eradication is based on a pre-treatment antibiotic resistance test using stool or stomach biopsy samples[18,19]. However, tissue culture based antibiotic resistance testing for *H. pylori* is not ideal in that it is costly, time consuming, and not all of the antibiotics used in the regimen can be tested. Instead, dual priming oligonucleotide polymerase chain reaction (DPO-PCR) has been used[20-22]. DPO-PCR tests are cost effective and less time consuming than tissue culture based tests[23,24]. However, DPO-PCR test is currently only available for clarithromycin (CLR) resistance testing, as a method for rapid metronidazole (MTZ) resistance testing for *H. pylori* has been invented clinically. However, Korea is a region with high CLR resistance. In fact, the resistance rate has gradually increased, from 22.9% in 2003–2005 to 37.0% in 2007–2009, with the major barriers for *H. pylori* eradication success being CLR resistance, prompting clinical data to be accumulated using DPO-PCR tests in *H. pylori* eradication regimens[9,25].

Unfortunately, there is little data on the efficacy and safety profiles of the TT regimen compared to the concomitant therapy (CT) regimen in first-line *H. pylori* eradication.

Herein, we investigated the efficacy (ESR) and safety profiles (treatment-related side effect events) of the TT regimen as compared to those of CT in patients with treatment-naïve *H. pylori* infections in Korea, where the CLR resistance rate is high (> 15%).

## MATERIALS AND METHODS

### *Institutional review board approval*

The Institutional Review Board of the Gil Medical Center (GMC) reviewed the study protocol and ethics. This study was conducted in accordance with the Declaration of Helsinki, and the study protocol was approved by the ethics committee of the GMC.

### *Enrolled study population*

We enrolled patients (> 18 years) with evidence of an *H. pylori* infection who were treatment naïve for *H. pylori* eradication that visited the GMC (Incheon, Korea) between March 2016 and October 2020. Exclusion criteria for this study were as follows: (1) Patients under 18 years of age; (2) Patients with a history of previous eradication; (3) Patients with a history of an allergy to any medication used in this study; (4) Patients with any operation history regarding the stomach; (5) Seriously ill patients with critical medical history [heart failure (≥ New York Heart Association class II), severe respiratory illness, decompensated liver cirrhosis, terminal or supportive care stage of malignancy, *etc.*]; (6) Patients who could not afford to revisit the hospital for follow-up after medication; and (7) Patients who could not take medication orally.

Enrolled patients were randomly assigned to either the TT group or the CT group in a 1 to 1 manner and their compliance rates, ESRs, and treatment-related side effect rates were assessed (Figure 1).

### *Diagnosis of H. pylori infection*

All patients underwent an upper gastrointestinal endoscopy and showed positive results through a rapid urease test, Giemsa stain, or DPO-PCR.

### *PCR guided CLR resistance test for TT group*

We used DPO-PCR to detect the presence of either a 2142G or 2143G point mutation on 23S rRNA. The point mutation in the V domain of 23S rRNA has been depicted as a major risk factor for CLR resistance against *H. pylori*, and 2142G and 2143G are the most frequent sites for 23S rRNA point mutations. The wild type for both 2142G and 2143 G mutations was defined as CLR sensitive.

### *Eradication regimen for TT group, and CT group*

For the TT group, after the DPO-PCR test, patients who were deemed CLR resistant were administered a bismuth-containing quadruple combination (PBMT), and those who were CLR sensitive with a standard pancreatic adenocarcinoma (PAC) regimen (Figure 1). The bismuth-containing quadruple regimen consisted of 30 mg of lansoprazole twice daily + 500 mg MTZ twice daily + 300 mg bismuthate four times daily + 500 mg tetracycline four times daily for 10 d. The PAC regimen consisted of 30

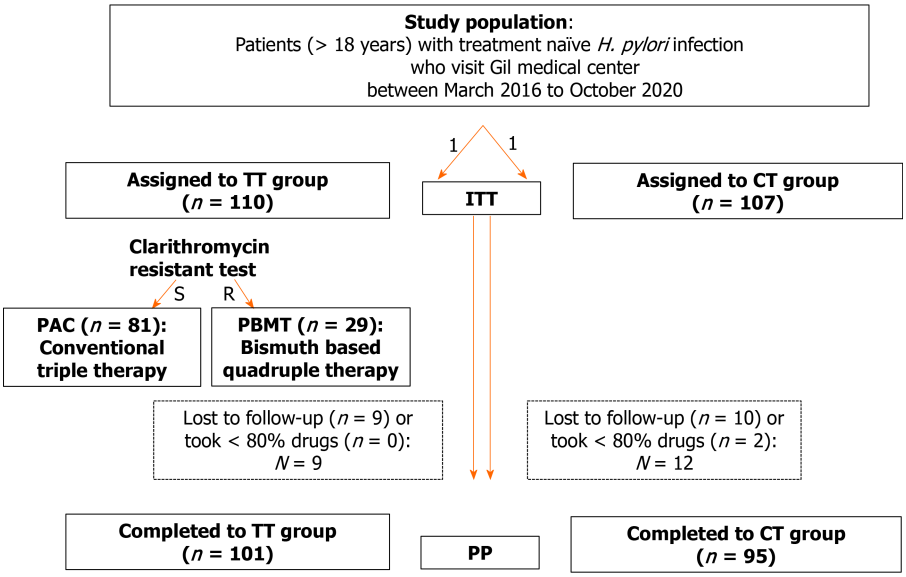


**Figure 1 Flow chart.** TT: Tailored therapy; CT: Concomitant regimen; S: Sensitive; R: Resistance; PAC: Pancreatic adenocarcinoma; PBMT: Bismuth-containing quadruple combination; ITT: Intention-to-treat; PP: Per-protocol; *H. pylori*: *Helicobacter pylori*.

mg lansoprazole + 500 mg CLR + 1000 mg amoxicillin (AMX), administered twice daily for 14 d.

The CT regimen consisted of 30 mg lansoprazole twice a day + 1 g AMX twice a day + 500 mg MTZ twice a day + 500 mg CLR twice a day for 10 d.

### *Follow up strategy, and outcome interpretation (efficacy and safety profiles)*

Four weeks after finishing their eradication medication, patients were recommended to visit the GMC and undergo the $^{13}$C-ure breath test (UBT; UBiTkit; Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) with a cut-off value of delta $^{13}CO2 < 2.5‰$ to evaluate eradication success. During this visit, the patients reported treatment-related side effects and drug compliance rates were also recorded by the physician.

### *Definition for treatment-related adverse events*

Physicians interviewed enrolled patients regarding treatment-related adverse events at follow up. Patients were asked for their treatment-related adverse effects type as both of open-ended, and closed-ended questions. Items for closed-ended questions regarding treatment-related side effect types were as follows: (1) Taste disturbance; (2) Nausea/vomiting; (3) Diarrhea/Loose stool/constipation; (4) Abdominal discomfort, dyspepsia; (5) General weakness, myalgia; (6) Dizziness, head ache; and (7) Skin rash.

The degree of treatment-related side effect were classified as 'mild', 'moderate', and 'severe' events according to the degree of tolerance of patients' daily activities as follows: No adverse events; mild (without limitation in daily activities); moderate (partly limited daily activities); and severe (completely limited daily activities). Patients were instructed to visit hospital immediately when any severe adverse events occurred.

### *Definition for treatment compliance*

Treatment compliance was defined according to the status of the consumption of the prescribed drugs through personal interviews at the follow-up visit. The compliance level was investigated through patients' self-reported questionnaire, and consumed/remained medication pill counts. Patients who consumed > 80% of the scheduled prescription were classified as having good compliance.

### *Statistics*

Treatment outcomes (efficacy profiles and safety profiles) were analyzed using an ITT analysis and PP analysis. In the ITT analysis, after excluding patients meeting the exclusion criteria, all of the enrolled study population were included. In PP analyses, patients who were lost to follow-up or those with poor compliance (< 80%) were excluded. Categorical variables were analyzed as percentiles and compared between the TT and CT groups using the chi-square test. Continuous variables were

represented as mean ± SD and were compared between groups using Student's t-test. Statistical significance was set to $P < 0.05$. The Statistical Package for the Social Sciences (SPSS) software (version 22.0; IBM Corp., Armonk, NY, United States) was used for statistical analyses.

## RESULTS

### Clinical characteristics

Of the 217 patients with a treatment-naive *H. pylori* infection, 110 [men: $n = 55$ (50.00%); mean age: 58.66 ± 13.03] were treated with TT, and 107 with CT [men: $n = 52$ (48.60%); mean age: 56.67 ± 10.88] (Table 1). The rates of endoscopy, smoking, drinking, and comorbidity status were not significantly different between the groups (Table 1).

After evaluating CLR resistance status using DPO-PCR test, of the 101 patients who were initially allocated to the TT group, 29 (26.36%) tested positive for CLR-resistant *H. pylori*, while 81 (73.64%) were classified as CLR-sensitive (Table 1). Among the 29 patients with CLR-resistant *H. pylori*, four patients showed a A2142G point mutation, while the other 25 patients showed a A2143G point mutation (Table 1).

### Follow up loss rate and compliance status of TT vs CT group

The follow-up loss (TT *vs* CT; 8.18% *vs* 9.35%, $P = 0.95$) and poor compliance rates (0% *vs* 1.87%, $P = 0.33$) were not significantly different between the groups (Table 1).

### Efficacy profiles (ESR) of TT vs CT group

In the ITT protocol, ESR after treatment was not significantly different between the groups (82.73% *vs* 82.24%, $P = 0.95$) (Table 2).

Additionally, in the PP protocol, the TT group showed a lower ESR than the CT group (90.10% *vs* 91.58%, $P = 0.72$), but the difference was not statistically significant (Table 2).

### ESR in TT group according to DPO-PCR results

When we determined ESR in the TT group according to the DPO-PCR results, a total of 72 patients showed CLR sensitive results (treated with PAC regimen), with the ESR for those patients being 80.25%, and 87.84% in the ITT and PP analyses, respectively (Table 3). Four patients showed an A2142G point mutation with ESR of (3/4) 75.00% and (3/3) 100% in the ITT and PP analyses, respectively (Table 3). Twenty-five patients with an A2143G point mutation showed an ESR of 92.00% and 95.83% in ITT and PP analyses, respectively (Table 3).

### Patient reported treatment-related side effect profiles of TT vs CT group

Treatment-related and patient-reported side effect events were significantly lower in the TT group than in the CT group (22.77% *vs* 50.52%, $P < 0.001$) (Table 4).

The most common side effect was nausea/vomiting [$n = 9$ (10.23%)] in the TT group and taste disturbance [$n = 18$ (18.56%)] in the CT group (Table 4).

## DISCUSSION

In this study, we investigated the efficacy (ESR) and safety profiles of TT as a first-line *H. pylori* eradication regimen compared to those of CT in Korea, where the CLR resistance rate is increasing (> 15%). According to our study results, the ESR between the TT and CT groups was not statistically different between the groups. However, the TT group showed a significantly lower treatment-related side effect rate as compared to that of the CT regimen. Considering that lower exposure to antibiotics and appropriate drug use are the best policies for reducing the spread of antibiotic-resistant bacteria[26,27], the TT regimen might be a promising *H. pylori* eradication strategy in an era where increased risk for antibiotic resistance has become a huge medical burden, as is the case in Korea.

Even though the CT regimen for *H. pylori* eradication has shown high ESR in Korea until recently, when compared to that of conventional TT, and even sequential treatment, several concerns arise with this regimen[14,28-34]. First, since the CT regimen includes multiple antibiotic medications for *H. pylori* eradication such as CLR, AMX, and MTZ, there is a possibility of antibiotic overuse[30,31,35]. Given that

**Table 1 Baseline characteristics of the study population**

| | TT group (*n* = 110) | CT group (*n* = 107) | *P* value |
|---|---|---|---|
| Age, mean ± SD (yr) | 58.66 ± 13.03 | 56.67 ± 10.88 | 0.22 |
| Men, *n* (%) | 55 (50.00) | 52 (48.60) | 0.89 |
| Body mass index ($m^2$/kg) | 24.31 ± 3.23 | 23.87 ± 2.91 | 0.35 |
| Smoking, *n* (%) | 24 (22.82) | 22 (20.59) | 0.74 |
| Drinking, *n* (%) | 30 (30.00) | 28 (26.17) | 0.39 |
| Comorbidity | | | 0.81 |
| Hypertension, *n* (%) | 26 (23.64) | 23 (21.50) | |
| Diabetes mellitus, *n* (%) | 22 (20.00) | 25 (23.36) | |
| Cardiovascular disease, *n* (%) | 2 (1.82) | 2 (1.86) | |
| Reasons for eradication | | | 0.37 |
| Peptic ulcer disease | 49 (44.54) | 43 (40.19) | |
| Post ESD due to EGC | 4 (3.63) | 2 (1.87) | |
| MALToma | 0 (0.00) | 1 (0.93) | |
| Chronic atrophic gastritis with intestinal metaplasia | 57 (51.82) | 61 (57.01) | |
| Clarithromycin resistance diagnosed by DPO-PCR | | | |
| No, *n* (%) | 81 (73.64) | | |
| A2142G positive, *n* (%) | 4 (3.64) | | |
| A2143G positive, *n* (%) | 25 (22.73) | | |
| Follow up loss, *n* (%) | 9 (8.18) | 10 (9.35) | 0.95 |
| Poor compliance, *n* (%) | 0 (0.00) | 2 (0.02) | 0.30 |

TT: Tailored therapy; CT: Concomitant therapy; DPO-PCR: Dual priming oligonucleotide polymerase chain reaction; ESD: Endoscopic submucosal dissection; EGC: Early gastric cancer; MALToma: Mucosa associated lymphoid tissue lymphoma.

**Table 2 *Helicobacter pylori* eradication success rates**

| Eradication rate | TT group (*n* = 110) | CT group (*n* = 107) | *P* value |
|---|---|---|---|
| Intention-to-treat | 91/110 (82.73) | 88/107 (82.24) | 0.95 |
| Per-protocol | 91/101 (90.10) | 87/95[1] (91.58) | 0.72 |

[1]Of 107 patients who were initially allocated into concomitant therapy group, 10 were lost to follow-up, and two patients showed poor compliance for taking medication (one patient showed eradication success, and the other eradication failure). Therefore, a total of 95 patients were included in our per-protocol analysis. TT: Tailored therapy; CT: Concomitant therapy.

indiscriminate misuse of antibiotics is related to the emergence of multidrug resistance bacteria, not just limited to *H. pylori*, appropriate use of antibiotics based on drug sensitivity analysis has been emphasized in solving the current multidrug resistance problems of bacteria[27,35]. Even if the CT regimen is effective in *H. pylori* eradication, there is room for antibiotic overuse, which might result in violating antibiotic stewardship, thus physicians should only use a CT regimen with extreme caution[13,27,30,36,37]. Second, because the CT regimen contains multiple antibiotic options, higher incidences of treatment-related side effects, which are related to poor treatment compliance, and ultimately result in eradication failure, have been reported in previous studies[31,32,38,39]. According to our findings, the CT group showed a statistically higher incidence of treatment-related side effects than the TT group.

The paradigm shift from empirically chosen eradication policy to bacteria-specific targeted therapy in *H. pylori* eradication, TT, originated from the rise of precision medicine and ab accumulation of data on resistance mechanisms of *H. pylori* infection [11,15,16,25,37,40-42]. According to a meta-analysis by Venerito *et al*[43] that analyzed

**Table 3 Eradication success rate in tailored therapy group according to dual priming oligonucleotide polymerase chain reaction results**

| Eradication rate | No mutation | A2142G positive | A2143G positive |
|---|---|---|---|
| Number of patients who initially enrolled | 81 | 4 | 25 |
| Number of patients with eradication failure | 9 | 0 | 1 |
| Number of patients lost to follow up | 7 | 1 | 1 |
| Number of patients with poor compliance | 0 | 0 | 0 |
| Eradication success rate | | | |
| Intention-to-treat | 65/81 (80.25%) | 3/4 (75.00%) | 23/25 (92.00%) |
| Per-protocol | 65/74 (87.84%) | 3/3 (100%) | 23/24 (95.83%) |

**Table 4 Treatment related adverse events, *n* (%)**

| | TT group (*n* = 101[1]) | CT group (*n* = 97[2]) | *P* value |
|---|---|---|---|
| Eradication related Side effects | | | < 0.001 |
| No | 78 (77.23) | 48 (49.48) | |
| Yes | 23 (22.77) | 49 (50.52) | |
| Taste disturbance | 2 (1.98) | 18 (18.56) | |
| Nausea/vomiting | 10 (9.90) | 15 (15.46) | |
| Diarrhea/loose stool/constipation | 6 (5.94) | 9 (9.28) | |
| Abdominal discomfort, dyspepsia | 3 (2.97) | 3 (3.09) | |
| General weakness, myalgia | 1 (0.99) | 1 (1.03) | |
| Dizziness, headache | 1 (0.99) | 2 (2.06) | |
| Skin rash | 0 (0.00) | 1 (1.03) | |

[1]Of the 110 patients who were initially allocated into the tailored therapy (TT) group, 9 were not followed up. Therefore, a total of 101 patients were included in the TT group.
[2]Of the 107 patients who were initially allocated into the concomitant therapy (CT) group, 10 were lost to follow up. Therefore, a total of 97 patients were included in the CT group, regardless of compliance status. TT: Tailored therapy; CT: Concomitant therapy.

the effect of antibiotic sensitivity of *H. pylori* on ESR, a conventional triple regimen yielded an ESR of 80%–95% among CLR-sensitive strains, but dropped to 0%–48% among CLR resistant strains[43,44]. Therefore, CLR resistance significantly affected the ESR of the conventional triple regimen[43]. However, MTZ resistance was not significantly associated with the ESR of PBMT even in MTZ-resistant strains as compared to that in MTZ-sensitive strains[43]. Therefore, confirming CLR susceptibility before eradication in order to use a tailored eradication was closely associated with an improved ESR as compared to that of the empirically chosen conventional triple regimen, regardless of the MTZ resistance test results[ 19-21,41,45-47].

To date, several antibiotic resistance mechanisms of *H. pylori* infection have been proposed[48]. First, antimicrobial genes that are expressed as key targeted structures, such as cell membranes, nucleic acids, DNA gyrases, DNA-dependent RNA polymerases, and redox enzymes, mutate to evade antibiotics, such as quinolone series resistant *H. pylori*. Second, the efflux systems of *H. pylori* change to prohibit the intracellular accumulation of antibiotics. Third, *H. pylori* enzymes that inactivate antibiotic compounds are activated or produced. Among these mechanisms, the CLR resistance phenomenon for *H. pylori* infection mainly originates from the mutation of the cellular target genes, especially associated with protein translation in the V domain of 23S rRNA (A2143G, A2142C, A2142G, A2143C, *etc.*)[48-50]. In our study, we used the DPO-PCR test for CLR resistance detection, which specifically targeted the A2143G and A2142G point mutations. Among the 101 patients in the TT group, 72 of them showed CLR sensitivity, while 29 showed CLR resistance. Even among patients with a negative DPO-PCR test result (*n* = 72), who were classified with wild-type *H. pylori* strains against CLR, and prescribed the conventional triple regimen, the ESRs were

70.83% and 78.46% in the ITT and PP analyses, respectively. This phenomenon might have resulted from mutations in the V domain of 23S rRNA other than A2142G and A2143G point mutations, which were not detected in the DPO-PCR test we used. Considering cost effectiveness, we targeted *H. pylori* strains with the most common site of point mutations resulting in CLR resistance. It is possible that the *H. pylori* strains classified as wild type through the DPO-PCR test were not really wild type. However, among the patients who showed a 2142G or A 2143 G point mutation *via* the DPO-PCR test, the ESR of the PBMT regimen was 100% and 90.48%, respectively, in the PP analysis. Efforts to develop cost-effective and multiple point mutation detection kits for *H. pylori* should be continued in order to improve the ESR of TT[23,24,51].

Even though there is limited data on the efficacy of the TT regimen in *H. pylori* treatment policy, there have been several studies assessing TT efficacy profiles compared to that of the CT regimen[45,52,53]. For example, Ong *et al*[52] conducted a randomized controlled study comparing the treatment outcomes of TT regimen *vs* CT regimen, and reported that in ITT analyses, the TT regimen group showed a higher eradication rate compared the CT regimen group[52]. As for treatment-related side effect events, the TT group also showed fewer events during treatment than the CT group. While this study was conducted in Pusan, Korea, which is far from Incheon, Korea, where the GMC is located, similar treatment outcomes were induced even though the CLR resistance rates differed between the cities.

Furthermore, Lesprit *et al*[27] reported that a tailored *H. pylori* eradication strategy based on the presence of a 23S ribosomal RNA point mutation that causes CAM resistance in patients with *H. pylori* infection is more cost effective than empirical treatment[27]. Kim *et al*[25] also conducted an economic modeling study comparing TR based on DPO-PCR and empirical treatment.

Even efficacy of TT regimen in treating *H. pylori* infection has been evaluated, cost-effectiveness of TT regimen should be evaluated to be widely used in clinical practice in Korea. Tailored regimen needs additional diagnostic procedure of antibiotic resistance test such as DPO-PCR. Even it varies depending on insurance coverages, DPO-PCR costs approximately $55.24 more than rapid urease test in Korea[23]. In this regards, several previous studies investigated medical costs of *H. pylori* tailored eradication strategy as compared to empirical first line eradication strategy (CLR based triple regimen) in Korea[23]. Cho *et al*[23] reported that it is acceptable level of predictive additional costs (only an extra $3.96 *per* eradicated patient) for tailored *H. pylori* eradication strategy with DPO-CPR as compared to CLR based conventional triple therapy[23]. In our study, we compared efficacy and safety level of TT *vs* CT regimen, and should discuss the medical cost effectiveness of TT *vs* CT regimen. However, there has been little data to investigate medical cost of TT strategy as compared to CT regimen. One would say medical cost of TT regimen might be much higher than that of CT regimen, since ESR of empirical CT regimen is generally much higher than empirical CLR based triple regimen. Not just considering each patient's medical cost during *Helicobacter* eradication, but also given that worrisome issues on increased prevalence of drug resistance bacteria in worldwide, tailored approaches in treating *H. pylori* infection should be considered following the major principle of antibiotic use guidelines, antibiotic sensitivity result-based treatment.

### Limits of the study

There are several limitations to this study. First, although we prospectively and randomly assigned patients to either the TT or CT group, we retrospectively reviewed the data. Second, since this study was conducted in a tertiary center located in Incheon, Korea, we applied our research results to other regions with caution. In a previous study, researchers investigated the CLR resistance rate for *H. pylori* infection in Incheon, and reported a CLR resistance rate of approximately 30%[54]. Although the aforementioned study was not conducted in our hospital, the GMC, the CLR resistance for *H. pylori* infection is similar to our study. Given that different regions might differ in *H. pylori* infection status and antibiotic resistance status, more multicenter and multinational studies are needed. Third, since we did not culture *H. pylori* for the antibiotic sensitivity test, but instead replaced it with a DPO-PCR test, and are therefore subject to the pit falls of a DPO-PCR. Nevertheless, in a previous study, the DPO-PCR test was validated alongside a culture-based antibiotic resistance test and showed approximately 98% accuracy[20,55].

## CONCLUSION

Despite the aforementioned limitations, this study focused on the efficacy and safety profiles of the TT regimen compared to those of the CT regimen in a relatively large dataset in Korea. For the rapidly rising antibiotic resistance rate, the most active countermeasure is to perform an antibiotic resistance test for the strain and actively select the appropriate antibiotic and then decide on the treatment.

In conclusion, according to our study results, the TT regimen showed promising results in terms of efficacy and safety profiles for the first-line regimen of *H. pylori* eradication as compared to those of the CT regimen. Therefore the DPO-based TT regimen might be a successful option for *H. pylori* eradication, especially when physicians are confronted with increased antibiotic resistance rates for *H. pylori* eradication.

## ARTICLE HIGHLIGHTS

### *Research background*

Antibiotic resistance to *Helicobacter pylori (H. pylori*) infection has been an emerging issue in the clinical field. Recently, to overcome this problem, an antibiotic sensitivity-based tailored therapy (TT) for *H. pylori* infection has got attention.

### *Research motivation*

However, there is limited data regarding efficacy of TT strategy in treatment of *H. pylori* infection in Korea as compared to that of concomitant therapy (CT) regimen.

### *Research objectives*

To investigate the efficacy and safety profiles of TT for *H. pylori* infection treatment compared to a non-bismuth quadruple therapy, CT.

### *Research methods*

We included treatment naive *H. pylori* infection patients (> 18 years) who visited the Gil Medical Center between March 2016 and October 2020. After randomly assigned to either the TT or CT treatment group in 1 to 1 manner, patient compliance, eradication success rate (ESR), and patient-reported side effects profiles were compared between the two groups. For the TT group, a dual priming oligonucleotide polymerase chain reaction (DPO-PCR) test, which detected A2142G and/or A2143G point mutations, and a clarithromycin (CLR) resistance test were performed. Patients in the CLR-resistant group were treated with a bismuth-containing quadruple combination therapy, while those with sensitive results were treated with the standard triple regimen.

### *Research results*

Of the 217 patients with a treatment naive *H. pylori* infection, 110 patients [mean age: 58.66 ± 13.03, men, $n$ = 55 (50%)] were treated with TT, and 107 patients [mean age: 56.67 ± 10.88, men, $n$ = 52 (48.60%)] were treated with CT. The compliance (TT *vs* CT, 100% *vs* 98.13%, $P$ = 0.30), and follow-up loss rates (8.18% *vs* 9.35%, $P$ = 0.95) were not significantly different between the groups. The ESR after treatment was also not statistically different between the groups (TT *vs* CT, 82.73% *vs* 82.24%, $P$ = 0.95). However, the treatment-related and patient-reported side effects were significantly lower in the TT group than in the CT group (22.77% *vs* 50.52%, $P$ < 0.001).

### *Research conclusions*

The DPO-based TT regimen shows promising results in efficacy and safety profiles as a first-line *Helicobacter* eradication regimen in Korea, especially when physicians are confronted with increased antibiotic resistance rates.

### *Research perspectives*

The DPO-based TT regimen might role as a first-line *Helicobacter* eradication regimen with similar efficacy and safety profiles as compared to CT regimen.

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ORIGINAL ARTICLE

**Retrospective Study**

# Histological differentiation impacts the tumor immune microenvironment in gastric carcinoma: Relation to the immune cycle

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## Abstract

### *BACKGROUND*

Various histological types of gastric carcinomas (GCs) differ in terms of their pathogenesis and their preexisting background, both of which could impact the tumor immune microenvironment (TIME). However, the current understanding of the immune contexture of GC is far from complete.

### *AIM*

To clarify the tumor-host immune interplay through histopathological features and the tumor immune cycle concept.

who provided written informed consent for participation in research before their surgery were included in the study.

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

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

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*METHODS*

In total, 50 GC cases were examined (15 cases of diffuse GC, 31 patients with intestinal-type GC and 4 cases of mucinous GC). The immunophenotype of GC was assessed and classified as immune desert (ID), immune excluded (IE) or inflamed (Inf) according to CD8+ cell count and spatial pattern. In addition, CD68+ and CD163+ macrophages and programmed death-ligand 1 (PD-L1) expression were estimated.

*RESULTS*

We found that GCs with different histological differentiation demonstrated distinct immune contexture. Most intestinal-type GCs had inflamed TIMEs rich in both CD8+ cells and macrophages. In contrast, more aggressive diffuse-type GC more often possessed ID characteristics with few CD8+ lymphocytes but abundant CD68+ macrophages, while mucinous GC had an IE-TIME with a prevalence of CD68+ macrophages and CD8+ lymphocytes in the peritumor stroma. PD-L1 expression prevailed mostly in intestinal-type Inf-GC, with numerous CD163+ cells observed. Therefore, GCs of different histological patterns have specific mechanisms of immune escape. While intestinal-type GC was more often related to PD-L1 expression, diffuse and mucinous GCs possessing more aggressive behavior demonstrated low immunogenicity and a lack of tumor antigen recognition or immune cell recruitment into the tumor clusters.

*CONCLUSION*

These data help to clarify the links between tumor histogenesis and immunogenicity for a better understanding of GC biology and more tailored patient management.

**Key Words:** Gastric carcinoma; Tumor immune microenvironment; Tumor infiltrating lymphocytes; Tumor associated macrophages

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**Core Tip:** In this study, we investigated the tumor-host interplay in gastric carcinoma (GC) through the tumor immune cycle concept. Histologically different GCs vary in immunogenicity and differ in tumor-infiltrating lymphocyte and macrophage densities. Intestinal GC demonstrated predominantly inflamed tumor immune microenvironment and frequent programmed death-ligand 1 expression. In contrast, more aggressive diffuse and mucinous GCs possessed low immunogenicity with a lack of cancer antigen recognition and trafficking. These data help to clarify the links between tumor histogenesis and immunogenicity, offering a better understanding of GC biology and the ability to provide more tailored patient management.

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## INTRODUCTION

Gastric carcinoma (GC) is one of the leading fatal malignancies worldwide[1]. Currently, GC prognosis and patient management are based on the UICC/American Joint Committee on Cancer (AJCC) tumour-node-metastasis staging system[2]. When assessing tumour prognosis, GC histopathological features are also significant[3]. The Laurén pathohistological classification is the most widely used in clinical practice because it reflects the GC morphology, specifies the tumour biology and behaviour

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and is helpful for treatment decisions[3,4]. However, GC patients with the same TNM stages and similar histopathological features may differ in outcomes[5]. Therefore, the current approach provides incomplete information for patient prognostic stratification and treatment decisions[5]. Recent large-scale studies, including genomic, transcriptomic and proteomic investigations, defined several molecular classifications for GC [6]. The Cancer Genome Atlas Research Network Group[7] suggested a molecular classification that divides GC into four main subtypes, including Epstein-Barr virus (EBV)-positive, microsatellite unstable (MSI), genomically stable and GC with chromosomal instability[8]. At the same time, the Asian Cancer Research Group established another classification with the following types: MSI, microsatellite stable (MSS) with epithelial-mesenchymal transition, MSS/*TP53*+ and MSS/*TP53*−[9]. However, unlike breast cancer, GC molecular classifications have not yet been connected to treatment options[10].

A growing body of evidence has demonstrated the clinical importance of immune cells for tumour prognosis and immunotherapy sensitivity[11]. Tumour-infiltrating immune cells, including neutrophils, macrophages, dendritic cells and different types of T cells, are major contributors to the tumour immune microenvironment (TIME), which modulates tumour development and progression[11]. Previous studies have demonstrated a correlation between the number of tumour-infiltrating lymphocytes (TILs) and the survival of patients with GC[12]. In addition, tumour-associated macrophages (TAMs) are known as alternative regulators of tumour cell proliferation, angiogenesis and tissue remodelling, thereby facilitating metastasis formation in GC [13]. A promising approach is to consider the tumour-host interplay through the mechanisms of tumour immunology and steps of tumour antigen recognition, T-cell activation and tumour cell killing[14].

However, the connections between the histopathological patterns and immune contexture of GC are not well established. This study aims to clarify the interplay among tumour-host immunity, histopathological features and the tumour immune cycle.

## MATERIALS AND METHODS

### Case selection and patient characteristics

This paper presents a retrospective study that includes 50 cases of primary gastric adenocarcinoma naïve to preoperative chemotherapy or radiotherapy (Table 1). We excluded patients who suffered from other primary malignancies or had a primary tumour of unclear pathological types as well as patients with a known familial or hereditary history of GC. There were 29 males (58%) and 21 females (42%) aged 55.1 ± 3.76 and 45.7 ± 3.59 years, respectively.

The parameters evaluated for each patient included sex, age, tumor location, depth of invasion, grade of differentiation, and tumor staging. Tumor staging was based on the 8$^{th}$ AJCC criteria[15]. Tumor histological type was assessed according to Lauren's classification[3,16]. Among the enrolled cases, there were 15 cases of diffuse GC and 35 patients with intestinal-type GC. The group with intestinal GC included 4 cases of mucinous GC and 31 nonmucinous adenocarcinomas. As mucinous GCs are rare and have a poor prognosis, we assessed them separately.

### Tissue processing and immunohistochemistry

The tissues taken after surgery were fixed in 10% neutral buffered formalin and processed. Histological slides were stained by hematoxylin and eosin, as well as by PAS with Alcian blue. For each tumor, the histological subtype and grade were assessed. For immunohistochemistry (IHC), serial sections 4 μm in thickness were used. Tissues were deparaffinized and hydrated. Endogenous peroxidase activity was blocked using 3% methanol in hydrogen peroxide. Next, antigen retrieval in a water bath at 98 °C was performed using Tris EDTA or citrate buffer (pH 6), followed by incubation with primary antibodies. After washing, labeled polymer secondary antibodies (Envision Detection System, Dako) were added to the slides. Peroxidase activity was detected using diaminobenzidine (DAB)–tetrahydrochloride liquid plus Chromogen System (Dako) substrate. The reaction was stopped with distilled water, after which sections were counterstained with hematoxylin and mounted in Richard-Allan Scientific Mounting Medium (Thermo Fisher).

The following antibodies were used for IHC: CD3 (DAKO; polyclonal) for the entire lineage of T lymphocytes; CD8 (DAKO; Clone C8/144B) for cytotoxic T cells as effector cells of cell-mediated antitumor immunity; and CD68 (DAKO, Clone KP1) and CD163

**Table 1 Clinical-pathological features of the patients**

| Characteristics | Intestinal GC | Mucinous GC | Diffuse GC | Total | *P* value |
|---|---|---|---|---|---|
| Number | 31 | 4 | 15 | 50 | |
| Age | 56.3 11.2 | 51.3 7.37 | 46.0 18.3 | 52.8 13.7 | 0.174 |
| Sex, *n* (%) | | | | | 0.738 |
| Males | 18 (58.1) | 3 (75) | 8 (53.5) | 29 (58) | |
| Females | 13 (41.9) | 1 (25) | 7 (46.7) | 21 (42) | |
| Stage, *n* (%) | | | | | 0.33 |
| Stage 2 | 2 (6.5) | 0 | 0 | 2 (4) | |
| Stage 3 | 13 (41.9) | 1 (25) | 6 (40) | 20 (40) | |
| Stage 4 | 16 (51.6) | 3 (75) | 9 (60) | 28 (56) | |
| Grade, *n* (%) | | - | - | | |
| G1 | 5 (16.1) | | | | |
| G2 | 11 (35.5) | | | | |
| G3 | 15 (48.4) | | | | |
| MMR status | | | | | |
| MSI | 2 | - | - | 2 | |
| MSS | 29 | 4 | 15 | 48 | |

GC: Gastric carcinoma; MMR: Mismatch repair; MSI: Microsatellite unstable; MSS: Microsatellite stable.

(Cell Marque, Clone MRQ-26) to visualize TAMs of M1 and M2 phenotypes. In addition, mismatch repair (MMR) deficiency was assessed using antibodies against MLH1 (Clone ES05, DAKO), MSH2 (Clone FE11, DAKO), PMS2 (Clone EP51, DAKO), and MSH6 (Clone EP49, DAKO). Tumors with a lack of MMR enzyme expression in the tumor cell nuclei were defined as MMR deficient or microsatellite unstable (MSI). Staining of stromal and immune cell nuclei was considered a positive control. Additionally, programmed death-ligand 1 (PD-L1) [PDL Cell Signaling, clone E1L3N(R)] expression was detected immunohistochemically. Human tonsil tissue was used as a positive control.

### *Methodology of tumor-host immunity assessment*

To interpret the tumour-host interplay and mechanisms of immune escape, we assessed the TIME according to the immune cycle concept[17]. The following types of TIME were considered: Immune desert, immune-excluded and inflamed. The immune desert (ID) type demonstrates a lack of pre-existing immunity and a few T-cells inside and around the tumour. The immune-excluded (IE) type shows prominent peritumour infiltration but sparse intratumour T lymphocytes. The inflamed (Inf) TIME possesses high lymphocyte infiltration, thus reflecting the activation of antitumour T cells with improper functioning.

When assessing different immune cells, we examined their number and spatial distribution. The primary histopathological assessment was performed microscopically by two independent pathologists. In addition, digital images of the sections were captured using a digital slide scanner (3DHISTECH, Budapest, Hungary) and assessed blindly. The density of CD8+ cells was counted inside the tumour clusters (TCs) and in the peritumour stroma (TS) in 10 visual fields corresponding to "hot spots", with further quantification *per* 1 $mm^2$.

The number of immunopositive cells was assessed as both continuous and dichotomized variables using cut-off values (84 cells *per* $mm^2$ as a median) to separate low-density from high-density results. The ID type of TIME was defined in cases with low CD8+ infiltration in both the TC and TS. The cases of the IE immunophenotype included tumours with a low CD8+ quantity in the TC compartment and high CD8+ infiltration in the TS compartment. GC with high CD8+ cell infiltration in the TC and TS was classified as belonging to the Inf immunophenotype. The same approach was used to evaluate the TAM distribution by counting CD68+ and CD163+ cells in the TC

and TS. Immune cells in vessels, submucosal lymphatic areas, and necrosis/necrosis adjacent areas were not counted in this study. The expression of PD-L1 was evaluated using the combined positive score (CPS) according to the percentage of stained tumour or immune (lymphocytes and macrophages) cells, with a positive score defined at CPS ≥ 1%[16].

### Statistical analysis

The descriptive statistics included count and frequencies for categorical variables and the mean ± SEM with 95% confidence interval (CI) for continuous variables. Normally distributed continuous variables were analysed with unpaired sample *t*-tests. Nonnormally distributed continuous variables were assessed with nonparametric Mann-Whitney *U*-tests. Chi-square or Fisher's exact test was used to compare categorical variables, whereas one-way analysis of variance was used to compare the continuous variables. Correlations were assessed using the Pearson correlation coefficient. Statistical assessment of the data was carried out using the MedCalc software package. Statistical significance was accepted at $P < 0.05$.

## RESULTS

We did not find sex-related differences in the frequency of various histological types of GCs. However, significant differences were observed in the age of patients with diffuse and intestinal GC types, which were 41.1 ± 6.51 (95%CI: 25.2-57.1) and 53.3 ± 3.07 (46.8-59.8) years old, respectively. However, age differences were not observed between patients with mucinous and nonmucinous intestinal GC.

### TIL and TAM densities varied in GC of different histological types

The assessment of GC immune profiles revealed that CD3+ and CD8+ lymphocyte counts varied in GCs with different histological types. However, CD8+ cell density did not correlate with the tumour grade ($P = 0.669$) or stage ($P = 0.560$). GCs of various histological differentiation types differed in the density of TILs (Figure 1 and Table 2). The number of CD3+ and CD8+ lymphocytes in intestinal-type GC was significantly higher than that in diffuse and mucinous GCs ($P < 0.001$). Importantly, mucinous GCs demonstrated prominent heterogeneity of immune cell infiltration, with few cells within tumour clusters and a higher density around them.

Compared with the common concept that TAMs correspond to M2 macrophages, in GCs, we found that M1 macrophages prevailed over the M2 type. Significant differences were not observed in CD68+ macrophage infiltration in the peritumour stroma with regard to the histological subtypes of GC ($P = 0.471$). However, the number of CD68+ cells within the tumour cluster was higher in intestinal and diffuse GC than in mucinous GC ($P < 0.001$). Moreover, M1 macrophages were more numerous than CD8+ cells in both intra- and peritumour areas of most observed tumours, and they were even more conspicuous in diffuse and mucinous GC. In contrast, CD163+ cells were less frequent and demonstrated specific associations with distinct histological types. M2 macrophages were few in mucinous and diffuse tumours; however, CD163+ cells were much more numerous in intestinal-type GCs ($P = 0.032$). Notably, the M2 macrophage number was comparable to the CD8+ cell count in all GC types. Notably, GCs with a poor prognosis (mucinous and diffuse type) demonstrated a proinflammatory macrophage phenotype in which the number of CD68+ cells was several times higher than that of M2-type macrophages ($P < 0.001$).

Therefore, CD68+ macrophages were the predominant type of immune cells in GCs of all histological types. The density of TILs demonstrated a relationship with the distinct immune contexture. Intestinal-type GCs were rich in CD68+, CD8+ and CD163+ cells. Diffuse GCs were heavily infiltrated by CD68+ cells but low in CD8+- and CD163+ cells. In mucinous GCs, CD68+ cells were the most abundant and prevailed over CD8+ and CD163+ cells, although the immune cell number was significantly lower within the TC than in the peritumor stroma.

### Immunophenotyping GC of different histological types

The histological types of GCs correlated with distinct immunophenotypes ($P < 0.001$) assessed in line with the immune cycle concept (Table 3). There were 10 cases of ID (20%), 13 tumors with IE (26%) and 27 cases with Inf-TIME (54%). Notably, the majority of intestinal-type GCs had an inflammatory TIME (25 of 31 patients; 80.7%). The rest of the patients with intestinal-type GCs showed ID (1 of 31; 3.2%) or IE TIME (5 of 31; 16.1%). More than half of diffuse-type GCs had ID TIME (8 of 15 patients;

**Table 2 Immune cells number in gastric carcinoma of different histological types and tumor immune microenvironment**

| Characteristics | CD8+ cells | | CD68+ cells | | CD163+ cells | |
|---|---|---|---|---|---|---|
| | TC | TS | TC | TS | TC | TS |
| **Histological type** | | | | | | |
| Intestinal | 214 ± 44.9 | 202 ± 14.7 | 303 ± 21.7 | 244 ± 12.4 | 173 ± 17.3 | 170 ± 9.67 |
| | 124-305 | 173-232 | 259-347 | 219-269 | 138-208 | 151-190 |
| Diffuse | 49.5 ± 6.63 | 66.0 ± 6.03 | 339 ± 23.6 | 225 ± 23.8 | 51.7 ± 8.60 | 55.5 ± 8.94 |
| | 36.0-63.1 | 43.6-98.3 | 291-388 | 176-274 | 34.1-69.4 | 37.2-73.9 |
| Mucinous | 7.53 ± 2.50 | 52.5 ± 37.5 | 101 ± 59.0 | 219 ± 16.2 | 12.5 ± 11.8 | 62.5 ± 12.5 |
| | 2.26-34.3 | 23.9-82.9 | 64-125 | 126-264 | 6.32-78.2 | 36-121 |
| | $P = 0.045$ | $P = 0.059$ | $P = 0.071$ | $P = 0.471$ | $P = 0.032$ | $P = 0.011$ |
| **TIME type** | | | | | | |
| ID | 43.7 ± 4.05 | 43.2 ± 5.93 | 367.5 ± 36.9 | 213.1 ± 35.9 | 72.5 ± 18.3 | 48.7 ± 10.5 |
| | 35.1-52.3 | 30.6-55.7 | 288-446 | 135-290 | 33.2-111 | 26.2-71.2 |
| IE | 19.4 ± 3.43 | 112 ± 15.2 | 157.3 ± 29.6 | 266.6 ± 25.8 | 55.5 ± 13.6 | 119.5 ± 16.3 |
| | 12.2-26.7 | 80.1-144 | 94.5-220 | 211-321 | 26.5-84.4 | 84.9-154.1 |
| Inflamed | 229 ± 44.9 | 190 ± 16.5 | 352.8 ± 15.6 | 232.6 ± 13.7 | 165.6 ± 18.2 | 151.3 ± 12.1 |
| | 138-319 | 156-223 | 321-384 | 204-260 | 128-202 | 126-175 |
| | $P = 0.042$ | $P = 0.004$ | $P = 0.674$ | $P = 0.060$ | $P = 0.024$ | $P = 0.011$ |
| Status PD-L1 expression | | | | | | |
| Positive | 341 ± 72.3 | 204 ± 52.7 | 430 ± 27.7 | 200 ± 8.19 | 231 ± 54.3 | 135 ± 29.5 |
| | 66.3-488 | 58.4-350 | 341-518 | 174-226 | 58.4-404 | 41.3-229 |
| Negative | 49.0 ± 13.1 | 116 ± 20.7 | 261 ± 41.4 | 279 ± 37.9 | 96.3 ± 28.0 | 131 ± 28.2 |
| | 20.1-77.9 | 72.0-159 | 169-352 | 195-362 | 34.6-157 | 69.5-193 |
| | $P = 0.010$ | $P = 0.075$ | $P = 0.252$ | $P = 0.715$ | $P = 0.032$ | $P = 0.260$ |

TC: Tumor clusters; TS: Peritumor stroma; TIME: Tumor immune microenvironment; PD-L1: Programmed death-ligand 1; ID: Immune desert; IE: Immune-excluded.

53.3%). The rest were IE (5 of 15; 33.3%) or had an inflammatory TIME (2 of 15; 13.3%). Most mucinous GCs (3 of 4; 75%) were IE TIME, and one case represented the ID immunophenotype. We did not find any statistically significant relationship between TIME and tumor grade ($P = 0.523$) or stage ($P = 0.756$). There were two intestinal-type GCs with MMR deficiency. Both cases were of inflamed-TIME and did not differ significantly in terms of immune cell patterns from other intestinal-type GCs. Therefore, GCs of different histological types illustrated the prevalence of distinct immunophenotypes. Inflamed TIME was more common for intestinal GCs, IE TIME prevailed in mucinous adenocarcinomas, and ID TIME was more typical for diffuse-type GC.

### *Tumor-infiltrating macrophages in GCs with different TIMEs*

Naturally, ID-type GCs demonstrated a low number of CD8+ cells with a similar rate of M2 macrophages. At the same time, the count of CD68+ cells was several times higher than the lymphocyte density ($P < 0.001$). In contrast, GCs with the IE immunophenotype had a centrifugal pattern of immune cell distribution with a twofold lower CD68+ cell count inside tumour clusters compared to peritumour areas ($P < 0.05$). Although CD68+ macrophages were the most abundant immune cells in both the intratumour and peritumour compartments of IE-GCs, their number was twice as low as that in carcinomas with ID and inflammatory TIME ($P < 0.001$). Notably, GCs of the inflamed TIME demonstrated the highest density of CD8+ lymphocytes and CD163+ macrophages in both the tumour cluster and peritumour stroma compared to GCs with other immunophenotypes (Table 2).

**Table 3 Relationship between immunophenotype and histological pattern of gastric carcinoma**

| Characteristics | ID TIME | IE TIME | Inf TIME | Total number |
|---|---|---|---|---|
| **Histological types of GC, *n* (%)** | | | | |
| Intestinal | 1 (3.2) | 5 (16.1) | 25 (80.7) | 31 (62) |
| Diffuse | 8 (53.3) | 5 (33.3) | 2 (13.3) | 15 (30) |
| Mucinous | 1 (25) | 3 (75) | 0 | 4 (8) |
| | | | | $P < 0.001$ |
| **PD-L1 expression status, *n* (%)** | | | | |
| PD-L1 positive GCs | 1 (5.6) | 0 | 8 (94.4) | 9 (18) |
| PD-L1 negative GCs | 9 (21.9) | 13 (31.8) | 19 (46.3) | 41 (82) |
| **Total number** | 10 | 13 | 27 | 50 |
| | | | | $P < 0.001$ |

TIME: Tumor immune microenvironment; PD-L1: Programmed death-ligand 1; GC: Gastric carcinoma; ID: Immune desert; IE: Immune-excluded.

We did not find any tight association between the number of CD68+ cells and the T lymphocyte infiltration pattern. GCs of ID and inflamed TIME demonstrated the highest number of intratumor CD68+ cells with a smaller amount in peritumor stroma (Figure 2). In contrast, the CD163+ cell number was more tightly associated with GC morphology and TIL patterns. M2 macrophages were the most numerous in intestinal-type GC with an inflamed TIME (Table 2). In addition, their amount was associated with PD-L1 expression.

### *PD-L1 expression in GCs with different immunophenotypes*

To clarify the potential mechanisms of GC immune evasion and its relationship with the TIME, we evaluated PD-L1 expression. There were 9 PD-L1-positive GCs of 50 observed cases (18%), and 8 of them were intestinal-type GCs (8 of 9). We did not find a significant relationship between PD-L1 expression and variables such as age, sex, pathological stage and GC histological type and grade. However, PD-L1 expression was related to TIME type ($P = 0.05$). Most cases of PD-L1 positivity were associated with an inflamed TIME (Table 3 and Figure 3). For tumour-infiltrating immune cells, PD-L1 positivity correlated with high CD8+ cell counts in TCs ($P = 0.005$) and CD163+ macrophages ($P = 0.032$) but not CD68+ density. Additionally, there were two intestinal-type GCs with Inf-TIME demonstrating MMR deficiency, and one of those tumours was PD-L1 positive. Thus, PD-L1 expression was found mostly in GC with an inflamed TIME, intestinal histology and a high density of CD8+ and CD163+ cells. Preexisting chronic inflammation and M2 macrophage polarization may be related to the activation of immune escape mechanisms in GC with an inflamed TIME.

## DISCUSSION

The results of the study showed a close relationship between GC histological differentiation and immunophenotype. GCs of distinct histological types differed in terms of the density and spatial patterning of lymphocytes and macrophages, thus reflecting the divergence of the TIME among GCs. Several years ago, three broad classes of the TIME (namely, ID, IE, and Inflamed), representing alterations in consequent steps of the tumour immune cycle, were proposed[17]. According to this concept, ID TIME is characterized by a low number of CD8+ cells caused by poor tumour immunogenicity. This type was typical for diffuse carcinoma and could be due to low mutational load or a lack of antigen release. Alternatively, dendritic cell insufficiency or disrupted costimulatory interactions can diminish antigen presentation and T cell activation[12]. The immune-excluded phenotype, which is more common for mucinous GCs, was associated with the abundance of CD8+ cells in the peritumour stroma; however, T-cytotoxic cells were not able to reach tumour cells. Such a failure can be due to altered mechanisms of T lymphocyte recruitment inside the tumour, which can be caused by the lack of chemokines or low levels of chemokine receptors[18,19]. In contrast, the

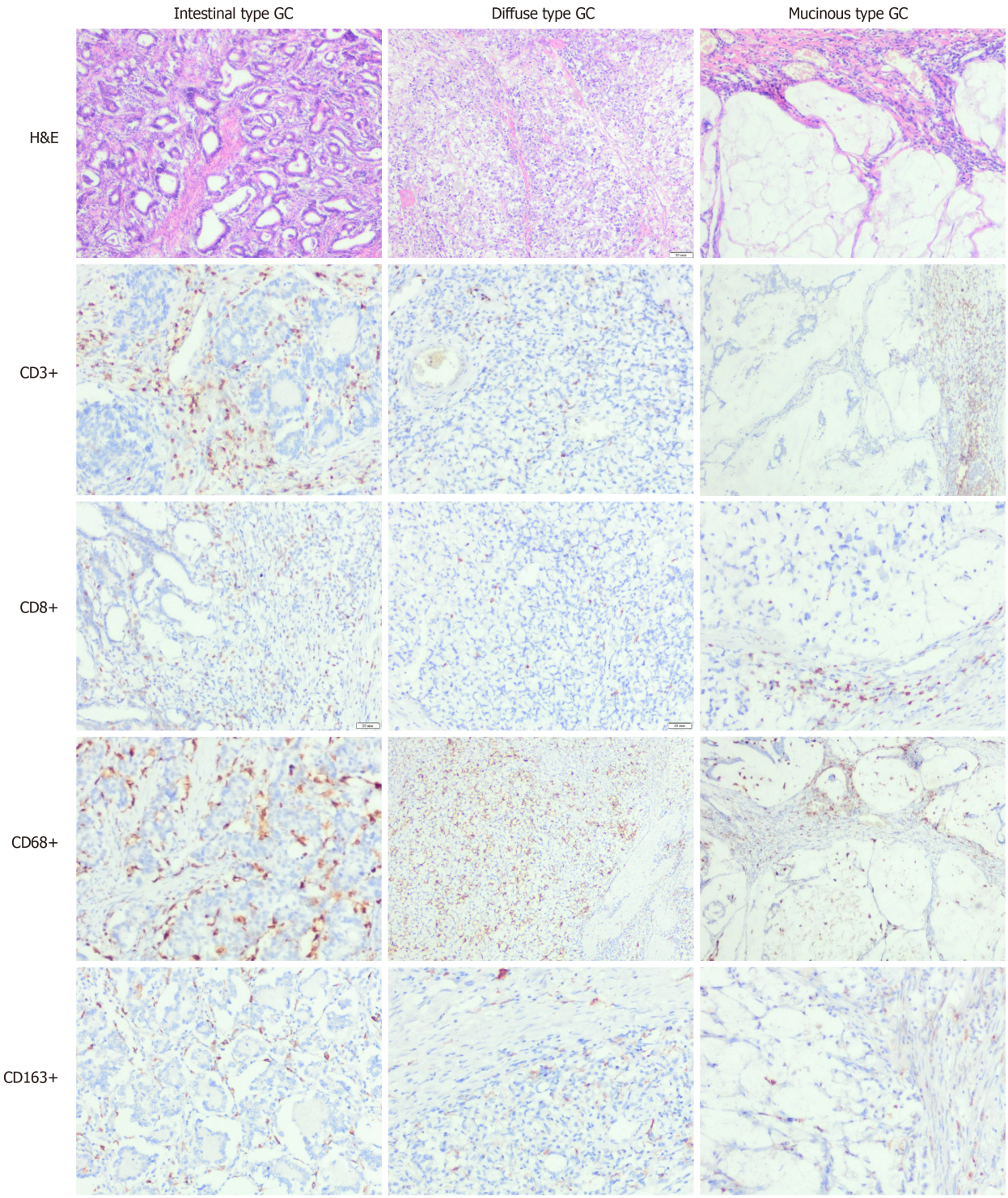


**Figure 1 Density of immune cells in gastric carcinoma of different histological types.** H&E and immunohistochemistry (× 100). Figures demonstrate differences in infiltration of various histological type gastric carcinoma by immune cells, including entire population of T-lymphocytes (CD3), T-cytotoxic cells (CD8), M1 and M2 macrophages (CD68 and CD163 respectively). GC: Gastric carcinoma.

inflammatory TIME predominated in intestinal-type GCs and was rich in lymphocytes infiltrating tumour clusters. Although these tumours demonstrated an "immune hot" phenotype, it seems that the functioning of TILs was improper[20,21]. In addition, the elevated expression of immunosuppressive molecules, such as VEGF and TGF-β, can make antitumour immunity ineffective[14,21]. Therefore, cancer cells of various differentiation types use specific strategies of immune escape and can block either the early steps of the antitumour response or the final mechanisms of cancer cell killing[22,23].

Why mucinous and diffuse GCs exhibit more invasive behavior remains unclear. It might be related to low cell differentiation, which is associated with a higher rate of proliferation, cell motility and cancer dispersion[24]. However, there is an alternative

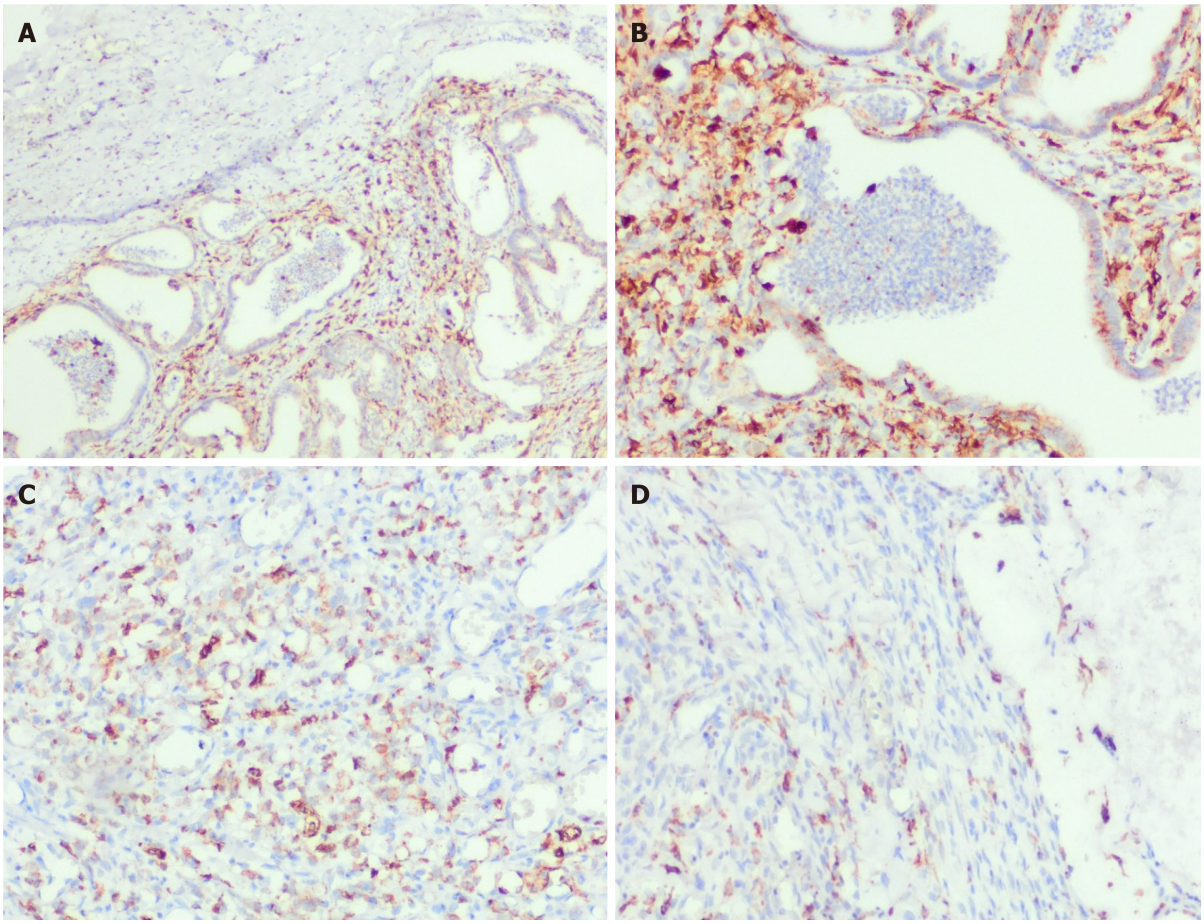


**Figure 2 Density and spatial distribution of macrophages in gastric carcinoma of different histological types.** Immunohistochemistry for CD68. A and B: Intestinal type gastric carcinoma (GC) of inflamed tumor immune microenvironment (TIME) (A) with high count of CD68+ cells forming dense meshwork (B) inside the tumor. (A: × 100, B: × 200); C: High number of intratumor CD68+ cells of diffuse type GC of ID TIME, × 200; D: Prevalence of CD68+ cells in peritumor stroma of mucinous GC of IE TIME, × 200.

explanation based on immune-mediated mechanisms. Papadopoulos *et al*[25] stated that mucin interferes with the inflammatory response and immunological recognition of tumor cells[25]. Our study supports this hypothesis, demonstrating the disruption of either antigen-presenting cell functioning or CD8+ cell trafficking into the tumor in diffuse and mucinous GCs. As there are no molecular targets identified for diffuse and mucinous GCs, it seems that elucidation of their immunosuppressive potential is a promising issue for further investigation.

In contrast, intestinal-type GC demonstrated a predominantly inflamed TIME that looks natural because it arose from preexisting immunological impairments[26,27]. The chronic inflammatory response is believed to be required for a sequence of epithelial transformations called the Correa cascade, which includes multifocal atrophic gastritis, intestinal metaplasia, dysplasia, and cancer[28-31]. Despite the high density of TILs, the inflamed TIME is often associated with the arrest of antitumour immunity[17,32], which can be provoked by various factors, such as the lack of a major histocompatibility complex in tumour cells, the impact of immunosuppressive immune cells (T-regulatory lymphocytes and myeloid-derived suppressor cells), or the expression of immune checkpoints[33]. Previously, an association between PD-L1 expression and MSI and EBV molecular subtypes was noticed[18]. In this study, we found a link between PD-L1 expression and inflamed TIME predominating in intestinal-type GCs. It is also worth noting that most PD-L1-positive cases were related to the high density of M2 macrophages, which could reflect the role of M2 macrophages in immune escape mechanisms.

Finally, this study revealed the prevalence of CD68+ macrophages in the TIME of GCs. Macrophages are thought to be key players in the innate immune response that could modulate chronic inflammatory responses and carcinogenesis. After monocyte recruitment and transformation, macrophages adjust to particular environmental conditions and adopt either proinflammatory (M1) or anti-inflammatory (M2) phenotypes[34]. It is widely accepted that cancer-associated macrophages are

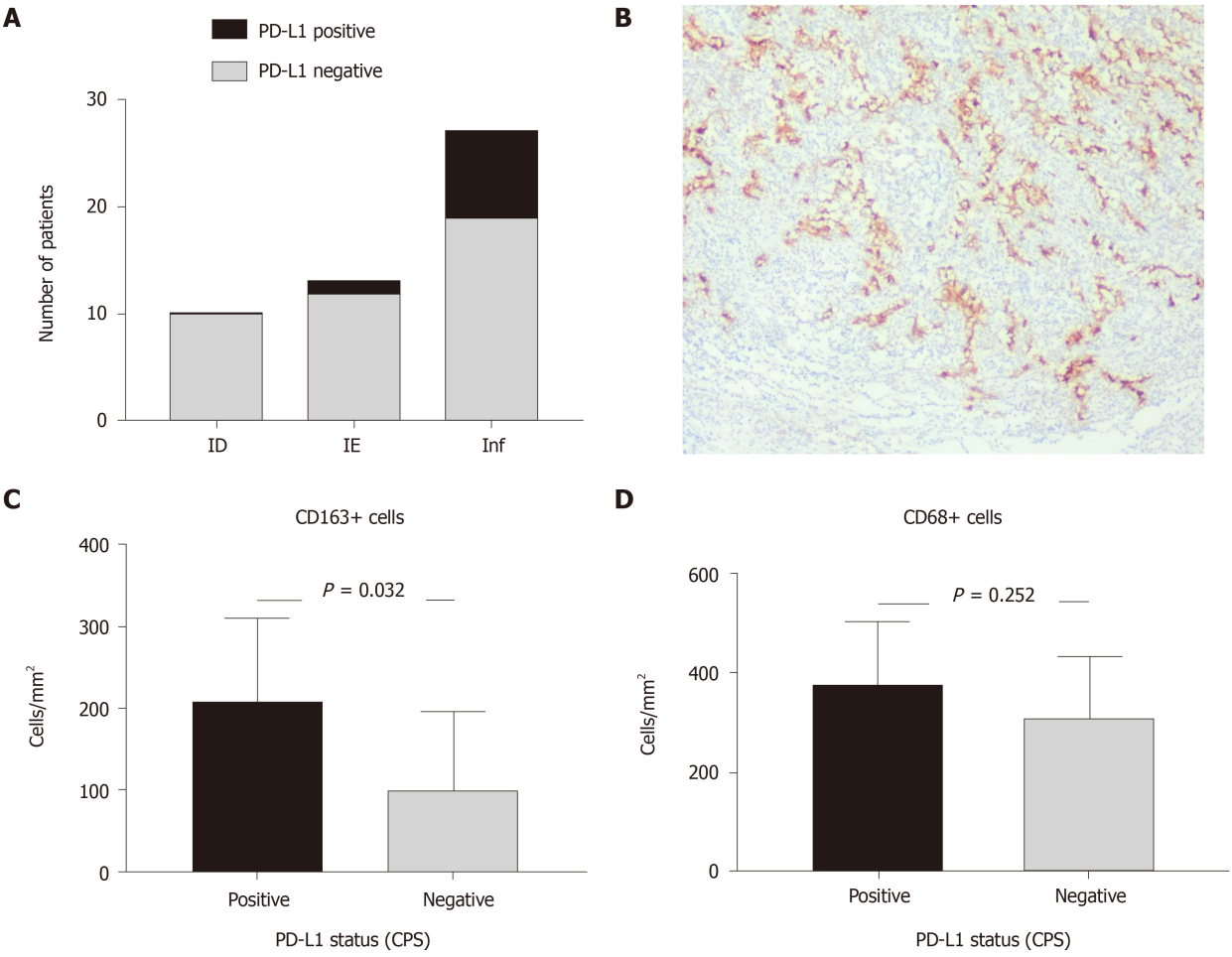


**Figure 3 Relationship between programmed death-ligand 1 expression, gastric carcinoma immunophenotype and number of M1 and M2 macrophages.** A: Frequency of programmed death-ligand 1 (PD-L1) expression in gastric carcinoma (GC) of different tumor immune microenvironment (TIME); B: PD-L1 expression in tumor cells of Inflamed TIME GC, immunohistochemistry for PD-L1, × 50; C and D: number of M1 (C) and M2 (D)-macrophages in GC regarding PD-L1 expression. PD-L1: Programmed death-ligand 1; CPS: Combined positive score.

represented by the M2 type, which contributes to the suppression of adaptive immunity, tissue remodelling, angiogenesis and tumour growth[33]. In contrast, in this study, we found that GCs were associated with the prevalence of M1 macrophages possessing proinflammatory features. CD68+ cells were the predominant cell type of the TIME and were much more numerous than lymphocytes, particularly in diffuse GCs. On the one hand, this finding demonstrates the role of innate *vs* adaptive immune cell balance in carcinomas of various aggressiveness levels. On the other hand, it conflicts with the concept of the high proinflammatory and antitumour activity of M1 macrophages because a high number of these macrophages can improve patient outcomes. However, in diffuse GC, the loss of cadherin expression in cancer cells combined with M1 macrophage activity (reactive oxygen species, causing additional DNA damage and mutagenesis, and matrix-degrading enzymes) could build an effective strategy against adverse biological cancer behaviour. This hypothesis motivates a greater understanding of the role of M1 macrophages in gastric cancer prognostication.

Our study is limited by its retrospective design and descriptive nature as well as the small number of cases for each tumour subtype, and these limitations preclude multivariate analyses of the immunologic variables and clinical outcomes. In addition, the cloning of antibodies used in the study to evaluate PD-L1 expression is not currently used in clinical practice to select gastric cancer patients for immunotherapy.

## CONCLUSION

In this study, we found a relationship between the histological and immunological

features of GC. Various histological types of GC demonstrate distinct immunophenotypes that could be related to different pathways defining tumor immunogenicity and mechanisms of immune evasion. Diffuse and mucinous GCs have low immunogenicity, which is associated with a lack of antitumor immunity activation or T cell recruitment but dense infiltration by CD68+ macrophages. In contrast, intestinal-type GCs exhibit a predominantly inflamed TIME associated with M2 macrophage polarization and PD-L1 expression.

## ARTICLE HIGHLIGHTS

### *Research background*

Various histological types of gastric carcinomas (GCs) differ in terms of their pathogenesis and preexisting background, both of which could impact the tumour immune microenvironment (TIME). However, the current understanding of the immune contexture of GC is far from complete.

### *Research motivation*

The data can help to clarify the links between tumor histogenesis and immunogenicity, offering a better understanding of GC biology and the ability to provide more tailored patient management.

### *Research objectives*

This retrospective study aimed to clarify the tumor-host immune interplay through histopathological features and the tumor immune cycle concept.

### *Research methods*

Of 50 GC cases were examined (15 cases of diffuse GC, 31 patients with intestinal-type GC and 4 cases of mucinous GC). The immunophenotype of GC was assessed and classified as immune desert (ID), immune excluded (IE) or inflamed (Inf) according to CD8+ cell count and spatial pattern. In addition, CD68+ and CD163+ macrophages and programmed death-ligand 1 (PD-L1) expression were estimated.

### *Research results*

GCs with different histological differentiation demonstrated distinct immune contexture. Most intestinal-type GCs had inflamed TIMEs rich in both CD8+ cells and macrophages. In contrast, more aggressive diffuse-type GC more often possessed ID characteristics with few CD8+ lymphocytes but abundant CD68+ macrophages, while mucinous GC had an IE-TIME with a prevalence of CD68+ macrophages and CD8+ lymphocytes in the peritumor stroma. PD-L1 expression prevailed mostly in intestinal-type Inf-GC, with numerous CD163+ cells observed.

### *Research conclusions*

GCs of different histological patterns have specific mechanisms of immune escape. While intestinal-type GC was more often related to PD-L1 expression, diffuse and mucinous GCs possessing more aggressive behavior demonstrated low immunogenicity and a lack of tumor antigen recognition or immune cell recruitment into the tumor clusters.

### *Research perspectives*

Assessment of tumor immune microenvironment in complex with the molecular landscape will provide a deeper understanding of the links between tumor histogenesis and immunogenicity for a better understanding of GC biology and more tailored patient management.

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ORIGINAL ARTICLE

**Observational Study**

# Prediction of the severity of colorectal lesion by fecal hemoglobin concentration observed during previous test in the French screening program

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**Informed consent statement:** Patients were not required to give informed consent to the study because the analysis used anonymous data that was obtained

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## Abstract

### *BACKGROUND*

The rate of positive tests using fecal immunochemical test (FIT) does not decrease with subsequent campaigns, but the positive predictive value of advanced neoplasia significantly decreases in subsequent campaign after a first negative test. A relationship between the fecal hemoglobin concentration (Fhb) and the opportunity to detect a colorectal cancer in subsequent campaign has been shown.

### *AIM*

To predict the severity of colorectal lesions based on Fhb measured during previous colorectal cancer screening campaign.

### *METHODS*

This etiological study included 293750 patients aged 50-74, living in Auvergne-Rhône-Alpes (France). These patients completed at least two FIT [$test_{(-1)}$ and $test_{(0)}$] between June 2015 and December 2019. Delay between $test_{(-1)}$ and $test_{(0)}$ was > 1

after each patient agreed to participate in screening campaigns.

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**Data sharing statement:** Data and materials are available when requested by e-mail. However, each request will be processed in accordance with French legislation on the availability of research data.

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year and test$_{(-1)}$ result was negative (< 150 ngHb/mL). The severity of colorectal lesions diagnosed at test$_{(0)}$ was described according to Fhb measured at test$_{(-1)}$ [Fhb$_{(-1)}$]. The relationship between the severity classified in seven ordinal categories and the predictive factors was analyzed in an ordered multivariate polytomous regression model.

*RESULTS*

The test$_{(0)}$ positive rate was 4.0%, and the colonoscopy completion rate was 97.1% in 11594 patients who showed a positive test$_{(0)}$. The colonoscopy detection rate was 77.7% in those 11254 patients who underwent a colonoscopy. A total of 8748 colorectal lesions were detected (including 2182 low-risk-polyps, 2400 high-risk-polyp, and 502 colorectal cancer). The colonoscopy detection rate varied significantly with Fhb$_{(-1)}$ [0 ngHb/mL: 75.6%, (0-50 ngHb/mL): 77.3%, (50-100 ngHb/mL): 88.7%, (100-150 ngHb/mL): 90.3%; $P$ = 0.001]. People with a Fhb$_{(-1)}$ within (100-150 ngHb/mL) ($P$ = 0.001) were 2.6 (2.2; 3.0) times more likely to have a high severity level compared to those having a Fhb$_{(-1)}$ value of zero. This risk was reduced by 20% in patients aged 55-59 compared to those aged < 55 [adjusted odds ratio: 0.8 (0.6; 1.0)].

*CONCLUSION*

The study showed that higher Fhb$_{(-1)}$ is correlated to an increased risk of severity of colorectal lesions. This risk of severity increased among first-time participants (age < 55) and the elderly (≥ 70). To avoid the loss of chance in these age groups, the FIT positivity threshold should be reduced to 100 ngHb/mL. The other alternative would be to reduce the time between the two tests in these age groups from the current 2 years to 1 year.

**Key Words:** Colorectal cancer screening; Fecal immunochemical test; Fecal hemoglobin concentration; Colorectal lesion severity

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**Core Tip:** The study showed that the severity of the colonic lesions increases with a high concentration of fecal hemoglobin measured in previous test. The elderly (≥ 70 years) had a high proportion of positive colonoscopy when the fecal hemoglobin concentration measured in previous campaign was between 100 and 150 ngHb/mL. Younger patients (age < 54) were likely to have a high-severity neoplasia. Given these results, the recommendation to reduce the FIT positivity threshold to 100 ngHb/mL for first-time participants and the elderly (aged ≥ 70) should attract the attention of the decision-making authority.

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## INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death[1]. To address these major public health issues, several countries have launched nationwide CRC screening program (CRCSP) in order to reduce the incidence and mortality of CRC.

In 2015, in France, the fecal immunochemical test (FIT) OC-Sensor® was introduced as part of the CRCSP based on its performance to detect advanced adenoma and CRC [2-5]. The positivity threshold has been set at 150 ngHb/mL (30 μgHb/g) of stool. Anyone who tested negative was re-invited for testing 2 years later. Anyone who tested positive was advised to complete a colonoscopy. Several studies showed a

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reduction in the CRC incidence several years after FIT introduction in the CRCSP[6,7].

Some colonic polyps such as adenomatous and serrated polyps exhibit a malignant potential, while others do not (hyperplastic, post-inflammatory, hamartomatous)[8]. Surveillance of polyp characteristic over time should lead to a significant increase in the clinical performance of the detection of polyps with high malignant potential[9]. The variability in growth behavior supports the hypothesis that some polyps continue to grow, other are remaining stable, whereas some polyps regress over time. Some authors have reported that advanced adenoma may have the potential to grow faster than non-advanced adenoma, while most small polyps remain stable or are regress over time[10,11].

It was shown that the rate of positive test results with FIT does not decrease during the subsequent campaigns, but the positive predictive value for advanced neoplasia, especially for CRC, significantly decreased in participants during the subsequent campaign after a first negative test[12,13]. A relationship between the fecal hemoglobin concentration (Fhb) and the risk to detect a CRC or an advanced adenoma has also been shown[14-21], but the severity risk of the colorectal lesions according to the earlier Fhb was poorly described.

Looking for less invasive methods for monitoring patients at moderate risk for CRC, this study aims to predict the severity of colorectal lesions based on the Fhb measured during a previous colorectal cancer screening test.

## MATERIALS AND METHODS

### Study design and population

This etiological study included 293750 patients living in one of the six French departments (Ain, Ardèche, Drôme, Isère, Savoie, & Haute-Savoie) of the Auvergne-Rhône-Alpes region. These patients were aged between 50 and 74 and had completed at least two FIT screening tests between June 1, 2015 and December 31, 2019. The delay between the last test [$test_{(0)}$] and the penultimate test [$test_{(-1)}$] was > 1 year, with a negative $test_{(-1)}$ result (< 150 ngHb/mL). The diagnosis of a colorectal lesion as well as its severity were described according to the Fhb measured at $test_{(-1)}$ [$Fhb_{(-1)}$].

All study data were extracted on the same date (November 30, 2020) from departmental databases. These databases were regularly enriched by socio-demographic data, diagnosis (colonoscopy, histopathology), and follow-up data provided by partners (Health Insurance Plans, Medical Information Services, Gastroenterologists, Surgeons & GPs).

### Population and design

In each selected department, the CRCSP campaigns were organized according to the CRCSP national specifications[22,23]. As a reminder, this nationwide screening program started in 2009, and the CRCSP target was every 2 years in an eligible population, *i.e.* asymptomatic patients aged 50 to 74, with no risk factors other than their age (according to the CRCSP national specification). This program was based on the guaiac fecal occult blood test (gFOBT) or hemoccult© II, which has been replaced by the FIT in 2015. The CRC screening test was a two-step method: The first step consisted in the completion of a FIT, the second-step consisted in the completion of a colonoscopy in case of positive FIT. The positivity threshold of the test was set at 150 ngHb/mL of stool. In case of normal colonoscopy, the patient received an invitation to the CRCSP after 5 years. In case of positive colonoscopy (high risk polyps or cancer) the patient was excluded from the nationwide program.

### Operational definition of variables and descriptive analysis

As a study result criterion, $test_{(0)}$ was positive at the threshold of 150 ngHb/mL. The value of the Fhb measured during this $test_{(0)}$ [$Fhb_{(0)}$] was treated as a discrete variable (0 ngHb/mL, 0-50 ngHb/mL, 50–100 ngHb/mL, 100-150 ngHb/mL, 150-300 ngHb/mL, > 300 ngHb/mL). The colonoscopy completion rate was defined by the proportion of patients who had a colonoscopy (complete or incomplete) among those with a positive $test_{(0)}$ result. Colonoscopy was considered positive when a colorectal neoplasia was diagnosed by the gastroenterologist or by a cytopathological examination of the specimens. In the CRCSP, no specific training other than specialized training as an endoscopic physician was required for the practice of colonoscopy by gastroenterologists. The colonoscopy detection rate was defined by the proportion of positive colonoscopies among those performed (complete or incomplete) after a positive test. In the event of a positive result, the diagnostic course was analyzed in terms of types of

diagnosed lesions including: Low risk polyps (LRP), high risk polyps (HRP), unspecified-polyp (UP), and CRC. HRP included: Adenomas ≥ 10 mm (except hyperplastic polyps), serrated adenomas, adenomas with high grade dysplasia, and villous or tubulo-villous adenomas. For these colorectal tumors detected, five localizations were described during colonoscopy: Cecum, right colon (ascending colon and right angle), transverse colon, left colon (left angle and descending colon), and rectosigmoid (rectum and sigmoid colon). The diagnoses associated with CRC and polyps or adenoma were those related to C18–C20 and D12 of the 10$^{th}$ version of the World Health Organization International Classification of Diseases[24]. CRC lesions were described by stage of severity, using the tumor, node, and metastasis (TNM) classification based on tumor size, lymph node involvement, and the possible presence of metastases[25]: CRC Stage-0 ($pTisN_0M_0$), Stage-I ($pT_{1-2}N_0M_0$), Stage-II ($pT_3N_0M_0$ or $pT_4N_0M_0$), Stage-III ($pT_1$-$T_2N_1M_0$ or $pT_1N_2M_0$ or $pT_3$-$T_4N_1M_0$ or $pT_2$-$T_3N_2M_0$ or $pT_4N_2M_0$), and Stage-IV (any T, any N, $M_1$). The size of the polyp, its dysplasia, its cytopathological aspect, and the TNM classification were used to define a scale of severity of colorectal lesions in 7 ordinal categories: Severity level-0 (LRP), level -1 (HRP), level-2 (Stage-0), level-3 (Stage-I), level-4 (Stage-II), level-5 (Stage-III), and level-6 (Stage-IV). The main studied factors were the value of $Fhb_{(-1)}$ and the variation of the Fhb between $test_{(-1)}$ and $test_{(0)}$. The value of $Fhb_{(-1)}$ expressed in ngHb/mL (0, 0-50, 50-100, 100-150) and its variation in ngHb/mL (≤ 0, 0-50, 50-100, 100-150, 150-300, ≥ 300) were treated as discrete variables. The cofactors studied were: (1) the participation in at least one gFOBT campaign (new participant: -the person under 50-years-old at the time the gFOBT was used in the program-, No-gFOBT: -the person has never completed a gFOBT despite being often invited to the gFOBT campaigns-, Yes-gFOBT: -the person completed at least one gFOBT campaign-); (2) the age at the time of performing $test_{(-1)}$ (50-54, 55-59, 60-64, 65-69, ≥ 70-years-old); (3) the gender (female *vs* male); (4) the delay (mo) between $test_{(-1)}$ and $test_{(0)}$ (≤ 24, 25-30, > 30) and; (5) the number of tests completed before the $test_{(-1)}$ (0, 1, 2, 3, ≥ 4).

### Statistical analysis

The main characteristics were described in frequencies for qualitative variables and in mean ± SD for quantitative variables. Proportions were compared using Pearson Chi-2 test or Fisher's exact test when appropriate.

The relationship between the diagnosis of neoplasia (positive colonoscopy *vs* negative colonoscopy) and the predictive factors [$Fhb_{(-1)}$, participation in at least one gFOBT campaign, age at $test_{(-1)}$, gender, delay between $test_{(-1)}$ and $test_{(0)}$, number of tests completed before $test_{(-1)}$] was analyzed in a multivariate logistic regression model, with the estimation of the adjusted odds ratio (OR) and its 95% confidence interval (CI). For the construction of the multivariate model, all the adjustment covariates regardless of the strength of association in univariate analysis were used. In addition, a strong correlation existed between several covariates [age, delay, number of tests completed before $test_{(-1)}$, participation in at least one gFOBT campaign], the model was extended to the terms of interaction between these covariates. Only the significant interaction terms ($P < 0.05$ in univariate analysis) were retained in the final model evaluated by the likelihood ratio test. Positive tests without colonoscopy at the time of the study ($n = 340$) were excluded from this logistic regression analysis.

The relationship between the severity of the lesions (ordinal variable; 0 to 6) and the predictive factors [$Fhb_{(-1)}$, participation in at least one gFOBT campaign, age at $test_{(-1)}$, gender, delay between $test_{(-1)}$ and $test_{(0)}$, number of tests completed before $test_{(-1)}$] was analyzed in an ordered, multivariate polytomous regression model, with the estimation of the risk and its 95%CI. All the adjustment covariates regardless of the strength of association in univariate analysis were used in the multivariate model. In addition, the model was extended to terms of interaction between covariates with a strong correlation (cited above). Only the significant interaction terms ($P < 0.05$ during a univariate analysis) were retained in the final model evaluated by the likelihood ratio test. In this analysis, the unspecified polyps ($n = 3664$) were classified as the polyps not at risk. Similarly, cancers without any precision (unknown/unspecified, $n = 101$) on the TNM classification were classified as tumors *in situ*. As a reminder, negative colonoscopies ($n = 2846$) were excluded from this ordered, univariate, and multivariate polytomous regression. The differences were significant at the 5% level with version 13 of the STATA software (College Station, TX, United States).

### Ethical considerations

Before analysis, all data were anonymized. The screening database had a favorable opinion from the institution that oversees the ethics of data collection ("Commission nationale de l'Informatique et des libertés")[26]. According to the current French

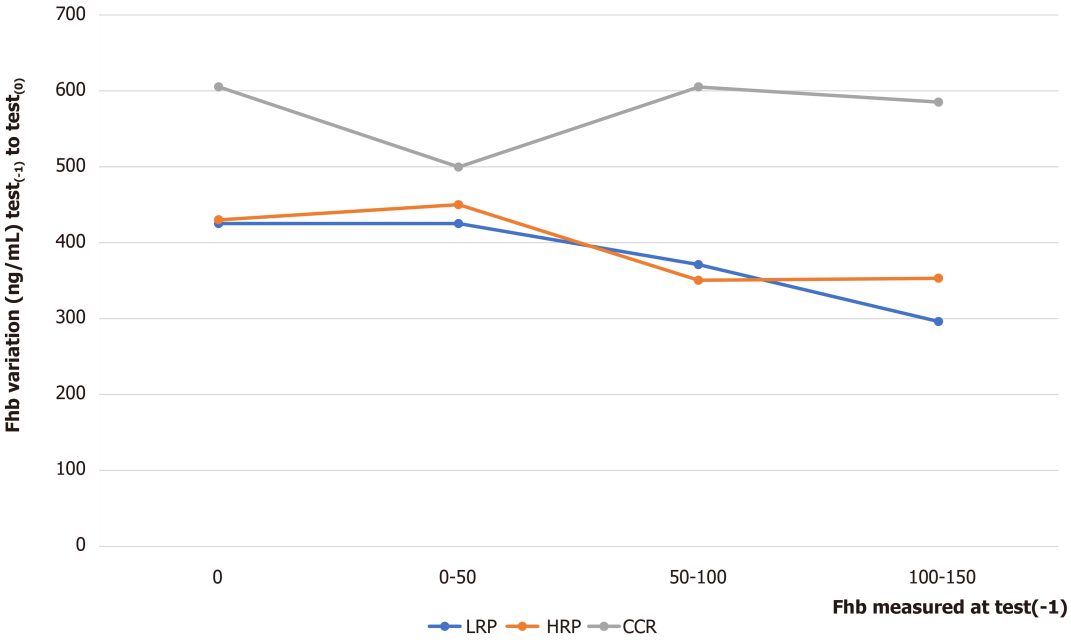


**Figure 1 Type of colorectal lesion according to fecal hemoglobin concentration variation (ng/mL) between $test_{(-1)}$ and $test_{(0)}$ and fecal hemoglobin concentration measured at $test_{(-1)}$.** Fhb: Fecal hemoglobin; LRP: Low risk polyps; HRP: High risk polyps.

legislation, a study that does not change the care of patients did not require the opinion of the Clinical Research Centers Ethics Committee. This article does not contain any studies with human participants performed by any of the authors. This study does not involve human participants, and an informed consent was therefore not required. This article does not contain any studies with animals performed by any of the authors.

## RESULTS

A total of 575587 patients completed at less one FIT between 2015 and 2019. Among them, 281837 (49.0%) people were excluded from this etiological analysis because they had completed only one FIT during the study duration. Finally, our study focused on the 293750 people who completed at least two FIT such as $test_{(-1)}$ and $test_{(0)}$. With a mean age of 61.1 ± 6.7 when completing the $test_{(-1)}$, the positivity rate of the $test_{(0)}$ was 4.0% in these 293750 people. This positivity rate was significantly higher ($P < 0.001$) among patients who have a $Fhb_{(-1)}$ between 100 and 150 ngHb/mL (Table 1).

A total of 282156 people (*i.e.* 96.2% of the sample) had a negative $test_{(0)}$ [$Fhb_{(0)} < 150$ ngHb/mL]. The colonoscopy completion rate was globally estimated at 97.1% in 1594 people who had a positive $test_{(0)}$ [$Fhb_{(0)} \geq 150$ ngHb/mL]. The colonoscopy detection rate was 77.7% among those 11254 people who had a colonoscopy, with a total of 8745 colonic lesions detected (including 2182 LRP, 2400 HRP, 502 CRC). This colonoscopy detection rate varied significantly ($P < 10^{-3}$) with the $Fhb_{(-1)}$ [0: 75.6%, (0-50): 77.3%, (50-100): 88.7%, (100-150): 90.3%; $P < 0.001$) (Table 1).

In total, 27.4% of the lesions detected (*i.e.* 2395 cases) were detected in people who completed a $test_{(0)}$ more than 30 mo after the $test_{(-1)}$. However, these 2395 lesions were mostly polyps compared to the lesions detected in people who completed a $test_{(0)}$ within a reasonable delay between $test_{(0)}$ and $test_{(-1)}$ (delay ≤ 24 m -LRP: 63.8%, HRP: 30.3%, CRC: 5.9%-; delay in 24-30 m -LRP: 64.2%, HRP: 29.3%, CRC: 6.5%-; delay > 30 m -LRP: 74.1%, HRP: 21.9%, CRC: 4.0%-; $P = 0.0001$).

For the same value of the previous Fhb [$Fhb_{(-1)}$], the colonoscopy detection rate was not significantly different between the positive values of $Fhb_{(0)}$ [*i.e.* $Fhb_{(-1)} = 0$ & $Fhb_{(0)} =$ (150-300): rate = 75.5% *vs* $Fhb_{(-1)} = 0$ & $Fhb_{(0)} \geq 300$: rate = 75.7%; $P = 0.8$). However, the colonoscopy detection rate was significantly higher when the $Fhb_{(0)}$ was higher, from 75.6% when $Fhb_{(0)}$ was 0 ngHb/mL to 90.3% at (100-150) ngHb/mL (Table 2).

Regardless of the age group, the colonoscopy detection rate was significantly different between the values of $Fhb_{(-1)}$ [*i.e.* age ≥ 70 years: $Fhb_{(-1)} = 0$: rate = 79.1%, $Fhb_{(-1)} =$ (0-50): Rate = 75.9%, $Fhb_{(-1)} =$ (50-100): Rate = 88.2%, $Fhb_{(-1)} =$ (100-150): Rate = 93.8%;

**Table 1 Result of the last test completed, based on the characteristics of the study population**

| Characteristics | Result of the last test [test$_{(0)}$] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Positive test and colonoscopy completion rate | | | Colonoscopy detection rate | | Number of the colorectal lesions | | | | | | | | | |
| | Nb. (%T+) | *P* value[1] | Number T+ (% Colo in T+) | Number Colo (% Positive Colo) | *P* value[1] | All lesions | Polyps | | | CRC | | | | | |
| | | | | | | | LRP | HRP | UP | S-0 | S-I | S-II | S-III | S-IV | USC |
| Overall | 293750 (4.0) | | 11594 (97.1) | 11254 (77.7) | | 8748 | 2182 | 2400 | 3664 | 133 | 130 | 53 | 64 | 21 | 101 |
| Fhb$_{(-1)}$ (ngHb/mL) | | $< 10^{-3}$ | | | $< 10^{-3}$ | | | | | | | | | | |
| 0 | 280012 (3.5) | | 9655 (97.0) | 9368 (75.6) | | 7085 | 1890 | 1769 | 3070 | 85 | 99 | 31 | 47 | 15 | 79 |
| 0-50 | 1680 (10.4) | | 174 (98.9) | 172 (77.3) | | 133 | 27 | 41 | 56 | 3 | 4 | 1 | 0 | 0 | 1 |
| 50–100 | 8776 (13.0) | | 1144 (96.9) | 1108 (88.7) | | 983 | 177 | 360 | 370 | 25 | 16 | 12 | 9 | 2 | 12 |
| 100-150 | 3282 (18.9) | | 621 (97.6) | 606 (90.3) | | 547 | 88 | 230 | 168 | 20 | 11 | 9 | 8 | 4 | 9 |
| gFOBT campaign participation | | $< 10^{-3}$ | | | 0.03 | | | | | | | | | | |
| Yes-gFOBT | 213476 (4.2) | | 8874 (97.0) | 8605 (77.8) | | 6695 | 1760 | 1906 | 2603 | 112 | 109 | 44 | 59 | 17 | 85 |
| New entrant | 40199 (2.9) | | 1156 (97.5) | 1127 (75.1) | | 846 | 168 | 203 | 456 | 8 | 6 | 0 | 0 | 1 | 4 |
| No-gFOBT | 40075 (3.9) | | 1564 (97.3) | 1522 (79.3) | | 1207 | 254 | 291 | 605 | 13 | 15 | 9 | 5 | 3 | 12 |
| Age (yr) at test$_{(-1)}$ | | $< 10^{-3}$ | | | $< 10^{-3}$ | | | | | | | | | | |
| 50-54 | 57625 (3.0) | | 1727 (97.9) | 1690 (73.7) | | 1245 | 276 | 348 | 582 | 10 | 14 | 1 | 6 | 1 | 7 |
| 55-59 | 62489 (3.5) | | 2166 (97.3) | 2107 (75.6) | | 1592 | 389 | 445 | 687 | 21 | 19 | 4 | 7 | 3 | 17 |
| 60-64 | 64141 (3.9) | | 2514 (97.4) | 2449 (78.4) | | 1920 | 509 | 503 | 807 | 28 | 19 | 17 | 15 | 7 | 15 |
| 65-69 | 63915 (4.7) | | 2997 (97.0) | 2907 (78.7) | | 2287 | 592 | 647 | 886 | 38 | 54 | 14 | 17 | 5 | 34 |
| ≥ 70 | 45580 (4.8) | | 2190 (95.9) | 2101 (81.1) | | 1704 | 416 | 457 | 702 | 36 | 24 | 17 | 19 | 5 | 28 |
| Gender | | $< 10^{-3}$ | | | $< 10^{-3}$ | | | | | | | | | | |
| Female | 160181 (3.3) | | 5343 (97.1) | 5188 (72.8) | | 3778 | 962 | 937 | 1663 | 59 | 51 | 15 | 33 | 11 | 47 |
| Male | 133569 (4.7) | | 6251 (97.0) | 6066 (81.9) | | 4970 | 1220 | 1,463 | 2001 | 74 | 79 | 38 | 31 | 10 | 54 |
| Delay (mo) between test$_{(-1)}$ and test$_{(0)}$ | | $< 10^{-3}$ | | | $< 10^{-3}$ | | | | | | | | | | |
| ≤ 24 | 40422 (5.2) | | 2111 (94.3) | 1991 (72.2) | | 1437 | 435 | 435 | 482 | 21 | 23 | 12 | 12 | 0 | 17 |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 24-30 | 163470 (4.0) | | 6538 (97.7) | 6390 (76.9) | | 4916 | 1322 | 1440 | 1833 | 99 | 83 | 29 | 39 | 14 | 57 |
| > 30 | 89858 (3.3) | | 2945 (97.6) | 2873 (83.4) | | 2395 | 425 | 525 | 1349 | 13 | 24 | 12 | 13 | 7 | 27 |
| Nb. of tests completed before test$_{(-1)}$ | | $< 10^{-3}$ | | | 0.01 | | | | | | | | | | |
| 0 | 68839 (3.5) | | 2421 (97.1) | 2351 (76.1) | | 1790 | 396 | 465 | 855 | 19 | 21 | 9 | 5 | 4 | 16 |
| 1 | 54790 (3.7) | | 2044 (97.7) | 1996 (77.6) | | 1548 | 364 | 432 | 667 | 20 | 26 | 6 | 13 | 6 | 14 |
| 2 | 55794 (4.0) | | 2245 (97.1) | 2180 (76.6) | | 1670 | 434 | 472 | 678 | 25 | 17 | 11 | 12 | 2 | 19 |
| 3 | 56716 (4.4) | | 2514 (97.3) | 2445 (77.9) | | 1905 | 553 | 562 | 649 | 34 | 37 | 13 | 24 | 5 | 28 |
| 4 | 57611 (4.1) | | 2370 (96.3) | 2282 (80.4) | | 1835 | 435 | 469 | 815 | 35 | 29 | 14 | 10 | 4 | 24 |

[1]Pearson Chi-2 test or Fisher's exact test. Fhb$_{(0)}$: Fecal hemoglobin measured at test$_{(0)}$ (last screening test); Fhb$_{(-1)}$: Fecal hemoglobin measured at test$_{(-1)}$ (penultimate test); CRC: Colorectal cancer; Colo: Colonoscopy; gFOBT: Guaiac fecal occult blood test; HRP: High risk polyps; LRP: Low risk polyps; UP: Unspecified-polyp; USC: Unspecified stage of colorectal cancer; S-0: Colorectal cancer Stage-0 ($pTisN_0M_0$), S-I: Stage-I ($pT_{1-2}N_0M_0$); S-II: Stage-II ($pT_3N_0M_0$ or $pT_4N_0M_0$); S-III: Stage-III ($pT_1$-$T_2N_1M_0$ or $pT_1N_2M_0$ or $pT_3$-$T_4N_1M_0$ or $pT_2$-$T_3N_2M_0$ or $pT_4N_2M_0$); S-IV: Stage-IV (any T, any N, $M_1$); T+: Positive test.

$P$ = 0.001). In the age groups of 65-69 years and ≥ 70 years, the proportion of CRC among colorectal lesions was significantly higher when the Fhb$_{(-1)}$ was between 100 and 150 [*i.e.* age in 65-69 years: Fhb$_{(-1)}$ = 0: %CRC = 6.2, Fhb$_{(-1)}$ = (0-50): %CRC = 8.2, Fhb$_{(-1)}$ = (50-100): %CRC = 8.1, Fhb$_{(-1)}$ = (100-150): %CRC = 15.8; $P$ = 0.001) (Table 3).

Overall, the proportion of recto-sigmoid lesions was 57.2% among the 5100 lesions for which the location was provided. The proportion of CRC among these colorectal lesions was significantly higher ($P$ = 0.01) in cecal localization compared to other colonic locations. However, this increase in the proportion of CRC among the cecal lesions was not statistically significant regardless of the stratum combining Fhb$_{(-1)}$ and Fhb$_{(0)}$ (Table 4).

Regardless of the Fhb$_{(-1)}$ modalities [except for modality (0-50)], the variation in Fhb between test$_{(0)}$ and test$_{(-1)}$ was significantly greater when the lesion was CRC colorectal cancer. For this modality (0-50), the number of lesions was relatively lower (CRC = 9, LRP = 27, UP = 56, HRP = 41) (Figure 1).

The multivariate logistic regression included 11254 people who completed a colonoscopy. This analysis shows that people with a Fhb$_{(-1)}$ in (100-150) were 2.9 times more likely to be diagnosed with a colorectal lesion in test$_{(0)}$ compared to those having a Fhb$_{(-1)}$ value of zero (adjusted OR = 2.9; 95%CI: 2.2-3.8). This risk of detecting a neoplastic lesion increased significantly with the age, the gender, the delay between test$_{(-1)}$ and test$_{(0)}$, and the number of tests performed before test$_{(-1)}$ (Table 5).

The ordered polytomous regression included 8748 people having exhaustive information on the colorectal lesion. The analysis showed that people with a Fhb$_{(-1)}$ in (100-150) ($P$ = 0.001) were 2.6 (2.2; 3.0) times more likely to have a high severity level, compared to those having a Fhb$_{(-1)}$ value of zero. The risk to have a high severity level

**Table 2 Colonoscopy detection rate, number and severity of colorectal lesions according to the previous rate and the last rate of fecal hemoglobin**

| $Fhb_{(0)}$ by $Fhb_{(-1)}$ | | | Colonoscopy detection rate | | Number of colorectal lesions by Severity | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $Fhb_{(-1)}$ (ngHb/mL) | $Fhb_{(0)}$ (ngHb/mL) | Number (% in subtotal) | Number Colo (%Positive Colo) | *P* value[1] | Nb. of colorectal lesions | Polyps | | | CRC | | | | | |
| | | | | | | LRP | HRP | UP | S-0 | S-I | S-II | S-III | S-IV | USC |
| 0 | | | | 0.8 | | | | | | | | | | |
| | 0 | 257589 (92.0) | | | | | | | | | | | | |
| | 0-50 | 1805 (0.6) | | | | | | | | | | | | |
| | 50-100 | 8275 (3.0) | | | | | | | | | | | | |
| | 100-149 | 2688 (1.0) | | | | | | | | | | | | |
| | 150-300 | 4903 (1.7) | 4778 (75.5) | | 3609 | 980 | 918 | 1601 | 35 | 38 | 5 | 6 | 2 | 24 |
| | ≥ 300 | 4752 (1.7) | 4590 (75.7) | | 3476 | 910 | 851 | 1469 | 50 | 61 | 26 | 41 | 13 | 55 |
| | Subtotal | 280012 (100.0) | 9368 (75.6) | | 7085 | 1890 | 1769 | 3070 | 85 | 99 | 31 | 47 | 15 | 79 |
| 0–50 | | | | 0.3 | | | | | | | | | | |
| | 0 | 1328 (79.1) | | | | | | | | | | | | |
| | 0-50 | 30 (1.8) | | | | | | | | | | | | |
| | 50-100 | 112 (6.7) | | | | | | | | | | | | |
| | 100-149 | 36 (2.1) | | | | | | | | | | | | |
| | 150-300 | 86 (5.1) | 85 (74.1) | | 63 | 17 | 18 | 24 | 1 | 2 | 1 | 0 | 0 | 0 |
| | ≥ 300 | 88 (5.2) | 87 (80.5) | | 70 | 10 | 23 | 32 | 2 | 2 | 0 | 0 | 0 | 1 |
| | Subtotal | 1680 (100.0) | 172 (77.3) | | 133 | 27 | 41 | 56 | 3 | 4 | 1 | 0 | 0 | 1 |
| 50–100 | | | | 0.7 | | | | | | | | | | |
| | 0 | 6589 (75.1) | | | | | | | | | | | | |
| | 0-50 | 106 (1.2) | | | | | | | | | | | | |
| | 50-100 | 700 (7.9) | | | | | | | | | | | | |
| | 100-149 | 237 (2.7) | | | | | | | | | | | | |
| | 150-300 | 540 (6.2) | 525 (88.4) | | 464 | 97 | 174 | 175 | 7 | 4 | 2 | 2 | 0 | 3 |
| | ≥ 300 | 604 (6.9) | 583 (89.0) | | 519 | 80 | 186 | 195 | 18 | 12 | 10 | 7 | 2 | 9 |
| | Subtotal | 8776 (100.0) | 1108 (88.7) | | 983 | 177 | 360 | 370 | 25 | 16 | 12 | 9 | 2 | 12 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 100–149 | | | | 0.3 | | | | | | | | | |
| | 0 | 2244 (68.4) | | | | | | | | | | | |
| | 0-50 | 49 (1.5) | | | | | | | | | | | |
| | 50-100 | 259 (7.9) | | | | | | | | | | | |
| | 100-149 | 109 (3.3) | | | | | | | | | | | |
| | 150-300 | 293 (8.9) | 286 (88.8) | 254 | 52 | 103 | 87 | 4 | 2 | 3 | 1 | 0 | 2 |
| | ≥ 300 | 328 (10.0) | 320 (91.6) | 293 | 36 | 127 | 81 | 16 | 9 | 6 | 7 | 4 | 7 |
| | Subtotal | 3282 (100.0) | 606 (90.3) | 547 | 88 | 230 | 168 | 20 | 11 | 9 | 8 | 4 | 9 |

[1]Pearson Chi-2 test or Fisher's exact test. $Fhb_{(0)}$: Fecal hemoglobin measured at $test_{(0)}$ (last screening test); $Fhb_{(-1)}$: Fecal hemoglobin measured at $test_{(-1)}$ (penultimate test); CRC: Colorectal cancer; Colo: Colonoscopy; HRP: High risk polyps; LRP: Low risk polyps; UP: Unspecified-polyp; USC: Unspecified stage of colorectal cancer; S-0: Colorectal cancer Stage-0 ($pTisN_0M_0$), S-I: Stage-I ($pT_{1-2}N_0M_0$); S-II: Stage-II ($pT_3N_0M_0$ or $pT_4N_0M_0$); S-III: Stage-III ($pT_1$-$T_2N_1M_0$ or $pT_1N_2M_0$ or $pT_3$-$T_4N_1M_0$ or $pT_2$-$T_3N_2M_0$ or $pT_4N_2M_0$); S-IV: Stage-IV (any T, any N, $M_1$).

was higher in male compared to female ($P$ = 0.001). This risk was reduced by 20% in age group 55-59 years compared to age group < 55 years [adjusted OR: 0.8 (0.6; 1.0), $P$ = 0.02] (Table 5).

## DISCUSSION

This study showed that the risk of detecting a colorectal lesion during a campaign was proportional to the Fhb observed in the previous test completed. Above all, it highlighted that an increase in the probability of detecting a colorectal neoplasia with a high level of severity was proportional to the Fhb observed in the previous test completed. These probabilities varied with the socio-demographic characteristics, especially with age.

The proportion of positive tests and the colonoscopy detection rate increased proportionally with the $Fhb_{(-1)}$. Furthermore, the detection of colorectal lesions according to the variation in the Fhb between $test_{(-1)}$ and $test_{(0)}$ allows to support an association between the detection of cancerous lesions and the strong Fhb variations between two consecutive tests. These results agree with previous data already described in the literature. Furthermore, the increased risk of detecting a neoplasia during a new screening 2 years after a negative screening test result has already been reported in Ireland[14] and recently in the Ile-de-France region[27]. These authors observed variable proportions of pathologies (advanced adenomas or CRC) during a subsequent campaign and hypothesized that these lesions could have been diagnosed during the previous campaign if the FIT positivity threshold was not relatively high. However, they admitted that lowering the positivity threshold would both allow the

**Table 3 Colonoscopy detection rate and proportion of colorectal cancer among colorectal lesions according to the previous rate of fecal hemoglobin and the age at the time of the previous test**

| $Fhb_{(-1)}$ by age at $test_{(-1)}$ | | Colonoscopy detection rate and CRC proportion | | | |
|---|---|---|---|---|---|
| | | Number Colo (%Positive colo) | *P* value[1] | Number of lesions (%CRC) | *P* value[2] |
| Age (yr) at $test_{(-1)}$ | $Fhb_{(-1)}$ (ngHb/mL) | | | | |
| Overall | | 11254 (77.7) | | 8748 (5.7) | |
| 50-54 | | | $<10^{-3}$ | | 0.7 |
| | 0 | 1471 (71.6) | | 1053 (3.1) | |
| | 0-50 | 21 (71.4) | | 15 (0.0) | |
| | 50-100 | 130 (90.8) | | 118 (2.5) | |
| | 100-150 | 68 (86.8) | | 59 (5.1) | |
| | Subtotal | 1690 (73.7) | | 1245 (3.1) | |
| 55-59 | | | $< 10^{-3}$ | | 0.1 |
| | 0 | 1797 (73.7) | | 1324 (3.9) | |
| | 0-50 | 31 (67.7) | | 21 (4.8) | |
| | 50-100 | 178 (87.1) | | 155 (7.1) | |
| | 100-150 | 101 (91.1) | | 92 (7.6) | |
| | Subtotal | 2107 (75.6) | | 1592 (4.5) | |
| 60-64 | | | $< 10^{-3}$ | | 0.05 |
| | 0 | 2044 (76.4) | | 1562 (4.6) | |
| | 0-50 | 37 (70.3) | | 26 (7.7) | |
| | 50-100 | 247 (90.3) | | 223 (8.1) | |
| | 100-150 | 121 (90.1) | | 109 (8.3) | |
| | Subtotal | 2449 (78.4) | | 1920 (5.3) | |
| 65-69 | | | $< 10^{-3}$ | | 0.001 |
| | 0 | 2373 (76.5) | | 1815 (6.2) | |
| | 0-50 | 54 (90.7) | | 49 (8.2) | |
| | 50-100 | 308 (88) | | 271 (8.1) | |
| | 100-150 | 172 (88.4) | | 152 (15.8) | |
| | Subtotal | 2907 (78.7) | | 2287 (7.1) | |
| ≥70 | | | $< 10^{-3}$ | | 0.01 |
| | 0 | 1683 (79.1) | | 1331 (6.5) | |
| | 0-50 | 29 (75.9) | | 22 (9.1) | |
| | 50-100 | 245 (88.2) | | 216 (10.2) | |
| | 100-150 | 144 (93.8) | | 135 (13.3) | |
| | Subtotal | 2101 (81.1) | | 1704 (7.6) | |

[1]Pearson Chi-2 test.

[2]Fisher's exact test. CRC: Colorectal cancer; Colo: Colonoscopy; $Fhb_{(-1)}$: Fecal hemoglobin measured at $test_{(-1)}$ (penultimate test).

diagnosis of lesions that could be serious only 2 years later and create an unsustainable endoscopy referral burden[14].

The current positivity threshold in the French screening program induces a considerable loss of chance. Indeed, in most countries, the threshold of FIT positivity is chosen in part to adapt to the offer of colonoscopies. The need to readjust this strategy in patients with a Fhb between 100 and 150 ngHb/mL should be assessed[27]. This

**Table 4 Number of colorectal lesions and the proportion of colorectal cancer among colorectal lesions by localization, according to the previous rate and the last rate of fecal hemoglobin**

| $Fhb_{(0)}$ by $Fhb_{(-1)}$ | | Colorectal lesion number (%Specified localization) | Number of colorectal lesions by localization and proportion of CRC | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Specified localization: number (% CRC) | Cecum: number (% CRC) | Right colon: number (% CRC) | Transverse colon: number (%CRC) | Left colon: number (%CRC) | Rectosigmoid: number (%CRC) | *P* value[1] |
| $Fhb_{(-1)}$ (ngHb/mL) | $Fhb_{(0)}$ (ngHb/mL) | | | | | | | | |
| Overall | 150-300 | 4390 (57.0) | 2500 (5.4) | 195 (6.7) | 490 (5.3) | 221 (3.2) | 252 (3.2) | 1342 (6.1) | 0.2 |
| | > 300 | 4358 (59.7) | 2600 (13.4) | 179 (16.8) | 432 (11.8) | 176 (11.4) | 235 (11.1) | 1578 (14.1) | 0.3 |
| | Total | 8748 (58.3) | 5100 (9.5) | 374 (11.5) | 922 (8.4) | 397 (6.8) | 487 (7.0) | 2920 (10.4) | 0.01 |
| 0 | 150-300 | 3609 (55.7) | 2011 (5.3) | 154 (7.8) | 391 (5.1) | 176 (2.8) | 200 (3.0) | 1090 (5.8) | 0.2 |
| | > 300 | 3476 (58.2) | 2022 (12.0) | 145 (14.5) | 355 (10.1) | 139 (8.6) | 185 (10.3) | 1198 (12.9) | 0.3 |
| | Subtotal | 7085 (56.9) | 4033 (8.6) | 299 (11.0) | 746 (7.5) | 315 (5.4) | 385 (6.5) | 2288 (9.5) | 0.02 |
| 0-50 | 150-300 | 63 (61.9) | 39 (10.3) | 2 (0.0) | 6 (16.7) | 4 (0.0) | 8 (12.5) | 19 (10.5) | 1.0 |
| | > 300 | 70 (55.7) | 39 (12.8) | 2 (0.0) | 4 (25.0) | 2 (0.0) | 3 (0.0) | 28 (14.3) | 0.8 |
| | Subtotal | 133 (58.7) | 78 (11.5) | 4 (0.0) | 10 (20) | 6 (0.0) | 11 (9.1) | 47 (12.8) | 0.9 |
| 50-100 | 150-300 | 464 (62.1) | 288 (5.6) | 24 (0.0) | 66 (4.6) | 23 (4.4) | 23 (0.0) | 152 (7.9) | 0.5 |
| | > 300 | 519 (63.0) | 327 (17.1) | 22 (27.3) | 46 (17.4) | 21 (23.8) | 29 (10.3) | 209 (16.3) | 0.5 |
| | Subtotal | 983 (62.6) | 615 (11.7) | 46 (13.0) | 112 (9.8) | 44 (13.6) | 52 (5.8) | 361 (12.7) | 0.6 |
| 100-149 | 150-300 | 254 (63.8) | 162 (6.2) | 15 (6.7) | 27 (7.4) | 18 (5.6) | 21 (4.8) | 81 (6.2) | 1.0 |
| | > 300 | 293 (72.4) | 212 (21.7) | 10 (30.0) | 27 (22.2) | 14 (21.4) | 18 (22.2) | 143 (21.0) | 1.0 |
| | Subtotal | 547 (68.4) | 374 (15) | 25 (16.0) | 54 (14.8) | 32 (12.5) | 39 (12.8) | 224 (15.6) | 1.0 |

[1]Pearson Chi-2 test or Fisher's exact test. CRC: Colorectal cancer; $Fhb_{(0)}$: Fecal hemoglobin measured at $test_{(0)}$ (last screening test); $Fhb_{(-1)}$: Fecal hemoglobin measured at $test_{(-1)}$ (penultimate test).

study highlights the need for a strategy taking into account the age of people participating in the screening campaign. The elderly (≥ 70 years) had a high proportion of positive colonoscopy when the Fhb measured in the previous campaign was between 100 and 150 ngHb/mL. Younger people (< 54 years) had a likelihood of having high-severity neoplasia. Given these results, the recommendation to reduce the FIT positivity threshold to 100 ngHb/mL for first-time arrivals and the elderly (≥ 70 years) should attract the attention of the French health authority.

Another alternative would be to reduce the delay between two tests for these first-time participants and the elderly (age ≥ 70 years) from the current 2 years to 1 year. This alternative could have its main justification in the enthusiasm of the elderly towards screening campaigns in France, described in a previous study[28]. The reduction in the time between two tests has the advantage of allowing the recovery of false negative results that a decrease in the positivity threshold cannot recover. Indeed, after finding that the Fhb was less than 4 μgHb/g in 94.0% of false negative individuals, Ibañez-Sanz *et al*[29] concluded that the decrease in the positivity threshold of the FIT does not increase the detection rate of advanced neoplasia but may increase costs and potential adverse effects. Whatever strategy chosen, it should also include the 65-69-age group, which accounts for more than 33.3% of the CRCs detected and presents a significantly increased proportion of CRC when $Fhb_{(-1)}$ was between 100 and 150.

In terms of the location of colorectal lesions, it has been argued that FITs are possibly less effective at detecting lesions located in the proximal colon than distally [30]. Digby *et al*[19] showed that 77.8% of adenomas and 69.2% of cancers were located in the distal colon. The results of this study are consistent with this proximal location. However, in the Ibañez-Sanz study[29], about 60% of the lesions were localized in the proximal colon, while the expected percentage was 30%.

**Table 5 Analysis of the relationship between the diagnosis and severity of colorectal lesions and the predictive factors**

| Predictive factors | Risk analysis of colorectal lesions in a logistic regression model | | | | Risk analysis of the severity of colorectal lesions in an ordered polytomous regression model | | | |
|---|---|---|---|---|---|---|---|---|
| | Univariate | | Multivariate | | Univariate | | Multivariate | |
| | Unadjusted OR (95%CI) | *P* value[1] | Adjusted OR (95%CI) | *P* value[1] | Unadjusted OR (95%CI) | *P* value[2] | Adjusted OR (95%CI) | *P* value[2] |
| $Fhb_{-1}$ (ngHb/mL) (Ref.: 0) | | | | | | | | |
| 0-50 | 1.1 (0.8; 1.6) | 0.6 | 1.1 (0.8; 1.6) | 0.5 | 1.4 (1.0; 2.0) | 0.1 | 1.3 (0.9; 1.8) | 0.1 |
| 50-100 | 2.5 (2.1; 3.1) | $< 10^{-3}$ | 2.4 (2.0; 3.0) | $< 10^{-3}$ | 1.8 (1.6; 2.1) | $< 10^{-3}$ | 1.8 (1.6; 2.0) | $< 10^{-3}$ |
| 100-150 | 3.0 (2.3; 3.9) | $< 10^{-3}$ | 2.9 (2.2; 3.8) | $< 10^{-3}$ | 2.6 (2.2; 3.1) | $< 10^{-3}$ | 2.6 (2.2; 3.0) | $< 10^{-3}$ |
| gFOBT campaign participation (Ref.: Yes-gFOBT) | | | | | | | | |
| New entrant | 0.9 (0.7; 1.0) | 0.04 | 3.3 (2.2; 5.0) | $< 10^{-3}$ | 0.6 (0.6; 0.8) | $< 10^{-3}$ | 0.1 (0.1; 0.2) | $< 10^{-3}$ |
| No-gFOBT | 1.1 (1.0; 1.2) | 0.2 | 2.8 (1.9; 4.1) | $< 10^{-3}$ | 0.8 (0.7; 0.9) | $< 10^{-3}$ | 0.2 (0.1; 0.3) | $< 10^{-3}$ |
| Age (yr) at $test_{(-1)}$ (Ref.: 50-54) | | | | | | | | |
| 55-59 | 1.1 (1.0; 1.3) | 0.2 | 1.3 (1.0; 1.6) | 0.04 | 1.1 (0.9; 1.3) | 0.4 | 0.8 (0.6; 1.0) | 0.02 |
| 60-64 | 1.3 (1.1; 1.5) | $< 10^{-3}$ | 1.4 (1.2; 1.8) | 0.001 | 1.0 (0.9; 1.2) | 0.5 | 0.7 (0.6; 0.9) | 0.004 |
| 65-69 | 1.3 (1.1; 1.5) | $< 10^{-3}$ | 1.4 (1.1; 1.8) | 0.002 | 1.3 (1.1; 1.5) | 0.002 | 0.9 (0.7; 1.1) | 0.2 |
| ≥ 70 | 1.5 (1.3; 1.8) | $< 10^{-3}$ | 1.7 (1.3; 2.1) | $< 10^{-3}$ | 1.2 (1.0; 1.4) | 0.01 | 0.8 (0.6; 1.0) | 0.07 |
| Gender (Ref.: Female) | | | | | | | | |
| Male | 1.7 (1.5; 1.9) | $< 10^{-3}$ | 1.7 (1.5; 1.8) | $< 10^{-3}$ | 1.2 (1.1; 1.3) | 0.002 | 1.2 (1.1; 1.3) | $< 10^{-3}$ |
| Delay (mo) between $test_{(-1)}$ and $test_0$ (Ref.: < 24) | | | | | | | | |
| 24-30 | 1.3 (1.1; 1.4) | $< 10^{-3}$ | 1.3 (1.1; 1.5) | $< 10^{-3}$ | 1.0 (0.9; 1.1) | 0.9 | 1.0 (0.9; 1.1) | 0.9 |
| > 30 | 1.9 (1.7; 2.2) | $< 10^{-3}$ | 2.1 (1.7; 2.6) | $< 10^{-3}$ | 0.6 (0.5; 0.7) | $< 10^{-3}$ | 0.6 (0.5; 0.7) | $< 10^{-3}$ |
| Number of tests completed before $test_{(-1)}$ (Ref.: 0) | | | | | | | | |
| 1 | 1.1 (0.9; 1.2) | 0.3 | 2.7 (1.9; 3.9) | $< 10^{-3}$ | 1.2 (1.0; 1.4) | 0.03 | 0.3 (0.2; 0.4) | $< 10^{-3}$ |
| 2 | 1.0 (0.9; 1.2) | 0.7 | 2.7 (1.8; 4.1) | $< 10^{-3}$ | 1.2 (1.0; 1.3) | 0.03 | 0.2 (0.1; 0.3) | $< 10^{-3}$ |
| 3 | 1.1 (1.0; 1.3) | 0.1 | 2.9 (1.9; 4.4) | $< 10^{-3}$ | 1.4 (1.2; 1.6) | $< 10^{-3}$ | 0.3 (0.2; 0.4) | $< 10^{-3}$ |
| 4 | 1.3 (1.1; 1.5) | $< 10^{-3}$ | 3.5 (2.3; 5.2) | $< 10^{-3}$ | 1.1 (1.0; 1.3) | 0.1 | 0.2 (0.1; 0.3) | $< 10^{-3}$ |

[1]*P* values were estimated from likelihood ratio tests based on data from 11254 people who completed a colonoscopy.

[2]*P* values were estimated from likelihood ratio tests based on data from 8748 people diagnosed with colorectal lesion after colonoscopy. $Fhb_{(-1)}$: Fecal hemoglobin measured at $test_{(-1)}$ (penultimate test); gFOBT: Guaiac fecal occult blood test; OR: Odds ratio; CI: Confidence interval.

The optimal interval for CRC screening using FIT remains unclear[5]. In terms of the impact of the delay between the tests on the risk of detection of a colorectal lesion, the results of this study are not consistent with Van Roon's analysis, certainly because of the lower test positivity threshold (≥ 50 ng/mL) in their study carried out in a small sample (7501 people)[31]. In addition, this study found an unexpected reverse direction in the analysis of the lesion severity risk according to the delay between $test_{(0)}$ and $test_{(-1)}$. This paradoxical reduction in the risk of severity could be explained by the high proportion of polyps among the lesions detected in patients with an abnormally long delay between $test_{(0)}$ and $test_{(-1)}$. We can also hypothesize that some patients with a time between the two tests greater than 2 years may be symptomatic and therefore will not appear in the screening program as mentioned by Liao *et al*[21].

### Limitations of the study

The main limitation of this study is the amount of missing data, especially on the stages of the lesions and their colonic locations. However, this is a consequence inherent to retrospective studies that cannot question the results of this study. In addition, the absence of a cancer registry in the "Auvergne Rhône-Alpes Region" does not allow interval cancers to be included in this study.

## CONCLUSION

An increased risk of severity of the colorectal lesion was observed in proportion to the increase in $Fhb_{(-1)}$. This risk of severity varied with the socio-demographic characteristics of the patients, especially among first-time participants. An increased colonoscopy detection rate was observed in the elderly in correlation with the increase in $Fhb_{(-1)}$. According to these results, the FIT positivity threshold should be reduced to 100 ngHb/mL for first-time participants and patients aged ≥ 70. The other alternative should be to reduce the delay between the two tests for these first-time participants and the elderly (age ≥ 70) from the current 2 years to 1 year.

## ARTICLE HIGHLIGHTS

### Research background

The rate of positive tests using fecal immunochemical test (FIT) does not decrease with subsequent campaigns, but the positive predictive value of advanced neoplasia significantly decreases in subsequent campaign after a first negative test. A relationship between the fecal hemoglobin concentration (Fhb) and the opportunity to detect a colorectal cancer in subsequent campaign has been shown.

### Research motivation

In this period of implementation of the optimization strategies of the French program, our motivation was to alert the health authority on the severity of the lesions not diagnosed because of the high positivity threshold of the current screening FIT.

### Research objectives

Our objective was to predict the severity of colorectal lesions based on Fhb measured during previous colorectal cancer screening campaign.

### Research methods

The etiological study included 293750 patients aged 50-74, living in Auvergne-Rhône-Alpes (France). These patients completed at least two FIT [$test_{(-1)}$ and $test_{(0)}$] between June 2015 and December 2019. Delay between $test_{(-1)}$ and $test_{(0)}$ was > 1 year, and $test_{(-1)}$ result was negative (< 150 ngHb/mL). The severity of colorectal lesions diagnosed at $test_{(0)}$ was described according to Fhb measured at $test_{(-1)}$ [$Fhb_{(-1)}$]. The relationship between the severity classified in seven ordinal categories and the predictive factors was analyzed in an ordered multivariate polytomous regression model.

### Research results

The $test_{(0)}$ positive rate was 4.0% and the colonoscopy completion rate was 97.1% in 11594 patients who showed a positive $test_{(0)}$. The colonoscopy detection rate was 77.7% in those 11254 patients who underwent a colonoscopy. In total, 8748 colorectal lesions were detected (including 2182 low-risk-polyps, 2400 high-risk-polyp and 502 colorectal cancer). The colonoscopy detection rate varied significantly with $Fhb_{(-1)}$ [0 ngHb/mL: 75.6%, (0-50): 77.3%, (50-100): 88.7%, (100-150): 90.3%; $P$ = 0.001). People with a $Fhb_{(-1)}$ within (100-150 ngHb/mL) ($P$ = 0.001) were 2.6 (2.2; 3.0) times more likely to have a high severity level compared to those having a $Fhb_{(-1)}$ value of zero. This severity risk was reduced by 20% in patients aged 55-59 compared to those aged < 55 [adjusted odds ratio: 0.8 (0.6; 1.0)].

### Research conclusions

The study showed that higher $Fhb_{(-1)}$ is correlated to an increased risk of severity of colorectal lesions. This risk of severity increased among first-time participants (age < 55) and the elderly (≥ 70). To avoid the loss of chance in these age groups, the FIT

positivity threshold should be reduced to 100 ngHb/mL. The other alternative would be to reduce the time between the two tests in these age groups from the current 2 years to 1 year.

### *Research perspectives*

At the end of this study, we aim to conduct an experiment with a screening program considering the age of patients and the previous values of the fecal hemoglobin concentration.

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CASE REPORT

# Plexiform angiomyxoid myofibroblastic tumor treated by endoscopic submucosal dissection: A case report and review of the literature

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**Author contributions:** Wu JD reviewed the literature and contributed to manuscript drafting; Xu FH and Chen YX were the patient's primary doctors and substantially contributed to the conception and design of the study and acquisition of the data; Luo C and Zhang L substantially contributed to the data acquisition; Hou XH and Song J were responsible for the revision of the manuscript for important intellectual content.

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## Abstract

### *BACKGROUND*

Plexiform angiomyxoid myofibroblastic tumor (PAMT) is a rare mesenchymal tumor characterized by multiple nodular plexiform growth patterns and an immunophenotype with myofibroblasts. The pathological characteristics, immunohistochemistry, diagnostic criteria, differential diagnosis, and gene-level changes of PAMT have been reported in many studies. At present, the main treatment for PAMT in the reported cases is surgery; only eight cases were treated *via* endoscopy (excluding 1 thoracoscopic resection), and the lesions were all smaller than 5 cm. There are no reports on the prognosis and follow-up of young patients with lesion sizes reaching 5 cm who undergo endoscopic submucosal dissection (ESD). Herein, we present the first case of a young patient with a lesion size reaching 5 cm who was diagnosed with PAMT *via* endoscopic submucosal dissection.

### *CASE SUMMARY*

A 15-year-old young man with upper abdominal pain for 2 years presented to the Gastroenterology Department of our hospital. Painless gastroscopy showed a semicircular bulge approximately 5 cm in size in the lesser curvature near the cardia of the fundus; the surface was eroded, and shallow ulcers had formed. The pathological manifestations of the biopsy were spindle cell proliferative lesions with interstitial mucinous changes, and the surface mucosa showed chronic inflammatory changes with active lesions; immunohistochemistry showed smooth muscle actin (SMA) (+), CD117 (-), CD34 (-), DOG-1 (-), S-100 (-), and Ki67 (LI: < 1%). We performed ESD on the patient. The lesion that we removed was 5 cm × 4 cm × 2 cm in size. Pathologically, the resected tissue displayed typical manifestations, such as fat spindle-shaped fibroblasts and myofibroblast-like cells showing irregular nodular hyperplasia. Immunohistochemistry staining of the tumor cells revealed the following: CD34 (partially +), SMA (weakly +), CD117 (-), DOG-1 (-),

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S-100 (-), SDHB (+), PCK (-), and Ki67 (labelling index: 2%). There was no recurrence or metastasis during the 3-mo follow-up after the operation, and the treatment effect was good. We also performed a review of the literature on the clinical manifestations, pathological features, immunohistochemistry, and differential diagnosis of PAMT.

*CONCLUSION*

At present, the diagnostic criteria for PAMT are relatively clear, but the pathogenesis and genetic changes require further study. PAMT is benign in nature, and these patients are less likely to experience local or metastatic recurrence. The main treatment is still surgery if the lesion is in the stomach. Partial gastrectomy and distal gastrectomy are the most frequently performed surgical treatments for PAMT, followed by local resection, subtotal gastrectomy, and wedge resection. But for comprehensive evaluation of the disease, ESD can be considered a suitable method to avoid excessive treatment.

**Key Words:** Plexiform angiomyxoid myofibroblastic tumor; Endoscopic submucosal dissection; Stomach; Cardia-preserving; Benign; Case report

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**Core Tip:** Plexiform angiomyxoid myofibroblastic tumor (PAMT) is a rare mesenchymal tumor. The main treatment for PAMT in the reported cases is surgery. We present a rare case of PAMT in a young patient who underwent endoscopic submucosal dissection (ESD). The biopsied and resected tissue showed interstitial mucinous changes and myofibroblast-like cells showing irregular nodular hyperplasia, and immunohistochemistry revealed that the tissue was SMA (+). There was no recurrence during the 3-mo follow-up after ESD, and the treatment effect was good. For comprehensive evaluation of the disease, ESD can be considered a suitable method to avoid excessive treatment.

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## INTRODUCTION

Plexiform angiomyxoid myofibroblastic tumor (PAMT), also termed plexiform fibromyxoma (PF), is a rare benign tumor of the gastrointestinal tract that was first proposed by Takahashi *et al*[1] in 2007. This tumor occurs mainly in the antrum of the stomach and shows unique histological features, including myxoid extracellular matrix and ovoid or spindle cells as a plexiform intramural growth pattern. Tumor cells show differentiation into myofibroblasts with SMA expression and have limited cytological atypia[2]. Although some reports showed extragastric expansion and vascular infiltration, no local metastasis or distant recurrence after resection was reported. The main treatment for PAMT in the reported cases is surgery. Herein, we present the first case of a young patient with a lesion size reaching 5 cm who was diagnosed with PAMT *via* endoscopic submucosal dissection.

## CASE PRESENTATION

### *Chief complaints*

A 15-year-old patient arrived at the Gastroenterology Department of our hospital complaining of abdominal pain for 2 years.

### History of present illness

The patient had intermittent abdominal pain over the past 2 years without special treatment.

### History of past illness

The patient had no previous medical history.

### Personal and family history

The patient never smoked or drank. He had no history of food or drug allergy and no family history of gastrointestinal tumors.

### Physical examination

The temperature of the patient was 36.5 °C, heart rate was 73 beats per minute, breathing rate was 16 beats per minute, and blood pressure was 130/79 mmHg. Examination of the chest, lung, and abdomen showed no abnormalities.

### Laboratory examinations

The patient's alkaline phosphatase level was 197 U/L, glutamyl transfer peptidase was 6 U/L, uric acid was 431.6 μmol/L, and phosphorus was 1.41 mmol/L. Other routine blood tests, routine urine tests, routine stool tests, and biochemical and erythrocyte sedimentation rates were all normal.

### Imaging examinations

Computed tomography (CT) of the lungs and abdomen revealed no obvious abnormalities in both the lungs and mediastinum, but a space-occupying lesion in the gastric fundus area. Our clinical diagnostic consideration was gastric stromal tumor.

### Further diagnostic work-up

Painless gastroscopy showed a semicircular bulge approximately 5 cm in size in the lesser curvature near the cardia of the fundus; the surface was eroded, and shallow ulcers had formed. A biopsy sample was taken from the surface of the lesion (Figure 1). Endoscopic ultrasonography revealed that the lesion originated from the submucosal layer, which was heterogeneous and hyperechoic, and the posterior muscularis propria and serosal surface were present. The blood supply to the lesion was abundant by Doppler ultrasound (Figure 2). Pathologically, the biopsy sample revealed spindle cell proliferative lesions with interstitial mucinous changes, and the surface mucosa showed chronic inflammatory changes with active lesions (Figure 3). Immunohistochemistry showed smooth muscle actin (SMA) (+), CD117 (-), CD34 (-), DOG-1 (-), S-100 (-), and Ki67 (labelling index: < 1%).

## FINAL DIAGNOSIS

Based on the pathological results and clinical results, the final diagnosis was PAMT of the stomach.

## TREATMENT

Because the lesion was near the cardiac area and the patient was young, we decided to remove the lesion by endoscopy. We performed ESD of the lesion, and as expected, the operation procedure was very difficult. The method of ESD was described everywhere. Simply, we marked the extent of the lesion first. Then, we performed submucosal injections and incised the mucosa at the outer edge of the lesion with a Dual knife and IT knife. After separating the submucosa, the lesion was exposed and dissected with the aid of the snare traction. After nearly 6 h of effort, we resected the lesion successfully (Figure 4A). The blood vessels of the lesion were treated with hemostatic agents during and after the operation. There was no active bleeding or perforation after washing. The cardia was preserved, and the lesion that we removed was 5 cm × 4 cm × 2 cm in size (Figure 4B).

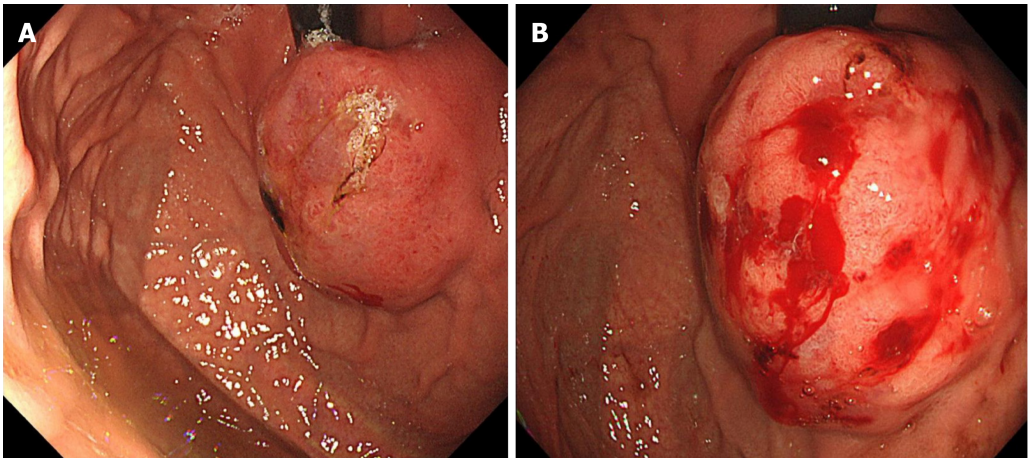


**Figure 1 Lesions under endoscopy before and after biopsy.** A: Image of the lesion before biopsy; B: Image of the lesion after biopsy.

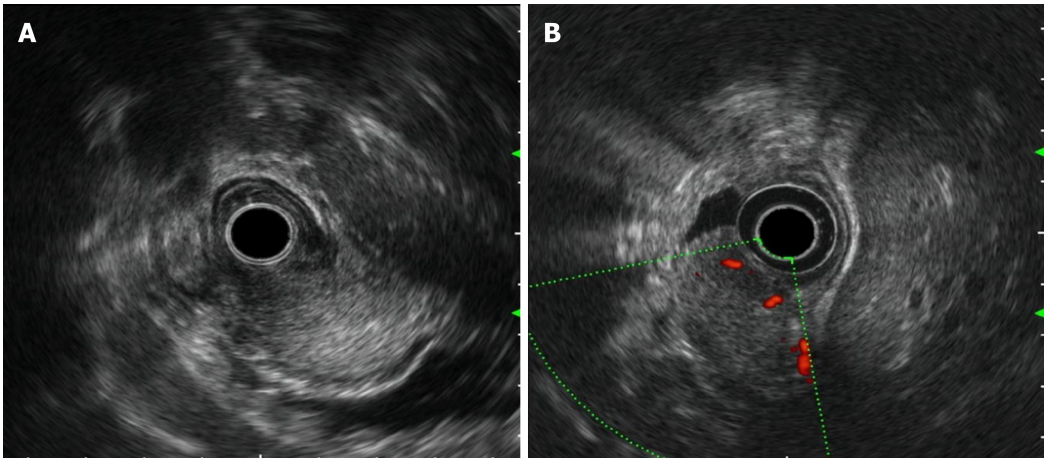


**Figure 2 Rich blood flow in the lesion under Doppler ultrasound.** A: Endoscopic ultrasonography revealed that the lesion originated from the submucosal layer, which was heterogeneous and hyperechoic, and the posterior muscularis propria and serosal surface were present; B: The blood flow was abundant on Doppler ultrasound.

## OUTCOME AND FOLLOW-UP

The patient had sudden abdominal pain on the second day after the operation, and the upper abdomen showed signs of abdominal muscle tension, tenderness, and rebound pain. The results of emergency CT examination showed perforation of the gastrointestinal tract and abdominal effusion. The patient was treated by gastrointestinal decompression, anti-inflammatory therapy, acid suppression, fluid rehydration, and pain relief. On the third day after the operation, the patient was accompanied by fever, and the abdominal symptoms and peritoneal irritation did not improve. Therefore, the patient underwent laparoscopic gastric perforation repair, intestinal adhesion release, and abdominal drainage operation. During the operation, there was a 1-cm perforation in the lower anterior wall of the gastric cardia, and the surrounding tissues were congested and edema. A large abscess cavity formed with the spleen and the left lobe of the liver under the diaphragm. More purulent fluid can be seen in the liver, right paracolic sulcus, intestines, and pelvis. We cleaned the abdominal cavity, sutured the perforation, and strengthened the seromuscular layer. The patient fully recovered without any symptoms and was discharged after 14 d. There was no recurrence during the 3-mo follow-up after ESD (Figure 4C). The pathology of the resected tissue showed typical manifestations, such as abundant slender blood vessels, rich mucus-like mesenchyme, and spindle-shaped, fat spindle-shaped fibroblasts and myofibroblast-like cells showing irregular nodular hyperplasia (Figure 5). Immunohistochemistry staining of tumor cells revealed the following: CD34 (partially +), SMA (weakly +), CD117 (-), DOG-1 (-), S-100 (-), SDHB (+), PCK (-), and Ki67 (labelling index: 2%).

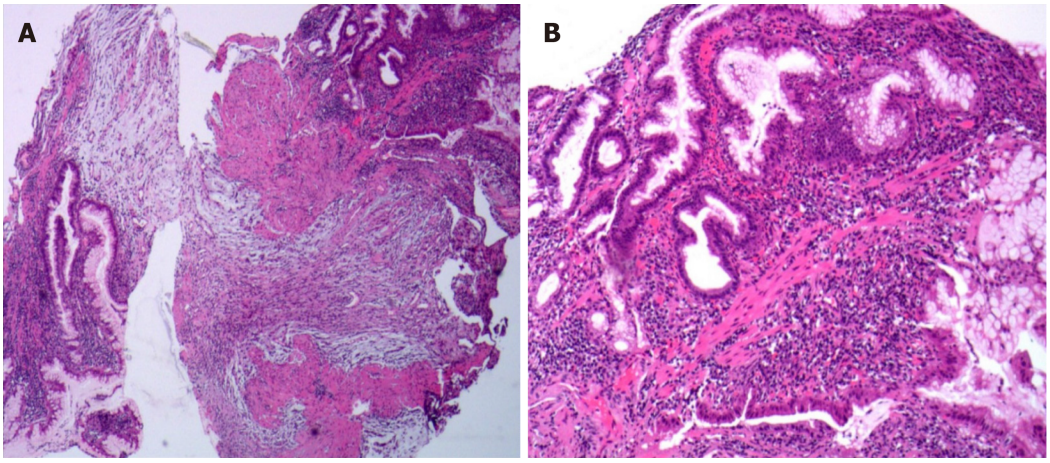


**Figure 3 Hematoxylin and eosin staining of the biopsy revealed spindle cell proliferative lesions with interstitial mucinous changes, and the surface mucosa showed chronic inflammatory changes with active lesions.** A: Magnification: 10 ×; B: Magnification: 40 ×.

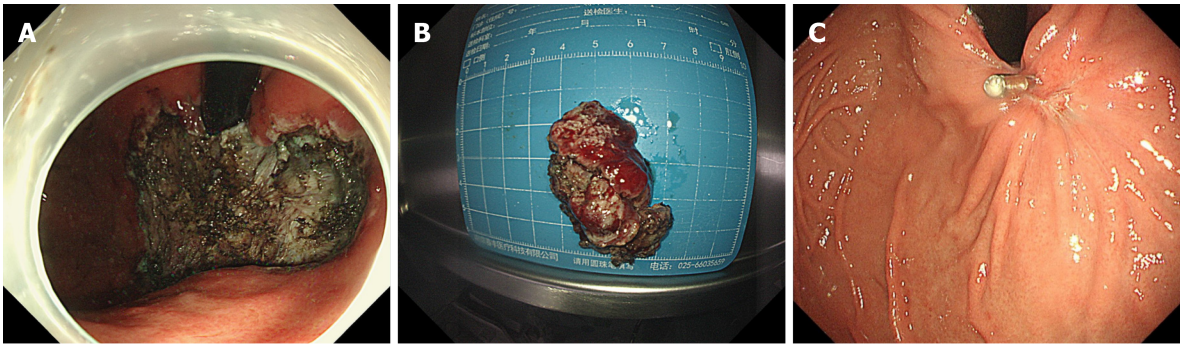


**Figure 4 Images of peeled tissue, lesion size, and clipping under endoscopy.** A: The wound after endoscopic submucosal dissection (ESD); B: Resected tissue: 5 cm × 4 cm × 2 cm; C: The ulcer scar of the wound 3 mo after ESD.

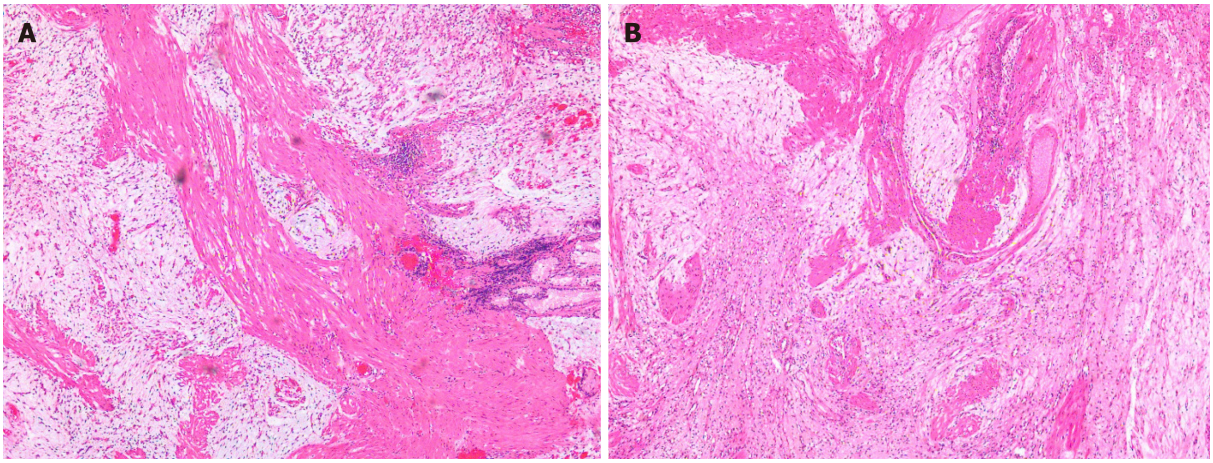


**Figure 5 Abundant slender blood vessels, rich mucus-like mesenchyme and spindle-shaped, fat spindle-shaped fibroblasts, and myofibroblast-like cells showing irregular nodular hyperplasia.** A: Magnification: 20 ×; B: Magnification: 10 ×.

## DISCUSSION

In 2007, Takahashi *et al*[1] first reported a rare gastric mesenchymal tissue tumor and named it PAMT[1,4]. Miettinen *et al*[3] first coined the term PF for this type of lesion in 2009, and subsequently, the term was adopted in WHO 2010 classification of digestive system tumors in the chapter "Mesenchymal Tumours of the Stomach," which was wrote in collaboration with Miettinen[23]. The term PF was used due to its cellular

architecture and fibromyxoid nature, allowing nonprofessionals to recognize that it is a benign disease when encountering this spectrum of lesions. Despite that the WHO classification established the nomenclature, many authors still believe that the term PAMT better describes the histogenesis and histology of such tumors[16,26,27].

Since then, until 2019, only 121 additional cases have been published in the literature[5], and two more cases have been added in 2020. Miettinen *et al*[3] reported that the incidence of gastric PAMT is very low, less than 1/150 of gastrointestinal stromal tumors (GISTs). The range in patient ages is broad, from 5 to 81 years old (average age, 43.17 ± 18.00 years; median age, 46 years). The patients were mostly middle-aged and elderly people, and the peak was usually between 30 and 60 years old[4]. Lesions are frequently located in the gastric antrum but occasionally located in the small intestine, other parts of the stomach, large intestine, mediastinum, and esophagus[24]. According to a recent review report, the maximum diameter of the tumor ranged from 0.8-17 cm, with a mean size of 4.81 ± 3.30 cm and median size of 4.0 cm[4].

Although PAMT is benign, it shows extensive vascular proliferation; therefore, clinical manifestations can be accidental discovery, general gastrointestinal symptoms, and gastrointestinal bleeding. Typical PAMT manifests as general gastrointestinal symptoms, including abdominal pain, abdominal distension, indigestion, loss of appetite, heartburn, diarrhea, and epigastric discomfort. Manifestations of gastrointestinal bleeding are also common and can lead to gastrointestinal bleeding-related manifestations such as anemia, melena, and hematemesis[6-9]. Some patients experience dull upper abdominal pain, along with the presence of a lump in the right hypochondrium[24].

The currently reported cases of PAMT have unique characteristics: (1) The tumor can be polypoid, nodular, or cystic. PAMT forms a leaf-like intramural/submucosal mass, which is light pink, brown, or red in color, usually shiny and mucinous, and very hard. The margins usually have a good outline, such as polypoid, nodular, and cystic lesions, but they are not completely different[1,3,6,13-16,21,25]. The mass is usually visible endoscopically[5,16,21,25] and radiologically[14,21,25]. The tumor originates from the submucosa or muscularis propria and can infiltrate into other tissue layers, protruding from the mucosa or serous membrane. The surface can be accompanied by ulcers and erosions; (2) histologically, tumor tissue is mainly composed of bland ovoid to spindle-shaped fibroblast-like cells, abundant thin-walled blood vessels, and fibrous mucous matrix. Tumor cells are arranged in an irregular plexiform or multinodular pattern, and are separated by the abundant mucus-like or mucous-fibrous matrix around the small blood vessels. These features are helpful to differentiate PF from other gastrointestinal mesenchymal tumors, such as myxoid GISTs, but further differentiation and confirmation of the diagnosis can be identified by immunostaining[3]. The myxoid matrix is always positive for Alcian blue. The tumor cells show monomorphous oval nuclei containing fuzzy nucleoli and fine chromatin, surrounded by mild eosinophilic cytoplasm with indistinct boundary. Nucleolus and chromatin are delicate and fuzzy[4]. Some cases showed vascular and lymphatic infiltration[3,28], but metastasis or recurrence did not occur after surgery; and (3) in immunohistochemistry and special staining of PAMT, vimentin, SMA, muscle-specific actin, and Alcian blue are generally positive[10], indicating the fibroblastic, myofibroblastic, and smooth-muscle-cell characteristics of PAMT. Current research reports show that vimentin, SMA, and MSA have a high sensitivity and specificity for the diagnosis of PAMT, mainly because they are important markers of smooth muscle tumors and mesenchymal tissues. The immunochemical staining indicators in some specimens such as H-caldesmon, calponin, and desmin were partially positive[1,3,11-17], suggesting possible myofibroblastic differentiation, while these stains have been negative in other specimens[1,3,18-21]. For the muscular lineage toward terminal muscle cell differentiation, desmin and caldesmon are more specific markers. These markers show limited and focal reactive results in PAMT, which is consistent with the myofibroblastic spectrum of PF cell development[4], but they are also expressed in desmoid-type fibromatosis. Equivocal results were found for estrogen receptor (ER) and progesterone receptor (PR) staining. Some case reports have shown that specimens are focally or diffusely positive for PR but negative for ER. Thus, hormone therapy is also a direction to consider, but it may be of little significance[24]. For c-kit, pancytokeratin, S-100 protein, B-catenin, neurofilament, cytokeratin, DOG-1, CD34, epithelial membrane antigen, and ALK-1, all reported cases have negative results[10]. Ki-67 staining commonly illustrates very low proliferation rates, mostly < 2%. CD34 is not usually expressed in leiomyomas or desmoid-type fibromatoses; however, it is normally expressed in endothelial cells, embryonic cells of the hematopoietic system, and solitary fibrous tumors. S100 protein can be expressed

in spindle cell neoplasms of the gastrointestinal (GI) tract, a characteristic of schwannomas. Combination of these markers can help make a differential diagnosis of spindle cell tumors in the GI tract[25]. B-catenin and ALK-1 are always negative in PAMT. CD117 or DOG1 is usually negative in PAMT but positive in GISTs, which do not show a unique plexiform wall growth pattern; thus, it can be separated from PAMT.

The diagnosis of PAMT depends on pathological and immunochemical examinations, and typical microscopic manifestations under high magnification should be observed, including bland ovoid to spindle-shaped fibroblast-like cells, abundant thin-walled blood vessels, and fibrous mucous matrix. Clinicians generally speculate that PAMT is benign based on the limited atypicality of tumor cells and low mitotic rate [22]. Vimentin, SMA, and muscle-specific actin were at least partially positive; Alcian blue was positive; and pancytokeratin, c-kit, DOG-1, CD34, S-100 protein, B-catenin, and ALK-1 were negative[4]. In our case, the tumor was positive for SMA, partly positive for CD34, and negative for DOG-1, S-100, PCK, and Ki67 (labelling index: 2%), which immunohistochemically confirmed PAMT.

For genes, mutations in *c-kit* and *PDGFRA* genes are important and characteristic in GISTs[29], but no studies have reported mutations in these two genes in PAMT. Therefore, PAMT can be further distinguished from GISTs in terms of gene mutations. Some cases have been described as having a repeated translocation, t(11;12)(q11;q13), involving the long noncoding gene metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) and the gene glioma-associated oncogene homologue 1 (*GLI1*). Through repeated *MALAT1-GLI1* translocation or *GLI1* upregulation, *GLI1* overexpression depicts a different pathogenic subgroup of plexiform fibromyxomas with activation of the Sonic Hedgehog signaling pathway. Inappropriate reactivation of the Hedgehog signaling pathway is responsible for the formation and progression of several cancers[30]. In addition to the canonical pathway of Hedgehog signaling, some canonical pathways that may be important for the development of gastrointestinal tumors has been recently described, including a noncanonical, Patched-dependent, and Smoothened-independent pathway[37]. Base on the molecular pathway of PF development, neoplasms with GLI1 oncogenesis may be sensitive to Hedgehog pathway inhibitors, targeting either Patched, Smoothened, GLI1, or other Hedgehog pathway components[31].

For the differential diagnosis of myxoid mesenchymal tumors in the gastrointestinal tract, the main considerations include GIST, leiomyomata, solitary fibrous tumor, leiomyosarcoma (LMS), desmoid tumor, inflammatory fibroid polyp, neurilemmoma, inflammatory myofibroblastic tumor, low-grade fibromyxoid sarcoma, Plexiform nerve fibroma, myxoma and, in women, myxoid low-grade endometrial stromal sarcoma[1,3,11-13,32]. PF is positive for SMA, DES, and vimentin and negative for c-kit, CD34, S-100, DOG1, and CD117. However, GIST may have CD117, CD34, and DOG-1 immunoreactivity and c-kit or PDGFRA mutation. Leiomyoma can be differentiated by the bundle like and braided arrangement of tumor tissue and the eosinophilic cytoplasm of fusiform tumor cells. SMA, MSA, desmin, and caldesmon were positive in leiomyoma, but desmin was negative in PAMT. Myxoid and leiomyoma have positive reaction for SMA, desmin, and caldesmon, and Plexiform neurofibroma have positive reaction for S-100 protein. Solitary fibrous tumors are positive for CD34, and consist of altered hypercellular and hypocellular areas, deposits of dense keloid-type collagen, and hemangiopericytoma-like areas[25].

The main treatment for PAMT is surgery. Current literature reviews up to 2019 reporting on 121 patients demonstrated no local or metastatic reoccurrence among the 84 patients who were followed[4,5]. Some reports also presented cases of PAMT treated *via* endoscopy, but the lesions were smaller than 5 cm[20,33-36]. Our case is the ninth case of endoscopic resection, and it is the largest mass that has been removed endoscopically so far. The lesion reached 5 cm × 4 cm × 2 cm in size and was near the cardia. The diseased tissue was completely removed, and the cardia was preserved. Our patient had no recurrence or metastasis during the 3-mo follow-up after the operation, and the treatment effect was good.

## CONCLUSION

At present, the diagnostic criteria for PAMT are relatively clear, but the pathogenesis and genetic changes require further study. PAMT is benign in nature, and these patients are less likely to experience local or metastatic recurrence. The main treatment is still surgery, and when the lesion is in the stomach, partial gastrectomy and distal

gastrectomy are the most commonly performed surgical treatments for PAMT, followed by local gastrectomy, subtotal gastrectomy, and wedge-shaped gastrectomy. But for comprehensive evaluation of the disease, ESD can be considered a suitable method to avoid excessive treatment.

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