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Contents

Monthly Volume 9 Number 5 May 15, 2017

FIELD OF VISION

194 Molecular classifications of gastric cancers: Novel insights and possible future applications

Garattini SK, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, Negri FV, Berenato R, Ermacora P, Cardellino GG, Giovannoni M, Pella N, Scartozzi M, Antonuzzo L, Silvestris N, Fasola G, Aprile G

ORIGINAL ARTICLE

Basic Study

209 Extramural vascular invasion and response to neoadjuvant chemoradiotherapy in rectal cancer: Influence of the CpG island methylator phenotype

Williamson JS, Jones HG, Williams N, Griffiths AP, Jenkins G, Beynon J, Harris DA

Observational Study

218 Critical evaluation of contemporary management in a new Pelvic Exenteration Unit: The first 25 consecutive cases

Chew MH, Yeh YT, Toh EL, Sumarli SA, Chew GK, Lee LS, Tan MH, Hennedige TP, Ng SY, Lee SK, Chong TT, Abdullah HR, Goh TLH, Rasheed MZ, Tan KC, Tang CL

Prospective Study

228 Introduction of laparoscopic gastrectomy for gastric cancer in a Western tertiary referral centre: A prospective cost analysis during the learning curve

Tegels JJ, Silvius CE, Spauwen FE, Hulsewé KW, Hoofwijk AG, Stoot JH

Contents

World Journal of Gastrointestinal Oncology
Volume 9 Number 5 May 15, 2017

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

WJGO covers topics concerning carcinogenesis, tumorigenesis, metastasis, diagnosis, prevention, prognosis, clinical manifestations, nutritional support, molecular mechanisms, and therapy of benign and malignant tumors of the digestive tract. The current columns of *WJGO* include editorial, frontier, diagnostic advances, therapeutics advances, field of vision, mini-reviews, review, topic highlight, medical ethics, original articles, case report, clinical case conference (Clinicopathological conference), and autobiography. Priority publication will be given to articles concerning diagnosis and treatment of gastrointestinal oncology diseases. The following aspects are covered: Clinical diagnosis, laboratory diagnosis, differential diagnosis, imaging tests, pathological diagnosis, molecular biological diagnosis, immunological diagnosis, genetic diagnosis, functional diagnostics, and physical diagnosis; and comprehensive therapy, drug therapy, surgical therapy, interventional treatment, minimally invasive therapy, and robot-assisted therapy.

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FIELD OF VISION

Molecular classifications of gastric cancers: Novel insights and possible future applications

Silvio Ken Garattini, Debora Basile, Monica Cattaneo, Valentina Fanotto, Elena Ongaro, Marta Bonotto, Francesca V Negri, Rosa Berenato, Paola Ermacora, Giovanni Gerardo Cardellino, Mariella Giovannoni, Nicoletta Pella, Mario Scartozzi, Lorenzo Antonuzzo, Nicola Silvestris, Gianpiero Fasola, Giuseppe Aprile

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Abstract

Despite some notable advances in the systemic management of gastric cancer (GC), the prognosis of patients with advanced disease remains overall poor and their chance of cure is anecdotic. In a molecularly selected population, a median overall survival of 13.8 mo has been reached with the use of human epidermal growth factor 2 (HER2) inhibitors in combination with chemotherapy, which has soon after become the standard of care for patients with HER2-overexpressing GC. Moreover, oncologists have recognized the clinical utility of conceiving cancers as a collection of different molecularly-driven entities rather than a single disease. Several molecular drivers have been identified as having crucial roles in other tumors and new molecular classifications have been recently proposed for gastric cancer as well. Not only these classifications allow the identification of different tumor subtypes with unique features, but also they serve as springboard for the development of different therapeutic strategies. Hopefully, the application of standard systemic chemotherapy, specific

targeted agents, immunotherapy or even surgery in specific cancer subgroups will help maximizing treatment outcomes and will avoid treating patients with minimal chance to respond, therefore diluting the average benefit. In this review, we aim at elucidating the aspects of GC molecular subtypes, and the possible future applications of such molecular analyses.

Key words: Molecular biology; Immunotherapy; Gastric cancer; Classification; Targeted therapy

Core tip: TCGA individuates four molecular subtypes: Chromosomal instability, microsatellite instability, genomically stable and Epstein-Barr virus positive tumors. Asian Cancer Research Group classification partially overlaps with the previous one. Although not prospectively validated, these novel classifications suggest that different subtypes of gastric cancer might be treated with specific therapeutic strategies in the near future.

Garattini SK, Basile D, Cattaneo M, Fanotto V, Ongaro E, Bonotto M, Negri FV, Berenato R, Ermacora P, Cardellino GG, Giovannoni M, Pella N, Scartozzi M, Antonuzzo L, Silvestris N, Fasola G, Aprile G. Molecular classifications of gastric cancers: Novel insights and possible future applications. *World J Gastrointest Oncol* 2017; 9(5): 194-208 Available from: URL: http://www.wjgnet.com/1948-5204/full/v9/i5/194.htm DOI: http://dx.doi.org/10.4251/wjgo.v9.i5.194

INTRODUCTION

Gastric cancer (GC) is among the most common malignancies worldwide and the second leading cause of cancer related deaths[1]. In fact, it represents the fifth most commonly diagnosed cancer (6.8% of oncologic diagnoses) resulting in an annual estimated incidence of 18 cases out of 100000 individuals among men and 9 out of 100000 for women[2].

The mainstay of first-line therapy for GC is still represented by a chemotherapy backbone composed by platinum compounds and fluoropyrimidines resulting in a median overall survival (OS) of about 11 mo. Still, the disappointing 5-year survival rate is estimated to be about 25%-30% and slightly higher for some Asian experience. Historically, many attempts have been made in order to re-classify gastric cancer with the aim of clustering some new subgroups that could have different prognostic and predictive value: Anatomical classification (Borrmann classification and Siewert and Stein classification), histological classification (WHO classification and Lauren's classification), and extent of disease (early gastric cancer *vs* advanced cancer).

The first effective molecular novelty came from the TOGA trial which demonstrated a significant improvement in OS with the addition of trastuzumab to chemotherapy when compared to chemotherapy alone in patients with HER2 overexpressing GCs (13.8 mo *vs* 11 mo, respectively; $P = 0.046$)[3]. Another clue to the "heterogeneity theory" comes from the observation that Asian patients demonstrate different pattern of disease and outcomes if compared to the Caucasian western population included in the largest trials.

Nowadays, with mounting biological information available, almost every solid cancer type is considered as a "collection" of multiple very molecularly heterogeneous diseases. Very important advances have been made in the molecular classification of breast cancers[4], lung tumors (by the identification of some tyrosine-kinase-inhibitor targetable subtypes), colorectal adenocarcinomas (predictive and prognostic classes sorted by mutations in *RAS* and *BRAF* genes), and malignant melanoma (identification of *BRAF* codon 600 mutation).

Nevertheless, the poor anatomical and molecular selections of GC patients entering clinical trials have potentially limited the effect of many therapeutic agents including chemotherapy, antiangiogenic drugs and the newly tested immune-modulators. In fact, the benefit of those drugs may have been diluted when tested in the overall population. Recently something has changed the way of thinking GC starting from the TCGA group publication appeared in 2014[5].

A more profound understanding of the molecular clustering of stomach cancer could give us the chance to obtain new insights into prognostic and predictive categorization of this cancer and could definitely provide the scientific knowledge for developing modernly conceived clinical trials that could maximize the effect of novel agents in the proper patient population, avoiding the use of costly drugs in non-stratified populations.

Finally, the aim of this review is to give a general picture of the current knowledge of the emerging molecular classification of GC and to explore the new possibilities connected to the latest discoveries made on the extreme heterogeneity of this disease.

THE IMPORTANCE AND LIMITATIONS OF MOLECULAR CLASSIFICATIONS

The first attempt to generate a comprehensive molecular classification for GC was made in 2013 by Singapore Researchers[6]. They identified three main types of gastric cancer, namely proliferative (characterized by high genomic instability and *TP53* mutation), metabolic (more sensitive to 5-FU therapy) and mesenchymal (stem cell-like tumors sensitive to PIK3CA-mTOR pathway inhibitors), based on genome expression. Soon after the TCGA research group published a classification dividing GCs into four main subgroups clustered on the basis of six different molecular biology approaches: Copy number variation (CNV) analysis, exome sequencing analysis, DNA methylation profile, mRNA sequencing, micro-RNA (miRNA) sequencing and reverse phase protein array[5]. The result

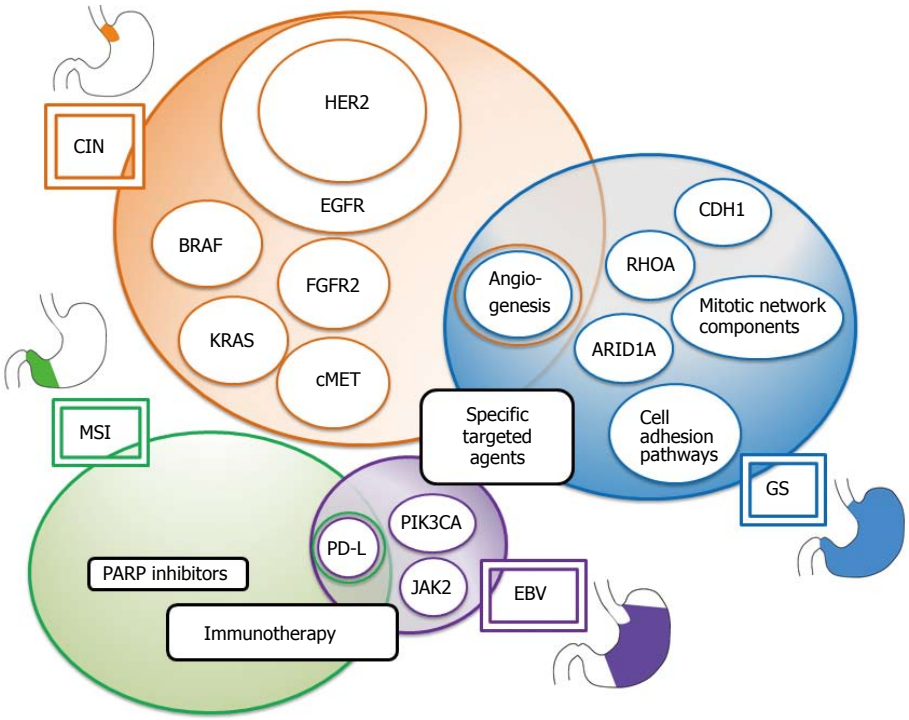

Figure 1 Four molecular subtypes of gastric cancer (chromosomal instability, genomical stability, microsatellite instability, and Epstein-Barr virus) are represented. Particular anatomic distribution and prospective therapeutic strategies. The areas represent the epidemiologic extent of each of the subtypes. On the side of each subtype the anatomical distribution is displayed. CIN: Chromosomal instability; GS: Genomical stability; MSI: Microsatellite instability; EBV: Epstein-Barr virus.

is the subdivision of GC into four genomic subtypes: Epstein-Barr virus (EBV) positive cancers (9% of all gastric tumors with frequent *PIK3CA* mutation and PD-L1/PD-L2 overexpression), Microsatellite Instability tumors (MSI, representing 22% and hypermutated), chromosomal instability (CIN, 50%, predominantly junctional, *TP53* mutated with RTK-RAS activation, with a high rate of CNV) and Genomically Stable (GS, 20%, presenting mutation in motility and adhesion molecules). Specific TCGA molecular subtypes are represented in Figure 1.

In the meantime, the Asian Cancer Research Group (ACRG) too proposed a novel molecular classification[7], and the resulting taxonomy divided GCs into: Mesenchymal subgroup (MSS/EMT, characterized by hallmarks of epithelial-to-mesenchymal transition), Microsatellite Instability subgroup (MSI), Microsatellite Stable *TP53* positive (MSS/TP53$^+$, somehow overlapping with EBV type of TCGA classification) and Microsatellite Stable *TP53*-tumors (MSS/TP53$^-$, overlapping with CIN by TCGA).

These novel classifications create a new paradigm in the definition of cancer biology and allow the identification of relevant genomic subsets by using different techniques such as genomic screenings, functional studies and molecular or epigenetic characterization. However, some limitations should also be openly recognized. First, these classifications are based on a highly complex methodology and currently they should not be replicated in standard laboratories lacking in the uttermost technologies. Attempts towards simplification are ongoing although results may not fully capture the underpinning complexity of the disease. Second, these classifications lack of a prospective validation on a large scale, including patients with different ethnicity and age. Third, the two proposed classifications have more differences than similarities; in particular, they are different in terms of demographics, baseline molecular mechanisms, driver genes, and association with prognosis. Moreover, there are notable dissimilarities in the distribution of Lauren's diffuse subtype among the different subgroups. Since different molecular subgroups may be identified across a number of independent gene expression profile studies, a collaborative international effort is warranted to aggregate a consensus classification. Fourth, the follow-up of included patients is limited, factor that may decrease their prognostic power, and subgroups were evaluated on resected specimens, with different prevalence of subgroups between localized, locally advanced and advanced settings. Fifth, both classifications insist on epithelial cells, but none of them take into account the active, nonmalignant stromal cells. Actually, not only gene expression profiles deriving from stromal tissues may influence assignment to a specific molecular category, thus creating interpretative troubles[8], but also novel stromal-based distinctive signatures have been proposed and related to the predominant cancer phenotype[9].

GC WITH CHROMOSOMAL INSTABILITY

CIN subtype represents approximately 50% of GCs[10] and it mostly occurs in the esophagogastric junction (EGJ)/cardia. CIN GC is related to intestinal type histology,

to copy number gains of chromosomes 8q, 17q and 20q, while, gains at 12q and 13q are associated with diffuse GC[11]. Interestingly, CIN showed elevated frequency in the EGJ/cardia, as demonstrated in TCGA characterization (65%, $P = 0.012$). CIN is characterized by somatic mutations at cytogenetic level, particularly involving loci that control mitotic checkpoints, thus gatekeeper and caretaker genes implicated in carcinogenesis. CIN comprises both altered DNA copy number and structural abnormalities in some chromosomal regions. Those alterations could result in gain or loss of whole chromosomes[12] (aneuploidy), non-reciprocal translocations, amplifications, deletion or the loss of one allele with loss of heterozygosis. Altogether, CIN results in the loss or gain of function of some "key genes", including oncogenes and tumor suppressor genes that may be efficaciously targeted by specific inhibitor molecules[13]. Notably, CIN GC is enriched in mutations in *TP53* gene and receptor tyrosine kinases (RTKs), furthermore it shows amplifications of cell cycle genes (Cyclin E1, Cyclin D1, and Cyclin-dependent kinase 6)[14].

Evaluation of the biological characteristics among CIN cancers demonstrated that *TP53* mutations occurs in 71% of GCs[5]. Furthermore, CIN also display amplification in oncogene pathways such as RTK/RAS/MAPK signaling, including HER2, BRAF, epidermal growth factor (EGFR), MET, FGFR2, RAS[5,15].

A recent work reviewed the pathogenic and molecular similarities between gastric intestinal-type adenocarcinoma and esophageal adenocarcinoma (EAC)[16], suggesting that treatment of EAC should recall that of gastric adenocarcinoma rather than being similar to the approach used for upper esophageal cancers (mostly squamous). In fact, not only EAC may arise from progenitor cells deriving from the cardia of the stomach but also the majority of EAC express a chromosomal instability that closely resembles the one found in CIN GC. All these findings suggest both the need for better subtyping esophageal cancers and the opportunity of developing specific therapeutics strategies in this disease as well.

HER2

The proto-oncogene HER2 is a member of the EGF receptor family with tyrosine kinase activity. It is known that HER2 positivity may vary depending on the primary tumour location as well as on the histotype of gastric cancer. Indeed, *HER2* overexpression/amplification is detected in more than 30% of the tumours arising from the gastroesophageal junction whereas less than 20% of tumours in the gastric body are HER2-positive. In addition, intestinal and diffuse histotype display a rate of HER2 positivity of 34% and 6% respectively[17]. HER2 plays a key role in a large number of cellular processes, including cell differentiation, proliferation, motility and signal transduction. After the combination of chemotherapy and HER2 targeted therapy with trastuzumab had defined a new standard of care for HER2-positive metastatic GC[3,18,19], other HER2 inhibitors were tested.

Lapatinib, a multi-kinase inhibitor, was evaluated in two randomized phase Ⅲ trials enrolling GC patients with advanced disease. The LOGiC trial tested the efficacy of lapatinib in combination with capecitabine plus oxaliplatin given upfront. The addition of lapatinib did not significantly increase OS [12.2 mo *vs* 10.5 mo, hazard ratio (HR) 0.91, $P = 0.349$], although progression-free survival (PFS) was longer (6.0 mo *vs* 5.4 mo, HR 0.82, $P = 0.0381$) and objective response rate (ORR) was higher (53% *vs* 39%, $P = 0.0031$) in the lapatinib arm[19]. The TyTAN trial randomized 261 Asian patients to receive lapatinib plus paclitaxel or paclitaxel alone in second-line treatment. Disappointingly, no marked survival differences between treatment groups were noted: Median OS (11.0 mo *vs* 8.9 mo, $P = 0.1044$) and PFS (5.4 mo *vs* 4.4 mo, $P = 0.2441$). Overall, 15 patients (6%) had previously received trastuzumab, 8 in the lapatinib/paclitaxel arm and 7 in the paclitaxel alone arm[20].

JACOB, a large randomized phase Ⅲ trial designed to test the efficacy of pertuzumab in combination with trastuzumab and standard chemotherapy (cisplatin plus fluoropyrimidine) has recently completed the accrual[21]. Results of the trial are eagerly awaited. Novel anti-HER2 drugs have been developed to try to overcome secondary trastuzumab resistance, as in the case of trastuzumab-emtansine (T-DM1). Data from phase Ⅲ GATSBY trial were recently presented concluding that TDM-1 did not improve patients' outcome compared to second-line taxanes at the 2015 clinical cut-off[22].

The majority of gastric cancer patients who achieve an initial response to trastuzumab-based regimens develop resistance within 7 mo[23]. These unsatisfactory results may be attributed to primary (*de novo*) or secondary (acquired) resistance to the HER2-targeted therapy. Therefore, as it happened for breast cancer, the onset of trastuzumab resistance has been investigated also in gastric cancer, showing several molecular mechanisms underlying the acquired resistance to HER2 inhibitors[24]. Lee *et al*[25] identified that *HER2*-amplified GC patients have diverse pattern of various concurrent molecular events. Zuo *et al*[26] employed the human gastric carcinoma cell line NCI-N87 with high HER2 expression to create trastuzumab-resistant NCI-N87/TR cells by stepwise exposure to increasing doses of trastuzumab. They showed that activation of the PI3K-AKT signalling pathway downstream of HER2 was one of the major mechanisms leading to resistance of NCI-N87/TR gastric cancer cells to trastuzumab, which was probably associated with *PTEN* gene down-regulation and mutation, as well as with over-activity of the IGF-1R signalling pathway[26]. The study conducted by Piro *et al*[27] identified the FGFR3/AKT axis as an escape pathway responsible for trastuzumab resistance in gastric cancer, indicating that the inhibition of FGFR3 could be a potential strategy to modulate this resistance. Recently, Arienti *et al*[28] explored the role of the IQ-domain GTPase-activating protein 1 (IQGAP1), a multifunctional scaffold protein, which interacts with diverse proteins to regulate cell adhesion and cell migration. IQGAP1 governs HER-2 expression, phosphorylation and signalling in breast cancer cell lines[29], it is overexpressed in aggressive form

of gastric cancer[30] and its overexpression is correlated with trastuzumab-induced resistance in breast cancer cell lines[31]. The study of Arienti *et al*[28] revealed that high IQGAP1 expression leads to resistance to trastuzumab in gastric cancer; in addition, they found two new mutations of the *HER2* gene that may be correlated with acquired resistance to the drug. Moreover, a functional cross-talk between the receptor tyrosine kinase MET and HER family members has been reported in the context of the acquisition of aggressive phenotypes[32]. The hepatocyte growth factor (HGF) mediated activation of MET may also causeresistance to lapatinib in HER2-amplified GC cell lines by stimulating downstream signalling[33]. De Silva *et al*[34] confirmed *in vitro* that MET is likely to be a significant mechanism of lapatinib resistance *in vivo*. Finally, we recently showed that HER2 loss may be associated with acquired resistance to first-line trastuzumab-based treatment in patients with initially HER2-positive GC[35]. All these evidences enhance the complex cross-talk between HER2 and its downstream pathway and stress the importance of further elucidating the strategies to overcome resistance to HER2-targeted therapy. Indeed, identifying the mechanisms underlying treatment resistance would increase the benefit from HER2-targeted therapy in patients with HER2-positive gastric cancer. Certainly, development of inhibitors targeting multiple receptors or common downstream signalling proteins deserves further investigation.

EGFR

The EGFR (or ERBB1) belongs to RTKs and it is the second most frequent RTK playing a key role in GC initiation and progression. Despite the wide use of anti-EGFR monoclonal antibodies in colorectal cancer, demonstration of efficacy in GC has not yet been provided. EGFR overexpression has been reported in 24%-27% of all gastric adenocarcinomas[36]. Several studies have evaluated the efficacy and safety of different anti-EGFR therapy, based on preclinical data[37]. The phase Ⅲ EXPAND trial evaluated the addition of cetuximab to first-line capecitabine and cisplatin in a non-selected cohort of GC patients. This trial showed no significant advantage in median PFS (4.4 mo *vs* 5.6 mo in favor of control arm, P = 0.32)[38]. The REAL-3 phase Ⅲ trial evaluated the addition of panitumumab to epirubicin, oxaliplatin, and capecitabine (EOC). It demonstrated that the addition of panitumumab is detrimental as to OS (11.3 mo for EOC and 8.8 mo for EOC plus panitumumab, HR 1.37, 95%CI:1.07-1.76, P = 0.013)[39]. These disappointing results have been confirmed with another anti-EGFR drug, nimotuzumab[40]. The failure of anti-EGFR monoclonal antibodies in advanced GC may lie in the lack of a proper selection, as happened to the patients treated in the aforementioned trials. A recent publication from Birkman *et al*[41] studied the prevalence of *EGFR* overexpression/genomic amplification in gastric intestinal-type adenocarcinoma. In this work, 220 paraffin-embedded samples of GC were collected with the aim of elucidating the prevalence of *EGFR* overexpression/amplification, the *HER2* overexpression/amplification and the combination of the previous two. Interestingly, EGFR overexpression was more frequent in intestinal-type GCs (32.7% of the specimens) and its genomic amplification was demonstrated in 14.1% of the patients. It has also been shown that *EGFR* amplification was associated to a deeper tumor invasion (pT3-4 *vs* pT1-2, OR 2.15, P = 0.029). This unfavourable clinical feature correlated also to a shortened time to cancer recurrence (P = 0.026) and cancer specific survival (P = 0.033). Furthermore, *HER2* overexpression/amplification has been shown to be less frequent when compared to *EGFR* overexpression/amplification and *EGFR/HER2* co-amplification (3.6% of the cases), indicating that these two different populations may bear specific genomic alterations potentially approachable with different treatments. All these data strongly suggest that modern trials should be designed with a careful stratification according to *EGFR* amplification to properly assess the clinical effectiveness of anti-EGFR drugs in GC patients.

RAS and BRAF

KRAS mutation occurs in less than 5% of GC and may have a negative prognostic value in GC patients. KRAS activates critical pathways involved in carcinogenesis and tumor progression, such as PI3K-Akt, RAF, MEK-extracellular signal regulated kinase and NF-kB. However, no target therapies are currently approved for this molecular aberration[42]. Other drugs, such as MEK inhibitors were tested in *KRAS* mutated cancer cell lines with promising results. Since preclinical study suggested that the combination of MEK-inhibitors and PI3K or BCL-XL inhibitors may be efficacious in *KRAS* mutant lung cancer patients[43], it would be intriguing to evaluate MEK inhibitors in monotherapy or in combination with PI3K inhibitors or BCL-XL in GC patients who carry this mutation. In GC patients**,** *BRAF* mutations are rare (2.2% in TCGA database) and are mostly represented by *BRAF* V599M[42]. The role of this mutation in GC is yet to be assessed.

FGFR2

FGFR2 amplification is associated with tumor cell proliferation and survival of GC cell lines and indicates poor prognosis. In the TCGA classification, approximately 9% of CIN GC patients had *FGFR2* gene amplification. Several drugs and studies targeting this mutation are ongoing[5]. A phase Ⅱ randomized trial is evaluating the activity of AZD4547 (a FGFR 1-2 and 3 inhibitor) compared to paclitaxel in second-line treatment. Other ongoing trials are testing dovitinib in *FGFR2* amplified GC patients or in combination with docetaxel[18].

C-MET

Mesenchymal epithelial transition factor (MET) alteration was rarely observed in GC (8%)[44]. MET is an RTK that interacts with its native ligand HGF. Deregulated expression of C-MET in GC has been related to worse

prognosis. In fact, the HGF/c-MET signal is involved in cancer growth, invasion, angiogenesis, anti-apoptosis and epithelial to mesenchymal transition[45]. Two monoclonal antibodies, rilotumumab (an anti-HGF antibody) and onartuzumab (an anti c-MET antibody) were tested. In a phase Ⅰb/Ⅱ study, rilotumumab was effective and it improved PFS[46]. Based on these data, the phase Ⅲ RILOMET-1 trial, conducted on selected *c-MET* amplified patients, evaluated OS and ORR in the experimental arm with rilotumumab plus ECX compared to control arm with placebo plus ECX. The trial results were negative, and demonstrated that rilotumumab does not improve survival[47]. A similar phase Ⅲ study called RILOMET-2 is ongoing for Asian patients in the same setting[48].

Onartuzumab, a monoclonal antibody directed to c-MET, was tested in MET-Gastric study, in which patients were randomized to receive FOLFOX alone or in combination with onartuzumab. Once again, results were negative (OS: 11.0 mo in the experimental arm *vs* 11.3 mo in the control arm, HR = 0.82, *P* = 0.24)[49]. Recently results on a specific MET kinase inhibitor have been presented at ASCO 2016[50]. For the first time AMG337 was tested, in a phase Ⅰ study, in humans with solid tumors: 51 patients were treated and among them 10 had *MET*-amplified gastrointestinal cancers: 4 partial responses and 1 complete response were observed. At the end of the study a maximum tolerated dose of 300 mg was reached. Although an expansion phase on *MET*-amplified patients was on the way, it was early interrupted for excess of toxicity. Despite these negative results, the interest on c-MET as a potential molecular target for novel therapies has not vanished, since better molecular selection of the patients and optimal combination/drugs may finally achieve the expected results.

VEGF and VEGFR-2

Another frequently amplified gene in CIN subtype is *VEGF*, a mediator of angiogenesis that is essential for cancer growth and metastasis as it ensures oxygen and nutrients supply to proliferating cancer cells[51]. Bevacizumab, a monoclonal antibody that targets VEGF, was tested in the AVAGAST trial. This study did not meet its primary endpoint of improved OS (median OS 12.1 mo *vs* 10.1 mo, HR 0.87 95%CI: 0.73-1.03, *P* = 0.1), but improvements in median PFS and tumor response rate were reported[52]. Similarly, the AVATAR trial showed no survival benefit with antiangiogenic therapy added to cisplatin and capecitabine-based regimens (HR 1.1)[53]. Although the addition of bevacizumab to standard therapy showed disappointing results, antiangiogenic strategy was further investigated beyond first line treatment. Ramucirumab, a fully human monoclonal IgG directed against VEGFR-2, was evaluated both as single agent and in combination with chemotherapy[54-56]. In the REGARD trial, ramucirumab demonstrated a statistically significant improvement when compared to the best supportive care in pretreated GC patients with advanced disease (OS: 5.2 mo *vs* 3.8 mo respectively, HR = 0.776; *P* = 0.047)[54]. In the RAINBOW trial, patients were randomized to receive paclitaxel with or without ramucirumab. Median OS was 9.63 mo for the combination therapy and 7.36 mo for paclitaxel alone (HR = 0.807, 95%CI: 0.678-0.962; *P* = 0.017)[55]. Recently, a novel VEGFR-2 tyrosine kinase inhibitor, apatinib, was evaluated in Asian patients who had previously received 2 or 3 lines of chemotherapy[57]. Patients exposed to apatinib had an improved median OS (6.5 mo *vs* 4.7 mo; HR = 0.709; 95%CI: 0.537-0.937; *P* = 0.156) and median PFS (2.6 mo *vs* 1.8 mo; HR = 0.444; 95%CI: 0.331-0.595; *P* < 0.001) compared to patients who received placebo. Therefore, multitarget TKIs represent another potential approach to block angiogenesis by simultaneously targeting VEGFR and other signaling pathways. Notably, the role of antiangiogenic strategy seems to gain importance in subsequent lines of treatment, but its role in first-line therapy is still unclear. An ongoing randomized phase Ⅲ trial is assessing the potential survival benefit of ramucirumab in combination with cisplatin and capecitabine given upfront[56].

GC WITH MICROSATELLITE INSTABILITY

According to the TCGA's molecular classification, the enrichment for microsatellite instability (MSI) characterizes a distinct molecular subgroup of GC. MSI occurs in about 15%-30% of GCs, and more frequently correlates with intestinal histotype, location in the distal part of the stomach, female gender and older age at diagnosis[5,58,59].

MSI is a genetic alteration consisting of the expansion or contraction of regions of repetitive nucleotide sequences, called microsatellites. The alteration is triggered by a dysfunction of DNA mismatch repair (MMR) enzymes, caused by mutations in one of several different DNA mismatch repair genes (*i.e., MLH1* or *MSH2*). In a single cell, bi-allelic inactivation of *MMR* genes causes an increased mutation rate (genomic instability) due to the failure of DNA mismatch repair that usually occurs during normal DNA synthesis[60].

Defective DNA mismatch repair is the hallmark of Lynch syndrome. Moreover, approximately 15% of sporadic colorectal cancers also displays MSI since both alleles of a *MMR* gene are inactivated[61]. Different *MMR* genes are probably involved in MSI-high (MSI-H) sporadic gastric cancer without *MLH1* hypermethylation, which represents the main mechanism leading to MMR deficiency in MSI GC[62,63].

MSI-H colorectal cancer have better prognosis compared to MSI low, and should not receive adjuvant chemotherapy with fluoropyrimidine after resection for stage Ⅱ disease[64]. In gastric cancer, 5-FU is frequently used and information about sensitivity to this agent may be very useful. A meta-analysis of Zhu *et al*[65] showed a 37% mortality risk reduction and improved median OS in patients with MSI-H compared to MSI-L(low) or microsatellite stable (MSS) GC patients. The relationship between MMRd, MSI and survival has been examined in patients with resectable GC randomized to surgery alone or perioperative chemotherapy within the MRC MAGIC

trial. MSI and *MLH1* deficiency was associated with a better outcome in patients treated with surgery alone while it had a negative prognostic effect in those treated with chemotherapy[62].

Despite MSI cases generally lack of targetable amplifications, mutation in *PIK3CA*, *ERBB3, ERB22* and *EGFR* are noted[5,59]; *BRAF* V600E mutations, commonly seen in MSI colorectal cancer, are absent in MSI GC[5]. However, the predictive role of these mutations in MSI GC population is uncertain. The combination of olaparib with paclitaxel as second-line therapy was found to be more active compared with paclitaxel alone in patients with metastatic or recurrent GC. Although the trial did not meet its primary endpoint (namely PFS), olaparib prolonged survival in patients with low levels of ataxia telangiectasia mutated, a key activator of DNA damage response[66]. A phase Ⅲ trial in this setting is under way and detailed analysis in MSI GC could be attractive.

The hypothesis of an increased activity of immunotherapy in MSI non-colorectal cancer has recently generated interest. In fact, the increased number of somatic mutations may amplify the number of neoantigens, thus stimulating the immune system and conferring higher sensitivity to PD-1 blockade to tumor[67,68]. Interestingly, the tendency to have a lymphocytic infiltrate, observed in MSI tumors, likely reflects immune activation of T-cells directed against tumor-specific carboxy-terminal frameshift peptides that are associated with MSI[69]. In addition to that, genomic aberrations in tumor cells lead to aberrant PD-L1 expression, suggesting a predictive role for MSI.

MSI has already been reported as a strong predictive factor for the use of immune check-point inhibitors in the treatment of patients with colorectal cancer[70]. The immune-related objective response rate and immune-related 6-mo PFS rate were 40% and 78%, respectively, for patients with dMMR and 0% and 11% for those with MMR-proficient cancer, with a higher median PFS and survival in the cohort with dMMR colorectal cancers *vs* 2.2 and 5.0 mo, respectively, in the cohort with MMR-proficient tumors. Le *et al*[68] enrolled 41 consecutive patients (9 patients with MMR deficient solid tumors other than colorectal cancer, only 1 patient with GC) to explore the activity of PD-1 blockade according to MMR status in non colorectal cancer too. Although data are not ready for clinical application, 30% of GC have been shown to present with a burden of nonsynonymous mutations that may define who are the optimal candidates for immune checkpoint inhibitors treatment[71]. Of note, a phase 2 study of pembrolizumab in subjects with advanced gastric or gastroesophageal junction adenocarcinoma who progressed after first-line therapy with platinum and fluoropyrimidine is currently recruiting participants[72]. Muro *et al*[73] have recently reported the activity of pembrolizumab in GC in a phase Ⅰ trial. The authors showed a decrease in tumor burden in 41% of the study patients. The ORR was 32% in Asian patients and 30% in non-Asian patients[73]. A phase 2 trial of nivolumab or nivolumab plus ipilimumab is recruiting patients to evaluate the response to checkpoint inhibitors in MSI-H gastrointestinal cancers[74]. Interestingly, a preventive vaccine, set-up using neopeptides frequently affecting MSI tumorigenesis, has been shown to delay the onset of dMMR tumors. It remains to be proven if vaccination against these neopeptides might be a promising approach for novel adjuvant treatment strategies in patients with MSI-H tumors[75].

GC WITH GENOMIC STABILITY

GS GCs account for around 20% of all the tumors analyzed by the TCGA project. This subtype occurs with equal frequency in males and females. GS gastric tumors are enriched for the diffuse histological variant [58% according to Lauren's classification) and for the poor cohesive variant (58% according to World Health Organization (WHO) classification]. One quarter of GS GCs arise in the antrum, about 20% in the gastroesophageal junction/cardia, and approximately 15% in the gastric body/fundus. The principal somatic genomic alterations observed in GS gastric tumors involve *CDH1*, *ARID1A* and *RHOA*. In addition, a recurrent interchromosomal translocation (between *CLDN18* and *ARHGAP26*) implicated in cell motility was found in GS gastric tumors[5].

CDH1

The *CDH1* gene is located on chromosome 16q22.1 and encodes E-cadherin, which belongs to the cadherin superfamily of calcium-dependent cell adhesion molecules. E-cadherin plays a well-documented role in the progression of epithelial cancers. Inactivating mutations in the *CDH1* gene are frequently found in gastric cancer, especially in hereditary diffuse gastric cancer[76]. *CDH1* promoter methylation is also frequently found in sporadic gastric cancer[77]. During epithelial tumorigenesis, the protein is downregulated and E-cadherin has been categorized as a tumor suppressor gene[78]. Li *et al*[79] reported that in diffuse-type GC, *CDH1* mutation is associated with shortened patients survival, independently from disease stage. In the analysis of the TGCA Research Network *CDH1* somatic mutations were enriched in the GS subtype (37% of cases). Therefore, the prognostic value of *CDH1* as well as its potential as therapeutic target in gastric cancer has yet to be fully understood and explored.

ARID1A

Inactivating mutations of *ARID1A* were found in GS gastric cancer, as in the EBV-subtype[5]. The *ARID1A* gene, located in chromosome 1p35.3, encodes adenine-thymine-rich interactive domain-containing protein 1A, which participates in chromatin remodeling, therefore is involved in regulating cellular processes including DNA repair, differentiation, and development[80]. As shown by Wang *et al*[81], loss of *ARID1A* expression was significantly correlated with tumor stage and grade; moreover, it was also significantly correlated with poor survival in GC patients. Restoring *ARID1A* expression in gastric cancer cells significantly inhibited cell proliferation and colony formation, whereas silencing *ARID1A* expression

in gastric epithelial cell lines significantly enhanced cell growth rate[81].

RHOA

Rho belongs to the Ras-related family of small molecular weight GTP-binding proteins, and it works as a molecular switch between the GDP-bound inactive form and the GTP-bound active form[82]. It regulates cytoskeletal organization, cell adhesion, intracellular membrane trafficking, gene transcription, apoptosis, and cell cycle progression[83]; moreover, it activates STAT3 to promote tumorigenesis[84]. RhoA plays a role in these processes through a variety of effectors including ROCK1, mDia and protein kinase N[85]. mDia is involved in nucleation and polymerization of actin filaments, while ROCK intervenes in induction of actinomyosin bundles and contractility. The balance between mDia and ROCK regulates cell morphogenesis, adhesion, and motility activities. In addition, the Rho-ROCK pathway is involved in Ras-mediated transformation, the amoeboid movement of tumor cells in the three-dimensional matrix, and transmigration of tumor cells through the mesothelial monolayer[86]. According to the TCGA, RHOA mutations were clustered in two adjacent amino-terminal regions that are predicted to be at the interface of RHOA with ROCK1 and other effectors, leading to a modulation of signaling downstream of RHOA[5]. Interestingly, diffuse-type GCs, characterized by malignant phenotype and stromal differentiation, frequently have gain-of-function mutations of RHOA[87].

The TCGA network discovered a recurrent interchromosomal translocation between claudin 18 (*CLDN18*) and Rho GTPase-activating protein 6 (*ARHGAP26*), resulting in the *CLDN18-ARHGAP26* fusion gene, which primarily occurs in GS GC[5]. ARHGAP26 (also known as GTPase Regulator Associated with Focal Adhesion Kinase, GRAF) is a GTPase-activating protein that facilitates conversion of RHO GTPases to the GDP state and has been implicated in enhancing cellular motility[88]. CLDN18 is a component of the tight junction adhesion structures[89]. Yao *et al*[90] showed that expression of *CLDN18-ARHGAP26* fusion gene in gastric epithelial cells resulted in epithelial–mesenchymal transition, which is indicative of cell transformation in cancer development. A recent trial tested IMAB362, a chimeric IgG1 antibody against CLDN18.2 showing clinical activity in patients with 2 + /3 + immunostaining[91].

The *CLDN18–ARHGAP* fusions were mutually exclusive with *RHOA* mutations; within the GS subtype, 30% of cases had either *RHOA* or *CLDN18–ARHGAP* alterations[5].

Given the role of RHOA in cell motility, modulation of RHOA may contribute to the disparate growth patterns and lack of cellular cohesion that are hallmarks of diffuse tumors.

Rho/Rho-kinase inhibitors have been explored as putative therapeutic targets in various diseases, including cancers[92]. The development of drugs that inhibit Rho GTPase signaling would be of great potential in this setting.

Other notable patterns

The GS subtype exhibited elevated expression of cell adhesion pathways, including the B1/B3 integrins, syndecan-1-mediated signaling, and angiogenesis-related pathways. Also in the GS subtype, hierarchical clustering of samples and pathways revealed several notable patterns, including elevated expression of mitotic network components such as AURKA/B and E2F, targets of MYC activation, FOXM1 and PLK1 signaling and DNA damage response pathways[5]. Specific inhibitors of AURKA are currently under investigation in phase Ⅰ/Ⅱ clinical trials in advanced GC[93]. PLKs, mitotic kinases of the polo family, play a pivotal role in the normal cell cycle, and their overexpression is involved in the pathogenesis of multiple human cancers[94]. PLK1 is overexpressed in approximately 80% of human tumors, including gastric cancer, and it is associated with poor prognosis[94]. Currently, inhibitors of PLK1 are being developed[95]. In a phase Ⅰ trials enrolling patients with advanced solid cancers, including gastric cancer, volasertib, a potent and selective PLK inhibitor that induces mitotic arrest and apoptosis, demonstrated anti-cancer activity with a manageable safety profile[96].

EBV ASSOCIATED GC

Latent EBV infection is associated with about 10% of GCs, as demonstrated by *in situ* hybridization EBV encoded miRNA detection, by whole genome sequencing or by PCR EBV genome detection[5].

EBV associated GC has been related to different epidemiological and clinico-pathological features. In a meta-analysis of 39 case-control studies, Bae *et al*[97] investigated the strength of association between EBV infection and GC risk, and showed a 10 fold increase (95%CI: 5.89-17.29). It was also reported that there is a higher risk of EBV associated GC in Far East Asia if compared to Europe[98].

In a meta-analysis of 70 studies the pooled prevalence of EBV-positive GC resulted 8.7% (95%CI: 7.5%-10.0%) with similar distributions across the three analyzed geographic regions (America, Asia and Europe). Moreover, a two-fold difference in male/female ratio favored men as to prevalence of EBV positive GC. The antral location was less frequently associated with EBV infection when compared to other types. In contrast, there was no statistically significant difference in the proportion of EBV-positive disease between intestinal (9.5%; 95%CI: 7.2%-12.5%) and diffuse (7.6%; 95%CI: 5.7%-10.3%) histology[98].

In addition, EBV-positive GC was more prevalent in younger patients compared to older subjects[99].

As to possible therapeutic approaches, Kim *et al*[100] observed that EBV infected GC patients had a higher rate of alteration in pathways related to immune response which may also be related to a more favorable prognosis

in these patients. According to *TCGA*, *PD-L1* gene was frequently amplified in EBV-positive GC, adding proofs to the hypothesis of higher immunogenicity of this class of GC. Based on the evidence that 15% of EBV positive GC harbor amplification of chromosomal region 9p24.1, the locus of PD-L1 and PD-L2, potential role of PD-L1 expression in EBV-positive GC was investigated in a study[101]. In EBV-associated GC, PD-L1 expression was present in 50% (16/32) and 94% (30/32) of tumor and immune cells, respectively. In contrast, EBV-negative GC showed a lower PD-L1 expression (10% and 39% of tumor and immune cells, respectively, $P < 0.001$), thus providing a further rationale for testing PD-1 expression in this GC subtype to potentially identify a predictive response factor for immunomodulatory therapeutic strategies.

Besides PD-L1 and PD-L2 expression, *PIK3CA* mutations, DNA hypermethylation, and *JAK2* mutations are also present[5]. In a large retrospective study, 855 GC specimens were analyzed to verify protein expression levels and prognostic values of *PIK3CA*, JAK2, PD-L1 and PD-L2. Only 59 samples were found to be EBV positive. *PIK3CA* and PD-L2 were more highly expressed in EBV positive GC than in negative ones, but no prognostic value of *PIK3CA*, JAK2, PD-L1 or PD-L2 was found. No differences in JAK2, PD-L1 or PD-L2 expression were seen between EBV positive and negative cases. Moreover, the expression of *PIK3CA*, JAK2, PD-L1 or PD-L2 was not significantly associated with any clinico-pathological feature, maybe due to the small number of EBV-associated GC cases, and the prognostic value of these mutations remains uncertain[102].

THE ACRG CLASSIFICATION

The ACRG proposed a different molecular classification for gastric cancer in 2015[7]. This classification has some overlapping features with the one proposed by TCGA even though some differences can be highlighted. The clustering process included a first subdivision into MSI (22.7%, better prognosis, mainly intestinal type) and EMT tumours (15.3%, worse prognosis mainly diffused type) with two exclusive gene expression profiles, the first characterized by the loss of function of genes involved in the MMR and the second by alterations in cell adhesion, angiogenesis, and motility. Notably, the MSI subtype was associated with a hypermutation in genes such as: *KRAS* (23.3%), PI3K-PTEN-mTOR pathway (42%), *ALK* (16.3%) *ARID1A* (44.2%), *ERBB2* (16.3%) and *ERBB3* (14%). The remaining tumours were further divided into MSS/TP53$^+$ (26.3%, P53 function intact) and MSS/TP53$^-$ (35.7%, loss of oncosuppressor function). In terms of survival, the MSI subtype showed the best overall prognosis, followed by MSS/TP53$^+$, MSS/TP53$^-$ and MSS/EMT. The MSI/TP53$^+$ subtype was more frequently associated with EBV infection if compared to the other groups and showed an active *TP53* pathway and a higher prevalence (compared to MSI/TP53$^-$) of *APC*, *ARID1A*, *KRAS*, *PI3KCA*, and *SMAD4* mutations. Finally, the MSI/TP53$^-$ subtype showed the highest prevalence of *TP53* mutations, relevant copy number variations (CNVs), a greater aneuploidy and recurrent focal amplifications in *MDM2, ROBO2, GATA6, MYC. ERBB2, EGFR, CCNE1* and *CCND1*. These latter two amplifications were mutually exclusive, so they could be considered driver alterations.

A comparison of the ACRG categories with the TCGA subtypes showed similarities in the tumors with MSI, while GS was approximated to MSS/EMT, EBV to MSS/TP53$^+$, and CIN to MSS/TP53$^-$. Nevertheless, in the TCGA cohort the EBV positive cancers represented a separated subgroup (with a favourable phenotype), whereas in the ACRG classification EBV infection occurred more frequently in the MSS/TP53$^+$ subtype, without CNVs, hypermethylation or hypermutation. Moreover, *PI3KCA* and *ARID1A* mutations were more prevalent in EBV$^+$ gastric cancers compared to MSS subtypes.

Although both the MSS/EMT and the GS molecular subgroups included tumors with a prevalent diffuse histology, the TCGA classification showed a lower percentage of Lauren's diffuse subtype compared to the ACRG database (24% *vs* 45% respectively); additionally, *CDH1* and *RHOA* mutations did not appear prevalent in the MSS/EMT subgroup, unlike the GS subtype. Finally, GS tumours were also present in the ACRG MSS/EMT, MSS/Tp53 + and MSS/tp53$^-$ molecular subgroups. All these findings showed that the GS and the MSS/EMT subgroups were not equivalent.

The comparison of the CIN TCGA subtype to ACRG MSS/TP53$^-$ subtype showed that the first is quite homogeneously distributed in the subtypes classified by ACRG.

Overall survival associations were weaker when using the TCGA genomic scheme in the ACRG cohort compared to the original prognosis trends: While the MSI subtype showed a better prognosis in both classifications, there were no differences in prognosis in CIN and GS subtypes when they were identified based on application of the TCGA classification on the ACRG patient population.

CONCLUSION

While the advent of novel molecular classifications has faded the "one size fit-all" era, a more profound understanding of the underpinning tumour biology has set the dawn of a more contemporary clinical approach called precision medicine. At present, the two aforementioned genomic classifications of GC represent the state-of-the-art achieved so far. Somehow it is possible to find an overlap between the TCGA and ACRG subtypes even though some difference can still be found. Emerging data clearly individuate a category of GC characterized by MSI that may benefit from immunotherapeutic approaches. For this subgroup, with good prognosis, the development of anti PD-1/PD-L1 drugs could be the leading research avenue. High mutational burden is also a driving feature of EBV positive GC that could be targeted with immunotherapy as

Table 1 Clinical outcomes of recent trials in gastric and esophagogastric adenocarcinomas

Trial name	Phase of study	Line of treatment	Selected biomarker	Treatment arms	*n*	Primary endpoint	Outcomes
CIN							
TOGA[3]	Ⅲ	First	HER2 expression/ amplification	CF/CX CF/CX + trastuzumab	296 298	OS	OS: 13.8 mo *vs* 11.1 mo (HR = 0.74, *P* = 0.005) PFS: 6.7 mo *vs* 5.5 mo (HR = 0.71, *P* = 0.0002) ORR: 47% *vs* 35% (*P* = 0.001)
LOGiC[19]	Ⅲ	First	HER2 expression/ amplification	CapeOX CapeOx + lapatinib	273 272	OS	OS: 12.2 mo *vs* 10.5 mo (HR = 0.91, *P* = 0.34) PFS: 6.0 mo *vs* 5.4 mo (HR = 0.82, *P* = 0.038) ORR: 53% *vs* 39% (*P* = 0.003)
TyTAN[20]	Ⅲ	Second	HER2 amplification by FISH	Paclitaxel Paclitaxel + lapatinib	129 132	OS	OS: 11.0 mo *vs* 8.9 mo (HR = 0.84, *P* = 0.104) PFS: 5.4 mo *vs* 4.4 mo (HR = 0.85, *P* = 0.244) ORR: 27% *vs* 9% (*P* < 0.001)
JACOB[21]	Ⅲ	First	HER2 expression/ amplification	Pertuzumab + tFP Placebo + tFP		OS	Ongoing
GATSBY[22]	Ⅱ / Ⅲ	Second	HER2 expression/ amplification	TAX T-DM1	117 228	OS	OS: 8.6 mo *vs* 7.9 mo (HR = 1.15, *P* = 0.86) PFS: 2.9 mo *vs* 2.7 mo (HR = 1.13, *P* = 0.31) ORR: 19.6% *vs* 20.6%
EXPAND[38]	Ⅲ	First	Unselected	CX CX + cetuximab	449 445	PFS	OS: 10.7 mo *vs* 9.4 mo (HR = 1.0, *P* = 0.95) PFS: 5.6 mo *vs* 4.4 mo (HR = 1.09, *P* = 0.32)
REAL-3[39]	Ⅲ	First	Unselected	EOC EOC + panitumumab	275 278	OS	OS: 11.3 mo *vs* 8.8 mo (HR = 1.37, *P* = 0.013) PFS: 7.4 mo *vs* 6.0 mo (HR = 1.22, *P* = 0.068) ORR: 42% *vs* 46% (*P* = 0.42)
RILOMET -1[47]	Ⅲ	First	MET positive by IHC HER2 negative	ECX ECX + rilotumumab	305 304	OS	OS: 11.5 mo *vs* 9.6 mo (HR = 1.37, *P* = 0.016) PFS: 5.7 mo *vs* 5.7 mo (HR = 1.30, *P* = 0.016) ORR: 39.2% *vs* 30% (OR = 0.67, *P* = 0.027)
METGastric[49]	Ⅲ	First	MET positive by IHC HER2 negative	mFOLFOX mFOLFOX + ornatuzumab	562	OS	OS: 11.3 mo *vs* 11.0 mo (HR = 0.82, *P* = 0.244) PFS: 6.8 mo *vs* 6.7 mo (HR = 0.90, *P* = 0.429) ORR: 41% *vs* 46% (*P* = 0.253)
AVAGAST[52]	Ⅲ	First	Unselected	CX CX + bevacizumab	387 387	OS	OS: 10.1 mo *vs* 12.1 mo (HR = 0.87, *P* = 0.1) PFS: 5.3 mo *vs* 6.7 mo (HR = 0.80, *P* = 0.037) ORR: 37.4% *vs* 46.0% (*P* = 0.03)
AVATAR[53]	Ⅲ	First	Unselected	CX CX + bevacizumab	102 100	OS	OS: 11.4 mo *vs* 10.5 mo (HR = 1.11, *P* = 0.55) PFS: 6.0 mo *vs* 6.3 mo (HR = 0.89, *P* = 0.47) ORR: 34% *vs* 41% (*P* = 0.35)
REGARD[54]	Ⅲ	Progression after TP	Unselected	BSC BSC + ramucirumab	117 238	OS	OS: 3.8 mo *vs* 5.2 mo (HR = 0.77, *P* = 0.047) PFS: 1.3 mo *vs* 2.1 mo (HR = 0.48, *P* < 0.001)
RAINBOW[55]	Ⅲ	Second	Unselected	Paclitaxel Paclitaxel + ramucirumab	335 330	OS	OS: 7.4 mo *vs* 9.6 mo (HR = 0.80, *P* = 0.017) PFS: 2.9 mo *vs* 4.4 mo (HR = 0.63, *P* < 0.0001)
Apatinib[57]	Ⅲ	Third or more	Unselected	Placebo Apatinib	91 176	OS	OS: 4.7 mo *vs* 6.5 mo (HR = 0.70, *P* = 0.015) PFS: 1.8 mo *vs* 2.6 mo (HR = 0.44, *P* < 0.001) ORR: 0% *vs* 2.84% (*P* = 0.16)
MSI							
NCT01063517[66]	Ⅱ	Second	ATM expression	Paclitaxel Paclitaxel + olaparib	62 61	PFS	OS: 8.3 mo *vs* 13.1 mo (HR = 0.56, *P* = 0.01) PFS: 3.55 mo *vs* 3.91 mo (HR = 0.80, *P* = 0.13)
NCT02589496	Ⅱ	Second	Unselected	Pembrolizumab		RR	Ongoing
GS							
FAST[91]	Ⅱ	First	CLDN18.2	EOX EOX + IMAB362	161	PFS	OS: 8.7 mo *vs* 12.5 mo (HR = 0.5) PFS: 5.7 mo *vs* 7.9 mo (HR = 0.5, *P* = 0.001)

Most significant target-oriented phase Ⅱ and phase Ⅲ trials are presented. In the table are shown in order: name of the trial, phase of the study, line of treatment, biomarker selection, treatment arms, number of enrolled patients, primary endpoint and key outcome results. tPF: Trastuzumab + Platinum + fluorouraci; PF: Platinum + fluoropyrimidine; TAX: Taxane, CF: Cisplatin + fluorouracil; CX: Cisplatin + capecitabine; EOC (or ECX): Epirubicin + oxaliplatin + capecitabine; BSC: Best supportive care; CIN: Chromosomal instability; GS: Genomical stability; MSI: Microsatellite instability.

efficaciously as in MSI tumours.

It is also possible to clearly segregate another class of GC classified either as GS or MMS/EMT, in which the prevalent deregulation is represented by EMT pathway alterations. Development of inhibitors of HGF/c-Met pathway, Rho/Rho-kinase, AURKA/AURKB, PLK1 could be a strategy adopted in the near future.

The category corresponding to CIN, and partially to MSS/TP53$^-$, represents a cluster of GC with high CNV variation leading to deregulation of specific biological targets such as receptors and kinases. Since these driver alterations are mostly mutually exclusive, they could be easily targeted using specific monoclonal antibodies or TKIs. On the other side, tumour heterogeneity may limit the efficacy of targeted strategies through alternative mechanisms of primary and acquired resistance[103].

The overall landscape is complex and our knowledge on this topic is still just at the starting point and novel trials should be designed accordingly (Table 1)[3,19-22,38,39,47,49,52-55,57,66,91]. Doubtlessly, dissecting and genotyping different

tumour subtypes and setting apart patients with different diseases will represent the future of gastrointestinal oncology. The key landmark comprehensive efforts made by TCGA and ACRG have just paved the way for precision oncology.

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ORIGINAL ARTICLE

Basic Study

Extramural vascular invasion and response to neoadjuvant chemoradiotherapy in rectal cancer: Influence of the CpG island methylator phenotype

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Author contributions: All authors substantially contributed to the conception and design of the study as well as drafting and approving the final manuscript; Williamson JS and Jones HG contributed to the acquisition of data from experimental studies; all authors contributed to the analysis and interpretation of data.

Institutional review board statement: All patients in this study were treated in the South West Wales Oncology Centre (Singleton Hospital, Swansea, United Kingdom) and ethical approval for this study was granted by South West Wales REC (Project Ref No:11/WA/0256). Consent was not required in accordance with the Human Tissue Act 2004 (chapter 30).

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Abstract

AIM

To identify whether CpG island methylator phenotype (CIMP) is predictive of response to neoadjuvant chemoradiotherapy (NACRT) and outcomes in rectal cancer.

METHODS

Patients undergoing NACRT and surgical resection for rectal cancer in a tertiary referral centre between 2002-2011 were identified. Pre-treatment tumour biopsies were analysed for CIMP status (high, intermediate or low) using methylation specific PCR. *KRAS* and *BRAF* status were also determined using pyrosequencing analysis. Clinical information was extracted from case records and cancer services databases. Response to radiotherapy was measured by tumour regression scores determined upon

histological examination of the resected specimen. The relationship between these molecular features, response to NACRT and oncological outcomes were analysed.

RESULTS

There were 160 patients analysed with a median follow-up time of 46.4 mo. Twenty-one (13%) patients demonstrated high levels of CIMP methylation (CIMP-H) and this was significantly associated with increased risk of extramural vascular invasion (EMVI) compared with CIMP-L [8/21 (38%) *vs* 15/99 (15%), $P = 0.028$]. CIMP status was not related to tumour regression after radiotherapy or survival, however EMVI was significantly associated with adverse survival ($P < 0.001$). Intermediate CIMP status was significantly associated with *KRAS* mutation ($P = 0.01$). There were 14 (9%) patients with a pathological complete response (pCR) compared to 116 (73%) patients having no or minimal regression after neoadjuvant chemoradiotherapy. Those patients with pCR had median survival of 106 mo compared to 65.8 mo with minimal regression, although this was not statistically significant ($P = 0.26$). Binary logistic regression analysis of the relationship between EMVI and other prognostic features revealed, EMVI positivity was associated with poor overall survival, advanced "T" stage and CIMP-H but not nodal status, age, sex, *KRAS* mutation status and presence of local or systemic recurrence.

CONCLUSION

We report a novel association of pre-treatment characterisation of CIMP-H with EMVI status which has prognostic implications and is not readily detectable on pre-treatment histological examination.

Key words: Rectal cancer; CpG islands; Methylation

Core tip: There is wide and unpredictable response of rectal cancer to neoadjuvant therapy which carries significant side effects and relies on limited pre-treatment risk stratification. Methylation specific PCR was used to determine CpG island Methylator phenotype (CIMP) status in 160 rectal cancers and compared with response to therapy, clinical and pathological outcomes. CIMP status was not directly related to tumour regression but was related to extramural vascular invasion which confers an adverse survival risk.

Williamson JS, Jones HG, Williams N, Griffiths AP, Jenkins G, Beynon J, Harris DA. Extramural vascular invasion and response to neoadjuvant chemoradiotherapy in rectal cancer: Influence of the CpG island methylator phenotype. *World J Gastrointest Oncol* 2017; 9(5): 209-217 Available from: URL: http://www.wjgnet.com/1948-5204/full/v9/i5/209.htm DOI: http://dx.doi.org/10.4251/wjgo.v9.i5.209

INTRODUCTION

Locally advanced rectal cancer is usually treated with neoadjuvant chemoradiotherapy to downstage and/or downsize the tumour prior to surgery[1,2]. The response of rectal cancer to neoadjuvant therapy varies significantly between patients. The most successful outcome is a pathological complete response (pCR) in which no viable tumour cells are seen upon subsequent histological examination of the resected bowel. In this scenario patients have a significantly improved 5-year survival of up to 85%-100%, although any residual lymph-nodal involvement is associated with a significantly worse survival despite complete local tumour regression[3]. This compares favourably with those showing minimal response to radiotherapy who may expect 5-year survival of between 55%-66%[4].

pCR occurs in between 10%-20% of patients undergoing neoadjuvant chemoradiation therapy[4-6], however up to 30% of patients do not show any response[7]. Furthermore, those patients not responding to neoadjuvant treatment risk progression of their disease with either local progression or distant metastases during preoperative treatment. The use of imaging technology including magnetic resonance imaging (MRI) and endorectal ultrasound are not sufficiently reliable[8,9] to be implemented as a sole means of discriminating between those with pCR and those without.

The adverse prognostic value of extramural vascular invasion (EMVI) is well established and is known to be associated with poor survival[10], increased risk of local recurrence[11] and death[11-14]. Furthermore, the presence of EMVI has a relative risk of 3.7 for the development of systemic recurrence when detectable on preoperative MRI scanning[15]. The role of EMVI in directing treatment is relatively new and not well established. In particular, National Institute of Health and Care Excellence recommend that EMVI may confer a higher risk of recurrence in stage Ⅱ rectal cancers and suggest adjuvant chemotherapy may be considered in those patients with EMVI where the relative benefits of this treatment are not otherwise clear[16]. EMVI status may also influence the decision to offer neoadjuvant radiotherapy, as it has been demonstrated that chemoradiation (CRT) can cause vessel fibrosis in EMVI-positive tumours, which may influence survival outcomes[17].

EMVI is detectable in rectal cancer patients on MRI, however, sensitivity and specificity are relatively low at 62% and 88% respectively[17]. It is therefore important that not only is EMVI accurately characterised but should be available early to inform decisions regarding neoadjuvant chemoradiotherapy and influence overall treatment outcomes.

Developments in genetics and epigenetics lend support to the notion that tumours display characteristic clinicopathological and morphological features depending on the nature of specific combinations of molecular pat-

terns[18]. In particular, the CpG island methylator phenotype (CIMP), which may account for up to 20% of all colorectal cancers[19,20], is associated with differences in tumour location, patient gender and association with characteristic gene mutations including *KRAS*, *BRAF* and *p53*[18], although this relationship has not been explored in EMVI. CpG islands are typically short (300-3000 base pairs) Cytosine-Guanine phosphodiester bonded sequences found in or around the promoter region of a gene where they are usually unmethylated if the genes are expressed. The CIMP phenotype is characterised by epigenetic DNA hyper-methylation and consequent suppression of key genes important in controlling cell growth and survival, which is associated with poor survival in rectal cancer[21,22]. It is becoming increasingly clear that epigenetic factors affecting specific gene promoter regions (CpG islands) can be equally as important as genetic alterations in all disease processes, as these can affect every component of gene regulation. Previous work has demonstrated that genetic factors such as *KRAS* mutation has an inverse relationship with EMVI[23] but little is known of the influence of epigenetic factors in the development of EMVI. The purpose of this study was to explore the relationship between CIMP and response to chemoradiotherapy and EMVI in rectal cancer.

MATERIALS AND METHODS

Patients undergoing neoadjuvant chemoradiotherapy and subsequent surgical resection for rectal adenocarcinoma with curative intent were identified from a prospectively maintained pathology database of all colorectal cancers between the years 2002 and 2011. All patients underwent endoscopic diagnostic biopsy in order to confirm histological evidence of rectal adenocarcinoma prior to treatment. After pre-treatment staging with thoracic-abdominal-pelvic computed tomography (CT), pelvic MRI, clinical examination under anaesthesia (EUA) and in some cases endorectal ultrasound (ERUS), patients were discussed by the multidisciplinary team and offered neoadjuvant chemoradiotherapy according to the local protocol. Local indications for neoadjuvant CRT were extensive mesorectal or pelvic sidewall nodal disease, predicted mesorectal fascia involvement by tumour and/or lymph nodes based on MRI imaging, or clinical fixity of tumour to surrounding structures. After a 6 to 8 wk period following completion of chemoradiotherapy patients underwent restaging investigations (MRI, CT, ERUS and/or EUA) to assess response to treatment and to plan surgical resection. Standardised surgical techniques to maximise complete excision were used including total mesorectal excision and extralevator pelvic floor excision. In some cases multivisceral resection was required for tumours beyond conventional planes. Neoadjuvant radiotherapy in all cases was administered at South West Wales Oncology Centre (Singleton Hospital, Swansea, United Kingdom) and delivered with concurrent 5-fluoroUracil (Capecitebine) according to local protocol.

Pre-treatment biopsy specimens stained with Haematoxylin and Eosin were examined by a consultant histopathologist to ensure they contained at least 60% adenocarcinoma tissue. Post treatment resection specimens were examined by two consultant histopathologists who were blinded to patient details and recorded their reports conforming to the Royal College of Pathologists colorectal cancer data set (2nd edition 2007) on separate sheets which were stored in a locked cabinet and not seen by other investigators until the data analysis stage. If the reports given by pathologists differed, a third pathologist would be asked to give an opinion and the final report reflected the consensus. When examining tumour regression scores, to ensure there was agreement between the two pathologists scoring the regression, Cohen's kappa statistic was utilised to measure agreement between both raters. For the Royal College of Pathologists tumour regression score there was almost perfect agreement ($k = 0.856$ $P < 0.001$). Patients not completing a full course of neoadjuvant CRT or those not proceeding to surgery were excluded from this study. Patients with rectosigmoid junction tumours, history of inflammatory bowel disease or known high risk genetic predisposition to colorectal cancer (familial adenomatous polyposis or Lynch syndrome) and those undergoing treatment for recurrent cancer were also excluded.

Demographic and clinical outcome data for patients in this study were gathered from patients' case notes, clinic letters and computerised patient hospital records. Patients with local and systemic recurrence were also identified in this way. To identify patients who had died following their treatment, the NHS Wales Informatics Service (Myrddin) database was utilised which records the date of death for each patient if this has occurred. Overall survival, local and systemic recurrence free survival were calculated from the date of surgical resection until either the date of death or the date that recurrence was confirmed clinically, radiologically or histopathologically. If no death or recurrence had occurred, the reference date of last known follow-up was used to calculate survival. These data were also cross referenced against the Cancer Information Network System Cymru database which records data for all patients undergoing cancer treatment in South Wales to ensure its accuracy. Ethical approval for this study was granted by South West Wales REC (Project Ref No.:11/WA/0256). Consent was not required in accordance with the Human Tissue Act 2004 (chapter 30).

DNA extraction

Formalin fixed paraffin embedded pre-treatment biopsy specimens were utilised for this study. Several representative 5 μm sections of the biopsy were cut and mounted unstained onto glass slides and DNA from these tissues was obtained using the MasterPure Complete DNA and RNA purification kit (Epicentre, Illumina, WI, United States).

The quantity and quality of DNA was measured at absorbance between 230 nm and 320 nm using spectrophotometry (Nanodrop ND-1000, Software v3.1.2, Thermoscientific, DE, United States). DNA quantity was

calculated by multiplying the measured concentration following spectrophotometry at 260 nm with the dilution factor. DNA was diluted to a working concentration of 20 ng/μL. Purity was further analysed by calculating the absorbance at 260 nm to absorbance at 280 nm ratio.

Bisulfite conversion and methylation specific PCR

Methylation specific PCR is accomplished by performing bisulfite conversion of genomic DNA (Imprint DNA Modification Kit, Sigma Aldrich, United States). The PCR products were resolved using gel electrophoresis on a 30% polyacrylamide gel. Depending on the methylation status of each CpG island, each patient could be classified as one of three epigenotypes; CIMP-High, Intermediate or Low using a two panel approach[24,25]. The first panel consists of SOCS1, MINT-1 and hMLH, which are associated strongly with CIMP-H. The second panel consist of NEUROG1, THBD, HAND1, ADAMTS1, IGFBP3. CIMP status could then be determined using the following system: (1) CIMP-High if $\geqslant$ 2/3 group 1 markers methylated; (2) CIMP-Intermediate if < 2/3 group 1 but $\geqslant$ 3/5 group 2 methylated; and (3) CIMP-Low if < 2/3 group 1 and < 3/5 group 2 methylated.

KRAS and BRAF mutational analysis

Pyrosequencing analysis was performed in collaboration with the Leeds Cancer Research United Kingdom Centre, (Leeds Institute of Cancer Studies and Pathology, Clinical Sciences Building, level 6, St. James's University Hospital, Leeds, LS9 7TF). Pyrosequencing conditions used were as previously published by this group[26]. Substitution and insertion/deletion mutations in *KRAS* codon 12, 13 and 61 and *BRAF*-600 were examined for all specimens using this method.

Definitions

Tumours were defined as low (0-5 cm from anal verge), mid (5-10 cm) or high (10-15 cm) rectal based on pre-operative rigid sigmoidoscopy and according to where the majority of the tumour was located. Predicted circumferential resection margin (CRM) involvement was defined by the presence of tumour foci (primary, nodal or extranodal deposit) within 1 mm of the mesorectal fascia or cylindrical resection margin for low tumours. An involved CRM was defined pathologically as tumour within 1 mm of the CRM. The original definition of EMVI describes "a rounded mass of tumour in an endothelium-lined space either surrounded by a rim of smooth muscle or containing red blood cells[27]". More recent definitions suggest venous invasion may also be suspected when a rounded or elongated tumour profile is identified adjacent to an artery, especially when no separate accompanying vein can be identified (the "orphan" artery sign), or where smooth tongues of tumour extend into pericolic/perirectal fat ("protruding tongue" sign)[28].

Statistical analysis

Statistical analysis was performed using SPSS v.18 Chicago: SPSS Inc. Data was tested for normality using a Kolomogorov-Smirnov test, and a Student's *t*-test was for analysis of normally distributed continuous data. Categorical variables were compared using χ^2 or Fishers exact test where expected frequencies were less than 10. Relationship between independent variables and time to event was compared using Kaplan-Meier methodology using the Log Rank test to determine significance. Multivariable analysis was performed using bivariate logistical regression and Cox Proportional Hazards modelling. Statistical significance was assumed at the 5% level.

RESULTS

Patient and tumour characteristics

There were 160 patients included in this study. There were 113 (71%) males and 47 (29%) females and the average age by the time of surgery was 65.4 years. By the time of this analysis, 53 (33%) patients had died and the median time from surgery to death was 26.2 mo (IQR 11.9-48.5).

Of the surviving patients, the median follow-up time from surgery was 46.4 mo (IQR 33.8-56.0). Local recurrence data were available for 152 patients and of these, 8 (5%) had evidence of local recurrence a median of 19.7 mo after surgery. Systemic recurrence data were available for 151 patients and of these, 37 (25%) had evidence of systemic recurrence at median 16.3 mo after surgery. Overall survival for all patients was estimated using Kaplan-Meier analysis at 73.3 mo (95%CI: 63.3-83.2). 4 (3%) patients had an involved CRM which was related to worse overall survival (74.1 mo *vs* 37.2 mo, $P = 0.047$).

There were 14 (9%) patients with a pCR compared to 116 (73%) patients having no or minimal regression after neoadjuvant chemoradiotherapy. Of those undergoing pCR, 8 were male, 6 were female and had a mean age of 66 years. None of the pCR patients demonstrated CIMP-H, whereas 2 were CIMP-I and 12 were CIMP-L. Those patients with pCR had median survival of 106 mo compared to 65.8 mo with minimal regression, although this was not statistically significant ($P = 0.26$). There were 52 patients (33%) with demonstrable *KRAS* mutation, but only a single *BRAF* mutation was detected in the study sample.

CIMP status analysis

CIMP status was determined in all patients, 21 (13%) were CIMP-H, 40 (25%) were CIMP-I and 99 (62%) were CIMP-L. Comparison of patient characteristics by CIMP status revealed no differences in mean age, gender, "T" or "N" stage, presence of systemic or local recurrence, CRM involvement, survival or tumour regression scores. Sub-analysis of individual CIMP markers with tumour regression scores revealed no significant differences. However, CIMP-H was significantly related to EMVI positivity with 8/21 (38%) CIMP-H patients demonstrating

Table 1 Comparison of pathological features by CpG island methylator phenotype status

	CIMP-H	CIMP-I	CIMP-L	*P* value
Mean age	66	69.2	63.9	
Sex				
Female	5	14	28	
Male	16	26	71	
ypT stage				
0 or pCR	2	2	16	
1	3	1	7	
2	2	10	20	
3	11	24	48	
4	3	3	8	
ypN stage				
0	11	27	65	
1	6	8	21	
2	4	5	13	
Systemic recurrence				
Absent	14	30	66	
Present	5	2	22	
Local recurrence				
Absent	20	37	87	
Present	0	2	6	
EMVI				
Negative	13	33	84	
Positive	8	7	15	CIMP-L *vs* CIMP-H *P* = 0.028
KRAS status				
Wildtype	15	20	73	
Mutant	6	20	26	KRAS Mut + CIMP-I *P* = 0.01
CRM				
Not involved	21	39	95	
Involved	0	0	4	
RC path score				
1 (pCR)	0	2	12	
2	6	9	14	
3	15	29	73	
Total	21	40	99	

CRM: Circumferential resection margin; CIMP: CpG island methylator phenotype; EMVI: Extramural vascular invasion; pCR: Pathological complete response.

EMVI compared with 15/99 (15%) who were CIMP-L. (CIMP-H/ EMVI$^+$ 38% *vs* CIMP-L/EMVI$^+$ 15%, Fishers exact, P = 0.028). Furthermore, a higher proportion of CIMP-I patients demonstrated *KRAS* mutation than other CIMP groups [CIMP-I + *KRAS* mutation 20/40 (50%) *vs* CIMP-H/L + *KRAS* mutation 32/120 (27%), Fishers exact, P = 0.01] (Table 1).

None of 21 (0%) patients with CIMP-H tumours experienced a pCR compared with 12/99 (12%) CIMP L patients, however this was not statistically significant (Fishers exact = 0.12). There were 30 (19%) patients with EMVI-positivity on histopathological examination of the specimen. This was associated with a significant reduction in median overall survival (83.8 mo *vs* 43.9 mo, P < 0.001, Figure 1).

No patient with pCR displayed EMVI, whereas 29 (25%) with RC Path score of 3 (minimal regression) displayed EMVI (P = 0.039, Table 2).

Multivariable analysis

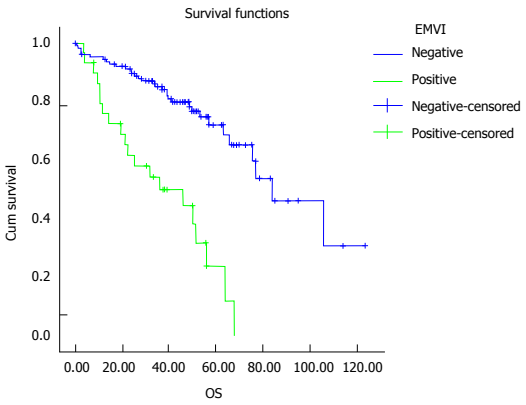
Cox hazard regression analysis revealed that EMVI-positivity was the only factor that was significantly related to adverse survival (Table 3).

Binary logistic regression analysis of the relationship between EMVI and other prognostic features revealed, EMVI positivity was associated with poor overall survival, advanced "T" stage and CIMP-H but not nodal status, age, sex, *KRAS* mutation status and presence of local or systemic recurrence (Table 4).

Table 2 Tumour regression scores (Royal College Pathologists data set) by extramural vascular invasion status

	EMVI +	EMVI-	*P* value
RC Path 1 (pCR)	0	14	0.039
RC Path 2	1	28	
RC Path 3	29	88	

EMVI: Extramural vascular invasion; pCR: Pathological complete response.



No at risk (mo)	0	0-20	20-40	40-60	60-80	80-100	100-120
EMVI negative	130	114	77	23	8	3	1
EMVI positive	30	19	9	2	1	0	0

Figure 1 Overall survival by extramural vascular invasion positivity. Positive *vs* negative, P < 0.001. EMVI: Extramural vascular invasion; OS: Overall survival.

DISCUSSION

CIMP as a prognostic marker

CIMP-positivity has been implicated as an adverse survival predictor in patients with colorectal cancer[29-31], however, the majority of studies investigating survival outcomes in relation to methylation status regard colon and rectal cancers as one entity. Most investigators identify CIMP as an adverse prognostic feature, particularly in colorectal cancer taken as a whole and this was also corroborated by a recent meta-analysis including all colorectal sub sites, which found shorter survival in CIMP positive patients[32,33].

The current understanding of the role of CIMP in colorectal cancer is that tumours with a greater level of CpG island methylation (CIMP-High or CIMP +) have distinct molecular and clinical characteristics compared to low levels of CpG methylation (CIMP-Low or CIMP -)[34]. There is some evidence that CIMP-Positivity is related to shorter overall survival[35] and disease free survival[36], however the populations in these studies generally lack

Table 3 Multivariable analysis of pathological and molecular variables against overall survival

	Wald statistic	OR	95%CI (lower)	95%CI (upper)	*P* value
T stage	1.735	1.392	0.851	2.279	0.188
N stage	0.268	0.857	0.479	1.535	0.605
EMVI	9.422	4.041	1.657	9.857	0.002
CIMP status	0.982	0.791	0.498	1.257	0.322
KRAS status	2.162	1.740	0.832	3.640	0.141
Sex	0.439	0.764	0.344	1.695	0.508
Local recurrence	0.861	1.763	0.532	5.839	0.353
Systemic recurrence	2.165	1.729	0.834	3.584	0.141
Tumour regression (pCR)	0.052	0.793	0.109	5.785	0.819
Involved CRM	0.146	1.339	0.299	6.002	0.703

CRM: Circumferential resection margin; CIMP: CpG island methylator phenotype; EMVI: Extramural vascular invasion; pCR: Pathological complete response.

Table 4 Binary logistic regression analysis; extramural vascular invasion positivity against overall survival and other pathological, demographic and molecular features

	OR	95%CI (lower)	95%CI (upper)	*P* value
Overall survival	0.936	0.893	0.981	0.006
T stage	7.764	1.749	34.463	0.007
N stage	2.552	0.851	7.651	0.095
Age	1.024	0.969	1.081	0.405
Systemic recurrence	0.865	0.200	3.749	0.846
Sex	0.564	0.119	2.668	0.470
Local recurrence	1.841	0.193	17.562	0.596
Involved CRM	0.276	0.009	8.376	0.459
KRAS mutation	1.577	0.389	6.391	0.524
CIMP-H	6.368	1.091	37.162	0.040

CRM: Circumferential resection margin; CIMP: CpG island methylator phenotype.

homogeneity of factors such as *KRAS* and *BRAF* mutation status, MSI status and tumour stage[34].

The present study did not demonstrate any relationship between CIMP status and survival. CIMP status was however significantly associated with EMVI positivity which itself was associated with worse survival. Therefore it is likely that the relative contribution of these phenomena to prognosis is more complex than previously understood and should be studied in more detail and with particular distinction of rectal cancers from colon cancers.

Predicting response to chemoradiotherapy

Relatively few studies have studied the role of CIMP as a predictive marker of rectal cancer response to neoadjuvant chemoradiotherapy. A factor that complicates the evidence is that there is no agreed definition on CIMP classification, and therefore widely ranging and contradicting results are found in the literature. Our research did not find that CIMP status was a predictor of response to chemoradiotherapy, although others have found that detecting the methylation status of individual gene promoter-regions affected the response to neoadjuvant treatment.

Ebert *et al*[37] examined a total of 294 patients with colorectal cancer undergoing neoadjuvant chemotherapy (5-fluorouracil, oxaliplatin and irinotecan), and analysed the expression, methylation and function of the *TFAP2E* gene. They demonstrated that hypermethylation of the promoter regions of *TFAP2E* was associated with down-regulation of the gene, and the subsequent up-regulation of a down-stream target. Furthermore, *TFAP2E* hypermethylation was a marker of 5-fluorouracil resistance in CRC in this study, but there was no effect on response to treatment with oxaliplatin or irinotecan.

CIMP and KRAS mutation

Ogino *et al*[38] examined methylation in 840 colorectal cancers led to the proposal that a further subset of intermediate methylation associated tumours exist but which do not fulfil the criteria for CIMP-High. These tumours (termed CIMP-intermediate) were independently associated with male gender and *KRAS* mutation. The three epigenotype model was further supported by Yagi *et al*[24], who used a large scale mass spectrometry analysis and hierarchical clustering to identify two panels of markers, the first to identify CIMP-High tumours and then a second panel to distinguish between CIMP-intermediate and low tumours. In our research, CIMP-I had a significant association with *KRAS*-mutation compared to CIMP-H or CIMP-L tumours ($P = 0.01$), confirming this association in our patients, although no difference with regards to survival was demonstrated.

CIMP classification and EMVI status

The adverse prognostic value of EMVI is well established

and is known to be associated with poor survival[10] and has a relative risk of 3.7 for the development of systemic recurrence when detectable on preoperative MRI scanning[15]. This is supported by data from the present study, which revealed significantly decreased survival with EMVI.

EMVI was also associated with a lack of response to neoadjuvant chemoradiotherapy. If EMVI is present before treatment and is absent after treatment, then this would indicate a response, whereas failure of EMVI to regress would indicate a lack of response. However, the presence of EMVI is not currently detectable on histological analysis of pre-treatment biopsy specimens. In the present study, a novel association between EMVI and CIMP-H status was identified. This finding does provide a novel insight into potential mechanisms for the association of poor survival with CIMP-H seen in other studies.

There are several mechanisms which may explain the link between CpG island hypermethylation and EMVI. For example, angiogenesis and subsequent local invasion of colorectal tumours has previously been linked to hypermethylation and silencing of micro-RNA-126 (miRNA-126), which is associated with up-regulation of vascular endothelial growth factor and subsequent increased likelihood tumour invasion[39]. Other research has suggested that silencing the gene that codes for E-Cadherin (a molecule that forms the adherens junctions between normal cells, preventing spread of tumour cells across the epithelial basement membrane)[40] is associated with increased risk of EMVI and reduced response to neoadjuvant chemoradiotherapy and worse survival in rectal cancers[41]. Finally, the invasion of cancer cells into the surrounding extracellular matrix depends on the function of matrix metalloproteinases (MMPS), which are themselves regulated by tissue inhibitors of matrix metalloproteinases (TIMPS). *In vitro* and animal studies have demonstrated that aberrant epigenotypes affecting the MMP/TIMPS axis can lead to increased tumour invasion and migration *in vitro* and increased tumourigenesis and therapeutic reversal of this aberrant methylation can suppress these tumourigenic phenomenon[42,43].

Given that CIMP is deemed to represent a phenotypic hypermethylated state, it is likely that the presence of the CIMP-H state explains the association of EMVI-positivity and poor survival seen in rectal cancer patients. The detection of a hypermethylated state in individual gene promoter regions may well further our understanding of the response to chemoradiotherapy in the future.

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COMMENTS

Background

There is wide variation in response to neoadjuvant chemoradiotherapy (NACRT) in rectal cancer, which has a significant impact on survival. There is currently no reliable means to predict response to NACRT, which carries significant side effects. The CpG island methylator phenotype (CIMP) is characterised by epigenetic DNA hyper-methylation and suppression of key genes controlling cell growth and survival and occurs in approximately 20% of colorectal cancers. The role of CIMP status in the prognosis and response of rectal cancer to neoadjuvant therapy is not well understood but evidence is emerging that it may be an adverse prognostic indicator.

Research frontiers

Previous studies have demonstrated an association of high levels of CIMP associated methylation with adverse survival and differential responses to neoadjuvant treatment where methylation is seen in specific genes in rectal cancer, however, the mechanism and exact nature of this association is not clear.

Innovations and breakthroughs

This study reports a novel association of CIMP related methylation with extra mural vascular invasion which represents an adverse prognostic indicator and provides a novel insight into potential mechanisms for the association of poor survival with CIMP H which may be related to epigenetic silencing of the normal inhibitory mechanisms which prevent cell migration, proliferation and vascular invasion.

Applications

Extramural vascular invasion (EMVI) has recently been associated with adverse survival and risk of metastasis and although it features in the National Institute of Health and Care Excellence United Kingdom guidelines for the treatment of rectal cancer, suggesting that short course neoadjuvant therapy should be considered in these patients on this basis, the current guidelines concede that the risks and benefits in this group are unclear and further research is needed. Indeed the prediction of EMVI on preoperative imaging is notoriously difficult and non-reproducible. EMVI is detectable in rectal cancer patients on magnetic resonance imaging, however, sensitivity and specificity are relatively low at 62% and 88% respectively and it is possible that in future, CIMP status could be used to enhance preoperative EMVI detection and subsequent risk stratification.

Terminology

CpG islands are typically short (300-3000 base pairs) Cytosine-Guanine phosphodiester bonded sequences found in or around the promoter region of a gene where they are usually unmethylated if the genes are expressed. The CIMP phenotype is characterised by epigenetic DNA hyper-methylation and consequent suppression of key genes important in controlling cell growth and survival. High levels of CIMP associated methylation (deemed CIMP-High), are associated with poor survival in rectal cancer. Extramural vascular invasion of a tumour is defined as “a rounded mass of tumour in an endothelium-lined space either surrounded by a rim of smooth muscle or containing red blood cells”. Venous invasion may also be suspected when a rounded or elongated tumour profile is identified adjacent to an artery, especially when no separate accompanying vein can be identified or where smooth tongues of tumour extend into pericolic/perirectal fat.

Peer-review

The authors aimed to identify whether CIMP status is predictive of response to neoadjuvant chemoradiotherapy and outcomes in rectal cancer. They found

that a novel association of CIMP status with extramural vascular invasion which represents an adverse prognostic indicator and provides a novel insight into potential mechanisms for the association of poor survival with CIMP-H rectal cancers. The study is well-designed and presented. The results are all clear and understandable, the descriptions of methods and materials are also clear.

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ORIGINAL ARTICLE

Observational Study

Critical evaluation of contemporary management in a new Pelvic Exenteration Unit: The first 25 consecutive cases

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Abstract

AIM

To critically appraise short-term outcomes in patients

treated in a new Pelvic Exenteration (PE) Unit.

METHODS

This retrospective observational study was conducted by analysing prospectively collected data for the first 25 patients (16 males, 9 females) who underwent PE for advanced pelvic tumours in our PE Unit between January 2012 and October 2016. Data evaluated included age, co-morbidities, American Society of Anesthesiologists (ASA) score, Eastern Cooperative Oncology Group (ECOG) status, preoperative adjuvant treatment, intra-operative blood loss, procedural duration, perioperative adverse event, lengths of intensive care unit (ICU) stay and hospital stay, and oncological outcome. Quantitative data were summarized as percentage or median and range, and statistically assessed by the χ^2 test or Fisher's exact test, as applicable.

RESULTS

All 25 patients received comprehensive preoperative assessment *via* our dedicated multidisciplinary team approach. Long-course neoadjuvant chemoradiotherapy was provided, if indicated. The median age of the patients was 61.9-year-old. The median ASA and ECOG scores were 2 and 0, respectively. The indications for PE were locally invasive rectal adenocarcinoma (n = 13), advanced colonic adenocarcinoma (n = 5), recurrent cervical carcinoma (n = 3) and malignant sacral chordoma (n = 3). The procedures comprised 10 total PEs, 4 anterior PEs, 7 posterior PEs and 4 isolated lateral PEs. The median follow-up period was 17.6 mo. The median operative time was 11.5 h. The median volume of blood loss was 3306 mL, and the median volume of red cell transfusion was 1475 mL. The median lengths of ICU stay and of hospital stay were 1 d and 21 d, respectively. There was no case of mortality related to surgery. There were a total of 20 surgical morbidities, which occurred in 12 patients. The majority of the complications were grade 2 Clavien-Dindo. Only 2 patients experienced grade 3 Clavien-Dindo complications, and both required procedural interventions. One patient experienced grade 4a Clavien-Dindo complication, requiring temporary renal dialysis without long-term disability. The R0 resection rate was 64%. There were 7 post-exenteration recurrences during the follow-up period. No statistically significant relationship was found among histological origin of tumour, microscopic resection margin status and post-operative recurrence (P = 0.67). Four patients died from sequelae of recurrent disease during follow-up.

CONCLUSION

By utilizing modern assessment and surgical techniques, our PE Unit can manage complex pelvic cancers with acceptable morbidities, zero-rate mortality and equivalent oncologic outcomes.

Key words: Colorectal cancer; Advanced pelvic tumour; Sacrectomy; Oncological outcome; Pelvic exenteration; Chordoma

Core tip: Pelvic exenteration surgery was introduced by Brunswick in 1948 as a palliative treatment for advanced pelvic tumour, which carries high morbidity and mortality rates. However, decades of medical evolution in preoperative imaging, adjuvant therapy, better anatomical knowledge of the pelvis and modernized surgical techniques has made this procedure safe and effective for treating complex pelvic tumours. This study describes and demonstrates how our new Pelvic Exenteration Unit utilises the advantage of modern assessment and contemporary surgical techniques to achieve excellent outcomes.

Chew MH, Yeh YT, Toh EL, Sumarli SA, Chew GK, Lee LS, Tan MH, Hennedige TP, Ng SY, Lee SK, Chong TT, Abdullah HR, Goh TLH, Rasheed MZ, Tan KC, Tang CL. Critical evaluation of contemporary management in a new Pelvic Exenteration Unit: The first 25 consecutive cases. *World J Gastrointest Oncol* 2017; 9(5): 218-227 Available from: URL: http://www.wjgnet.com/1948-5204/full/v9/i5/218.htm DOI: http://dx.doi.org/10.4251/wjgo.v9.i5.218

INTRODUCTION

Surgeries for advanced pelvic tumours constitute technical challenges. Despite better understanding of the pelvic anatomy due to superior imaging modalities, the resection of tumours and extirpation of any contiguous organs continue to be associated with considerable morbidity and risks. In addition, tumours originating from the rectum, gynaecological organs or urological organs behave differently and indications of surgery for each require multidisciplinary coordination and evaluation.

Pelvic exenteration (PE) surgery was first introduced by Brunswick[1] in 1948 but was associated with a high morbidity rate, a perioperative mortality rate of 23%, and a poor postoperative quality of life. As such, a non-surgical approach with chemotherapy and radiotherapy has traditionally been offered to the majority of the patients with pelvic tumours. These approaches may provide transient relief of symptoms but as the disease progress, many of the patients suffer from refractory pain, obstruction, bleeding, malodourous fistulating or erosive malignant cutaneous lesions, and pelvic sepsis. Survival may be increased up to 12-14 mo but remains poor, with < 4% of patients surviving beyond 4 years[2-5].

As a result of better patient selection, perioperative adjuvant chemotherapy and irradiation, careful planning and multidisciplinary involvement as well as advances in surgical techniques in the modern era, PE has become accepted as a procedure that can maintain adequate local disease control, prolong survival and achieve potential cure for advanced pelvic tumours. The most significant advances in surgical techniques have allowed for achievement of an R0 resection, as demonstrated by large-scale reviews which predominantly investigated for the locally-advanced and recurrent types of rectal cancers[6-8].

Accomplishing an R0 resection requires complete or partial removal of the pelvic vessels, muscles, ligaments and bony structures-including the ileum, ischium, pubic rami, sacrum or coccyx-as well as pelvic viscera. Experience gained over the years has led to acceptable morbidity risks and a low mortality rate. In a systematic review, Heriot *et al*[6] reported exenteration-related morbidity and mortality rates of 27% and 0.6% respectively. Similar trends were found in an Australian study of 148 patients who underwent PE, which reported a 0% 30-d mortality rate and good postoperative quality of life[9].

The development of a dedicated PE Surgical Unit in our institution was borne from recognition of the advantages afforded by an aggressive approach to tackling these advanced pelvic tumours. Nonetheless, the initial phase of conceptualization necessitated discussion on the understanding of pelvic cancer biology and pathophysiology among the various subspecialties, as well as of the appropriate surgical indications. The core members of this PE Unit included: A colorectal surgeon, who had received comprehensive training in PE; a gynaecologist, who specialized in gynaecological malignancies; an urologist, who specialized in urological cancers; and a team of experienced anaesthesiologists. Other subspecialty surgeons-including plastic, vascular and orthopaedic surgeons-were referred on an *ad hoc* basis. While the concept of PE surgery was not new to this Unit at its inception, the latest surgical techniques for achieving R0 margins had only recently been introduced into its practice.

This article reports our systematic evaluation of the short-term oncological outcomes achieved by the newly established PE group using modern techniques.

MATERIALS AND METHODS

Definitions

Definitions of the PE surgeries described herein correspond to those published in a 2013 systematic review from Yang *et al*[10], and include.

Total (T)PE

Whereby rectum, distal colon, genitourinary viscera, internal reproductive organs, draining lymph nodes and pelvic peritoneum are removed. If a sacrectomy is performed, it is specified as TPE with sacrectomy.

Anterior PE

Whereby upper rectum, reproductive organs and bladder are removed. The lower rectum may be spared or a perineal excision may be performed.

Posterior PE

Whereby the rectum and reproductive organs are removed. The bladder may be spared. If a sacrectomy or coccygectomy was performed, it is specified.

Lateral PE

Whereby a lateral pelvic node dissection is performed, with *en bloc* resection of all involved structures, including viscera and vascular structures. If the sciatic nerve can be preserved, its perineural sheath is excised.

Study design

After approval was obtained by the Institutional Review Board of our hospital, a retrospective review of patient records was conducted to identify the first consecutive 25 patients who underwent PE through our new PE Unit. No exclusion criteria were applied. These patients had been treated between January 2012 and October 2016, and all had received or were undergoing follow-up consisting of 3-mo outpatient clinic visits for at least 2 years following the surgery. The follow-up routine included monitoring of carcinoembryonic antigen level (each clinic visit) and computed tomography (CT) chest, abdomen and pelvis scans (once annually for the first 2 years). No patient was lost to follow-up.

Data were expressed as median, maximum range and minimum range due to smaller sample size. Statistical analysis was performed by the Microsoft Excel 2010 software, with Fisher's exact test used to determine significance, indicated by *P* value.

Patient selection

All 25 patients had been evaluated by the multidisciplinary team of the PE Unit, which included medical and radiation oncologists as well as surgeons. For each case, all findings from imaging modalities had been retrieved and carefully re-evaluated by a dedicated radiologist. The extent of local regional disease, as well as the potential for distant metastatic disease, had been determined, with the plan for multi-visceral resection and its approach being formulated accordingly.

Patients considered for surgical resection were those who had no evidence of metastatic disease, had good performance status, and represented those who the multidisciplinary team deemed that the ability to achieve a R0 resection was possible. Patients who did not meet operative criteria were those with either unresectable metastatic disease (for who surgery was performed with palliative intent) or unresectable large volume disease, or who were deemed physically or psychosocially unfit for extensive surgery.

Typically, in our institute, patients with primary advanced colorectal cancer undergo long-course neoadjuvant chemoradiotherapy. Upfront surgery is planned only in cases with prior chemoradiotherapy treatment for other cancers (*e.g.,* prostate) or with cancers unlikely to benefit from neoadjuvant therapy (*e.g.,* chordomas). A delay of 8-12 wk after neoadjuvant chemoradiation treatment is routinely advocated to achieve maximum down-staging. Repeat imaging is usually performed at 4 wk after completion of the neoadjuvant treatment, in order to determine response. The organs and planes involved before commencing neoadjuvant treatment are resected, as well, in order to ensure negative margin.

The entire team of specialty surgeons and anaesthetists assigned to the case would perform preoperative

counselling in their respective area of resection or reconstruction. The counselling process involved appropriate patient-level explanations on the probability of achieving an R0 resection, the survival benefit post-PE, the morbidity and mortality risks associated with organ-specific resection or reconstruction, the anaesthetic risks and the financial implications. Stoma care and potential need of postoperative rehabilitation were also discussed with both the patient and any caregivers. The surgical candidate was also advised of the potential need for 2-4 wk postoperative inpatient hospital stay, including 1-2 d in the intensive care unit (ICU). The usual consultation process takes 4-6 wk. The majority of that time is allotted to allow patients to decide whether they are keen on the procedure and to come to accept the need for stoma; only after these issues are resolved can the patient provide final consent.

Surgical approach

PE cases are highly heterogeneous, and the surgery types vary considerably; however, our PE Unit adheres to certain principles for all cases. All patients undergo oral bowel preparation, as well as mechanical thromboprophylaxis, prior to surgery. Chemical thromboprophylaxis is not routinely administrated, with respect to the potential high-risk of bleeding related to the extra-fascial plane dissection requirement.

All of the 25 cases assessed in this study had dedicated anaesthetists and underwent the PE in the Lloyd-Davis position. For those patients requiring a high sacrectomy (S2 and above), a combined anterior and posterior jack-knife approach was used. After laparotomy and adhesiolysis, any suspicious peritoneal nodules were biopsied and sent for frozen section. Positivity for peritoneal disease would have precluded curative resection, triggering abandonment of the procedure; however, none of the cases in our series showed positivity or peritoneal recurrence during the surgical exploration.

In all of the 25 cases, *en bloc* resection was the surgical aim. The surgical planes had been determined preoperatively by consensus among all involved surgeons. If an organ was abutting the tumour, *en bloc* resection was performed. There was no attempt in any case of a trial of dissection for organ preservation to prevent tumour spillage. Ureteric stents were not routinely inserted if bladder or ureteric resection was planned.

The standard approach of anterior or posterior PE, in our PE Unit, is to mobilise the central pelvic compartment (*i.e.,* the rectum) immediately after ligating the inferior mesenteric vessels and performing transection of the distal sigmoid colon. The dissection continues along the total mesorectal excision (TME) plane, if feasible, and down to the pelvic floor. The dissection stops at the level of the organ involving the tumour. In pelvises restrained by adhesions or tumour, extra-fascial plane dissections are performed, but only after vascular control is obtained. Many of the 25 cases described herein necessitated cranial-to-caudal anterior compartment mobilization (*i.e.,* urogenital and gynecological organs) and transection, specifically at the urethra or vagina, before the final transection of the rectum.

In our PE Unit, frozen section is utilized to confirm clear histopathology margins in areas associated with perioperative doubt. Advanced energy medical devices are commonly employed for TME mobilization and pelvic wall dissection, in order to reduce blood loss. The appropriate laparoscopic lengths of these devices are determined according to the narrow-width and deep-depth of the pelvis.

The technique for lateral PE utilized in our case series to achieve clear margins was that described by Höckel *et al*[11] and Austin *et al*[12]. The anatomic approach of this technique reaches the plane lateral to the internal iliac vessels. Vascular control of common iliac vessels and external iliac vessels is first achieved with vessel loops, and the external iliac vessels are mobilized to allow easy access to the obturator canal. The internal iliac artery is usually ligated first, before the internal iliac vein is accessed and ligated. All subsequent distal branches are suture ligated. These internal iliac vessels are then resected *en bloc* with the tumour specimen.

In our case series, the external iliac artery resection was performed only after a graft from the common iliac to the femoral artery was created. In addition, all sciatic nerves were preserved, but the perineural sheath was resected *en bloc*, if required. Lateral node dissection was also performed if there were suspicious nodes noted preoperatively, or if the tumour extended to the area of the lateral pelvic sidewall. This dissection would commence from the aortoiliac bifurcation, proceed down to the nodes around the common and external iliac vessels, and down to the origin of the internal iliac vessels and the obturator canal (Figure 1).

In our PE Unit, for sacrectomies, the abdominal approach is used for low sacrectomy (S3/S4), as described by Solomon *et al*[13]. For the combined abdominal-perineal approach, the abdominal phase incorporates complete mobilization of the posterior plane, up to 1 cm from the level of the sacrectomy. Ligation of the various internal iliac vessel branches, particularly the sacral, visceral and gluteal veins, is performed. Preservation of the upper sacral nerves is paramount, and all presacral fascia and piriformis muscles are dissected free. The perineal phase begins with an elliptical skin incision, which is followed by dissection below the coccyx and up to the level of the S2/S3 junction posteriorly, with the gluteal muscles and sacrococcygeous ligaments being dissected free. The sacrectomy is then performed by 20-mm osteotome, applied transabdominally, in a medial to lateral manner; this is carried out with a surgical assistant located at the perineum and placing an osteotome below the sacrum to prevent damage or button-holing of the perineal skin (Figure 2). For our cases, the perineal defect was reconstructed by the plastic surgeon using either primary closure and biological mesh reinforcement or myocutaneous pedicle flap.

For high sacrectomy (S1/S2), the orthopaedic team

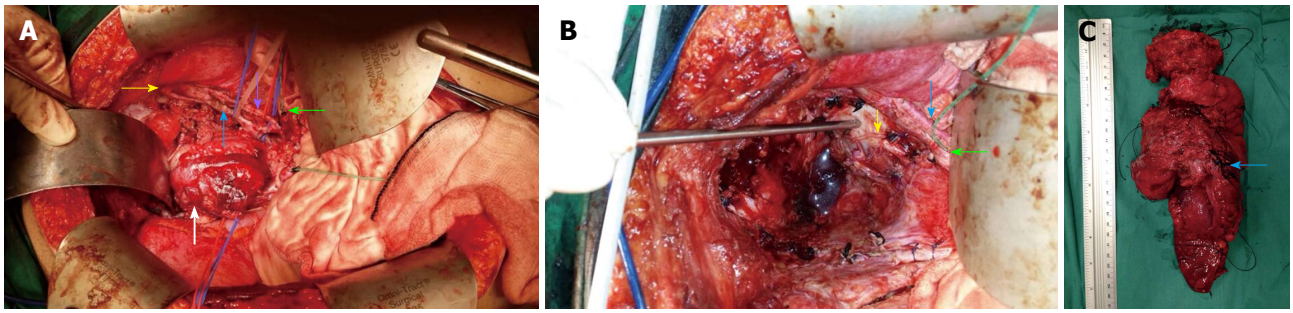

Figure 1 Total pelvic and lateral exenteration. A: A Deaver retractor was placed caudally (White arrow: Pelvic tumour; Yellow arrow: Right obturator nerve; Blue and purple arrows: Right internal iliac vein and artery respectively; Green arrow: Transected right distal ureter at pelvic brim with infant feeding tube inserted for intraoperative urinary diversion); B: Post-exenteration view showing the right internal iliac vessels, obturator nerve and pelvic lymph nodes excised and the metal vacuum tube pointed at exposed pelvic bone (Yellow arrow: Sciatic nerve; Blue arrow: Right external iliac vessels; Green arrow: Transected right distal ureter); C: Cicatrising tumour specimen showing invasion into bladder and right pelvic sidewall (Blue arrow: Right internal iliac vessels).

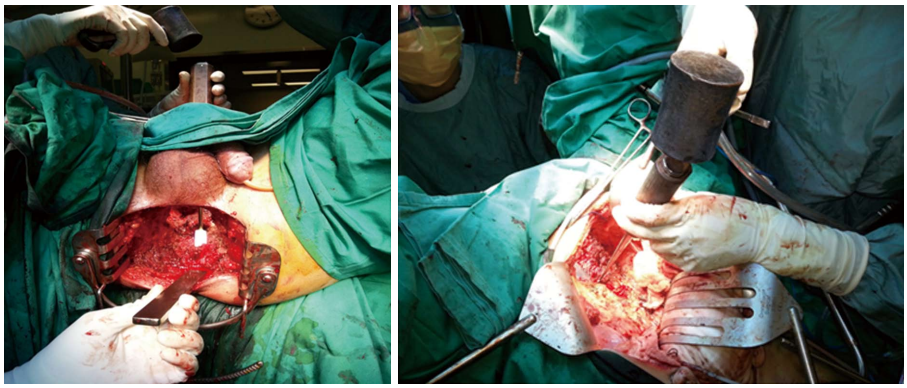

Figure 2 Demonstration of abdominal perineal approach for level S3 sacrectomy. The left panel (perineal view) demonstrates placement of the osteotomes posterior to the sacral bone in order to protect perineal skin while the surgeon transects the sacrum at S3 level in the right panel (abdominal view).

conducts the surgery with the patient in a prone jack-knife position. This procedure is performed only after complete mobilization of all vascular structures and organs off of the sacrum, down to the coccyx. Following ligation of all posterior internal iliac branches and completion of mobilization as described above, a penny towel pack is able to be placed between the sacrum and iliac vessels. The osteotomy site is marked anteriorly, using a drill. A myocutaneous flap is mobilized and tucked deep in the pelvis, a stoma is "matured" if necessary, and finally the abdomen is closed. After turning the patient to prone position, an incision is made down to the level of the sacrectomy and then transected with *en bloc* resection of the tumour. Reconstruction of the defect is then completed using the flap.

In our PE unit, an ileal conduit is commonly performed as the means of permanent urinary diversion. To avoid urinary complications, it is essential to have technical collaboration between the colorectal surgeons and the urologists. The most important technical step in ileal conduit formation is to ensure delicate handling of the ureters and ileum; the former must be meticulously mobilised with care to preserve ureteric vascularity. Transection of the ureters is performed as distal as possible, without compromising the oncological outcome. Ureteroenteric anastomosis is methodically performed, in order to achieve good tissue vascularity, in a tension-free manner and without malrotation of the ureters. These concepts are crucial to prevent urinary anastomotic leaks, conduit ischaemia and late ureteric strictures, while balancing the need for an adequate resection margin.

RESULTS

Patient demographics

Twenty-five consecutive cases were evaluated. The patient demographics and indications for surgeries are summarized in Table 1. The median length of follow-up period was 17.6 mo (range: 6.3-39.0 mo). The most common indications for PE were locally invasive rectal adenocarcinomas (13 cases, including 9 primary and 4 recurrent), followed by advanced colonic adenocarcinomas (5 cases, including 3 primary and 2 recurrent), recurrent cervical carcinomas (3 cases) and malignant sacral chordomas (3 cases). There were 10 TPEs performed, and the majority of these cases were combined with lateral PEs. Three out of those 10 TPE cases also had sacrectomy. Except for 4 isolated lateral PEs, anterior (1 of 4) and posterior PEs (5 of 7) were commonly performed in conjunction with lateral PEs. R0 resection was achieved in 16 cases (64%). These results are summarised in Table 2.

Table 1 Characteristics of patients who underwent pelvic exenteration

Variable	
Sex, *n* (%)	
Male	16 (64)
Female	9 (36)
Age, *n* (%)	Median, 61.9 yr (range, 30-72)
ASA score, *n* (%)	Ⅰ: 11 (44)
	Ⅱ: 13 (52)
	Ⅲ: 1 (4)
	Median, 2
ECOG status	0: 22 (88)
	1: 2 (8)
	3: 1 (4)
	Median, 0
Co-morbidities, *n* (%)	
Hypertension	6 (24)
Diabetes mellitus	5 (20)
Hyperlipidaemia	8 (32)
Ischaemic heart disease	1 (4)
Primary cancer type (*n* = 16)	
Colorectal	12
Chordoma	3
Gynaecological	1
Recurrent cancer type (*n* = 9)	
Colorectal	6
Gynaecological	3

ASA: American Society of Anesthesiologists; ECOG: Eastern Cooperative Oncology Group.

Morbidity and mortality

The median operative time was 11.5 h (range: 6.3-16.8 h). The median volume of blood loss was 3306 mL (range: 650-11000 mL), and the median volume of red cell transfusion was 1475 mL (range: 222-5565 mL). Of note, procedures combined with lateral PEs had higher blood loss (median: 2500 mL, range: 650-11000 mL). The highest blood loss in our series was 11 L, which occurred in a rectal cancer patient with 2nd occurrence of left pelvic wall nodal recurrence, and on who an isolated lateral PE was performed. This surgery was the 3rd procedure after initial ultra-low anterior resection and followed a prior attempt at lateral node dissection. After extensive adhesiolysis, the left pelvic nodal recurrence was resected *en bloc* with left distal ureter and internal iliac artery and vein. A segment of left external iliac vein was resected for margin, and a prosthetic graft reconstruction was made from common iliac to left femoral vein. The left ureter was reconstructed and re-implanted with a Boari flap.

The median length of ICU stay was 1 d (range: 0-8 d), and the median length of hospital stay was 21 d (range: 8-136 d). There was no perioperative mortality. The postoperative complications are summarized in Table 3. A total of 20 complications occurred in 12 patients. Three patients (12%) experienced major complications, including 2 patients (8%) with grade 3 Clavien-Dindo postoperative complications, which required further invasive interventions. The first patient with high body mass index (BMI) underwent redo-laparotomy for a torn ileal conduit mesentery bleed on postoperative day 1. The second patient, also with high BMI, underwent vertical rectus abdominis myocutaneous flap reconstruction and developed a postoperative large infective seroma in the abdominal wound site, which required percutaneous drainage on postoperative day 24. There was only one patient who required temporary renal dialysis (grade 4A Clavien-Dindo) following TPE with ileal conduit reconstruction, but no revision surgery was needed; the causes of acute renal failure were multifactorial, but did not include the newly-constructed ileal conduit. This patient's renal function gradually recovered, without long-term disability. The remaining 9 patients had grade 2 complications, which required pharmacological interventions.

Table 2 Pre-operative and operative treatment details

Incidence of neoadjuvant chemoradiation	
Primary cancer	
Colorectal	75%
Chordoma	0%
Gynaecological	0%
Recurrent cancer	
Colorectal	67%
Gynaecological	0%
Operative procedure, *n* (%)	
Total PE	1 (4)
Total PE with lateral exenteration	9 (36)
Anterior PE	3 (12)
Anterior and Lateral PE	1 (4)
Posterior PE	2 (8)
Posterior and Lateral PE	5 (20)
Lateral PE	4 (16)
Sacrectomy combined with any above PE procedures	9 (36)

PE: Pelvic exenteration.

Table 3 Clavien-Dindo classification of surgical complications, *n* = 12

Grade	Feature	*n*
2	Wound infection	6
	Urinary tract infection	4
	Venous access infection	4
	Prolonged ileus	1
	Deep vein thrombosis	1
	Acute myocardial infarction	1
3	Postoperative bleeding: Re-laparotomy	1
	Donor site-infected seroma percutaneous drainage	1
4	Temporary renal dialysis	1
Total adverse events		20

Short-term oncological outcome

During the study period, 18 out of 25 patients were in remission. There were 7 (30.4%) post-PE recurrences that presented during follow-up, and these included 2 with local regional recurrence, 2 with distant metastasis, and 3 with both regional and distant recurrences. The histopathological origin of cancer and postoperative microscopic margin status for each of these cases are

Table 4 Characteristics of post-pelvic exenteration recurrent diseases

Pre-PE status	Histology origin	Regional recurrence	Distant metastasis	Regional and distant	R0	R1
Primary	Colonic	0	0	1	1	0
Primary	Rectal	0	1	1	0	2
Primary	Sacral chordoma	1	0	0	1	0
Recurrent	Rectal	1	0	0	0	1
Recurrent	Cervical	0	1	1	2	0
Total		2	2	3	4	3

Both microscopic resection margin status and pre-exenteration primary or recurrent tumours do not show any statistically significant influence on post-exenteration recurrence (P = 0.67). PE: Pelvic Exenteration.

summarized in Table 4. There were no statistically significant relationships among microscopic resection margin status, histopathological origin of tumour and postoperative recurrence (P = 0.67); these results may, however, simply reflect the small size cohort of this study. Among these 7 cases, 4 of the patients died during follow-up. Two of the patients' deaths were attributed to cardiopulmonary failure from systemic disease burden. The remaining 2 patients' deaths were related to sepsis secondary to locoregional recurrences, with 1 having developed urosepsis from ileal conduit malignant stricture and the other having developed pelvic sepsis from malignant pelvic floor fistula. The overall median survival from surgery to death was 12 mo (range: 6.1-17.0 mo).

DISCUSSION

PE surgery has evolved over the decades. Brunschwig[1] originally developed PE as a palliative intervention, but-as detailed in the Introduction-the procedure had high morbidity and mortality rates and poor long-term outcome. These drawbacks precluded its widespread application by surgeons and acceptance by patients; and, despite its potentially life-saving benefits, this psychological and physical taxing operative procedure was considered with even more caution. However, constant evolution in chemoradiation interventions and surgical techniques, as well as better patient selection, have increased the safely of this procedure when performed by an experienced multidisciplinary team. Now, besides the survival benefits, there are also marked improvements to patients' quality of life.

Studies have shown that the oncological benefit of PE is best when a negative pathological margin can be achieved[2,10,14-16]. To assess our short experience using a multidisciplinary team approach for PE surgery, the outcomes of a series of 25 consecutive patients were evaluated based on morbidity, mortality and recurrence. A systematic review performed by Young *et al*[9], which incorporated 23 studies and 1049 patients as a benchmark, noted a 73% R0 resection rate (range: 42%-100%). In that same review, the median perioperative mortality rate was low, at 2.2%, with the majority ranging from 0% to 25%. Our case series demonstrated comparable outcomes, namely 64% R0 resection rate and 0% in-hospital or 30-d perioperative mortality rates. The postoperative complication rate in our case series was 48% but the actual serious morbidity (grades 3 and 4 Clavien-Dindo) was 12%, and two-third of the adverse events in our case series were grade 2 Clavien-Dindo that necessitated pharmacological treatment alone. This finding is comparable to the median rate of 57% that was reported from the systematic review[9]. Short-term follow-up in our case series found a recurrence rate of 28%. There was, however, no statistically significant relationship among pathological resection margin status and post-exenteration recurrence in our study; since this is likely due to a small sample size, we must await our series to expand further before survival benefit can be commented on.

While our case series was large enough to generally assess the learning curve of our PE Unit, our procedures were highly heterogeneous and included complex lateral and posterior PEs that are not commonly performed. The results provide validation that these techniques applied for PE surgery allow for good short-term outcomes; yet, the authors acknowledge that achieving better outcomes would rely also on better decision-making and patient selection. One of the first criteria of patient selection for such extensive surgery is physical fitness and minimal co-morbidities. In our study cohort, the median age of patients was 62-year-old, and the oldest patient was 72-year-old (who underwent surgery for sacral chordoma). In general, our patients were fit; the median ASA score was 2 and the median ECOG score was 0. The one exception was a 42-year-old woman, who underwent the surgery despite being ECOG grade 3 status due to a symptomatic pelvic recurrence that caused significant disability.

The post-surgery social aspects are other important issues that must be considered in the decision-making process. Many patients are reluctant to accept the physical, psychological and financial sacrifices required for the surgery. It is not uncommon that a patient ends up with two permanent ostomies and are then unable to overcome the perceived lack of independence and social stigma. In our case series, multiple consultations were required in order to obtain the appropriate informed consent from the patient and caregiver, with the time frame often being 4-6 wk.

It was crucial in our preoperative planning that attempts were made to obtain histological proof of the tumour

before PE, especially for cases of recurrent disease. This was achieved *via* endoscopic or percutaneous biopsy for accessible tumours. We also had to perform an open biopsy for 1 patient. Yet, this approach was considered especially important to aid in planning of the extra-fascial planes and because dissection is meant to avoid opening up of tumour planes and subsequent spillage of tumour cells. Obtainment of intraoperative biopsies of the tumours and subsequent frozen section histology can take time before proceeding to a PE, creating anxiety and uncertainty in both the patient and relatives, ultimately making the logistic planning of a multidisciplinary surgery difficult and inefficient. A confirmed preoperative diagnosis allows the patient to be convinced of the necessity of such extensive surgery and may avoid any potential medico-legal pitfalls.

Proper preoperative planning is necessary, with adequate time set aside for preanaesthetic assessment, a dedicated operative theatre list, invasive intraoperative haemodynamic monitoring and Level 1 rapid transfuser device set-up, if necessary. Adequate blood and ICU resources must be ensured before the operation commences. This operative planning incurs costs as well. Therefore, success of the programme long-term would also require cost-conscious practices or may negate support from the administrative side for these highly expensive and complex procedures.

For R0 resections, magnetic resonance imaging (MRI) has been shown to be a valuable tool to identify the anatomy of involved organs and to guide the extent of resection and reconstruction options, especially when reviewed by an experienced radiologist. In an expert's hand, the radiological accuracy of rectal cancer staging improves in sensitivity (from 77% to 96%) and specificity (from 40% to 74%)[17]. We have had the benefit in our team of a dedicated radiologist who specializes in evaluating all images after initial reporting. The key questions asked include the likelihood of involvement of contiguous organs, the presence of undiagnosed peritoneal disease, and, often, the difference between post-radiation fibrosis *vs* tumour. This is especially pertinent to determine if a low sacrectomy will be required to treat advanced or recurrent rectal cancers. On MRI of a previously irradiated rectal cancer, it can be difficult-even for an expert-to differentiate between viable residual tumour and post-treatment fibrosis[17]. In these instances, as well as when indeterminate loco-regional or systemic organ or nodal disease is encountered on anatomical imaging, the fluorodeoxyglucose positron emission tomography (PET) with CT scan can be utilized. PET CT scan has reported sensitivity of 91% and specificity of 76% for colorectal metastatic lesions, and sensitivity of 91% and specificity of 91% for colorectal recurrence[18]. In addition, PET CT scan can guide the surgical decision for pelvic lymph node dissection to avoid pelvic autonomic nerve injury or late lower limb lymphedema.

MRI PET scan has been introduced for rectal cancer, and shown improved accuracy of T-staging for cases in which standalone MRI and PET CT failed to define the nature of an avid lesion. A small case series study has shown promising results regarding the use of MRI PET as compared to PET CT, with a true positive rate of 86% for the former *vs* 71% for the latter in overall TNM staging[19]. MRI PET is not readily available in our practice; however, it may represent the next-generation of preoperative imaging for PE planning. In the case of isolated pelvic sidewall or nodal recurrence, where the tumour is not accessible for biopsy and the disease is not apparent, serial imaging and tumour marker surveillance should be conducted after endoscopic re-assessment (if accessible) for anastomosis or luminal recurrence.

For the future of PE surgery, there are proposals to adopt laparoscopic or robotic techniques, especially for colorectal and gynaecological malignancies, due to the potential benefit of the minimally invasive nature of these surgeries. There are some published reports of laparoscopic-assisted anterior PE or TPE in highly selected patients with rectal or gynaecological cancers[20-23]. The preliminary data have shown minimal blood loss, short hospital stays, low morbidity rates, and non-comprising short-term oncological outcome. The first report of robotic PE in advanced rectal cancer patients was published by Shin *et al*[24] in 2014. The authors reported on 3 consecutive male patients with locally advanced rectal cancer involving prostate and seminal vesicles. The robotic approach was performed with reduced operative time and blood loss. Except for one minor vesical-urethral anastomosis leak requiring temporary suprapubic cystostomy, there were no other major surgical complications. Oncologic outcomes were also favourable in that study. These reports have highlighted the possibilities of minimally invasive surgery in the setting of complex PE. However, the small cohorts on which they are based consist of highly selected patients who have participated in short-term follow-up, and wide adaptation of this novel approach will require larger clinical trials.

Our PE Unit has demonstrated a safe and effective approach to manage complex pelvic cancers, with acceptable morbidity rates, zero-rate mortality and equivalent oncologic outcomes. The success of managing this group of patients was made possible by careful patient selection, detailed preoperative planning, multidisciplinary teamwork and an adaptation of modern operative techniques and technologies.

COMMENTS

Background

Advanced pelvic tumour is a debilitating illness, which poses a formidable surgical challenge. Chemotherapy and radiotherapy often improve the symptoms, but the results are transient. As the disease progress to the terminal stage, many patients suffer from refractory pain, bleeding, malodourous fistula or pelvic sepsis. Pelvic exenteration (PE) is a combination of numerous extensive surgical procedures that aims to remove all the diseased organs in order to achieve a negative resection margin. This complex intervention is

currently the only curative option for advanced pelvic tumour.

Research frontiers

PE has long been associated with high morbidity and mortality rates. However, adaptation of contemporary perioperative medical care approaches and innovative surgical techniques has allowed PE to emerge as the mainstream intervention, offering a good curative rate with low morbidity and mortality rates in selected patients with locally advanced pelvic tumours. Due to the substantial postoperative physiological disturbances associated with PE and the need to attain a negative margin, the focus of recent research has been to identify the suitable patient through comprehensive preoperative screening, detailed radiological staging, and adjuvant downstaging chemoradiotherapy. In addition, the development of methodological lateral PE and abdominal-approach sacrectomy has helped to improve the oncological outcome.

Innovations and breakthroughs

In this study, the authors describe their initial experience and treatment strategy in a newly-established PE Unit that achieves low morbidity, zero-rate mortality and acceptable R0 resection rate. The short-term result is equivalent to other reports in the recent literature. The authors attribute this success to a dedicated multidisciplinary team, state-of-the art perioperative care and modern operative techniques.

Applications

This study provides a descriptive patient selection criteria, perioperative non-surgical treatment strategy, and operative techniques that will help to reduce postoperative PE complications and achieve good oncological outcomes.

Terminology

PE is a generic description of combined surgical procedures that were developed to remove the advanced pelvic tumour. Often, the advanced pelvic tumour has invaded into contiguous organs adjacent to the tumour origin, and therefore multiple surgical procedures are utilised in order to resect all diseased organs and achieve negative pathological resection margin. PE can be subgrouped into four types according to pelvic organs that are resected. Anterior PE involves removal of the upper rectum and genitourinary organs. Posterior PE involves removal of the rectum and reproductive organs, but spares the bladder. Total PE is defined as removal of the rectum, distal colon, genitourinary viscera, internal reproductive organs, draining lymph nodes and pelvic peritoneum. Lateral PE involves removal of the lateral pelvic lymph nodes along with diseased vascular and neural structures. After extensive resection, it is common to combine further procedures, such as permanent faecal or urinary diversion and perineal reconstruction, in order to maintain the physiology and to close the muscular defect.

Peer-review

The newly-established PE Unit reported by the authors offers state-of-the-art exenteration service in Singapore. This study confirms that the modern perioperative treatment strategy and multidisciplinary approach produced excellent short-term outcomes in the first 25 consecutive cases.

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ORIGINAL ARTICLE

Prospective Study

Introduction of laparoscopic gastrectomy for gastric cancer in a Western tertiary referral centre: A prospective cost analysis during the learning curve

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Author contributions: Stoot JH, Tegels JJ, Hoofwijk AG and Hulsewé KW did the study conceptualization; Tegels JJ, Spauwen FE and Silvius CE were responsible for data acquisition; Tegels JJ, Spauwen FE, Silvius CE and Stoot JH performed quality control of data and algorithms; Tegels JJ, Spauwen FE, Silvius CE, Hulsewé KW, Hoofwijk AG and Stoot JH performed data analysis interpretation; Tegels JJ performed statistical analysis; Tegels JJ, Spauwen FE and Silvius CE performed manuscript preparation; Tegels JJ was resposible for manuscript edition and first and final draft writing; Tegels JJ, Spauwen FE, Silvius CE, Hoofwijk AG, Hulsewé KW and Stoot JH performed manuscript reviews, significant writing contributions and reviewed and approved the final version of the manuscript.

Institutional review board statement: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Approval for this study was granted by the local Institutional Review Board/Ethical Standards Committee at Zuyderland Medical Centre before the start of this study. No informed consent was required for study participation as per Institutional Review Board/Ethical Standards Committee at Zuyderland Medical Centre.

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Abstract

AIM

To evaluate the costs of the introduction of a laparoscopic surgery program for gastric cancer in a Western community training hospital and tertiary referral centre for gastric cancer surgery.

METHODS

All patients who underwent surgery for gastric cancer with curative intent in 2013 and 2014 were prospectively included. Primary outcomes were costs regarding surgery and hospital stay.

RESULTS

Laparoscopic gastrectomy was used in 52 patients [mean age 68 years (± 9, range 50 to 87) years] and open gastrectomy was used in 25 patients [mean age 70 years (± 10, range 46 to 85)]. Mean costs (in euro's) of surgical instrumentation were significantly higher for laparo-

scopic surgery: 2270 ± 670 *vs* 1181 ± 680 in the open approach ($P < 0.001$). Costs of theatre use were higher in the laparoscopic group: mean 3818 ± 865 *vs* 2545 ± 1268 in the open surgery ($P < 0.001$). Total costs of hospitalization (*i.e.*, costs of surgery and admission) were not different between laparoscopic and open surgery, 8187 ± 4864 and 6152 ± 2680 respectively ($P = 0.729$). Mean length of hospital stay was 9 ± 12 d in the laparoscopic group *vs* 14 ± 14 d in the open group ($P = 0.044$).

CONCLUSION

The introduction of laparoscopic gastrectomy for gastric cancer coincided with higher costs for theatre use and surgical instrumentation compared to the open technique. Total costs were not significantly different due to shorter length of stay and less intensive care unit (ICU) admissions and shorter ICU stay in the laparoscopic group.

Key words: Laparoscopic surgery; Healthcare costs; Gastric cancer

Core tip: The introduction of laparoscopic surgery for gastric cancer did not seem to result in increased costs as compared to open gastrectomy for gastric cancer. Despite higher operating room costs (longer operating time and more costly operating room materials) costs were similar between the open and laparoscopic group due to reduced length of stay and complication rate in laparoscopic gastrectomy patients.

Tegels JJ, Silvius CE, Spauwen FE, Hulsewé KW, Hoofwijk AG, Stoot JH. Introduction of laparoscopic gastrectomy for gastric cancer in a Western tertiary referral centre: A prospective cost analysis during the learning curve. *World J Gastrointest Oncol* 2017; 9(5): 228-234 Available from: URL: http://www.wjgnet.com/1948-5204/full/v9/i5/228.htm DOI: http://dx.doi.org/10.4251/wjgo.v9.i5.228

INTRODUCTION

In patients with gastric cancer, surgical resection is the only treatment that can offer cure or increase long-term survival[1]. Laparoscopic surgery for gastric cancer has gained popularity despite initial concerns regarding safety and oncological adequacy[2]. Studies conducted in South Korea and Japan reported that laparoscopic gastrectomy is comparable to open gastrectomy with regard to surgical and oncological outcomes[2-4]. A meta-analysis by Memon *et al*[4] showed that laparoscopic procedures are associated with less blood loss but longer operation time. Many studies have reported outcomes of laparoscopic surgery for early gastric cancer (EGC), but several authors have shown that a laparoscopic approach can also be used in cases of advanced gastric cancer[5-7]. This makes it a potentially important strategy in Europe where the vast majority of patients present at stage Ⅱ or higher as opposed to Asian countries where EGC is far more common[8].

In the current economic climate, governmental organizations and health insurance companies have a major influence on the regulation of costs in healthcare. Moreover, surgeons often have to prove that new techniques are cost-effective for hospital organizations. To the best of our knowledge, no cost-analysis studies have yet been performed concerning the introduction of the laparoscopic procedure for gastric resections[2]. The aim of this study was to evaluate the costs of the introduction of a laparoscopic surgery program for gastric cancer in a Western community training hospital and tertiary referral centre for gastric cancer surgery.

MATERIALS AND METHODS

Patients

Introduction of laparoscopic gastrectomy was started in January 2013. All consecutive patients with gastric adenocarcinoma eligible for curative surgery from January 2013 to December 2014 were included. Whether the patient would undergo laparoscopic or open surgery depended on the surgeon's experience in laparoscopic gastric surgery and surgeon preferences. Patients who underwent multivisceral resections were not included in this study. All data were collected in a prospective database. If non-equal groups were obtained, a consecutive number of patients who underwent open gastrectomy with curative intent would be retrospectively included to create two groups of equal size. All data, including intraoperatively used materials (*e.g.,* electrosurgical devices, staplers, suture materials and reusable instruments) were all available through the hospitals fully digitized patient information system, also for retrospectively included patients. This observational study collected data concerning direct hospital-related costs and complication rates and admission length. The charlson comorbidity index (CCI) was used to classify comorbidities in patients[9]. Patients received care as usual. Approval for this study was obtained from the medical ethics committee.

Preoperative stage

Preoperatively all patients underwent gastroesophageal endoscopy and biopsies were taken to confirm the diagnosis. Further preoperative staging was done with computed tomography (CT) of the abdomen and chest. Magnetic resonance imaging and/or positron emission tomography/CT imaging was selectively performed when liver lesions were visible on CT-imaging. Multidisciplinary consensus regarding the treatment was obtained in all cases. Neo-adjuvant chemotherapy was administered whenever patient condition and comorbidities would

Table 1 Costs of disposable instruments for laparoscopic surgery and open surgery

Item	Price
Laparoscopic surgery	
Ligasure Impact 5 mm, Medtronic, Ireland[1]	€ 448.22
Ligasure Impact 10 mm, Medtronic, Ireland[1]	€ 376.00
Autosuture endobag, endocatch, Medtronic, Ireland[1]	€ 63.56
Autosuture EndoGIA 12 mm, Medtronic, Ireland[1]	€ 203.00
Reload EndoGIA purple 45, Medtronic, Ireland[1]	€ 173.00
Reload EndoGIA purple 60, Medtronic, Ireland[1]	€ 176.00
Reload EndoGIA Gold 45, Medtronic, Ireland[1]	€ 181.00
Endopaddle 12 mm, Medtronic, Ireland[1]	€ 81.84
Alexis small/medium, applied medical	€ 31.00
Endoshear 5 mm, Medtronic, Ireland[1]	€ 73.50
EEA XL, Covidien United States	€ 439.62
EEA Orvil, Covidien United States	€ 94.20
Bladeless trocar 5 mm, Medtronic, Ireland[1]	€ 48.88
Bladeless trocar 5-12 mm, Medtronic, Ireland[1]	€ 48.88
Blunt trocar 5-12 mm, Medtronic, Ireland[1]	€ 47.81
Pyramidal bladed trocar 10-15 mm, Medtronic, Ireland[1]	€ 80.43
Hem-o-lok L filling, Weck United States	€ 23.00
Hem-o-lok XL filling, Weck United States	€ 23.00
Open surgery	
Ligasure Impact, Medtronic, Ireland[1]	€ 343.80
Purssting stapler, Medtronic, Ireland[1]	€ 57.34
TA Green 30, Medtronic, Ireland[1]	€ 106.30
Reload TA Green 30, Medtronic, Ireland[1]	€ 60.50
TA Green 60, Medtronic, Ireland[1]	€ 110.09
Reload TA Green 60, Medtronic, Ireland[1]	€ 65.00
GIA Blue 60, Medtronic, Ireland[1]	€ 119.68
Reload GIA Blue 60, Medtronic, Ireland[1]	€ 75.51
GIA Blue 80, Medtronic, Ireland[1]	€ 147.48
Reload GIA Blue 80, Medtronic, Ireland[1]	€ 81.74
GIA Green 80, Medtronic, Ireland[1]	€ 148.96
Reload GIA Green 80, Medtronic, Ireland[1]	€ 81.74
CEEA 21, Medtronic, Ireland[1]	€ 408.97
CEEA 25, Medtronic, Ireland[1]	€ 384.48
CEEA 28, Medtronic, Ireland[1]	€ 388.33
CEEA 25 XL, Medtronic, Ireland[1]	€ 439.62

[1]Formerly Covidien United States.

allow.

Outcome measurement

Primary outcomes included costs regarding surgery and hospital stay. Costs were obtained from the Financial Controllers of the involved departments. Data (*e.g.*, duration of operation, use of disposables) were collected prospectively. For patients who were retrospectively included all data were available through the electronic patient record system. This system also recorded which disposable and reusable operating theatre materials were used during surgery. Costs of ward stay were 180 euro's per day; ICU admission was 665 euro's per day. Costs of operating theatre use were hourly rates for surgery and anesthesiology combined at 800 euro's per hour. Sterilization costs of reusable instruments were also accounted for and varied for different types of surgical sets that were used. All used instruments, disposable and reusable, were noted during the procedure by one of the operating room nurses on a prepared list that was provided to prospectively collect data. Costs of the disposable instruments for the laparoscopic and open surgery are shown in Table 1. Postoperatively all patients were admitted to the recovery ward before they were transferred to the general ward. For laparoscopic and open gastrectomy, costs of both disposable and reusable instruments, operating theatre use, ICU stay and hospital stay were calculated separately.

Secondary outcomes were estimated blood loss, duration of operation, length of ICU stay, length of hospital stay, anastomotic leakage rate and complications. Complications were graded according to Clavien-Dindo classification: Grade 3a (*i.e.,* complication requiring reintervention) or greater was considered a major complication[10]. Tumour stage was classified in line with the American Joint Committee on Cancer tumor node metastasis 5th edition.

Surgical technique and postoperative care

Open surgery was performed by three surgeons prior to introduction of laparoscopic surgery for gastric cancer. All three had extensive experience in open surgery for gastric cancer.

Laparoscopic procedures were performed by two the abovementioned three surgeons. Both surgeons also had extensive prior expertise in laparoscopic surgery for other gastrointestinal malignancies mainly colorectal surgery and gastric GIST tumours. Prior to introducing laparoscopic surgery for gastric cancer, specific expertise and proficiency was obtained by the laparoscopic surgeons by taking expert courses in the Netherlands and Singapore.

Surgical resections for gastric malignancy were defined as either distal or total gastrectomies. The type of resection performed depended on the localization and depth of invasion of the tumour. In both open and laparoscopic surgery, a standard D2 or D1+ lymph node dissection (dissection of group 1 and number 8a and 9 lymph nodes) was performed in accordance with Dutch guidelines. Continuity of the gastrointestinal tract in subtotal gastrectomies was restored either by a Billroth-Ⅱ or a Roux-en-Y reconstruction. In the case of a total gastrectomy a Roux-en-Y reconstruction was always performed. Patients were not routinely admitted to the ICU postoperatively. ICU admission was always for complication management (*e.g.*, sepsis, pulmonary complications).

Postoperative care of both open and laparoscopic patients included several aspects of a multimodal perioperative Enhanced Recovery After Surgery (ERAS) program for gastrointestinal cancer[11]. These include early enteral feeding (*i.e.,* resumption of liquids on postoperative day one) and early mobilization. Follow-up of the patients after discharge was performed periodically. Follow-up consisted of physical examination, blood tests, and CT-imaging if indicated.

Statistical analysis

Data were analyzed using SPSS 20 (IBM Corp. Armonk,

Table 2 Baseline characteristics *n* (%)

	Open gastrectomy (*n* = 25, 32%)	Laparoscopic gastrectomy (*n* = 52, 68%)	*P* value
Age	70.0 (± 10, 46-85)	68 ± 9, 50-87)	0.470
Sex (male/female)	17/8	32/20	0.623
BMI[1]	25 ± 4, 18-36	25 ± 5, 15-38	0.824
CCI[2]			0.158
0-2	16 (64)	27 (52)	
3-4	7(28)	11 (21)	
> 4	2 (8)	14 (27)	
Tumour stage[3]			0.681
0	0 (0.0)	2 (4)	
1^1	4 (16)	11 (21)	
1^2	6 (24)	15 (29)	
2	5 (20)	4 (8)	
3^1	4 (16)	10 (19)	
3^2	3 (12)	4 (8)	
4	3 (12)	6 (11)	
Subtotal gastrectomy	16 (64)	38 (73)	0.436
Total gastrectomy	9 (36)	14 (27)	
Neoadjuvant chemotherapy	11 (44)	36 (69)	0.046

[1]BMI: Body mass index in kg/cm^2; [2]CCI: Charlson comorbidity index; [3]In accordance with tumor node metastasis 5th edition.

NY). Continuous variables were expressed as mean ± SD or mean (range) if appropriate. χ^2 tests were used to compare the difference in frequencies of categorical variables. To compare the means of two independent samples, *t*-tests and non-parametric tests were used. The threshold for statistical significance was set at a *P*-value of < 0.05.

RESULTS

Baseline characteristics

A total of 77 patients underwent gastrectomy with curative intent from January 2013 to December 2014. The laparoscopic approach was used in 52 (68%) patients. The open approach was used in 25 (32%) patients. There were no statistically significant differences in sex, age, CCI (*i.e.,* comorbidities) or tumour stage. Patients undergoing laparoscopic gastrectomy had significantly more frequently received neoadjuvant chemotherapy (69% *vs* 44%, *P* = 0.046) (Table 2).

A consecutive series of 30 patients who underwent open surgery were included retrospectively, these patients underwent surgery between May 2012 and January 2013. These patients did not differ from patients who underwent open surgery in the prospective series with regards to the baseline characteristics mentioned in the prospective group.

Primary outcome

The costs (in euro's) of surgical instrumentation were significantly higher for laparoscopic surgery compared to open gastrectomy, 2270 ± 670 and 1181 ± 680 respectively, *P* < 0.001 (Table 3). Also, the costs of theatre use were significantly higher in the laparoscopic group compared to open gastrectomy, 3819 (± 865) and 2545 ± 1268 respectively, *P* < 0.001. Costs of general ward stay were significantly lower in the laparoscopic group compared to open gastrectomy, 1381 ± 1298 and 2218 ± 1810 respectively, *P* = 0.023. ICU stay and total admission costs (*i.e.,* ward stay and ICU stay combined) were not significantly different. The total costs of admission and surgery did not significantly differ between open and laparoscopic gastrectomy, 7672 ± 8064 and 8187 ± 4863 respectively, *P* = 0.729.

Table 3 Primary outcome, costs of surgery, hospital admission and intensive care unit stay

Costs (in euro's)	Open gastrectomy (*n* = 25, 32.5%)	Laparoscopic gastrectomy (*n* = 52, 67.5%)	*P* value
Surgical instrumentation	1181 ± 680	2270 ± 670	< 0.001
Operating theatre use	2545 ± 1268	3819 ± 865	< 0.001
Ward stay	2218 ± 1810	1381 ± 1298	0.023
ICU stay	1729 ± 6499	716 ± 3299	0.366
Admission	3947 ± 6719	2097 ± 4419	0.153
Total costs	7673 ± 8064	8187 ± 4864	0.729

ICU: Intensive care unit.

When a retrospective consecutive series of open gastrectomies was included to obtain equal sized groups (*i.e.*, 55 open *vs* 52 laparoscopic gastrectomies), total admission costs were significantly lower in the laparoscopy group, 2097 ± 4420 *vs* 4611 ± 7991, *P* = 0.048. Costs difference of total hospitalisation (*i.e,* operating theatre and ward stay) between open and laparoscpic gastrectomy was smaller at 8187 ± 4868 for laparoscopic patients *vs* 7915 ± 8653 for patients who underwent open surgery, *P* = 0.843.

Secondary outcomes

Comparison between the two techniques showed that total theatre time utilized was 191 min ± 95 for the open procedure and 286 min ± 65 for the laparoscopic gastric resection (*P* < 0.001) (Table 4). Results for secondary outcome parameters are listed in Table 4. Mean intraoperative blood loss was significantly less in the laparoscopic gastrectomy group (267 mL *vs* 592 mL, *P* = 0.002). In three cases, the laparoscopic approach was converted to an open procedure. In one case this was due to a splenic rupture, which was caused during laparoscopic surgery. In the other two patients the reason of conversion to an open procedure was a limited view of suspected ingrowth of tumour in the pancreas.

Laparoscopic gastrectomy was associated with a lower rate of overall complications and major complications, 16 (31%) *vs* 15 (60%), *P* = 0.025 and 6 (12%) *vs* 7 (28%), *P* = 0.104 respectively. Anastomotic leakage rates were higher in patients undergoing open gastrectomy than laparoscopic gastrectomy 2 (12%) and 2 (4%) respectively, *P* = 0.322. The differences in major complications and anastomotic leakage rates were not statistically significant in the prospective series. Also, patients who underwent laparoscopic resection had a shorter length of hospital stay and ICU stay (Table 4).

Table 4 Secondary outcome parameters

	Open gastrectomy (n = 25, 32.5%)	Laparoscopic gastrectomy (n = 52, 67.5%)	P value
Intraoperative blood loss (mL)	592 (± 529, 100-2500)	267 (± 316, 20-2000)[1]	0.002
OR time (min)	191 (± 95, range 95-554)	286 (± 65, range 207-597)	< 0.001
Lymph node yield (n)	25 (± 10, 751)	26 (± 8, 10-47)	0.651
Any complication	15 (60%)	16 (31%)	0.025
Grade Clavien-Dindo ⩾ 3a	7 (28%)	6 (12%)	0.104
Anastomotic leakage	3 (12%)	2 (4%)	0.322
Mean length of stay (d)	15 (± 14, 5-59)	9 (± 12, 2-84)	0.044
Mean ICU stay (d)	3 (± 10, 0-49)	1 (± 5, 0-35)	0.366
Readmission	4 (16%)	6 (12%)	0.720

[1]Five missing values for intraoperative blood loss in laparoscopic group. ICU: Intensive care unit.

When comparing equal sized groups (*i.e.*, 55 open and 52 laparoscopic gastrectomies), significantly more major complications occur in the open surgery group, 17 (31%), compared to the laparoscopic group, 6 (12%), P = 0.019. Also, the anastomotic leakage rate was significantly higher in the open surgery group at 10 (18.2%) compared to 2 (4%) in the laparoscopic group, P = 0.029.

In the prospective series two patients died after surgery 1 (4%) after open gastrectomy and 1 (2%) after laparoscopic gastrectomy. In the total series (*i.e.,* including the restrospective series of open gastrectomies) four patients (7.3%) died after open gastrectomy, three died after septicemia from anastomotic leakage with one patient who also had a concurrent pancreatic leakage. One patient died of a severe aspiration pneumonia. One patient (1.9%) died after a laparoscopic gastrectomy from intestinal ischemia of the right and transverse colon.

Both techniques had a similar lymph node yield: mean 29 ± 10 and 26 ± 8.5 for open and laparoscopic gastrectomy respectively (P = 0.103). There were three cases of microscopically irradical resection: One in the open group and two in laparoscopic gastrectomy group (P = 0.614). Analysis of equal sized groups (*i.e.,* 55 open *vs* 52 laparoscopic gastrectomies) resulted in similar results for the abovementioned secondary outcome parameters.

DISCUSSION

The aim of this study was to evaluate the costs of laparoscopic surgery for gastric cancer during the introduction of this new technique in a tertiary referral centre. The results show a significant increase in costs of surgery associated with the laparoscopic procedure. These costs are mainly due to increased use of (non-) disposable instrumentation and theatre time. The secondary outcomes suggest that laparoscopic gastrectomy is safe. This is represented by less blood loss, and less (major) post-operative complications in laparoscopic surgery. With regards to oncological safety the number of harvested lymph nodes and microscopically irradical resections were equal in laparoscopic and open surgery. Only two patients died in this study, one following open and one following laparoscopic gastrectomy.

This study was conducted at the time when laparoscopic approach was introduced in our tertiary referral hospital for gastric cancer. The complexity of the laparoscopic approach is one of the reasons for a more time-consuming procedure. As surgeons gain experience, operative time is expected to decrease and theatre costs (at an hourly rate) will decline. Moreover, knowledge of the postoperative care on the clinical wards and safety of earlier discharge (ERAS) for patients who underwent laparoscopic as well as open surgery may help reduce hospital stay. This study shows positive results with regards to financial aspects of laparoscopic surgery even during the introduction and learning curve phase of its introduction.

Even though the duration of operation is expected to decline, the longer operative time compared to open surgery will probably remain. This has been shown in larger meta-analyses with weighted mean differences ranging from + 48 to + 82 min of longer operative time for laparoscopy[12-14].

These meta-analyses also show several other advantages of laparoscopic surgery compared to open surgery such as significantly shorter hospital stay (2.5-3.6 d) and significantly lower complication rates[13,14]. These differences can be expected to be associated with lower costs. Moreover, laparoscopic gastrectomy has been shown to be associated with improved quality of life[15]. Studies in liver surgery, pancreatectomy and wedge resections for gastrointestinal tumours, have shown that laparoscopic surgery has the same advantages discussed above compared to open surgery (*e.g.,* shorter hospital stay, less intraoperative blood loss, decreased medical complications and no differences in operative mortality)[16-18]. For pancreatic and wedge resections this was performed at the cost of a longer operative time and a more expensive procedure due to costly surgical instruments[16,17]. In these studies increased costs associated with the procedure and instrumentation are offset by a reduction in other costs (*e.g.,* shorter hospital stay). This possibly makes laparoscopy a viable and cost effective option.

Another potential cost benefit of laparoscopic surgery could be found in long term complications of open abdominal surgery. Incidence of incisional hernia can be expected to be much lower in laparoscopic surgery compared to patients who underwent midline laparotomy. Therefore costs of treating incisional hernia might be

lower in laparoscopic compared to open surgery for gastric cancer.

Multimodal fast-track programs such as ERAS could further decrease hospital stay and complication rates and therefore costs. A fast-track program in laparoscopic gastrectomy for gastric cancer has been shown to be associated with decreased hospital stay and costs[19].

One of the main limitations of this study is its non-randomized design. Therefore a selection bias cannot be excluded. Also the non-equal sized groups is a consequence of this fact. By partially retrospectively studying prospectively maintained digital registration data of used materials an effort could be made to compare equal sized groups. Most data and all costs-related data regarding laparoscopic procedures however were collected prospectively. Despite this, statistically significant differences were shown for the primary and secondary outcomes. No definitive conclusions can be drawn with regard to aspects such as postoperative complications and long term oncological safety. However, secondary outcomes show differences in favor of laparoscopic surgery. These are in line with other studies and show a shorter length-of hospital stay and fewer complications. Another limitation is that only patients who underwent surgery with curative intent for gastric adenocarcinoma were included. No conclusions can be drawn with regard to costs of palliative resections.

In conclusion, during the introduction of a laparoscopic gastrectomy programme for gastric cancer costs for theatre use and surgical instrumentation were higher compared to the open technique but overall costs were similar due to reduced length of stay and lower complications rates (and therefore lower ICU admission rates and costs). Similar results regarding surgical safety, feasibility and post-operative complications between laparoscopic and open gastrectomy were found. Larger prospective studies will be needed to determine cost effectiveness of laparoscopic surgery for gastric cancer.

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COMMENTS

Background

Laparoscopic surgery for gastric cancer has gained popularity despite initial concerns regarding safety and oncological adequacy. Studies conducted in Korea and Japan reported that laparoscopic gastrectomy is comparable to open gastrectomy with regard to surgical and oncological outcomes.

Research frontiers

A meta-analysis by Memon *et al* showed that laparoscopic procedures are associated with less blood loss but longer operation time. Many studies have reported outcomes of laparoscopic surgery for early gastric cancer, but several authors have shown that a laparoscopic approach can also be used in cases of advanced gastric cancer.

Innovations and breakthroughs

The authors to evaluate the costs of the introduction of a laparoscopic surgery program for gastric cancer in a Western community training hospital and tertiary referral centre for gastric cancer surgery.

Applications

Larger prospective studies will be needed to determine cost effectiveness of laparoscopic surgery for gastric cancer.

Peer-review

An interesting manuscript describing cost analysis for laparoscopic gastric cancer surgery. It contains an important message for gastric cancer surgeons, to provide proof in favor of laparoscopic surgery for fellow surgeons, hospital directory boards and insurance compagnies. Nice short and concise manuscript.

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