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REVIEW

# Robotic rectal surgery: State of the art

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## Abstract

Laparoscopic rectal surgery has demonstrated its superiority over the open approach, however it still has some technical limitations that lead to the development of robotic platforms. Nevertheless the literature on this topic is rapidly expanding there is still no consensus about benefits of robotic rectal cancer surgery over the laparoscopic one. For this reason a review of all the literature examining robotic surgery for rectal cancer was performed. Two reviewers independently conducted a search of electronic databases (PubMed and EMBASE) using the key words "rectum", "rectal", "cancer", "laparoscopy", "robot". After the initial screen of 266 articles, 43 papers were selected for review. A total of 3013 patients were included in the review. The most commonly performed intervention was low anterior resection (1450 patients, 48.1%), followed by anterior resections (997 patients, 33%), ultra-low anterior resections (393 patients, 13%) and abdominoperineal resections (173 patients, 5.7%). Robotic rectal surgery seems to offer potential advantages especially in low anterior resections with lower conversions rates and better preservation of the autonomic function. Quality of mesorectum and status of and circumferential resection margins are similar to those obtained with conventional laparoscopy even if robotic rectal surgery is undoubtedly associated with longer operative times. This review demonstrated that robotic rectal surgery is both safe and feasible but there is no evidence of its superiority over laparoscopy in terms of postoperative, clinical outcomes and incidence of complications. In conclusion robotic rectal surgery seems to overcome some of

technical limitations of conventional laparoscopic surgery especially for tumors requiring low and ultra-low anterior resections but this technical improvement seems not to provide, until now, any significant clinical advantages to the patients.

**Key words:** Robotic surgery; Robotic rectal surgery; DaVinci rectal surgery; Robotic rectal cancer; Robotics for rectal cancer; Robotic rectal resection



**Core tip:** Laparoscopic rectal surgery has progressively expanded. However it has some technical limitations. The need to overcome these limitations leads to the development of robotic platforms. Although the positive feedback is by the surgeons, there is still no evidence in literature about the superiority of robotic rectal surgery when compared to traditional laparoscopy.

Staderini F, Foppa C, Minuzzo A, Badii B, Qirici E, Trallori G, Mallardi B, Lami G, Macrì G, Bonanomi A, Bagnoli S, Perigli G, Cianchi F. Robotic rectal surgery: State of the art. *World J Gastrointest Oncol* 2016; 8(11): 757-771 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i11/757.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i11.757

## INTRODUCTION

Laparoscopic colorectal surgery has progressively expanded since a number of randomized controlled trials (RCTs)[1-3], review articles[4,5], meta-analysis[6] and case series[7] have demonstrated its better postoperative outcomes when compared to open surgery. However, laparoscopic surgery has some technical limitations such as poor ergonomics, 2-dimension view, coning and fulcrum effect, that may influence surgery in narrow anatomical fields such as in the pelvis during rectal surgery. The need to overcome these limitations leads to the development of robotic platforms. The da Vinci robotic surgical system is the only totally robotic platform available. After approval by Food and Drug Administration in 2000, its use progressively spreaded as demonstrated by the increasing number of publications. Three-D high definition vision, wrist-like movement of instruments (endowrist™), stable camera holding, motion filter for tremor-free surgery and improved ergonomics for the surgeon are the advantages of the robotic system that may make rectal surgery more affordable and theoretically should provide better outcomes for the patient. Although the positive feedback is by the surgeons, there is still no evidence in literature about the superiority of robotic rectal surgery when compared to traditional laparoscopy. The aim of this study was to review the rapidly expanding literature in order to focus on the current state and assess any benefits of robotic rectal cancer surgery.

## RESEARCH AND LITERATURE

A review of the literature examining robotic surgery for rectal cancer during the period from 2000 to 2015 was performed. Two reviewers independently conducted a search of electronic databases (PubMed and EMBASE) using the key words "rectum", "rectal", "cancer", "laparoscopy", "robot". The reference lists provided by the identified articles were additionally hand-searched to prevent article loss by the search strategy. This method of cross-references was continued until no further relevant publications were identified. The last search was performed on December 2015. Inclusion criteria were prospective, retrospective, randomized, comparative studies about robotic rectal surgery for cancer including anterior resections, low anterior resections, ultralow anterior resections, abdominoperineal resections, proctectomies, proctocolectomies. Exclusion criteria were: Abstracts, letters, editorials, technical notes, expert opinions, reviews, meta-analysis, studies reporting benign pathologies, studies in which the outcomes and parameters of patients were not clearly reported, studies in which it was not possible to extract the appropriate data from the published results, overlap between authors and centers in the published literature, non-English language papers.

The literature search yielded 266 papers, the process is listed in Figure 1. After the 1st filtering, the remaining 60 studies were 33 comparative, 26 case series, and 1 RCT. Then 17 studies were excluded due to duplicated data. They were 7 comparative and 9 case series. After this process a total of 43 papers, 27 comparative including only 1 RCT and 16 case series were included and reviewed.

## STUDIES OVERVIEW

The number of publications about robotic rectal surgery for cancer has been constantly increasing. Among the papers we included there was only 1 paper per year published in 2006, 2007, 2008, 3 papers in 2009, 2 in 2010, 5 per year in 2011 and 2012, 10 in 2013 and 15 in 2014. With regard to the nationality of the 1st author there were 16 studies in the South Korea (37.2%), 11 in the United States (25.5%), 4 in Italy (9.3%), 2 in Turkey (4.6%), 2 in the Singapore (4.6%), 1 in Japan (2.3%), 1 in Denmark (2.3%), 1 in Spain (2.3%), 1 in Romania (2.3%), 1 in Brazil (2.3%), 1 in Canada (2.3%), 1 in Taiwan (2.3%), 1 in China (2.3%) (Table 1).

### *Surgical technique*

A total of 3013 robotic operations were performed. Sixteen studies[10,12,14,16,17,22,23,25,27,28,37,38,40-42,48] (1257 patients) reported a totally robotic procedure which was carried out with either a single[10,16,17,22,23,25,27,28,37,38,40-42,48] or a double docking[12,28] technique. In 22 studies[8,13,15,18,20,21,25,26,30-34,36,39,43-47,49,50] (1384 patients) an

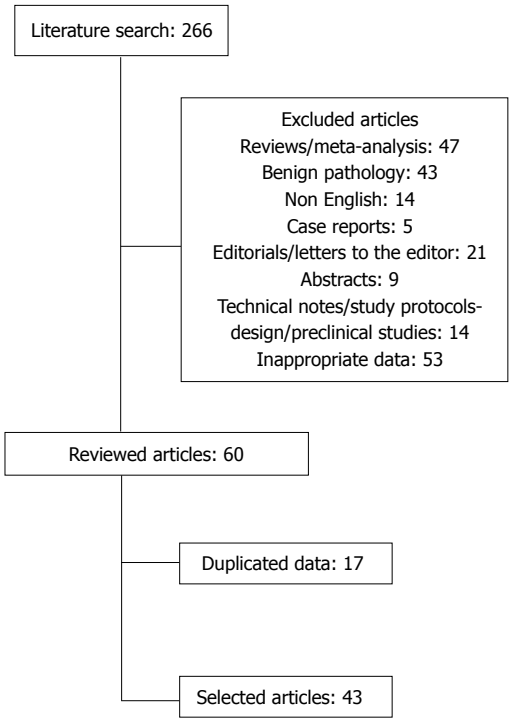


**Figure 1 Flow diagram of literature search.**

hybrid robotic technique was performed: The inferior mesenteric vessels ligation and splenic flexure mobilization were performed laparoscopically whereas pelvic dissection and total mesorectal excision were performed robotically. In 5 studies[9,11,19,29,35] (372 patients) the robotic technique was not specified. Laparoscopic procedures described in the 27 comparative studies[8-33] were performed in the same manner as robotic surgery using laparoscopic instruments (Table 1).

### *Demographics and preoperative data*

Most of patients were male (1911, 63%), the mean age was 58, the mean BMI was 26.6. Nine hundred-eight patients (20%) underwent a neoadjuvant chemotherapy, 71 (2.3%) a neoadjuvant chemo-radiotherapy and 8 (0.2%) radiotherapy only. With regard to the type of operation, 1450 (48.1%) were low anterior resections, 997 (33%) were anterior resections (AR), 393 (13%) ultra-low anterior resections (ULAR) and 173 (5.7%) abdominoperineal resections (APR). In the studies where the type of operation was not specified and where it was stated that a TME was performed[27,29,41] we assumed that all operations were low anterior resections (LAR) (Table 2)

### *Operative data*

The mean robotic operative time ranged from 202 min[31] to 485.8 min[17]. For the 1345 laparoscopic patients in the selected comparative studies the mean operative time ranged from 158.1[30] to 374.3 min[17]. This difference was statistically significant in 12 comparative studies[10,14,17-24,27,28,30] with a longer time for robotic surgery. Levic *et al*[9] were the only authors that reported a longer laparoscopic operative time ($P$ = 0.055), but all interventions were performed with a single port technique (Table 3).

The estimated blood loss (EBL) was not reported in 14 studies. The mean value ranged from 17 mL[36] to 280 mL[14] with the robotic approach and from 59.2[18] to 271.4[15] in the laparoscopic group. Among 16 comparative studies[8-10,12-15,17,19-21,23,24,29,31,33] that evaluated the EBL only Kang *et al*[23] and Erguner *et al*[21] reported a significantly lower EBL with the robotic approach when compared to the laparoscopic one.

Thirty seven studies reported the conversion rate to open surgery. Three[8,22,31] out of 22 comparative studies[8-15,17,19-25,28-33] showed a significantly lower conversion rate in the robotic series when compared to laparoscopy. The difference in overall conversion rate reported by Ielpo *et al*[14] was not statistically significant. However, when data were analyzed according to the tumor location (upper, mid, lower rectum), the conversion rates between robotic and laparoscopic procedures for lower rectal cancers were respectively 1.8% and 9.2% ($P$ = 0.04).

The rate of patients that underwent a protective ileostomy creation ranged from 0%[30] to 100%[10] both in the robotic and laparoscopic group. The difference in protective ileostomy creation was statistically significant in 5 studies. Kuo *et al*[17] reported a lower rate in the robotic *vs* the laparoscopic group whereas Saklani *et al*[19], Erguner *et al*[21], Kim *et al*[25], Baek *et al*[29] showed a lower rate in the laparoscopic *vs* the robotic group.

### *Postoperative data*

The mean postoperative day to first flatus ranged from 1.9[48] to 3.2[30] d in the robotic cases and from 2.4[23] to 3.4[17] in the laparoscopic ones. No statistically significant difference between robotic and laparoscopic cases was reported in any of the articles reviewed (Table 4).

The day of first postoperative liquid diet was available in 11 studies[6,22,27,29,34,36,43,45,47,48,50] ranging from 1[16] to 3.9[45] d in the robotic cases. Only two[22,29] comparative studies reported the first postoperative liquid diet in their robotic and laparoscopic series, in one[22] of these the difference was statistically significant in favour of robotic surgery (3 d *vs* 5 d, $P$ = 0.005).

The day of first postoperative solid diet was available in 11 studies[8,10,13,17,19,23-25,30,34,37] ranging from 2.58[10] to 7.5[18] d in the robotic cases and from 2.48[10] to 7.7[18] d in laparoscopic cases. Among 9 comparative studies[8,10,13,17,19,23-25,30] only Kang *et al*[23] reported a significant earlier oral intake in the robotic group (4.5 d *vs* 5.2 d. $P$ = 0.004) when compared to the laparoscopic one.

The mean length of hospital stay ranged from 4.5[33] to 14.2[17] and from 3.6[33] to 15.1[17] d after robotic and laparoscopic surgery respectively. Among 8 comparative studies, Tam *et al*[15], Levic *et al*[9] and Park *et al*[30] reported a shorter length of stay in their laparoscopic series whereas 5[8,22-24,32] studies reported a significant

**Table 1 Studies overview**

| Ref. | Year | Country | Study design | Surgical technique | Platform | No. of pts Robot | No. of pts Lap | No. of pts Open |
|---|---|---|---|---|---|---|---|---|
| Park *et al*[8] | 2015 | South Korea | Comparative | Hybrid | DV | 133 | 84 | |
| Levic *et al*[9] | 2014 | Denmark | Comparative | NS | DV | 56 | 36 | |
| Yoo *et al*[10] | 2014 | South Korea | Comparative | Tot rob | NS | 44 | 26 | |
| Koh *et al*[11] | 2014 | Singapore | Comparative | NS | NS | 19 | 19 | |
| Melich *et al*[12] | 2014 | Canada | Comparative | Tot rob | DV | 92 | 106 | |
| Barnajian *et al*[13] | 2014 | United States | Comparative | Hybrid | DV-S | 20 | 20 | 20 |
| Ielpo *et al*[14] | 2014 | Spain | Comparative | Tot rob | NS | 56 | 87 | |
| Tam *et al*[15] | 2014 | United States | Comparative | Hybrid | DV | 21 | 21 | |
| Ghezzi *et al*[16] | 2014 | Brazil | Comparative | Tot rob | DV-S | 65 | | 109 |
| Kuo *et al*[17] | 2014 | Taiwan | Comparative | Tot rob | DV | 36 | 28 | |
| Park *et al*[18] | 2014 | South Korea | Comparative | Hybrid | DV | 32 | 32 | |
| Saklani *et al*[19] | 2013 | South Korea | Comparative | NS | NS | 74 | 64 | |
| Fernandez *et al*[20] | 2013 | United States | Comparative | Hybrid | DV-S | 13 | 59 | |
| Erguner *et al*[21] | 2013 | Turkey | Comparative | Hybrid | NS | 27 | 37 | |
| D'Annibale *et al*[22] | 2013 | Italy | Comparative | Tot rob | DV-S | 50 | 50 | |
| Kang *et al*[23] | 2013 | South Korea | Comparative | Tot rob | NS | 165 | 165 | 165 |
| Park *et al*[24] | 2013 | South Korea | Comparative | Hybrid | DV | 40 | 40 | |
| Kim *et al*[25] | 2012 | South Korea | Comparative | Tot rob | DV | 62 | 147 | |
| Kim *et al*[26] | 2012 | South Korea | Comparative | Hybrid | DV | 30 | 39 | |
| Bertani *et al*[27] | 2011 | Italy | Comparative | Tot rob | DV | 52 | | 34 |
| Kwak *et al*[28] | 2011 | South Korea | Comparative | Tot rob | DV | 59 | 59 | |
| Baek *et al*[29] | 2011 | United States | Comparative | NS | NS | 41 | 41 | |
| Park *et al*[30] | 2011 | South Korea | Comparative | Hybrid | DV | 52 | 123 | 88 |
| Patriti *et al*[31] | 2009 | Italy | Comparative | Hybrid | DV | 29 | 37 | |
| Baik *et al*[32] | 2008 | South Korea | Comparative | Hybrid | DV | 18 | 18 | |
| Pigazzi *et al*[33] | 2006 | United States | Comparative | Hybrid | DV | 6 | 6 | |
| Parisi *et al*[34] | 2014 | Italy | Case series | Hybrid | DV Si | 40 | | |
| Baek *et al*[35] | 2014 | South Korea | Case series | NS | NS | 182 | | |
| Shiomi *et al*[36] | 2014 | Japan | Case series | Hybrid | DV | 113 | | |
| Kim *et al*[37] | 2014 | South Korea | Case series | Tot rob | DV-S | 200 | | |
| Stănciulea *et al*[38] | 2013 | Romania | Case series | Tot rob | DV-Si | 100 | | |
| Zawadzki *et al*[39] | 2013 | United States | Case series | Hybrid | DV | 77 | | |
| Sng *et al*[40] | 2013 | South Korea | Case series | Tot rob | DV-S | 197 | | |
| Du *et al*[41] | 2013 | China | Case series | Tot rob | DV | 22 | | |
| Alimoglu *et al*[42] | 2012 | Turkey | Case series | Tot rob | DV | 7 | | |
| Akmal *et al*[43] | 2012 | United States | Case series | Hybrid | DV | 80 | | |
| Park *et al*[44] | 2012 | United States | Case series | Hybrid | DV-S | 30 | | |
| Kang *et al*[45] | 2011 | South Korea | Case series | Hybrid | DV | 389 | | |
| deSouza *et al*[46] | 2010 | United States | Case series | Hybrid | DV | 44 | | |
| Pigazzi *et al*[47] | 2010 | United States | Case series | Hybrid | DV | 143 | | |
| Choi *et al*[48] | 2009 | South Korea | Case series | Tot rob | DV | 50 | | |
| Ng *et al*[49] | 2009 | Singapore | Case series | Hybrid | DV | 8 | | |
| Hellan *et al*[50] | 2007 | United States | Case series | Hybrid | DV | 39 | | |

Tot rob: Totally Robotic; DV: Da Vinci; NS: Not specified.

shorter length of stay after robotic surgery.

No statistically significant differences in the overall 30 d mortality between the robotic and laparoscopic approach was found among 15 comparative studies[8-11,13,14,19-24,29-31] (0.10% and 0.45% respectively).

Twenty-three studies reported the reintervention rate. In the robotic series it ranged from 0%[8,22,32,33,42,48] to 15%[20] whereas it ranged from 0%[32,33] to 15.7%[11] after laparoscopic surgery. The most common cause of reintervention was anastomotic leak in both the robotic and laparoscopic groups. No statistically significant differences were found in any of the 12 comparative studies[11-15,20-24,32,33].

The overall complication rate in the robotic and laparoscopic groups was 24.5% and 27.7% respectively. No significant differences in this parameter were reported between the robotic and laparoscopic series[8-11,13-15,19-25,28-33]. The most frequent complication in both the robotic and laparoscopic cases was anastomotic leak followed by bowel obstruction and urinary complications (Table 5). Thirteen studies[10,18,19,22-24,26,31,37,38,40,44,45] reported urinary and sexual dysfunction after rectal surgery, 9 of these were comparative. Park *et al*[18] reported an earlier and significant restoration of erectile function after robotic surgery when compared to the laparoscopic one. Kim *et al*[26] observed an earlier recover of urinary function after robotic intervention within six months from the operation ($P$ = 0.001). After 6 mo the difference was no more statistically significant.

Table 6 shows the studies which classified complications according to the Clavien Dindo Scoring System. Clavien-Dindo 1 and 2 were the most frequent

**Table 2 Demographics and preoperative data**

| Ref. | M/F | Age | BMI | ASA | | | | Preop CHT | Type of operation | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | | AR | LAR | ULAR | APR |
| Park *et al*[8] | 86/47 | 59.2 (32-86) | 23.1 (14.6-32.8) | 94 | 31 | 8 | 0 | 15 | 100 | 33 | 0 | 0 |
| Levic *et al*[9] | 34/22 | 65 (23-83) | 24.8 (16-34.5) | 17 | 35 | 4 | 0 | 15 | 0 | 41[1] | 0 | 15 |
| Yoo *et al*[10] | 35/9 | 59.77 (+ 12.33) | 24.13 (+ 3.33) | 26 | 17 | 1 | 0 | 24 | 0 | 0 | 44 | 0 |
| Koh *et al*[11] | 15/4 | 62 (47-92) | - | 5 | 14 | 0 | 0 | 8 | 0 | 0 | 17 | 2 |
| Melich *et al*[12] | 52/40 | 60 (57.7-62.2) | 23.1 (22.5-23.7) | 1 (1-3) | | | | 13 | 0 | 92 | 0 | 0 |
| Barnajian *et al*[13] | 12/8 | 62 (44-82) | 22 (18-31) | 0 | 4 | 16 | 0 | 10 | 0 | 15 | 0 | 5 |
| Ielpo *et al*[14] | 25/31 | 43.4 (+ 11) | 22.8 (+ 2.5) | 11 | 32 | 11 | 0 | 46 | 0 | 40 | 1 | 15 |
| Tam *et al*[15] | 10/11 | 60 (41-73) | 25 (20-37) | _ | _ | _ | _ | 18 | 11 | 1 | 4 | 5 |
| Ghezzi *et al*[16] | 41/24 | 61 | 24.7 | 12 | 49 | 4 | 0 | 47 | 0 | 44 | 11 | 10[2] |
| Kuo *et al*[17] | 21/15 | 55.9 (30-89) | - | 0 | 33 | 3 | 0 | 28 | 0 | 0 | 36 | 0 |
| Park *et al*[18] | 32/0 | - | 23.8 | - | - | - | - | 15 (+ RT) | 0 | 22 | 9 | 1 |
| Saklani *et al*[19] | 50/24 | 59.6 (32-85) | 23.4 (16.9-29.8) | 50 | 24 | 0 | 0 | 74 | 0 | 46 | 26 | 2 |
| Fernandez *et al*[20] | 13/0 | 67.9 (+ 2.1) | - | 0 | 0 | 11 | 2 | 10 | 0 | 5 | 0 | 8 |
| Erguner *et al*[21] | 14/13 | 54 (24-78) | 28.3 (19.8-30.8) | - | - | - | - | 4 | 0 | 27 | 0 | 0 |
| D'Annibale *et al*[22] | 30/20 | 66 (+ 12.1) | - | - | - | - | - | 34 (+ RT) | 17 | 33 | 0 | 0 |
| Kang *et al*[23] | 104/61 | 61.2 (+ 11.4) | 23.1 (+ 2.8) | 109 | 56 | 0 | 0 | 39 | 165[3] | 0 | 0 | 0 |
| Park *et al*[24] | 41/21 | 56 | 24.2 | 33 | 28 | 1 | 0 | 9 | 0 | 51 | 10 | 1 |
| Kim *et al*[25] | 28/12 | 57.3 | 23.9 | 27 | 9 | 4 | 0 | 32 | 0 | 0 | 40 | 0 |
| Kim *et al*[26] | 18/12 | 54.13 (+ 8.52) | 24.36 (+ 2.4) | 29 | 1 | 0 | 0 | 10 | 29 | 1[3] | 0 | 0 |
| Bertani *et al*[27] | 31/21 | 59.6 (+ 11.6) | 24.8 (+ 3.62) | 49 | | | 3 | 24 | 0 | 52 | 0 | 0 |
| Kwak *et al*[28] | 39/20 | 60 (53-68) | 23.3 (21.8-25.2) | 28 | 27 | 4 | 0 | 8 (RT) | 0 | 54 | 5 | 0 |
| Baek *et al*[29] | 25/16 | 63.6 (48-87) | - | 0 | 18 | 22 | 1 | 33 | 0 | 33 | 2 | 6 |
| Park *et al*[30] | 28/24 | 57.3 | 23.7 | 21 | 26 | 5 | 0 | 12 (+ RT) | 52 | 0 | 0 | 0 |
| Patriti *et al*[31] | 11/18 | 68 | 24 | 2 | 13 | 14 | 0 | 7 (+ RT) | 29 | 0 | 0 | 0 |
| Baik *et al*[32] | 14/4 | 57.3 (37-79) | 22.8 (19.4-31.7) | 12 | 6 | 0 | 0 | - | 18 | 0 | 0 | 0 |
| Pigazzi *et al*[33] | 2/4 | 60 (42-78) | 31 (25-36) | 0 | 2 | 4 | 0 | 2 | 0 | 6 | 0 | 0 |
| Parisi et al[34] | 19/21 | 67 (39-86) | 25.22 (18.36-33.20) | 20 | 14 | 6 | 0 | 17 | 0 | 35 | 0 | 5 |
| Baek *et al*[35] | 117/65 | 57.6 (26-78) | 23.4 (14.8-30.5) | 111 | 65 | 6 | 0 | 50 | 0 | 182 | 0 | 0 |
| Shiomi *et al*[36] | 78/35 | 64 (23-84) | 23.4 (16.7-30.6) | 39 | 74 | 0 | 0 | 3 | 11 | 71 | 23 | 8 |
| Kim *et al*[37] | 134/66 | 58.15 | 23.85 | - | - | - | - | 43 | 0 | 200 | 0 | 0 |
| Stănciulea *et al*[38] | 66/34 | 62 (32-84) | 26 (16.4-38) | - | - | - | - | 58 | 30 | 39 | 8 | 23 |
| Zawadzki *et al*[39] | 45/32 | 60.1 (34-82) | 28 (18-43) | 62 | 15 | 0 | 48 | 0 | 68 | 9 | 0 | 0 |
| Sng *et al*[40] | 131/66 | 60 (20-89) | 23.5 (16.9-33.1) | 117 | 71 | 9 | 0 | 54 | 3 | 126 | 55 | 13 |
| Du *et al*[41] | 14/8 | 56.4 (+ 7.8) | 22.5 (+ 2.1) | - | - | - | - | - | 0 | 22 | 0 | 0 |
| Alimoglu *et al*[42] | 5/2 | 52.9 (32-88) | - | - | - | - | - | 4 | 0 | 0 | 0 | 7 |
| Akmal *et al*[43] | 50/30 | 60.35 (24-85) | 27.2 (18-44) | 0 | 37 | 39 | 4 | 62 | 0 | 40 | 21 | 19 |
| Park *et al*[44] | 16/14 | 58 | 27.6 | 0 | 12 | 18 | 0 | 20 | 0 | 5 | 19 | 6 |
| Kang *et al*[45] | 252/137 | 59 (26-86) | - | 280 | 107 | 2 | 0 | 72 | 382 | 1[3] | 0 | 6 |
| deSouza *et al*[46] | 28/16 | 63 | - | 4 | 27 | 13 | 0 | 31 | 0 | 30 | 6 | 8 |
| Pigazzi *et al*[47] | 87/56 | 62 (26-87) | 26.5 (16.5-44) | 0 | 0 | 57 | 93 (+ RT) | 0 | 80 | 32 | 31 | 0 |
| Choi *et al*[48] | 32/18 | 58.5 (30-82) | 23.2 (19.4-29.2) | 27 | 19 | 4 | 0 | 3 (+ RT) | 0 | 40 | 8 | 2 |
| Ng *et al*[49] | 5/3 | 55 (42-80) | - | - | - | - | - | - | 2 | 0 | 6 | 0 |
| Hellan *et al*[50] | 21/18 | 58 (26-84) | 26 (16-44) | 0 | 0 | | 17 | 33 | 0 | 22 | 11 | 6 |

[1]9 hartmann; [2]1 Posterior pelvic exenteration; [3]1 hartmann. AR: Anterior resections; ULAR: Ultra-low anterior resections; APR: Abdominoperineal resections; CHT: Chemotheraypy; BMI: Body mass index; ASA: American society anesthesiologists.

complications in both groups (13.8% robotic *vs* 12.4% laparoscopic).

### *Oncological outcome*

The mean number of harvested nodes ranged from 10[14] to 20.6[48] and from 9[14] to 21[10] in the robotic and laparoscopic cases respectively. Three of 22 comparatives[8-15,17,19-25,28-33] studies reported a statistically significant difference in the number of harvested nodes between the robotic and laparoscopic approach: Levic *et al*[9] and D'Annibale *et al*[22] showed an higher number of examined nodes after robotic surgery whereas Yoo *et al*[10] showed an higher number of examined nodes after laparoscopic surgery (Table 7).

The mean length of distal resection margins after robotic rectal surgery was available in 20 studies[8-10,13,15-17,19,21-38,40,41,43,45,48,50]. It ranged from 13.3 mm[10] to 460 mm[15]. Tumor involvement rate of distal margins was available 21 studies[8,9,11,12,15,17,20,21,23,25,26,28-30,34,36,37,39,46,48,50] and ranged from 0%[8,15,17,20,21,25,26,28-30,34,36,37,48,50] to 2.6%[39] of patients. An involvement of distal resection margin was found in 6 (0.47%) out of 1257 patients operated on with the robotic technique.

The mean length of distal resection margins after laparoscopic rectal surgery was available in 19[8-10,13,15,17,19,21-26,28-33] studies. It ranged from 13 mm[25] to 510 mm[15]. The involvement of distal margins was available in 14 studies[8,9,11,12,15,17,20,21,23,25,26,28-30] and ranged from 0%[8,9,11,12,15,21,23,25,26,28-30] to 5%[15] of patients. A distal margin positivity was reported in 3 (0.3%) out of 857

**Table 3 Operative data**

| Ref. | Patients | Mesorectum | Technique | Mean operative time (min) | EBL (mL) | Conversion to open (%) | Stoma (%) |
|---|---|---|---|---|---|---|---|
| Park *et al*[8] | 133 | RME | Hybrid | 205.7 (109-505) | 77.6 (0-700) | 0 (0) | 29 (21.8) |
| | 84 | LME | Tot lap | 208.8 (94-407) | 82.3 (0-1100) | 6 (7.1) | 20 (23.8) |
| Levic *et al*[9] | 56 | RME | NS | 247 (135-111)[1] | 50 (0-400)[1] | 3 (5.4) | 31 (55.3) |
| | 36 | LME | SP | 295 (108-465)[1] | 35 (0-400)[1] | 0 (0) | 9 (25) |
| Yoo *et al*[10] | 44 | RME | Tot rob | 316.43 (+ 65.11) | 239.77 (+ 278.61) | 0 (0) | 44 (100) |
| | 26 | LME | Tot lap | 286.77 (+ 51.46) | 215.38 (+ 247.29) | 0 (0) | 26 (100) |
| Koh *et al*[11] | 19 | RME | NS | 390 (289-771)[1] | - | 1 (5.2) | 17 (89) |
| | 19 | LME | HAL | 225 (130-495)[1] | - | 5 (26.3) | 0 (0) |
| Melich *et al*[12] | 92 | RME | Tot rob | 285 (266-305) | 201 (165-237) | 1 (1.1) | - |
| | 106 | LME | Tot lap | 262 (252-272) | 232 (191-272) | 4 (3.8) | - |
| Barnajian *et al*[13] | 20 | RME | Hybrid | 240 (150-540)[1] | 125 (50-650)[1] | 0 (0) | 11 (55) |
| | 20 | LME | Tot lap | 180 (140-480)[1] | 175 (50-900)[1] | 2 (10.5) | 11 (55) |
| | 20 | OME | Open | 240 (115-475)[1] | 250 (50-800)[1] | na | 12 (60) |
| Ielpo *et al*[14] | 56 | RME | Tot rob | 309 (150-540) | 280 (0-4000) | 2 (3.5) | 28 (50) |
| | 87 | LME | Tot lap | 252 (180-420) | 240 (0-4000) | 10 (11.5) | 53 (60.9) |
| Tam *et al*[15] | 21 | RME | Hybrid | 274.8 (189-449) | 252.6 (30-2000) | 1 (4.7) | 13 (62) |
| | 21 | LME | Tot lap | 236.3 (171-360) | 271.4 (50-1200) | 0 (0) | 11 (52) |
| Ghezzi *et al*[16] | 65 | RME | Tot rob | 299 (+ 58) | 0 (0-175)[1] | 1 (1.5) | 51 (91.1) |
| | 109 | OME | Open | 207 (+ 56.5) | 150 (0-400)[1] | na | 66 (63.3) |
| Kuo *et al*[17] | 36 | RME | NS | 485.8 (315-720) | 80 (30-200) | 0 (0) | 7 (19.4) |
| | 28 | LME | Tot lap | 374.3 (210-570) | 103.6 (30-250) | 0 (0) | 13 (46.4) |
| Park *et al*[18] | 32 | RME | Hybrid | - | - | - | 3 (9.4) |
| | 32 | LME | Tot lap | - | - | - | 3 (9.4) |
| Saklani *et al*[19] | 74 | RME | NS | 365.2 (150-710) | 180 (0-1100) | 1 (1.4) | 53 (71.6) |
| | 64 | LME | Tot lap | 311.6 (180-530) | 210 (0-1200) | 4 (6.3) | 35 (54.7) |
| Fernandez *et al*[20] | 13 | RME | Hybrid | 528 (416-700)[1] | 157 (50-550)[1] | 1 (8) | - |
| | 59 | LME | HAL | 344 (183-735)[1] | 200 (25-1500)[1] | 10 (17) | - |
| Erguner *et al*[21] | 27 | RME | Hybrid | 280 (175-480) | 50 (20-100) | 0 (0) | 19 (70.3) |
| | 37 | LME | Tot lap | 190 (110-300) | 125 (50-400) | 0 (0) | 13 (35.1) |
| D'Annibale *et al*[22] | 50 | RME | Tot rob | 270 (240-315)[1] | - | 0 (0) | - |
| | 50 | LME | Tot lap | 280 (240-350)[1] | - | 6 (12) | - |
| Kang *et al*[23] | 165 | RME | Tot rob | 309.7 (+ 115.2) | 133 (+ 192.3) | 1 (0.6) | 41 (25) |
| | 165 | LME | Tot lap | 277.8 (+ 81.9) | 140.1 (+ 216.4) | 3 (1.8) | 43 (27.2) |
| | 165 | OME | Open | 252.6 (+ 88.1) | 275.4 (+ 368.4) | na | 47 (31.8) |
| Kim *et al*[25] | 62 | RME | Tot rob | 390 (+ 97) | - | 3 (4.8) | 22 (35.5) |
| | 147 | LME | Tot lap | 285 (+ 80) | - | 5 (3.4) | 34 (23.1) |
| Park *et al*[24] | 40 | RME | Hybrid | 235.5 (+ 57.5) | 45.7 (+ 40) | 0 (0) | 14 (35) |
| | 40 | LME | Tot lap | 185.4 (+ 72.8) | 59.2 (+ 35.8) | 0 (0) | 6 (15) |
| Kim *et al*[26] | 30 | RME | Hybrid | - | - | - | - |
| | 39 | LME | Tot lap | - | - | - | - |
| Bertani *et al*[27] | 52 | RME | Tot rob | 260 (190-570) | 100 (50-1000) | - | - |
| | 34 | OME | Tot lap | 164 (100-350) | 120 (50-2000) | - | - |
| Kwak *et al*[28] | 59 | RME | Tot rob | 270 (241-325)[1] | - | 0 (0) | 25 (42.4) |
| | 59 | LME | Tot lap | 228 (177-254)[1] | - | 2 (3.4) | 26 (44.1) |
| Baek *et al*[29] | 41 | RME | NS | 296 (150-520) | 200 (20-2000)[1] | 3 (7.3) | 33 (94.3) |
| | 41 | LME | NS | 315 (174-584) | 300 (17-1000)[1] | 9 (22) | 14 (40) |
| Park *et al*[30] | 52 | RME | Hybrid | 232.6 (+ 54.2) | - | 0 (0) | 1 (1.9) |
| | 123 | LME | Tot lap | 158.1 (+ 49.2) | - | 0 (0) | 5 (4.1) |
| | 88 | OME | Open | 233.8 (+ 59.2) | - | na | 4 (4.5) |
| Patriti *et al*[31] | 29 | RME | Hybrid | 202 (+ 12) | 137.4 (+ 156) | 0 (0) | 0 (0) |
| | 37 | LME | Tot lap | 208 (+ 7) | 127 (+ 169) | 7 (19) | 0 (0) |
| Baik *et al*[32] | 18 | RME | Hybrid | 217.1 (149-315) | - | 0 (0) | - |
| | 18 | LME | Tot lap | 204.3 (114-297) | - | 2 (11) | - |
| Pigazzi *et al*[33] | 6 | RME | Hybrid | 264 (192-318) | 104 (50-200) | 0 (0) | - |
| | 6 | LME | Tot lap | 258 (198-312) | 150 (50-300) | 0 (0) | - |
| Parisi *et al*[34] | 40 | RME | Hybrid | 340 (235-460)[1] | 50 (20-250)[1] | 0 (0) | 22 (55) |
| Baek *et al*[35] | 182 | RME | NS | - | - | - | - |
| Shiomi *et al*[36] | 113 | RME | Hybrid | 302 (135-683)[1] | 17 (0-690)[1] | 0 (0) | - |
| Kim *et al*[37] | 200 | RME | Tot rob | 308.3 | - | 1 (0.5) | 9 (4.5) |
| Stănciulea *et al*[38] | 100 | RME | Tot rob | - | 150 (0-250)[1] | 4 (4) | 64 (64) |
| Zawadzki *et al*[39] | 77 | RME | Hybrid | 327 (178-510)[1] | 189 (30-1000)[1] | 3 (3.9) | 53 (69) |
| Sng *et al*[40] | 197 | RME | Tot rob | 278.7 (145-515) | < 50 (50-1500)[1] | 0 (0) | - |
| Du *et al*[41] | 22 | RME | Tot rob | 220 (152-286) | 33 (10-70) | 0 (0) | - |
| Alimoglu *et al*[42] | 7 | RME | Tot rob | - | - | 0 (0) | - |
| Akmal *et al*[43] | 80 | RME | Hybrid | 303.5 | - | 4 (5) | 46 (57.5) |
| Park *et al*[44] | 30 | RME | Hybrid | 369 (306-410)[1] | 100 (75-200)[1] | - | - |
| Kang *et al*[45] | 389 | RME | Hybrid | 322.35 | - | 3 (0.7) | 93 (24) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| deSouza *et al*[46] | 44 | RME | Hybrid | 347 (155-510)[1] | 150 (50-1000)[1] | - | 34 (77.2) |
| Pigazzi *et al*[47] | 143 | RME | Hybrid | 297 (90-660) | 283 (0-6000) | 7 (4.9) | 71 (50) |
| Choi *et al*[48] | 50 | RME | T Tot rob | 304.8 (190-485) | - | 0 (0) | 16 (32) |
| Ng *et al*[49] | 8 | RME | Hybrid | 278.7 (145-515) | - | 0 (0) | 6 (75) |
| Hellan *et al*[50] | 39 | RME | Hybrid | 285 (180-540)[1] | 200 (25-6000)[1] | 1 (2.5) | 4 (10.2) |

Tot rob: Totally robotic; Tot lap: Totally laparoscopic; HAL: Hand assisted laparoscopy; SP: Single port; NS: Not specified. [1]Median. EBL: Estimated blood loss; RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision.

**Table 4 Postop data**

| Ref. | Pts | Mesorectum | Flatus (POD) | Liquid diet (POD) | Solid diet (POD) | Length of stay (d) | 30 d mortality (%) | Reinterventions (%) | |
|---|---|---|---|---|---|---|---|---|---|
| Park *et al*[8] | 133 | RME | 2.42 (1-6) | - | 4.92 (3-11) | 5.86 (4-14) | 0 (0) | - | |
| | 84 | LME | 2.47 (1-6) | - | 5.19 (2-11) | 6.54 (3-25) | 0 (0) | - | |
| Levic *et al*[9] | 56 | RME | - | - | - | 8 (4-100) | 0 (0) | - | |
| | 36 | LME | - | - | - | 7 (3-51) | 2 (5.6) | - | |
| Yoo *et al*[10] | 44 | RME | - | - | 2.58 (+ 1.62) | 11.41 (+ 5.56) | 0 (0) | - | |
| | 26 | LME | - | - | 2.48 (+ 1.53) | 11.04 (+ 6.33) | 0 (0) | - | |
| Koh *et al*[11] | 19 | RME | - | - | - | 7 (4-21)[1] | 0 (0) | 1 (5.2) | Bleeding |
| | 19 | LME | - | - | - | 6 (4-28)[1] | 0 (0) | 3 (15.7) | Adhesive SBO, colonic infarction, anastomotic leak |
| Melich *et al*[12] | 92 | RME | - | - | - | 9.6 (8.3-11) | - | 6 (6.5) | 6 leak/abscess |
| | 106 | LME | - | - | - | 9.9 (8.5-11.3) | - | 5 (4.7) | 4 leak/abscess, 1 obstruction due to adhesions |
| Barnajian *et al*[13] | 20 | RME | 3 (1-8)[1] | - | 4 (2-9)[1] | 6 (4-31)[1] | 0 (0) | 2 (10) | Presacral bleeding, pelvic abscess |
| | 20 | LME | 4 (3-13)[1] | - | 4 (4-14)[1] | 7 (5-36)[1] | 0 (0) | 1 (5) | Pancreatic tail injury |
| | 20 | OME | 4 (2-8)[1] | - | 4.5 (2-9)[1] | 7 (3-16)[1] | 0 (0) | 2 (10) | Presacral bleeding, enterotomy |
| Ielpo *et al*[14] | 56 | RME | - | - | - | 13 (5-60) | 0 (0) | 3 (5.3) | NS |
| | 87 | LME | - | - | - | 10 (5-16) | 0 (0) | 3 (3.4) | NS |
| Tam *et al*[15] | 21 | RME | - | - | - | 8.7 (4-23) | - | 0 (0) | |
| | 21 | LME | - | - | - | 6 (3-14) | - | 1 (5) | Bleeding |
| Ghezzi *et al*[16] | 65 | RME | 2 (1-2) | 1 (1-2) | - | 6 (5-8)[1] | 0 (0) | 3 (4.6) | NS |
| | 109 | OME | 3 (2-5) | 5 (4-6) | - | 9 (8-10)[1] | 0 (0) | 2 (1.8) | NS |
| Kuo *et al*[17] | 36 | RME | 2.9 (1-6) | - | 6.4 (4-12) | 14.2 (9-27) | - | - | |
| | 28 | LME | 3.4 (1-11) | - | 5.8 (3-16) | 15.1 (7-57) | - | - | |
| Park *et al*[18] | 32 | RME | - | - | - | - | - | - | |
| | 32 | LME | - | - | - | - | - | - | |
| Saklani *et al*[19] | 74 | RME | 2.45 (1-10) | - | 4.6 (2-13) | 8 (4-21) | 0 (0) | - | |
| | 64 | LME | 2.48 (1-6) | - | 5.1 (2-14) | 9.2 (5-29) | 0 (0) | - | |
| Fernandez *et al*[20] | 13 | RME | - | - | - | 13[1] | 0 (0) | 2 (15) | SBO |
| | 59 | LME | - | - | - | 8[1] | 1 (2) | 7 (12) | NS |
| Erguner *et al*[21] | 27 | RME | - | - | - | - | 1 (3.7) | 1 (3.7) | Colonic necrosis |
| | 37 | LME | - | - | - | - | 1 (2.7) | 3 (8.1) | 1 ileostomy retraction, 2 anastomotic leak |
| D'Annibale *et al*[22] | 50 | RME | - | 3 (3-5)[1] | - | 8 (7-11)[1] | 0 (0) | 0 (0) | |
| | 50 | LME | - | 5 (4-6)[1] | - | 10 (8-14)[1] | 0 (0) | 3 (6) | Anastomotic leak |
| Kang *et al*[23] | 165 | RME | 2.2 (+ 1.1) | - | 4.5 (+ 1.9) | 10.8 (+ 5.5) | 0 (0) | 15 (9) | NS |
| | 165 | LME | 2.4 (+ 1.2) | - | 5.2 (+ 2.4) | 13.5 (+ 9.2) | 0 (0) | 5 (15) | NS |
| | 165 | OME | 3 (+ 1.4) | - | 6.4 (+ 2.5) | 16 (+ 8.6) | 0 (0) | 9 (5.4) | NS |
| Kim *et al*[25] | 62 | RME | - | - | 6 (+ 5) | 12 (+ 6) | - | - | |
| | 147 | LME | - | - | 7 (+ 5) | 14 (+ 9) | - | - | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Park *et al*[24] | 40 | RME | 2.4 (+ 1.6) | - | 7.5 (+ 3.5) | 10.6 (+ 4.2) | 0 (0) | 2 (5) | Anastomotic leak |
| | 40 | LME | 2.5 (+ 1.3) | - | 7.7 (+ 2.3) | 11.3 (+ 3.6) | 0 (0) | 1 (2.5) | Anastomotic leak |
| Kim *et al*[26] | 30 | RME | - | - | - | - | - | - | |
| | 39 | LME | - | - | - | - | - | - | |
| Bertani *et al*[27] | 52 | RME | 2 (1-5) | 2 (1-13) | - | 6 (4-51)[1] | - | 2 (4) | |
| | 34 | OME | 3 (1-9) | 3 (2-12) | - | 7 (4-24)[1] | - | 0 (0) | |
| Kwak *et al*[28] | 59 | RME | - | - | - | - | - | - | |
| | 59 | LME | - | - | - | - | - | - | |
| Baek *et al*[29] | 41 | RME | - | 2.3 (1-13) | - | 6.5 (2-33) | 0 (0) | - | |
| | 41 | LME | - | 2.4 (1-9) | - | 6.6 (3-20) | 0 (0) | - | |
| Park *et al*[30] | 52 | RME | 3.2 (+ 1.8) | - | 6.7 (+ 3.8) | 10.4 (+ 4.7) | 0 (0) | - | |
| | 123 | LME | 3 (+ 1.1) | - | 6.1 (+ 2.7) | 9.8 (+ 3.8) | 0 (0) | - | |
| | 88 | OME | 4.4 (+ 3) | - | 7.6 (+ 3.3) | 12.8 (+ 7.1) | 1 (1.1) | - | |
| Patriti *et al*[31] | 29 | RME | - | - | - | 11.9 (6-29) | 0 (0) | - | - |
| | 37 | LME | - | - | - | 9.6 (5-37) | 0 (0) | - | - |
| Baik *et al*[32] | 18 | RME | 1.8 (1-2)[1] | - | - | 6.9 (5-10)[1] | - | 0 (0) | |
| | 18 | LME | 2.4 (1-6)[1] | - | - | 8.7 (6-12)[1] | - | 0 (0) | |
| Pigazzi *et al*[33] | 6 | RME | - | - | - | 4.5 (3-11) | - | 0 (0) | |
| | 6 | LME | - | - | - | 3.6 (3-6) | - | 0 (0) | |
| Parisi *et al*[34] | 40 | RME | 1 (1-3)[1] | 1 (1-5)[1] | 2 (2-6)[1] | 5 (3-18)[1] | 0 (0) | 1 (2.5) | Anastomotic leak |
| Baek *et al*[35] | 182 | RME | - | - | - | - | - | - | - |
| Shiomi *et al*[36] | 113 | RME | 2 (1-3)[1] | 3 (3-7)[1] | - | 7 (6-24)[1] | 0 (0) | 2 (1.8) | Anastomotic leak |
| Kim *et al*[37] | 200 | RME | 2.4 | - | 5 | 10.7 | | 16 (8) | ns |
| Stănciulea *et al*[38] | 100 | RME | - | - | - | 10 (6-38)[1] | - | 6 (6) | 3 anastomotic leak, 1 bowel obstruction, 1 bleeding, 1 bowel injury |
| Zawadzki *et al*[39] | 77 | RME | - | - | - | 6.4 (3-26) | 0 (0) | 3 (3.9) | Anastomotic leak |
| Sng *et al*[40] | 197 | RME | - | - | - | 9 (5-122)[1] | - | - | |
| Du *et al*[41] | 22 | RME | 2.6 (1.41-4.37)[1] | - | - | 7.8 (7-13)[1] | - | - | |
| Alimoglu *et al*[42] | 7 | RME | - | - | - | 8.1 (5-10)[1] | 0 (0) | 0 (0) | |
| Akmal *et al*[43] | 80 | RME | - | 2.75 (1-19) | - | 7.55 (2-33) | 0 (0) | - | |
| Park *et al*[44] | 30 | RME | - | - | - | 4 (3-6)[1] | 0 (0) | - | |
| Kang *et al*[45] | 389 | RME | 2.3 | 3.9 | - | 13.5 | 0 (0) | 36 (9.2) | ns |
| deSouza *et al*[46] | 44 | RME | - | - | - | 5 (3-36)[1] | 1 (0.46) | 2 (0.92) | 1 anastomotic leak |
| Pigazzi *et al*[47] | 143 | RME | - | 2.7 (1-19) | - | 8.3 (2-33) | 0 (0) | - | |
| Choi *et al*[48] | 50 | RME | 1.9 (1-3) | 2.6 (2-12) | - | 9.2 (5-24) | - | 0 (0) | |
| Ng *et al*[49] | 8 | RME | - | - | - | 5 (4-30)[1] | 0 (0) | - | - |
| Hellan *et al*[50] | 39 | RME | - | 2 (1-11)[1] | - | 4 (2-22)[1] | 0 (0) | 4 (10.3) | Anastomotic leak |

[1]Values are expressed as mean, solid diet includes soft diet. SBO: Small bowel obstruction; RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision; POD: Post operative day.

patients. Among the 19 comparative[8-10,13,15,17,19,21-26,28-33] studies only Park *et al*[24] reported a longer distal margin in the robotic than in the laparoscopic group ($P$ = 0.04). No significant difference in distal margins tumor involvement was reported when the robotic and laparoscopic approaches were compared.

Mean circumferential resection margins (CRM) after robotic rectal surgery were reported in 9 studies[9,13,17,21,25,30,43,44,47] ranging from 1.8 mm[43] to 11 mm[44]. CRM tumor involvement was available in 32 studies[8,10-12,14-17,19,20,22-30,35-37,39,40,42,44-50] and ranged from 0%[15,16,20,22,36,42,44,46,49,50] to 11.1%[17] of patients with a 2.94 overall rate (76 out of 2583 patients).

Mean CRM after laparoscopic rectal surgery were reported in 6[9,13,17,21,25,30] comparative studies. It ranged from 4 mm[21] to 8.2 mm[30]. CRM involvement was reported in 17 studies[8,10-12,14,15,17,19,20,22-26,28-30] and occurred in 51 out of 1158 patients (4.4%) Where the 2 procedures where compared only D'Annibale *et al*[22] observed a significantly greater number of patients with positive CRM in the laparoscopic series when compared with the robotic one.

Only in 11 papers[9,11,13,20,21,26,32,34,36,41,44] reported the quality of mesorectum. Complete mesorectum excision ranged from 100%[11,36] to 60%[9] in the robotic series and from 100%[11] to 40.6%[9] after laparoscopy. Total mesorectal excision was achieved in 83.62% of robotic cases *vs* 77.22% of laparoscopic ones. None of the 7 comparative studies showed a significant difference in the quality of mesorectum between the 2 procedures.

**Table 5 Complications according to Clavien Dindo classification**

| Ref. | Pts | Mesorectum | Complicated pts (%) | 1 (%) | 2 (%) | 3 (%) | | 4 (%) | 5 (%) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3a | 3b | | |
| Park *et al*[8] | 133 | RME | 26 (19.5) | 11 (42.3) | 5 (19.2) | 9 (34.6) | | 1 (3.8) | |
| | 84 | LME | 19 (22.6) | 7 (36.8) | 4 (21) | 6 (31.6) | | 2 (10.5) | |
| Yoo *et al*[10] | 44 | RME | 17 (38.6) | 13 (76.5) | | | 4 (23.5) | | |
| | 26 | LME | 7 (26.9) | 5 (71.4) | | | 2 (28.5) | | |
| Koh *et al*[11] | 19 | RME | 3 (15.7) | 2 (66.7) | | | 1 (33.3) | | |
| | 19 | LME | 7 (36.8) | 4 (57) | | | 3 (43) | | |
| Melich *et al*[12] | 92 | RME | 17 (18.4) | 11 (64.7) | | | 6 (35.3) | | |
| | 106 | LME | 18 (17) | 13 (72.2) | | | 5 (27.8) | | |
| Barnajian *et al*[13] | 20 | RME | 8 (40) | | 3 | 3 (37.5) | 2 (25) | | |
| | 20 | LME | 4 (10) | 2 | | 1 | 1 | | |
| | 20 | OME | 8 (40) | | 5 | | 2 | 1 (33.3) | |
| Ielpo *et al*[14] | 56 | RME | 15 (26.8) | 11 (73.3) | | | 4 (26.7) | | |
| | 87 | LME | 20 (23) | 15 (75) | | | 5 (25) | | |
| Ghezzi *et al*[16] | 65 | RME | 27 (41.5) | 22 (81.5) | | | 5 (18.5) | | |
| | 109 | OME | 45 (41.3) | 38 (84.5) | | | 7 (15.5) | | |
| Kuo *et al*[17] | 36 | RME | 11 (30.5) | 4 (36.3) | | 3 (27.2) | 4 (36.3) | | |
| | 28 | LME | 14 (50) | 11 (78.6) | | 1 (7) | 2 (14.2) | | |
| Fernandez *et al*[20] | 13 | RME | | | | | 2 | | |
| | 59 | LME | | | | | | | |
| Erguner *et al*[21] | 27 | RME | 3 (11.1) | 2 (66.7) | | | 1 (33.3) | | |
| | 37 | LME | 8 (21.6) | 5 (62.5) | | | 3 (37.5) | | |
| D'Annibale *et al*[22] | 50 | RME | 5 (10) | 5 (100) | | | | | |
| | 50 | LME | 10 (20) | 7 (70) | | | 3 (30) | | |
| Kang *et al*[23] | 165 | RME | 34 (20.6) | 16 (47.1) | | | 3 (8.8) | | |
| | 165 | LME | 46 (27.9) | 20 (43.5) | | | 1 (2.2) | | |
| | 165 | OME | 41 (24.8) | 30 (73.2) | | | 2 (4.9) | | |
| Park *et al*[24] | 40 | RME | 6 (15) | 4 (66.7) | | | 2 (33.3) | | |
| | 40 | LME | 5 (12.5) | 4 (80) | | | 1 (20) | | |
| Park *et al*[30] | 52 | RME | 10 (19.2) | 6 (60) | | | 4 (40) | | |
| | 123 | LME | 15 (12.2) | 9 (60) | | | 6 (40) | | |
| | 88 | OME | 18 (20.5) | 9 (50) | | | 9 (50) | | |
| Baik *et al*[32] | 18 | RME | 4 (22.2) | 3 (75) | 1 (25) | | | | |
| | 18 | LME | 1 (5.5) | | 1 (100) | | | | |
| Pigazzi *et al*[33] | 6 | RME | 1 (16.6) | | 1 (100) | | | | |
| | 6 | LME | 1 (16.6) | | | 1 (100) | | | |
| Parisi *et al*[34] | 40 | RME | 4 (10) | 1 (25) | 1 (25) | | 2 (50) | | |
| Shiomi *et al*[36] | 113 | RME | 23 (20.3) | 10 (43.5) | 10 (43.5) | 1 (4.3) | 2 (8.7) | | |
| Kim *et al*[37] | 200 | RME | | | | 16 (59.2) | | | |
| Stănciulea *et al*[38] | 100 | RME | 18 (18) | | 10 (55.5) | 2 (5.5) | 6 (38.9) | | |
| Zawadzki *et al*[39] | 77 | RME | | | 2 | 3 | | | |
| Sng *et al*[40] | 197 | RME | 74 (37) | 58 (78.3) | 5 (6.8) | 9 (12.1) | 1 (1.3) | 1 (1.3) | |
| Du *et al*[41] | 22 (4.5) | RME | 1 (4.5) | 1 (100) | 0 | | | | |
| Alimoglu *et al*[42] | 7 | RME | 2 (28.5) | 2 (100) | | | | | |
| Kang *et al*[45] | 389 | RME | 74 (19) | 34 (45.9) | 4 (5.4) | 36 (48.6) | | | |
| deSouza *et al*[46] | 44 | RME | 19 (43) | 15 (79) | 1 (5.2) | 1 (5.2) | 1 (5.2) | 1 (5.2) | |
| Choi *et al*[48] | 50 | RME | 9 (18) | | 4 (44.4) | 5 (55.5) | | | |
| Hellan *et al*[50] | 39 | RME | 15 (38.4) | 11 (73.3) | 4 (26.7) | | | | |

RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision.

### *Short-term oncologic outcomes*

Only 11 authors[8-10,16,19,25,28,31,38,42,47] reported short term oncologic outcomes (Table 8). The main drawback is the heterogeneity of the length of follow up ranging from 1 mo[9,42] to 80 mo[8] making results difficult to compare. The disease free survival in the laparoscopic group ranged from 75%[10] to 89.2%[31] with local recurrence ranging from 0%[9,42] to 16.6%[8] and an overall survival ranging from 88.5%[10] to 98%[24]. The disease free survival in the robotic group ranged from 70.4%[16] to 100%[31,42] with local recurrence ranging from 0%[9,31,42] to 12.8%[10] and an overall survival ranging from 85%[16] to 100%[42].

## CONCLUSION AND DISCUSSION

Robotic rectal surgery is constantly increasing over the years. Previous reviews have already demonstrated its safety and feasibility[51-53], although there are not published studies demonstrating its superiority over the laparoscopic approach mainly due to the lack of randomized control trials. This lack of evidence about the effectiveness of robotic rectal surgery is in contrast with the overall opinion of surgeons that report an easier surgical approach especially to narrow and difficult anatomic spaces such as the pelvis. Several authors[52-54] reported 3D high definition vision, wrist-like movement

**Table 6 Short term oncologic outcomes**

| Ref. | Pts | Mesorectum | DSF% (yr) | LR (%) | Distant metastases (%) | OS % (yr) | F-u mo (median) |
|---|---|---|---|---|---|---|---|
| Park *et al*[8] | 133 | RME | 81.9 (5) | 3 (2.3) | 16 (12) | 92.8 (5) | 58 (4-80) |
| | 84 | LME | 78.7 (5) | 1 (1.2) | 14 (16.6) | 93.5 (5) | 58 (4-80) |
| Levic *et al*[9] | 56 | RME | | 0 (0) | 8 (14.3) | | 12 (1-31) |
| | 36 | LME | | 0 (0) | 2 (5.6) | | 10 (1-33) |
| Yoo *et al*[10] | 43[1] | RME | 76.7 (3) | 6 (12.8) | | 95.2 (3) | 33.9 (4.4-61.3) |
| | 26 | LME | 75 (3) | 2 (8.3) | | 88.5 (3) | 36.5 (3.7-69.9) |
| Ghezzi *et al*[16] | 65 | RME | 73.2 (5) | 2 (3.2) | 19 (29.6) | 85 (5) | 60 |
| | 109 | OME | 69.5 (5) | 17.5 (16.1) | 26 (24.2) | 76.1 (5) | 60 |
| Saklani *et al*[19] | 74 | RME | 77.7 (3) | 2 (2.7) | | 90 (3) | 30.1 (11-61)[2] |
| | 64 | LME | 78.8 (3) | 4 (6.3) | | 92.1 (3) | 30.1 (11-61)[2] |
| Kim *et al*[25] | 62 | RME | | 0 (0) | 3 (4.2) | 98 (1.5) | 17.4 |
| | 147 | LME | | 1 (0.7) | 8 (5.4) | 98 (1.7) | 20.6 |
| Kwak *et al*[28] | 59 | RME | | 1 (1.8) | 2 (3.6) | | 17 (11-25) |
| | 59 | LME | | 1 (1.9) | 2 (3.7) | | 13 (9-22) |
| Patriti *et al*[31] | 29 | RME | 100 (3) | 0 (0) | 0 (0) | 96.6 (2.4) | 29.2[2] |
| | 37 | LME | 83.7 (3) | 2 (5.4) | 4 (6) | 97.2 (1.5) | 18.7[2] |
| Stănciulea *et al*[38] | 100 | RME | | 2 (2) | | 90 (3) | 24 (9-63) |
| Alimoglu *et al*[42] | 7 | RME | 100 (1) | 0 (0) | 0 (0) | 100 (1) | 12 (6-21)[2] |
| Pigazzi *et al*[47] | 143 | RME | 77.6 (3) | 2 (1.4) | 13 (9) | 97 (3) | 17.4 (0.1-52.5)[2] |

[1]1 patient excluded (palliative ISR); [2]Mean. DSF: Disease free survival rate; LR: Local recurrence; OS: Overall survival; RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision.

**Table 7 Histopathological data**

| Ref. | Pts | Mesorectum | Harvested nodes | Quality of mesorectum (complete) | Proximal margin (mm) | Distal margin (mm) | Distal margin + (%) | CRM (mm) | CRM + (%) | pTpN stage (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | 0 | 1 | 2 | 3 | 4 |
| Park *et al*[8] | 133 | RME | 16.34 (2-43) | - | 111.7 (40-350) | 27.5 (10-140) | 0 (0) | - | 9 (6.8) | 0 (0) | 49 (36.8) | 36 (27.1) | 48 (36.1) | 0 (0) |
| | 84 | LME | 16.63 (2-49) | - | 105.1 (40-340) | 28.7 (10-90) | 0 (0) | - | 6 (7.1) | 0 (0) | 22 (26.2) | 28 (33.3) | 34 (40.5) | 0 (0) |
| Levic *et al*[9] | 56 | RME | 21 (7-83)[1] | 34 | - | 30 (5-80) | 1 (0.56) | 9 (0-60)[1] | - | 3 (5.4) | 12 (21.4) | 20 (35.7) | 21 (37.5) | 0 (0) |
| | 36 | LME | 13 (3-33)[1] | 26 | - | 30 (5-75) | 0 (0) | 10 (1-43)[1] | - | 1 (2.8) | 6 (16.7) | 15 (41.7) | 14 (38.8) | 0 (0) |
| Yoo *et al*[10] | 44 | RME | 13.93 (+ 9.27) | - | 225.2 (+ 102.5) | 13.3 (+ 9.7) | - | - | 4 (9.1) | 5 (11.4) | 14 (31.8) | 11 (25) | 9 (20.5) | 5 (11.4) |
| | 26 | LME | 21.42 (+ 15.71) | - | 208.4 (+ 89.5) | 16.7 (+ 30) | - | - | 5 (19.2) | 1 (3.8) | 7 (26.9) | 8 (30.8) | 8 (30.8) | 2 (7.7) |
| Koh *et al*[11] | 19 | RME | 16 (4-24)[1] | 19 | - | - | 1 (5.2) | - | 1 (5.2) | 2(10.5) | 3 (15.7) | 4 (21) | 9 (47.3) | 1 (5.2) |
| | 19 | LME | 14 (5-27)[1] | 19 | - | - | 0 (0) | - | 0 (0) | 0 (0) | 5 (26.3) | 4 (21) | 9 (47.3) | 1 (5.2) |
| Melich *et al*[12] | 92 | RME | 17.2 (15-19.5) | - | - | - | 1 (1.1) | - | 3 (3.3) | - | - | - | - | - |
| | 106 | LME | 16.3 (14.4-18.1) | - | - | - | 0 (0) | - | 3 (2.8) | - | - | - | - | - |
| Barnajian *et al*[13] | 20 | RME | 14 (3-22)[1] | 16 | - | 20.5 (5-50)[1] | - | 10.5 (1-30)[1] | - | 0 (0) | 6 (40) | 4 (25) | 10 (35) | 0 (0) |
| | 20 | LME | 11 (4-18)[1] | 19 | - | 21.5 (1-55)[1] | - | 4 (0-30)[1] | - | 0 (0) | 7 (35) | 3 (15) | 10 (50) | 0 (0) |
| | 20 | OME | 12 (4-20)[1] | 19 | - | 20.5 (1-45)[1] | - | 8 (0-30)[1] | - | 0 (0) | 8 (40) | 3 (15) | 9 (45) | 0 (0) |
| Ielpo *et al*[14] | 56 | RME | 10 (0-29) | - | - | - | - | - | 2 (3.6) | 0 (0) | 14 (25) | 21 (37.5) | 21 (37.5) | 0 (0) |
| | 87 | LME | 9 (0-17) | - | - | - | - | - | 2 (2.3) | 0 (0) | 19 (21.8) | 38 (43.6) | 30 (34.5) | 0 (0) |
| Tam *et al*[15] | 21 | RME | 19.7 (8-40) | - | - | 460 (10-180) | 0 (0) | - | 0 (0) | 2 (10) | 5 (24) | 4 (19) | 9 (43) | 1 (5) |
| | 21 | LME | 14.8 (8-21) | - | - | 510 (5-80) | 1 (5) | - | 1 (5%) | 3 (14) | 7 (33) | 4 (19) | 7 (33) | 0 (0) |
| Ghezzi *et al*[16] | 65 | RME | 20.1 | - | - | 27 (16-44) | - | - | 0 (0) | 10 (15.4) | 5 (7.7) | 17 (26.2) | 27 (41.5) | 6 (9.2) |
| | 109 | OME | 14.1 | - | - | 22 (15-30) | - | - | 2 (1.8) | 15 (13.8) | 10 (9.2) | 38 (34.9) | 42 (38.5) | 4 (3.7) |
| Kuo *et al*[17] | 36 | RME | 14 (2-33) | - | - | 22 (4-42) | 0 (0) | 6.7 (0-18) | 4 (11.1) | 7 (19.4) | 4 (11.1) | 11 (30.5) | 14 (38.8) | 0 (0) |
| | 28 | LME | 13.9 (3-31) | - | - | 17.9 (1-60) | 1 (3.6) | 7 (0-16) | 4 (14.2) | 6 (21.4) | 2 (7.1) | 8 (28.6) | 12 (42.8) | 0 (0) |
| Park *et al*[18] | 32 | RME | - | - | - | - | - | - | - | - | - | - | - | - |
| | 32 | LME | - | - | - | - | - | - | - | - | - | - | - | - |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saklani *et al*[19] | 74 | RME | 11.6 (1-36) | - | 128 (50-240) | 17 (1-60) | - | - | 3 (4) | 18 (24.3) | 16 (21.6) | 22 (29.7) | 18 (24.3) | 0 (0) |
| | 64 | LME | 14.7 (1-27) | - | 140 (55-280) | 22 (2-70) | - | - | 1 (1.6) | 8 (12.5) | 13 (20.3) | 23 (35.9) | 20 (31.3) | 0 (0) |
| Fernandez *et al*[20] | 13 | RME | 16 | 9 | - | - | 0 (0) | - | 0 (0) | | - | - | - | - |
| | 59 | LME | 20 | 24 | - | - | 1 (2) | - | 1 (2) | | - | - | - | - |
| Erguner *et al*[21] | 27 | RME | 16 (3-38) | 19 | 120 (40-180) | 40 (30-80) | 0 (0) | 4 (2-8) | - | 0 (0) | 15 (55.5) | 11 (40.7) | 1 (3.7) | 0 (0) |
| | 37 | LME | 16 (3-31) | 17 | 140 (45-230) | 25 (5-50) | 0 (0) | 4 (1-10) | - | 0 (0) | 17 (46) | 16 (43.2) | 4 (10.8) | 0 (0) |
| D'Annibale *et al*[22] | 50 | RME | 16.5 (11-44) | - | - | 30 (20-70) | - | - | 0 (0) | - | - | - | - | - |
| | 50 | LME | 13.8 (4-29) | - | - | 30 (10-60) | - | - | 6 (12) | - | - | - | - | - |
| Kang *et al*[23] | 165 | RME | 15 (+ 9.4) | - | 120 (+ 49) | 19 (+ 14) | 0 (0) | - | 7 (4.2) | 4 (2.4) | 56 (33.9) | 51 (30.9) | 54 (32.7) | 0 (0) |
| | 165 | LME | 15.6 (+ 9.1) | - | 113 (+ 51) | 20 (+ 17) | 0 (0) | - | 11 (6.7) | 9 (5.4) | 55 (33.1) | 47 (28.5) | 54 (32.7) | 0 (0) |
| | 165 | OME | 17.4 (+ 10.9) | - | 114 (+ 55) | 22 (+ 17) | 0 (0) | - | 17 (10.3) | 14 (8.5) | 55 (33.3) | 41 (24.8) | 55 (33.3) | 0 (0) |
| Kim *et al*[25] | 62 | RME | 16 (+ 10) | - | - | 30 (+ 14) | - | - | 2 (3.2) | 4 (6.5) | 17 (27.4) | 16 (25.8) | 24 (38.7) | 0 (0) |
| | 147 | LME | 16 (+ 9) | - | - | 25 (+ 16) | - | - | 4 (2.7) | 6 (4.1) | 55 (37.7) | 35 (24) | 46 (31.5) | 4 (2.7) |
| Park *et al*[24] | 40 | RME | 12.9 (+7.5) | - | 198 (+ 69) | 14 (+ 9) | 0 (0) | 6.2 (4.7) | 3 (7.5) | 0 (0) | 19 (47.5) | 9 (22.5) | 11 (27.7) | 1 (2.5) |
| | 40 | LME | 13.3 (+8.6) | - | 213 (+ 139) | 13 (+ 9) | 0 (0) | 6.9 (5.1) | 2 (5) | 0 (0) | 13 (32.5) | 15 (37.5) | 11 (27.5) | 1 (2.5) |
| Kim *et al*[26] | 30 | RME | - | 29 | - | 27.9 (+ 10.2) | 0 (0) | - | 2 (6) | - | - | - | - | - |
| | 39 | LME | - | 37 | - | 28.6 (+ 13.6) | 0 (0) | - | 1 (2.5) | - | - | - | - | - |
| Bertani *et al*[27] | 52 | RME | 20.5 (5-43)[1] | - | - | 26 (1-70) | - | - | 2 (4) | - | - | - | - | - |
| | 34 | OME | 16 (6-46)[1] | - | - | 26 (1-80) | - | - | 2 (6) | - | - | - | - | |
| Kwak *et al*[28] | 59 | RME | 20 (12-27)[1] | - | - | 22 (15-30) | 0 (0) | - | 1 (1.7) | 3 (5.1) | 16 (27.1) | 23 (39) | 13 (22) | 4 (6.8) |
| | 59 | LME | 21 (14-28)[1] | - | - | 20 (12-35) | 0 (0) | - | 0 (0) | 3 (5.1) | 16 (27.1) | 23 (39) | 12 (20.3) | 5 (8.5) |
| Baek *et al*[29] | 41 | RME | 13.1 (3.33) | - | - | 36 (4-100) | 0 (0) | - | 1 (2.4) | 7 (17.1) | 12 (29.3) | 4 (9.8) | 15 (36.6) | 3 (7.3) |
| | 41 | LME | 16.2 (5-39) | - | - | 38 (4-110) | 0 (0) | - | 2 (4.9) | 3 (7.3) | 15 (36.6) | 3 (7.3) | 19 (46.3) | 1 (2.4) |
| Park *et al*[30] | 52 | RME | 19.4 (+ 10.2) | - | 165 (+ 60) | 28 (+ 19) | 0 (0) | 7.9 (+ 4.5) | 1 (1.9) | 0 (0) | 15 (28.8) | 15 (28.8) | 22 (42.3) | 0 (0) |
| | 123 | LME | 15.9 (+ 10.1) | - | 169 (+ 84) | 32 (+ 21) | 0 (0) | 8.2 (+ 5.8) | 3 (2.4) | 0 (0) | 34 (27.6) | 52 (42.3) | 37 (30.1) | 0 (0) |
| | 88 | OME | 18.5 (+ 10.9) | - | 124 (+ 66) | 23 (+ 15) | 0 (0) | 8.5 (+ 5.7) | 2 (2.3) | 0 (0) | 27 (30.7) | 32 (36.4) | 29 (33) | 0 (0) |
| Patriti *et al*[31] | 29 | RME | 10.3 (+ 4) | - | - | 21 (+ 9) | - | - | - | 0 (0) | 11 (38) | 9 (31) | 7 (24.1) | 2 (6.9) |
| | 37 | LME | 11.2 (+ 5) | - | - | 45 (+ 72) | - | - | - | 0 (0) | 17 (46) | 8 (21.6) | 10 (27.2) | 2 (5.4) |
| Baik *et al*[32] | 18 | RME | 20 (6-49) | 17 | 109 (75-200) | 40 (10-55) | - | | - | 0 (0) | 5 (27.8) | 4 (22.2) | 9 (50) | 0 (0) |
| | 18 | LME | 17.4 (9-42) | 13 | 103 (55-85) | 37 (15-60) | - | - | - | 0 (0) | 5 (27.8) | 4 (22.2) | 9 (50) | 0 (0) |
| Pigazzi *et al*[33] | 6 | RME | 14 (9-28) | - | - | 38 (18-90) | - | - | - | - | - | - | - | - |
| | 6 | LME | 17 (9-39) | - | - | 35 (22-50) | - | - | - | - | - | - | - | - |
| Parisi *et al*[34] | 40 | RME | 19 (6-35)[1] | 32 | 118.5 (65-390)[1] | 40 (20-80)[1] | 0 (0) | - | - | 2 (5) | 10 (25) | 9 (22.5) | 19 (47.5) | 0 (0) |
| Baek *et al*[35] | 182 | RME | 14.8 (2-47) | - | - | 22 (+ 14.3) | - | - | 10 (5.5) | 5 (2.7) | 57 (31.3) | 52 (28.5) | 62 (34) | 6 (3.3) |
| Shiomi *et al*[36] | 113 | RME | 32 (11-112)[1] | 113 | 180 (65-376) | 26 (5-100) | 0 (0) | - | 0 (0) | 5 (4.4) | 35 (31) | 28 (24.7) | 38 (33.6) | 7 (6.2) |
| Kim *et al*[37] | 200 | RME | 16.1 | - | 132.5 | 22 | 0 (0) | - | 2 (1) | - | - | - | - | - |
| Stănciulea *et al*[38] | 100 | RME | 14 (4-32)[1] | - | - | 30 (2-70)[1] | - | - | - | 5 (5) | 24 (24) | 43 (43) | 21 (21) | 7 (7) |
| Stănciulea *et al*[38] | 77 | RME | 12.9 (3-45) | - | - | - | 2 (2.6) | - | 1 (1.2) | 26 (34) | 8 (10) | 15 (19) | 26 (34) | 2 (3) |
| Sng *et al*[40] | 197 | RME | 16 (1-80)[1] | - | - | 17 (0-8.3)[1] | - | - | 2 (2.5) | - | - | - | - | - |
| Du *et al*[41] | 22 | RME | 14.3 (8-27)[1] | 19 | - | 26 (10-55) | - | - | - | 0 (0) | 1 (4.5) | 9 (40.9) | 12 (54.5) | 0 (0) |
| Alimoglu *et al*[42] | 7 | RME | 16 (14-21) | - | - | - | - | - | 0 (0) | 0 (0) | 3 (42.8) | 1 (14.2) | 3 (42.8) | 0 (0) |
| Akmal *et al*[43] | 80 | RME | 14.2 (2-33) | - | - | 32.5 (2-100) | - | 1.8 (0-45) | - | 15 (18.8) | 20 (25) | 12 (15) | 27 (33.8) | 5 (6.3) |
| Park *et al*[44] | 30 | RME | 20 (14-25)[1] | 25 | - | - | - | 11 (5-20) | 0 (0) | 6 (20) | 7 (23.3) | 4 (13.3) | 10 (33.3) | 3 (10) |
| Kang *et al*[45] | 389 | RME | 15.7 (+ 10) | - | 11.7 | 2.15 | - | - | 14 (3.6) | 24 (6.2) | 122 (31.4) | 103 (26.5) | 140 (36) | 0 (0) |
| deSouza *et al*[46] | 44 | RME | 14 (5-45) | - | - | - | 1 (2.7) | - | 0 (0) | 4 (9.1) | 14 (31.8) | 15 (34.1) | 8 (18.2) | 3 (6.8) |
| Pigazzi *et al*[47] | 143 | RME | 14.1 (1-39) | - | - | 29 (0-100) | - | 19 (1-45) | 1 (0.7) | 18 (12.6) | 36 (25.2) | 36 (25.2) | 53 (37) | 0 (0) |
| Choi *et al*[48] | 50 | RME | 20.6 (6-48) | - | - | 19 (5-45) | 0 (0) | - | 1 (2) | 0 (0) | 10 (20) | 19 (38) | 19 (38) | 2 (4) |
| Ng *et al*[49] | 8 | RME | 15 (2-26)[1] | - | - | - | - | - | 0 (0) | 0 (0) | 3 (37.5) | 2 (25) | 2 (25) | 0 |
| Hellan *et al*[50] | 39 | RME | 13 (7-28)[1] | - | - | 26.5 (4-75)[1] | 0 (0) | - | 0 (0) | 8 (20.5) | 13 (33.3) | 4 (10.3) | 13 (33.3) | 1 (2.6) |

[1]Median. RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision.

**Table 8 Short term oncologic outcomes**

| Ref. | Pts | Mesorectum | DSF% (yr) | LR (%) | Distant mtx (%) | OS % (yr) | F-u mo (median) |
|---|---|---|---|---|---|---|---|
| Park *et al*[8] | 133 | RME | 81.9 (5) | 3 (2.3) | 16 (12) | 92.8 (5) | 58 (4-80) |
| | 84 | LME | 78.7 (5) | 1 (1.2) | 14 (16.6) | 93.5 (5) | 58 (4-80) |
| Levic *et al*[9] | 56 | RME | | 0 (0) | 8 (14.3) | | 12 (1-31) |
| | 36 | LME | | 0 (0) | 2 (5.6) | | 10 (1-33) |
| Yoo *et al*[10] | 43[1] | RME | 76.7 (3) | 6 (12.8) | | 95.2 (3) | 33.9 (4.4-61.3) |
| | 26 | LME | 75 (3) | 2 (8.3) | | 88.5 (3) | 36.5 (3.7-69.9) |
| Ghezzi *et al*[16] | 65 | RME | 73.2 (5) | 2 (3.2) | 19 (29.6) | 85 (5) | 60 |
| | 109 | OME | 69.5 (5) | 17.5 (16.1) | 26 (24.2) | 76.1 (5) | 60 |
| Saklani *et al*[19] | 74 | RME | 77.7 (3) | 2 (2.7) | | 90 (3) | 30.1 (11-61)[2] |
| | 64 | LME | 78.8 (3) | 4 (6.3) | | 92.1 (3) | 30.1 (11-61)[2] |
| Kim *et al*[25] | 62 | RME | | 0 (0) | 3 (4.2) | 98 (1.5) | 17.4 |
| | 147 | LME | | 1 (0.7) | 8 (5.4) | 98 (1.7) | 20.6 |
| Kwak *et al*[28] | 59 | RME | | 1 (1.8) | 2 (3.6) | | 17 (11-25) |
| | 59 | LME | | 1 (1.9) | 2 (3.7) | | 13 (9-22) |
| Patriti *et al*[31] | 29 | RME | 100 (3) | 0 (0) | 0 (0) | 96.6 (2.4) | 29.22 |
| | 37 | LME | 83.7 (3) | 2 (5.4) | 4 (6) | 97.2 (1.5) | 18.72 |
| Stănciulea *et al*[38] | 100 | RME | | 2 (2) | | 90 (3) | 24 (9-63) |
| Alimoglu *et al*[42] | 7 | RME | 100 (1) | 0 (0) | 0 (0) | 100 (1) | 12 (6-21)[2] |
| Pigazzi *et al*[47] | 143 | RME | 77.6 (3) | 2 (1.4) | 13 (9) | 97 (3) | 17.4 (0.1-52.5)[2] |

[1]1 patient excluded (palliative ISR); [2]Mean. DSF: Disease free survival rate; RME: Robotic mesorectal excision; LME: Laparoscopic mesorectal excision; OME: Open mesorectal excision.

of instruments (endowrist™), stable camera holding, motion filter for tremor-free surgery and improved ergonomics as major improvements in rectal surgery but it seems that these technical benefits have not reflected better clinical outcomes yet. This review aimed to analyze robotic rectal surgery from the first report to nowadays in order to focus on the current state and assess any benefits of robotic rectal surgery and its evolution through the years.

A well-established finding of this review is the longer operative time of robotic surgery when compared to the laparoscopic one. This is most likely due not to longer dissection but to non-surgical technical time. In fact in the totally robotic approach the docking and undocking has to be performed twice and in the hybrid approach there is the need to switch from laparoscopy to robot. A totally robotic technique without undocking is feasible, but this approach is technically much more difficult and as a consequence, a longer operative time is needed[10,12,14,16,17,22-24,27,28,37,38,40-42,48]. Traditionally, longer operative time is related with increased morbidity, most likely related to the difficulty of the operation[53]. However prolonged times in robotic surgery are not associated with an increased complication rate as demonstrated by this review and previously published review and meta-analysis[55].

In our review 2[21,23], out of 16 comparative studies reported a significantly lower estimated blood loss after robotic rectal surgery confirming that there is still no evidence that robotic rectal surgery for cancer may be associated with a lower intraoperative blood loss.

As regards convertion rates to open surgery, 3[8,22,31] out of 22 comparative studies reported significant lower complication rates in robotic patients. Many authors associated these results to better visualization, 3D view, endowrist™ technology and stable camera holding resulting in an easier dissection in narrow anatomical fields such as the pelvis[56]. Even the results reported by Ielpo *et al*[14] suggest that the robotic approach has lower conversion rates when the tumor location requests a low anterior resection and as a consequence, when the operations is technically more challenging. Since converted cases are associated to greater morbidity and tumor recurrence[57], robotic surgery could provide better oncologic long term results as well as a decreased perioperative morbidity.

The difference in protective ileostomy creation observed in this review can be related to several factors: The surgeon's habit, the tumor location, the surgeon's learning curve. Moreover, a trend toward an increasing stoma creation after robotic surgery could have been verified because of the initial worries about the new technique. On the bases of our findings the robotic approach seems associated with a higher rate of protective stoma creation.

One of the main benefits of minimally invasive surgery is the early recover. In this review we were unable to draw definitive results about any benefit of the robotic technique over conventional laparoscopy. Length of hospital stay, day of 1$^{st}$ flatus, 1$^{st}$ solid diet and 1$^{st}$ liquid diet were substantially similar in both the robotic and laparoscopic series even if some authors reported some advantages for either the robotic or the laparoscopic technique[8,9,15,22-24,30,32].

Anastomotic leak is the most severe surgical complication in rectal surgery. Well known risk factors for anastomotic leak are represented by cancers located less than 6 cm from anal verge, neoadjuvant radiochemotherapy, obesity and intraoperative blood transfusions[58-63]. In this review the overall anastomotic leak rates in the robotic and laparoscopic series were similar (7.3% *vs* 7.6%) with no comparative study

reporting any significant difference between the 2 types of procedure. All together these results demonstrate that robotic surgery does not reduce the anastomotic leak rate. Nevertheless results of comparative studies are contradictory since 9[11,15,19,20-22,23,25,30] of these reported less anastomotic leaks in the robotic group and 9[8-10,14,17,24,28,29,31] in the laparoscopic one, but none of these results was significant. Looking at intraoperative complications, only Levic *et al*[9] reported a significant, higher rate in the robotic patients (4.48% *vs* 0%). However it must be considered that in this study there were more obese patients in the robotic group and all robotic and laparoscopic operations were performed in 2 different hospitals.

The number of harvested and examined lymph nodes is pivotal in the postoperative tumor staging whose accuracy increases with the number of nodes retrieved within the surgical specimen. The robotic platform with its 3D high definition vision and wrist-like movement of instruments should improve the lymph nodes retrieving. Nevertheless, the difference between the mean harvested lymph nodes in the robotic and laparoscopic series was not substantial in our review (15.1 *vs* 15.7 respectively) and only 2 authors[9,10] reported a significant higher number of retrieved lymph nodes in the robotic group.

The length of tumor involvement of both the distal and circumferential resection margins is considered an important parameter in evaluating the treatment of rectal cancer. Findings from the present review seems to determinate the lack of any advantages of robotic surgery over the laparoscopic approach. This issue might be explained by the likely surgeon's trend to prefer robotic approach in more advanced and distal tumors because of the theoretical superiority of this technique in pelvic dissection. In this review indeed 7 authors[10,11,15,20,22,25,31] reported a significant lower distance of the tumor from anal verge when the robotic approach was compared with the laparoscopic one. Two comparative studies[13,22] reported even a significant wider CRM in their robotic series when compared to the laparoscopic ones. However a possible bias in the evaluation of this parameter is the non-uniform recording of data: some authors report median values, others the mean values making data not comparable. Even definition of circumferential resection margin is still not clear as it is currently considered as positive as positive if < 1 mm[8,11,14,19,24,25,30,35,64] by some authors and < 2 mm[10,12,15-17,20,22,23,26-29,36,37,39,40,42,44-50] by others.

Thanks to its technical characteristics the robot platform should help in performing total and complete mesorectal excision that is an important target in rectal surgery since it potentially reflects the radicality of the operation. Unfortunately even if this is a major parameter in evaluating the radicality of the intervention, only 11 out of 43 studies in this review have addressed this important parameter. On the basis of our results any superiority of robotic mesorectal excision over the laparoscopic one cannot be demonstrated.

Robotic surgery may help in the identification and preservation of autonomic nerves due to high definition 3D image. Common sites of potential nerve damage are the superior hypogastric plexus, leading to ejaculation dysfunction in males and impaired lubrification in females, and the pelvic splanchnic nerve/pelvic plexus leading to erectile dysfunction in men. According to results of the CLASSIC trial[59] the risk of an autonomic injury with sexual dysfunction in males is significantly higher in laparoscopic surgery when compared to the open approach. The perceived advantages of robotic surgery may translate to decreased incidence of urinary dysfunction and erectile dysfunction in males. Although some preliminary results suggested that robotic assisted rectal surgery is superior to conventional laparoscopic surgery in preventing sexual or urinary dysfunction[63,64], we cannot provide definitive results since only few studies addressed this issue with high heterogeneity in the scores systems used for the analysis. Furthermore not all the patients in the studies agreed in answering questionnaires and this could lead to a possible type Ⅱ error. Some authors[26,18] reported an earlier recovery of erectile, sexual desire and urinary function when the robotic group was compared with the laparoscopic one but they did not report any difference in long-term follow-up.

In conclusion, results from the present review show that robotic surgery is as feasible and safe as conventional laparoscopy in the treatment of rectal cancer, with the only drawback of longer operative time. The magnified view, the improved ergonomics and dexterity might improve the diffusion of minimally invasive approach in the treatment of rectal cancer. Potential clinical benefits of the robotic technique must be demonstrated, if any, only by RCTs.

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REVIEW

# Molecular predictive markers in tumors of the gastrointestinal tract

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## Abstract

Gastrointestinal malignancies are among the leading causes of cancer-related deaths worldwide. Like all human malignancies they are characterized by accumulation of mutations which lead to inactivation of tumor suppressor genes or activation of oncogenes. Advances in Molecular Biology techniques have allowed for more accurate analysis of tumors' genetic profiling using new breakthrough technologies such as next generation sequencing (NGS), leading to the development of targeted therapeutical approaches based upon biomarker-selection. During the last 10 years tremendous advances in the development of targeted therapies for patients with advanced cancer have been made, thus various targeted agents, associated with predictive biomarkers, have been developed or are in development for the treatment of patients with gastrointestinal cancer patients. This review summarizes the advances in the field of molecular biomarkers in tumors of the gastrointestinal tract, with focus on the available NGS platforms that enable comprehensive tumor molecular profile analysis.

**Key words:** Predictive biomarkers; Targeted therapy; Next generation sequencing; Gastrointestinal tract; Somatic mutations; Liquid biopsy



**Core tip:** Gastrointestinal cancers are among the leading causes of cancer morbidity and mortality worldwide. So far, various targeted agents associated with predictive biomarkers are available or are under development for the selection of treatment in patients with gastrointestinal cancer. Advances in high-throughput technologies such as next generation sequencing and the use of noninvasive

materials for tumor characterization, such as liquid biopsies, will facilitate tumor molecular profiling and lead to the establishment of further targeted treatment therapies.

Papadopoulou E, Metaxa-Mariatou V, Tsaousis G, Tsoulos N, Tsirigoti A, Efstathiadou C, Apessos A, Agiannitopoulos K, Pepe G, Bourkoula E, Nasioulas G. Molecular predictive markers in tumors of the gastrointestinal tract. *World J Gastrointest Oncol* 2016; 8(11): 772-785 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i11/772.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i11.772

## INTRODUCTION

The comprehension of the importance of tumor biology has led to the development of new drugs that target specific molecules involved in carcinogenesis. The efficacy of such targeted therapies often depends on the presence or absence of gene alterations that encode for the protein-target or for proteins involved in the molecular pathway targeted by the specific medication. This targeted therapeutical approach is based on the tumor's molecular analysis in order to select patients with increased probability to respond to the treatment given. Advances in Molecular Biology techniques have permitted comprehensive tumor genomic profiling using new breakthrough technologies such as next generation sequencing (NGS)[1-3].

Nowadays, biomarkers are used in the management of patients with cancer and can be divided into predictive and prognostic. Prognostic biomarkers are defined as those that provide information on the possible outcome of cancer in a particular patient regardless of treatment. Predictive biomarkers provide information on the potential benefit of the administrated treatment (whether this relates to the tumor's volume shrinkage or survival). Predictive biomarkers can be used to identify subpopulations of patients that are likely to respond to a particular treatment[1]. They can be subdivided in positive and negative predictive biomarkers. The first are used for positive selection of patients who are likely to benefit from targeted therapy, whereas the latter for resistance prediction[1].

The number of genes involved in targeted therapy (predictive biomarkers), is increasing continuously. The simultaneous analysis of these biomarkers is feasible using molecular biology technologies that allow accurate, fast and cost effective genomic analysis with limited requirements concerning the quantity of the biological material used[1,4]. NGS has all the features required to carry out such analysis and provides simultaneous information on a large number of actionable alterations in tumor tissues and thus a more precise molecular characterization of the tumor. The massive amount of genetic information produced is the main advantage of this technology. However, it also constitutes its main challenge, requiring usage of appropriate software and bioinformatics tools, along with web-based tools for data analysis, management and interpretation[5].

The human gastrointestinal (GI) tract is an organ system which includes all structures between mouth and anus and is divided into upper (buccal cavity, pharynx, esophagus, stomach and duodenum) and lower (small and large intestine) GI tracts. GI cancers are complex diseases and refer to malignant conditions that affect the digestive system. The current review will focus on the advances in the field of molecular biomarkers and the application of high throughput technologies, in the most common tumors of the gastrointestinal tract.

### *Esophageal cancer*

Esophageal cancer is one of the most aggressive malignancies with a rapidly increasing incidence rate in the recent decades. There are two predominant histological types: Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) of the distal esophagus and the gastroesophageal junction. Smoking and heavy alcohol consumption are associated with increased risk of ESCC, while gastroesophageal reflux disease and Barrett esophagus may of increase the risk of EAC[6].

*TP53* mutations are identified in about 50% of esophageal cancers and are associated with poorer survival[7]. Apart from mutations in *TP53* ESCC and EAC seem to differ significantly in the genetic alterations pattern. Agrawal *et al*[8] using NGS reported a substantial disparity in the spectrum of mutations, with more insertions/deletions in ESCCs, A:T>C:G transversions in EACs, and C:G>G:C transversions in ESCCs. Inactivating mutations of *NOTCH1* are identified in about 20% of ESCCs but not in EACs. Somatic aberrations in EACs are mainly identified in the Wnt, cell cycle and Notch pathways[9,10]. A number of genes that can be used as predictive markers for targeted therapy have been explored for somatic mutations in esophageal adenocarcinoma, including genes of the RAF/MEK/ERK (MAPK) kinase pathway such as *EGFR, BRAF, KRAS, PIK3CA*[11]. However the reported frequency of somatic mutations identified appears to be low and this is obvious when accessing data from the Catalogue of Somatic Mutations in Cancer database (COSMIC, cancer.sanger.ac.uk) (Figure 1), which is currently the most comprehensive global resource accessing the world literature on somatic mutations in human cancer[12]. In a recent study, NGS-based comprehensive genomic profiling was used to analyze ESCC and EAC tumors[13]. The analysis showed that the esophageal histotypes differ significantly in genomic alterations profile, with *KRAS* and *ERBB2* far more frequently altered in EAC compared to ESCC. In contrast, genes of the mechanistic target of rapamycin (MTOR) pathway (*PIK3CA* and *PTEN*) and *NOTCH1* are more frequently altered in ESCC compared to EAC. They also have different amplification patterns (Figure 2).

ESCC and EAC also differ in the gene amplification

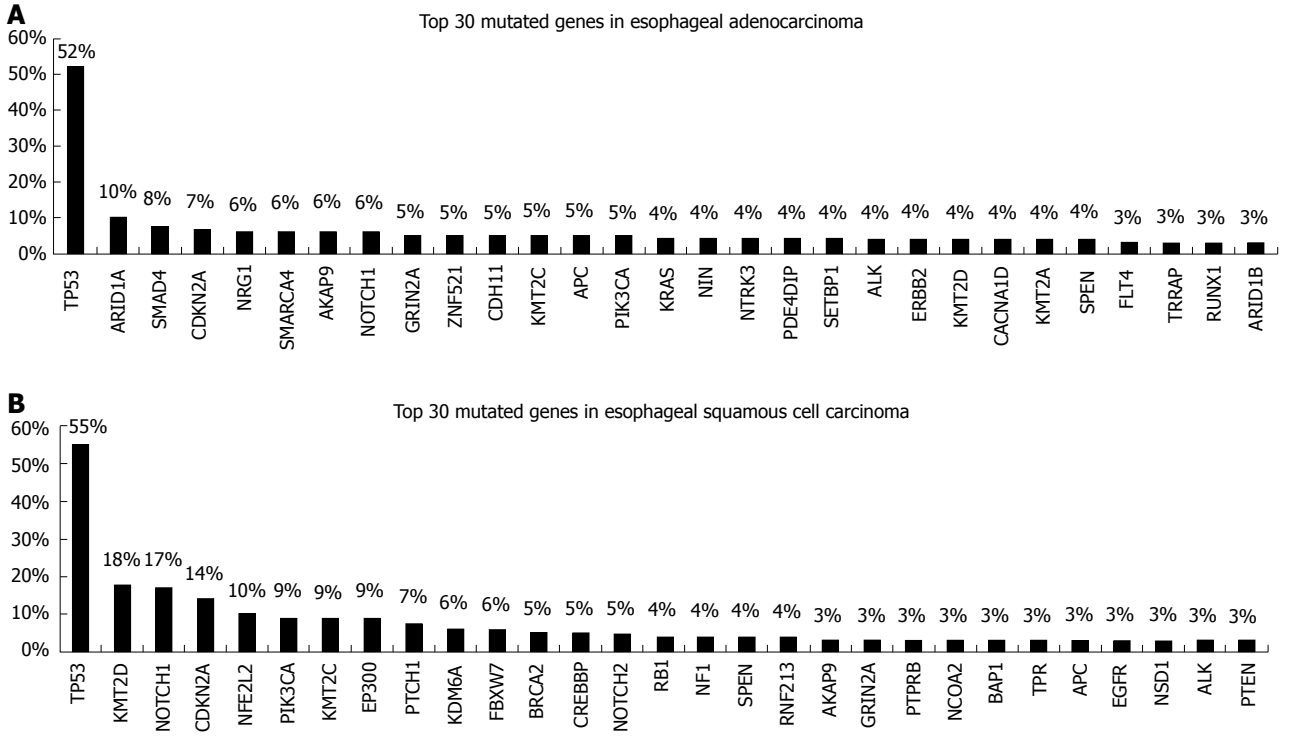


**Figure 1 Bar chart showing the most frequently mutated genes in esophageal cancer according to catalogue of somatic mutations in cancer database.** In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the most frequently mutated genes are listed. A: Top 30 mutated genes in esophageal adenocarcinoma; B: Top 30 mutated genes in esophageal squamous cell carcinoma.

and/or protein (over)expression of the receptor tyrosin kinases (RTKs) EGFR and HER2 making them possible prognostic markers and as therapeutic targets[7,14]. EGFR is frequently overexpressed in ESCCs, while HER2 overexpression occurs mainly in EACs. Thus the trastuzumab-platinum regimen is currently used for the 15% of the EACs patients that test positive for HER2 (ERBB2) amplification or overexpression[13,14].

Numerous preclinical studies addressed EGFR and HER2 inhibition in esophageal cancer cell lines and there are various phase Ⅱ/Ⅲ clinical trials testing EGFR, HER2, and VEGF targeting therapies for esophageal cancer[7,15]. However, the results obtained to date do not allow the use of these agents in clinical practice. Upon trial completion several clinical studies have concluded, that in order to select patients who will respond to RTK-targeted therapy, there is a need for molecular patient stratification before treatment.

In a disease with historically poor outcomes and limited options, comprehensive genomic profiling of relapsed and refractory cancers, including distinct evaluation for EAC and ESCC has led to promising information suggesting targeted therapies for future consideration.

### *Gastric cancer*

Gastric cancer (GC) develops from the inner lining of the stomach and is a very aggressive malignancy, with poor prognosis and very high cancer related mortality. The high mortality rate is largely due to the late stages of cancer diagnosis and to the lack of effective medical treatment for advanced stages of this disease[16,17]. The majority of these cancers are adenocarcinomas and can be further classified as diffuse (poorly differentiated) or intestinal (well-differentiated) types that have distinct molecular profiles[16,17].

Concerning the causes of GC, it can be viewed as a multifactorial disease since many inherited and environmental factors play a role in its development. Infectious agents such as Helicobacter pylori and EBV, dietary habits and the genetic background are considered as causative agents[17].

Given the variety of causes of the disease, it is not surprising that these tumors present a high level of biological heterogeneity, with distinct molecular profile for each patient. Genetic and epigenetic alterations play important role in GCs therefore, targeted therapy based on the biology of the individual patient could improve treatment outcome[17-19].

ERBB2 amplifications occur frequently in gastric tumors (2%-27%)[12,20]. Trastuzumab, a monoclonal antibody against HER2/neu receptor, was the first targeted agent to be used in the treatment of ERBB2-positive advanced gastric and gastroesophageal junction (GEJ) adenocarcinoma[20]. Several molecular targeted agents associated with a survival benefit in other cancer types are now under clinical investigation for the treatment of gastric cancer, including inhibitors of EGFR, MET, FGFR, VEGF, and PI3K[18,20]. Additionally, *CDH1* gene mutations at the somatic level are considered of prognostic significance[19].

Several studies have investigated gastric cancer's

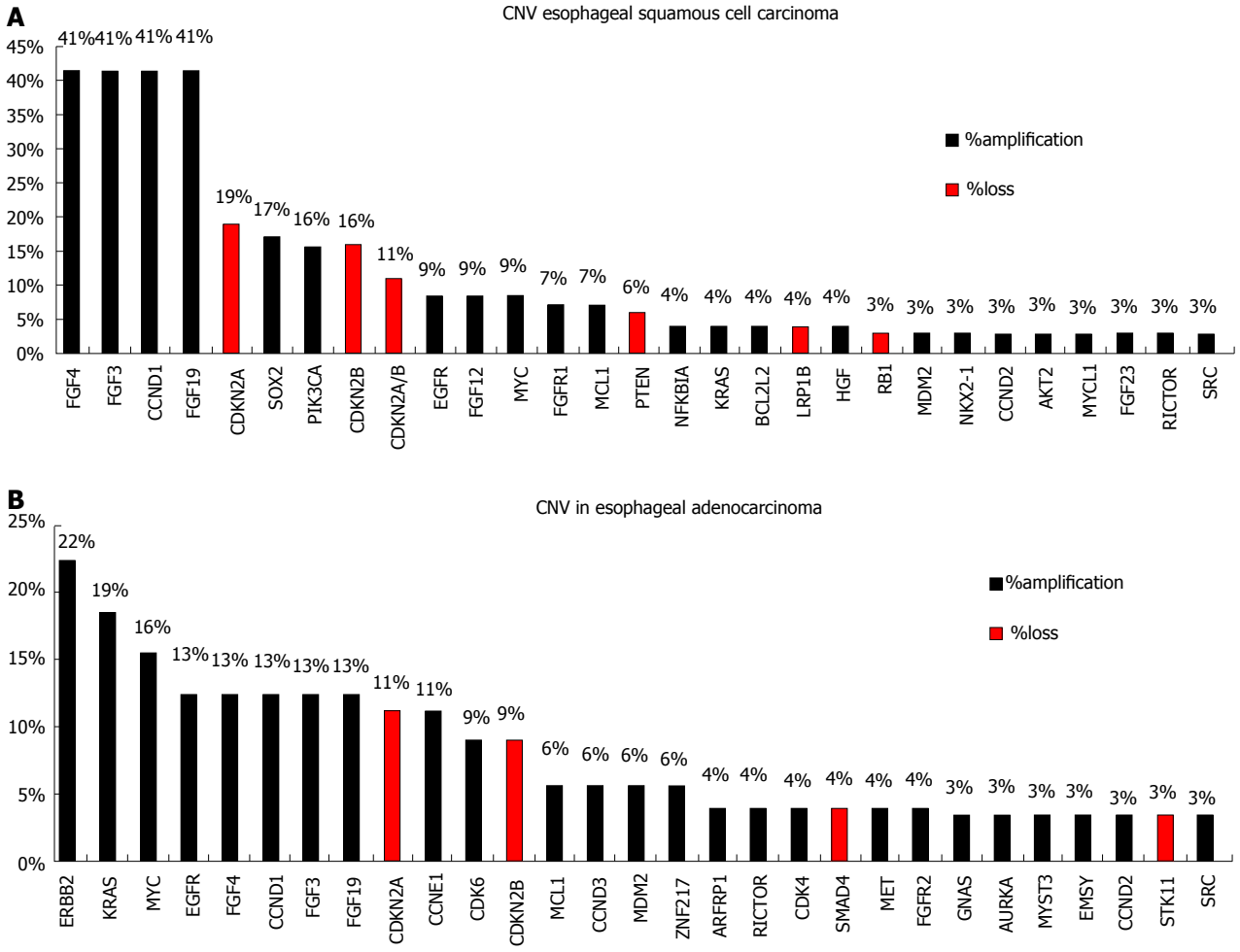


**Figure 2 Bar chart showing the copy number variations in esophageal cancer according to the study performed[13].** In the Y-axis the percentage of CNV frequency is represented. In the X-axis the most frequently altered genes are listed. A: Genes most commonly affected by CNV (amplification or loss) in esophageal adenocarcinoma; B: Genes most commonly affected by CNV (amplification or loss) in esophageal squamous cell carcinoma. CNV: Copy number variation.

molecular profile using whole genome as well as targeted NGS approaches[19,21-23]. The presence of somatic mutations and copy number variations (CNV) in many cancer driver genes has been revealed. Among the cancer genes frequently mutated in gastric cancer *P53*, *ARD1A*, *CDH1*, *PIK3CA*, *APC, CTNNB1, ERBB3, ATM, KRAS* are the most important prognostic and/or predictive markers (Figure 3)[12]. Consequently, molecular profile-directed therapy seems to be a promising strategy for the improvement of standard chemotherapy effectiveness.

CNVs have been observed for HER2, FGFR2, and MET that represent viable treatment targets for which therapeutics are already approved or are currently under investigation[24] (Figure 4).

The high heterogeneity of these tumors triggered scientists to attempt their molecular characterization. In a study conducted by The Cancer Genome Atlas, molecular classification four major genomic subtypes of gastric cancer were defined: EBV-infected tumors; MSI tumors; genomically stable tumors; and chromosomally unstable tumors[21].

In a recent study, Li *et al*[19], using whole genome NGS data were able to classify gastric cancers into regular (86.8%) and hyper-mutated (13.2%) subtypes based on mutation burden. Additionally, in the "regular" mutated cohort a further classification, using 40 significantly mutated genes, could be obtained, separating the patients to S1 and S2 subtypes with distinct prognostic outcomes.

### *Gastrointestinal stromal tumors*

Gastrointestinal stromal tumors (GISTs) are rare tumors of the gastrointestinal tract. They are mesenchymal in origin and are characterized by overexpression of the KIT protein[25]. Morphological diagnosis based on microscopic examination is the standard for GIST diagnosis. They occur anywhere within the GI tract, but they are most common in the stomach (60%) or small intestine (30%)[26]. Their diagnosis is based on the expression of the transmembrane tyrosine kinase (TK) receptor, KIT, since 95% of GISTs express CD 117 antibody. In 80% of the cases, somatic mutations in the *cKIT* gene are observed, resulting in constitute receptor activation. Additionally, in 5%-10% of the cases without *cKIT* mutations, the TK receptor *PDGFRA* is

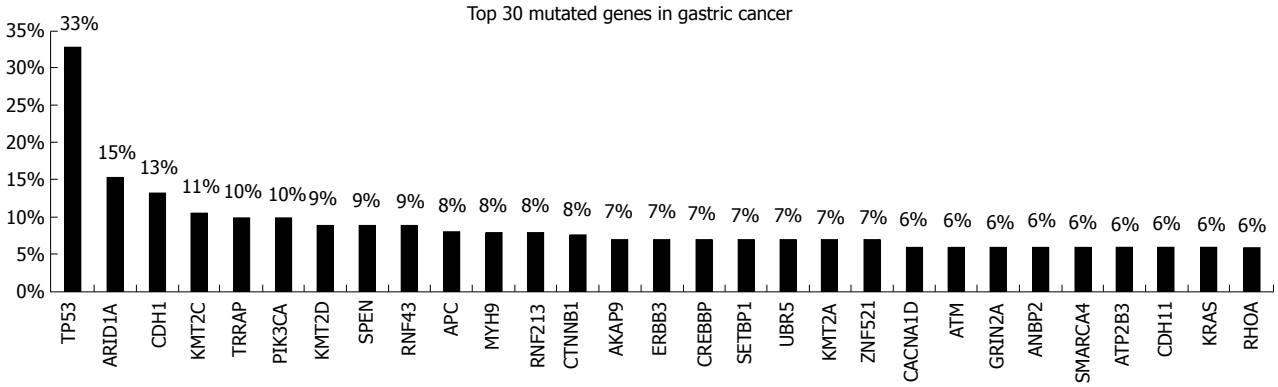


**Figure 3 Bar chart showing the most frequently mutated genes in gastric cancer according to catalogue of somatic mutations in cancer database.** In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the genes are listed.

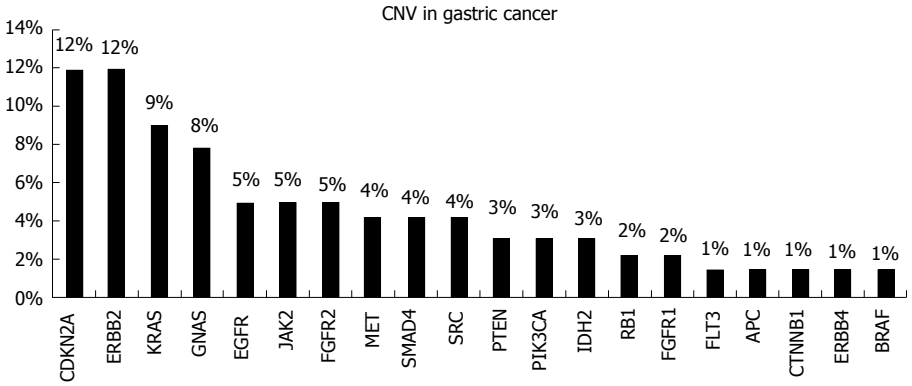


**Figure 4 Copy number variation in the most important treatment targetable genes in gastric cancer.** In the Y-axis the percentage of observed CNV is represented. In the X-axis the genes are listed. CNV: Copy number variation.

mutated[27]. The mutation spectrum of these tumors is very limited as we observe in COSMIC database (Figure 5)[12]. Tyrosine kinase inhibitors (TKIs) like imatinib, sunitinib, and more recently regorafenib, have proven effectiveness in suppressing the growth of metastatic GISTs, allowing patients to live far longer than during the previous era of ineffective chemotherapy[28-30]. The response to targeted therapy with TKIs is mainly dependent on the presence and type of mutation. Patients with mutations in exon 11 of the *cKIT* are highly responsive to imatinib, while the presence of a mutation in exon 9 of this gene implies intermediate response rates and necessitates a double dose of drug administration. Furthermore, resistance mutations to imatinib are also observed in the *cKIT/PDGFRA* genes. These mutations can be present in the primary tumor or arise as a result of the drug administration (secondary mutations)[29].

### *Colorectal cancer*

The cancer of colon and rectum (colorectal, CRC) is the third most common cancer worldwide with 95% of these tumors being classified as adenocarcinomas. It's a leading cause of cancer related deaths; however, colorectal cancer mortality is declining in the last decades, mainly due to early diagnosis and the presence of new therapy strategies[31,32]. This malignancy is one of the first paradigms of the benefits that can be derived from the application of personalized treatment in cancer therapy[33-35].

Genetic alterations in colorectal cancer include mainly single-base substitutions (SBS). Nevertheless, small insertions and deletions (indels), amplifications, homozygous deletions and translocations can also be observed[36].

Five hundred and seventy-two cancer relevant genes are included in the Catalogue of Somatic Mutations in Cancer (COSMIC, cancer.sanger.ac.uk)[12]. Somatic mutations in CRC cancer are observed in the majority of these genes. The pattern of genomic alterations was identified through Massively Parallel Sequencing studies, revealing the inter- and intra-tumor genetic heterogeneity of these tumors[37-39]. Apart from single base substitutions, gene amplifications are also observed (Figure 6). Mutations in many important biologic pathways occur. In Table 1 the frequency of mutations in important molecular signaling pathways and the related therapies are represented. The gene mutation frequency is calculated using data from samples analysed by whole genome screening in the COSMIC database. The information concerning the therapies targeting each pathway was retrieved from MyCancer Genome knowledge database (www.mycancergenome.org/), that provides reliable information concerning

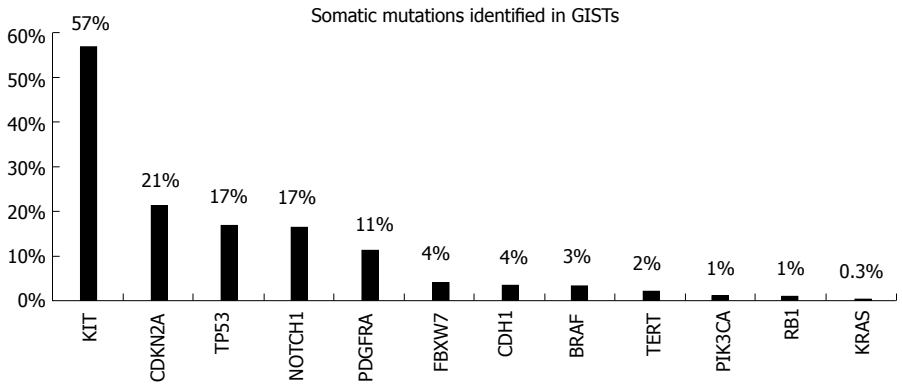


**Figure 5 Bar chart showing the most frequently mutated genes in gastrointestinal stromal tumors according to catalogue of somatic mutations in cancer database.** In the Y-axis the percentage of observed mutation frequency is represented. In the X-axis the genes are listed. GIST: Gastrointestinal stromal tumors.

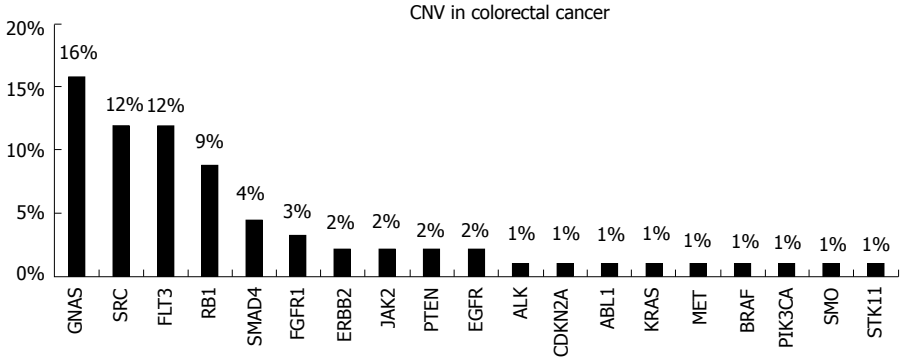


**Figure 6 Copy number variation in the most important treatment targetable genes in colorectal cancer.** CNV: Copy number variation.

important cancer related genes and their correlation with treatment options (Table 1)[40].

The *RAS* proto-oncogenes (*HRAS, KRAS* and *NRAS*) encode a family of highly homologous proteins. They participate in a signal transduction cascade, namely the RAS/RAF/MEK/ERK pathway, which regulates the growth and survival properties of the cells. They are controlled by extracellular signals transmitted by the transmembrane receptor tyrosine kinase (TK), EGFR[34]. This TK and the RAS/RAF/MEK/ERK pathways it controls, play an important role in colorectal carcinogenesis, making it a good target for biological therapy of this disease.

Two monoclonal antibodies were designed as effective inhibitors of EGFR. Cetuximab (Erbitux, Merck KgaA, Darmstadt, Germany) is a chimeric mouse/human antibody, and Panitumumab (Vectibix, Amgen Thousand Oaks, CA, United States), is a fully human antibody[33-35]. They both target the extracellular domain of the EGFR protein and compete with ligands, blocking ligand induced intracellular signal transmission. However, anti-EGFR treatment is not effective in patients harboring activating mutations in genes that participate in the intracellular transduction RAS/RAF/MEK/ERK pathway. This is due to the constitutive, independent of ligand, activation of the mutated proteins[33].

In total, activating mutations in the *RAS* genes, mainly in codons 12, 13 or 61, occur in approximately 20% of all human cancers. Mutations in *KRAS* account for about 85% of all *RAS* mutations in human tumors, *NRAS* for about 15%, and *HRAS* for less than 1%[41,42]. Which particular *RAS* gene is mutated seems to be tumor specific. Colonic, pancreatic and lung cancers have high frequencies of *KRAS* mutations[41,42].

Acquired mutations in *KRAS* and *NRAS* are commonly used to identify colorectal cancer patients who are unlikely to benefit from anti-EGFR therapy. Approximately 40% of colorectal cancer tumors harbor mutations in the *KRAS* gene, with the majority of the mutations occurring in codons 12, 13 and 61. In 5% of the colorectal cancer cases a mutation occurs in the *NRAS* gene[35,41].

Another important gene of the RAS/RAF/MEK/ERK pathway is *BRAF*. Mutations in the *BRAF* gene (exons 11 and 15) have been detected in about 12% of colorectal cancers and are mutually exclusive with *RAS* mutations[12,43,44]. The *BRAF* activating aberrations, result in constitutive BRAF kinase activity, ERK signaling, proliferation and transformation[44]. The majority of *BRAF* mutations are observed in exon 15 (codon 600) and a minority of mutations are observed in exon 11[44,45].

Several studies have reported that patients with metastatic CRC (mCRC) that harbor *BRAF* mutations do not respond to the anti-EGFR antibody agents cetuximab or panitumumab[43,46,47]. However it is unclear if the presence of *BRAF* mutations in CRC cancer can be

**Table 1 Overall gene mutation frequency in each molecular signaling pathway**

| Biologic pathway | Frequency of mutation in genes involved in each pathway | Therapies that target the pathway |
|---|---|---|
| Beta-catenin/WNT signaling | 75% | FZD, GSK inhibitors |
| Cell cycle control | 68% | CDK, CDK1, CDK2, CDK4/6 inhibitors |
| Receptor tyrosine kinase/growth factor signaling | 67% | Therapeutic antibodies/tyrosine kinase inhibitors |
| MAP kinase signaling | 61% | BRAF, ERK, MEK AND SRC inhibitors |
| PI3K/AKT1/MTOR | 52% | Allosteric mTORC1 inhibitors/mTORC1/2 catalytic inhibitors |
| DNA damage/repair | 48% | PARP INHIBITORS |
| TGFbeta signaling | 37% | TGFBR1 inhibitors |
| Chromatin remodeling/DNA methylation | 32% | DNMT inhibitors, Histone deactylase |
| Immune checkpoints | 26% | Anti-CTLA4 antibodies, anti-PD-1 antibodies, Anti-PD-L1 antibodies, Immunotherapies |
| JAK/STAT signaling | 23% | JAK inhibitors |
| Hedgehog signaling | 12% | SMO inhibitors |

The information concerning therapies that target each molecular pathway was retrieved for MyCancer Genome Site (Available from: URL: http//www.mycancergenome.org/).

used as a predictive marker or if it has only a prognostic value, independent of treatment, since different studies arrive at controversial conclusions concerning its clinical significance[45,48].

The *PIK3CA* gene encodes the catalytic subunit of phosphatidylinositol 3-kinase while belongs to a family of lipid kinases. These kinases regulate a diverse range of cellular processes including cell proliferation, adhesion, survival, and migration[49]. Mutations in *PIK-3CA* stimulate downstream AKT-mTOR signaling pathways, thereby promoting growth-factor independent growth, cell invasion and metastasis. *PIK3CA* mutations have been reported in multiple malignancies, including approximately 25% of gastric, 4% of lung, 25% of breast, and 20% of colorectal cancers[50]. The majority (80%) of *PIK3CA* mutations cluster in 2 "hotspot" regions, the helical domain (exon 9) and the kinase domain (exon 20). Concomitant *PIK3CA* mutations in exons 9 and 20 seem to be linked to significantly worse cancer-specific survival[51]. *PIK3CA* mutations may also be associated with clinical resistance to EGFR-targeted monoclonal antibodies, but there have been conflicting results[52-55]. A meta-analysis comprising 864 patients, from 11 studies, with colorectal cancer treated with cetuximab or panitumumab-based therapy showed that *PIK3CA* mutations, particularly in exon 20, are significantly associated with worse response and shorter progression-free and overall survival[51]. Somatic *PIK3CA* mutations have also been associated with superior colorectal cancer-specific survival in patients who regularly intake aspirin after diagnosis[56]. *PIK3CA* activating mutations may also predict sensitivity to inhibitors of the PI3K-AKT-mTOR pathway[57]. Inhibitors of mTOR, PI3K, and AKT, alone or in combination with other therapies are in clinical trials in solid tumors[58,59].

A number of rare gene mutations occurring in the PI3K/AKT/mTOR pathway are potentially actionable in colorectal cancer. PTEN is a key negative regulator of the PI3K pathway. *PTEN* gene mutations occur in about 5% of colorectal cancers[12,60,61]. *PTEN* inactivating mutations and *PTEN* loss have as a consequence the upregulation of the PI3K/ AKT pathway[54,61].

Currently, the prognostic and predictive significance of *PTEN* mutations or *PTEN* loss of expression is under investigation. In retrospective studies, *PTEN* loss was associated with decreased sensitivity of colorectal cancer tumors to anti-EGFR antibodies[60-62]. Preclinical data and *in vitro* studies suggest that it may be associated with sensitivity to PI3K and mTOR inhibitors. Based on these data, several PI3K and mTOR inhibitors are currently in clinical trials for the treatment of patients with PTEN-deficient cancers[61,63].

AKT (Protein kinase B, PKB) is a serine/threonine kinase that is encoded by three genes AKT1, 2 and 3. Somatic mutations in the *AKT1* gene occur in colorectal cancer in about 1% of the cases according to the COSMIC database[12]. The only mutation observed is the activating mutation *E17K*, which is also observed in other types of cancer[64]. AKT1 is a critical component of the PI3K/AKT/mTOR pathway, thus it has become an attractive target for therapeutic intervention[49,65]. *AKT1 E17K* mutations have also been associated with primary resistance to cetuximab[66].

In colorectal cancer, DNA mismatch repair (MMR) system deficiency occurs frequently and leading to microsatellite instability (MSI). These are small changes in the DNA sequence that occur during DNA replication and are usually additions or deletions of one or two nucleotide bases[67,68]. This phenomenon is most common in areas of the genome that contain repetitive DNA sequences with a repeat unit, from one to four bases, and are known as Microsatellite regions[67,69]. The presence of microsatellite instability (MSI High) is a good prognostic marker[67,70]. It is found in 90% of cases of tumors arising patients with hereditary Lynch syndrome and in 10%-15% of the sporadic cancers[71]. Sporadic MSI-H tumors can be distinguished from the hereditary ones through somatic mutation analysis of the *BRAF* gene or loss of *MLH1* expression[72,73]. Somatic mutations in the *BRAF* gene occurs only in sporadic MSI-H tumors but not in Lynch-associated CRC cancers. Similarly, *hMLH1* promoter methylation rarely occurs in

Lynch syndrome-associated cancers, while is common in sporadic MSI-high cancers[73].

Recent studies have indicated that MSI high tumors, both sporadic and hereditary, are less aggressive and are related with low probability of lymph node and distant recurrences[70]. In addition they respond differently to chemotherapy, since they are less sensitive to Topoisomerase inhibitors and to the treatment with 5-fluorouracil[74-76]. Additionally, it has been proposed that MMR-deficient tumors are more responsive to PD-1 blockade than the mismatch repair-proficient tumors[77].

Gene expression profiling (GEP) is an emerging tool which aims to identify differentially expressed subsets of genes (gene signatures) in groups of patients with distinct clinical outcomes. Several commercial GEP tests are currently available for stage Ⅱ/Ⅲa colorectal cancer patients. Oncotype DX® Colon Cancer Assay (Genomic Health, Inc., Redwood City, CA) and ColoPrint (Agendia, BV, Amsterdam, Holland) are the most promising gene signature tests[78]. Both tests provide a risk of recurrence, but OncotypeDx has the advantage of being applicable to Formalin Fixed Paraffin Embedded (FFPE) tissue for analysis, while ColoPrint requires fresh tissue which is not easily available. Oncotype DX® Colon Cancer Assay is a quantitative reverse transcription polymerase chain reaction (RT-qPCR) assay on RNA extracted from FFPE tumor tissue, used to assess risk of recurrence in stage Ⅱ colon cancer patients at three years after surgery[79-81]. The test uses gene expression profiling of 12 genes that include seven prognostic genes and five reference genes, in order to provide a Recurrence Score (RS). The RS allows patients and physicians to determine the risk of developing a distant metastasis. In a retrospectively performed study by Yothers *et al*[82], RS was the strongest predictor of disease recurrence independent of other factors, such as T-stage, mismatch repair status, number of nodes examined, tumor grade, and lymphovascular invasion. The greatest utility of this test seems to be in the prediction of recurrence risk in T3, mismatch repair-proficient (MMR-P) stage Ⅱ colon cancer patients[82]. However, it has also been validated in stage Ⅲ patients with very promising results[83]. The continuous RS predicted recurrence as well as disease free survival (DFS) and overall survival (OS) in all three patient subgroups (stage Ⅱ, ⅢA/B, and ⅢC). The use of this assay could lead to overall reduction in adjuvant chemotherapy use in this subgroup of stage Ⅱ/Ⅲ colon cancer patients[83].

## LIQUID BIOPSY

Until recently, the best material for somatic mutation analysis was considered formalin fixed paraffin embedded (FFPE) tumor tissue. FFPE tissue is a widely available material, easy to use and maintain. In addition, the cancer tissue can be selected and mutation analysis can be performed without contamination by normal tissues[42]. This increases the sensitivity of mutation detection assays which is very important because, due to tumor heterogeneity, somatic mutations can sometimes be present at a very low percentage. However, FFPE tissue material also has several disadvantages[84,85]. First of all in some cases it is not available. This is the case of non-operable tumors. Furthermore, the examination of a limited tumor area present in a paraffin block doesn't take into account tumor molecular heterogeneity and does not necessarily reflect the molecular profile of other tumors or metastasis that are eventually present in the patient's body[85]. Additionally, the genetic material obtained, due to the paraffinization process, is sometimes of very bad quality and not suitable for molecular analysis[86]. Most importantly, tumor molecular profile is altered mainly following therapy and those alterations cannot be detected by analyzing the primary tumor material[87].

Nowadays, the presence of cell-free tumor derived nucleic acids (ctDNA/ctRNA) in cancer patients body fluids (plasma, serum, Broncho-alveolar, urine, stool, *etc.*) is well documented[84]. The term Liquid Biopsy has emerged indicating the use of these noninvasive materials for tumor characterization. The mutation status detected in a liquid biopsy reflects the status present in the patient's tumor. Furthermore Liquid Biopsy analyses take into account intra-tumor or inter metastatic heterogeneity and could eventually detect more tumor alterations compared with the analysis of a specific area in a FFPE tissue[83,84]. A variety of sensitive methods can be used for the detection of ctDNA in plasma samples, including digital PCR, Real time PCR, Arms PCR and NGS[84].

The utility of liquid biopsy analysis has been proven in many studies that used ctDNA for the detection of tumor specific alterations in plasma with prognostic and/or predictive significance[87-98]. A liquid biopsy analysis can be performed before treatment as well as for patients monitoring during therapy. It is also very helpful in the detection of secondary mutations that arise due to targeted therapy. The detection of secondary mutations in plasma can modify the treatment strategy for those patients (Table 2).

## NGS METHODOLOGIES

NGS is a general term referring to all post-Sanger sequencing technologies that are able to massively sequence millions of DNA segments[99,100]. The goal of these technologies is to increase sequencing capacity and speed at a lower cost. Furthermore, the sensitivity obtained is superior to that of the conventional sequencing technology, making possible the detection of mutations that are present at very low percentages in a background of normal DNA, which is very important for somatic mutation detection. Currently the most widely used platforms are those offered by Illumina, Inc. (United Sates); Thermo Fisher Scientific, Inc. (United

**Table 2 Gene mutations identified in ctDNA of patients with tumors of the gastrointestinal tract and their correlation with possible clinical implications**

| Gene | Mutation type | Tumor type | Possible clinical implications | Ref. |
|---|---|---|---|---|
| *KRAS/NRAS* | Point mutation/amplification | Colorectal/pancreatic cancer | Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors | [88-90] |
| *BRAF* | Point mutation | Colorectal cancer | Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors | [88,91] |
| *MET* | Amplification/alteration | Colorectal/esophageal cancer | Resistance to anti-EGFR therapy/sensitivity to MEK inhibitors | [92-94] |
| *HER2* | Amplification | Colorectal/gastric cancer | Resistance to anti-EGFR therapy/sensitivity to Anti HER2 inhibitors | [95,96] |
| *EGFR* | Point mutation | Colorectal/pancreatic cancer | Panitumumab | [97,98] |
| *PIK3CA* | Point mutation | Colorectal/pancreatic cancer | mTOR inhibitor | [89] |
| *Ckit* | Point mutations | GISTS | Imatinib or dose escalation or alternative TKIs | [99,100] |
| *PDGFRA* | Point mutations | GISTS | Imatinib or dose escalation or alternative TKIs | [99,100] |

TKI: Tyrosine kinase inhibitors; GIST: Gastrointestinal stromal tumors; EGFR: Epidermal growth factor receptor; mTOR: Mechanistic target of rapamycin.

Sates) and Roche Holding AG (Switzerland)[101-103]. The first NGS platform was created by Roche and used emulsion PCR (emPCR) to clonally amplify the fragments that are then sequenced *via* sequencing-by-synthesis (SBS) technology[101]. The Illumina platform is currently widely used in the NGS market and involves bridge amplification, of a solid surface-bound DNA, to clonally amplify the fragments that are then sequenced using SBS chemistry[102]. Unlike the previous two technologies, the Life technology platform uses Ion semiconductor sequencing, instead of fluorescence based sequencing, detecting the protons released as nucleotides are incorporated during synthesis[103,104].

The past years have seen an accelerating outbreak of publications in which NGS is applied for a variety of goals such as full-genome resequencing or more targeted mutation detection. Worldwide collaborative efforts, such as COSMIC database, International Genome Consortium (ICGC) and The Cancer Genome Atlas (TCGA) project, enabled to catalogue NGS data of thousands of cancer genomes across many disease types[105,106]. Targeted NGS, involving gene panels, is a quicker and cost effective alternative to whole genome sequencing or exome sequencing. Targeted NGS panels for somatic mutation detection include actionable cancer genes and allow the determination of the patient's tumor molecular profile. The goal of their use is to increase the percentage of patients with detected actionable alterations and with copy number data, allowing them to be included in clinical trials[107-109].

Such panels are currently available or can be custom made. They exhibit high rates of sensitivity, specificity and repeatability; therefore they are optimal for diagnostic use. Benchtop NGS sequencers are now offered by both Illumina (MiSeq) and Thermo Fisher (PGM™ and Ion Proton™). The availability of the equipment required and the cost effectiveness of the analysis allows its implementation in local specialized laboratories[108,109]. However, the reliability of these tests should be reassured. Thus, NGS performing laboratories should have specialized personnel and equipment which will provide adequate data analysis management and interpretation with the aid of appropriate software and bioinformatics tools. Importantly, these tests should be operated under the guidelines of a quality assurance system[110,111].

Concerning the selection of the appropriate sequencing platform, it should be based on the individual laboratory's needs. All NGS platforms have advantages and disadvantages and the choice of the platform used should be based on the application for which it is required. For example, the MiSeq platform (Illumina) has lower error rates especially in the homo-polymer regions compared to both Ion Proton and PGM (Life Technology). However it requires higher DNA concentration and quality, which is not always available when the starting material is FFPE tissue. On the other hand, the NGS platforms offered by Thermo Fisher provide a fast and cost-effective sequencing solution with good analytical performance. Additionally, they more compatible with low DNA concentrations and partially degraded poor quality DNA from FFPE samples[107-109,112]. Consequently, they provide an attractive option of clinical utility for the detection of cancer hotspot mutation analysis.

## CONCLUSION

This review summarizes the use of biomarkers in the most common cancers of the GI tract. They are used for positive selection of patients who are likely to benefit from targeted therapy or for resistance prediction. Biomarker based targeted treatment is established in a subset of patients with gastrointestinal cancer. Meta-analysis studies have shown that biomarker based treatment is a promising approach and is associated with improved treatment outcome[113-115]. However, ongoing clinical trials, identification of novel biomarkers as well as further advances in high-throughput technologies will hopefully result in further development of therapeutic targets, treatment strategies and improved survival for these patients in the near future.

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# Clinical impact of chemotherapy to improve tumor microenvironment of pancreatic cancer

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## Abstract

A perioperative multimodal strategy including combination chemotherapy and radiotherapy, in addition to surgical resection, has been acknowledged to improve patient prognosis. However chemotherapy has not been actively applied as an immunomodulating modality because of concerns about various immunosuppressive effects. It has recently been shown that certain chemotherapeutic agents could modify tumor microenvironment and host immune responses through several underlying mechanisms such as immunogenic cell death, local T-cell infiltration and also the eradication of immune-suppressing regulatory cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells. With the better understanding of the cell components in the tumor microenvironment and the effect of chemotherapy to improve tumor microenvironment, it has been gradually clear that the chemotherapeutic agents is two-edged sword to have both immune promoting and suppressing effects. The cellular components of the tumor microenvironment include infiltrating T lymphocytes, dendritic cells, regulatory T cells, tumor associated macrophages, myeloid derived suppressor cells and cancer associated fibroblasts. Based on the better understanding of tumor microenvironment following chemotherapy, the treatment protocol could be modified as personalized medicine and the prognosis of pancreas cancer would be more improved utilizing multimodal chemotherapy. Here we review the recent advances of chemotherapy to improve tumor microenvironment of pancreatic cancer, introducing the unique feature of tumor microenvironment of pancreatic cancer, interaction between anti-cancer reagents and these constituting cells and future prospects.

**Key words:** Pancreas cancer; Microenvironment; Chemotherapy; Immune cells; Immunomodulation



**Core tip:** It has been gradually clear that the chemotherapeutic agents are two-edged sword to have both immune promoting and suppressing effects. The cellular

components of the tumor microenvironment including infiltrating T lymphocytes, dendritic cells, regulatory T cells, tumor associated macrophages, myeloid derived suppressor cells and cancer associated fibroblasts could be improved. Based on the better understanding of tumor microenvironment following chemotherapy, the treatment protocol could be modified as personalized medicine and the prognosis of pancreas cancer would be more improved utilizing multimodal treatment strategy.

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## INTRODUCTION

Pancreatic carcinoma is an extremely aggressive malignant tumor and the fifth leading cause of death worldwide and is expected to be the second by 2030 in Western countries[1,2]. The only curative option is surgical resection, but the 5-year overall survival (OS) rate still needs to be improved from the current 10%-15% even after curative resection[1,3]. A perioperative multimodal strategy including combination chemotherapy and radiotherapy, in addition to surgical resection, has been acknowledged to improve patient prognosis. New cytotoxic agents such as gemcitabine, Tegafur-gimeracil-oteracil potassium (TS-1) and combination chemotherapy with 5-fluorouracil (5-FU), oxaliplatin, and irinotecan along with perioperative chemoradiotherapy before and after surgery have recently been widely investigated[4,5]. Chemotherapy, usually a standard treatment option for cancer, has not been actively applied as an immune-modulating modality because of concerns about various immunosuppressive effects. However, certain chemotherapeutic agents have recently been shown to improve host immune responses and even break immune tolerance[6]. Several underlying mechanisms have been clarified, including immunogenic cell death[7,8], local T-cell infiltration and also the eradication of immune-suppressing regulatory cells such as regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), all of which are associated with cells in the tumor microenvironment[9]. On the other hand, care must be taken that chemotherapy-induced cancer metastasis does occur during treatment through non-immunological pathways[10].

We review recent advances in chemotherapeutic regimens to improve the tumor microenvironment for pancreatic cancer, and introduce unique features of the tumor microenvironment for pancreatic cancer, interactions between anti-cancer reagents and the constituent cells, and future prospects.

## OVERVIEW OF STANDARD CHEMOTHERAPY AND CHEMORADIATION THERAPY FOR PANCREATIC CANCER

Although the only curative option is surgical resection, with the advances in perioperative strategy for pancreatic carcinoma, many cytotoxic agents have proven effective in treating this disease. Chemoradiation therapy had been also adopted aiming at locoregional response and additional effects outside the field of irradiation (abscopal effects)[11].

Representative cytotoxic agents include historical 5-FU monotherapy, gemcitabine monotherapy, and gemcitabine-based combination therapies[4]. The following randomized controlled trials are investigations recently undertaken try to improve the chemotherapeutic strategy for pancreatic cancer. Burris *et al*[12] had shown for the first time that gemcitabine was superior to 5-FU in terms of overall survival, thus suggesting gemcitabine as a key drug in advanced pancreatic cancer. In 2011, FOLFILINOX (5-FU, leucovorin, irinotecan and oxaliplatin) had been shown to have survival benefit over gemcitabine alone in patients with metastatic pancreas cancer[13]. Recently Nab-paclitaxel plus gemcitabine have been reported to have superior efficacy compared with gemcitabine monotherapy in metastatic pancreas cancer (MPACT trial)[14]. However, MPACT trial, consisting of Gem + Nab-paclitaxel had OS of 8.5 mo compared to 6.7 mo in patients treated with gemcitabine alone) suggesting minimal improvement of survival by current chemotherapy regimens and requiring for further developments.

Taken together, overall survival of patients with metastatic disease extended to nearly 1 year from around 6 mo in the preceding two decades, thanks to recent therapeutic advances. However, although these reagents are promising, median progression-free survival remains limited and the 5-year survival rate of patients is still unsatisfactory, at around 15%-20%, even with these multimodal treatment strategies. This is due in part to dose limiting toxicity of side effects such as neuropathy and bone marrow suppression and due also to chemoresistance, relapse and metastasis even after surgical resection.

## TUMOR MICROENVIRONMENT OF PANCREATIC CANCER

Tumor cells alone were initially considered the specific target of chemotherapy, leading to a focus on the cytotoxicity of agents inhibiting DNA repair, tubulin formation and cell proliferation[15]. However, recent research has identified the tumor microenvironment as comprising tumor cells, host immune cells such as T cells, Tregs and MDSCs, and cancer-associated fibroblasts or stromal cells that support or suppress each

**Table 1 Targets of chemotherapy to improve tumor microenvironment**

| Cellular components | Target molecules | Chemotherapeutic agents | Underlying mechanism | Ref. |
|---|---|---|---|---|
| TIL | CD4, CD8 positive T lymphocytes | GEM, TS-1, | Increase lymphocyte infiltration | [22] |
| | RAS/MAPK | MEK inhibitor, PD1/PDL1 immune checkpoint inhibitors | | [24] |
| DC | | GEM | Proliferation of DC and CTL | [26] |
| Treg | CD4 and FoxP3 positive T lymphocytes | GEM, cyclophosphamide | Depletion | [21] |
| MDSC | CCL2, CCR2, GMCSF | GEM, 5-FU | Increase differentiation | [34,35] |
| | | | Depletion | [5] |
| CAF | Palladin positive fibroblasts | GEM | Depletion | [38] |
| | mTOR/4E-BP1 pathway | GEM, Pasireotide | Reduce tumor growth and chemoresistance | [39] |

TIL: Tumor infiltrating lymphocytes; DC: Dendritic cells; Treg: Regulatory T cells; TAM: Tumor associating macrophages; MDSC: Myeloid derived suppressor cells; CAF: Cancer associated fibroblasts; Gem: Gemcitabine; 5-FU: 5-fluorouracil.

other[9]. Each of these cellular components contributes to treatment response and patient prognosis, with tumor cells forming a network through direct interactions and cytokines providing important signals to initiate cell invasion into vessels and lymph nodes, leading to distant metastasis. Desmosomes are also one of the specific features of pancreas carcinoma that make drug delivery so difficult and prevent immune cells from infiltrating to tumor nests[16].

These evidences collectively indicate that tumor cells are thought to grow, interacting with the microenvironment, highlighting the need to clarify the specific mechanisms by which each chemotherapeutic agent improves the tumor microenvironment to contribute to treatment efficacy.

The following sections are arranged to describe recent evidence for the effects of chemotherapeutic agents on the cellular components of the tumor microenvironment (Table 1).

## INFILTRATING T LYMPHOCYTES

A number of reports have suggested that the accumulation of CD4 and CD8 lymphocytes in solid tumors offers a good prognostic indicator for patient survival[17,18]. In terms of pancreatic cancer, Tewari *et al*[19] demonstrated a positive correlation between prognosis and the presence of tumor infiltrating T cells. Although the clinical relevance differs among types of cancer, in association with the HLA class I expression level[20], some agents have been reported to induce T-cell infiltration into pancreas cancers[21]. Homma *et al*[22] showed that $CD4^+$ and $CD8^+$ cells were significantly increased after neoadjuvant chemotherapy comprising gemcitabine and TS-1 followed by radiotherapy (NACRT), and a high accumulation of $CD4^+$ cells offered a good prognostic marker for pancreas carcinoma after NACRT. Teng *et al*[23] recently classified the types of tumor microenvironment based on the presence or absence of T-cell infiltration and expression of PD1 along with patient prognosis.

Loi *et al*[24] recently suggested that therapeutic cooperation of MEK and PD-1/PD-L1 immune check point inhibitors could increase tumor-infiltrating lymphocytes through RAS/MAPK pathways in breast cancer. Great expectations are held for increased control of the tumor microenvironment, especially with tumor-infiltrating lymphocytes enabling further improvements in patient prognosis associated with immune check point inhibitors.

## DENDRITIC CELLS

Dendritic cells are the most potent antigen presenting cells and play a crucial part in the initiation, programing, and regulation of antitumor immunity, directing cytotoxic T lymphocytes and natural killer cells to become potent antitumor effectors capable of eradicating malignant cells[25,26]. Recently it had been reported that dendritic cells are impaired in number and display maturation defects disable to function as antigen presenting cells in pancreatic cancer due to the inflammation of the disease[27]. Meanwhile, chemotherapy can promote immunogenic cell apoptosis enhancing immunogenicity and mediating efficient phagocytosis by dendritic cell[7]. Moreover, gemcitabine can enhance the cross presentation of tumor associated antigens by dendritic cells and as well as inducing the proliferation of DC and CTL[26]. Those strategies utilizing chemotherapeutic agents might be useful to overcome negative microenvironment.

## REGULATORY T CELLS

Tregs are defined as T cells expressing both CD4 and forkhead box P3 (FoxP3), and are usually associated with poor prognosis and immunosuppression in various cancers. Transcriptional FoxP3 is a crucial intracellular marker and also a key developmental and functional factor for $CD4^+FoxP3^+$ Tregs[28]. In terms of pancreatic cancer, multimodal chemotherapy including GEM, cyclophosphamide, and taxane has been demonstrated to decrease Tregs in the tumor microenvironment[5]. Low Treg percentage in circulation at 1 year after PC resection had been correlated with improved survival[29]. We have also previously shown that neoadjuvant treatment of pancreatic ductal adenocarcinoma with chemotherapy and chemoradiotherapy can alter the

local Treg balance in favor of antitumor immunity in resected human sections[21]. Another paper by Keenan *et al*[30] showed that immunization of mice with Listeria Monocytegenes engineered to express k-ras along with depletion of Treg cells reduced progression of early stages PanINs. Also, Shibuya *et al*[31] recently reported that CD8 effector T cells show marked accumulation in the tumor microenvironment, but are suppressed by Tregs and PD-L1 expressed on T cells. These findings have therefore led to expectations for novel strategies of multimodal chemotherapy in combination with immune checkpoint inhibitors reducing Tregs.

## TUMOR-ASSOCIATED MACROPHAGES

Tumor-associated macrophages (TAMs) are derived from $CCR2^+$ monocytes in the spleen and peripheral blood, infiltrating into the tumor and developing into macrophages on stimulation by the releasing hormone CCL2 and colony-stimulating factor 1 (CSF-1)[32,33]. TAMs have recently been reported to limit the effects of chemotherapy and promote tumor chemoresistance[34]. Michem et al. reported that targeting TAMs by inhibiting CSF1R or C-C chemokine receptor 2 (CCR2) could decrease the number of pancreatic tumor-initiating cells and improve chemotherapeutic efficacy *in vivo*. The Denargo group reported that the combination of cytotoxic chemotherapy and blockade of CSF1R, which is prominently expressed by monocytes, Mo-MDSC and macrophages, resulted in improved anti-tumor T-cell responses[32]. Furthermore, Sanford *et al*[33] reported that the CCL2/CCR2 chemokine axis plays a crucial role in the recruitment of inflammatory monocytes from bone marrow to peripheral sites of inflammation and an increased ratio of inflammatory monocytes in blood compared to bone marrow offers a novel predictor of decreased patient survival following tumor resection. These lines of evidence clearly show that chemotherapy combined with chemokine blockade might reduce the chemoresistance associated with the exclusion of TAMs.

## MYELOID DERIVE SUPPRESSOR CELLS

MDSCs are heterogeneous populations of immune cells derived from progenitor cells in bone marrow. MDSCs with a phenotype of $CD33^+HLA\text{-}DR^{-/low}$ that are lineage-negative ($CD14^-$, $CD15^-$) are well described as immunosuppressive in cancer patients contributing to tumor progression by damping T-cell immunity and promoting angiogenesis[35,36]. Many chemotherapeutic drugs have long been thought to exclude MDSCs from various cancers. Zheng *et al*[5] showed that GEM and 5-FU have a direct killing effect on MDSCs. In contrast, Takeuchi *et al*[36] reported that GEM could increase MDSC numbers through increases in GM-CSF levels, converting M2 macrophages into suppressive MDSCs. Therefore MDSC in peripheral blood might be a possible predictive biomarker of chemotherapy failure in PC patients[37]. Also, GEM and 5-FU have been reported to activate NLRP3 inflammasomes in MDSCs, leading to interleukin-1β release, which restrains their antitumor efficacy[38]. More recently, Hu *et al*[39] reported TNFB2R as important for its suppressive function. Uniquely, Sanford *et al*[40] recently reported the clinical utility of zoledronic acid. This agent is usually utilized to improve calcium imbalances in patients osteoporosis, but also prevents tumor-mediated myelopoiesis associated with the generation of MDSC. Further studies are warranted to adjust the balance between direct reduction of MDSCs and indirect promotion of MDSCs by chemotherapy in combination with the multimodal strategies described above.

## CANCER-ASSOCIATED FIBROBLASTS

Fibrous stroma associated with cancer in the tumor micro environment has increasingly been recognized as involving cancer-associated fibroblasts (CAFs). These cells are reported to contribute to poorer survival in various tumors, including pancreatic ductal adenocarcinoma, which has been reported to contain large numbers of CAFs[41]. The characteristically dense desmosome in pancreatic cancer acts as a barrier to drug delivery, thus contributing to chemoresistance[4]. Among the many markers of CAFs, Sato *et al*[41] reported that palladin, a CAF marker, could represent an independent marker of poor prognosis and a biomarker to predict the efficiency of chemotherapy or even disease recurrence. Duluc *et al*[42] recently revealed one of the underlying mechanisms abrogating pancreatic cancer chemoresistance through the mTOR/4E-BP1 pathway, allowing GEM-based chemotherapy combined with sst1 receptor-activating pasireotide to reduce tumor growth and chemoresistance. This kind of anti-stromal targeted therapy could be expected in addition to host immune cell-targeted therapy, as an adjunct to direct killing of cancer cells.

## DISCUSSION

With our developing understanding of the cell components in the tumor microenvironment and the effects of chemotherapy in improving this environment, chemotherapeutic agents have gradually been revealed to represent a two-edged sword with effects that both promote and suppress immunity[5]. Such therapies deplete one factor of immune suppression while at the same time inducing another mechanism to inhibit host immune responses. Some experimental data in this paper show that these problems might be overcome by multimodal combination chemoimmunotherapy in addition to standard chemotherapy, blocking antibodies for cytokine release or utilizing immune checkpoint inhibitors. Beaty *et al*[43] reported combining chemotherapy with the agonist CD40, as a member of the TNF receptor superfamily, for surgically incurable PDA and observed tumor regression in some patients. Takeuchi *et al*[36] likewise reported that anti-GM-CSF

antibody blocking could accelerate the formation of immunosuppressive myeloid cells in the tissue microenvironment of human pancreatic cancer.

Chemotherapeutic protocols including timing and dose might also be further explored and modified based on both reductions in tumor size and the induction of anti-tumor-specific immunity. Metronomic chemotherapy or low-dose chemotherapy has been reported to induce anti-tumor T-cell immunity *in vivo*[44]. One of the underlying mechanisms might be that such low-toxicity doses of cytotoxic agents induce minimal suppression of tumor cells while concomitantly inducing minimal suppression of immune-promoting cells based on altered immune balance.

Lastly, future evidence should be accumulated regarding these balances in the tumor microenvironment during multimodal chemotherapy by measuring biomarkers locally and systematically. Biopsy specimens provide information of infiltrating T lymphocyte levels in the tumor microenvironment, offering possible predictors of beneficial response to chemotherapy in breast and pancreas cancers. SPARC expression levels in the stroma could represent a target for nab-paclitaxel. Although data must continue to be accumulated, miRNA might reflect changes in immune balances and predict the efficacy of chemoimmunotherapy[45]. Taking all these lines of evidences together in combination with the properties of emerging agents, current problems seem likely to be overcome, at least in part, and the prognosis of pancreas cancer can be expected to continue improving in the coming decades.

## CONCLUSION

The chemotherapeutic agents have both immune promoting and suppressing effects in the tumor microenvironment of pancreatic cancer. Based on the better understanding of tumor microenvironment following chemotherapy, the treatment protocol could be modified as personalized medicine and the prognosis of pancreas cancer would be more improved utilizing multimodal chemotherapy.

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MINIREVIEWS

# Current noninvasive tests for colorectal cancer screening: An overview of colorectal cancer screening tests

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## Abstract

Colorectal cancer (CRC) has become the third most common cancer in the world. Screening has been shown to be an effective way to identify early CRC and precancerous lesions, and to reduce its morbidity and mortality. Several types of noninvasive tests have been developed for CRC screening, including the fecal occult blood test (FOBT), the fecal immunochemical test (FIT), the fecal-based DNA test and the blood-based DNA test (the SEPT9 assay). FIT has replaced FOBT and become the major screening test due to high sensitivity, specificity and low costs. The fecal DNA test exhibited higher sensitivity than FIT but its current cost is high for a screening assay. The SEPT9 assay showed good compliance while its performance in screening needs further improvements. These tests exhibited distinct sensitivity and specificity in screening for CRC and adenoma. This article will focus on the performance of the current noninvasive *in vitro* diagnostic tests that have been used for CRC screening. The merits and drawbacks for these screening methods will also be compared regarding the techniques, usage and costs. We hope this review can provide suggestions for both the public and clinicians in choosing the appropriate method for CRC screening.

**Key words:** Colorectal cancer; Adenoma; Fecal immunochemical test; Fecal DNA; SEPT9; Septin 9



**Core tip:** The choice of colorectal cancer (CRC) screening methods is crucial for screening validity and compliance. Currently, the fecal immunochemical test (FIT), fecal DNA and the blood-based SEPT9 assays are the three *in vitro* diagnostic tests for CRC screening. In this article, we reviewed the current application of the three types of assays and compared their performance

in CRC screening. FIT is still the cheapest method with high screening validity, and fecal DNA tests also exhibit high validity but its price is high. In contrast, the SEPT9 assay showed high compliance with acceptable performance. The choice of screening test may depend on the balance of performance, compliance and costs.

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## INTRODUCTION

Colorectal cancer (CRC) has become the second and the third leading cause of new cancer cases in Europe and in the United States, respectively[1]. There were approximately 142820 new cases with 50830 deaths in the United States in 2013, and approximately 447000 new cases of CRC and 215000 deaths in European countries in 2012[1,2]. The new cases for CRC are approximately 400000 in China in 2012, and it has become the third leading cause of death in the country[3].

Regular screening can achieve early CRC detection and early treatment. However, 60%-70% of patients are found at middle- or late-stage CRC when they are diagnosed[4]. Approximately 60% CRC deaths could be avoided and the average 5-year survival rate could be increased from 46% to 73% if healthy people carry out a regular periodic screening each year[5]. Therefore, an effective early screening method for CRC can reduce CRC morbidity and mortality.

There are four *in vitro* diagnostic (IVD) screening method currently available for CRC screening, including the fecal occult blood test (FOBT), the fecal immunochemical test (FIT), the fecal DNA test and the plasma SEPT9 gene methylation test. This review will provide a detailed analysis on the performance of these tests, and compare their merits and drawbacks in CRC screening. It is our aim for this review that the public and the professionals can choose the appropriate methods for CRC screening.

## STOOL-BASED TESTS FOR CRC SCREENING

### *The FIT test*

The guaiac FOBT test (gFOBT) has been used for a long time as a screening test for CRC. It exhibited a sensitivity of 12.9%-79.4% with a specificity of 86.7%-97.7% for CRC screening in many studies[6-13]. However, its sensitivity and specificity for CRC detection is lower than the more specific FIT (previous called iFOBT) test. This is because the gFOBT relies on peroxidase-like activity between heme and guaiac, which can be affected by many factors in daily diet without distinguishment between upper and lower gastrointestinal (GI) tract bleeding, while the FIT test targets the hemoglobin in the lower GI tract, as hemoglobin from upper GI tract will be degraded when it arrives at lower GI tract. This characteristic allows FIT test to specifically detect the bleeding from lower GI tract, and therefore detect the diseases with bleeding, such as adenoma, polyps, inflammatory diseases and CRC, *etc.* As the gFOBT test has many drawbacks in CRC screening, FIT is used more commonly in current CRC screening. We therefore focus on the performance of FIT test in this review.

The performance of FIT test in CRC screening in asymptomatic, average-risk adults has been listed in Table 1. Data from 19 studies showed that the overall sensitivity for CRC was 0.79 (95%CI: 0.69-0.86) and the overall specificity was 0.94 (95%CI: 0.92-0.95)[12,14-31]. This includes a total of 113360 subjects with 437 CRC cases confirmed by colonoscopy or 2-year follow-up. As the overall sensitivity and specificity are satisfactory for a cancer screening test with low costs, FIT is currently the most commonly-used method for CRC screening. The overall CRC positivity rate of 0.39% (437/113360) appeared to be significantly lower than the other two screening reports with asymptomatic, average-risk adults using fecal DNA (0.65%, 65/9989; $\chi^2$ = 15.93, $P$ < 0.001)[32] and *SEPT9* gene methylation assay (0.67%, 53/7941; $\chi^2$ =14.66, $P$ < 0.001)[33], respectively, indicating that the use of 2-year follow-up as a confirmatory methods may result in underestimation of CRC cases.

The use of qualitative FIT or quantitative FIT has always been an issue in choosing the FIT test for screening. A strip test (colloidal gold immunochromatographic method) is currently the main technique for qualitative FIT. It does not need specific instruments and the interpretation of test results relies on human recognition of test bands, although instruments are available to digitize the chrominance of the bands. In contrast, immunoturbidimetry is the main method for quantitative FIT, and the current devices include automated instrument for samples processing and colorimetry. Therefore, the current qualitative FIT appears to be faster, more convenient, less costly while more subjective than the quantitative FIT.

The performance between the qualitative and quantitative FIT showed significant differences. As shown in Table 1, the overall sensitivity of the qualitative FIT was 0.82[12,15-21], which was significantly higher that of the quantitative FIT (0.73) ($\chi^2$ =3.933, $P$ = 0.047)[22-31], while the qualitative FIT exhibited significantly lower specificity than the quantitative FIT (0.93 *vs* 0.95) ($\chi^2$ = 81.64, $P$ < 0.001), although the difference was small. This comparison needs to be interpreted with caution, as different studies used different cutoff values and resulted in distinct sensitivity and specificity. Ideally, they should be compared under the same cutoff value so that the sensitivity and specificity can be directly

**Table 1 The sensitivity and specificity of qualitative and quantitative fecal immunochemical test**

| Qualitative FIT | | | | | Quantitative FIT | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Ref. | Total cases | CRC cases | Sensitivity | Specificity | Ref. | Total cases | CRC cases | Sensitivity | Specificity |
| Allison *et al*[12], 1996 | 7493 | 35 | 0.69 | 0.94 | Sohn *et al*[22], 2005 | 3794 | 12 | 0.25 | 0.99 |
| Allison *et al*[15], 2007 | 5356 | 14 | 0.86 | 0.97 | Levi *et al*[23], 2011 | 1204 | 6 | 1.00 | 0.88 |
| Cheng *et al*[16], 2002 | 7411 | 16 | 0.88 | 0.91 | Levi *et al*[24], 2007 | 80 | 3 | 0.67 | 0.83 |
| Nakama *et al*[17], 1999 | 4611 | 18 | 0.56 | 0.97 | Morikawa *et al*[25], 2005 | 21805 | 79 | 0.66 | 0.95 |
| Nakama *et al*[18], 1996 | 3365 | 12 | 0.83 | 0.96 | Launoy *et al*[26], 2005 | 7421 | 28 | 0.86 | 0.94 |
| Parra-Blanco *et al*[19], 2010 | 1756 | 14 | 1.00 | 0.93 | Itoh *et al*[27], 1996 | 27860 | 89 | 0.87 | 0.95 |
| Chiu *et al*[20], 2013 | 8822 | 13 | 0.85 | 0.92 | Nakazato *et al*[28], 2006 | 3090 | 19 | 0.53 | 0.87 |
| Chiang *et al*[21], 2011 | 2796 | 28 | 0.96 | 0.87 | Park *et al*[29], 2010 | 770 | 13 | 0.77 | 0.94 |
| | | | | | de Wijkerslooth *et al*[30], 2012 | 1256 | 8 | 0.75 | 0.95 |
| | | | | | Brenner *et al*[31], 2013 | 2235 | 15 | 0.73 | 0.96 |
| | | | | | Brenner *et al*[31], 2013 | 2235 | 15 | 0.60 | 0.95 |
| Overall (pooled data) | 41610 | 150 | 0.82 | 0.93 | | 71750 | 287 | 0.73 | 0.95 |

CRC: Colorectal cancer; FIT: Fecal immunochemical test.

**Table 2 Comparison of sensitivity and specificity between Cologuard and fecal immunochemical test**

| | Pathological categories | Cologuard | FIT |
|---|---|---|---|
| Sensitivity[32] | CRC | 92.3% | 73.8% |
| | Advanced precancerous lesions | 42.4% | 23.8% |
| | Polyps with high-grade dysplasia | 69.2% | 46.2% |
| | Serrated sessile polyps | 42.4% | 5.1% |
| Specificity[32] | Nonadvanced or negative findings | 86.6% | 94.9% |
| | Negative results on colonoscopy | 89.8% | 96.4% |

CRC: Colorectal cancer; FIT: Fecal immunochemical test.

compared. The pooled data analyzed here provides a reference for comparing the two types of FIT tests. It can be suggested that the quantitative FIT may be a good choice for CRC screening tests that do not need high accuracy or are performed in hospitals where automated instruments are not available.

However, it should be mentioned that the cutoff value for qualitative FIT is preset, while the cutoff value for quantitative FIT can be adjusted to balance the sensitivity with specificity. Therefore, the data format for qualitative FIT is "positive" or "negative" without traceability, while the results from quantitative FIT are digitized with traceability. This is extremely useful when the relationship between the amount of bleeding in a certain disease and the population/personal information (such as diet, age, habit, sex, *etc.*) is investigated. Future model for predicting CRC incidence might partially relies on the data from quantitative FIT.

### *The fecal DNA test*

The detection of abnormal DNA or epigenetic markers from colorectal lesions is based on natural exfoliation of cancerous or precancerous cells into the colorectal tract. The fecal DNA test aims at detecting the DNA mutations, microsatellite instability, impaired DNA mismatch repair and abnormal methylation. There are many studies focusing on the detection of CRC by fecal DNA markers[34,35], and the overall sensitivity for CRC detection by various fecal DNA marker combinations ranged from 53% to 87%, with specificities beyond 76%[34,35]. Although there are a large number of fecal DNA markers available in these studies, the first commercial fecal DNA test was not available until the approval of Cologuard (Exact Sciences, Madison, WI, United States) by the United States FDA in 2014. Imperiale *et al*[32] published the leading study on Cologuard in 2014. By randomizing subjects to Cologuard or FIT screening, it showed that the sensitivity of Cologuard was superior to that of FIT in CRC, advanced precancerous lesions, polyps with high-grade dysplasia and serrated sessile polyps, while its specificity appeared to be lower than that of FIT (Table 2).

The Cologuard DNA test includes quantitative molecular assays for KRAS mutations, aberrant NDRG4 and BMP3 methylation, and β-actin, plus a hemoglobin immunoassay. As the hemoglobin immunoassay is essentially a FIT test, Cologuard is actually a combination of gene mutation, methylation and occult blood tests. The multitarget stool DNA test provides a new way that combines various detecting technology to detect CRC and early colorectal lesions with high sensitivity and specificity. The high detection of precancerous lesions, HGD and serrated sessile polyps is extremely useful for a screening test, as these lesions may develop into CRC if they are not resected. The only obstacle for broad application of Cologuard is the cost, as the detection of multitargets increased the cost of the test. Its current expense of $599 per test is high for a routine screening assay.

## BLOOD-BASED TESTS FOR CRC SCREENING

### *The plasma SEPT9 gene methylation assay*

An ideal screening test for cancer could be a simple blood test in the foreseeable future. The plasma *SEPT9* gene methylation test Epi proColon (Epigenomics AG, Berlin, Germany) is currently the only commercially

**Table 3 The reported positive detection rate for each colorectal cancer stage using 1/3 algorithm**

| Ref. | Positive detection rate for each colorectal cancer stage | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| deVos *et al*[38], 2009 | 52.6% (10/19) | 75.0% (30/40) | 77.8% (21/27) | 100.0% (4/4) |
| Warren *et al*[40], 2011 | 71.4% (5/7) | 90.3% (28/31) | 100.0% (7/7) | 100% (5/5) |
| Tóth *et al*[41], 2012 | 84.0% (21/25) | 100.0% (14/14) | 100.0% (35/35) | 100.0% (18/18) |
| Lee *et al*[47], 2013 | 30.8% (8/26) | 36.7% (11/30) | 25.0% (7/28) | 64.7% (11/17) |
| Johnson *et al*[44], 2014 | 61.5% (16/26) | 80.0% (16/20) | 65.2% (15/23) | 92.3% (12/13) |
| Pooled positive detection rate | 58.3% (60/103) | 73.3% (99/135) | 70.8% (85/120) | 87.7% (50/57) |

**Table 4 The reported positive detection rate for each colorectal cancer stage using 2/3 algorithm**

| Ref. | Positive detection rate for each colorectal cancer stage | | | |
|---|---|---|---|---|
| | I | II | III | IV |
| Grützmann *et al*[37], 2008 | 50.0% (11/22) | 69.4% (25/36) | 79% (42/53) | 91% (10/11) |
| deVos *et al*[38], 2009 | 26.3% (5/19) | 60.0% (24/40) | 66.7% (18/27) | 75.0% (3/4) |
| Tóth *et al*[41], 2012 | 60.0% (15/25) | 92.8% (13/14) | 81.6% (31/35) | 77.8% (14/18) |
| Jin *et al*[46], 2015 | 66.7% (12/18) | 82.6% (19/23) | 84.1% (37/44) | 100.0% (5/5) |
| Pooled positive detection rate | 51.2% (43/84) | 71.7% (81/113) | 80.5% (128/159) | 84.2% (32/38) |

available blood-test for CRC early detection and screening, and was approved recently by the United States FDA as a CRC screening test for average-risk population over 50 years old. Many clinical studies have proved the test to be a method with acceptable sensitivity and specificity for CRC detection[33,36-49]. The test was firstly developed by Lofton-Day *et al*[36] in 2008 as a research kit, and was commercialized by Epigenomics AG as its first generation assay Epi proColon 1.0. At the same time, ARUP lab also developed its SEPT9 assay as a lab-developed test[40]. Abbott developed its real-time mS9 CRC assay, but there was only one report on its performance and the sensitivity of 36.3% was much lower than other SEPT9 tests[47]. The 2nd generation test (Epi proColon 2.0) was launched in 2011-2012 with better performance. Till today, most reports on the SEPT9 assay appeared to be case-control study or cohort study investigating the test performance in selected population, exhibiting a sensitivity of 36.6%-95.6% with a specificity of 81.5%-99.0% using 1/3, 2/3, 1/2 or 1/1 algorithm[33,36-49]. In contrast, there is only one study (the PRESEPT trial) investigating the application of the assay in CRC screening in average-risk population, exhibiting a sensitivity of 48.2% and 68.2% with a specificity of 91.5% and 80.0% using 1/2 or 1/3 algorithm, respectively[33,43].

Detection of early stage CRC is crucial for early intervention and reduction of mortality. The positive detection rate (PDR) of the SEPT9 assay for stage I, II, III and IV was 26.3%-84.0%, 36.7%-100.0%, 25.0%-100.0% and 64.7%-100.0%, respectively, depending on different algorithm, exhibiting a huge variation for each stage. As 1/3 and 2/3 algorithm are the most commonly used methods for result interpretation, we calculated the PDR for each stage using the two algorithms. The pooled PDR for stage I, II, III and IV was 58.3%, 73.3%, 70.8% and 87.7%, respectively, using 1/3 algorithm (Table 3), and was 51.2%, 71.7%, 80.5% and 84.2%, respectively, using 2/3 algorithm (Table 4)[36-47]. No statistical difference in PDR in any stage between the two algorithms has been found. It can be clearly seen that the PDR for early stage CRC (stage I) was above 50% and fell into 70%-80% for stage II and III, which is acceptable for a blood-based CRC test. However, these PDRs were from case-control or cohort studies, and more studies should be performed at screening settings.

Although the SEPT9 assay was designed for CRC detection, researchers also studied its detection sensitivity for precancerous adenoma. The pooled PDR for non-advanced adenoma and advanced adenoma (AA) was 10.0% and 18.2%, respectively, from six studies, in which the PDR for AA was significantly higher than the PDR of normal control group (11.8%, $\chi^2$ test, $P < 0.001$)[30,33,37,40,43,46]. However, as PDR of 18.2% was still too low for an effective test, the SEPT9 assay may not be applicable in adenoma detection.

The SEPT9 assay exhibited high compliance in screening. One recent report showed that 63% of subjects in a CRC screening study refused colonoscopy. 97% of subjects who refused colonoscopy accepted a noninvasive screening test, and 83% chose the SEPT9 test and 15% chose FIT test. The majority of patients who refused colonoscopy chose the SEPT9 assay due to its convenience and less time-consuming procedure[50].

### *CEA and other serum glycoprotein markers*

CEA and carbohydrate antigen 199 (CA199) are the two most common serum-based glycoprotein CRC markers, however, they are not appropriate for CRC screening due to their low sensitivity and the lack of CRC specificity, especially for early-stage CRC[41,51-53]. For example, CEA test exhibited a sensitivity of 40.9%-51.8% and a specificity of 85.2%-95% for CRC detection in three studies[41,51,52]. Therefore, it is more appropriate to be used in monitoring the CRC recurrence or response from

**Table 5 Sensitivity and specificity of fecal immunochemical test, fecal DNA and SEPT9 tests in colorectal cancer and advanced adenoma screening**

| | FIT[12,15-31] | Fecal DNA[32] | SEPT9[43] |
|---|---|---|---|
| Sensitivity (CRC) | 79% | 92% | 68% |
| Specificity | 94% | 87% | 80% |
| Sensitivity (AA) | 24% | 42% | 18% |

FIT: Fecal immunochemical test; CRC: Colorectal cancer; AA: Advanced adenoma.

**Table 6 Positive detection rate of SEPT9, fecal immunochemical test and carcino-embryonic antigen tests and various combined tests**

| SEPT9 alone | FIT alone | CEA alone | SEPT9 + FIT | SEPT9 + CEA | FIT + CEA | SEPT9 + FIT + CEA |
|---|---|---|---|---|---|---|
| 77.00% | 74.6% | 41.3%[e] | 94.4%[c] | 86.4%[c] | 84.5% | 97.2%e |
| | (NS) | | | | (NS) | |
| (181/235) | (53/71) | (97/235) | (67/71) | (203/235) | (60/71) | (69/71) |

[c]$P < 0.01$; [e]$P < 0.001$ *vs* SEPT9 alone. FIT: Fecal immunochemical test; CEA: Carcino-embryonic antigen; NS: Not significant.

patients to surgical or systemic therapy, rather than screening[53].

The main drawback of serum glycoprotein markers in CRC screening is that the sensitivity and specificity of any single marker is not high enough to make it a reliable indicator. These markers have been found in various cancers other than CRC with low sensitivity for early stage lesions. Combined use of multiple markers may be a way to achieve diagnostic significance in CRC detection. In one report, five glycoprotein markers, including CEA, CA199, CA242, CA72-4, and CA125, are used together as indicators for CRC. It showed that the sensitivity of any single marker was low (18.8%–52.2%) for detecting CRC in stages Ⅰ and Ⅱ, while the combination of the five exhibited a sensitivity of 85.3% at the specificity of 95%[54].

## COMPARISON OF NONINVASIVE TESTS FOR CRC SCREENING

The sensitivity for CRC and AA, and the specificity in asymptomatic average-risk population for FIT, fecal DNA and SEPT9 tests are shown in Table 5. It can be seen that the fecal DNA test exhibited the best performance in terms of sensitivity for CRC and AA, while its specificity was slightly lower than that of the FIT. It is noteworthy that the fecal DNA test can detect 42% AA, which may reduce the number of subjects progressing to CRC, *i.e.*, reducing the CRC morbidity. The SEPT9 assay is the only blood-base CRC screening assay currently. Although its screening performance was not satisfactory at the moment, it showed very high compliance[50]. The blood-based CRC screening assay may be more popular if the sensitivity and specificity in screening setting could be improved to the level of those in case-control studies (ideally sensitivity > 70% and specificity > 90% for CRC screening).

The current costs for FIT, fecal DNA and the SEPT9 test are $10-50, $599 and approximately $170, respectively. As the recommended screening frequency for FIT, fecal DNA and the SEPT9 is once per year, once per three years and once per year, respectively, FIT might be the cheapest test considering the balance between performance and costs. However, the quality adjusted life year of the three tests should be compared under the same setting to evaluate the cost-effectiveness of them, although some studies have been performed for each individual method in different settings, such as different health systems.

## CRC SCREENING WITH COMBINED TESTS

The combination of fecal DNA (mutation and methylation) with a hemoglobin immunoassay in Cologuard has provided a good example for CRC screening when multiple markers are analyzed together to enhance the detection sensitivity. There are merits and drawbacks for this strategy. First, combination of multiple markers enhances sensitivity at the price of reducing specificity. The number of false positive cases will increase with the increased number of markers. Therefore, to identify the markers with high sensitivity and specificity and to find the best combination of markers remain a challenge for combined screening test development. Ideally, the number of markers should be kept to minimum, while the sensitivity and specificity can be balanced to provide the best performance. Secondly, the detection of multiple markers with distinct methods increases the technical difficulties in an assay. For example, the detection of mutation in Cologuard may use sequencing or PCR method, while the detection of methylation needs to use the methylation specific PCR method containing bisulfite conversion. In contrast, immunoassay is used in the detection of hemoglobin. Furthermore, the sample preparation procedure may also be different for detecting different abnormalities. Therefore, a good combined test needs not only optimization of each individual test, but also an accurate algorithm to maximize the performance of each test. The optimization and interpretation of the combined test must come from clinical trials with large number of cases. Thirdly, a screening test should be accurate, fast, convenient, simple and cheap. These features allow large-scale screening in a certain period of time, and allow easy test in areas where test instruments are not available. In addition, low costs ensure screening tests for average-risk population, in which the CRC incidence could be lower than 1% in people over 50 years old. All the above considerations need to be addressed in future development of combined screening test.

As FIT, SEPT9 and CEA tests are all CRC detection tests with high specificity, the combination of them may provide higher sensitivity with no significant compromise

in specificity. We recently tested this assumption in an opportunistic screening setting, in which blood and stool samples were collected from outpatients and inpatients coming to the GI departments of three Chinese hospitals[55]. Table 6 shows the test results from the screening. SEPT9, FIT or CEA alone detected 77.0%, 74.6% and 41.3% of CRC cases, respectively, while the combination of the three increased the sensitivity to 97.2%, and SEPT9 plus FIT exhibited a sensitivity of 94.4%. Since CEA is more sensitive to late-stage CRC than early-stage CRC, and no significant difference was found between SEPT9 + FIT+ CEA and SEPT9 + FIT, we recommend SEPT9 + FIT as a routine method for CRC screening.

## CONCLUSION

The FIT, fecal DNA and the SEPT9 tests are IVD tests currently used for CRC screening. FIT tests exhibited satisfactory sensitivity and specificity with low costs and therefore become the major screening test for CRC at the moment. The sensitivity of the fecal DNA test appeared to be very high due to combination of multiple methods while its high cost is an obstacle preventing the test from broad use. Both sensitivity and specificity for the SEPT9 test in CRC screening were lower than those of the FIT and fecal DNA test, but it showed high compliance with promising future if its accuracy can be improved. Combined tests with multiple markers should be a future direction in CRC screening, however, some hurdles, such as technical integration, test/interpretation optimization, and high costs, etc, need to be overcome before they can be used in large-scale CRC screening aiming at asymptomatic average-risk population.

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CASE REPORT

# Case of pseudo-Meigs' syndrome caused by gastric cancer-related metastatic ovarian tumor with prolonged survival

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Author contributions: Okamoto M, Maeda K, Yanagitani A and Tanaka K equally discussed the clinical manifestations; Maeda K, Yanagitani A and Tanaka K reviewed the manuscript critically; Okamoto M wrote the paper.

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## Abstract

A 48-year-old woman presented with bilateral enlarged ovaries, ascites, bilateral pleural effusion, and advanced gastric cancer. Pleural fluid cytology did not reveal malignant cells. Oophorectomy, performed as a palliative procedure, was followed by rapid resolution of the pleural effusion and ascites. The patient was diagnosed with pseudo-Meigs' syndrome, and underwent chemotherapy followed by partial gastrectomy. At the last follow-up, 84 mo following oophorectomy, she was alive, and free of disease recurrence, despite not receiving any further treatment. Pseudo-Meigs' syndrome should be considered in patients with bilateral ovarian tumors, ascites and pleural effusion, and treatment such as oophorectomy may result in symptomatic improvement and better prognosis in similar patients.

**Key words:** Pseudo-Meigs' syndrome; Ovarian tumor; Gastric cancer; Pleural effusion; Ascites



**Core tip:** In general, the prognosis of gastric cancer with distant metastases is poor. On the other hand, oophorectomy for gastric cancer-related metastatic ovarian tumors may improve survival, especially in the absence of metastasis to other organs. We here report a long-term survival case of pseudo-Meigs' syndrome caused by gastric cancer following oophorectomy. We conclude that pseudo-Meigs' syndrome should be considered in patients with gastric cancer with enlarged

ovaries, pleural effusion, and ascites.

Okamoto M, Maeda K, Yanagitani A, Tanaka K. Case of pseudo-Meigs' syndrome caused by gastric cancer-related metastatic ovarian tumor with prolonged survival. *World J Gastrointest Oncol* 2016; 8(11): 801-804 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i11/801.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i11.801

## INTRODUCTION

Meigs' syndrome is defined as the presence of pleural effusion and ascites in association with benign ovarian tumors such as fibromas. In these patients, pleural effusion and ascites typically disappear following oophorectomy. Pseudo-Meigs' syndrome is similar to Meigs' syndrome, except that the ovarian tumor may be malignant rather than benign. Pseudo-Meigs' syndrome caused by gastric cancer with metastatic ovarian tumors is very rare. Herein, we report a case of pseudo-Meigs' syndrome caused by gastric cancer with metastatic ovarian tumors, with prolonged survival following oophorectomy.

## CASE REPORT

A 48-year-old woman was admitted to our hospital with a 3-mo history of lower abdominal fullness. She had no significant medical history. She smoked half a pack of cigarettes and consumed 1 L of beer a day for 28 years. On physical examination, her height and weight were 161 cm and 49 kg, respectively. Her blood pressure (103/60 mmHg), pulse rate (92 beats/min), body temperature (37.3 ℃), and arterial oxygen saturation at room air (96%) were almost normal. The right chest breath sounds were decreased, her abdomen was distended, and a hard fist-sized mass was palpable in her lower abdomen. Her serum levels of carbohydrate antigen 19-9 and carbohydrate antigen 125 (CA 125) were elevated at 170.4 U/mL (normal, < 35 U/mL) and 897 U/mL (normal, < 37 U/mL), respectively. Computed tomography demonstrated bilateral enlarged ovaries, ascites, and bilateral pleural effusion (Figure 1). Upper gastrointestinal endoscopy revealed an ulcerated lesion with raised margins on the greater curvature of the body of the stomach (Figure 2). Biopsy of this lesion confirmed the diagnosis of poorly differentiated adenocarcinoma. Based on the above findings, a diagnosis of gastric cancer with metastatic ovarian tumors, malignant ascites, and pleural effusion, was made, although pleural fluid cytology failed to identify any malignant cells. Consequently, bilateral oophorectomy was performed as a palliative procedure. The resected ovarian tumors measured 15 cm (right) and 8 cm (left) in diameter. The tumors were solid with multiple mucus-containing cysts (Figure 3A and B). There was no evidence of tumor dissemination in the abdomen and the ascitic fluid was negative for cancer cells on cytology. Histological examination of the resected ovarian specimens confirmed that the tumors comprised poorly differentiated adenocarcinoma similar to the gastric tumor (Figure 3C), suggesting that the bilateral ovarian tumors were secondary to the gastric cancer (Krukenberg tumors).

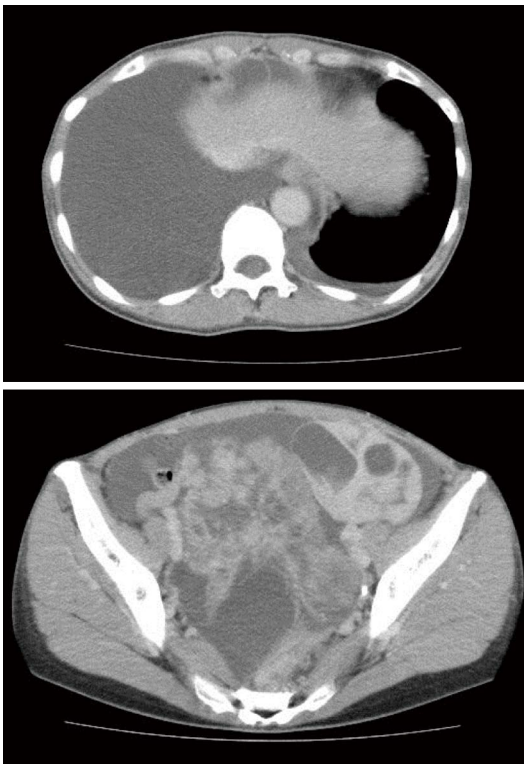

**Figure 1 Whole body computed tomography-scan demonstrating bilateral enlarged ovaries, ascites and bilateral pleural effusion.**

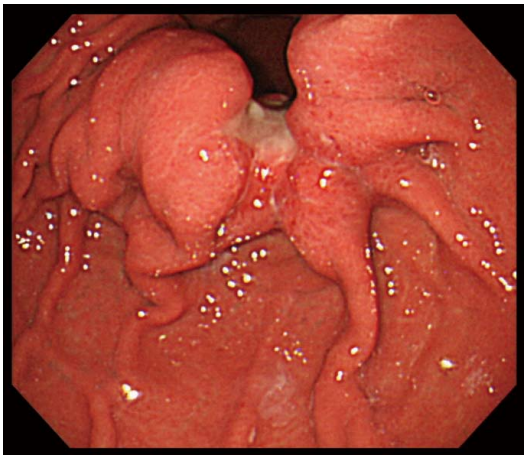

**Figure 2 Upper gastrointestinal endoscopy revealing an ulcerated lesion with raised margins on the greater curvature of the body of the stomach.**

The patient's pleural effusion and ascites resolved completely within 2 wk of her surgery. In view of these findings, we considered a diagnosis of pseudo-Meigs' syndrome. Following the oophorectomy, she received chemotherapy with docetaxel and S-1, with one course comprising docetaxel 40 mg/m$^2$ as an intravenous

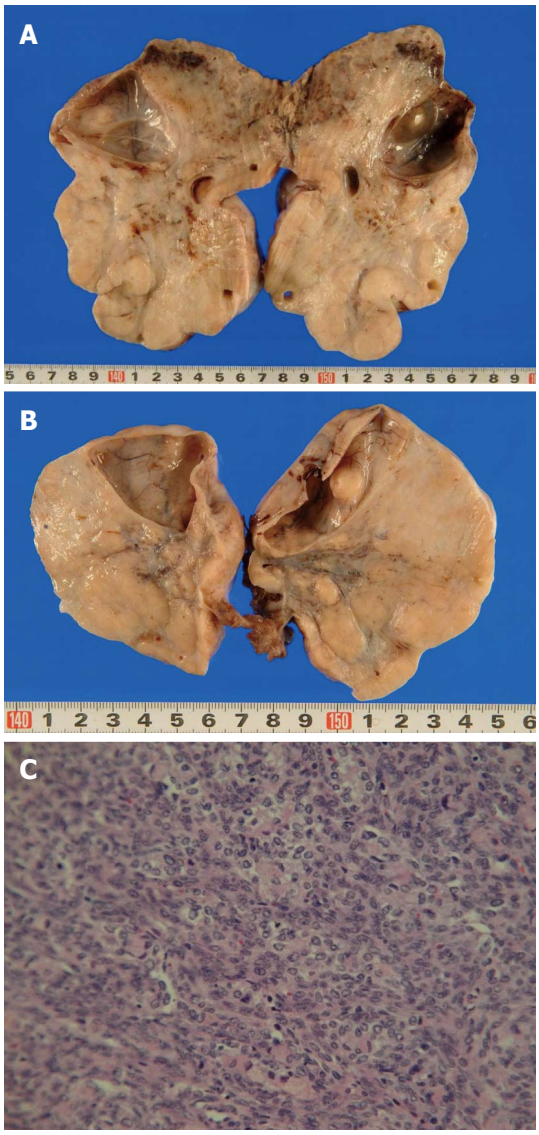


**Figure 3 Resected ovarian tumors measured 15 cm (right) (A) and 8 cm (left) (B) in diameter.** The tumors were solid with multiple mucus-containing cysts. Histological examination of the resected ovarian specimens confirmed that the tumors were composed of poorly differentiated adenocarcinoma similar to the gastric tumor (C).

infusion on day 1 and oral S-1 80 mg/m$^2$ on days 1-14 of a 21-d cycle. After 10 cycles of chemotherapy, approximately 9 mo after her first hospital visit, since no further metastases were detected, she underwent distal gastrectomy. The final pathological diagnosis was Stage Ⅳ [pT3, pN1, cM1(Ovary)] according to the TNM classification of gastric carcinoma (UICC fifth edition). At the last follow-up, 84 mo following oophorectomy, she was alive and free of disease recurrence, despite not receiving any further treatment.

## DISCUSSION

In 1937, Meigs and Cass[1] first reported a case series of seven patients with ovarian fibroma with pleural effusion and ascites, in whom the pleural effusion and ascites disappeared following removal of the ovarian tumors. Subsequently, Rhoads et al. reported similar cases and coined the term "Meigs' syndrome"[1,2]. A similar presentation associated with primary malignant or metastatic ovarian tumors, instead of benign ovarian tumors, is referred to as pseudo-Meigs' syndrome[3].

Ascites may be secondary to ovarian tumors or fluid secretion from the peritoneum, develop as a result of tumor stimulation or in response to cytokines, or may be secondary to tumor-related lymphatic obstruction[4]. Pleural effusion is explained as occurring secondary to the movement of ascitic fluid to the pleural cavity *via* transdiaphragmatic lymphatics and diaphragmatic foramen[5]. It is uncommon for malignant tumor cells to be identified in the pleural or ascitic fluid in patients with pseudo-Meigs' syndrome.

Metastatic ovarian cancer comprises 6%-30% of all ovarian malignancies. The most common primary sites are the gastrointestinal tract, breast, and reproductive organs. In Japan, gastric cancer is the most common primary site because of its relatively high prevalence[6]. Pseudo-Meigs' syndrome is more frequently caused by primary ovarian malignant tumors than ovarian tumors metastases from gastrointestinal cancer, and gastric cancer as the primary source for pseudo-Meigs' syndrome is particularly rare. In fact, to our knowledge, only 10 cases have been described so far, including five in the literature[5,7-10] and five in Japanese conference proceedings. The mean age of the patients in the published reports was 51.8 (range, 32-76) years. Two and three cases had unilateral and bilateral ovarian metastases, respectively, and the ovarian tumor size ranged from 10 to 25 cm in diameter. Pleural effusion was bilateral in three cases and unilateral in two. Moreover, the serum CA 125 levels are often elevated in patients with Meigs' syndrome and decrease following oophorectomy, as in the present case. Benjapibal *et al*[11] explained that CA 125 may be produced from the peritoneal epithelium by a biomedical factor, secondary to mechanical irritation by a large tumor, or owing to an increase in the intraperitoneal pressure related to the large volume of ascites.

The prognosis of gastric cancer with distant metastasis is poor, with a median survival time of 8.6-13.8 mo when treated by chemotherapy alone or in combination with molecular targeted therapy[12]. On the other hand, Lu *et al*[13] reported that oophorectomy for gastric cancer-related metastatic ovarian tumors may improve survival, especially in the absence of metastasis to other organs. The overall survival times of patients who did and did not undergo metastasectomy were reported as 14.1 and 8 mo, respectively, in their study. Furthermore, Brieau *et al*[14] reported that oophorectomy along with current chemotherapy regimens, such as taxane- or platinum-based therapy, improved survival even if the patients had extra-ovarian metastatic sites. In the present case, we selected a docetaxel and S-1 combination regimen with the expectation of efficacy for non-measurable lesions other than in the ovaries[15,16].

The increased survival of the patient reported herein may be owing to her oophorectomy in conjunction with chemotherapy. Accordingly, this case emphasizes the need to be aware of pseudo-Meigs' syndrome, and supports the recommendation of oophorectomy in cases where metastases are limited to the ovaries.

## COMMENTS

### *Case characteristics*

A 48-year-old woman with a 3-mo history of lower abdominal fullness.

### *Clinical diagnosis*

Gastric cancer with metastatic ovarian tumors, malignant ascites, and pleural effusion.

### *Differential diagnosis*

Gastric cancer with primary ovarian cancer, reactive ascites and, pleural effusion.

### *Laboratory diagnosis*

The serum levels of carbohydrate antigen 19-9 and carbohydrate antigen 125 were elevated, at 170.4 U/mL (normal, < 35 U/mL) and 897 U/mL (normal, < 37 U/mL), respectively.

### *Imaging diagnosis*

Computed tomography demonstrated bilateral enlarged ovaries, ascites, and bilateral pleural effusion.

### *Pathological diagnosis*

The resected ovarian specimens comprised poorly differentiated adenocarcinoma, similar to the gastric tumor, suggesting that the bilateral ovarian tumors were secondary to the gastric cancer; there was no evidence of tumor dissemination in the abdomen and the ascitic fluid was negative for cancer cells on cytology.

### *Treatment*

Oophorectomy and gastrectomy in conjunction with chemotherapy.

### *Related reports*

Gastric cancer, as the primary source for pseudo-Meigs' syndrome, is particularly rare. This case is one of only a few reports of pseudo-Meigs' syndrome caused by gastric cancer with prolonged survival.

### *Term explanation*

Meigs' syndrome is defined as the presence of pleural effusion and ascites in association with benign ovarian tumors such as fibromas. In these patients, pleural effusion and ascites typically disappear following oophorectomy. Pseudo-Meigs' syndrome is similar to Meigs' syndrome, except that the ovarian tumor may be malignant rather than benign.

### *Experiences and lessons*

The authors experienced a long-term survival case of pseudo-Meigs' syndrome caused by gastric cancer. If the metastases are limited to the ovaries, it is important not to automatically assume that the tumor is unresectable.

### *Peer-review*

This is an interesting case report in terms of the disappearance of ascites and pleural effusion after oophorectomy, and prolonged survival despite of the extent of the disease at diagnosis.

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