Comparison of clinical outcomes of single-incision versus multi-port laparoscopic surgery for rectosigmoid or upper rectal cancer

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Abstract

Background: The clinical impact of single-incision laparoscopic surgery (SILS) for rectal cancer is unclear. However, SILS is a technically challenging procedure for novice surgeons. The aim of this study was to evaluate clinical outcomes of SILS for rectal cancer compared with multi-port laparoscopic surgery (MPLS).

Patients and Methods: We retrospectively analyzed 357 consecutive patients with stage I–III rectal cancer located in the rectosigmoid or upper rectum who underwent SILS or MPLS between January 2012 and December 2016, using propensity score-matched analysis.

Results: After propensity score-matching, we enrolled 204 patients (n=102 per group). Before matching, significant group-dependent differences were observed in tumor location (p < 0.001). After matching, preoperative clinical factors were similar between groups. SILS was successful in 73.5% of cases, an additional port was required in 23.5% and 2.9% were converted to open surgery. Compared to the MPLS group, the SILS group showed shorter operative time (192 vs. 211 min, p=0.015) and shorter postoperative hospital stay (9 vs. 11 days, p=0.038). Other operative factors and morbidity rates did not differ significantly between groups. The number of harvested lymph nodes was smaller in the SILS group (24) than in the MPLS group (27, p=0.008). Postoperative recurrence did not differ between groups before or after matching. No significant differences in 3-year disease-free, 3-year local recurrence-free or 5-year overall survival were found between groups.

Conclusions: SILS is safe, feasible and offers satisfactory oncological outcomes in selected patients with rectosigmoid or upper rectal cancer.

Introduction

Single incision laparoscopic surgery (SILS) for colon disease is a recent advance in minimally invasive techniques, offering benefits in short-term surgical outcomes including cosmetic results [1], postoperative pain [2, 3], and postoperative recovery [2, 3]. In addition, SILS is feasible and safe for colon cancer in terms of short-term [3–7] and long-term oncological outcomes [5–10].

On the other hand, the utility of SILS for rectal cancer is unknown. SILS for rectal cancer is somewhat technically challenging in terms of mobilization from the pelvic floor and division of the rectum with a laparoscopic stapling device through the umbilical port [11, 12]. Several retrospective studies have reported the safety and feasibility of SILS in selected patients with rectal cancer [13–17], but evidence in support of SILS for rectal cancer remains lacking and the clinical impact is unclear. The aim of this study was thus to evaluate short- and long-term outcomes of SILS compared with multi-port laparoscopic surgery (MPLS) for rectal cancer.

Patients And Methods
Patient populations and surgeons

The medical records of all patients with histologically confirmed rectal cancer at Osaka Police Hospital were entered into a database of prospectively collected data. Between January 2012 and December 2016, a total of 455 consecutive patients were diagnosed with rectal cancer, located in the rectosigmoid or upper rectum [18]. Tumors were located between the height of the sacral promontory and the peritoneal reflection in all cases [18]. The lower edge of the tumor was 7–15 cm from the anal verge in all cases. Tumor location was established preoperatively by barium enema, colonoscopy, or pelvic computed tomography. Patients with tumors located below the peritoneal reflection were excluded from this study.

Among these patients, 2 patients were excluded because they did not undergo primary tumor resection. Thirty-five patients underwent open surgery. Fifty-six patients were diagnosed with stage IV disease. Five patients who underwent simultaneous resection of another cancer were excluded from this study (gastric cancer, n = 2; hepatocellular carcinoma, n = 1; ascending colon cancer, n = 2). A final total of 357 patients who underwent laparoscopic surgery for stage I–III rectal cancer were included in this study.

In Osaka Police Hospital, the first case of SILS for rectal cancer was performed in January 2012. Since then, the indications for SILS have gradually been expanded to include advanced cancers, and SILS has been used for select patients with rectal cancer. Patients are given a sheet describing the differences between conventional MPLS and SILS, and receive a thorough explanation of each operative procedure. All patients agreed to undergo SILS, and provided written informed consent. All operations were performed by 3 surgeons, 2 of whom had completed fellowship training in advanced minimally invasive surgical techniques and had routinely been performing SILS procedures for colorectal disease. The remaining surgeon performed SILS under the guidance of one of the two fully trained surgeons.

Data collection

Patient age, sex, body mass index (BMI), Eastern Cooperative Oncology Group performance status (ECOG-PS), American Society of Anesthesiologists (ASA) score, previous abdominal surgery, tumor location, and clinical TNM classification were obtained from the medical records. Categorization of the primary tumor localization was performed according to the surgical records and pathological reports. Postoperative complications were classified according to the Clavien-Dindo classification [19]. Infectious complications comprised abscess, colitis, urinary tract infection, nephritis, catheter-related infection, or cholecystitis (Table 3). Operative mortality was defined as death during the same admission or within 30 days of surgery. All patients were followed up for at least 30 days after surgery. This study was approved by the institutional review board of Osaka Police Hospital (approval no. 21-1412).

Postoperative follow-up

Adjuvant chemotherapy was recommended for all patients with high-risk Stage II or III disease. Physical examination was performed along with carcinoembryonic antigen and cancer antigen 19–9 assays every 3 months, computed tomography every 6 months, and colonoscopy every year postoperatively.
Patients with suspected tumor recurrence went through additional evaluations, and all patients with resectable recurrent tumors underwent repeat surgery.

**Statistical methods**

Prior to propensity score-matching, the t test or Wilcoxon rank-sum test was used for continuous variables, and the $\chi^2$ test or Fisher’s exact test was applied for categorical variables. Propensity score-matching was then applied to minimize the possibility of selection bias and to adjust for significant differences in the baseline characteristics of patients (Fig. 1). The first step in the matching process was to complete a multivariate logistic regression analysis to obtain propensity scores. Seven covariates that might affect short- and long-term outcomes for SILS were included in the model for calculating the propensity score, as follows: age, sex, BMI, ECOG-PS, ASA score, tumor location, and clinical TNM classification. The next step was a 1:1 matching process, using calipers set at 0.2. This propensity score-matching was used to evaluate the effects of SILS on surgical and pathological outcomes. After propensity score-matching, baseline characteristics, including covariates not entered into the propensity score model, were compared between groups using bivariate analyses.

Data are presented as median and interquartile range (IQR) for continuous variables and as the frequency and percentage for categorical variables. The $\chi^2$ test was used for comparisons of categorical variables. Student’s t test was used to determine the significance of differences between continuous variables. Survival curves were calculated using the Kaplan-Meier method and were then compared by log-rank testing. Values of $p < 0.05$ were considered statistically significant. All statistical analyses were performed using JMP version 14.0 software (SAS Institute, Cary, NC, USA).

**Results**

**Baseline patient profiles**

An overview of the study is shown in Fig. 1. Among 455 consecutive patients who underwent SILS for rectal cancer, 357 patients underwent laparoscopic rectal surgery. Of these, 159 patients underwent SILS and 198 underwent MPLS. Table 1 lists the demographic characteristics of the overall cohort and for propensity score-matched patients. Before matching, the proportion of patients with rectosigmoid cancer was significantly higher in the SILS group ($p < 0.001$). After matching, 102 matched pairs were selected. Baseline characteristics of patients were conserved between the two matched groups. None of the patients in the overall or matched cohorts had received preoperative chemotherapy or chemoradiotherapy.

**Comparison of short-term outcomes between groups**

Table 2 summarizes the details of operative findings between groups. After matching, median operative time was significantly longer in the MPLS group (211 min) than in the SILS group (192 min; $p = 0.015$).
No relevant differences were found between groups in terms of procedure, blood loss, extent of lymph node dissection, multivisceral resection rate, or rate of conversion to open surgery. In the SILS group, 24 patients (23.5%) required an additional port.

Table 3 depicts the postoperative complications that occurred in each group. The rate of Clavien-Dindo grade ≥ 2 did not differ between groups after matching (p = 0.523), and no significant differences in rates of postoperative complications (bleeding, anastomotic leakage, wound infection, bowel obstruction, pneumonia, or infectious complications) were observed. Perioperative death was recorded in 1 patient in each group, due to pneumonia in the MPLS group and sepsis in the SILS group. The rate of reoperation was similar between groups (prior to matching: p = 1; after matching: p = 0.119). Median duration of hospital stay was significantly shorter in the SILS group than in the MPLS group (p = 0.038).

The pathological features and oncologic outcomes are summarized in Table 4. Median number of harvested lymph nodes was significantly lower in the SILS group (24 nodes) than in the MPLS group (27 nodes, p = 0.008). Radial margin positivity was found in 1 patient in the SILS group and 2 patients in the MPLS group. Tumor size, histology, proximal margin, distal margin, lymphatic invasion, venous invasion, lymph node metastasis, and pathological TNM stage showed similar results in both groups.

**Comparison of long-term oncological outcomes between groups**

Median follow-up was 61.4 months in the SILS group and 61.0 months in the MPLS group (p = 0.211). Before matching, 3-year disease-free survival rate was significantly higher in the SILS group (88.2%) than in the MPLS group (78.8%, p = 0.005) (Fig. 2a) and 3-year local recurrence rate was lower in the SILS group (1.3%) than in the MPLS group (6.4%, p = 0.020) (Fig. 3a). The 5-year overall survival was similar between groups (p = 0.130) (Fig. 4a). After matching, no significant differences were observed between groups in terms of 3-year disease-free survival rate (89.9% vs. 88.4%, p = 0.519) (Fig. 2b), 3-year local recurrence rate (2.1% in each group, p = 0.632) (Fig. 3b), or 5-year overall survival rate (87.9% vs. 87.5%, p = 0.980) (Fig. 4b).

**Discussion**

The present study appears to be the first to compare clinical outcomes between SILS and MPLS for rectosigmoid or upper rectal cancer patients. The results suggest that, in selected patients with rectal cancer, SILS can be performed safely (as per the 73.5% SILS completion rate) and yields adequate short-term surgical outcomes (e.g., morbidity 23.5%, mortality 1.0%). In terms of oncological outcomes, we achieved a 99% R0 resection rate, and satisfactory 3-year disease-free, 3-year local recurrence-free, and 5-year overall survival rates in patients with rectosigmoid or upper rectal cancer who underwent SILS.

In this study, SILS was successfully performed in 73.5% of the matched cohort. In a previous systematic review of SILS for colorectal cancer [20], the rate of conversion to open surgery was 0.92% and 13.3% of patients who underwent SILS procedures required insertion of an additional port to allow completion of
the operation. Gash et al. reported that the conversion rate of SILS for rectal cancer was 8% [11]. A few studies have concluded that single-incision plus one port laparoscopic rectal resection for rectal cancer is safe and feasible [12]. Surgeons sometimes encounter technical difficulties in patients with rectal cancer, including mobilization and division of the rectum, and these problems might be overcome by adding a port [12, 21]. In our study, 24 patients (23.5%) required an additional port because division of the rectum through the umbilical port was difficult, and such cases could be completed with SILS plus one port. We suggest that inserting an additional port is a reasonable option when pure SILS is judged as technically difficult.

The operative findings, including procedure, blood loss, extent of lymph node dissection, and multivisceral resection rate, were comparable, regardless of the surgical approach, to those of conventional laparoscopic surgery for rectal cancer in recent randomized control trials [22–25]. In addition, operative time was significantly shorter in the SILS group than in the MPLS group (p = 0.015), even though SILS is technically difficult. This difference was attributed to improvements in surgeon skills. Postoperative complication rate did not differ between groups, and results were comparable with findings from previous studies [22–25]. The duration of postoperative hospital stay was significantly shorter in the SILS group than in the MPLS group (p = 0.038). Recent studies have indicated a median postoperative hospital stay of 8 days [22, 23], whereas the median postoperative hospital stay was 9 days in our SILS group and 11 days in our MPLS group. This result is likely associated with the efforts of the Japanese health insurance system to maintain low medical costs. Although this study analyzed only 102 patients and was retrospective in nature, our results with SILS show high reliability in terms of successful completion rate, operative time and postoperative hospital stay in patients with rectal cancer.

In cancer treatment, oncological clearance must take precedence over cosmetic advantages or reduced invasiveness. In our study, the number of harvested lymph nodes was significantly less in the SILS group than in the MPLS group (p = 0.008). However, oncological outcomes including number of harvested lymph nodes, proximal dissection margin, distal dissection margin, and residual tumor status, regardless of surgical approach, were comparable to the oncological outcomes of randomized control trials comparing open surgery and MPLS for rectal cancer [26–28]. For the present study, the 3-year disease-free and 5-year overall survival rates in the SILS group were 89.9% and 87.9%, respectively. In previous studies, neoadjuvant therapy was introduced in 50–100% of patients [26–28], whereas no patients in our study received such treatments. Nevertheless, the 3-year local recurrence rate was 2.1%, regardless of approach. The utility of chemoradiotherapy for patients with rectal cancer involving the rectosigmoid or upper rectum remains controversial, due to the low incidence of local recurrence after surgery alone [29, 30]. At our hospital, complete surgical resection is the first treatment for rectosigmoid or upper rectal cancer, and we believe that neoadjuvant therapy is not needed for rectosigmoid or upper rectal cancers.

Our study showed several limitations warranting consideration. First, data were obtained retrospectively from a single, high-volume center. To overcome this limitation, we matched cases using several clinical variables, balancing groups and reducing selection bias. However, the potential for selection bias remains, despite the propensity score-matching. Second, in this study, patients with rectal cancer located
at the rectosigmoid or upper rectum, 7–15 cm from the anal verge, were eligible. In Japan, lateral lymph node dissection is indicated when the lower border of the tumor is located distal to the peritoneal reflection and the tumor has invaded beyond the muscularis propria [31]. As lateral lymph node dissection by SILS is technically more challenging, performing SILS for a patient who requires lateral lymph node dissection is considered inappropriate, so patients with tumors located below the peritoneal reflection were excluded from our study, which may have contributed to our good long-term oncological outcomes. Third, BMI in our cohort was typical of a Japanese population, which may have significantly affected the feasibility of SILS. Fourth, this study investigated proximal, distal, and radial margins as pathological parameters, but not circumferential resection margin, in accordance with Japanese practice guidelines [18]. Despite these limitations, we consider that this analysis using propensity score-matching confirmed SILS as safe and feasible for rectosigmoid or upper rectal cancer. Further analysis is needed to validate our results, and long-term oncological outcome need to be evaluated in randomized clinical trials.

Conclusions

SILS is safe, feasible and can provide satisfactory oncological outcomes in select patients with rectosigmoid or upper rectal cancer.

Declarations

Disclaimer:

The present manuscript is an original contribution not previously published and not under consideration for publication elsewhere, and, if accepted, will not be published anywhere in similar form, in any language. All authors have read and approved the manuscript, and the study was approved by the institutional research ethics committee.

Contributions of each author:

MT and YS conceived and designed the study. MT, YS, MO, KI, AN, MI, TM and HA acquired the data. MT and YS analyzed and interpreted the data. MT drafted the manuscript. YS, MO, KI, AN, MI, TM and HA critically revised the article. MT, YS, MO, KI, AN, MI, TM and HA approved the final version of the manuscript to be published.

Conflicts of Interest and Financial Disclosure:

Mitsuyoshi Tei, Yozo Suzuki, Masahisa Ohtsuka, Kazuya Iwamoto, Atsushi Naito, Mitsunobu Imasato, Tsunekazu Mizushima, and Hiroki Akamatsu have no conflicts of interest or financial ties to disclose.

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Tables
| Table 1                                                                 |
|----------------------------------------------------------------------|
| Demographic characteristics of patients                              |

|                                | Overall (n = 357) | Propensity score-matched pairs (n = 204) |
|--------------------------------|------------------|----------------------------------------|
|                                | MPS (n = 198)     | SPS (n = 159)                          | MPS (n = 102) | SPS (n = 102) |
| Age, years, median (IQR)       | 68 (60–73)       | 67 (61–74)                             | 67 (59–72)    | 55 (61–76)    | 0.632       |
| Gender, male, n (%)            | 124 (62.6)       | 95 (59.8)                              | 60 (58.8)     | 64 (62.8)     | 0.579       |
| BMI, kg/m², median (IQR)       | 22.1 (19.7–23.8) | 22.7 (20.3–26.4)                      | 22.4 (19.7–24.0) | 22.7 (20.0–24.3) | 0.093       |
| ECOG-PS, 0 or 1, n (%)         | 191 (96.5)       | 153 (96.2)                             | 99 (97.1)     | 96 (94.1)     | 0.905       |
| ASA score, 3>, n (%)           | 27 (13.6)        | 23 (14.5)                              | 13 (12.8)     | 15 (14.7)     | 0.823       |
| Tumor location, n (%)          | 84 (42.4)        | 123 (77.4)                             | <0.001        | 71 (69.6)     | 73 (71.6)   | 0.878       |
| Clinical TNM stage, n (%)      | 53 (26.8)        | 51 (32.1)                              | 26 (25.5)     | 31 (30.4)     | 0.534       |
| I                              | 69 (34.9)        | 53 (33.3)                              | 34 (33.3)     | 34 (33.3)     |              |
| II                             | 76 (38.4)        | 55 (34.6)                              | 42 (41.2)     | 37 (36.3)     |              |
| III                            |                  |                                        |              |              |              |

ECOG-PS, Eastern Cooperative Oncology Group Performance Status Scale; ASA score, American Society of Anesthesiologists Score; BMI, Body Mass Index; *Comorbidities: Cardiac = ischemic disease, chronic heart failure and cardiomyopathy, excluded hypertension; Pulmonary = asthma, chronic obstructive pulmonary disease, and interstitial pneumonia Cerebrovascular = history of transient ischemic attacks and cerebrovascular event with or without neurological deficit.
Table 2
Operative findings

| Procedure | Overall (n = 357) | Propensity score-matched pairs (n = 204) |
|-----------|------------------|-----------------------------------------|
|           | MPS (n = 198)    | SPS (n = 159)                           | P value | MPS (n = 102) | SPS (n = 102) | P value |
| HAR       | 72 (36.4)        | 126 (79.2)                              | .001    | 64 (62.8)     | 71 (69.6)     | .299    |
| LAR       | 114 (57.6)       | 32 (20.1)                               |         | 34 (33.3)     | 30 (29.4)     |         |
| Hartmann  | 9 (4.5)          | 1 (0.6)                                 |         | 4 (3.9)       | 1 (1.0)       |         |
| APR       | 3 (0.5)          | 0                                        |         | 0             | 0             |         |
| Stoma creation, n (%) | 25 (12.6) | 2 (1.3) | < 0.001 | 8 (7.8) | 2 (2.0) | 0.101 |
| Blood loss, ml, median (IQR) | 5 (5–100) | 5 (5–50) | < 0.001 | 5 (5–52) | 5 (5–50) | 0.277 |
| Operative time, minutes, median (IQR) | 223 (182–273) | 182 (150–224) | < 0.001 | 211 (180–259) | 192 (162–220) | 0.015 |
| Extent of lymph node dissection, D3, n (%) | 155 (78.3) | 127 (79.9) | 0.794 | 80 (78.54) | 81 (79.4) | 1 |
| Multivisceral resection, n (%) | 8 (4.0) | 8 (5.0) | 0.798 | 4 (3.9) | 7 (6.9) | 0.537 |
| SPS completion, n (%) | - | 125 (78.6) | - | - | 75 (73.5) | - |
| Conversion to open surgery, n (%) | 7 (3.5) | 5 (3.1) | 3 (2.9) | 3 (2.9) | 1 |
| Required an additional port, n (%) | - | 29 (18.2) | - | - | 24 (23.5) | |
| Clavien-Dindo classification (grade ≥ 2), n (%) | Overall (n = 357) | Propensity score-matched pairs (n = 204) |
|---------------------------------------------|------------------|------------------------------------------|
|                                            | MPS (n = 198)    | SPS (n = 159) | P value | MPS (n = 102) | SPS (n = 102) | P value |
| Bleeding                                   | 2 (1.0)          | 1 (0.6)     | 1       | 2 (2.0)       | 0            | 0.498   |
| Anastomotic leakage                        | 29 (14.7)        | 14 (8.8)    | 0.103   | 12 (11.8)     | 12 (11.8)    | 1       |
| Wound infection                            | 26 (13.1)        | 12 (7.6)    | 0.119   | 8 (7.8)       | 8 (7.8)      | 1       |
| Bowel obstruction                           | 11 (5.6)         | 5 (3.1)     | 0.314   | 5 (4.9)       | 4 (3.9)      | 1       |
| Pneumonia                                  | 4 (2.0)          | 3 (1.9)     | 1       | 3 (2.9)       | 3 (2.9)      | 1       |
| *infectious complications                  | 17 (8.6)         | 11 (6.9)    | 0.693   | 6 (5.9)       | 6 (5.9)      | 1       |
| Perioperative death                         | 2 (1.0)          | 1 (0.6)     | 1       | 1 (1.0)       | 1 (1.0)      | 1       |
| Re-operation                               | 8 (4.0)          | 6 (3.8)     | 1       | 6 (5.9)       | 1 (1.0)      | 0.119   |
| Overall complication                       | 60 (30.3)        | 32 (20.1)   | 0.038   | 29 (28.4)     | 24 (23.5)    | 0.523   |
| Postoperative hospital stay, days, median (IQR) | 12 (9–20) | 9 (8–12) | < 0.001 | 11 (8–18) | 9 (8–13) | 0.038 |

*infectious complications = abscess, colitis, urinary tract infection, nephritis, catheter-related infection, cholecystitis
Table 4
Pathological features and oncologic outcomes

|                                   | Overall (n = 357) | Propensity score-matched pairs (n = 204) |
|-----------------------------------|-------------------|----------------------------------------|
|                                   | MPS (n = 198)     | SPS (n = 159)                          | P value | MPS (n = 102)     | SPS (n = 102)                          | P value |
| Tumor size, mm, median (IQR)      | 41 (30–60)        | 38 (25–50)                             | 0.017   | 41 (25–56)        | 37 (28–50)                             | 0.240   |
| Histology, tub                    | 190 (96.0)        | 156 (98.1)                             | 0.358   | 97 (95.1)         | 100 (98.0)                             | 0.445   |
| Proximal margin, mm, median (IQR) | 120 (95–125)      | 115 (95–120)                           | 0.694   | 110 (90–105)      | 110 (95–110)                           | 0.447   |
| Distal margin, mm, median (IQR)   | 35 (24–36)        | 30 (25–36)                             | 0.781   | 30 (25–35)        | 30 (24–32)                             | 0.745   |
| Number of harvested lymph nodes, median (IQR) | 25 (18–32) | 22 (15–30)                             | 0.004   | 27 (19–35)        | 24 (15–29)                             | 0.008   |
| Ly, positive                      | 135 (68.2)        | 99 (62.3)                              | 0.263   | 69 (67.6)         | 64 (62.7)                              | 0.467   |
| V, positive                       | 132 (66.7)        | 106 (66.7)                             | 1       | 65 (63.7)         | 67 (65.7)                              | 0.884   |
| Positive lymph node metastasis, n (%) | 87 (43.9) | 69 (43.4)                              | 1       | 41 (40.2)         | 42 (41.2)                              | 1       |
| Positive radial margin, n (%)     | 4 (2.0)           | 1 (0.6)                                | 0.387   | 2 (2.0)           | 1 (1.0)                                | 1       |
| pTNM stage, n (%)                 | 54 (27.3)         | 49 (30.8)                              | 0.336   | 34 (33.3)         | 33 (32.4)                              | 0.786   |
| I                                 | 59 (29.8)         | 41 (25.8)                              |         | 28 (27.5)         | 28 (27.5)                              |         |
| II                                | 82 (41.4)         | 69 (43.4)                              |         |                   |                                       |         |
| III                               | 3 (1.5)           | 0                                      |         | 39 (38.2)         | 41 (40.2)                              |         |
| IV                                |                   |                                        |         | 1 (1.0)           | 0                                      |         |
|                      | Overall (n = 357) | Propensity score-matched pairs (n = 204) |
|----------------------|-------------------|------------------------------------------|
| Recurrence, n (%)    | 15 (7.6)          | 6 (5.9)                                  |
| Liver                | 14 (7.1)          | 6 (5.9)                                  |
| Lung                 | 7 (3.5)           | 3 (2.9)                                  |
| Local                | 8 (4.0)           | 3 (2.9)                                  |
| Distant lymph node   | 3 (1.5)           | 2 (2.0)                                  |
| Others               | 41 (20.7)         | 18 (17.6)                                |
| Total                |                   | 21 (13.2)                                |

Figures

Figure 1
Flowchart of patients who underwent SILS for colorectal cancer

Figure 2
Kaplan-Meier analysis of the 3-year disease-free survival rate between groups

**Figure 3**

Kaplan-Meier analysis of the 3-year local recurrence rate between groups

**Fig. 4a**

**Fig. 4b**

Kaplan-Meier analysis of the 5-year overall survival rate between groups

**Figure 4**

Kaplan-Meier analysis of the 5-year overall survival rate between groups