Outcomes of marginal donors for lung transplantation after ex vivo lung perfusion: A systematic review and meta-analysis

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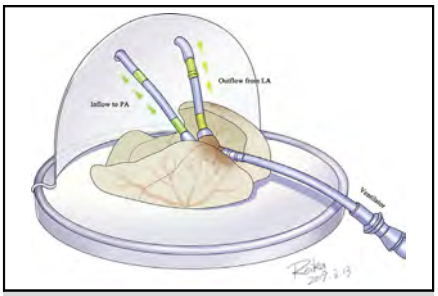
ABSTRACT

Objective: Ex vivo lung perfusion (EVLP) is reportedly a useful strategy that permits marginal donor lungs to be evaluated and reconditioned for successful lung transplantation (LTx). This systematic review and meta-analysis was performed to evaluate the outcomes of EVLP conducted for marginal donor lungs.

Methods: We searched PubMed, the Cochrane Library, and Embase to select studies describing the results of LTx following EVLP for marginal donor lungs compared with standard LTx without EVLP. We performed a meta-analysis to examine donor baseline characteristics, recipient baseline characteristics, and postoperative outcomes.

Results: Of 1380 studies, 8 studies involving 1191 patients met the inclusion criteria. Compared with the non-EVLP group (ie, standard LTx without EVLP), the EVLP group (ie, EVLP of marginal donors following LTx) had similar donor age and sex and recipient baseline age, sex, body mass index, bridge by ventilator/extracorporeal life support/extracorporeal membrane oxygenation, and rate of double LTx but more abnormal donor lung radiographs ($P = .0002$), a higher smoking history rate ($P = .03$), and worse donor arterial oxygen tension/inspired oxygen fraction ($P < .00001$). However, there were no significant differences in outcomes between the EVLP and non-EVLP groups with respect to the length of postoperative intubation, postoperative extracorporeal life support/extracorporeal membrane oxygenation use, length of intensive care unit stay, length of hospital stay, 72-hour primary graft dysfunction of grade 3, 30-day survival, or 1-year survival (all P values $> .05$).

Conclusions: Posttransplant outcomes were similar between EVLP-treated LTx and standard LTx without EVLP, although the quality of donor lungs was worse with EVLP-treated LTx. (J Thorac Cardiovasc Surg 2019;■:1-11)



Ex vivo lung perfusion is a useful strategy for marginal donor lungs.

Central Message

The outcomes after lung transplantation of ex vivo lung perfusion on marginal donor lungs are comparable with those of standard lung transplantation without ex vivo lung perfusion treatment.

Perspective

This study provides further confirmation that marginal donor lungs treated by ex vivo lung perfusion have outcomes comparable with those of standard donor lung transplantation, although the ex vivo lung perfusion group had worse quality of donor lungs than the group without ex vivo lung perfusion. It may improve donor lung use for successful transplantation.

See Commentary on page XXX.

Lung transplantation (LTx) has been regarded as the best and only effective therapy for patients with end-stage pulmonary disease. However, challenges have existed since the first clinically successful LTx was performed in 1983.[1] The major difficulty is the shortage of acceptable donor lungs with a low utilization rate of 17% to 27%.[2-4] After retrieval, hypothermic preservation is widely used to maintain the

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Abbreviations and Acronyms

BMI = body mass index
ECLS = extracorporeal life support
ECMO = extracorporeal membrane oxygenation
EVLP = ex vivo lung perfusion
ICU = intensive care unit
LTx = lung transplantation
OCS = Organ Care System
Pao_2/Fio_2 = arterial oxygen tension/inspired oxygen fraction
PGD = primary graft dysfunction

donor lungs, which inhibits cellular metabolism without evaluation and reconditioning before LTx.[5]

Ex vivo lung perfusion (EVLP) is a modern lung preservation technique before LTx. Three different EVLP techniques have been reported: the Lund, Toronto, and Organ Care System (OCS) (TransMedics, Andover, Mass) protocols.[6] Some differences exist among these protocols. The Lund and Toronto protocols use continuous flow perfusion in static devices. However, the OCS protocol uses pulsatile flow perfusion in a mobile device. With respect to the left atrium, the Lund and OCS protocols are open but the Toronto protocol is closed with 3- to 5-mm Hg left atrium pressure. The Lund and OCS protocols use a blood-based perfusate, but the Toronto protocol use an acellular perfusate.[6] Although these systems have some different characteristics, they are used for the same purposes to accurately assess and recondition lung quality.

Marginal donor lungs are lungs that may be transplantable but do not meet the criteria for ideal donor lungs, including a high arterial oxygen tension/inspired oxygen fraction (Pao_2/Fio_2) ratio $\geq$300 mm Hg, no infiltration on chest radiographs, clear bronchoscopic findings, and no smoking history.[7] Whether such marginal donor lungs transplanted with EVLP have different outcomes than lungs transplanted without EVLP remains unconfirmed. Although some previous studies have shown acceptable outcomes, they were small, primarily single-center studies.[7-15] We performed a systematic review and meta-analysis to test our hypothesis that outcomes may not significantly differ between marginal donor lungs transplanted with EVLP and acceptable donor lungs transplanted without EVLP.

MATERIALS AND METHODS

This systematic review and meta-analysis was conducted and reported according to the recommendations of the preferred reporting items for systematic reviews and meta-analyses checklist (http://www.prisma-statement.org/).[16]

Literature Search Strategy

PubMed, the Cochrane Library, and Embase were searched to select studies related to this meta-analysis. The last search was performed on December 31, 2018. The search terms were *lung transplantation*, *pulmonary transplantation*, *ex vivo lung perfusion*, *EVLP*, *extracorporeal lung perfusion*, and *ex vivo lung recondition*. Two authors (DT and YW) independently evaluated the qualification of the references. A third review author (HS) resolved any disagreements between the 2 review authors when necessary. The full text of the retrieved articles and their references were assessed to identify whether the studies contained useful information.

Study Selection Criteria

A relevant study in this systematic review and meta-analysis met the following criteria: original studies with $\geq$5 patients in each group, conducted on human subjects, full text written in English only, and reported outcomes of EVLP on marginal donors following LTx and standard LTx without EVLP. If overlapping data were presented in several publications from the same data source, only the most detailed and relevant articles were selected in this meta-analysis. Duplicate articles were excluded.

Selection Criteria and Data Appraisal

The first author, publication year, location, study design, EVLP protocol, number of patients, EVLP (LTx) utilization rate, donor age, donor sex, abnormal radiograph findings of the donor, donor smoking history, donor Pao_2/Fio_2, recipient age, recipient sex, LTx indication, double LTx, recipient bridge by a ventilator/extracorporeal membrane oxygenation (ECMO), and recipient body mass index (BMI) were extracted from each included study. The primary outcomes were 72-hour primary graft dysfunction (PGD) of grade 3, 30-day survival, and 1-year survival. The secondary outcomes were postoperative ECMO/extracorporeal life support (ECLS) use, length of postoperative ventilation, intensive care unit (ICU) stay, and hospital stay. We attempted to contact the authors of the selected articles to obtain relevant data. Data were extracted from figures by Engauge Digitizer 4.1 software (available at: http://markummitchell.github.io/engauge-digitizer)[17] or converted to the mean $\pm$ standard deviation according to previous studies.[18,19] We used the Newcastle-Ottawa Quality Assessment Scale for nonrandomized studies because its design, content, and ease of use are directed to the task of incorporating quality assessments into the interpretation of meta-analytic results. The scale includes 3 aspects (ie, selection, comparability, and outcome) and 8 items.[20] It ranges from 0 to 9 stars, and studies with a score $\geq$6 were considered to have adequate methodologic quality for inclusion. The existence of publication bias was evaluated in funnel plots.[16,21]

Statistical Analysis

Review Manager (RevMan) version 5.3.5 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) was used for this meta-analysis. For continuous data, the mean difference (MD) and 95% confidence interval (CI) were calculated. Dichotomous data were analyzed using the odds ratio (OR) with 95% CI. Data were graphically plotted using forest plots to evaluate the treatment effects and heterogeneity of the trials. Data were combined and analyzed using random effects (significant heterogeneity) or fixed effects (no significant heterogeneity). The Mantel-Haenszel method was used for dichotomous data, and the inverse-variance method was used for continuous data. Statistically significant heterogeneity was defined as a χ^2 P value $< .1$ or an I^2 statistic >50%.[22] A funnel plot estimating the precision of trials (plots of logarithm of OR for efficacy against the sample size) was examined for asymmetry to estimate publication bias.

RESULTS

Study Selection

A preferred reporting items for systematic reviews and meta-analyses flow diagram of the trial selection process

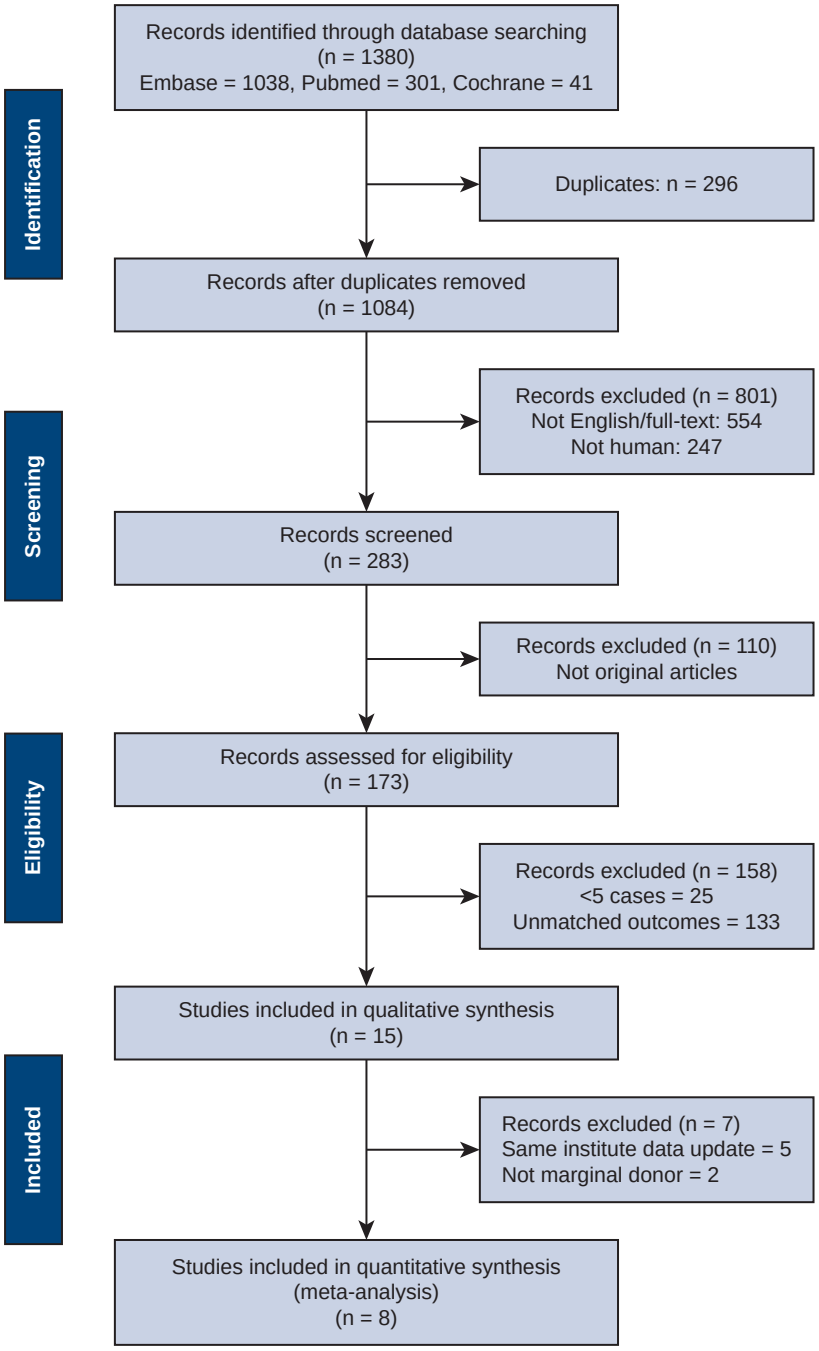


FIGURE 1. This systematic review and meta-analysis was conducted and reported according to the recommendations of the preferred reporting items for systematic reviews and meta-analyses checklist. In total, 1380 records were identified from all sources (Embase, PubMed, and Cochrane Library) and only 8 studies involving 1191 patients met all inclusion criteria and were selected for the meta-analysis.

and reasons for study exclusion are shown in detail in Figure 1. In total, 1380 records were identified from all sources (Embase, n = 1038; PubMed, n = 301; and Cochrane, n = 41). After excluding duplicates (296 records), 1084 titles and abstracts were screened for eligibility. Subsequently, after removal of non-English/and nonfull texts (554 records) and nonhuman studies (247 records), 283 remaining articles were screened for eligibility. Thereafter, only original articles were included in this meta-analysis. We also excluded studies that contained <5 patients (25 records) or unmatched outcomes (133 records) that did not show our primary outcomes. The 15 remaining studies were screened for qualitative synthesis; 5 records included data from the same institute

TABLE 1. Summary of donor baseline characteristics (ex vivo lung perfusion [EVLP] vs non-EVLP)

Authors, year	Location	Study design	EVLP protocol	EVLP/ non-EVLP*	EVLP/ total EVLP†	Percentage of DBD‡	Age (y)‡	Male sex‡	Abnormal radiograph of donor‡	Donor smoking‡	Donor Pao_2/FIo_2 (mm Hg)*
Aigner et al,[8] 2012	Austria	SP	Toronto	9/119	9/13	All	43.2 ± 14.1/ NR	NR	NR	NR	209.9 ± 46.2/ 452.9 ± 76.4
Boffin et al,[9] 2014	Italy	SR	Toronto	8/28	8/11	All	44.7 ± 16.2/ 43.3 ± 16.8	12.5/53.6	NR	25.0/72.7	338 ± 126/ 498 ± 62.5
Cypel et al,[7] 2012	Canada	SR	Toronto	50/253	50/58	56.0/94.8	45/45	NR	68.0/45.1	NR	334.6 ± 86.7/ 450.3 ± 59.4
Fisher et al,[11] 2016	United Kingdom	MP	Toronto and Lund	18/184	18/53	72.2/82.6	47.7 ± 10.7/ 43.6 ± 10.7	55.6/46.7	NR	NR	NR
Koch et al,[12] 2018	Germany	SP	Toronto	9/41	9/11	All	54 ± 14/ 54 ± 16	66.7/51.2	88.9/43.9	66.7/61.1	270 ± 74/ 413 ± 96
Sage et al,[14] 2014	France	MP	Toronto	31/81	31/32	All	47.1 ± 11.2/ 51.4 ± 9.5	NR	71.0/19.8	29.0/21.0	276.1 ± 58.9/ 512.5 ± 59.8
Valenza et al,[15] 2014	Italy	SP	Lund	7/28	7/8	All	54 ± 9/ 40 ± 15	NR	85.7/39.3	57.1/100	264 ± 78/ 453 ± 119
Nilsson et al,[13] 2018	Sweden	MP	Lund	54/271	54/61	All	NR	NR	NR	NR	229.5 ± 90.0/ NR

EVLP, Ex vivo lung perfusion; *DBD*, donation after brain death; Pao_2/FIo_2, arterial oxygen tension/inspired oxygen fraction; *SP*, single-center prospective; *NR*, not reported; *SR*, single-center retrospective; *MP*, multicenter prospective. *EVLP on marginal donor lungs following lung transplant versus standard lung transplant of lungs not treated with EVLP. †EVLP on marginal donor lungs following lung transplant versus all EVLP cases (use ratio). ‡EVLP versus non-EVLP with percentages.

and the same or some of the same participants[2,10,23-25]; and 2 studies evaluated EVLP for nonmarginal donors, including eligible donors only for a Pao_2/FIo_2 >300 mm Hg.[26,27] We selected the most detailed and relevant articles for this meta-analysis. Eight studies involving 1191 patients met all inclusion criteria and were selected for the meta-analysis.

Study Characteristics

All studies were published in English with full texts. Three studies were multicenter prospective studies.[11,13,14] Three studies were single-center prospective studies,[8,12,15] and the others were single-center retrospective studies.[7,9] One study was performed in Canada,[7] and the others were from Europe.[8,9,11-15] Eight studies involved 247 EVLP cases and a final total of 186 LTx EVLP cases. The use ratio was 75.3% with a range from 34.0%[11] to 96.9%.[10,14] All studies reported the indications for LTx, which were chronic obstructive pulmonary disease/emphysema,[7,8,10-14] idiopathic pulmonary fibrosis/pulmonary fibrosis/cystic fibrosis,[7-15] pulmonary artery hypertension,[7,10,13,28] interstitial lung disease,[11] bronchiolitis/α-1 antitrypsin deficiency,[10,11,13] and retransplantation.[12] The donor and recipient baseline characteristics among the 8 extracted studies selected in this meta-analysis are summarized in Tables 1 and 2.

Demographic Characteristics

The number of patients enrolled in each trial ranged from 35 to 303.[7,15,28] Of 1191 patients, 186 (15.6%) underwent EVLP followed by LTx, and 1005 (84.4%) underwent LTX without EVLP. All studies were published from 2012 to 2018 with cases collected from 2008 to 2017. The EVLP systems used in the individual studies were as follows. The Toronto protocol was used in the studies by Aigner and colleagues,[8] Boffini and colleagues,[9] Cypel and colleagues,[7] Koch and colleagues,[12] and Sage and colleagues.[14] The Lund protocol was used in the studies by Valenza and colleagues[15] and Nilsson and colleagues.[13] A hybrid system combining elements of both the Toronto and Lund protocols was used in the study by Fisher and colleagues.[11] All studies selected only donation after brain death, except 2 studies that showed the percentage of donation after brain death in EVLP and non-EVLP, 56.0%

TABLE 2. Summary of recipient baseline characteristics (ex vivo lung perfusion [EVLP] vs non-EVLP)

Authors, year	Age (y)*	Male sex†	Transplant indications*	Bridge by ventilator/ ECMO†	Recipient BMI*
Aigner et al,[8] 2012	50 ± 17/43 ± 15	NR	CF: 22.2%; IPF: 44.4%; COPD: 33.3%	NR	NR
Boffini et al[9] 2014	46.6 ± 9.8/51.7 ± 17.4	75.0/75.0	PF: 57%/61%	NR	24.8 ± 5.8/24.1 ± 5.8
Cypel et al,[7] 2012	56/56	NR	PF or PAH: 32%/38.7%; COPD/emphysema: 27.8%/35.8%	NR	NR
Fisher et al,[11] 2016	51.6 ± 12.1/50.5 ± 9.6	72.2/57.6	CF: 22.2%/25.5%; ILD: 38.9%/25.5%; Bronchiolitis: 5.6%/5.4%; Other: 0/4.9% IPF: 2/10; COPD: 8/22; CPFE: 1/3	88.9/63.0	22.7 ± 4.1/23.9 ± 3.5
Koch et al,[12] 2018	55 ± 7/55 ± 6	88.9/58.5	CF: 0/3; Retransplantation: 0/1; Sarcoidosis, histiocytosis X: 0/2	0/0	25 ± 4/23 ± 4
Sage et al,[14] 2014	40.1 ± 9.5/41 ± 9.9	35.5/48.1	CF: 48%/49%; COPD: 29%/20%; PF: 10%/15%; Other: 13%/16%	NR	NR
Valenza et al,[15] 2014	38 ± 15/49 ± 14	NR	CF: 36%/57%; PF: 40%/0; Other: 25%/43%	85.7/35.7	20 ± 2.2/22.6 ± 1.7
Nilsson et al,[13] 2018	52 ± 12/51 ± 13	NR	IPF: 24%/25%; PAH: 2%/6%; COPD: 33%/28%; α1 ATD: 6%/13%; CF: 20%/12%; Other:15%/16%	11.1/10.3	NR

ECMO, Extracorporeal membrane oxygenation; *BMI*, body mass index; *NR*, not reported; *CF*, cystic fibrosis; *IPF*, idiopathic pulmonary fibrosis; *COPD*, chronic obstructive pulmonary disease; *PF*, pulmonary fibrosis; *PAH*, pulmonary artery hypertension; *ILD*, interstitial lung disease; *CPFE*, combined pulmonary fibrosis and emphysema; *ATD*, antitrypsin deficiency. *EVLP on marginal donor lungs following lung transplant versus standard lung transplant without EVLP treatment. †EVLP versus non-EVLP with percentages.

and 94.8% (Cypel and colleagues[7]), and 72.2% and 82.6% (Fisher and colleagues[11]). Tables 1 and 2 show the donor and recipient clinical characteristics among the 8 studies included in this meta-analysis. Table 3 shows the posttransplant outcomes, which include postoperative ECMO use, length of postoperative ventilation, length of ICU stay, length of hospital stay, 72-hour PGD grade of 3, 30-day survival, and 1-year survival.

TABLE 3. Summary of postoperative outcomes (ex vivo lung perfusion [EVLP] vs non-EVLP)

Authors, year	Postoperative ECMO*	Length of postoperative ventilation (d)†	Length of ICU stay (d)†	Length of hospital stay (d)†	72-h PGD of grade 3*	30-d survival*	1-y survival*
Aigner et al,[8] 2012	NR	2.6 ± 1.9/4.6 ± 8.2	5.5 ± 1.6/9.7 ± 9.4	28.9 ± 16.9/27.8 ± 19.0	NR	100/95.8	77.8/84.0
Boffini e al,[9] 2014	25/17.9	NR	NR	NR	37.5/50.0	87.5/82.1	NR
Cypel et al,[7] 2012	2.0/2.8	10.6 ± 22.3/3.4 ± 7.5	12.2 ± 22.1/11.9 ± 45.5	30.8 ± 33.2/30.5 ± 53.0	2.0/8.7	96.0/96.4	88.0/86.2
Fisher et al,[11] 2016	38.9/3.3	17.8 ± 27.4/4.9 ± 17.0	25.6 ± 26.4/7.7 ± 18.5	37.4 ± 23.1/29.7 ± 17.9	27.8/17.4	33.3/96.7	66.7/79.9
Koch et al,[12] 2018	11.1/0	9.2 ± 12.8/5.2 ± 10.4	12.5 ± 13.4/18.9 ± 58.2	26 ± 16/19 ± 8	0/0	88.9/100	77.8/95.1
Sage et al,[14] 2014	NR	5.8 ± 10.5/3.1 ± 7.0	12.4 ± 10.5/7.2 ± 5.4	40.6 ± 17.8/38.1 ± 37.6	9.7/8.6	100/96.3	93.5/91.4
Valenza et al,[15] 2014	14.3/7.1	5.9 ± 5.5/3.5 ± 4.2	10.7 ± 4.8/5.9 ± 5.2	NR	28.6/32.1	100/100	57.1/78.6
Nilsson et al,[13] 2018	NR	3.8 ± 8.3/3.0 ± 16.8	8.9 ± 13.9/7.3 ± 27.4	35.8 ± 20.9/34.3 ± 45.2	NR	NR	87.0/83.0

ECMO, Extracorporeal membrane oxygenation; *ICU*, intensive care unit; *PGD*, primary graft dysfunction; *NR*, not reported. *EVLP versus non-EVLP with percentages. †EVLP on marginal donor lungs following LTx versus standard LTx of non-EVLP.

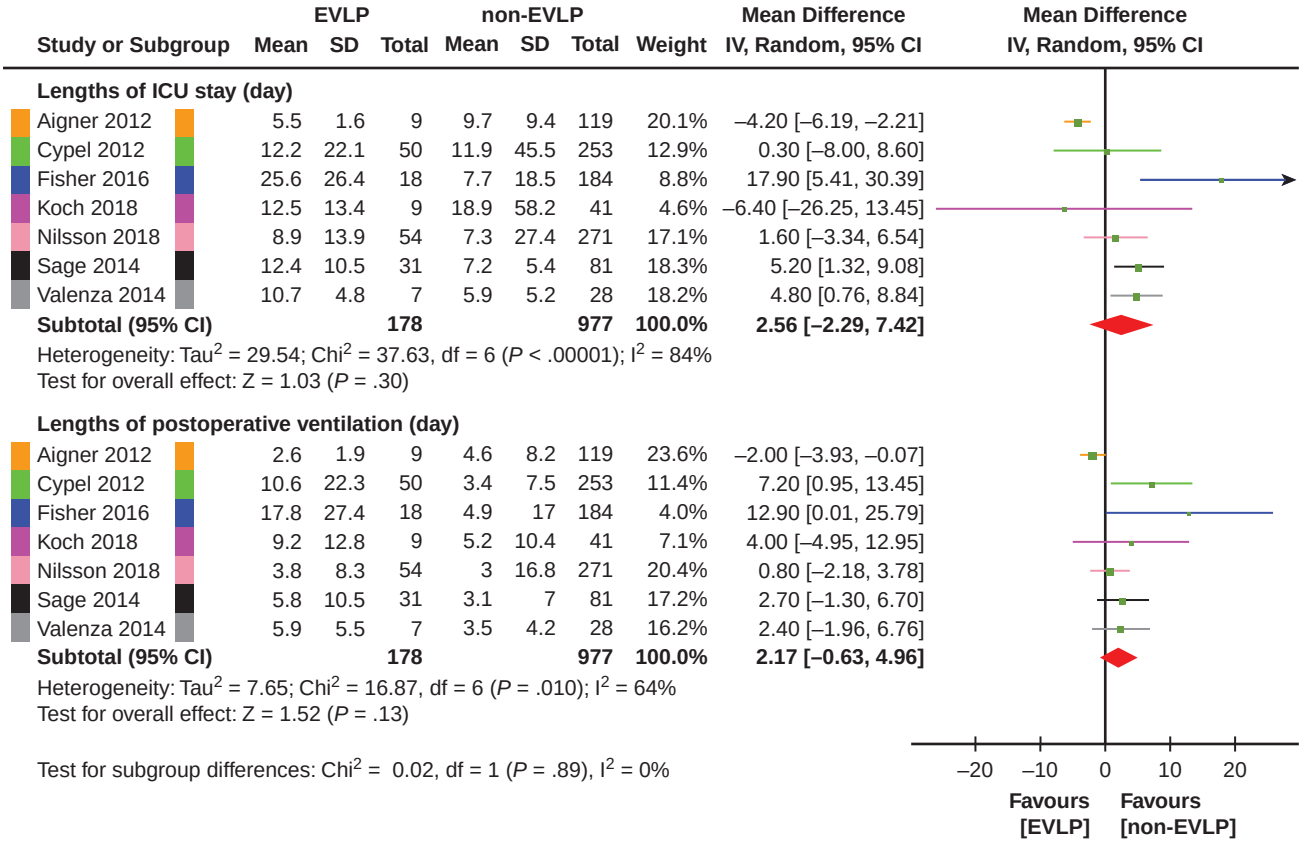


FIGURE 2. Forest plot of lengths of intensive care unit (*ICU*) stay and postoperative ventilation between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar lengths of ICU stay and postoperative ventilation between the EVLP and non-EVLP groups were shown in the global analysis ($P = .30$ and .13, respectively). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

Quality Assessment

The quality assessment results for the individual studies are shown in Table E1. We used the Newcastle-Ottawa Quality Assessment Scale for cohort studies. All studies scored either 6[8,11,14,15] or 7.[7,9,12,13]

Donor Baseline Characteristics

The donor baseline characteristics are summarized in Table 1 and Figures E1 through E4. The mean age of the EVLP donors ranged from 44.7[9] to 54.0[15] years, and the mean age of the non-EVLP donors ranged from 40.0[15] to 54[12] years. There was no significant difference in age (MD, 3.45; 95% CI, −3.30 to 10.20 years; $P = .32$) (Figure E1).[4,10-12,15] Three of 8 studies reported donor sex, and there was no significant difference in donor sex (male) (OR, 1.09; 95% CI, 0.63-1.88; $P = .76$) (Figure E2).[9-12] However, this meta-analysis demonstrated more abnormal donor lung radiograph findings (OR, 5.69; 95% CI, 2.28-14.19; $P = .0002$) (Figure E3),[7,12,14,15,28] more donors with a smoking history (OR, 3.36; 95% CI, 1.15-9.84; $P = .03$) (Figure E3),[9,12,14,15] and worse donor Pao_2/Fio_2 (MD, 182.78 mm Hg; 95% CI, 127.00-238.60 mm Hg; $P < .00001$) (Figure E4)[7-10,12,14,15] in the EVLP group. In all studies, abnormal donor lung radiograph findings were about 5.69 times (range, 2.59-10.22 times) more frequent and the donor Pao_2/Fio_2 ratio was about 182.78 mm Hg (range, 115.70-243.00 mm Hg) lower in the EVLP group than non-EVLP group.

Recipient Baseline Characteristics

The recipient baseline characteristics are summarized in Table 2 and Figures E5 through E8. The mean recipient age in the EVLP group ranged from 38.0[15] to 55.0[12] years, and that in the non-EVLP group ranged from 41.0[14] to 55.0[12] years. Four studies recorded both recipient sex and BMI in the EVLP group versus the non-EVLP group (male, 35.5%[14]-75%[9] vs 48.1%[14]-75%[9] and BMI, 20[15]-25[12] vs 22.6[15]-24.1[9], respectively). There were no significant differences between the 2 groups in age (MD, −0.09; 95% CI, −2.10 to 1.92 years; $P = .93$) (Figure E5),[8-15] male sex (OR, 1.01; 95% CI, 0.58-1.77;

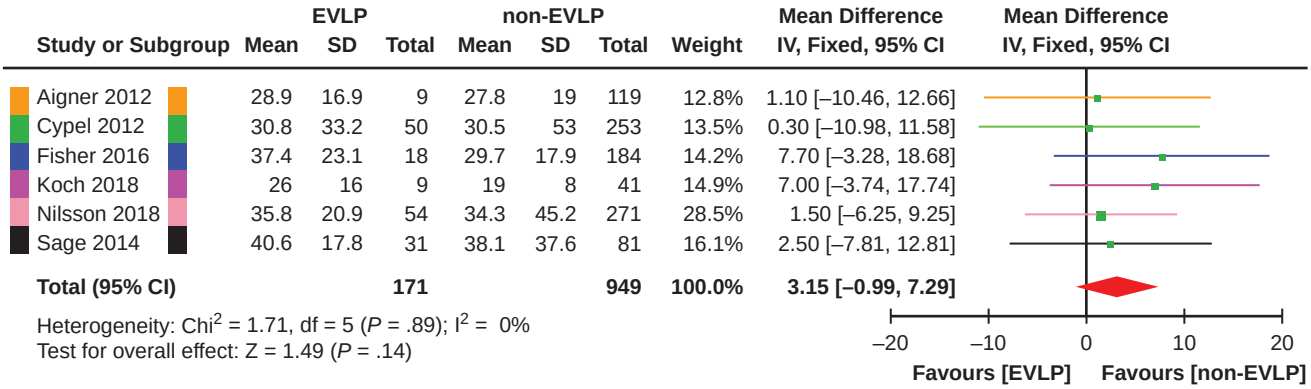


FIGURE 3. Forest plot of length of hospital stay between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. A similar length of hospital stay between the EVLP and non-EVLP groups was shown in the global analysis ($P = .14$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

$P = .96$) (Figure E6),[9,11,12,14] or recipient BMI (MD, −0.65; 95% CI, −2.71 to 1.40; $P = .53$) (Figure E7).[9,11,12,15] There were also no significant differences in the use of a bridge to LTx by a ventilator/ECLS/ECMO (OR, 2.96; 95% CI, 0.74-11.81; $P = .12$) (Figure E8)[11-13,15] or the number of double LTx (OR, 1.03; 95% CI, 0.28-3.73; $P = .97$) (Figure E8).[11-15]

Postoperative Outcomes

The postoperative outcomes are summarized in Table 3, Figure E9, and Figures 2 through 4. Five of 8 studies reported the rate of postoperative ECMO/ECLS use.[7,9,11,12,15] Six of 8 studies listed data on the length of hospital stay,[7,8,11-14] and 72-hour PGD grade of 3.[7,9,11,12,14,15,28] Seven of 8 studies reported length of postoperative ventilation,[7-9,11-15] length of ICU stay,[7,8,11-15] 30-day survival,[7-12,14,15] and 1-year survival.[7,8,11-15]

Postoperative ECMO/ECLS Use and Length of Ventilation

The rate of postoperative ECMO/ECLS use in the EVLP group was 3.72 times higher (95% CI, 0.83-16.66; $P = .09$) than that in the non-EVLP group (Figure E9).[7,9,11,12,15] Additionally, the length of ventilation was 2.17 days longer (95% CI, −0.63 to 4.96; $P = .13$) than that in the non-EVLP group (Figure 2).[7,8,11-15] However, there were no significant differences between the 2 groups in postoperative ECMO/ECLS use or length of ventilation. The statistical heterogeneity of postoperative ECMO/ECLS use ($I^2 = 62\%$) and length of ventilation ($I^2 = 64\%$) was considered high.

ICU Stay and Hospital Stay

The lengths of the ICU stay and hospital stay in the EVLP versus the non-EVLP group were 5.5 to 25.6 days versus 5.9 to 18.9 days and 26.0 to 40.6 days versus 19.0 to 38.1 days, respectively. Although the ICU stay and hospital stay were 2.56 days longer (95% CI, −2.29 to 7.42; $P = .30$) (Figure 2) and 3.15 days longer (95% CI, −0.99 to 7.29; $P = .14$) (Figure 3) in the EVLP than non-EVLP group, respectively, there were no significant differences in either outcome between the EVLP and non-EVLP groups.[7,8,11-15] The statistical heterogeneity of the length of ICU stay across studies was considered high ($I^2 = 84\%$), but there was no statistical heterogeneity in the length of hospital stay ($I^2 = 0\%$).

72-hour PGD of Grade 3, 30-Day and 1-Year Survival Rates

The 72-hour PGD of grade 3 and the 30-day and 1-year survival rates are summarized in Table 3 and Figure 4. There was no significant difference in 72-hour PGD of grade 3 (OR, 0.79; 95% CI, 0.42-1.50; $P = .47$),[7,9,11,12,14,15] 30-day survival (OR, 0.77; 95% CI, 0.32-1.82; $P = .55$)[7-9,11,12,14,15] or 1-year survival (OR, 0.89; 95% CI, 0.57-1.40; $P = .62$)[7,8,11-15] between the EVLP and non-EVLP groups (Figure 4). There was no statistical heterogeneity in 72-hour PGD of grade 3 or the 30-day and 1-year survival rates ($I^2 = 0\%$).

Assessment of Publication Bias

Funnel plots of the studies were used in the meta-analysis reporting on 72-hour PGD of grade 3 (Figure E10), 30-day survival (Figure E11), and 1-year survival (Figure E12) after EVLP of marginal donor lungs compared with non-EVLP of standard LTx. There was no evidence of publication bias or heterogeneity in the funnel plots of 72-hour PGD of grade 3, 30-day survival, and 1-year survival among the included studies (all $I^2 = 0\%$).

DISCUSSION

This meta-analysis provides further confirmation that marginal donor lungs treated by EVLP have outcomes

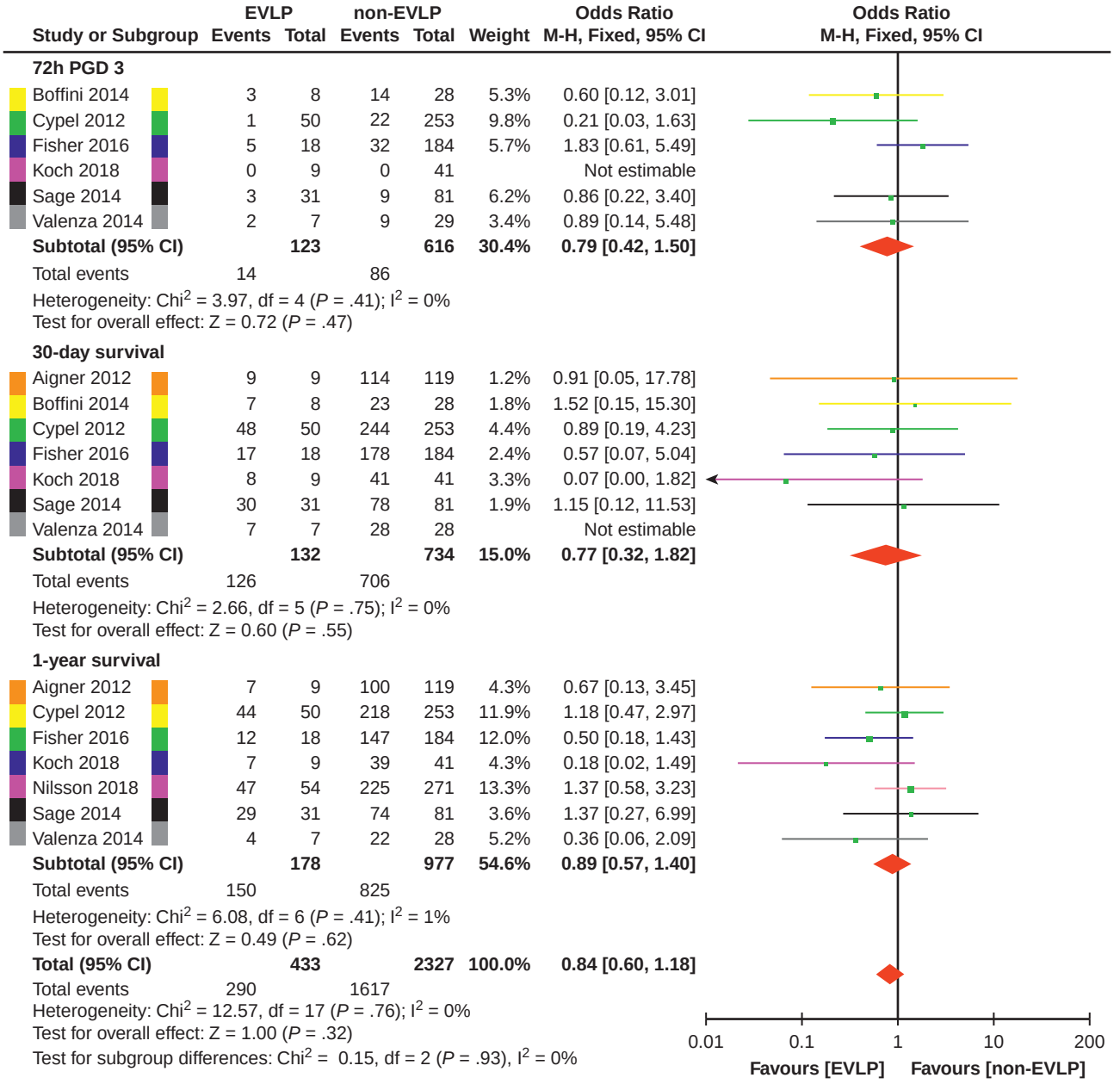


FIGURE 4. Forest plot of 72-hour primary graft dysfunction (*PGD*) of grade 3, 30-day survival and 1-year survival between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar 72-hour PGD of grade 3, 30-day survival and 1-year survival between the EVLP and non-EVLP groups was shown in the global analysis ($P > .05$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

comparable with those of standard donor LTx, which may improve donor lung utilization for successful transplantation.

Steen and colleagues[29,30] described the first clinical application in 2001 and updated the application of short-period EVLP of marginal donor lungs. In 2008, Cypel and colleagues[31] altered the EVLP system and technique, reporting that EVLP prevents ongoing injury associated with prolonged ischemia and accelerates lung recovery in pig donor lungs. Thereafter, this team reported the first prospective clinical trial using EVLP that demonstrated that extended acellular normothermic EVLP can yield early

outcomes similar to those of standard LTx without EVLP.[23] After that, the Toronto group evaluated 50 clinical cases and showed acceptable posttransplantation outcomes of marginal donor lungs treated by EVLP.[7,31] Other transplant centers throughout the world recently applied the Toronto strategy and achieved promising outcomes of LTx after EVLP-treated marginal donor lungs, which expanded the limited donor pool.[8,9,12,14] In 2012, Warnecke and colleagues[32] reported their first experience of EVLP with OCS in LTx, which was technically similar to previously described EVLP methods. Moreover, a case report has suggested OCS lungs are also capable of maintaining a safe and near-physiologic environment for more than 10 hours, even for marginal donor grafts.[33] The results of the Normothermic Ex Vivo Lung Perfusion as an Assessment of Extended/Marginal Donor Lungs trial, which is the first Food and Drug Administration-mandated multicenter, prospective clinical trial, recently showed that the early and midterm results were equivalent between patients who underwent EVLP-treated LTx and standard LTx without EVLP.[34] In the present meta-analysis, we did not include EVLP systems of OCS because no study of the OCS system met the study selection criteria. We did not distinguish the use of the Toronto and Lund systems, which shared the same purposes. Indeed, the characteristics of the donor lungs evaluated with these systems and the clinical outcomes were similar (Table E2).

The radiograph status and Pao_2/Fio_2 ratio are important criteria for evaluating the quality of donor lungs. An abnormal radiograph status or Pao_2/Fio_2 ratio often indicates low-quality donor lungs, especially a Pao_2/Fio_2 ratio <300 mm Hg. Zych and colleagues[35] reported that in LTx using donors with a Pao_2/Fio_2 ratio <300 mm Hg, PGD of grade 3 was observed significantly more frequently at 48 hours and 72 hours, contributing to early morbidity and mortality. In this meta-analysis, all studies that described a more frequent abnormal radiograph status or lower Pao_2/Fio_2 ratio in the EVLP than non-EVLP groups.

In this meta-analysis, treatment with ECMO after LTx in the EVLP group was performed in a similar proportion of patients undergoing EVLP versus non-EVLP strategies, which is consistent with previous studies.[7,9,15,28] As previous studies demonstrated,[7,8,28] the EVLP group tended to have a longer ventilation time than the non-EVLP group. However, this difference was not significant. Wallinder and colleagues[2] showed that the ventilation time was significantly longer in recipients in the EVLP group in their early report, but not in their recent study.[25] In this meta-analysis, the mechanical ventilation time was comparable between the EVLP group and conventional transplant group, which is consistent with previous studies.[7-9,11-15,25,28]

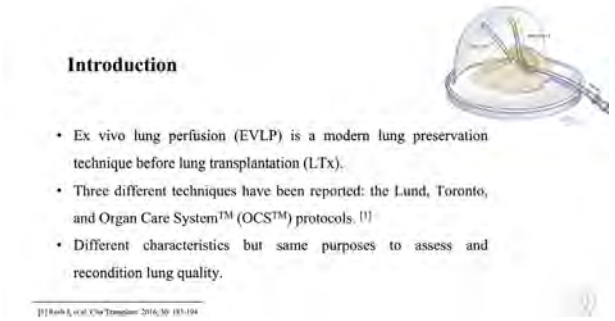
The length of the posttransplant ICU and hospital stay is critical for LTx recipients. Recipients may develop various complications such as severe catabolism, infection problems, and early graft rejection during their ICU and hospital stay. In the early outcomes reported by Wallinder and colleagues[2] the length of ICU stay was significantly longer in recipients of the EVLP group. However, this was not observed in their subsequent analysis.[25] In other previous studies, the lengths of the ICU and hospital stay were also similar between the EVLP and non-EVLP groups.[7,8,10-15,25,36] In the present meta-analysis, the length of ICU and hospital stay in the EVLP group were comparable with those in the non-EVLP group.

The incidence of PGD has also been compared between the EVLP group and non-EVLP group in previous studies.[7,9,11,12,14,15,23] However, none of the included studies described whether patients with posttransplant ECMO were automatically classified as having PGD of grade 3. The Toronto group performed a prospective trial of EVLP in clinical LTx and reported that the incidence of 72-hours PGD of grade 3 was only 15% compared with 30% in the standard LTx group ($P = .11$).[23] In the present meta-analysis, 6 of 8 studies provided data regarding 72-hour PGD of grade 3, and no significant differences were observed between the 2 groups.[7,9,11,12,14,15] Although EVLP lungs were more impaired than standard LTx lungs, the incidence of 72-hour PGD of grade 3 was similar in both groups.

Marginal donor lungs can be evaluated by EVLP, but whether they can be reconditioned for acceptable transplantation is unconfirmed. Although the Toronto group performed transplantations in sicker recipients, a low 30-day mortality rate of approximately 2% was shown in their previous studies, which is another advantage of EVLP.[7,23,28,31,36,37] Aigner and colleagues[8] showed that the 30-day survival rate in the EVLP group was 100% compared with 95.8% in the non-EVLP group ($P = .6$). In addition, the 1-year survival rate was >80% after LTx (available at http://www.ishlt.org) as reported during the past decade.[25] The previous studies demonstrated that the 1-year survival rate is almost equivalent in recipients of EVLP-treated marginal donor lungs versus standard LTx with EVLP.[7,14,25] In this meta-analysis, there was no significant difference in 30-day or 1-year survival between the EVLP and non-EVLP groups, which is consistent with previous studies.[7-15,24,25]

Study Limitations

Our study has some inevitable limitations. No studies included in this meta-analysis were randomized controlled trials. The potential problems and biases of the individual publications included in a meta-analysis may affect the pooled quality. Only 186 patients were pooled in the EVLP group; this is considered a relatively small number. Long-term survival and chronic lung allograft dysfunction were



VIDEO 1. Video abstract summarizing the systematic review and meta-analysis of outcomes of marginal donors for lung transplantation after ex vivo lung perfusion.Video available at: https://www.jtcvs.org/article/S0022-5223(19)31641-1/fulltext.

not assessed in this meta-analysis because of the short median follow-up duration in most of the publications. Institutional differences in EVLP protocols (Lund and Toronto) existed that may have resulted in different recipient outcomes. Additionally, only the number of patients with postoperative ECMO use and 72-hour PGD of grade 3 were reported in the included studies. No information was provided about whether patients with posttransplant ECMO were automatically classified as having PGD of grade 3 or ECMO use for cardiovascular support. In addition, the Donor Ex-Vivo Lung Perfusion in the United Kingdom lung transplantation study performed a hybrid system combining elements of both the Toronto and Lund protocols. Although the study was terminated because of slow recruitment and a concern about high levels of ECMO use, and although the sample size in the EVLP arm was too small to allow firm conclusions to be drawn, our inclusion criteria were met and our meta-analysis showed important data. We extrapolated some data from the survival curves or converted to the mean and standard deviation as in previous studies, which may have decreased accuracy and affected the final conclusion. Additionally, we selected only English-language publications and excluded unpublished and gray studies, which may have resulted in potential publication bias.

CONCLUSIONS

The outcomes after LTx of EVLP on marginal donor lungs are comparable with those of standard LTx without EVLP treatment. The present meta-analysis indicates that EVLP-treated LTx has posttransplant outcomes similar to those of standard LTx without EVLP, although the EVLP group had worse quality of donor lungs than the non-EVLP group (Video 1).

Conflict of Interest Statement

Authors have nothing to disclose with regard to commercial support.

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References

1. Cooper JD, Pearson FG, Patterson GA, Todd TR, Ginsberg RJ, Goldberg M, et al. Technique of successful lung transplantation in humans. *J Thorac Cardiovasc Surg*. 1987;93:173-81.
2. Wallinder A, Ricksten SE, Silverborn M, Hansson C, Riise GC, Liden H, et al. Early results in transplantation of initially rejected donor lungs after ex vivo lung perfusion: a case-control study. *Eur J Cardiothorac Surg*. 2014;45:40-4.
3. Klein AS, Messersmith EE, Ratner LE, Kochik R, Baliga PK, Ojo AO. Organ donation and utilization in the United States, 1999-2008. *Am J Transplant*. 2010;10:973-86.
4. Valapour M, Paulson K, Smith JM, Hertz MI, Skeans MA, Heubner BM, et al. OPTN/SRTR 2011 annual data report: lung. *Am J Transplant*. 2013;13:149-77.
5. Southard JH, Knes JM, Wang H, Hansen TN. Loss of NO-induced relaxation in abdominal aorta preserved in a CO-culture system. *Transplant Proc*. 2001;33:855-6.
6. Reeb J, Cypel M. Ex vivo lung perfusion. *Clin Transplant*. 2016;30:183-94.
7. Cypel M, Yeung JC, Machuca T, Chen M, Singer LG, Yasufuku K, et al. Experience with the first 50 ex vivo lung perfusions in clinical transplantation. *J Thorac Cardiovasc Surg*. 2012;144:1200-6.
8. Aigner C, Slama A, Hotzenecker K, Scheed A, Urbanek B, Schmid W, et al. Clinical ex vivo lung perfusion—pushing the limits. *Am J Transplant*. 2012;12:1839-47.
9. Boffini M, Ricci D, Bonato R, Fanelli V, Attisani M, Ribezzo M, et al. Incidence and severity of primary graft dysfunction after lung transplantation using rejected grafts reconditioned with ex vivo lung perfusion. *Eur J Cardiothorac Surg*. 2014;46:789-93.
10. Fildes JE, Archer LD, Blaikley J, Ball AL, Stone JP, Sjoberg T, et al. Clinical outcome of patients transplanted with marginal donor lungs via ex vivo lung perfusion compared to standard lung transplantation. *Transplantation*. 2015;99:1078-83.
11. Fisher A, Andreasson A, Chrysos A, Lally J, Mamasoula C, Exley C, et al. An observational study of donor ex vivo lung perfusion in UK lung transplantation: DEVELOP-UK. *Health Technol Assess*. 2016;20:1-276.
12. Koch A, Pizanis N, Olbertz C, Abou-Issa O, Taube C, Slama A, et al. One-year experience with ex vivo lung perfusion: preliminary results from a single center. *Int J Artif Organs*. 2018;41:460-6.
13. Nilsson T, Wallinder A, Henriksen I, Nilsson JC, Ricksten SE, Moller-Sorensen H, et al. Lung transplantation after ex vivo lung perfusion in two Scandinavian centres. *Eur J Cardiothorac Surg*. 2019;55:766-72.
14. Sage E, Mussot S, Trebbia G, Puyo P, Stern M, Dartevelle P, et al. Lung transplantation from initially rejected donors after ex vivo lung reconditioning: the French experience. *Eur J Cardiothorac Surg*. 2014;46:794-9.
15. Valenza F, Rosso L, Coppola S, Froio S, Palleschi A, Tosi D, et al. Ex vivo lung perfusion to improve donor lung function and increase the number of organs available for transplantation. *Transpl Int*. 2014;27:553-61.
16. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Reprint—preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Phys Ther*. 2009;89:873-80.
17. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*. 2007;8:16.
18. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res*. 2018;27:1785-805.
19. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. 2014;14:135.
20. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. 2010;25:603-5.
21. Sterne JA, Sutton AJ, Ioannidis JP, Terrin N, Jones DR, Lau J, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ*. 2011;343:d4002.
22. Bonilla L, Ben-Aharon I, Vidal L, Gafter-Gvili A, Leibovici L, Stemmer SM. Dose-dense chemotherapy in nonmetastatic breast cancer: a systematic review

and meta-analysis of randomized controlled trials. *J Natl Cancer Inst*. 2010;102:1845-54.
23. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med*. 2011;364:1431-40.
24. Tikkanen JM, Cypel M, Machuca TN, Azad S, Binnie M, Chow CW, et al. Functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation. *J Heart Lung Transplant*. 2015;34:547-56.
25. Wallinder A, Riise GC, Ricksten SE, Silverborn M, Dellgren G. Transplantation after ex vivo lung perfusion: a midterm follow-up. *J Heart Lung Transplant*. 2016;35:1303-10.
26. Slama A, Schillab L, Barta M, Benedek A, Mitterbauer A, Hoetzenecker K, et al. Standard donor lung procurement with normothermic ex vivo lung perfusion: a prospective randomized clinical trial. *J Heart Lung Transplant*. 2017;36:744-53.
27. Warnecke G, Van Raemdonck D, Smith MA, Massard G, Kukreja J, Rea F, et al. Normothermic ex-vivo preservation with the portable organ care system lung device for bilateral lung transplantation (INSPIRE): a randomised, open-label, non-inferiority, phase 3 study. *Lancet Respir Med*. 2018;6:357-67.
28. Cypel M, Keshavjee S. Extracorporeal membrane oxygenation as a bridge to lung transplantation. *ASAIO J*. 2012;58:441-2.
29. Steen S, Sjoberg T, Pierre L, Liao Q, Eriksson L, Algotsson L. Transplantation of lungs from a non-heart-beating donor. *Lancet*. 2001;357:825-9.
30. Ingemansson R, Eyjolfsson A, Mared L, Pierre L, Algotsson L, Ekmehag B, et al. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. *Ann Thorac Surg*. 2009;87:255-60.
31. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic ex vivo lung perfusion. *J Heart Lung Transplant*. 2008;27:1319-25.
32. Warnecke G, Moradiellos J, Tudorache I, Kuhn C, Avsar M, Wiegmann B, et al. Normothermic perfusion of donor lungs for preservation and assessment with the organ care system lung before bilateral transplantation: a pilot study of 12 patients. *Lancet*. 2012;380:1851-8.
33. Bozso S, Freed D, Nagendran J. Successful transplantation of extended criteria lungs after prolonged ex vivo lung perfusion performed on a portable device. *Transpl Int*. 2015;28:248-50.
34. Sanchez PG, Davis RD, D'Ovidio F, Cantu E, Weyant M, Camp P, et al. The NOVEL lung trial one-year outcomes. *J Heart Lung Transplant*. 2014;33:S71-2.
35. Zych B, Garcia Saez D, Sabashnikov A, De Robertis F, Amrani M, Bahrami T, et al. Lung transplantation from donors outside standard acceptability criteria—are they really marginal? *Transpl Int*. 2014;27:1183-91.
36. Cypel M, Rubacha M, Yeung J, Hirayama S, Torbicki K, Madonik M, et al. Normothermic ex vivo perfusion prevents lung injury compared to extended cold preservation for transplantation. *Am J Transplant*. 2009;9:2262-9.
37. Cypel M, Aigner C, Sage E, Machuca T, Slama A, Stern M, et al. Three center experience with clinical normothermic ex vivo lung perfusion. *J Heart Lung Transplant*. 2013;32:S16.

Key Words: marginal donor lung, lung transplantation, ex vivo lung perfusion, meta-analysis

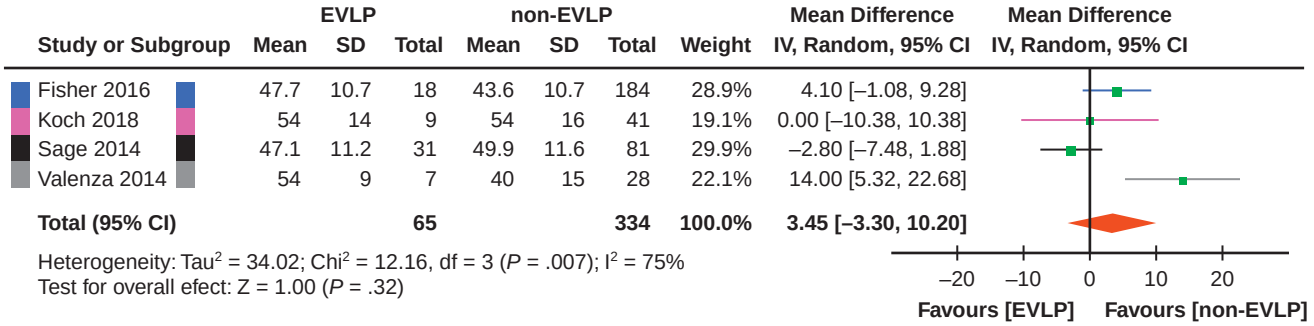

FIGURE E1. Forest plot of donor age between the ex vivo lung perfusion (*EVLP*) group and non-EVLP group. A similar donor age between the EVLP and non-EVLP groups was shown in the global analysis ($P = .32$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

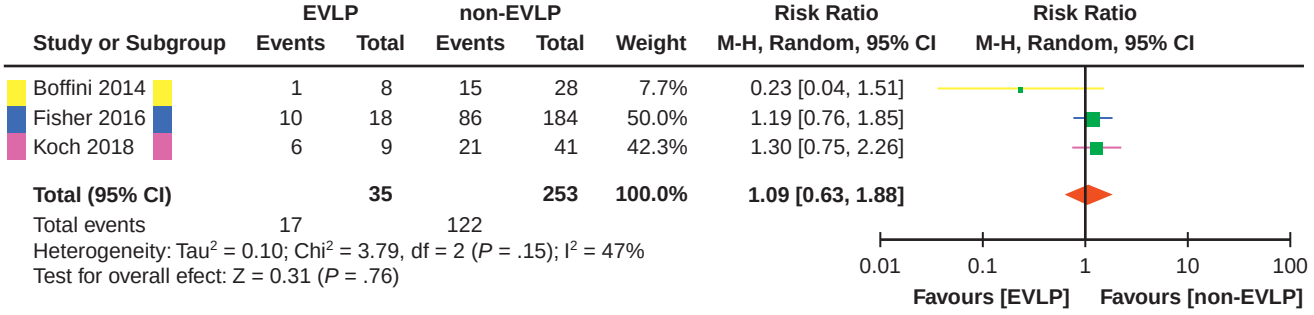

FIGURE E2. Forest plot of donor sex between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar donor sex between the EVLP and non-EVLP groups was shown in the global analysis ($P = .76$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

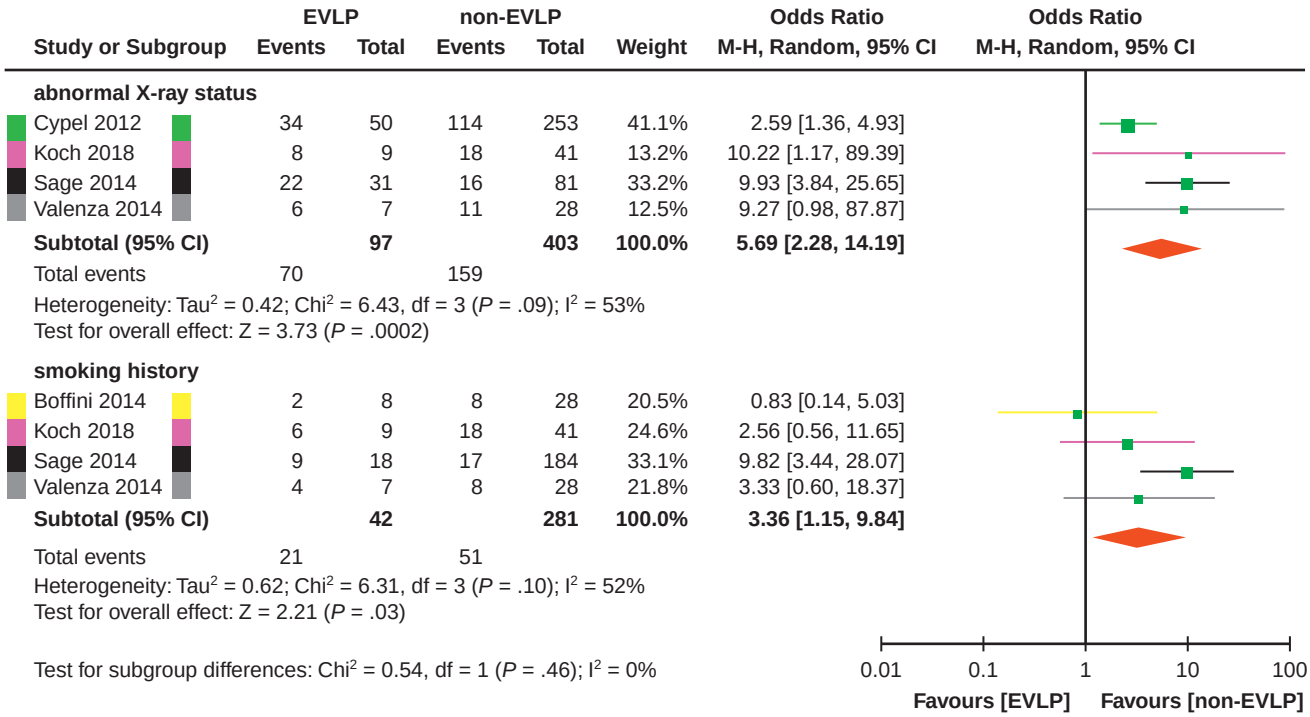

FIGURE E3. Forest plots of donor abnormal radiograph status and smoking history between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. In the global analysis, abnormal radiograph findings and a smoking history were more frequently found in the EVLP group ($P = .0002$ and .03, respectively). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

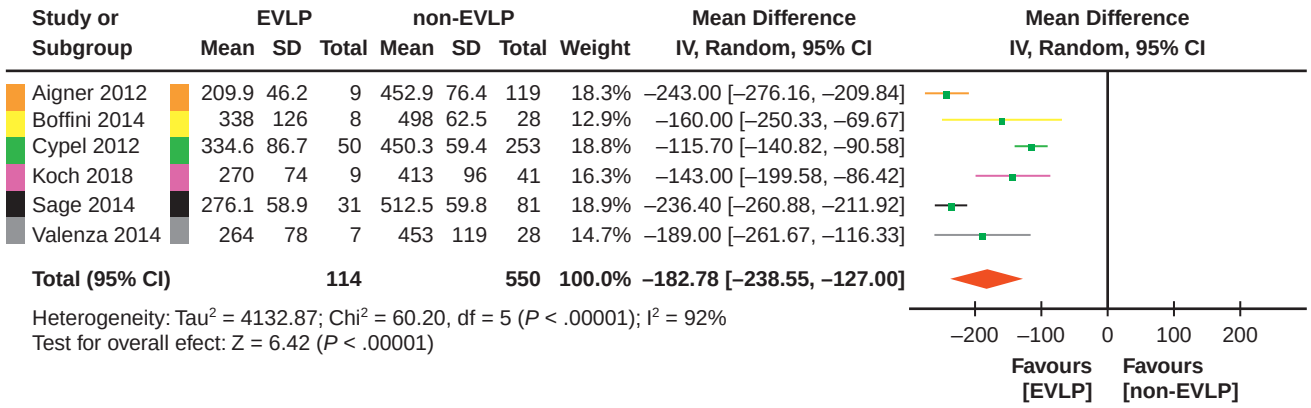

FIGURE E4. Forest plot of donor arterial oxygen tension/inspired oxygen fraction (Pao_2/FIo_2) ratio between the ex vivo lung perfusion (*EVLP*) group and non-EVLP group. In the global analysis, a worse Pao_2/FIo_2 ratio was shown in the EVLP group ($P < .00001$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

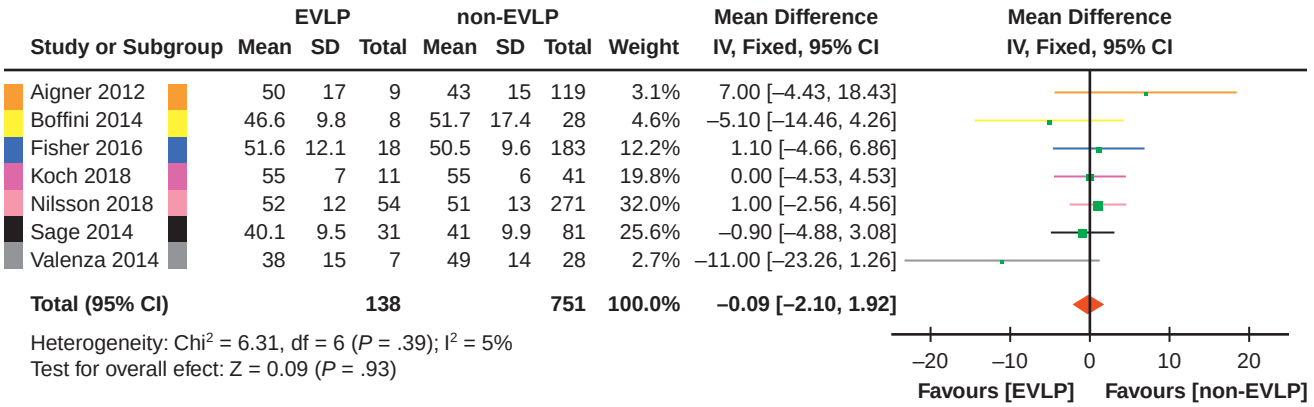

FIGURE E5. Forest plot of recipient age between the ex vivo lung perfusion (*EVLP*) group and non-EVLP group. A similar recipient age between the EVLP and non-EVLP groups was shown in the global analysis ($P = .93$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

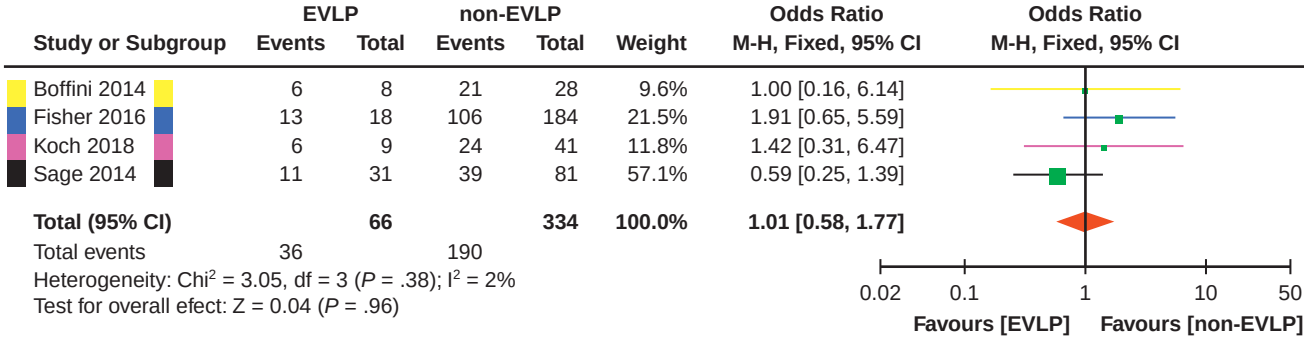

FIGURE E6. Forest plot of recipient sex between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar recipient sex between the EVLP and non-EVLP groups was shown in the global analysis ($P = .96$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

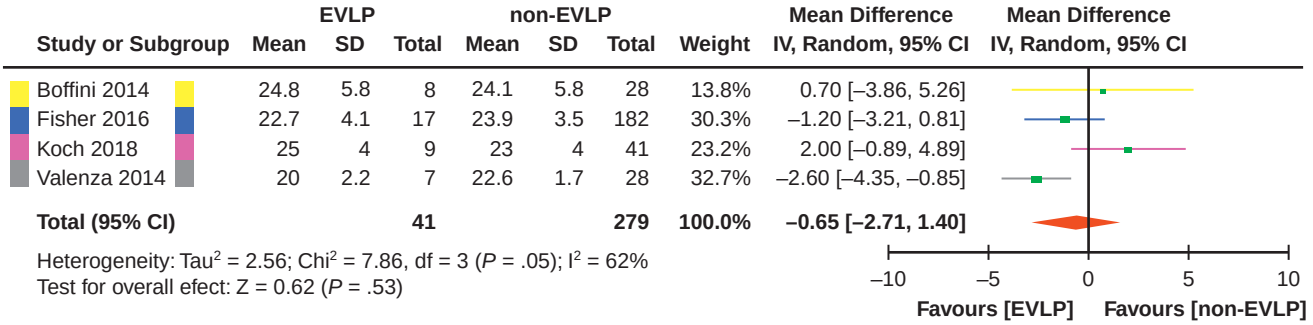

FIGURE E7. Forest plot of recipient body mass index (BMI) between EVLP group and non-EVLP group. A similar recipient BMI between the ex vivo lung perfusion (EVLP) and non-EVLP groups was shown in the global analysis ($P = .53$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (CI). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *SD*, Standard deviation; *IV*, inverse-variance.

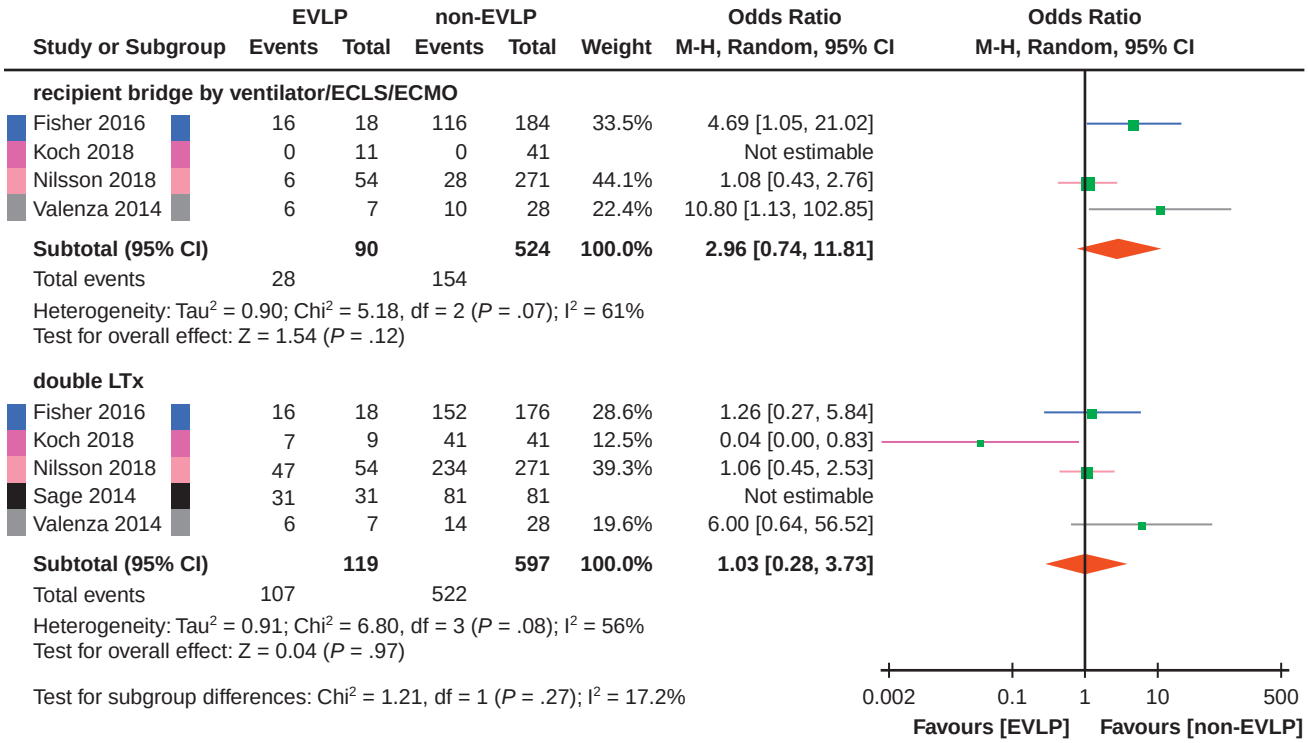

FIGURE E8. Forests plot of recipient bridge by ventilator/extracorporeal membrane oxygenation/extracorporeal life support (*ECMO/ECLS*) and double lung transplantation (*LTx*) between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar frequencies of recipient bridge by ventilator/ECLS/ECMO and double LTx between the EVLP and non-EVLP groups were shown in the global analysis ($P = .12$ and .97, respectively). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

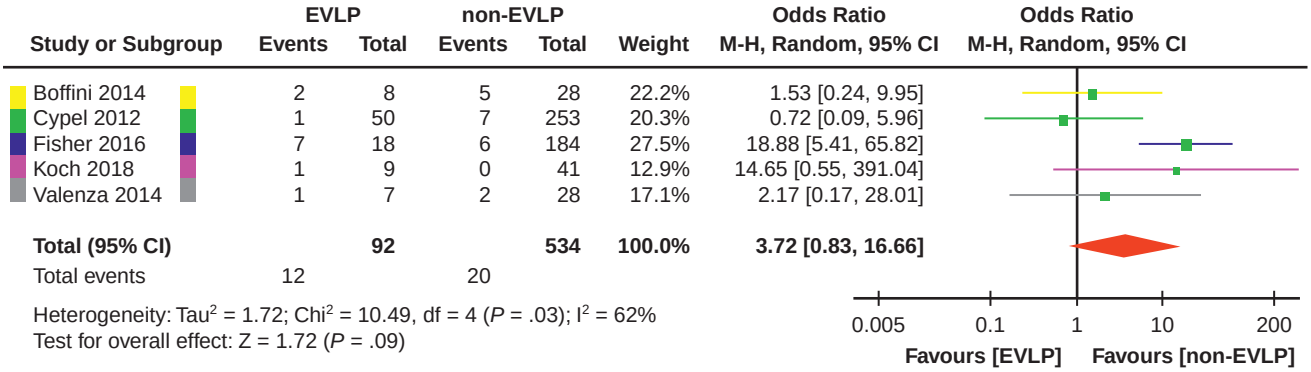

FIGURE E9. Forest plot of postoperative extracorporeal membrane oxygenation/extracorporeal life support (*ECMO/ECLS*) use between ex vivo lung perfusion (*EVLP*) group and non-EVLP group. Similar postoperative ECMO/ECLS use between the EVLP and non-EVLP groups was shown in the global analysis ($P = .09$). The *solid squares* indicate the mean difference and are proportional to the weights used in the meta-analysis. The *solid vertical line* indicates no effect. The *horizontal lines* represent the 95% confidence interval (*CI*). The *diamond* indicates the weighted mean difference, and the *lateral tips of the diamond* indicate the associated CI. *M-H*, Mantel-Haenszel.

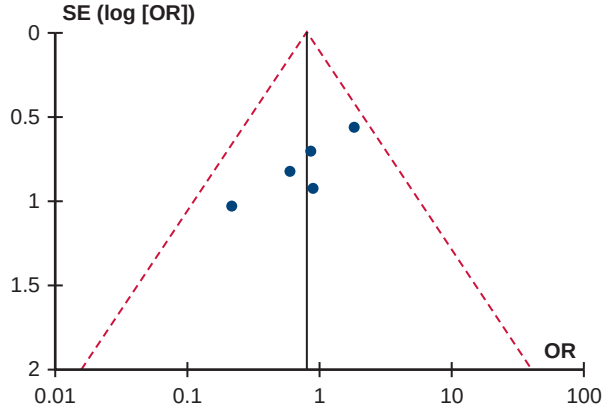

FIGURE E10. Funnel plot for the visual detection of systematic publication bias in 72-hour primary graft dysfunction (PGD) of grade 3. The studies between the *red lines* contained no publication bias. *SE*, Standard error; *OR*, odds ratio.

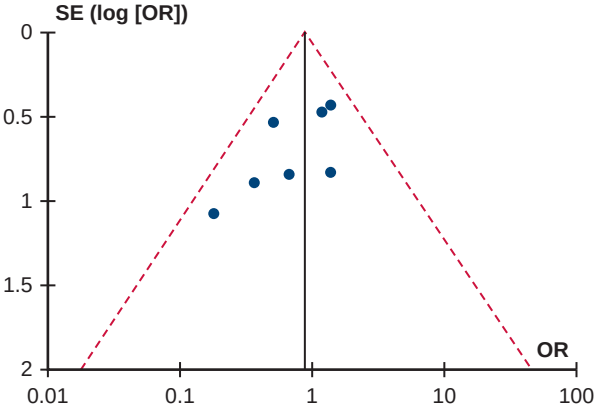

FIGURE E12. Funnel plot for the visual detection of systematic publication bias in 1-year survival. The studies between the *red lines* contained no publication bias. *SE*, Standard error; *OR*, odds ratio.

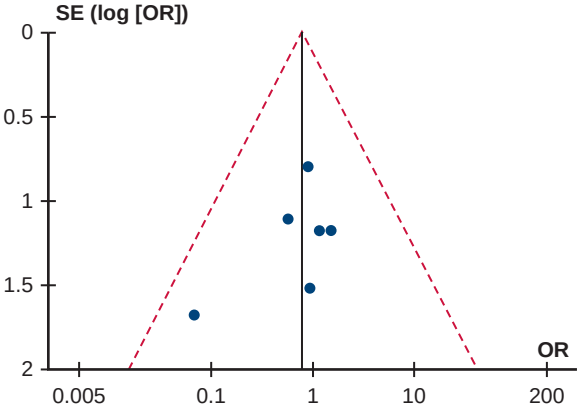

FIGURE E11. Funnel plot for the visual detection of systematic publication bias in 30-day survival. The studies between the *red lines* contained no publication bias. *SE*, Standard error; *OR*, odds ratio.

TABLE E1. Quality assessment of individual studies using the Newcastle-Ottawa Quality Assessment Scale score for cohort studies[18,22,23]

Study	Selection*				Comparability†		Outcome‡			Score
	1	2	3	4	1	2	1	2	3	
Aigner et al,[9] 2012	×	×	×	/	×	/	×	/	×	6
Boffini et al,[10] 2014	×	×	/	×	×	/	×	×	×	7
Cypel et al,[11] 2012	×	×	/	×	×	/	×	×	×	7
Fisher et al,[13] 2016	×	×	×	/	×	/	×	/	×	6
Koch et al,[14] 2018	×	×	×	/	×	/	×	×	×	7
Sage et al,[9] 2014	×	×	×	/	×	/	×	/	×	6
Valenza et al,[17] 2014	×	×	×	/	×	/	×	/	×	6
Nilsson et al,[15] 2018	×	×	×	/	×	/	×	×	×	7

×, Eligibility in NOS; /, dissatisfaction in NOS. *Contains: 1 = Is the case definition adequate? 2 = Representativeness of the cases; 3 = Selection of controls; and 4 = Definition of controls. †Contains: Comparability of cases and controls on the basis of the design or analysis. ‡Contains: 1 = Assessment of outcome; 2 = Was follow-up long enough for outcomes to occur? and 3 = Adequacy of follow-up of cohorts.

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TABLE E2. Summary of clinicopathologic outcomes in different ex vivo lung perfusion systems (Lund system vs Toronto system)

Outcomes	Total cases (Lund/Toronto)	Lund system	Toronto system	*P* value
Utilization rate	69/125			.583*
Yes		61 (88.4)	107 (85.6)	
No		8 (11.6)	18 (14.4)	
Abnormal radiograph of donor	7/90			.669†
Yes		6 (85.7)	64 (71.1)	
No		1 (14.3)	26 (28.9)	
Donor smoking	7/48			.408†
Yes		4 (57.1)	17 (35.4)	
No		3 (42.9)	31 (64.6)	
Postoperative ECMO	7/67			.400†
Yes		1 (14.3)	4 (6.0)	
No		6 (85.7)	63 (94.0)	
30-d survival	7/107			1.000†
Alive		7 (100)	103 (96.3)	
Dead		0 (0)	4 (3.7)	
1-y survival	61/99			.434†
Alive		56 (91.8)	87 (87.9)	
Dead		5 (8.2)	12 (12.1)	
72-h PGD 3	7/98			.110†
Yes		2 (28.6)	7 (7.1)	
No		5 (71.4)	91 (92.9)	

The data were extracted from the studies included in the present meta-analysis. Values are presented as n (%). *ECMO*, Extracorporeal membrane oxygenation; *PGD*, primary graft dysfunction. *χ^2 test. †Fisher exact test.

000 Outcomes of marginal donors for lung transplantation after ex vivo lung perfusion: A systematic review and meta-analysis

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The outcomes after lung transplantation of ex vivo lung perfusion on marginal donor lungs are comparable with those of standard lung transplantation without ex vivo lung perfusion treatment.

Differences Between Patients With Idiopathic Pleuroparenchymal Fibroelastosis and Those With Other Types of Idiopathic Interstitial Pneumonia in Candidates for Lung Transplants

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ABSTRACT

Introduction. The prognostic implications of having patients with idiopathic pleuroparenchymal fibroelastosis (IPPFE) on lung transplantation waiting lists have been unclear. In Japan, where a severe shortage of brain-dead donors remains a major limitation for organ transplantation, it is particularly important to predict the prognoses of patients when they are listed for transplantation. The purpose of this study was to investigate the characteristics of lung transplantation candidates with IPPFE and the influence of those characteristics on prognosis.

Methods. This was a retrospective review of 29 consecutive adult lung transplant candidates with idiopathic interstitial pneumonia between January 2014 and April 2018.

Results. Eight patients with IPPFE and 21 with other types of idiopathic interstitial pneumonia were included. Body mass index (median 17.1 kg/m^2 vs 23.5 kg/m^2, $P < .01$) and ratio of anteroposterior to transverse diameter of the thoracic cage were significantly lower (0.530 vs 0.583, $P = .02$) in the IPPFE group. Patients with a body mass index <20.0 kg/m^2 ($P = .02$), 6-minute walk distance <250.0 m ($P < .01$), ratio of PaO_2 to fraction of inspiratory oxygen <300.0 mm Hg ($P < .01$), and an inability to perform the diffusing capacity of carbon monoxide test ($P < .01$) had significantly shorter survival times in the other idiopathic interstitial pneumonia, but not in the IPPFE, group. Some patients with IPPFE survived for long enough to undergo transplantation.

Conclusions. Patients with IPPFE waiting for transplantation have some distinctive characteristics and should be retained on waiting lists to receive transplants.

In Japan, where a severe shortage of brain-dead donors is a major ongoing problem, over 100 people are added to the waiting list annually, whereas only around 50 to 60 brain-dead-donor lung transplants are performed annually [1,2]. Therefore, an upper age limit for transplant patients has been set in Japan. At the time of registration for transplant, candidates must be aged <55 years for bilateral lung transplantation and <60 years for single lung transplantation. Idiopathic interstitial pneumonia (IIP) is one of the major indications for transplantation in Japan, with approximately 20% of brain-dead-donor transplantations having been performed on patients with IIPs, whereas approximately 22% of patients listed for lung transplantation have IIPs [2,3]. In Japan, lungs from brain-dead donors are allocated primarily on the basis of accrued time on the waiting list; as of the end of 2018, patients with rapidly progressive disease are not prioritized.

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Idiopathic pleuroparenchymal fibroelastosis (IPPFE), a rare subtype of IIP, is characterized by fibrosis involving the pleura and subpleural lung parenchyma, predominantly in the upper lobes [4]. In general, patients with IIPs, especially those with idiopathic pulmonary fibrosis (IPF), have poor prognoses; those with IPPFE are thought to have even poorer prognoses [5]. Lung transplantation has been considered a therapeutic option for patients with advanced IPPFE; however, no official data are currently available regarding the number and detailed outcomes of lung transplantations in patients with IPPFE in Japan, and little is known regarding the prognostic factors and outcomes of those after listing for lung transplantation. This is partly because IPPFE was not distinguished correctly from other IIPs (oIIPs) until it was defined as a specific clinicopathologic entity in the updated 2013 classification of IIPs [4].

The clinical features of IPPFE reportedly differ from those of oIIPs; patients with IPPFE are often slender even before disease onset, experience weight loss, and develop a chest wall deformity known as"flat chest" [6]. Furthermore, patients with IPPFE reportedly tend to have the following clinicopathologic characteristics: a disproportionate reduction in forced vital capacity compared with diffusing capacity of carbon monoxide (DL_{CO}) together with relative preservation of the partial pressure of oxygen (PaO_2) in arterial blood accompanied by mild increases in partial pressure of carbon dioxide [6]. However, the influences of these characteristics on patients' prognoses while awaiting lung transplantation have not been investigated.

We hypothesized that patients with IPPFE have distinct characteristics and prognostic factors compared with those with oIIPs. The purpose of this study was to investigate the characteristics of IPPFE and their influence on the prognosis of lung transplantation candidates and compare them to those of patients with oIIPs.

MATERIALS AND METHODS

Patients

This was a retrospective review of 29 consecutive adult lung transplant candidates with IIP who were referred to the University of Tokyo Hospital and registered on the Japan Organ Transplant Network between January 2014 and April 2018. These candidates were selected on the basis of international guidelines [7]. This study was approved by the ethics committee of the University of Tokyo (2406-[5]).

Data Collection

Clinical data at the time of registration were collected from medical records and the lung transplantation registration database in our institute. The PaO_2 and partial pressure of carbon dioxide in arterial blood at rest were measured with supplemental oxygen via a nasal cannula if the patient had been receiving domiciliary oxygen therapy and the ratio of PaO_2 to fraction of inspiratory oxygen (P-to-F ratio) was used to evaluate oxygenation. The fraction of inspiratory oxygen was defined as 0.21 in room air, 0.24 with a 1.0 L/min nasal cannula, and an additional 0.04 per liter per minute. Mortality in patients with interstitial pneumonia (GAP index) was predicted using the method reported by Ley et al [8] and Ryerson et al [9]. Pulmonary artery pressure was measured by right heart catheterization. The ratio of the anteroposterior diameter of the thoracic cage (APDT) to its transverse diameter (TDT) was calculated to assess the flatness of the chest wall, measurements being taken at the level of the sixth ribs in the horizontal section of a computed tomography image as recommended by Harada et al [10]. The average of right and left APDT-to-TDT ratios was used in the analysis. The observation period was calculated from the day of the starting evaluation to list the patient to the date of last contact, transplantation, or death.

Diagnosis of IPPFE and IIPs

Diagnoses were obtained from the Japan Organ Transplant Network registry and made on the basis of previously reported guidelines or criteria. IPPFE was diagnosed by high-resolution computed tomography using previously reported criteria [11]. Patients with pleural thickening associated with subpleural fibrosis concentrated in the upper lobes with less marked lower lobe involvement were classified as having definite pleuroparenchymal fibroelastosis (PPFE), whereas those in whom these changes were not concentrated in the upper lobes (consistent with PPFE) were also classified into the IPPFE group. Patients with secondary PPFE, such as those with upper lobe fibrosis and a history of bone marrow or lung transplantation were excluded. IPF was diagnosed by high-resolution computed tomography or lung biopsy according to published guidelines [4,12,13], and oIIPs were diagnosed as previously described [4,13–15].

Statistical Analysis

Statistical analysis was performed using JMP software (version 13; SAS Institute, Cary, NC, United States). Data are presented as median, proportion, or range, unless otherwise stated. The Mann-Whitney U test was used for comparisons between 2 groups. Frequencies were compared using the Fischer exact test for categorical variables. Kaplan-Meier survival estimates were used to assess duration of survival and the log-rank test to compare survival rates between subgroups. Patients who had undergone lung transplantation or were alive awaiting transplantation at the end of the data collection period were treated as censored. All analyses were 2-tailed, and $P < .05$ was considered to denote statistical significance.

RESULTS

Characteristics and Clinical Data

Eight of the 29 lung transplant candidates with IIP were diagnosed as having IPPFE and 21 as having oIIPs, namely IPF (13), idiopathic non-specific interstitial pneumonia (5), and unclassifiable IIP (3). At the end of the data collection period, 5 patients (17%) had undergone lung transplantation, 14 (48%) had died before transplantation, and 10 (34%) were alive and waiting for a transplant.

Characteristics and clinical data according to diagnostic group at registration are shown in Table 1. Patients with IPPFE were more likely to be women (62% vs 10%, $P < .01$) and to have a significantly lower body mass index (BMI) than those in the oIIPs group (median 17.1 kg/m^2 vs 23.5 kg/m^2, $P < .01$). The P-to-F ratio did not significantly differ between the 2 groups (median 316 vs 347, $P = .64$).

Table 1. Characteristics and Clinical Data of Study Patients on Registration

Variables	All (n = 29)	IPPFE (n = 8)	oIIPs (n = 21)	*P* Value
Age, median (range), y	51 (26-65)	52.5 (40-59)	51 (26-65)	.51*
Male, No. (%)	22 (76)	3 (38)	19 (90)	<.01†
Ever smoker, No. (%)	17 (59)	4 (50)	13 (62)	.68†
BMI, median (range), kg/m^2	21.4 (14.0-34.4)	17.1 (14.0-20.3)	23.5 (16.1-34.4)	<.01*
≥20 kg/m^2, No. (%)	18 (62)	2 (25)	16 (76)	.03†
Long-term steroid usage, No. (%)	15 (52)	2 (25)	13 (62)	.11†
History of pneumothorax, No. (%)	12 (41)	5 (63)	7 (33)	.22†
Chronic pneumothorax, No. (%)	3 (10)	2 (25)	1 (5)	.18†
Domiciliary oxygen therapy, No. (%)	22 (76)	5 (63)	17 (81)	.36†
History of acute exacerbation, No. (%)	7 (24)	0 (0)	7 (33)	.14†
P-to-F ratio, median (range)	330 (165-607)	316 (244-500)	347 (165-607)	.64*
$PaCO_2$, median (range), mm Hg	40.7 (30.9-69.9)	44.7 (32.4-68.4)	40.0 (30.9-69.9)	.32*
%FVC, median (range), %	46.7 (17.1-103)	43.6 (17.1-68.3)	48.7 (30.4-103)	.17*
Could not perform, No. (%)	2 (7)	2 (25)	0 (0)	
%DLco, median (range), %	46.1 (25.1-83.7)	53.2 (45.3-83.7)	42.9 (25.1-60.1)	.05*
Could not perform, No. (%)	8 (28)	4 (50)	4 (19)	
GAP score, median (range)	4 (1-6)	3.5 (2-5)	4 (1-6)	.88*
6MWD, median (range), m	335 (50-555)	285 (60-555)	380 (50-535)	.13*
≥250 m, No. (%)	24 (83)	5 (63)	19 (90)	.11†
PAP, mean (range), mm Hg	17 (10-34)	16 (10-34)	17 (11-32)	.22*
≥25 mm Hg, No. (%)	3 (10)	1 (13)	2 (10)	1†
APDT-to-TDT ratio, median (range)	0.578 (0.484-0.716)	0.530 (0.484-0.593)	0.583 (0.500-0.716)	.02*
KL-6, median (range), U/mL	1021 (200-6703)	730 (200-2173)	1150 (254-6703)	.25*
SP-D, median (range), ng/mL	237.5 (119-851)	237.5 (133-851)	240.5 (119-589)	.78*
Observation period, median (range), d	403 (66-1050)	353 (66-681)	416 (86-1050)	.51*
Outcomes, No. (%)				
Death	14 (48)	4 (50)	10 (48)	
Transplantation	5 (17)	2 (25)	3 (14)	
Awaiting	10 (34)	2 (25)	8 (38)	

Abbreviations: 6MWD, 6-min walking distance; $\%DL_{CO}$, percent predicted diffusing capacity of the lung for carbon monoxide; %FVC, percent predicted forced vital capacity; APDT-to-TDT ratio, ratio of anteroposterior and transverse diameters of the thoracic cage; BMI, body mass index; IPPFE, idiopathic pleuroparenchimal fibroelastosis; KL-6, Krebs von den Lungen-6; oIIPs, other idiopathic interstitial pneumonias; P-to-F ratio, ratio of partial pressure of oxygen and fraction of inspiratory oxygen; $PaCO_2$, partial pressure of carbon dioxide; PAP, pulmonary artery pressure; SP-D, surfactant protein D.

*Continuous variables were compared using the Mann–Whitney U-test.

†Frequencies were compared using the Fischer's exact test for categorical variables.

Percent predicted DL_{CO} ($\%DL_{CO}$) was better preserved in the IPPFE than the oIIP group (53.2% vs 42.9%, $P = .05$); however, 4 patients in the IPPFE group (50%) were unable to perform this test because of their low vital capacity. The APDT-to-TDT ratio was significantly lower in the IPPFE group (0.530 vs 0.583, $P = .02$). Seven patients (33%) in the oIIP group had histories of acute exacerbations vs no patients in the IPPFE group ($P = .14$; nonsignificant).

Survival Analysis

We performed a survival analysis of the 8 candidates with IPPFE and 21 with oIIPs. Median duration was 353 days for the IPPFE group and 416 days for the oIIP group. Kaplan-Meier survival curves showed that the mortality rate did not differ significantly between the 2 groups (Fig 1). When patients were classified on the basis of the APDT-to-TDT ratio (cutoff 0.58), the APDT-to-TDT ratio was not significantly associated with survival in either diagnostic group (Fig 2). History of pneumothorax was not significantly associated with survival in either diagnostic group.

Kaplan-Meier survival curves showed that patients in the oIIP group with BMIs <20.0 kg/m^2, 6-minute walking distance (6MWD) <250.0 m, P-to-F ratio <300.0 mm Hg, and those who were unable to perform the DL_{CO} test had significantly shorter survival times. In contrast, these 4 characteristics were found to have no significant influence on mortality rate in the IPPFE group (Fig 3). Only 1 (20%) of the 5 patients with oIIPs and a BMI <20.0 kg/m^2 survived longer than 1 year, and all 5 patients died before transplantation. However, 3 of the 6 patients (50%) with IPPFE and a BMI <20.0 kg/m^2 survived more than 1 year, and 2 of the 6 patients (33%) underwent transplantation. Likewise, all patients with 6MWD <250.0 m in the oIIPs group died within a year, whereas 2 of the 3 patients (67%) in the IPPFE group lived longer, and 1 (33%) eventually underwent transplantation. Only 1 of the 7 patients with a P-to-F ratio <300.0 mm Hg in the oIIP group underwent transplantation, and 5 of the 7 (71%) died within 1 year; however, in the IPPFE group, 1 of 2 patients (50%) lived more than 1 year and was still alive at the end of the observation period. All but 1 of the patients in the oIIP group who were unable to perform the DL_{CO} test died within a year; the

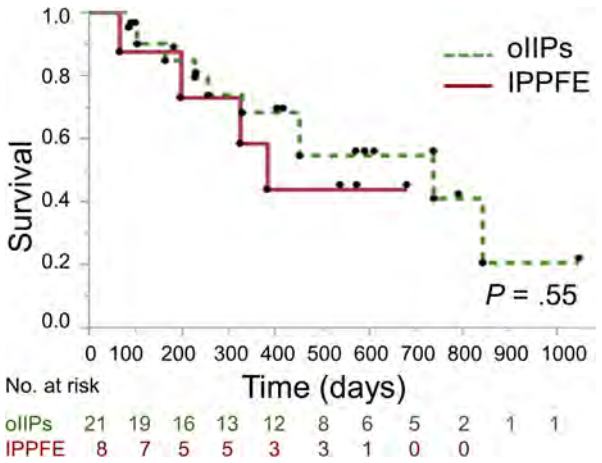

Fig 1. Kaplan–Meier survival curves for candidates with idiopathic interstitial pneumonia grouped by diagnosis: idiopathic pleuroparenchymal fibroelastosis (IPPFE, n = 8) and other idiopathic interstitial pneumonias (oIIPs, n = 21). The dots indicate censoring or death. $P = .55$ by log-rank test. IPPFE, idiopathic pleuroparenchymal fibroelastosis; oIIPs = other idiopathic interstitial pneumonias.

surviving 1 was censored on day 91. In contrast, in the IPPFE group, 1 patient (25%) who was unable to perform the DL_{CO} test was still awaiting lung transplantation on day 681 (Fig 3).

DISCUSSION

IPPFE has been thought to be rare. However, since the classification of IIPs was updated in 2013 [4], awareness of this condition has increased, and it was recently reported that it is not a rare form of IIP [16]. Indeed, our cohort of 29 patients with IIP included 8 with IPPFE (29%). Particularly in Japan, where the average waiting time for transplantation is still more than 800 days and the waiting list mortality rate is nearly 50% [1], it is increasingly important to predict the prognosis of patients with IPPFE on the waiting list and distinguish it from that of patients with oIIPs to enable optimal allocation of limited donor lungs.

In this study, patients with IPPFE showed clinical characteristics consistent with those reported previously [6,17], including lower BMI, higher incidence of flat chest, and better preserved $\%DL_{CO}$ than in patients with oIIPs. Importantly, the factors of a low BMI, short 6MWD, low P-to-F ratio, and an inability to perform the DL_{CO} test were not associated with poorer survival in the IPPFE group; however, they were in the oIIP group. In addition, some patients with IPPFE and these unfavorable factors survived longer than expected and eventually underwent successful lung transplantation, whereas almost all patients with oIIPs and these factors died while awaiting transplantation. These results suggest that the characteristics and prognostic factors of lung transplant candidates with IPPFE are distinct from those of candidates with oIIPs.

In this study, patients with IPPFE had significantly lower APDT-to-TDT ratios than those with oIIPs; a low APDT-to-TDT ratio is reportedly associated with poorer post-transplant pulmonary function [18]. However, the pretransplantation influence of this ratio has not previously been determined. In this study, the APDT-to-TDT ratio was not associated with mortality in candidates with lung transplantation. Thus, patients with a lower APDT-to-TDT ratio, which is generally considered to denote a more advanced stage of IPPFE, do not necessarily have a poorer prognosis and have the potential to undergo

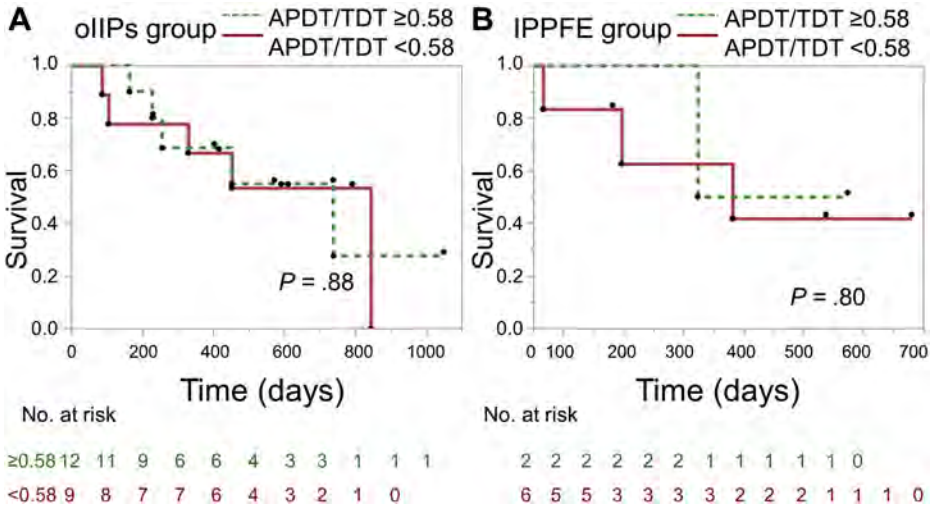

Fig 2. Kaplan-Meier survival curves grouped by APDT-to-TDT ratio. **(A)** Transplant candidates with oIIPs. **(B)** Transplant candidates with IPPFE. The dots indicate censoring or death. $P = .88$, $P = .80$ by log-rank test. APDT, anteroposterior diameter of the thoracic cage; IPPFE, idiopathic pleuroparenchymal fibroelastosis; oIIPs, other idiopathic interstitial pneumonias; TDT, transverse diameter of the thoracic cage.

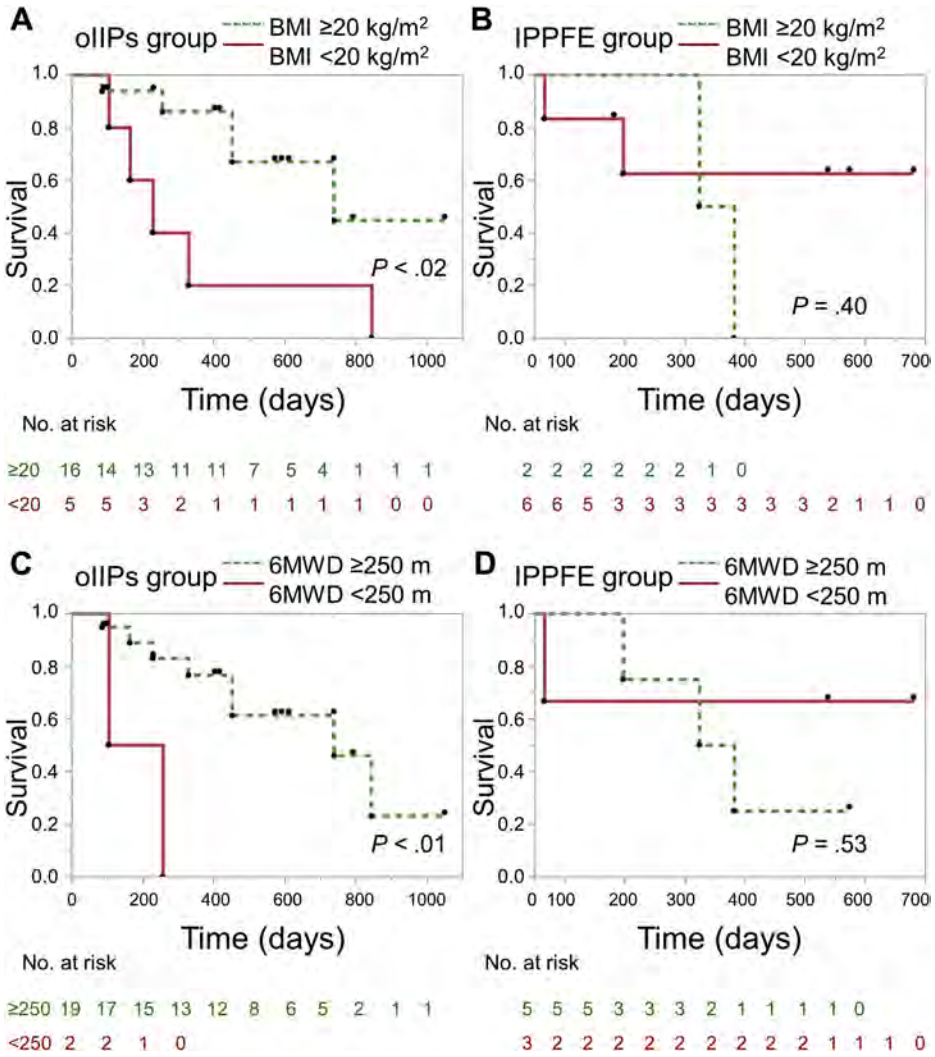

Fig 3. Kaplan–Meier survival curves according to various clinical variables. **(A)** Transplant candidates with oIIPs grouped by BMI. **(B)** Transplant candidates with IPPFE grouped by BMI. **(C)** Transplant candidates with oIIPs grouped by 6MWD. **(D)** Candidates with IPPFE grouped by 6MWD. **(E)** Transplant candidates with oIIPs grouped by P-to-F ratio. **(F)** Candidates with IPPFE grouped by P-to-F ratio. **(G)** Transplant candidates with oIIPs grouped by their ability to perform the DL_{CO} test. **(H)** Transplant candidates with IPPFE grouped by their ability to perform the DL_{CO} test. The dots indicate censoring or death. Survival curves were compared using log-rank tests. 6MWD, 6-min walking distance; DL_{CO}, diffusing capacity of the lung for carbon monoxide; BMI, body mass index; IPPFE, idiopathic pleuroparenchymal fibroelastosis; oIIPs, other idiopathic interstitial pneumonias; P-to-F ratio, the ratio of partial pressure of oxygen and fraction of inspiratory oxygen.

transplantation. Of note in this regard, patients with IPPFE intrinsically have a low APDT-to-TDT ratio (flat chest). Like other variables, including forced vital capacity and DL_{CO} [19], change in APDT-to-TDT ratio over time may be more important in predicting prognosis; further study is necessary.

Established prognostic factors for patients with IPF include BMI [20,21], 6MWD [21,22], PaO_2 [23], and DL_{CO} [21,24]; our findings are consistent with these. However, few studies on patients with IPPFE have been published. Hayashi et al [5] reported an association between BMI and outcome in patients with IPPFE. However, their study included mainly older patients (median age, 68.5 years) and was not limited to candidates for lung transplantation. To the best of our knowledge, no studies have yet reported the influence of 6MWD on survival in patients with IPPFE. PaO_2 in arterial blood is reportedly a prognostic factor of patients with IPPFE [4]; however, that study included patients presenting with IPPFE and was not limited to candidates for lung transplantation.

In our study, a low BMI, short 6MWD, low P-to-F ratio, and an inability to perform the DL_{CO} test were significantly associated with mortality in transplant candidates with oIIPs but not in those with IPPFE. Furthermore, our findings

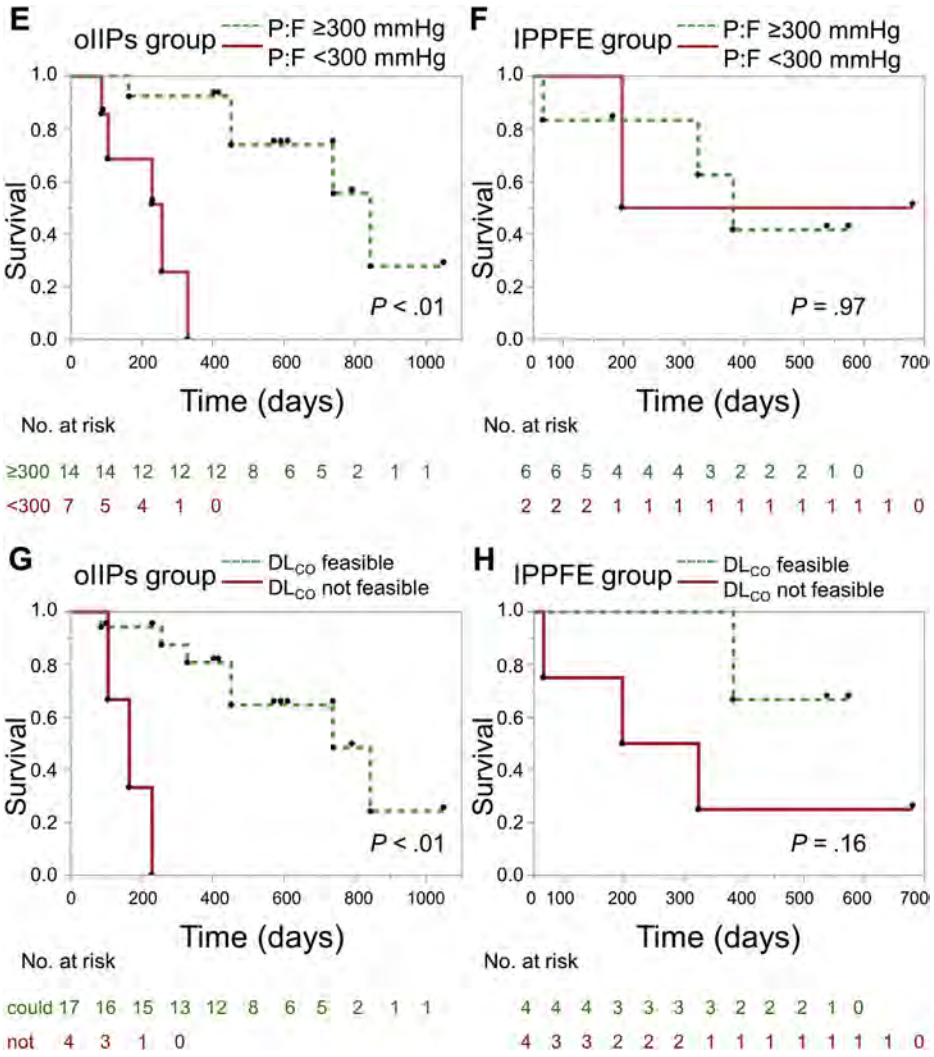

Fig 3. (*continued*).

suggest that a high proportion of patients with IPPFE and these characteristics survive for long enough to undergo transplantation. These differences between IPPFE and oIIPs may be partly attributable to specific characteristics of these diseases. Because patients with IPPFE have intrinsically lower body weights and candidates for lung transplantation are generally younger than patients with IPPFE, low BMI at first visit does not necessarily reflect the severity of IPPFE. Patients with IPPFE reportedly have relatively well preserved PaO_2 in the earlier stages of the disease [6]; whereas, candidates for lung transplantation are often in worse condition than those presenting for their first visit and are often receiving oxygen therapy. In our study, the reason for patients being unable to perform the DL_{CO} test was that their vital capacity was too low. Patients with IPPFE often have relatively better diffusion capacity than restrictive pulmonary function [6]. Thus, some patients who are unable to perform the DL_{CO} test may have preserved diffusion capacity.

In our study, overall survival did not differ significantly between patients with IPPFE and those with oIIPs. Shioya et al [25] reported that patients with a GAP index of II + III and IPPFE have worse survival than those with IPF. These researchers also showed that some patients with IPPFE have better survival than those with IPF later during follow-up and that the survival curve reaches a plateau; these findings are similar to ours (Figs 1, 3). Interestingly, they suggest that patients with IPPFE characteristically have poor survivals, yet despite this, some of them live for a long time.

Pneumothorax is reportedly a prognostic factor in patients with IPF [26]. In the present study, a history of pneumothorax was not associated with survival in patients with either IPPFE or oIIPs. This discrepancy may be explained by the different observation periods. Nishimoto et al's [26] study included development of pneumothorax after diagnosis, whereas ours included only a history of pneumothorax before registration on the waiting list.

Pulmonary hypertension is also reportedly a prognostic factor in patients with end-stage lung disease referred for lung transplantation [27]. In our study, only 1 patient with IPPFE and 2 with oIIPs had pulmonary hypertension at the time of listing. Some patients may have developed pulmonary hypertension after listing; however, these data were not systematically collected, preventing full evaluation of the impact of pulmonary hypertension in the present study.

Our study has several limitations. First, this was a retrospective and single-center study with too few patients to allow extensive multivariate analysis, thus some confounding factors may be present. For instance, although being a man is reportedly a prognostic factor in both IIPs and IPPFE [7,8,20], we could not perform survival analysis regarding sex because the sex distribution was lopsided. Given that IPPFE is a relatively rare subtype of IIPs, it would be difficult to perform a prospective study. Second, although some patients with IPPFE and unfavorable factors survived for long enough to undergo transplantation, their outcomes and quality of life after transplantation have not yet been investigated. A large multicenter study is desirable.

In conclusion, we found that patients with IPPFE on the waiting list for transplantation have some characteristics that differ from those of patients with oIIPs; those with IPPFE have a lower BMI and APDT-to-TDT ratio, and their $\%DL_{CO}$ is relatively well preserved. Furthermore, some patients with IPPFE live for long enough to undergo transplantation despite having unfavorable prognostic factors, such as a lower BMI, short 6MWD, low P-to-F ratio, and an inability to perform the DL_{CO} test. Therefore, care should be taken when evaluating patients with IPPFE to consider the possibility of lung transplantation.

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REFERENCES

[1] Date H. Current status and problems of lung transplantation in Japan. J Thorac Dis 2016;8:S631–6.

[2] Sumiko M, Japanese Society of Lung and Heart–Lung Transplantation. Registry of the Japanese Society of Lung and Heart-Lung Transplantation: official Japanese lung transplantation report. accessed 04.04.19, http://www2.idac.tohoku.ac.jp/dep/surg/shinpai/pg185.html; 2019.

[3] Japan Organ Transplant Network. Lungs. Number of patients listed for lung transplant. accessed 04.04.19; in Japanese], http://www.jotnw.or.jp/file_lib/pc/datafile_hope_detail_pdf/lungs0228txt_3.pdf; 2019.

[4] Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An official American Thoracic Society/European Respiratory Society statement: update of the international multidisciplinary classification of the idiopathic interstitial pneumonias. Am J Respir Crit Care Med 2013;188:733–48.

[5] Hayashi H, Nei T, Abe S, Saito Y, Kokuho N, Atsumi K, et al. Body mass index and arterial blood oxygenation as prognostic factors in patients with idiopathic pleuroparenchymal fibroelastosis. Sarcoidosis Vasc Diffus Lung Dis 2017;34:35–40.

[6] Bonifazi M, Montero MA, Renzoni EA. Idiopathic pleuroparenchymal fibroelastosis. Curr Pulmonol Reports 2017;6:9–15.

[7] Orens JB, Estenne M, Arcasoy S, Conte JV, Corris P, Egan JJ, et al. International guidelines for the selection of lung transplant candidates: 2006 update–a consensus report from the Pulmonary Scientific Council of the International Society for Heart and Lung Transplantation. J Hear Lung Transplant 2006;25:745–55.

[8] Ley B, Ryerson CJ, Vittinghoff E, Ryu JH, Tomassetti S, Lee JS. A multidimensional index and staging system for idiopathic pulmonary fibrosis. Ann Intern Med 2012;156:684–91.

[9] Ryerson CJ, Vittinghoff E, Ley B, Lee JS, Mooney JJ, Jones KD, et al. Predicting survival across chronic interstitial lung disease: The ILD-GAP model. Chest 2014;145:723–8.

[10] Harada T, Yoshida Y, Kitasato Y, Tsuruta N, Wakamatsu K, Hirota T, et al. The thoracic cage becomes flattened in the progression of pleuroparenchymal fibroelastosis. Eur Respir Rev 2014;23:263–6.

[11] Reddy TL, Tominaga M, Hansell DM, Von Der Thusen J, Rassl D, Parfrey H, et al. Pleuroparenchymal fibroelastosis: a spectrum of histopathological and imaging phenotypes. Eur Respir J 2012;40:377–85.

[12] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al. An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011;183:788–824.

[13] Sverzellati N, Lynch DA, Hansell DM, Johkoh T, King TE, Travis WD. American Thoracic Society–European Respiratory Society classification of the idiopathic interstitial pneumonias: advances in knowledge since 2002. RadioGraphics 2015;35:1849–71.

[14] Travis WD, Hunninghake G, King TE, Lynch DA, Colby TV, Galvin JR, et al. Idiopathic nonspecific interstitial pneumonia report of an American Thoracic Society Project. Am J Respir Crit Care Med 2008;177:1338–47.

[15] Skolnik K, Ryerson CJ. Unclassifiable interstitial lung disease: a review. Respirology 2016;21:51–6.

[16] Nakatani T, Arai T, Kitaichi M, Akira M, Tachibana K, Sugimoto C, et al. Pleuroparenchymal fibroelastosis from a consecutive database: a rare disease entity? Eur Respir J 2015;45:1183–6.

[17] Watanabe K. Pleuroparenchymal fibroelastosis: its clinical characteristics. Curr Respir Med Rev 2014;9:229–37.

[18] Miyoshi R, Chen-Yoshikawa TF, Takahagi A, Oshima Y, Hijiya K, Motoyama H, et al. Pulmonary function and exercise capacity in patients with flat chests after lung transplantation. Ann Thorac Surg 2017;104:1695–701.

[19] Du Bois RM, Weycker D, Albera C, Bradford WZ, Costabel U, Kartashov A, et al. Ascertainment of individual risk of mortality for patients with idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2011;184:459–66.

[20] Fernandez R, Safaeinili N, Kurihara C, Odell DD, Jain M, DeCamp MM, et al. Association of body mass index with lung transplantation survival in the United States following implementation of the lung allocation score. J Thorac Cardiovasc Surg 2018;155:1871–1879.e3.

[21] Ikezoe K, Handa T, Tanizawa K, Chen-Yoshikawa TF, Kubo T, Aoyama A, et al. Prognostic factors and outcomes in Japanese lung transplant candidates with interstitial lung disease. PLoS One 2017;12:1–14.

[22] Du Bois RM, Albera C, Bradford WZ, Costabel U, Leff JA, Noble PW, et al. 6-Minute walk distance is an independent predictor of mortality in patients with idiopathic pulmonary fibrosis. Eur Respir J 2014;43:1421–9.

[23] Kondoh Y, Taniguchi H, Kataoka K, Furukawa T, Ando M, Murotani K, et al. Disease severity staging system for idiopathic pulmonary fibrosis in Japan. Respirology 2017;22:1609–14.

[24] Bahmer T, Kirsten A-M, Waschki B, Rabe KF, Magnussen H, Kirsten D, et al. Prognosis and longitudinal changes of physical activity in idiopathic pulmonary fibrosis. BMC Pulm Med 2017;17:104. https://doi.org/10.1186/s12890-017-0444-0.

[25] Shioya M, Otsuka M, Yamada G, Umeda Y, Ikeda K, Nishikiori H, et al. Poorer prognosis of idiopathic pleuroparenchymal fibroelastosis compared with idiopathic pulmonary fibrosis in advanced stage. Can Respir J 2018;2018:6043053.

[26] Nishimoto K, Fujisawa T, Yoshimura K, Enomoto Y, Enomoto N, Nakamura Y, et al. The prognostic significance of pneumothorax in patients with idiopathic pulmonary fibrosis. Respirology 2018;23:519–25.

[27] Nowak J, Hudzik B, Niedziela J, Walczak P, Rozentryt P, Ochman M, et al. Prognostic value of mean, diastolic, and systolic pulmonary artery pressure in patients with end-stage lung disease referred for lung transplantation. Transplant Proc 2018;50:2048–52.

· 综述 ·

T_2 期食管癌环形肌与纵形肌细分的临床意义研究进展

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【摘要】 T_2 期食管癌是指食管肿瘤侵及食管固有肌层部分,其分期、治疗方式及预后较其他阶段的食管癌更笼统且具争议。食管癌的 TNM 分期系统在不断完善,但始终未对 T_2 期食管癌进一步细分,现有分期方式的准确性也受到质疑。准确的分期对 T_2 期食管癌的治疗方式及预后有着重要意义。虽然治疗方式在近几年已有明显改善,即从单纯手术治疗到以手术为主的综合治疗,从提倡术后化疗到术前新辅助治疗,但针对 T_2 期食管癌的治疗目前仍无定论。准确的分期和恰当的治疗方式对 T_2 期食管癌预后有较大影响。近年来有关 T_2 期食管癌的研究进展较快,研究内容也越来越深入,本文就 T_2 期食管癌的分期、治疗方式及预后的研究进展作一综述,探索 T_2 期食管癌亚分期的临床意义,为进一步提高 T_2 期食管癌分期的准确性,治疗方案的科学性及改善预后提供参考。

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Progress in the clinical significance of subclassifying the circular and longitudinal muscle layers in patients with T_2 esophageal cancer

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【**Abstract**】T_2 esophageal cancer refers to the invasion of esophageal tumors into the esophageal muscularis propria, of which staging, treatment and prognosis are more general and controversial than other stages of esophageal cancer. Although the TNM staging system for esophageal cancer has been continuously improved, T_2 esophageal cancer has not been further subclassified. The accuracy of the current staging method has also been questioned. Accurate staging has important significance for the treatment of T_2 esophageal cancer and prognosis. Although treatment has improved significantly in recent years, from surgery alone to surgery-based comprehensive treatment, from postoperative chemotherapy to preoperative neoadjuvant treatment, the treatment of T_2 esophageal cancer is still inconclusive. Accurate staging and proper treatment have a significant impact on the prognosis of T_2 esophageal cancer. In recent years, studies on T_2 esophageal cancer have progressed rapidly, and the content of research has become more and more profound. This article reviews the research progress on the staging, treatment and prognosis of T_2 esophageal cancer, providing reference for further improving the accuracy of T_2 stage esophageal cancer staging, the scientific treatment plan and prognosis.

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食管癌是最常见的恶性消化道肿瘤之一,其发病率和病死率分别居世界第八位和第六位[1]。中国作为食管癌的高发区,其病死率和发病率均占第四位[2]。近年来随着人们就诊意识的提高,防癌知识宣教的普及,高发地区食管癌发病率有降低趋势[3-4]。但早期食管癌的检出率明显升高,使得对早期食管癌的研究也越来越多且较深入。近年来有关 T_2 期食管癌的研究进展较快,研究内容也越来越深入,本文综述 T_2 期食管癌的分期、治疗方式及预后的研究进展,探索 T_2 期食管癌亚分期的临床意义,为进一步提高 T_2 期食管癌分期的准确性,治疗方案的科学性及改善预后提供参考。

临床分期

1. T_2 期亚分期

近年来关于 T_2 期食管癌的研究进展较快,食管癌 TNM 分期系统也在不断完善,最新 AJCC 和 UICC 联合发布的第八版食管癌分期系统将 T 分期分为 T_{1a}, T_{1b}, T_2, T_3, T_{4a} 和 T_{4b} 6 个层次,其中原发肿瘤侵入食管固有肌层被定义为 T_2 期食管癌,但国际上始终未对 T_2 期食管癌进行亚分期。

肿瘤侵犯食管固有肌层,解剖学上虽包括浅肌层(环形肌)和深肌层(纵形肌),但普遍认为两者临床影像学和病理学的特征性以及治疗方式并无明显区别,而且,目前还无循

证医学研究的大数据表明两者预后有何显著差异，因此国际上并未将 T 期食管癌进一步细分。为了评价对 T_2 期食管癌进行亚分期是否有助于决定食管癌的预后，Guo 等[5]回顾分析了 85 例经胸腔镜切除的 T_2 期食管癌的临床和病理特征，根据肌层浸润深度，分别将肿瘤侵入食管环形和纵形肌层定义为 T_{2a} 和 T_{2b} 疾病，结果发现 T_{2b} 患者淋巴结转移发生率明显高于 T_{2a} 患者（52.2% 对 30.8%，$P=0.047$），故 T_{2a} 患者比 T_{2b} 患者预后更差。*Kaplan-Meier* 法比较的预后因子生存差异结果也显示 T_{2a} 患者生存率高于 T_{2b} 患者（$P=0.017$）。为进一步检验 T_2 期患者的预后价值，Duan 等[6]采用类似方法，用单变量和多变量分析确定可能与生存相关的预后因子，并对 T_{2a}、T_{2b} 患者与同期 T_{1b}、T_3 患者的生存差异进行比较，发现 T_2 分期与食管癌患者术后预后差异显着和独立相关（$P=0.002$），T_2 期和淋巴结转移之间的高度相关也可以解释 T_2 期亚分期患者的生存差异（$P=0.044$），且 T_{2a} 患者的生存率接近于 T_{1b} 患者的生存率，T_{2b} 患者生存率更接近于 T_3 期患者（$P=0.000$），进一步强调了 T_2 期亚群的预后价值。

上述研究显示，T_2 亚分期（T_{2a} 和 T_{2b}）是 T_2 期食管癌的独立预后因素，有助于确定和改善食管癌患者的预后。AJCC 和 UICC 联合发布的食管癌分期系统是目前国际上最权威、使用最广泛的食管癌分期标准，但目前食管癌的 TNM 分期系统修订是存在争议的，基于大量食管癌患者资料库，食管分期系统也在不断完善，目前关于 T_2 期的研究大多是来自单一机构的回顾性研究，我们需要通过加大样本量及较长时间的随访进一步证实 T_2 亚分期的临床意义，为下一次 TNM 分期系统的修订提供参考依据。

2. 术前临床分期方式

临床分期是食管癌预处理评估中的关键步骤，对确定最佳治疗策略和预测治疗效果起着重要作用。虽然现有分期技术在 T_2 期食管癌分期方面已取得较大进展，但仍无法精准定位 T_2 期食管癌，许多报道强调了分期的准确性不高，且差异较大，有文献表明，高达 66% 的食管肿瘤浸润程度评定过高[7-8]。

CT 是在食管癌初步诊断后进行的一种重要的补充检查方式，检查部位以颈部、胸部、腹部为主，在 CT 中食管癌主要表现为食管壁增厚、管腔变小，可以为 T_2 期食管癌的周围脏器和可能的转移病灶判断提供初步依据。研究显示，CT 对肿瘤浸润深度分期的总体准确率为 50%~80%，淋巴结受累的准确率为 50%~70%[9]，但 CT 缺乏对 T_2 期肿瘤的准确分期定义，且多项研究表明 CT 在分期上的特异性和准确性低于其他分期方式，对微小转移的淋巴结灶也易做错误诊断，因此不足以在临床上提供 T_2 期准确分期。

FDG-PET 越来越多的被运用于食管癌初步分期，为分期提供了代谢活性检查的手段，即恶性肿瘤细胞摄取 PDG 后，磷酸化作用被暂时保存在细胞内，然后用 PET 检测食管 FDG 浓度增加的区域。FDG-PET 在鉴别区域转移方面已被证实相对 CT 有更高的准确性和特异性[10]，在检测远处转移方面也有较大作用，Lerut 等[11]研究对比了 FDG-PET 与 EUS 和 CT 联合使用对检测远处转移疾病的准确性，结果显示 FDG-PET有更高的准确率（86% 对 62%）。临床上更多使用 FDG-PET和 CT 联合来提高分期的准确性。

EUS 是一种微创方法，可以探测肿瘤的浸润深度和区域淋巴结状态，并提供了详细的食管壁图像，T_2 期食管癌在 EUS 中表现为肿瘤入侵第 4 超声层的代表固有肌层的低回声带，但并不完全浸透，利用回声波改变的层面来确定 T 分期。T 分期中，EUS 相对 CT 和 PET 其准确性、特异性更高，但 EUS 对 T_2 期疾病节点状态的判断表现极差，对 T_2 期疾病的准确率低至 31%[12-13]，这可能与肿瘤周围炎性改变及纤维化，解剖位置导致的超声定位不当以及肿瘤体积较大时超声频率相应衰减增加难以反映肿瘤全貌有关[14]。Rice 等[8]发现仅 13% 的 T_2 期食管癌患者被正确分期。Pech 等[15]研究也发现 EUS 对 T 分期的敏感性从 T_1 和 T_3 的 82%~83% 降至 T_2 期的 43%。但 EUS 引导 FNA 可以帮助确定淋巴结转移，Vazquez-Sequerios 等[16]研究发现，EUS 联合 FNA 后，敏感性和准确性分别增加至 93% 和 90%，目前这种优势对于 T_2 期疾病的判断是否适用还不确定，但建议在淋巴结转移判断中使用 EUS 引导 FNA 活检的方式，以提高临床分期的准确性，对制定诊疗方案具有重要意义。

TNM 分期是食管癌的独立预后因素，突出了对食管癌准确病理和临床分期的预后意义，治疗前无法准确分期可能会导致选择不太合适的治疗策略和更差的预后，对生存率有不利影响。因此，T_2 期食管癌的进一步分期和更多分期模式的探索应受到更多学者的关注。

预后因素

随着诊疗方式和技术水平的提高，食管癌的预后有了明显改善，但其结果不能令人满意，因此是备受关注的问题之一。大量研究表明，许多临床病理因素与食管癌预后有关[17-18]，而肿瘤分级、肿瘤浸润深度、有无淋巴结转移等通常作为 T_2 期食管癌患者预后的重要分析指标。

肿瘤分级是 T_2 期食管癌的独立预后因素之一，肿瘤细胞的分化程度可以反应其恶性程度，分化程度越低其恶性程度越高，越容易早期发生转移和术后复发，Dongrong 等[19]对肿瘤分级研究的生存曲线表明高分化肿瘤具有更好的预后，但 Guo 等[5]和 Hsu 等[20]的研究显示肿瘤分化与生存率无显著相关性。

肿瘤浸润程度作为预后因素之一是食管癌 TNM 分期的重要依据，并与淋巴结转移显著相关，是预测淋巴结转移和判断预后的重要指标，即肿瘤浸润深度越深，食管癌患者预后越差。这些因素很容易在术后病理标本中确定，但肿瘤浸润程度和淋巴结转移的确定对术前治疗决策的决定极其重要。浸润深度是由食管癌 TNM 分期系统中的 T 分类表示，T_2 期食管癌是肿瘤浸润到固有肌层，但目前国际上并未明确说明肿瘤浸润深度与患者危险分层有关。Rice 等[21]发现 T_2

期食管癌的淋巴结转移率为 43%，与 T_1 期患者相比，T_2 期患者有 6 倍的可能性患有 N_1 疾病。Guo 等[5]研究结果也显示肿瘤固有肌层浸润深度能独立预测食管癌患者的生存。但最近 Seder 等[22]在研究 T_2 期肿瘤浸润深度与长期生存率的关系时发现，食管腺癌患者总体(OS)和无病生存率(DFS)的单因素分析显示 OS 和 DFS 的 P 值分别为 0.42 和 0.34，多因素分析 P 值分别为 0.15 和 0.21，表明食管腺癌患者生存率与肌层浸润深度无关。

诊断时的淋巴结状态和分期被认为是食管癌最重要的预后因素，淋巴结转移是食管癌的主要转移方式，早期食管癌便可以通过黏膜下层互通交汇的淋巴回流系统和血管发生淋巴结转移，T_2 期食管癌肿瘤已浸润了黏膜下层，淋巴结转移的风险增加。在 Duan 等[6]回顾性分析 120 例 T_2 期食管癌患者临床病理特点的研究中，淋巴结转移率为 30.8%。T_2 期食管癌淋巴结转移百分比在不同的研究中存在差异，但大多数在 40% 左右[23]。作为食管癌恶化的评定指标之一，淋巴结转移数极大影响着患者预后，NCCN 指南建议，至少有 15 枚淋巴结应该切除，以便在没有进行归纳放化疗的情况下对接受食管切除术的患者进行充分的淋巴结分期。淋巴结转移使预后更差，有相当数量的 T_2 期患者在诊断中有阳性淋巴结，临床医师应该考虑阳性淋巴结的高风险进行分期治疗，还是采用诱导治疗，目前尚无定论。

综合治疗

T_2 期食管癌的最佳治疗策略一直没有定论[24]，但单纯的手术治疗已经不能达到最理想的效果，对于 T_2 期食管癌的治疗，术前新辅助治疗联合手术的系统性综合治疗模式越来越受到临床医师的关注[25-26]。新辅助治疗是指在实施食管癌手术前所做的全身治疗，目的是缩小肿瘤范围、清理已发生局部转移的癌细胞，利于后续的手术治疗。但新辅助化疗对于食管癌的治疗效果目前还存在争议，各临床试验结论差异较大。

多项随机对照研究证实了新辅助治疗对于食管癌患者生存时间的益处，新辅助放化疗已成为大部分西方国家局部晚期食管癌的标准治疗方案。Stiles 等[12]在对 $T_{2/3}$ 食管癌患者进行新辅助治疗的研究显示，患者总体生存率从 44.2% 提高至 56.2%（$P<0.05$）。Kountourakis 等[27]对 T_2 期食管癌患者的回顾性研究中，患者 5 年生存率为 64.1%，DFS 为 58.4%，相对行单纯手术有所提高。虽然新辅助化疗在一些研究中显示具有优势，但目前仍无大量证据证明术前放化疗和单纯手术在切除率、复发率和远期生存率等方面存在显著差异，如 Markar 等[28]的一项 T_2 期食管癌的多中心回顾性研究中显示，与单独手术相比，新辅助治疗对生存或复发无显著影响，手术切除原发肿瘤和区域淋巴结的手术方法可能足以控制局部区域疾病，额外使用新辅助疗法对 T_2 期食管癌患者预后无益处，Chen 等[29]的研究也表明单纯手术治疗利于 T_2 食管癌患者的预后。

新辅助治疗在临床实践中的使用越来越多，但由于随机试验的样本数量较少，食管癌患者的新辅助治疗还未得到充分的支持，新辅助治疗的模式还需要更多的研究作为理论支持。

小结与展望

T_2 期食管癌在治疗方面是一个特殊的实体，也是一个极具争议的话题，近几年对 T_2 期食管癌的研究进展较快，其管理系统也在不断完善。适当的分期方式和精准的分期对 T_2 期食管癌的预后和最佳治疗方式的确定有重要作用，T_2 期进一步分期对改善食管癌患者的预后有重要意义，但这需要更多的数据和研究作理论支撑，同时应积极地寻找特异、敏感的预后评价指标以提高食管癌疗效。从单一手术治疗到以手术为主的综合治疗，食管癌的治疗方式在不断优化，积极推动手术治疗、辅助治疗和新辅助治疗的联合治疗方式，以精准的分期治疗为依据，微创化、综合化、规范化、科学化的诊断治疗模式需要我们更多的探索。

参考文献

[1] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012 [J]. CA Cancer J Clin, 2015, 65(2): 87-108. doi: 10.3322/caac.21262.

[2] Lin Y, Totsuka Y, He Y, et al. Epidemiology of esophageal cancer in Japan and China[J]. J Epidemiol, 2013, 23(4): 233-242.

[3] 田东，贺巧，王洋，等. 食管癌高发区农村居民饮食状况及对食管癌认知调查[J]. 川北医学院学报, 2010, 25(2): 470-473. doi: 10.3969/j.issn.1005-3697.2010.05.026.
Tian D, He Q, Wang Y, et al. Survey on eating habits of rural residents in high-incidence area of esophageal cancer and their cognition of esophageal caner[J]. J North Sichuan Med Coll, 2010, 25(2): 470-473. doi: 10.3969/j.issn.1005-3697.2010.05.026.

[4] Tian D, Mo SJ, Han LK, et al. Investigation of dietary factors and esophageal cancer knowledge: comparison of rural residents in high- and low-incidence areas[J]. Sci Rep, 2018, 8(1): 4914. doi: 10.1038/s41598-018-23251-3.

[5] Guo W, Xiao HL, Ma Z, et al. Should stage T2 esophageal squamous cell carcinoma be subclassified? [J]. Ann Surg Oncol, 2014, 21(8): 2540-2545. doi: 10.1245/s10434-014-3636-3.

[6] Duan J, Deng T, Ying G, et al. Prognostic significance of the T2 substage in patients with esophageal squamous cell carcinoma[J]. Dis Esophagus, 2017, 30(4): 1-7. doi: 10.1093/dote/dow027.

[7] Crabtree TD, Kosinski AS, Puri V, et al. Evaluation of the reliability of clinical staging of T2 N0 esophageal cancer: a review of the Society of Thoracic Surgeons database[J]. Ann Thorac Surg, 2013, 96(2): 382-390. doi: 10.1016/j.athoracsur.2013.03.093.

[8] Rice TW, Mason DP, Murthy SC, et al. T2N0M0 esophageal cancer [J]. J Thorac Cardiovasc Surg, 2007, 133(2): 317-324.

[9] Sancheti M, Fernandez F. Management of T2 esophageal cancer[J]. Surg Clin North Am, 2012, 92(5): 1169-1178. doi: 10.1016/j.

suc. 2012. 07. 003.

[10] Wong R, Walker-Dilks C, Raifu A. Evidence-based guideline recommendations on the use of positron emission tomography imaging in oesophageal cancer[J]. Clin Oncol(R Coll Radiol), 2012, 24(2): 86-104. doi:10. 1016/j. clon. 2011. 09. 006.

[11] Lerut T, Flamen P, Ectors N, et al. Histopathologic validation of lymph node staging with FDG-PET scan in cancer of the esophagus and gastroesophageal junction: a prospective study based on primary surgery with extensive lymphadenectomy[J]. Ann Surg, 2000, 232(6): 743-752.

[12] Stiles BM, Mirza F, Coppolino A, et al. Clinical T2-T3N0M0 esophageal cancer: the risk of node positive disease[J]. Ann Thorac Surg, 2011, 92(2): 491-496. doi:10. 1016/j. athoracsur. 2011. 04. 004.

[13] Tekola BD, Sauer BG, Wang AY, et al. Accuracy of endoscopic ultrasound in the diagnosis of T2N0 esophageal cancer[J]. J Gastrointest Cancer, 2014, 45(3): 342-346. doi:10. 1007/s12029-014-9616-9.

[14] Kutup A, Vashist YK, Groth S, et al. Endoscopic ultrasound staging in gastric cancer: Does it help management decisions in the era of neoadjuvant treatment? [J]. Endoscopy, 2012, 44(6): 572-576. doi:10. 1055/s-0032-1308950.

[15] Pech O, Gunter E, Dusemund F, et al. Accuracy of endoscopic ultrasound in preoperative staging of esophageal cancer: results from a referral center for early esophageal cancer[J]. Endoscopy, 2010, 42(6): 456-461. doi:10. 1055/s-0029-1244022.

[16] Vazquez-Sequerios E, Norton ID, Clain JE, et al. Impact of EUS-guided fine-needle aspiration on lymph node staging in patients with esophageal carcinoma[J]. Gastrointest Endosc, 2001, 53(7):751-757.

[17] Hou X, Gu YK, Liu XW, et al. The impact of tumor cell differentiation on survival of patients with resectable esophageal squamous cell carcinomas[J]. Ann Surg Oncol, 2015, 22(3): 1008-1014. doi: 10. 1245/s10434-014-4067-x.

[18] Wu J, Chen QX, Teng LS, et al. Prognostic significance of positive circumferential resection margin in esophageal cancer: a systematic review and meta-analysis[J]. Ann Thorac Surg, 2014, 97(2): 446-453. doi:10. 1016/j. athoracsur. 2013. 10. 043.

[19] Situ D, Wang J, Lin P, et al. Do tumor location and grade affect survival in pT2N0M0 esophageal squamous cell carcinoma? [J]. J Thorac Cardiovasc Surg, 2013, 146(1): 45-51. doi:10. 1016/j. jtcvs. 2013. 01. 034.

[20] Hsu PK, Wu YC, Chou TY, et al. Comparison of the 6th and 7th editions of the American Joint Committee on Cancer tumor-node-metastasis staging system in patients with resected esophageal carcinoma[J]. Ann Thorac Surg, 2010, 89(4): 1024-1031. doi:10. 1016/j. athoracsur. 2010. 01. 017.

[21] Rice TW, Zuccaro G Jr, Adelstein DJ, et al. Esophageal carcinoma: depth of invasion is predictive of regional lymph node status[J]. Ann Thorac Surg, 1998, 65(3):787-792.

[22] Seder CW, Mahon B, Hennon M, et al. Depth of muscularis propria invasion does not prognosticate survival in T2 esophageal adenocarcinoma[J]. Anticancer Res, 2018, 38(4): 2195-2200.

[23] Chen J, Liu S, Pan J et al. The pattern and prevalence of lymphatic spread in thoracic oesophageal squamous cell carcinoma[J]. Eur J Cardiothorac Surg, 2009, 36(3): 480-486. doi:10. 1016/j. ejcts. 2009. 03. 056.

[24] Martin JT, Worni M, Zwischenberger JB, et al. The role of radiation therapy in resected T2N0 esophageal cancer: a populationbased analysis[J]. Ann Thorac Surg, 2013, 95(2): 453-458. doi:10. 1016/j. athoracsur. 2012. 08. 049.

[25] Zhang JQ, Hooker CM, Brock MV, et al. Neoadjuvant chemoradiation therapy is beneficial for clinical stage T2N0 esophageal cancer patients due to inaccurate preoperative staging[J]. Ann Thorac Surg, 2012, 93: 429-437.

[26] Dolan JP, Diggs BS, Luna RA, et al. Significant understaging is seen in clinically stage T2N0 esophageal cancer patients undergoing esophagectomy[J]. Dis Esophagus, 2016, 29(4): 320-325. doi: 10. 1111/dote. 12334.

[27] Kountourakis P, Correa AM, Hofstetter WL, et al. Combined modality therapy of cT2N0M0 esophageal cancer: the University of Texas M. D. Anderson cancer center experience[J]. Cancer, 2011, 117(5): 925-930. doi:10. 1002/cncr. 25651.

[28] Markar SR, Gronnier C, Pasquer A, et al. Role of neoadjuvant treatment in clinical T2N0M0 oesophageal cancer: results from a retrospective multi-center European study[J]. Eur J Cancer, 2016, 56: 59-68. doi:10. 1016/j. ejca. 2015. 11. 024.

[29] Chen WH, Chao YK, Chang HK, et al. Long-term outcomes following neoadjuvant chemoradiotherapy in patients with clinical T2N0 esophageal squamous cell carcinoma[J]. Dis Esophagus, 2012, 25(3): 250-255. doi:10. 1111/j. 1442-2050. 2011. 01243. x.

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Original Article

Clinical nomogram for lymph node metastasis in pathological T1 esophageal squamous cell carcinoma: a multicenter retrospective study

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Background: Endoscopic resection is increasingly used to treat pathological T1 (pT1) esophageal cancer (EC) patients. However, the procedures are limited by lymph node metastasis (LNM) and remain controversial. We aimed to construct a nomogram to predict the risk of LNM in patients with pT1 esophageal squamous cell carcinoma (ESCC).

Methods: A total of 243 patients with pT1 ESCC who underwent esophagectomy and lymph node dissection at two different institutes between February 2013 and June 2019 were analyzed retrospectively. Patients were categorized into the negative group and the positive group according to whether there was LNM. Risk factors for LNM were evaluated by univariate and multivariate analyses. The nomogram was used to estimate the individual risk of LNM.

Results: Forty-six (18.9%) of the 243 patients with pT1 ESCC exhibited LNM. The LNM rate in patients with stage T1a disease was 5.7% (5/88), and the rate in patients with stage T1b disease was 26.5% (41/155). Multivariable logistic regression analysis showed that tumor differentiation [odds ratio (OR) =1.942, 95% confidence interval (CI): 1.067–3.536, P=0.030], the T1 sub-stage (OR =4.750, 95% CI: 1.658–13.611, P=0.004), the preoperative alanine aminotransferase/aspartate aminotransferase ratio (LSR) (OR =5.371, 95% CI: 1.676–17.210, P=0.005), and the high-density lipoprotein cholesterol (HDL-C) level (OR =5.894, 95% CI: 1.917–18.124, P=0.002) were independent risk factors for LNM. The nomogram had relatively high accuracy, with an area under the receiver operating characteristic curve (AUC) of 0.803 (95% CI: 0.732–0.873). The calibration curve showed that the predicted probability of LNM was in good agreement with the actual probability.

Conclusions: Clinicopathological and hematological parameters of tumor differentiation, the T1 sub-stage, the preoperative LSR, and the HDL-C level may predict the risk of LNM in T1 ESCC. The risk of LNM can be predicted by the nomogram.

Keywords: Lymph node metastasis (LNM); nomogram; risk factors; T1; esophageal squamous cell carcinoma (ESCC)

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Introduction

Esophageal cancer (EC) is a common digestive tract cancer. The morbidity and mortality due to EC rank 7th and 6th worldwide, respectively (1). In recent years, with the improvement of individuals' awareness of the need to seek medical treatment, the improvement of dietary habits and the dissemination of cancer prevention knowledge, the incidence of EC in high-incidence areas has tended to decrease (2). Esophageal squamous cell carcinoma (ESCC) is the most common histological type of EC in China. The five-year survival rate for patients with ESCC diagnosed in an early stage is greater than 90.0% after curative treatment (3,4).

Radical esophagectomy and lymph node dissection are the gold standard of treatment. Owing to improvements in surgical instruments and technology, endoscopic submucosal dissection (ESD) and endoscopic mucosal resection (EMR) can be performed in patients with early-stage ESCC (5,6). A retrospective study showed that the endoscopic resection rates for stage T1a and stage T1b EC patients were 53.0% and 20.9%, respectively (7). The incidence of lymph node metastasis (LNM) in pathological T1 (pT1) ESCC is 7.0–16.0% in the mucosa and 16.0–38.2% in the submucosa (6,8,9). The higher risk of LNM in submucosa limits the application of endoscopic resection. In addition, previous studies have reported that the lymph node status is the most important prognostic factor in early-stage ESCC (4,8,10). Therefore, an accurate prediction of the risk of LNM in T1 ESCC significantly affects treatment decisions and prognostic predictions.

A few studies have reported that LNM in stage T1 EC is related to the depth of tumor invasion, degree of tumor differentiation, tumor location and tumor size (5,8,9). However, there is still controversy. In addition, previous studies indicated that the preoperative alanine aminotransferase/aspartate aminotransferase ratio (LSR) and preoperative high-density lipoprotein cholesterol (HDL-C) level were factors affecting the prognosis of ESCC (11-13). However, no previous studies have proven their relationship with LNM. We speculated that some preoperative hematological indicators could also reflect the lymph node status. In addition, it is essential to construct an effective model for the prediction of the risk of LNM to select the optimal treatment and lymphadenectomy strategy for ESCC.

Methods

Patients

This study was a retrospective study of data from 243 patients with pT1 ESCC who underwent esophagectomy at the Affiliated Hospital of North Sichuan Medical College and Nanchong Central Hospital from February 2013 to June 2019. The following criteria were used for inclusion in this study: (I) patients with primary ESCC; (II) patients who underwent McKeown esophagectomy (thoracotomy/video-assisted thoracic surgery) and three-field lymphadenectomy; and (III) reevaluation of the postoperative pathology showed that the tumor only infiltrated the mucosal layer or the submucosa. The following exclusion criteria were used: (I) patients with esophagogastric junction carcinoma; (II) patients who received preoperative neoadjuvant therapy; (III) patients with distant metastases; (IV) patients with any concurrent primary cancer of other organs; and (V) patients >80 years old. The Ethics Committees and Review Board of the Affiliated Hospital of North Sichuan Medical College approved the study, and the need for patient consent was waived.

Patients were categorized into the negative group and the positive group according to whether there was LNM. The following variables were extracted from the database: sex, age, tumor location, degree of tumor differentiation, T1 sub-stage, tumor size, carcinoembryonic antigen (CEA) level, neutrophil count, lymphocyte count, LSR and HDL-C level.

This study based the tumor dissection, pathological staging, and lymph node status on the American Joint Committee on Cancer (AJCC) & The Union for International Cancer Control (UICC) 8th edition EC TNM classification criteria (14). The lymph node metastasis ratio (LNMR) was calculated as follows: (number of pathologically confirmed LNM/total number of lymph nodes dissected) ×100%.

Surgical procedures

All patients underwent gastroscopy, upper gastrointestinal radiography and contrast-enhanced computed tomography (CT) of the neck, chest, and upper abdomen prior to surgery. Esophageal mucosa staining was performed in patients with unclear lesions, and esophageal biopsy was performed to confirm the preoperative diagnosis. No

preoperative neoadjuvant therapy was administered, and no contraindications for surgery were noted. All patients underwent McKeown esophagectomy with three-field lymphadenectomy.

Experienced pathologists completed the postoperative pathology reports. All specimens were analyzed for the depth of tumor invasion, degree of tumor differentiation, and the presence of lymphatic invasion. In patients with multifocal cancer, the lesion with the greatest invasion depth was chosen for the classification of tumor depth and the evaluation of lymph node status.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics (version 22.0 Inc., Chicago, IL, USA) and the R programming language (version 3.4.1, Vienna, Austria). Data are reported as the frequencies, means and medians with percentages. The chi-square test and Student's *t*-tests were performed in univariate analysis to determine the differences in parameters between the two groups. Factors found to be significant (P<0.050) in univariate analysis were included in the subsequent multivariate logistic regression analysis to identify the independent risk variables associated with LNM. The nomogram was constructed based on the results of the multivariate analysis and evaluated by the receiver operating characteristic (ROC) curve, the area under the ROC curve (AUC), and the calibration curve. The calibration curve was based on 1,000 bootstrap replicates (15). The odds ratio (OR) and 95% confidence interval (CI) were calculated. A P value <0.05 was considered statistically significant.

Results

Characteristics

A total of 243 patients were included in the analysis: 160 (65.8%) patients were male, and 83 (34.2%) patients were female. The median age was 64.6±7.59 years. The distribution of tumor locations in all patients was as follows: 41 (16.9%), 161 (66.2%) and 41 (16.9%) patients had EC in the upper, middle and lower esophagus, respectively. The distribution of the degree of tumor differentiation was as follows: 92 (37.9%), 130 (53.3%), and 21 (8.6%) had G1, G2 and G3 disease, respectively. The numbers of patients with stage T1a and T1b disease were 88 (36.2%) and 155 (63.8%), respectively. The mean tumor size, CEA level, neutrophil count, lymphocyte count, LSR and HDL-C level were 2.32±1.11 cm, 2.39±1.53 μg/L, 4.46±1.99 10^9/L, 1.63±0.57 10^9/L, 0.84±0.30 and 1.30±0.31 mmol/L, respectively. The clinicopathological and hematological characteristics of patients in the LNM-negative and LNM-positive groups are shown in *Table 1*.

The prevalence of LNM

Forty-six (18.9%) of the 243 pT1 ESCC patients exhibited LNM. The LNM rates in patients with T1a and T1b disease were 5.7% (5/88) and 26.5% (41/155), respectively. A total of 6,240 lymph nodes were dissected during surgery, with a mean of 27±6 lymph nodes. Two hundred sixty-two lymph nodes were metastatic. The LNMR was 4.2% (262/6,240). The LNMRs in patients with T1a and T1b disease were 0.8% (16/1,999) and 5.8% (246/4,241), respectively.

The risk factors for LNM

The results of the univariate analysis revealed that the factors affecting LNM in T1 ESCC were the degree of tumor differentiation, T1 sub-stage, tumor size, LSR and HDL-C level (P<0.050). There was no significant difference in sex, age, tumor location, CEA level, neutrophil count or lymphocyte count (P=0.349, 0.447, 0.325, 0.053, 0.222, 0.381 and 0.849, respectively) (*Table 1*).

Multivariate logistic regression analysis demonstrated that the independent risk factors for LNM were tumor differentiation (OR =1.942, 95% CI: 1.067–3.536, P=0.030), the T1 sub-stage (OR =4.750, 95% CI: 1.658–13.611, P=0.004), the LSR (OR =5.371, 95% CI: 1.676–17.210, P=0.005), and the HDL-C level (OR =5.894, 95% CI: 1.917–18.124, P=0.002) (*Table 2*).

Nomogram

The established nomogram allowed for the estimation of the individual risk of LNM (*Figure 1*). A total score was calculated based on the degree of tumor differentiation, T1 sub-stage, tumor size, the LSR and the HDL-C level. A total score could be easily calculated by summing each individual score, and by projecting the total score to the lower total point scale, we were able to predict the probability of LNM. It also illustrated the relative contribution of each factor to the overall risk for LNM.

Table 1 Main clinical characteristics and parameters in 243 patients with pT1 ESCC

Variable	All patients, N=243	LNM		P value
		Negative, N=197	Positive, N=46	
Sex				0.349[a]
Male	160 (65.8%)	127 (64.5%)	33 (71.7%)	
Female	83 (34.2%)	70 (35.5%)	13 (28.3%)	
Age (years)				0.447[a]
<60	58 (23.9%)	49 (24.9%)	9 (19.6%)	
≥60	185 (76.1%)	148 (75.1%)	37 (80.4%)	
Tumor location				0.325[a]
Upper	41 (16.9%)	35 (17.8%)	6 (13.0%)	
Middle	161 (66.2%)	132 (67.0%)	29 (63.0%)	
Lower	41 (16.9%)	30 (15.2%)	11 (23.9%)	
Degree of tumor differentiation				<0.001[a,*]
G1	92 (37.9%)	80 (40.6%)	12 (26.1%)	
G2	130 (53.5%)	107 (54.3%)	23 (50.0%)	
G3	21 (8.6%)	10 (5.1%)	11 (23.9%)	
T1 sub-stage				<0.001[a,*]
T1a	88 (36.2%)	83 (42.1%)	5 (10.9%)	
T1b	155 (63.8%)	114 (57.9%)	41 (89.1%)	
Tumor size (cm)				0.002[a,*]
<2	90 (37.0%)	82 (41.6%)	8 (17.4%)	
≥2	153 (63.0%)	115 (58.4%)	38 (82.6%)	
CEA (μg/L)	2.39±1.53	2.45±1.54	2.14±1.50	0.222[b]
Neutrophil (10^9/L)	4.46±1.99	4.52±2.09	4.23±1.48	0.381[b]
Lymphocyte (10^9/L)	1.63±0.57	1.63±0.58	1.65±0.53	0.849[b]
LSR	0.84±0.30	0.81±0.28	0.97±0.33	0.001[b,*]
HDL-C (mmol/L)	1.30±0.31	1.26±0.29	1.44±0.35	0.001[b,*]

*, P<0.05; [a], Chi-square test; [b], Student's test; LNM, lymph node metastasis; ESCC, esophageal squamous cell carcinoma; CEA, carcinoembryonic antigen; LSR, alanine aminotransferase/aspartate aminotransferase ratio; HDL-C, high-density lipoprotein cholesterol.

Table 2 Multivariate analysis of predictive factors for LNM in pT1 ESCC

Characteristic	B	OR	95% CI	P value
Degree of tumor differentiation (G1/G2/G3)	0.729	1.942	1.067–3.536	0.030*
T1 sub-stage (T1a/T1b)	0.664	4.750	1.658–13.611	0.004*
Tumor size (<2 cm/≥2 cm)	1.559	2.075	0.851–5.062	0.108
LSR	1.683	5.371	1.676–17.210	0.005*
HDL-C (mmol/L)	1.775	5.894	1.917–18.124	0.002*

*, P<0.05. LNM, lymph node metastasis; ESCC, esophageal squamous cell carcinoma; LSR, alanine aminotransferase/aspartate aminotransferase ratio; HDL-C, high-density lipoprotein cholesterol; B, regression coefficient; OR, odds ratio; CI, confidence interval.

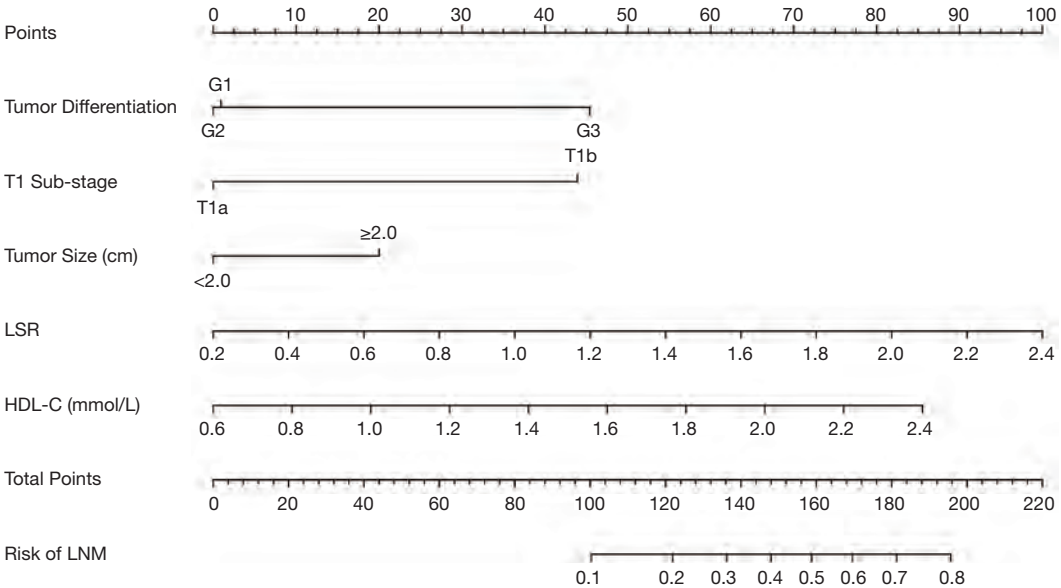

Figure 1 Nomogram predicting the risk of LNM in patients with T1 ESCC. LNM, lymph node metastasis; ESCC, esophageal squamous cell carcinoma; LSR, alanine aminotransferase/aspartate aminotransferase ratio; HDL-C, high-density lipoprotein cholesterol.

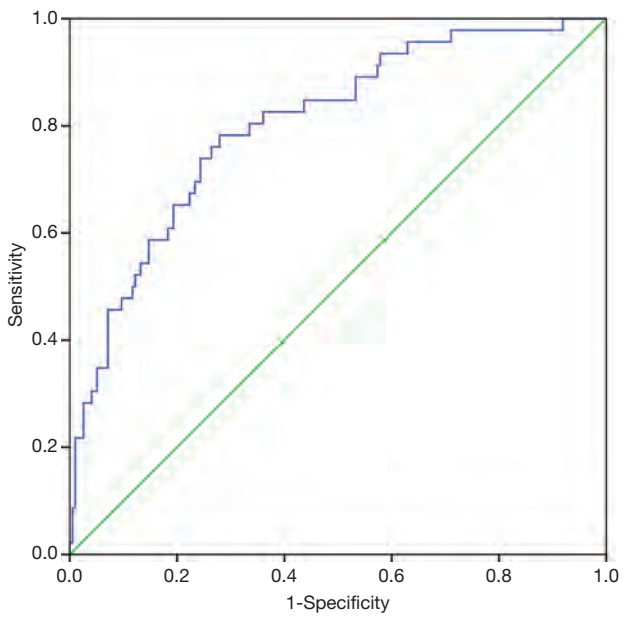

Figure 2 The receiver operating characteristic (ROC) curve for the nomogram. The C-index was 0.803 (95% CI: 0.732–0.873).

The ROC analysis is shown in *Figure 2*, which demonstrated that the nomogram had a robust discriminatory ability, with an AUC of 0.803 (95% CI: 0.732–0.873) (*Figure 2*). According to the calibration curve, the LNM probabilities predicted by the nomogram were consistent with the actual probabilities (*Figure 3*).

Discussion

Previous studies reported that the LNM rate in patients with T1a EC was 8.6–16.0%, and the rate in those with T1b was 16.0–34.3% (6,8,9,16). The differences in the incidences of LNM between reports may result from differences in the pathological type, sample size, method of lymph node dissection, and quality of the histopathological assessment of the resected samples (8).

The results of our study revealed an incidence of LNM of 5.7% (5/88) in stage T1a ESCC. This incidence was similar to that reported by Toshiaki *et al.* (17). The present study demonstrated an incidence of LNM of 26.5% (41/155) in stage T1b ESCC. A retrospective study of 295 patients who underwent surgery and/or ESD/EMR demonstrated that the T1b ESCC LNM rate was 34.3% (35/102) (16). This result may be partially attributed to the fact that both studies focused on the resection of lymph nodes and the evaluation of postoperative pathological sections, resulting in a higher LNM rate. However, Nentwich *et al.* (18) reported that the LNM rate in patients with T1b ESCC was 16.7% (5/30). The difference between these two results may be due to the larger sample size of our study and the fact that the patients underwent three-field lymphadenectomy.

Previous studies indicated that a worse degree of

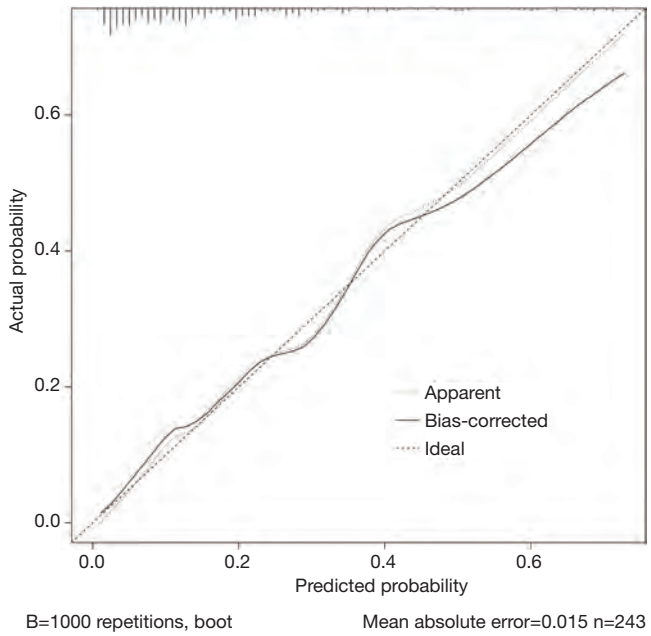

Figure 3 The calibration curves for the nomogram. The x-axis represents the predicted probability, and the y-axis represents the actual probability of LNM. LNM, lymph node metastasis.

differentiation of ESCC resulted in a higher LNM rate. Our results showed that the LNM rates of G1, G2 and G3 tumors were 13.0% (12/92), 17.7% (23/130), and 52.4% (11/21), respectively. Shen *et al.* (19) reported that the LNM rates of G1, G2 and G3 tumors were 6.1% (3/49), 17.2% (17/99) and 45.2% (33/73) respectively, which were similar to the values obtained in our study. Akutsu *et al.* (16) reported that the LNM rates of well/moderately differentiated and poorly differentiated tumors were 17.4% (36/207) and 35.1% (13/37), respectively, which was different from our results of 15.8% (35/222) and 52.4% (11/21). There were fewer patients with poorly differentiated tumors in our study, but we still found that patients with G3 tumor differentiation had a significantly higher risk of LNM. We also found that the LNM rate of G3 tumors was 2-3 times higher than that of G1-G2 tumors.

The depth of tumor invasion (T1b) was one of the risk factors that affected LNM in the present study ($P<0.05$). The incidence of LNM increased markedly after the tumor invaded through the mucosal layer to the submucosa (16). Endoscopic treatment is acceptable for patients with limited LNM and stage T1a disease (5). However, whether it is suitable for patients with a high risk of LNM and stage T1b disease remains controversial. Previous studies reported that the submucosa was divided into sm1, sm2 and sm3, and the risk of LNM in each layer was assessed to confirm the application of endoscopic resection in patients with stage T1b disease (5,6,20). However, preoperative examinations are suitable for patients with stage T1a and T1b disease, and it is difficult to further differentiate the T1b substage (10,18). Furthermore, the submucosa is a thin layer, and endoscopic resection has no absolute safety zone (9). The LNM rate of patients with disease extending into the submucosa in this study was 26.5%. There is a high risk of LNM when using endoscopic treatment in patients with stage T1b ESCC.

Tumor size is an important index that refers to the maximum diameter of the primary tumor, and it is easily measured before and during the operation (21). Duan *et al.* reported that a tumor size larger than 2.5 cm was a risk factor for LNM, and the LNM rates of tumors smaller than 2.5 cm and larger than 2.5 cm were 9.8% (8/82) and 27.9% (17/61), respectively (8). We used 2 cm as the threshold, and the results showed that the LNM rates of tumors smaller than 2 cm and larger than 2 cm were 8.9% (8/90) and 24.8% (38/153), respectively. The results were consistent despite the differences in tumor size thresholds. In our study, the chi-square test showed a statistically significant difference in tumor size between the LNM-negative and LNM-positive groups. However, multivariate regression analysis showed that a tumor size greater than 2 cm was not a risk factor for LNM, possibly due to the collinearity of the included indicators.

The alanine aminotransferase (ALT) level, aspartate aminotransferase (AST) level, and LSR are often used to assess liver damage, metabolic syndrome and cardiovascular disease (22). Previous studies have reported that patients with a high LSR have a good prognosis (12,13). The LSR may affect some proinflammatory mediators (e.g., CCL2, TNF, and IL-6) involved in carcinogenesis and tumor invasion and metastasis (13). One study reported that alcohol consumption and the AST/ALT ratio were independent risk factors for the incidence of EC in Korean men (23). However, whether the level of the LSR affects LNM in T1 ESCC has not been reported. Our results showed that patients with higher LSR exhibited a significantly higher risk of LNM. We hypothesized that the CCL1 in the lymphatic sinus is expressed in large amounts when tumor cells metastasize via the flow of the lymph, and the entry of tumor cells into the lymph nodes is controlled (24). The proinflammatory mediators TNF, IL-1β, and LPS increase CCL1 production and tumor cell

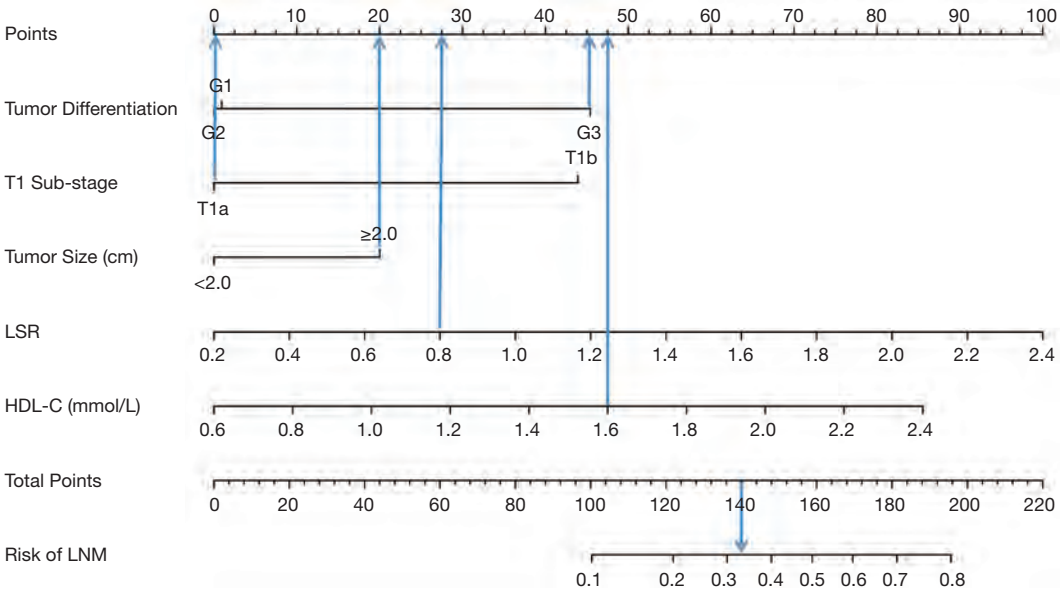

Figure 4 A clinical example of the use of the nomogram. The total score is 140=45+0+20+27.5+47.5, and the corresponding risk of LNM is 33%. LNM, lymph node metastasis; LSR, alanine aminotransferase/aspartate aminotransferase ratio; HDL-C, high-density lipoprotein cholesterol.

migration to lymphatic endothelial cells (24). The level of the LSR affects the functions of proinflammatory factors and chemokines and indirectly affects LNM.

HDL-C is an antiatherosclerotic lipoprotein that is considered a protective factor against coronary heart disease (25). Previous studies reported that EC patients with low levels of HDL-C exhibited a poor prognosis and that the HDL-C levels were significantly decreased in patients with cancer compared to normal human blood lipid levels (11,26). The reason for the reduced HDL-C level in cancer is that growing cancer cells require a large amount of cholesterol to synthesize new cell membranes. The activity of the HDL-C receptor is increased, and the outflow of intracellular cholesterol is increased, which reduces the amount of HDL-C in the serum (27). No previous studies have reported the relationship between serum HDL-C levels and LNM in ESCC. However, in a study on LNM in gastric cancer, we found that a low HDL-C level was a risk factor for LNM (28), which was contrary to our findings that a high HDL-C level was a risk factor in ESCC. We suspected that in ESCC and gastric cancer, the mechanism may be somewhat different. Of course, more mechanism studies are needed to verify this conjecture.

In addition, our study developed a nomogram to estimate the probability of LNM in patients with T1 ESCC. In our nomogram, the specific probability of LNM was predicted, and the discriminatory ability and calibration were determined. A previous study (8) developed a nomogram to predict the risk of LNM in patients with pT1 ESCC but did not evaluate its discriminatory ability and calibration. The discriminatory ability of the nomogram was determined by the AUC. The predicted and actual probabilities of LNM were compared in a calibration diagram (19,29). The AUC of our model was 0.803 (95% CI: 0.732–0.873), which proved that this model was highly accurate at predicting LNM. The calibration curve showed that the predicted probability of LNM was in good agreement with the actual probability. A total score was calculated from the 5 included parameters. The summarized total score indicates the probability of LNM. *Figure 4* shows a patient with poor tumor differentiation (G3), invasion into the mucosal layer (pT1a), a 3 cm tumor (≥2.0 cm), an LSR of 0.8 and an HDL-C level of 1.6 mmol/L. For this patient, the calculated total score was 140=45+0+20+27.5+47.5, and the corresponding risk of LNM was 33%.

Limitations

Some inevitable limitations were present in our study. First, this study was a retrospective study with some selection

bias. Second, our study found for the first time that the preoperative LSR and HDL-C level were independent risk factors for LNM. However, we have not identified the mechanism of these effect on LNM at the cellular and molecular levels, and further studies are needed. Third, our nomogram still needs to be validated in other databases due to the selected inclusion indicators and epidemiological differences.

Conclusions

Patients with pT1b ESCC exhibited a relatively high probability of LNM. The clinicopathological and hematological parameters of the degree of tumor differentiation, T1 sub-stage, preoperative LSR and HDL-C level may predict the risk of LNM in T1 ESCC. The risk of LNM in individuals can be predicted by the nomogram.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: All authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The Ethics Committees of the Affiliated Hospital of North Sichuan Medical College approved the study [No. 2018ER (R) 005] and the need for patient consent was waived.

References

1. Heymach J, Krilov L, Alberg A, et al. Clinical Cancer Advances 2018: Annual Report on Progress Against Cancer From the American Society of Clinical Oncology. J Clin Oncol 2018;36:1020-44.
2. Tian D, Mo SJ, Han LK, et al. Investigation of Dietary Factors and Esophageal Cancer Knowledge: Comparison of Rural Residents in High- and Low-incidence Areas. Sci Rep 2018;8:4914.
3. Ning B, Abdelfatah MM, Othman MO. Endoscopic submucosal dissection and endoscopic mucosal resection for early stage esophageal cancer. Ann Cardiothorac Surg 2017;6:88-98.
4. Minashi K, Nihei K, Mizusawa J, et al. Efficacy of Endoscopic Resection and Selective Chemoradiotherapy for Stage I Esophageal Squamous Cell Carcinoma. Gastroenterology 2019;157:382-90.e3.
5. Gamboa AM, Kim S, Force SD, et al. Treatment allocation in patients with early-stage esophageal adenocarcinoma: Prevalence and predictors of lymph node involvement. Cancer 2016;122:2150-7.
6. Weksler B, Kennedy KF, Sullivan JL. Using the National Cancer Database to create a scoring system that identifies patients with early-stage esophageal cancer at risk for nodal metastases. J Thorac Cardiovasc Surg 2017;154:1787-93.
7. Merkow RP, Bilimoria KY, Keswani RN, et al. Treatment trends, risk of lymph node metastasis, and outcomes for localized esophageal cancer. J Natl Cancer Inst 2014;106:1-8.
8. Duan XF, Tang P, Shang XB, et al. The prevalence of lymph node metastasis for pathological T1 esophageal cancer: a retrospective study of 143 cases. Surg Oncol 2018;27:1-6.
9. Gertler R, Stein HJ, Schuster T, et al. Prevalence and topography of lymph node metastases in early esophageal and gastric cancer. Ann Surg 2014;259:96-101.
10. Dubecz A, Kern M, Solymosi N, et al. Predictors of Lymph Node Metastasis in Surgically Resected T1 Esophageal Cancer. Ann Thorac Surg 2015;99:1879-85.
11. Chen P, Han L, Wang C, et al. Preoperative serum lipids as prognostic predictors in esophageal squamous cell carcinoma patients with esophagectomy. Oncotarget 2017;8:41605-19.
12. Chen SL, Li JP, Li LF, et al. Elevated Preoperative Serum Alanine Aminotransferase/Aspartate Aminotransferase

(ALT/AST) Ratio Is Associated with Better Prognosis in Patients Undergoing Curative Treatment for Gastric Adenocarcinoma. Int J Mol Sci 2016. doi: 10.3390/ijms17060911.
13. Huang H, Wang XP, Li XH, et al. Prognostic value of pretreatment serum alanine aminotransferase/aspartate aminotransferase (ALT/AST) ratio and gamma glutamyltransferase (GGT) in patients with esophageal squamous cell carcinoma. BMC Cancer 2017;17:544.
14. Rice TW, Gress DM, Patil DT, et al. Cancer of the esophagus and esophagogastric junction-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017;67:304-17.
15. Liu Y, Zou ZQ, Xiao J, et al. A nomogram prediction model for recurrent laryngeal nerve lymph node metastasis in thoracic oesophageal squamous cell carcinoma. J Thorac Dis 2019;11:2868-77.
16. Akutsu Y, Uesato M, Shuto K, et al. The overall prevalence of metastasis in T1 esophageal squamous cell carcinoma: a retrospective analysis of 295 patients. Ann Surg 2013;257:1032-8.
17. Tanaka T, Matono S, Mori N, et al. T1 squamous cell carcinoma of the esophagus: long-term outcomes and prognostic factors after esophagectomy. Ann Surg Oncol 2014;21:932-8.
18. Nentwich MF, von Loga K, Reeh M, et al. Depth of submucosal tumor infiltration and its relevance in lymphatic metastasis formation for T1b squamous cell and adenocarcinomas of the esophagus. J Gastrointest Surg 2014;18:242-9.
19. Shen W, Shen Y, Tan L, et al. A nomogram for predicting lymph node metastasis in surgically resected T1 esophageal squamous cell carcinoma. J Thorac Dis 2018;10:4178-85.
20. Manner H, Wetzka J, May A, et al. Early-stage adenocarcinoma of the esophagus with mid to deep submucosal invasion (pT1b sm2-3): the frequency of lymph-node metastasis depends on macroscopic and histological risk patterns. Dis Esophagus 2017;30:1-11.
21. Zhang H, Tang P, Miao X, et al. Does tumor size improve the accuracy of prognostic prediction in patients with esophageal squamous cell carcinoma after surgical resection? Oncotarget 2016;7:66623-34.
22. Oren R. Serum liver enzymes--should we count on them? Liver Int 2014;34:171-3.
23. Kimm H, Kim S, Jee SHJYMJ. The Independent Effects of Cigarette Smoking, Alcohol Consumption, and Serum Aspartate Aminotransferase on the Alanine Aminotransferase Ratio in Korean Men for the Risk for Esophageal Cancer. 2010;51:310-7.
24. Das S, Sarrou E, Podgrabinska S, et al. Tumor cell entry into the lymph node is controlled by CCL1 chemokine expressed by lymph node lymphatic sinuses. 2013;210:1509.
25. Liu YY, Lin SJ, Chen YY, et al. High-density lipoprotein cholesterol as a predictor of poor survival in patients with nasopharyngeal carcinoma. Oncotarget 2016;7:42978-87.
26. Wang XP, Li XH, Zhang L, et al. High level of serum apolipoprotein A-I is a favorable prognostic factor for overall survival in esophageal squamous cell carcinoma. BMC Cancer 2016;16:516.
27. Morin EE, Li XA, Schwendeman A. HDL in Endocrine Carcinomas: Biomarker, Drug Carrier, and Potential Therapeutic. Front Endocrinol (Lausanne) 2018;9:715.
28. Fan G, Hu D, Peng F, et al. Different Risk Profiles for the Postsurgical Prognosis of Gastric Cancer Patients with Different Blood Types: The FIESTA Study. J Cancer 2018;9:2885-94.
29. Semenkovich TR, Yan Y, Subramanian M, et al. A Clinical Nomogram for Predicting Node-positive Disease in Esophageal Cancer. Ann Surg 2019. [Epub ahead of print].

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ORIGINAL SCIENTIFIC REPORT

Depth of Invasion into the Circular and Longitudinal Muscle Layers in T2 Esophageal Squamous Cell Carcinoma Does Not Affect Prognosis or Lymph Node Metastasis: A Multicenter Retrospective Study

Dong Tian[1,2] · Heng Huang[3] · Yu-Shang Yang[1] · Kai-Yuan Jiang[3] · Xi He[4] · Xiao-Guang Guo[5] · Long-Qi Chen[1]

Abstract

Background Although a greater depth of tumor invasion is correlated with a poorer prognosis in esophageal squamous cell carcinoma (ESCC), it remains controversial whether T2 ESCC should be subclassified by circular and longitudinal muscle invasion. We conducted a multicenter retrospective study to evaluate the relationship between the depth of invasion and long-term outcome and to identify the clinical significance of subclassifying T2 ESCC.

Methods Patients with T2 ESCC who underwent esophagectomy at two different institutes between January 2009 and December 2017 were analyzed retrospectively. ESCC with circular and longitudinal muscle invasion was defined as T2 circular and T2 longitudinal ESCC, respectively. Survival outcomes and risk factors for lymph node metastasis (LNM) were evaluated by univariate and multivariate analyses. In addition, data from stage T1b ESCC cases during the same period were retrieved for use as a comparison cohort to evaluate the prognostic significance of the T2 substage.

Results A total of 536 T2 ESCC patients were eligible, and 192 (36%) patients developed LNM. No significant difference was found in general characteristics between the T2 circular and T2 longitudinal ESCC groups ($n = 219$ and $n = 317$, $P > 0.05$), except for tumor location ($P = 0.02$). The T2 substage was not significantly correlated with survival on univariate or multivariate analysis ($P = 0.30$ and $P = 0.34$, respectively). Multivariate analysis also indicated that the T2 substage was not an independent risk factor for LNM ($P = 0.15$). When patients with stage T1b ESCC were considered, their survival time was significantly different from that of patients with T2 circular and T2 longitudinal disease ($P = 0.01$).

Conclusions The depth of tumor invasion into the circular and longitudinal muscle layers in T2 ESCC does not affect the prognosis or risk of LNM.

Dong Tian and Heng Huang have contributed equally to this work.

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Introduction

Esophageal squamous cell carcinoma (ESCC) is a common malignant gastrointestinal tumor, with a morbidity and mortality rate ranking seventh and sixth, respectively, worldwide [1]. In recent years, the incidence of esophageal cancer (EC) in high-incidence areas has tended to decrease as residents' awareness of medical treatment has improved and as cancer prevention education has become popular [2].

T2 ESCC refers to tumor invasion of the esophageal muscularis propria, and the staging, treatment and prognosis of T2 ESCC are more controversial than those of other stages. Tumor invasion into the esophageal muscularis propria anatomically consists of the inner circular layer versus the outer longitudinal layer. Accurate staging has significance in choosing a treatment strategy for T2 EC, which can include surgery alone, surgery-based comprehensive treatment, postoperative chemotherapy and preoperative neoadjuvant treatment [3, 4]. However, many studies have emphasized the high inaccuracy of and large differences in staging methods. Some studies have indicated that the T2 esophageal tumor stage is overestimated up to 66% of cases [5, 6]. In addition, the TNM staging system for ESCC has been continuously improved as research on T2 ESCC has progressed rapidly, but this stage has not been further subclassified. In the latest 8th edition of the Union for International Cancer Control (UICC) and the American Joint Committee on Cancer (AJCC) cancer staging manual [7], the T classification includes T1a, T1b, T2, T3, T4a and T4b. It is generally accepted that there are no significant differences between the characteristics of videography and pathology clinically, including the treatment method, in T2 ESCC. Guo et al. [8] retrospectively evaluated the clinicopathological data of 85 patients with T2 ESCC and showed that the T2 substage was an independent prognostic factor for patients. Duan et al. [9] performed a similar study to support the hypotheses that T2 ESCC can be subclassified and that T2 circular patients have a superior prognosis. However, another study [10] found that the T2 substage did not correlate with the survival of T2 EC patients. The data from almost all of the above-mentioned studies were from single centers or small sample populations and thus do not provide authoritative evidence for identifying the value of the T2 substage.

The depth of tumor invasion is strongly correlated with lymph node metastasis (LNM) and survival in ESCC [11]. Additionally, the clinical significance of subclassifying circular and longitudinal muscle layer invasion in patients with T2 ESCC is controversial. We conducted a multicenter retrospective study to evaluate the correlation between invasion depth and long-term outcome and to identify the clinical significance of subclassifying T2 ESCC.

Materials and methods

Patients

Eligible patients with T2N0-3 ESCC who underwent esophagectomy at West China Hospital of Sichuan University and the Affiliated Hospital of North Sichuan Medical College between January 2009 and December 2017 were selected; their clinicopathological data had been routinely collected in an ongoing prospective registry and were analyzed retrospectively. The inclusion criteria for the patients were as follows: (1) pathologically confirmed T2N0-3 ESCC according to the 8th edition of the AJCC and UICC classification guidelines and (2) radical esophagectomy. The exclusion criteria were as follows: (1) age younger than 20 years; (2) esophageal adenocarcinoma/esophagogastric junction adenocarcinoma; (3) neoadjuvant therapy; and (4) other primary cancer during the follow-up period. In addition, data from stage T1b ESCC cases during the same period were retrieved for use as a comparison cohort to evaluate the prognostic significance of the T2 substage. This project, which was censored in July 2018, was authorized by the Ethics Committees of West China Hospital of Sichuan University (No. 201649), and the need for patient consent was waived.

Methods

Staging and surgery

Patients were staged preoperatively according to the 8th edition of the AJCC and UICC classification guidelines, and T2 ESCC was diagnosed using contrast-enhanced computed tomography (CT), endoscopic ultrasound (EUS), contrast esophagography, bronchoscopy and histology. According to the depth of tumor invasion, invasion of the circular layer was defined as stage T2 circular, and invasion of the longitudinal layer was defined as stage T2 longitudinal. Experienced thoracic surgeons from two different institutions initiated discussion of the preoperative staging results and performed transthoracic esophagectomy or combined thoracoabdominal esophagectomy with two- or three-field lymph node resection. Photomicrograph samples of tumor sections (an average of 15 sections were selected from each specimen) from each patient were assessed by an experienced pathologist (Xiao-Guang Guo, Department of Pathology, Nanchong Central Hospital) to determine the depth of invasion (circular vs longitudinal) accurately; the pathologist reviewed the slides from both

centers retrospectively, and relevant data were collected retrospectively.

Follow-up

After surgery, home visits and telephone interviews were used to analyze patients' quality of life and determine their living status. The patients were followed up at 3- to 6-month intervals during the first 2 years and then every 6–12 months until the last follow-up. Complete follow-up information until death or March 2019 was available for all patients. During each follow-up investigation, magnetic resonance imaging and histological examinations were performed in an outpatient clinic if clinical recurrence was suspected.

Clinical characteristics

Demographic and oncologic data were collected from the qualified patients, including sex, age, location, differentiation, tumor length, follow-up status, T stage, N stage, survival time, comorbidity, operation performed, postoperative hospital stay and so on. The survival time was calculated from the date of surgery to death or last documented follow-up. The tumor length was defined as the length measured by pathological sampling.

Statistical analysis

All clinicopathological data were entered into IBM SPSS Statistics, version 22.0, to be analyzed statistically. Statistical data are described as frequencies, means and medians with percentages. The Chi-squared test and Fisher's exact test were performed to determine differences between stage T2 circular and T2 longitudinal ESCC. The Kaplan–Meier method was used to compare survival for each prognostic factor, while survival was compared between groups by log-rank test. Cox proportional hazard regression analysis was performed to identify potential prognostic factors for overall survival (OS). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated for these features to quantify the strength of these associations. A logistic regression model was used to identify the factors associated with LNM. A P value < 0.05 was considered statistically significant.

Results

General characteristics

A total of 536 T2 ESCC patients met the inclusion criteria for the study between January 2009 and December 2017; 219 (41%) patients had T2 circular ESCC, and 317 (59%) had T2 longitudinal ESCC. A total of 399 (74%) patients were male, and 137 (26%) were female. The mean follow-up period and age was 25.4 ± 12.6 (range: 0–58) months and 61.7 ± 7.7 (range 38–82) years, respectively. A total of 102 (19%) patients died, and 192 (36%) patients developed LNM during the follow-up period. In the T1b cohort, a total of 84 (23.0%) patients developed LNM. More general features are listed in Table 1.

In this study, LNM occurred in 68 (31%) of the 219 patients with circular muscle invasion and 124 (39%) of the 317 patients with longitudinal muscle invasion, but the difference was not significant ($P = 0.24$). In addition, while there was no significant difference in age, sex, length, differentiation, comorbidity, operation performed or postoperative hospital stay ($P > 0.05$), there was a significant difference in tumor location ($P = 0.02$) between stage T2 circular and T2 longitudinal ESCC (Table 1).

Univariate and multivariate analyses of survival

The prognostic factors for OS are shown in Table 2. As demonstrated on univariate analysis, T2 substage, sex, age, location, length, differentiation and operation performed were not significantly associated with survival ($P = 0.30$, 0.83, 0.25, 0.22, 0.23, 0.56 and 0.07, respectively). However, N stage, comorbidity and postoperative hospital stay were significantly associated with survival ($P < 0.05$). Similar results were observed on multivariate analysis, which demonstrated that N stage (HR, 1.62; 95% CI, 1.03–2.54; $P = 0.03$) and comorbidity (HR, 1.56; 95% CI, 1.01–2.40; $P = 0.04$) were independent prognostic factors. Additionally, the evaluated OS for T2 circular and T2 longitudinal ESCC was 26.3 ± 13.0 months and 24.8 ± 12.2 months, with no significant difference ($P = 0.30$). Among the 536 patients, the OS was 25.4 ± 12.6 months (Fig. 1). The survival of patients with T1b ESCC was significantly different from that of patients with T2 circular and T2 longitudinal ESCC ($P = 0.01$), but the survival of patients with T2 circular ESCC was more similar to that of patients with T2 longitudinal ESCC than to that of patients with T1b ESCC (Fig. 2). The mean OS in the T1b, T2 circular and T2 longitudinal ESCC groups was 30.5 ± 22.5 months, 26.3 ± 13.0 months and 24.8 ± 12.2 months, respectively, with a significant difference in OS among the three groups ($P < 0.05$). Among the 902 patients, the OS was 27.5 ± 17.5 months. As shown in Table 3, the T2 substage was not significantly correlated with LNM on univariate or multivariate analysis (HR, 1.40; 95% CI, 0.97–2.01; $P = 0.07$ and HR, 1.33; 95% CI, 0.92–1.54; $P = 0.13$, respectively).

Discussion

It is universally acknowledged that the depth of tumor invasion is associated with patient prognosis and that the prognosis of patients with shallow invasion is better than that of patients with deep clinical invasion [12]. Despite this, T2 EC has not yet been further subclassified by the depth of invasion, i.e., invasion of the circular and longitudinal muscle layers, in the TNM staging system. Until now, there have been no large-sample, multicenter, evidence-based medical research studies indicating significant differences in the prognosis of T2 ESCC patients with different depths of tumor invasion. The current multicenter retrospective study aimed to identify whether the depth of tumor invasion (circular vs longitudinal) in T2 ESCC could affect prognosis and LNM and to determine the clinical significance of subclassifying T2 ESCC.

Table 1 General characteristics of T1 and T2 esophageal squamous cell carcinoma

Characteristic	Stage T2 circular (n = 219)[b]	Stage T2 longitudinal (n = 317)[b]	Stage T1b (n = 366)	P value[c]
Sex, n (%)				0.07[d]
Male	154 (70)	245 (77)	276 (75)	
Female	65 (30)	72 (23)	90 (25)	
Age, n (%)				0.29[d]
< 60 years	69 (32)	114 (36)	182 (50)	
60 years	150 (68)	203 (64)	184 (50)	
Location, n (%)				0.02[d,*]
Upper third	33 (15)	29 (9)	55 (15)	
Middle third	125 (57)	218 (69)	232 (63)	
Lower third	61 (28)	70 (22)	79 (22)	
Length, n (%)				0.33[d]
< 4 cm	106 (48)	140 (44)	282 (77)	
> 4 cm	113 (52)	177 (56)	84 (23)	
N-stage, n (%)[a]				0.25[e]
N0	151 (69)	193 (61)	282 (77)	
N1	48 (22)	87 (27)	58 (16)	
N2	16 (7)	26 (8)	22 (6)	
N3	4 (2)	11 (3)	4 (1)	
Comorbidity, n (%)				0.46[d]
Yes	76 (35)	120 (38)	79 (22)	
N0	143 (65)	197 (62)	287 (78)	
Differentiation, n (%)[a]				0.73[d]
G1	66 (30)	93 (29)	48 (13)	
G2	82 (38)	129 (41)	157 (43)	
G3	71 (32)	95 (30)	161 (44)	
Operation performed, n (%)				0.29[d]
Left thoracic approach	176 (80)	266 (84)	295 (81)	
Right thoracic approach	43 (20)	51 (16)	70 (19)	
Postoperative hospital stay, days				0.50[d]
<10 days	76 (35)	124 (39)	188 (51)	
10 days	143 (65)	193 (61)	178 (49)	

[a]The 8th edition of the UICC and AJCC cancer staging system

[b]The circular and longitudinal invasions were defined as T2 circular and T2 longitudinal stages

[c]Comparison between stages T2 circular and T2 longitudinal

[d]Chi-squared test was used

[e]Fisher's exact test was used

[*]$P < 0.05$

The depth of tumor invasion, represented by the T classification, is a significant index for predicting LNM and prognosis in EC [12]. As the depth of tumor invasion increases, the rate of LNM increases, generally with a poorer prognosis. The accuracy of the clinical T stage is crucial for determining the treatment strategy preoperatively. Accurate preoperative clinical staging provides the surgeon with more original information about the patient, which cannot be provided by pathological staging due to the large impact of chemoradiotherapy. However, there have been no studies specifically on the preoperative substaging of T2 ESCC. Some previous studies have emphasized that current staging methods are not accurate and vary widely, which indicates that additional studies and more sophisticated preoperative examination methods are required to identify T2 ESCC tumors [5, 6].

Many are concerned regarding the differences among and prognosis of patients with disease in different T2 substages. Guo et al. [8] found that the prognosis of patients with T2 circular ESCC was far superior to that of patients with T2 longitudinal ESCC ($P = 0.017$), and a significant difference in the risk of positive lymph nodes was observed ($P = 0.047$). Nevertheless, we could not determine whether there were differences in general characteristics between patients with T2 circular and T2 longitudinal ESCC in this study. Similarly, a study of 120 patients by Duan et al. [9] also emphasized the poor prognosis of T2 longitudinal patients ($P < 0.001$). However, the above studies were single-center studies with relatively small sample populations, which might have led to inaccurate results.

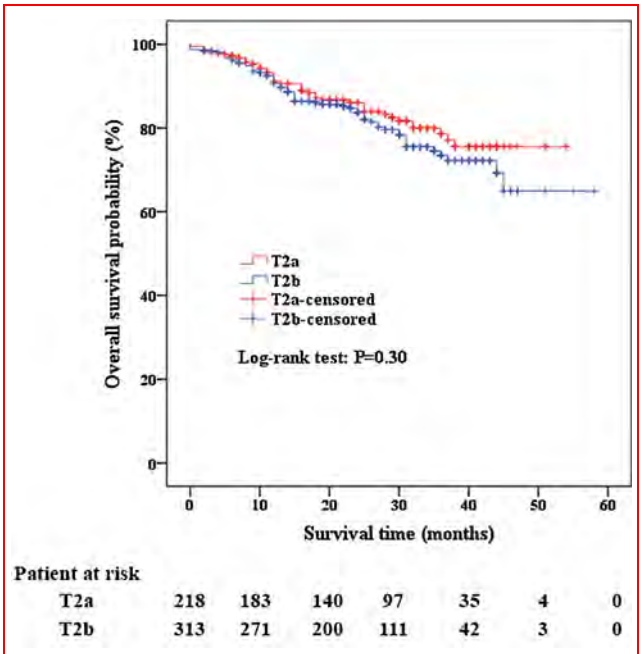

Fig. 1 Kaplan–Meier curves for T2 circular and T2 longitudinal of esophageal squamous cell carcinoma (ESCC) on overall survival. The mean overall survival (OS) in T2 circular and T2 longitudinal of ESCC was (26.3 ± 13.0) months and (24.8 ± 12.2) months. The difference of OS between two groups was not significant ($P = 0.30$). Of the 536 patients, the OS was (25.4 ± 12.6) months

Conversely, in our multicenter study with a larger sample population, we found that the T2 substage did not significantly influence OS ($P = 0.30$), which is completely different from the findings of the previous studies [10]. In another similar study, the depth of tumor invasion into the

Table 2 Univariate and multivariate analyses of overall survival in T2 esophageal squamous cell carcinoma

Characteristic	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
T2 substage (T2 circular/T2 longitudinal)[b]	1.24 (0.83–1.85)	0.30	1.22 (0.81–1.85)	0.34
N-stage (N0/N1/N2/N3)[a]	1.57 (1.01–2.45)	0.01*	1.62 (1.03–2.54)	0.03*
Sex (male/female)	1.05 (0.68–1.63)	0.83	1.19 (0.75–1.88)	0.47
Age (<60/60)	1.28 (0.84–1.96)	0.25	1.14 (0.73–1.77)	0.56
Location (U/M/L)	0.75 (0.42–1.35)	0.22	0.61 (0.33–1.13)	0.13
Length (<4 cm/4 cm)	1.27 (0.86–1.89)	0.23	1.22 (0.81–1.83)	0.33
Comorbidity (yes/no)	1.86 (1.26–2.75)	<0.001*	1.56 (1.01–2.40)	0.04*
Differentiation (G1/G2/G3)[a]	0.83 (0.51–1.35)	0.56	1.00 (0.59–1.69)	0.95
Operation performed (L/R)	0.58 (0.32–1.05)	0.07	0.60 (0.32–1.11)	0.10
Postoperative hospital stay (<10 days/10 days)	1.87 (1.19–2.94)	0.01*	1.64 (0.99–2.70)	0.05

U upper third, *M* middle third, *L* lower third, *L* left thoracic approach, *R* right thoracic approach, *HR* hazard ratio, *CI* confidence interval

[a]The 8th edition of the UICC and AJCC cancer staging system

[b]The circular and longitudinal invasions were defined as T2 circular and T2 longitudinal stages

*$P < 0.05$

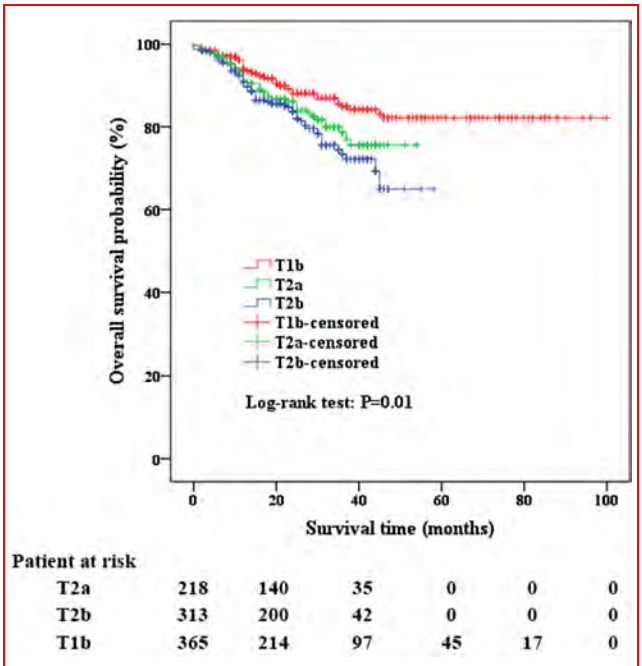

Fig. 2 Kaplan–Meier curves for T1b, T2 circular and T2 longitudinal of esophageal squamous cell carcinoma (ESCC) on overall survival. The mean overall survival (OS) in T1b, T2 circular and T2 longitudinal of ESCC was (30.5 ± 22.5) months, (26.3 ± 13.0) months and (24.8 ± 12.2) months. The differences of OS between two groups were significant (P = 0.01). Of the 902 patients, the OS was (27.5 ± 17.5) months

muscularis propria was not found to be associated with OS in patients with esophageal adenocarcinoma (P = 0.24). Reasonably, ESCC and adenocarcinoma occur in different types of patients and have different pathological features, tumor characteristics and staging systems. Nevertheless, this study provided a reference for our results that the T2 substage was not significantly favorable for prognosis.

LNM occurs through the lymphatic reflux system and blood vessels from the submucosal layer; furthermore, LNM can occur in early EC [13]. Many patients with T2 ESCC have positive lymph nodes at the time of diagnosis (approximately 40% in most studies) [14, 15]. Our results are consistent with those of previous studies, as the rate of LNM was 36%. We usually associate LNM with patient survival. In our study, we identified N stage (P = 0.03) as an independent prognostic factor, which is similar to the results of previous studies [8, 9, 16].

Rice et al. [11] found that patients with T2 ESCC had a sixfold increased chance of suffering from N1 disease compared with T1 patients. We speculated that LNM was closely related to the T2 substage in ESCC. In our cohort, LNM occurred in 124 (39%) patients with T2 longitudinal ESCC, which was slightly more than the 68 (31%) patients with T2 circular ESCC (P = 0.25). These results are consistent with those of a study by Christopher et al. (48% vs 32%, P = 0.16) [10]. With increasing tumor invasion depth, patients with T2 ESCC have a greater risk of LNM, but this finding does not suggest that T2 ESCC should be subclassified. However, the association between LNM and the T2 substage in previous studies was positive, and patients with T2 longitudinal ESCC had a higher incidence of positive lymph nodes than patients with T2 circular ESCC (52.2% vs 30.8%, 37.2% vs 19.0%, $P < 0.05$) [8, 9]. This apparent difference might be due to the size of the sample population. The fact that relatively few patients with T2 circular ESCC had positive lymph nodes in the

Table 3 Logistic regression analysis on the factors associated with lymph node metastasis

Characteristic	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
T2 substage (T2 circular/T2 longitudinal)[b]	1.40 (0.97–2.01)	0.07	1.32 (0.91–1.93)	0.15
Sex (male/female)	0.58 (0.38–0.88)	0.01*	0.63 (0.40–0.98)	0.04*
Age, year (<60/60)	0.86 (0.60–1.25)	0.44	0.86 (0.58–1.26)	0.43
Location (U/M/L)	1.73 (0.93–3.22)	0.04*	1.51 (0.79–2.89)	0.20
Length (<4 cm/4 cm)	1.51 (1.06–2.16)	0.02*	1.43 (0.99–2.06)	0.06
Comorbidity (yes/no)	1.21 (0.84–1.74)	0.31	1.48 (0.98–2.24)	0.06
Differentiation (G1/G2/G3)[a]	1.30 (0.84–2.02)	0.10	1.87 (1.13–3.11)	0.05*
Operation performed (L/R)	0.90 (0.56–1.44)	0.66	1.05 (0.64–1.72)	0.85
Postoperative hospital stay (<10 days/10 days)	0.90 (0.63–1.30)	0.58	0.98 (0.66–1.47)	0.93

U upper third, *M* middle third, *L* lower third, *L* left thoracic approach, *R* right thoracic approach, *OR* odds ratio, *CI* confidence interval

[a]The 8th edition of the UICC and AJCC cancer staging system

[b]The circular and longitudinal invasions were defined as T2 circular and T2 longitudinal stages

*$P < 0.05$

study by Duan et al. might also have caused this phenomenon. According to the logistic regression model, we further found that the T2 substage was not significantly correlated with LNM on univariate or multivariate analysis ($P > 0.05$), indicating that the depth of tumor invasion into the circular and longitudinal muscle layers in T2 ESCC did not affect LNM. In brief, clinicians are supposed to provide treatment or induction therapy protocols considering the high risk of LNM based on stage [17]. The relationship between LNM and the depth of tumor invasion requires more thorough research.

The optimal treatment strategy for T2 EC has not been determined [18]. The significance of stratifying patients with T2 ESCC is to identify differences between patients with T2 circular and T2 longitudinal ESCC and guide clinicians in selecting different treatment strategies for patients with various stages of disease. We found that patients with T1b ESCC had more favorable survival than those with T2 circular and T2 longitudinal ESCC and that the survival of patients with T2 circular ESCC was more similar to that of patients with T2 longitudinal ESCC than to that of patients with T1b ESCC ($P = 0.01$). This result emphasized that there was no significant reason to stratify the T2 stage into substages, which is very consistent with the authoritative principles and determination methods of the UICC and AJCC cancer staging system [7]. However, Duan et al. [9] demonstrated that the survival of patients with T2 circular ESCC was more similar to that of patients with T1b ESCC than to that of patients with T2 longitudinal ESCC ($P < 0.001$). Nevertheless, we could not determine prognostic value of the T2 substage from the above-mentioned evidence.

As reflected in our study, sex, age, differentiation, length, location, operation performed and postoperative length of hospital stay were not independent prognostic factors in patients with T2 substage ESCC ($P > 0.05$), while comorbidity was a prognostic factor ($P < 0.05$). We found that the presence of comorbidity significantly affected patient survival and was an independent prognostic factor of T2 ESCC. Thus, patients with comorbidities may have poor survival. Although tumor length is not a reference in the TNM staging system, Alexander et al. [19] showed that tumor length had a significant impact on prognosis in patients with EC. In our study, only tumor location was significantly different between stage T2 circular and T2 longitudinal EC, but it did not affect OS on univariate or multivariate analysis and could not serve as a reference for subclassifying T2 EC.

Several limitations to this study should be mentioned. Primarily, although this was a multicenter study with a large sample population, we could not exclude differences from other databases because our sample population was relatively small compared with the worldwide collaborative EC database. A study with a larger sample size may produce more accurate results. Second, our study was retrospective, with a relatively short follow-up duration and inherent error, and the results are less convincing than those of prospective studies. Longer follow-up durations should be used to obtain a more comprehensive five-year survival rate for more accurate survival information. Additionally, we did not analyze the accuracy of predictive clinical staging, which is more important and valuable in the treatment of patients. Finally, we did not evaluate recurrence or disease-free survival in our study, which are also important endpoints, especially regarding the outcomes of patients with malignant tumors.

In conclusion, the depth of tumor invasion into the circular and longitudinal muscle layers in T2 ESCC does not affect prognosis or LNM. It makes little sense to subclassify T2 ESCC by the depth of invasion into the circular and longitudinal muscle layers. Our study is consistent with the 8th edition of the TNM cancer staging system. Studies with more multicenter data, larger sample populations and longer follow-up periods are required to verify this conclusion.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Bray F, Ferlay J, Soerjomataram I et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68:394–424
2. Tian D, Mo SJ, Han LK et al (2018) Investigation of dietary factors and esophageal cancer knowledge: comparison of rural residents in high- and low-incidence areas. Sci Rep 8:4914
3. Zhang JQ, Hooker CM, Brock MV, et al (2012) Neoadjuvant chemoradiation therapy is beneficial for clinical stage T2 N0 esophageal cancer patients due to inaccurate preoperative staging. Ann Thorac Surg 93:429–435
4. Dolan JP, Kaur T, Diggs BS et al (2016) Significant understaging is seen in clinically staged T2N0 esophageal cancer patients undergoing esophagectomy. Dis Esophagus 29:320–325
5. Crabtree TD, Kosinski AS, Puri V et al (2013) Evaluation of the reliability of clinical staging of T2 N0 esophageal cancer: a

review of the society of thoracic surgeons database. Ann Thorac Surg 96:382–390
6. Rice TW, Mason DP, Murthy SC et al (2007) T2N0M0 esophageal cancer. J Thorac Cardiovasc Surg 133:317–324
7. Amin MB, Greene FL, Edge SB, et al (2017) The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin 67:93–99
8. Guo W, Xiao HL, Ma Z et al (2014) Should stage T2 esophageal squamous cell carcinoma be subclassified? Ann Surg Oncol 21:2540–2545
9. Duan J, Deng T, Ying G et al (2017) Prognostic significance of the T2 substage in patients with esophageal squamous cell carcinoma. Dis Esophagus 30:1–7
10. Seder CW, Mahon B, Hennon M et al (2018) Depth of muscularis propria invasion does not prognosticate survival in T2 esophageal adenocarcinoma. Anticancer Res 38:2195–2200
11. Rice TW, Zuccaro G Jr, Adelstein DJ et al (1998) Esophageal carcinoma: depth of tumor invasion is predictive of regional lymph node status. Ann Thorac Surg 65:787–792
12. Nentwich MF, von Loga K, Reeh M et al (2014) Depth of submucosal tumor infiltration and its relevance in lymphatic metastasis formation for T1b squamous cell and adenocarcinomas of the esophagus. J Gastrointest Surg Off J Soc Surg Aliment Tract 18:242–249
13. Endo M, Yoshino K, Kawano T et al (2000) Clinicopathologic analysis of lymph node metastasis in surgically resected superficial cancer of the thoracic esophagus. Dis Esophagus 13:125–129
14. Wagner TD, Khushalani N, Yang GY (2010) Clinical T2N0M0 carcinoma of thoracic esophagus. J Thorac Dis 2:36–42
15. Chen J, Liu S, Pan J et al (2009) The pattern and prevalence of lymphatic spread in thoracic oesophageal squamous cell carcinoma. Eur J Cardio Thorac Surg Off J Eur Assoc Cardio Thorac Surg 36:480–486
16. Schwarz RE, Smith DD (2007) Clinical impact of lymphadenectomy extent in resectable esophageal cancer. J Gastrointest Surg Off J Soc Surg Aliment Tract 11:1384–1393
17. Sancheti M, Fernandez F (2012) Management of T2 esophageal cancer. Surg Clin N Am 92:1169–1178
18. Martin JT, Worni M, Zwischenberger JB et al (2013) The role of radiation therapy in resected T2 N0 esophageal cancer: a population-based analysis. Ann Thorac Surg 95:453–458
19. Hollis AC, Quinn LM, Hodson J et al (2017) Prognostic significance of tumor length in patients receiving esophagectomy for esophageal cancer. J Surg Oncol 116:1114–1122

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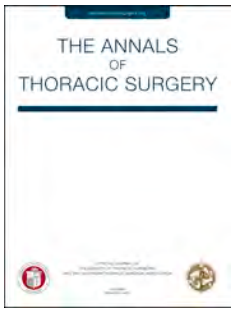

Lung volume reduction surgery: Only short-term evaluation is enough?

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Lung volume reduction surgery: Only short-term evaluation is enough?

To the Editor:

We read with great interest the article by Dr. Seadler et al. (1) assessing clinical and quality of life outcomes following lung volume reduction surgery (LVRS). This study demonstrated patients with emphysema refractory to medical therapy can benefit significantly from LVRS, which were consistent with a previous 10-year experienced study (2). For all we know, this study first conducted the correlation between the quality of life and pulmonary metrics, and assessed the feasible of using questionnaire as a potential substitute for actual pulmonary function testing. We congratulate Dr. Seadler et al. for this innovative and excellent study.

The following questions are proposed to win an opportunity to discuss with Dr. Seadler et al. Firstly, this study presents the long-term longitudinal data from 2007 to 2015. However, the follow-up outcomes were short-term. The National Emphysema Treatment Trial recommended two additional years of follow-up after LVRS provided valuable information regarding durability(3). Dr. Seadler et al. reported one-year results which may lead to lack of evidence for LVRS in this present study. Secondly, previous studies showed the forced expiratory volume in 1 second decreased gradually after attaining its maximal value at 3-6 months after operation with a subsequent decline toward preoperative levels (4) (5). However, this study only described the pulmonary functions of 1-year after LVRS without maximum or change trend of pulmonary functions, which may conduct an inaccuracy result.

Besides, a friendly reminding of typo in this manuscript is that the sum case number of “Smoking Pack year” in “Table 2” was equal to 120, not 121.

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References

1. Seadler B, Thuppal S, Rizvi N et al. Clinical and quality of life outcomes following lung volume reduction surgery. Ann Thorac Surg 2019.

2. Ginsburg ME, Thomashow BM, Bulman WA et al. The safety, efficacy, and durability of lung-volume reduction surgery: A 10-year experience. J Thorac Cardiovasc Surg 2016;151(3):717-724 e711.

3. Naunheim KS, Wood DE, Mohsenifar Z et al. Long-term follow-up of patients receiving lung-volume-reduction surgery versus medical therapy for severe emphysema by the national emphysema treatment trial research group. Ann Thorac Surg 2006;82(2):431-443.

4. J H, KE B, U S et al. Two years' outcome of lung volume reduction surgery in different morphologic emphysema types. The Annals of thoracic surgery 1999;68(5):1792-1798.

5. Weder W, Tutic M, Lardinois D et al. Persistent benefit from lung volume reduction surgery in patients with homogeneous emphysema. Ann Thorac Surg 2009;87(1):229-236; discussion 236-227.

Tumour size: a non-negligible prognostic factor for patients with thymoma

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Keywords: Thymoma • Tumour size • Prognostic factor

We read with great interest the well-written article by Okumura *et al.* [1] entitled 'Tumour size determines both recurrence-free survival and disease-specific survival after surgical treatment for thymoma'. This study demonstrated that tumour size in thymoma was a strong predictor of recurrence-free survival (RFS) and disease-specific survival (DSS), and should be considered when determining the treatment strategy for thymoma patients. Based on what we know, this was the first nationwide study that specifically evaluated the relationship between tumour size (maximum diameter) and prognosis only in thymoma. We congratulate Dr Okumura and his colleagues for this excellent and innovative study.

Tumour size as a prognostic factor can be found in lung cancer, breast cancer but has not been mentioned in current staging systems of thymic epithelial tumours. Data from International Thymic Malignancy Interest Group (ITMTG) found that tumour size was not associated with survival in patients with completely resected thymic tumours [2]. Similar results were obtained from the European Society of Thoracic Surgeons (ESTS) database, which demonstrated that tumour size was identified neither as a predictor of overall survival nor as a predictor of RFS, but as a predictor of incomplete resection and increased the risk of recurrence [3]. On the other hand, several studies have confirmed that tumour size is an important predictor of survival in thymic epithelial tumours. A review paper showed about 36% (4/11) of studies demonstrating that a smaller tumour can predict better survival [4]. Fukui *et al.* [5] found that patients with tumour size >4.0 cm showed worse RFS compared with those with a smaller size in completely resected thymic epithelial tumours. The study by Okumura *et al.* was in accordance with these studies which supported the finding that tumour size can affect the prognosis in patients with thymomas.

Controversy regarding these studies may arise from the different inclusion criteria. In Okumura *et al.*'s study, only thymoma was included which can prevent bias due to pathological heterogeneity. In addition, we also found that the definitions of RFS and DSS of this study were slightly different from the standard outcome measures for thymic malignancies which were developed by the ITMTG [6]. In the set of standards, RFS was defined as patients after successful curative treatment (R0 resection or radiographic chest X-ray after chemotherapy or radiotherapy), and DSS was defined as death from thymic malignancy, myasthenia gravis. In Okumura *et al.*'s study, RFS was defined as the time from surgery date to that of first recurrence after undergoing a macroscopic complete resection but not R0 resection. DSS was defined as the time from the surgery date to that of death from thymoma, but patients with myasthenia gravis were excluded. That may present differences between this study and results of ITMTG and ESTS on tumour size.

Taken together, we believe that tumour size is a significantly independent predictor of thymoma patients after surgical resection, and should remain a non-negligible clinical parameter in assessing surgically treated thymoma patients. As a result, it should be highlighted that routine assessment of tumour size should be incorporated into preoperative evaluation of thymoma patients.

Finally, as a friendly reminder, we want to point out that no '*P* value' was shown in Figure 4.

Conflict of interest: none declared.

REFERENCES

[1] Okumura M, Yoshino I, Yano M, Watanabe SI, Tsuboi M, Yoshida K *et al*. Tumour size determines both recurrence-free survival and disease-specific survival after surgical treatment for thymoma. Eur J Cardiothorac Surg 2019; doi:10.1093/ejcts/ezz001.

[2] Nicholson AG, Detterbeck FC, Marino M, Kim J, Stratton K, Giroux D *et al*. The IASLC/ITMIG Thymic Epithelial Tumors Staging Project: proposals for the T component for the forthcoming (8th) edition of the TNM classification of malignant tumors. J Thorac Oncol 2014;9:S73–80.

[3] Ruffini E, Detterbeck F, Van Raemdonck D, Rocco G, Thomas P, Weder W *et al*. Tumours of the thymus: a cohort study of prognostic factors from the European Society of Thoracic Surgeons database. Eur J Cardiothorac Surg 2014;46:361–8.

[4] Detterbeck F, Youssef S, Ruffini E, Okumura M. A review of prognostic factors in thymic malignancies. J Thorac Oncol 2011;6:S1698–704.

[5] Fukui T, Fukumoto K, Okasaka T, Kawaguchi K, Nakamura S, Hakiri S *et al*. Prognostic impact of tumour size in completely resected thymic epithelial tumours. Eur J Cardiothorac Surg 2016;50:1068–74.

[6] Huang J, Detterbeck FC, Wang Z, Loehrer PJ Sr. Standard outcome measures for thymic malignancies. J Thorac Oncol 2011;6:S1691–7.

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DISEASES OF THE ESOPHAGUS

ISDE The International Society for Diseases of the Esophagus

Original Article

Neoadjuvant chemotherapy with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable esophageal squamous cell carcinoma: a prospective nonrandomized trial for short-term outcomes

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SUMMARY. **Neoadjuvant chemotherapy (NAC) significantly extends survival in advanced esophageal squamous cell carcinoma (ESCC), but the short-term outcomes for cT4 ESCC remain controversial. Many NAC regimens have been previously reported, although no study has reported a regimen of irinotecan and nedaplatin for cT4 potential resectable ESCC. We evaluated the short-term outcomes of NAC with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable ESCC. A total of 51 patients with cT4 potentially resectable ESCC were eligible for this study. Twenty of these patients underwent NAC, and the other 31 patients underwent surgery alone. The toxicities and response of NAC were evaluated. The clinicopathologic characteristics, responses, toxicities, surgical outcomes, postoperative complications, and survival time between the two groups were analyzed. No significant differences were found in clinicopathologic characteristics between the groups ($P > 0.05$). The response rate of NAC was 75% (15/20). The differences in the long-axis diameter of the tumor and cT stage between pre- and post-NAC were significant ($P < 0.05$). Twenty-four toxic events occurred in 11 patients of the NAC group, and 20/24 of these were mild. The R0 resection rates in the NAC group and the surgery alone group were 85% and 64.5%, with no statistically significant difference ($P > 0.05$). Differences in the pathological T stage and pathological tumor-node-metastasis (TNM) stage were significant ($P < 0.05$). The overall survival (OS) time and mortality in the NAC group versus the surgery alone group were 31.57 ± 3.06 months versus 15.24 ± 1.46 months and 25% versus 61.3%, respectively. The differences in OS and mortality were significant ($P < 0.05$). The NAC group and R0 resection were significant and independent predictors of positive prognosis. NAC with irinotecan and nedaplatin in a single cycle followed by esophagectomy on cT4 resectable ESCC as a new NAC is safe and effective.**

KEY WORDS: **esophageal squamous cell carcinoma, irinotecan, nedaplatin, neoadjuvant chemotherapy, short-term outcome.**

INTRODUCTION

Esophageal cancer penetrates the esophageal wall and easily involves adjacent organs because no tunicae serosa is present.[1] Patients are usually diagnosed at an advanced stage due to this particular feature. An esophageal cancer involving adjacent structures (aorta, major airway, lung, diaphragm, pulmonary vein, pleural, and pericardium) is defined as T4 disease, which results in a poor R0 resection rate and survival time.[2-4]

Surgery alone may be performed in T4 patients, although its prognostic benefit and R0 resection rate remain dismal.[5-11] Matsubara *et al.*[12] concluded that patients with macroscopic-T4 but not pathologic-T4 tumors had favorable outcomes and that only patients with definitive evidence of unresectability should be excluded from esophagectomy. In addition, Tachibana *et al.*[13] and Chen *et al.*[14] demonstrated that esophagectomy of cT4 can achieve the best improvement in swallowing and the longest survival with an

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acceptable mortality rate. The optimal management for patients with potentially resectable cT4 esophageal squamous cell carcinoma (ESCC) remains unknown.

The overall survival (OS) time of cT4 ESCC has been improved by the development of multidisciplinary treatments, as reported in recent studies.[15-19] Many neoadjuvant chemotherapy (NAC) regimens have been reported in previous studies but were associated with different prognostic outcomes.[20-29]

Irinotecan has been administered as NAC to advanced esophageal cancer patients in previous trials. However, most of these treated cases were esophageal adenocarcinoma with a low pathologic complete response (pCR) rate. A response to this chemotherapy was found in ESCC, although the number of cases was limited.[30,31] We thus considered that the NAC regimen with irinotecan and nedaplatin would also be applicable in NAC regimens. Additionally, the short-term outcomes and treatment toxicities for cT4 ESCC remain controversial.

Most NAC requires two cycles of preoperative chemotherapy, but a single cycle has also conferred positive responses in some cases. Tumors rapidly develop resistance to chemotherapy, and responses are generally short lived.[32] Karagiannis *et al.*[33] showed that some NAC increases the risk of metastatic dissemination through a tumor microenvironment of metastasis (TMEM)-mediated mechanism, despite decreasing the tumor size. This may be due to drug resistance in NAC with lower doses or longer preoperative time intervals. Recently, Fujiwara *et al.*[34] compared the perioperative results and prognoses of patients who underwent complete (two cycles) or incomplete (single cycle) NAC because of adverse events or the patient's refusal of treatment. They found perioperative outcomes and long-term prognosis of patients with locally advanced ESCC were not significantly influenced, even if the patients did not receive a complete cycle of NAC. For these reasons, we hypothesized that one cycle of NAC as a pulse therapy may prevent drug resistance and the risk of metastatic dissemination. This one-cycle treatment may achieve a comparable effect to two or more NAC cycles because a similar total dose is used. If downstaging can be achieved by a single cycle, chemotherapy toxicities and relapse can be avoided.

In this study, we evaluated the short-term outcomes of a single cycle of irinotecan and cisplatin NAC followed by esophagectomy in cT4 potentially resectable ESCC patients.

PATIENTS AND METHODS

Patients

Between January 2014 and March 2017, 970 consecutive patients who were histologically diagnosed with ESCC and planned to undergo surgery presented at the Affiliated Hospital of North Sichuan Medical College. In total, 109 patients were defined as cT4 according to the TNM classification of the American Joint Committee on Cancer (AJCC) & The Union for International Cancer Control (UICC).[35] Of these 109 patients, 51 entered this prospective trial.

The criteria for inclusion of patients in this prospective trial were as follows: (1) ESCC in the thoracic esophagus, (2) cT4 according to the AJCC & UICC 8th edition classification, (3) expected survival time greater than 3 months, (4) general condition adequate to tolerate single-cycle NAC and/or esophagectomy, (5) evaluated as resectable esophageal cancer by pretreatment examinations, and (6) provided written informed consent.

Exclusion criteria were as follows: (1) received other treatment (chemotherapy/radiotherapy/palliative therapy) that may affect the prognosis or additional cycles of perioperative chemotherapy, (2) distant metastasis, (3) esophageal adenocarcinoma and esophagogastric junction carcinoma, (4) any concurrent primary cancer at other organs, (5) rejected operative surgery after NAC, (6) esophageal perforation or tracheoesophageal fistula, and (7) >80 years old.

The staging evaluation before treatment included the following procedures: (1) necessary general physical examination, (2) esophagogastroduodenoscopy (EGD) and biopsy, (3) contrast esophagography, (4) cervical and abdominal ultrasonography (US) and endoscopic ultrasonography (EUS), (5) contrast-enhanced computed tomography (CT) of the neck, chest, and upper abdomen, (6) bone scintigraphy, and 7) bronchoscopy performed only for the cancer in the upper or middle thoracic esophagus.

All patients were staged according to the AJCC & UICC criteria. cT4 was defined using contrast-enhanced CT, contrast esophagography, and bronchoscopy (upper or middle thoracic ESCC), and EUS. Lymphatic metastasis was assessed by morphology using cervical and abdominal US, EUS, and contrast-enhanced CT. Distant metastasis was determined using contrast-enhanced CT and bone scintigraphy. Esophageal cancer without distant metastasis invading the pleura, pericardium, diaphragm, and fat plane in the triangular space among the esophagus, aorta, and spine could be defined as a resectable cT4 tumor. However, if the aorta, trachea, and spine were invaded by esophageal cancer, surgery could not be performed.[35]

The excluded 58 patients included 20 with distant organ metastases, 14 who underwent previous chemotherapy and/or radiotherapy, 8 with a low performance status index for surgery or chemotherapy, 7 who were >80 years old, and 9 who refused esophagectomy after an active response to NAC.

This study was conducted with the approval of the Ethics Committee of the Affiliated Hospital of North Sichuan Medical College.

METHODS

Chemotherapy regimens

This was a prospective, nonrandomized trial that included patients who made an informed decision regarding whether to receive NAC followed by esophagectomy or esophagectomy alone. When patients decided to receive NAC followed by esophagectomy, they underwent the single cycle of NAC first. The chemotherapy regimen consisted of 120 mg/m^2 irinotecan (Jiangsu Hengrui Medicine Co., Ltd., Lianyungang, Jiangsu, P. R. China) on day 1, day 8, and day 15 for 3 hours as a drip intravenous infusion and 20 mg/m^2 nedaplatin (Qilu Pharmaceutical, Jinan, Shandong, P. R. China) administered intravenously from day 1 to day 5 for 1 hour combined with antiemetic and antimyelosuppression to alleviate toxicities. Then, patients in the NAC group received a subcutaneous injection of 6 mg PEG-rhG-CSF (Qilu Pharmaceutical, Jinan, Shandong, P. R. China) on day 15 to prevent myelosuppression. If the total cell counts of the bone marrow were $<$30,000/mm^3, surgery was delayed for 1 week or more. For patients with severe dysphagia, total parenteral nutrition was used, with or without additional oral administration of liquid nutrients. Soft or normal foods were given to patients if dysphagia improved. Examinations for staging evaluation before treatment were performed 4–6 weeks after NAC (before surgery).

Surgery

Esophagectomy was completed in both groups. NAC group patients underwent surgery 4–6 weeks after completing NAC. Before surgery, restaging evaluation was performed again, as performed prechemotherapy. Patients with esophageal cancer in the upper third of the thoracic esophagus underwent McKeown esophagectomy and were treated with three-field lymphadenectomy. The type of esophagectomy (Sweet, Ivor-Lewis, or McKeown esophagectomy) performed on middle third and lower third of the thoracic esophagus was at the discretion of the surgeon.

Clinical indexes

The database was queried to include all patients and the following variables: clinicopathologic characteristics, NAC-associated toxicities, postoperative complications, R0 resection, CR + PR and OS time. Survival time for all of the patients was calculated from the start of initial treatment until death from any cause or the final follow-up visit.

Evaluation of residual tumor (R) was classified as follows: R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; and R2, macroscopic residual tumor.[36] Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0 (NCI CTC v 4.0).[37]

Briefly, the responses were classified as follows: complete response (CR), complete disappearance of all clinical evidence of existing lesions during chemotherapy; partial response (PR), a decrease in tumor size of more than 30% during chemotherapy; progressive disease (PD), an increase in tumor size of more than 20% compared with the initial size; and stable disease (SD), any changes in tumor size that could be classified as neither a PR nor PD. Patients with a tumor showing a CR or PR were defined as major responders, and those with a tumor showing SD or PD were defined as nonresponders.[38]

Follow-up

Patients were followed up by monthly home visits or telephone interviews to determine their living conditions and to confirm they were alive. All of the patients were followed up until May 2017 or death. Patients were closely observed by general physical examination, contrast esophagography, cervical and abdominal US and EUS, contrast-enhanced CT of the cervical, chest and upper abdomen, and bone scintigraphy every 3 months and by EGD every 6 months after the surgery.

Statistical analysis

Follow-up data after treatment were available for all patients. Statistical analyses were performed with SPSS 22.0 (SPSS, Inc., Chicago, IL). Data were reported as the frequencies, means, and medians with percentages. The Chi-square test was used for comparison of the categorical variables. OS curves were plotted by the Kaplan-Meier method. Log-rank tests were applied to identify significant differences in survival among groups. We used the Cox proportional hazards model for multivariable OS analysis. Variables potentially related to the risk of OS with $P < 0.10$ on univariate analysis were included in the multivariate analysis. $P < 0.05$ was considered to indicate a statistically significant difference.

RESULTS

Clinicopathologic characteristics

Between January 2014 and March 2017, 51 patients were enrolled in this study. Twenty patients chose NAC with irinotecan and cisplatin in a single cycle followed by esophagectomy, and the other 31 patients

Table 1 Summary of patient clinicopathologic characteristics

Parameters	All patients ($N = 51$)	NAC ($N = 20$)	Surgery alone ($N = 31$)	P
Age (mean ± SD) year (range)	61.2 ± 6.57 (43–76)	60.3 ± 7.18 (43–69)	61.8 ± 6.20 (49–76)	0.44*
Gender				0.98**
Male	42(82.4%)	17(85%)	25(80.6%)	
Female	9(17.6%)	3(15%)	6(19.4%)	
Tumor localization				0.35**
Upper third	4(7.8%)	1(5%)	3(9.7%)	
Middle third	32(62.8%)	15(75%)	17(54.8%)	
Lower third	15(29.4%)	4(20%)	11(35.5%)	
Initial long-axis diameter of tumor (mean ± SD) cm (range)	5.3 ± 0.89 (3.5–8.0)	5.3 ± 0.95 (4–8)	5.2 ± 0.86 (3.5–7)	0.90*
BMI (mean ± SD) (range)	24.2 ± 6.57 (19.5–31.2)	24.2 ± 2.78 (19.5–31.2)		0.95*
Clinical N stage				0.33**
cN0	23(45.1%)	8(40.0%)	15(48.4%)	
cN1	17(33.3%)	9(45%)	8(25.8%)	
cN2	11(21.6%)	3(15%)	8(25.8%)	
cT4 invaded organs				0.66**
fat plane in triangular space†	17(33.3%)	6(30%)	11(35.5%)	
pleura	22(43.2%)	9(45%)	13(41.9%)	
pericardium	8(15.7%)	3(15%)	5(16.1%)	
diaphragm	4(7.8%)	2(10%)	2(6.5%)	

*Student's t test was used; **$\chi 2$ test or Fisher's exact test was used.
†The fat plane in the triangular space between the esophagus, aorta, and spine was obliterated.
BMI, body mass index; NAC, neoadjuvant chemotherapy.

underwent surgery alone. All NAC group patients completed the single-cycle regimen. All 51 patients had locally advanced potentially resectable cT4 SCC and underwent esophagectomy. Of the 51 patients, 42 (82.4%) were male, and 9 (17.6%) were female. The median patient age was 61.2 ± 6.57 years (range: 43–76 years). The tumor location of all patients was 4 (7.8%), 32 (62.8%), and 15 (29.4%) in the upper, middle, and lower third, respectively. The average length of the tumor before treatment was 5.25 ± 0.89 cm (range: 3.5–8.0 cm). The average body mass index (BMI) was 24.15 ± 2.68 (range: 19.49–31.22). No significant differences in gender distribution, age, tumor location, initial long-axis diameter of the tumor, and clinical N stage before treatment were observed between the NAC group and the surgery alone group ($P = 0.30$, $P = 0.46$, $P = 0.44$, $P = 0.44$, and $P = 0.09$, respectively) (Table 1).

Response to NAC

All of the NAC group patients underwent NAC with a single cycle of irinotecan and nedaplatin. Four to six weeks after the NAC regimen, there were 4 (20%) patients with CR, 11 (55%) patients with PR, and 5 (25%) patients with SD. None of the patients encountered PD. Accordingly, the response rate to the single-cycle NAC regimen was 75% (15/20). The long-axis diameter of the tumor before NAC was 5.3 ± 0.95 cm and 2.85 ± 2.05 cm 4–6 weeks after NAC. The differences in the long-axis diameter of the tumor and in the cT stage between pre-NAC and post-NAC were significant ($P < 0.05$). However, there was no significant difference in cN stage between pre-NAC and post-NAC ($P > 0.05$) (Table 2).

Toxicity

NAC with irinotecan and cisplatin in a single cycle was generally well tolerated. The overall toxicities experienced by the patients during chemotherapy are listed in Table 3. Twenty-four toxic events occurred in 11 patients (55%) of the NAC group and 20/24 of these were mild (grade 1–2). The toxic rates of leukopenia, nausea and vomiting, diarrhea, alopecia, and renal dysfunction were 35%, 30%, 40%, 5%, and 10%, respectively. The major toxicities were hematologic (leukopenia and neutropenia) and gastrointestinal reaction (nausea or/and vomiting, diarrhea), with 2 (10%) of the patients experiencing grade 3 or 4 leukopenia and neutropenia and 2 (10%) patients with grade 3 gastrointestinal reactions. All of the toxicities were within expectations and were manageable, and no treatment-related death occurred. No patient canceled their operation due to NAC toxicity.

Surgical outcome

As shown in Table 4, more than half of the patients in the NAC and surgery alone groups received the Ivor-Lewis or McKeown procedure (55% and 54.8%, respectively). Seventeen of the 20 patients (85%) in the NAC group received an R0 resection compared with 20 of the 31 patients (64.5%) in the surgery alone

Table 2 Response to NAC with irinotecan and nedaplatin in a single cycle

	Pre-NAC	Post-NAC	*P*
Long-axis diameter of tumor (mean ± SD) cm	(5.27 ± 0.95)	(2.85 ± 2.05)	0.00*
cT stage(%)†			0.00**
cT4	20(100%)	5(25%)	
Others	0(0%)	15(75%)	
cN stage(%)†			0.79**
cN0	8(40%)	10(50%)	
cN1	9(45%)	8(40%)	
cN2	3(15%)	2(10%)	

*Student's t test was used; ** $\chi 2$ test or Fisher's exact test was used.
†8th edition of the AJCC & UICC.
cN stage, clinical N stage; cT stage, clinical T stage; NAC, neoadjuvant chemotherapy.

Table 3 Toxicities experienced by the patients during NAC

	NCICTC version 4.0 common toxicity criteria					
	Grade 1	Grade 2	Grade 3	Grade 4	All grades (%)	Grade 3/4 (%)
Leukopenia	3	2	1	1	7(35%)	2(10%)
Nausea or vomiting	3	3	0	0	6(30%)	0(0%)
diarrhea	3	3	2	0	8(40%)	2(10%)
Alopecia	1	0	0	0	1(5%)	0(0%)
Renal dysfunction	1	1	0	0	2(10%)	0(0%)

NAC, neoadjuvant chemotherapy; NCICTC version 4.0, National Cancer Institute Common Toxicity Criteria, version 4.0.

group, and 50% and 48.4% of the patients had lymph node metastasis in the NAC group and the surgery alone group, respectively. The mean resected lymph nodes and metastatic lymph nodes in the NAC group versus the surgery alone group were 16.75 ± 3.63 versus 16.75 ± 3.63 and 1.55 ± 2.14 versus 1.55 ± 2.14, respectively. There were no significant differences in the type of esophagectomy, surgical radicality (R0 vs. R1 + R2), the nature of the lymph nodes, mean resected lymph nodes, mean metastatic lymph node, pathological N stage, or histopathological grading between the NAC group and surgery alone group ($P = 0.99$, $P = 0.20$, $P = 0.91$, $P = 0.12$, $P = 0.68$, $P = 0.88$ and $P = 0.99$, respectively). In addition, The R0 and R1 patients in the NAC group versus surgery group were 17 versus 20 and 2 versus 7, respectively. There was also no significant difference ($P = 0.27$) between the NAC group and the surgery group. However, the differences in pathological T stage and TNM stage between the NAC group and the surgery alone were significant ($P = 0.00$ and $P = 0.001$, respectively).

Postoperative complications

Nine postoperative events occurred in 5 patients (25%) of the NAC group, and 18 postoperative events occurred in 11 patients (35.5%) of the surgery alone group. The main complications in the two groups were infection complications (pyothorax, pneumonia, and surgical site infection), surgery-related complications (chylothorax, anastomotic leak, and recurrent nerve paralysis) and other complications (deep venous thrombosis and hypoproteinemia). There was no operative mortality patient in either group (postoperative within 30 days) but 1 (3.2%) hospital mortality (2 months after operation) patient in the surgery alone group. The patient died of anastomotic leak and pyothorax due to palliative resection (R2 resection). Regarding postoperative complications, the incidences of infection complications, surgery-related complications, and other complications for the NAC group versus the surgery alone group were 3 (15%) versus 7 (22.6%), 2 (10%) versus 5 (16.1%), and 4 (20%) versus 6 (19.4%), respectively. No differences were observed between the NAC and surgery alone groups (Table 5).

Short-term survival outcomes

With a median follow-up of 15.06 ± 8.52 months (range: 2–38 months), 24 (47.1%) out of 51 patients died of disease progression or postoperative complications, 5 (25%) in the NAC group, and 19 (61.3%) in the surgery alone group. To evaluate whether the NAC group and surgery alone group had different outcomes, the survival rates were compared. The median OS for all 51 patients was 22.89 ± 2.16 months. The OS was 31.57 ± 3.06 months in the NAC group versus 15.24 ± 1.46 months in the surgery alone group. The differences in mortality and OS between the NAC group and the surgery alone group were significant ($P = 0.01$ and $P = 0.001$, respectively). The Kaplan-Meier curves are shown in Figure 1.

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Table 4 Surgical outcome in the NAC group and surgery alone group

	NAC ($N = 20$)	Surgery alone ($N = 31$)	P
Type of esophagectomy			0.99*
Sweet (left chest)	9 (45%)	14 (45.2%)	
Ivor-Leiws or McKeown(right chest)	11 (55%)	17 (54.8%)	
Surgical radicality			0.11**
R0	17 (85%)	20 (64.5%)	
R1 + R2	3 (15%)†	11 (35.5%)‡	
Lymph node			0.91*
Node-negative cases(%)	10 (50%)	15 (48.4%)	
Node-positive cases(%)	10 (50%)	16 (51.6%)	
Mean resected lymph node	16.75 ± 3.63	14.32 ± 6.20	0.12**
Mean metastasis lymph node	1.55 ± 2.14	1.84 ± 2.58	0.68**
Pathological T stage§,¶			0.00*
pT0	4 (20%)	0 (0%)	
pT1	2 (10%)	0 (0%)	
pT2	7 (35%)	0 (0%)	
pT3	4 (20%)	6 (19.4%)	
pT4	3 (15%)††	25 (80.6%)‡‡	
Pathological N stage§,§§			0.88*
pN0	10 (50%)	15 (48.4%)	
pN1	6 (30%)	7 (22.6%)	
pN2	3 (15%)	7 (22.6%)	
pN3	1 (5%)	2 (6.4%)	
Pathological differentiation§			0.99*
Well differentiated	5 (25%)	8 (25.8%)	
Moderately differentiated	12 (60%)	18 (58.1%)	
Poorly differentiated	3 (15%)	5 (16.1%)	
Pathological stage§,¶¶			0.001*
pStage I	8 (40%)	0 (0%)	
pStage II	1 (5%)	1 (3.2%)	
pStage III	9 (45%)	17 (54.9%)	
pStage IV	2 (10%)	13 (41.9%)	

*$\chi 2$ test or Fisher's exact test was used; **Student's t test was used.
†Three patients were performed as R1/R2 resection due to pleura (1), pericardium (1), and diaphragm (1) invading and residual tumor existing, respectively; ‡Eleven patients were performed as R1/R2 resection due to aorta (6) and pleura (5) invading and residual tumor existing; §8th edition of the AJCC&UICC; ¶ypT for NAC group; ††Three patients were diagnosed as ypT4 due to pleura (1), pericardium (1) and diaphragm (1) invading, respectively; ‡‡patients were diagnosed as pT4 due to aorta (6), the fat plane in the triangular space among the esophagus, aorta and spine (7), pleura (10), pericardium (1) and diaphragm (1) invading, respectively; §§ypN for NAC group; ¶¶yp stage for NAC group.
NAC, neoadjuvant chemotherapy; R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; R2, macroscopic residual tumor.

Variables predicting short-term survival

According to the univariate analysis, treatment group ($P = 0.01$) and surgical radicality ($P = 0.00$) were significant prognostic factors. No significant differences in age, gender, long-axis diameter of the tumor, type of esophagectomy, postoperative complications, lymph node resection, lymph node metastasis, and BMI were observed ($P = 0.23$, $P = 0.57$, $P = 0.75$, $P = 0.51$, $P = 0.37$, $P = 0.36$, $P = 0.12$ and $P = 0.78$, respectively). The multivariate analysis also revealed that treatment group ($P = 0.007$) and surgical radicality ($P = 0.01$) were significant prognostic factors. There were no significant differences in age, gender, long-axis diameter of the tumor, type of esophagectomy, postoperative complications, lymph node resection, lymph node metastasis, and BMI ($P = 0.13$, $P = 0.10$, $P = 0.12$, $P = 0.06$, $P = 0.10$, $P = 0.73$, $P = 0.69$ and $P = 0.53$, respectively) (Table 6).

DISCUSSION

Esophageal cancer is one of the most aggressive and common cancers with a low 5-year survival rate after curative surgery.[39] To improve outcomes, current evidence supports the effects of NAC on patients with advanced esophageal cancer.[40] A recent study demonstrated that NAC improved R0 resection and OS compared with surgery alone, with a 12% decrease in the mortality hazard.[41] However, the optimal chemotherapy regimen for advanced esophageal cancer is also uncertain. Additionally, the results of the JCOG 9907[20] study aided in the approval of NAC with FP as a standard regimen in Japan. However, the response rate remained unsatisfactory at 38%.

One or more cycles of NAC were used in most previous studies with the expectation of more responders.[34,42] However, there were no significant differences in 5-year OS rate and median survival times

Table 5 Postoperative complications in the NAC group and surgery alone group

	NAC group ($n = 20$)	Surgery alone group ($n = 31$)	P^*
Total complications	5(25%)	11(35.5%)	0.43
Hospital mortality (> 30 days)	0	1(3.2%)	1.00
Infection complications	3(15%)	7(22.6%)	0.76
Pyothorax	0(0%)	2(6.5%)	
Pneumonia	2(10%)	3(9.7%)	
Surgical site infection	1(5%)	2(6.5%)	
Surgical-related complications	2(10%)	5(16.1%)	0.84
Chylothorax	0(0%)	1(3.2%)	
Anastomotic leak	1(5%)	2(6.5%)	
Recurrent nerve paralysis	1(5%)	2(6.5%)	
Other complications	4(20%)	6(19.4%)	1.00
DVT	1(5%)	2(6.5%)	
Hypoproteinemia	3(25%)	4(12.9%)	

*χ^2 test or Fisher's exact test was used.
DVT, deep venous thrombosis; NAC, neoadjuvant chemotherapy.

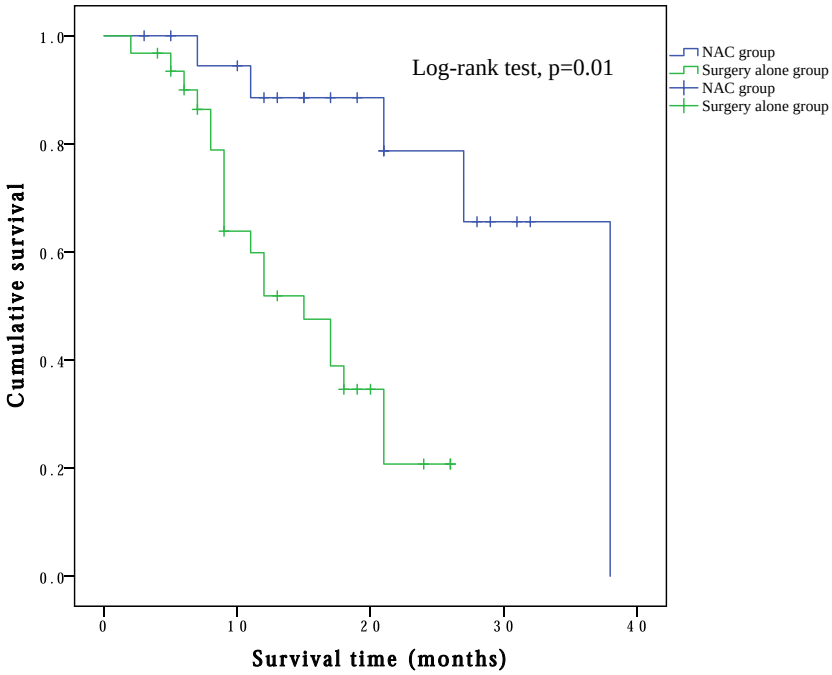

Fig. 1 Kaplan-Meier curves of NAC group and surgery alone group on survival outcomes. The mean overall survival period in NAC group and surgery alone group were (31.57 ± 3.06) months and (15.24 ± 1.46) months, respectively. The differences of OS between two groups were significant ($P < 0.05$)

between early and late responders.[15,42] Therefore, considering the waste of hospital costs and resources and the decreased toxicities, we used a single cycle NAC. In addition, tumors did not further progress during this shorter period.

Irinotecan (CPT-11, Camptosar), a semisynthetic camptothecin, is an inhibitor of the enzyme topoisomerase I. Irinotecan has emerged as a significant new chemotherapeutic agent with a broad spectrum of antitumor activity, including effectiveness against esophageal and gastric cancer.[30,43] Recently, irinotecan has often been preferred in advanced esophageal cancer as a preoperative chemoradiotherapy. Most of these cases were esophageal adenocarcinoma with a low pCR rate. A positive response was found in a limited number of ESCC cases in a previous trial.[44] Irinotecan plus a platinum-based chemotherapy regimen in advanced esophageal cancer corresponded to a total response rate of 57% (ESCC 66%), including a 6% complete response rate.[27] Nedaplatin (cis-diamine-glycolate platinum, CDGP) is a less nephrotoxic analog of CDDP, a second-generation platinum derivative that has shown potent antitumor activity against lung, testicular, esophageal,

Table 6 Univariate and multivariate analysis of prognostic factors according to OS

Prognostic factors	Univariate analysis		Multivariate analysis	
	HR(95%CI)	*P* value	HR(95%CI)	*P* value
Groups (NAC/Surgery alone)	0.211(0.061–0.730)	0.011	7.853(1.747–35.305)	0.007
Age (<60/≥60)	2.010(0.635–6.359)	0.232	0.445(0.155–1.274)	0.13
Gender (male/female)	0.661(0.155–2.813)	0.574	0.371(0.113–1.220)	0.10
Long-axis diameter of tumor (<5/≥5)	0.824(0.251–2.706)	0.749	0.396(0.123–1.277)	0.12
Type of esophagectomy (left/right chest)	1.455(0.480–4.409)	0.507	0.361(0.127–1.024)	0.06
Surgical radicality (R0/R1 + R2)	0.033(0.004–0.280)	0.000	6.157(1.457–26.024)	0.01
Postoperative complications (Yes/No)	0.583(0.177–1.924)	0.374	2.359(0.838–6.641)	0.10
Lymph node resection (<12/≥12)	1.917(0.460–7.831)	0.360	0.821(0.263–2.561)	0.73
Lymph node metastasis (+/−)	0.413(0.134–1.274)	0.121	1.327(0.325–5.412)	0.69
BMI (≤25/>25)	1.176(0.371–3.728)	0.782	0.529(0.742–1.882)	0.53

OS, overall survival; NAC, neoadjuvant chemotherapy; R0, no residual tumor; R1, suspicion of residual tumor or microscopic residual tumor; R2, macroscopic residual tumor.

gynecological, and head and neck cancers. Hydration is unnecessary for nedaplatin treatment.[45] Therefore, our regimen contained irinotecan and nedaplatin as a single cycle NAC with the expectation of better outcomes.

Clinicopathologic characteristics and response

In this study, there was no significant difference in the clinicopathologic characteristics before treatment between the NAC and surgery alone groups. The objective of NAC is to reduce the size of the primary lesion and control lymph node metastasis and micrometastasis to achieve downstaging so that a better outcome can be expected when surgical resection is performed.[46] In this study, the effective response rate was 75%. This rate was slightly higher than that reported in previous studies evaluating irinotecan-based regimens.[27,44] This may be due to the differences in cancer stage and details of the NAC regimen.

Four (20%) patients had T0N0M0 status, which was better than other NAC regimens.[21,28,47] However, there were no significant differences in pathological N and G status ($P > 0.05$). Motoori *et al.*[48] also reported a similar conclusion that there was no change in N stage after NAC. This finding may be partly attributed to the fact that we administered NAC to advanced ESCC patients mainly with clinically node-positive esophageal cancer. However, this protocol differed from other NAC regimens administered to locally advanced esophageal cancer patients with downstaging of either the T or N status.[29,45,49] The different responses between the T stage and the N stage may be due to the drug action mechanism and the characteristics of tumor invasion. The effects of the NAC regimen can also be assessed by the long-axis diameter of the tumor.[50] In this study, there was a significant difference between pre- and post-NAC on long-axis diameter of tumor, which showed the effective response of this NAC regimen ($P < 0.05$). Azria D *et al.*[51] obtained a significantly better prognosis in patients responding to NAC than nonresponders and surgery alone patients.

Toxicity

Although the outcomes of patients who received NAC were favorable, the major toxicities were hematologic (leukopenia and neutropenia), and gastrointestinal reactions (nausea or/and vomiting, diarrhea) and toxicities were major concerns. Frequencies of grade 3/4 leucopenia of 33.3% and of neutropenia of 90% were reported in other studies.[23,25,52–57] In our study, grade 3/4 toxicity developed in 4 (20%) of the 20 patients who underwent the single-cycle NAC. All of the toxicities were manageable, and none of the patients died of NAC-related causes. Our NAC regimen was notably milder than previous regimens, which may be due to the single cycle in our regimen and the prophylactic use of antiemetic and antimyelosuppression to alleviate toxicities.

Surgical outcome and complications

Previous studies demonstrated that NAC did not increase perioperative morbidity and mortality even in minimally invasive esophagectomy (MIE). The Medical Research Council Oesophageal Cancer Working Party reported that the total postoperative complication rates in NAC and surgery alone groups were 41% and 42%, respectively.[24,28,58,59] However, lower postoperative complication rates were observed in our study. Regarding the complication rates, there was no statistically significant difference between the two groups ($P > 0.05$). These results indicate that NAC with irinotecan and nedaplatin administered in a single cycle as a new NAC regimen followed by esophagectomy of cT4 resectable tumors is safe.

The rates of R0 resections in previous studies ranged from 76% to 100%.[60-62] These R0 resection rates were similar to that our study. Our NAC regimen achieved a satisfactory rate of 85% for R0 resection.

Chan *et al*.[63] reported that the R0 resection (curative resection) rate was about 80%, which was similar to that in our trial. Additionally, the Medical Research Council Oesophageal Cancer Working Party reported that the R0 resection rates in NAC and surgery alone groups were similar and did not significantly differ.[64] In our study, although the resection rate in the NAC group was better than that in the surgery alone group (85% vs. 64.5%), the difference between the rates was not significant ($P > 0.05$).

The mean numbers of lymph nodes sampled in the NAC group and the surgery alone group were less than those reported in a previous study.[65] The mean numbers of metastatic lymph nodes in our study had no significant difference between the NAC group and the surgery alone group ($P > 0.05$). Additionally, the number of cases of metastatic lymph nodes was greater than that reported in another study,[64] which reason may be due to different pathological stages.

Short-term survival outcomes

Our study results clearly showed that the NAC group and R0 resection were significant and independent predictors of positive prognosis in both univariate and multivariate analyses. According to the results of mortality and OS, there were better short-term survival outcomes in the NAC group than in the surgery alone group. The differences in mortality and OS between the NAC group and the surgery alone group were significant. Another study also showed a significant OS benefit for patients in the NAC group. The median OS time was 16 months in the NAC group compared with 12 months in the surgery alone group.[22] However, the results of a prospective randomized study in North America comparing NAC followed by surgery versus surgery alone showed no statistically significant differences in the median OS (14.9 months vs. 16.1 months).[65] The reason for these differences is unclear but may be due to different NAC regimens.

Another important prognostic factor was R0 resection, as shown in the univariate and multivariable analyses. Patients in the NAC group who underwent R0 resection demonstrated an improved survival time.[16,66–68] In our study, although the resection rate in the NAC group was better than that in the surgery alone group (85% vs. 64.5%), the difference between these rates was not significant ($P > 0.05$). This may be due to the limited number of cases in our study.

LIMITATIONS

Some inevitable limitations are present in this study. First, the sample size was considered small, although several comparisons reached statistical significance, which could be due to the time limitation and inclusion criteria. Further studies with larger sample sizes may lead to more accurate results. Second, the inclusion of patients from a single center limits the external generalizability of the results. Third, this study was not randomized. A randomized control trial could not be performed for patients with cT4 esophageal cancer because many patients refuse for their treatment to be determined randomly and because treatment arms are not always performed according to plan. We, therefore, allowed patients to choose whether to undergo NAC or surgery alone after informing the patients of the tumor staging, merits, and demerits of each treatment and the potential significance of the present trial. Finally, we did not evaluate the recurrence and disease-free survival in this study. As we know, it is also an important endpoint especially in the outcome of malignant tumor. Despite these limitations, this prospective nonrandomized study was based on a specific group of patients diagnosed with cT4 ESCC, and our treatments were protocol based, limiting potential bias.

CONCLUSIONS

NAC with irinotecan and nedaplatin in a single cycle as a new NAC regimen followed by esophagectomy on cT4 resectable ESCC is safe and effective.

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References

1 Shimada H, Shiratori T, Okazumi S *et al.* Have surgical outcomes of pathologic T4 esophageal squamous cell carcinoma really improved? Analysis of 268 cases during 45 years of experience. J Am Coll Surg 2008; 206: 48–56.
2 Tachimori Y, Ozawa S, Numasaki H *et al.* Comprehensive registry of esophageal cancer in Japan, 2009. Esophagus 2016; 13: 110–37.
3 Chak A, Canto M, Gerdes H *et al.* Prognosis of esophageal cancers preoperatively staged to be locally invasive (T4) by endoscopic ultrasound (EUS): a multicenter retrospective cohort study. Gastrointest Endosc 1995; 42: 501–6.
4 Gebski V, Burmeister B, Smithers B M *et al.* Survival benefits from neoadjuvant chemoradiotherapy or chemotherapy in oesophageal carcinoma: a meta-analysis. Lancet Oncol 2007; 8: 226–34.
5 Makino T, Doki Y. Treatment of T4 esophageal cancer. Definitive chemo-radiotherapy vs chemo-radiotherapy followed by surgery. Ann Thorac Cardiovasc Surg 2011; 17: 221–8.
6 Fujita H, Sueyoshi S, Tanaka T *et al.* Esophagectomy: is it necessary after chemoradiotherapy for a locally advanced T4 esophageal cancer? Prospective nonrandomized trial comparing

chemoradiotherapy with surgery versus without surgery. World J Surg 2005; 29: 25–31; discussion-1.
7 Brucher B L, Becker K, Lordick F *et al.* The clinical impact of histopathologic response assessment by residual tumor cell quantification in esophageal squamous cell carcinomas. Cancer 2006; 106: 2119–27.
8 Meguid R A, Hooker C M, Taylor J T *et al.* Recurrence after neoadjuvant chemoradiation and surgery for esophageal cancer: does the pattern of recurrence differ for patients with complete response and those with partial or no response? J Thorac Cardiovasc Surg 2009; 138: 1309–17.
9 Hsu F M, Lin C C, Lee J M *et al.* Improved local control by surgery and paclitaxel-based chemoradiation for esophageal squamous cell carcinoma: results of a retrospective non-randomized study. J Surg Oncol 2008; 98: 34–41.
10 Roof K S, Coen J, Lynch T J *et al.* Concurrent cisplatin, 5-FU, paclitaxel, and radiation therapy in patients with locally advanced esophageal cancer. J Radiat Oncol Biol Phys 2006; 65: 1120–8.
11 Donahue J M, Nichols F C, Li Z *et al.* Complete pathologic response after neoadjuvant chemoradiotherapy for esophageal cancer is associated with enhanced survival. Ann Thorac Surg 2009; 87: 392–9; discussion 8-9.
12 Matsubara T, Ueda M, Kokudo N, Takahashi T, Muto T, Yanagisawa A. Role of esophagectomy in treatment of esophageal carcinoma with clinical evidence of adjacent organ invasion. World J Surg 2001; 25: 279–84.
13 Tachibana M, Dhar D K, Kinugasa S *et al.* Surgical treatment for locally advanced (T4) squamous cell carcinoma of the thoracic esophagus. Dysphagia 2002; 17: 255–61.
14 Chen M F, Chen P T, Lu M S, Lee C P, Chen W C. Survival benefit of surgery to patients with esophageal squamous cell carcinoma. Sci Rep 2017; 7: 46139.
15 Akutsu Y, Kono T, Uesato M *et al.* Is the outcome of a salvage surgery for T4 thoracic esophageal squamous cell carcinoma really poor? World J Surg 2014; 38: 2891–7.
16 Shimoji H, Karimata H, Nagahama M, Nishimaki T. Induction chemotherapy or chemoradiotherapy followed by radical esophagectomy for T4 esophageal cancer: results of a prospective cohort study. World J Surg 2013; 37: 2180–8.
17 Pimiento J M, Weber J, Hoffe S E *et al.* Outcomes associated with surgery for T4 esophageal cancer. Ann Surg Oncol 2013; 20: 2706–12.
18 Seto Y, Chin K, Gomi K *et al.* Treatment of thoracic esophageal carcinoma invading adjacent structures. Cancer Sci 2007; 98: 937–42.
19 Kuroda J, Yoshida M, Kitajima M *et al.* Utility of preoperative chemoradiotherapy for advanced esophageal carcinoma. J Gastroenterol Hepatol 2012; 27: 88–94.
20 Ando N, Kato H, Igaki H *et al.* A randomized trial comparing postoperative adjuvant chemotherapy with cisplatin and 5-fluorouracil versus preoperative chemotherapy for localized advanced squamous cell carcinoma of the thoracic esophagus (JCOG9907). Ann Surg Oncol 2012; 19: 68–74.
21 Blum Murphy M A, Elimova E, Ajani J A. Current concepts and future potential in neoadjuvant chemotherapy for esophageal cancer. Expert Rev Gastroenterol Hepatol 2016; 10: 383–92.
22 Boonstra J J, Kok T C, Wijnhoven B P *et al.* Chemotherapy followed by surgery versus surgery alone in patients with resectable oesophageal squamous cell carcinoma: long-term results of a randomized controlled trial. BMC Cancer 2011; 11: 181.
23 Hara H, Tahara M, Daiko H *et al.* Phase II feasibility study of preoperative chemotherapy with docetaxel, cisplatin, and fluorouracil for esophageal squamous cell carcinoma. Cancer Sci 2013; 104: 1455–60.
24 Hirao M, Ando N, Tsujinaka T *et al.* Influence of preoperative chemotherapy for advanced thoracic oesophageal squamous cell carcinoma on perioperative complications. Br J Surg 2011; 98: 1735–41.
25 Hironaka S, Tsubosa Y, Mizusawa J *et al.* Phase I/II trial of 2-weekly docetaxel combined with cisplatin plus fluorouracil in metastatic esophageal cancer (JCOG0807). Cancer Sci 2014; 105: 1189–95.
26 Ilson D H. Phase II trial of weekly irinotecan/cisplatin in advanced esophageal cancer. Oncology (Williston Park) 2004; 18: 22–5.
27 Ilson D H, Saltz L, Enzinger P *et al.* Phase II trial of weekly irinotecan plus cisplatin in advanced esophageal cancer. J Clin Oncol 1999; 17: 3270–5.
28 Kelsen D P, Ginsberg R, Pajak T F *et al.* Chemotherapy followed by surgery compared with surgery alone for localized esophageal cancer. N Engl J Med 1998; 339: 1979–84.
29 Korst R J, Kansler A L, Port J L, Lee P C, Kerem Y, Altorki N K. Downstaging of T or N predicts long-term survival after preoperative chemotherapy and radical resection for esophageal carcinoma. Ann Thorac Surg 2006; 82: 480–5; discussion 4–5.
30 Ilson D H, Bains M, Kelsen D P *et al.* Phase I trial of escalating-dose irinotecan given weekly with cisplatin and concurrent radiotherapy in locally advanced esophageal cancer. J Clin Oncol 2003; 21: 2926–32.
31 Knox J J, Wong R, Visbal A L *et al.* Phase 2 trial of preoperative irinotecan plus cisplatin and conformal radiotherapy, followed by surgery for esophageal cancer. Cancer 2010; 116: 4023–32.
32 Janjigian Y Y, Ku G Y, Campbell J C *et al.* Phase II trial of cetuximab plus cisplatin and irinotecan in patients with cisplatin and irinotecan-refractory metastatic esophagogastric cancer. Am J Clin Oncol 2014; 37: 126–30.
33 Karagiannis G S, Pastoriza J M, Wang Y *et al.* Neoadjuvant chemotherapy induces breast cancer metastasis through a TMEM-mediated mechanism. Sci Transl Med 2017; 9: eaan0026.
34 Fujiwara Y, Lee S, Kishida S *et al.* Influence of incomplete neoadjuvant chemotherapy on esophageal carcinoma. Int J Clin Oncol 2018. doi: 10.1007/s10147-018-1291-6.
35 Rice T W, Gress D M, Patil D T, Hofstetter W L, Kelsen D P, Blackstone E H. Cancer of the esophagus and esophagogastric junction-Major changes in the American Joint Committee on Cancer eighth edition cancer staging manual. CA Cancer J Clin 2017; 67: 304–17.
36 Zagars G K, Ballo M T, Pisters P W, Pollock R E, Patel S R, Benjamin R S. Surgical margins and reresection in the management of patients with soft tissue sarcoma using conservative surgery and radiation therapy. Cancer 2003; 97: 2544–53.
37 Chen A P, Setser A, Anadkat M J *et al.* Grading dermatologic adverse events of cancer treatments: the Common Terminology Criteria for Adverse Events Version 4.0. J Am Acad Dermatol 2012; 67: 1025–39.
38 Karimata H, Shimoji H, Nishimaki T. Clinicopathological factors predicting R0 resection and long-term survival after esophagectomy in patients with T4 esophageal cancer undergoing induction chemotherapy or chemoradiotherapy. Surg Today 2015; 45: 479–86.
39 Mariette C, Finzi L, Fabre S, Balon J M, Van Seuningen I, Triboulet J P. Factors predictive of complete resection of operable esophageal cancer: a prospective study. Ann Thorac Surg 2003; 75: 1720–6.
40 Rigas J R, Dragnev K H, Bubis J A. Docetaxel in the treatment of esophageal cancer. Semin Oncol 2005; 32: 39–51.
41 Samson P, Robinson C, Bradley J *et al.* Neoadjuvant chemotherapy versus chemoradiation prior to esophagectomy: impact on rate of complete pathologic response and survival in esophageal cancer patients. J Thorac Oncol 2016; 11: 2227–37.
42 Chi Y K, Chen Y, Li X T, Sun Y S. Prognostic significance of the size and number of lymph nodes on pre and post neoadjuvant chemotherapy CT in patients with pN0 esophageal squamous cell carcinoma: a 5-year follow-up study. Oncotarget 2017; 8: 61662–73.
43 Ilson D H, Minsky B. Irinotecan in esophageal cancer. Oncology (Williston Park) 2003; 17: 32–6.
44 Lee M S, Mamon H J, Hong T S *et al.* Preoperative cetuximab, irinotecan, cisplatin, and radiation therapy for patients with locally advanced esophageal cancer. Oncologist 2013; 18: 281–7.
45 Takigawa N, Segawa Y, Ueoka H *et al.* Combination of nedaplatin and vindesine for treatment of relapsed or refractory non-small-cell lung cancer. Cancer Chemother Pharmacol 2000; 46: 272–8.
46 Ando N, Ozawa S, Kitajima M, Iizuka T. Chemotherapy and multimodality therapy in the treatment of esophageal cancer. Gan To Kagaku Ryoho 1995; 22: 869–76.
47 Law S, Fok M, Chow S, Chu K M, Wong J. Preoperative chemotherapy versus surgical therapy alone for squamous cell

carcinoma of the esophagus: a prospective randomized trial. J Thorac Cardiovasc Surg 1997; 114: 210–7.
48 Motoori M, Yano M, Yasuda T *et al.* Early response to neoadjuvant chemotherapy in advanced esophageal cancer evaluated by computed tomography predicts the utility of a second cycle of chemotherapy. Mol Clin Oncol 2013; 1: 521–6.
49 Hamai Y, Hihara J, Emi M *et al.* Results of neoadjuvant chemoradiotherapy with docetaxel and 5-Fluorouracil followed by esophagectomy to treat locally advanced esophageal cancer. Ann Thorac Surg 2015; 99: 1887–93.
50 Naritaka Y, Ogawa K, Shimakawa T *et al.* Case report: a young woman with advanced esophageal cancer showing pathological complete response to neoadjuvant chemotherapy (CDDP, 5-FU and ADM). Anticancer Res 2004; 24: 2385–9.
51 Azria D, Ychou M, Senesse P, Dubois J B. Neoadjuvant treatment of operable esophageal cancers. Gastroenterol Clin Biol 1999; 23: 1107–8.
52 Takahashi H, Arimura Y, Yamashita K *et al.* Phase I/II study of docetaxel/cisplatin/fluorouracil combination chemotherapy against metastatic esophageal squamous cell carcinoma. J Thorac Oncol 2010; 5: 122–8.
53 Osaka Y, Shinohara M, Hoshino S *et al.* Phase II study of combined chemotherapy with docetaxel, CDDP and 5-FU for highly advanced esophageal cancer. Anticancer Res 2011; 31: 633–8.
54 Yamasaki M, Miyata H, Tanaka K *et al.* Multicenter phase I/II study of docetaxel, cisplatin and fluorouracil combination chemotherapy in patients with advanced or recurrent squamous cell carcinoma of the esophagus. Oncology 2011; 80: 307–13.
55 Tamura S, Imano M, Takiuchi H *et al.* Phase II study of docetaxel, cisplatin and 5-fluorouracil (DCF) for metastatic esophageal cancer (OGSG 0403). Anticancer Res 2012; 32: 1403–8.
56 Ferri L E, Ades S, Alcindor T *et al.* Perioperative docetaxel, cisplatin, and 5-fluorouracil (DCF) for locally advanced esophageal and gastric adenocarcinoma: a multicenter phase II trial. Ann Oncol 2012; 23: 1512–7.
57 Watanabe M, Baba Y, Yoshida N *et al.* Outcomes of preoperative chemotherapy with docetaxel, cisplatin, and 5-fluorouracil followed by esophagectomy in patients with resectable node-positive esophageal cancer. Ann Surg Oncol 2014; 21: 2838–44.
58 Kranzfelder M, Schuster T, Geinitz H, Friess H, Buchler P. Meta-analysis of neoadjuvant treatment modalities and definitive non-surgical therapy for oesophageal squamous cell cancer. Br J Surg 2011; 98: 768–83.
59 Tanaka E, Okabe H, Tsunoda S *et al.* Feasibility of thoracoscopic esophagectomy after neoadjuvant chemotherapy. Asian J Endosc Surg 2012; 5: 111–7.
60 Teniere P, Hay J M, Fingerhut A, Fagniez P L. Postoperative radiation therapy does not increase survival after curative resection for squamous cell carcinoma of the middle and lower esophagus as shown by a multicenter controlled trial. French University Association for Surgical Research. Surg Gynecol Obstet 1991; 173: 123–30.
61 Pouliquen X, Levard H, Hay J M, McGee K, Fingerhut A, Langlois-Zantin O. 5-Fluorouracil and cisplatin therapy after palliative surgical resection of squamous cell carcinoma of the esophagus. Ann Surg 1996; 223: 127–33.
62 Ando N, Iizuka T, Ide H *et al.* Surgery plus chemotherapy compared with surgery alone for localized squamous cell carcinoma of the thoracic esophagus: a Japan clinical oncology group study–JCOG9204. J Clin Oncol 2003; 21: 4592–6.
63 Chan A C, Lee D W, Griffith J F *et al.* The clinical efficacy of neoadjuvant chemotherapy in squamous esophageal cancer: a prospective nonrandomized study of pulse and continuous-infusion regimens with cisplatin and 5-Fluorouracil. Ann Surg Oncol 2002; 9: 617–24.
64 Medical Research Council Oesophageal Cancer Working G. Surgical resection with or without preoperative chemotherapy in oesophageal cancer: a randomised controlled trial. Lancet North Am Ed 2002; 359: 1727–33.
65 Pennathur A, Luketich J D, Landreneau R J *et al.* Long-term results of a phase II trial of neoadjuvant chemotherapy followed by esophagectomy for locally advanced esophageal neoplasm. Ann Thorac Surg 2008; 85: 1930–7; discussion 6-7.
66 van Hagen P, Hulshof M C, van Lanschot J J *et al.* Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med 2012; 366: 2074–84.
67 Herskovic A, Russell W, Liptay M, Fidler M J, Al-Sarraf M. Esophageal carcinoma advances in treatment results for locally advanced disease: review. Ann Oncol 2012; 23: 1095–103.
68 Miyata H, Yamasaki M, Kurokawa Y *et al.* Clinical relevance of induction triplet chemotherapy for esophageal cancer invading adjacent organs. J Surg Oncol 2012; 106: 441–7.

EXPERIENCE FROM AN AMERICAN ASSOCIATION FOR THORACIC SURGERY FOUNDATION FOR THORACIC SURGERY TRAINING FELLOWSHIP: LUNG TRANSPLANTATION IN TORONTO GENERAL HOSPITAL

To the Editor:

Because I (D.T.) am extremely interested in lung transplantation, I applied for an American Association for Thoracic Surgery (AATS) Graham Foundation for Thoracic Surgery Training Fellowship. The goal of this fellowship is to provide an international training and educational experience for young thoracic surgeons from mainland China by having them spend a focused period of between 1 month and 1 year studying clinical techniques at a host training site in North America. Thanks to recommendation from Dr Hiroshi Date, chief of the thoracic surgery department at the Kyoto University Hospital, and acceptance by Dr Shaf Keshavjee, surgeon in chief of the University Health Network, I was fortunate to be selected by the AATS Graham Foundation to receive a Thoracic Surgery Training Fellowship, which took place with Dr Shaf Keshavjee at Toronto General Hospital (TGH) from January to March 2018.

TGH has a history of turning heparin, insulin, and pacemakers into world firsts. Beginning with the world's first successful lung transplant in 1983 and continuing with the first successful double-lung transplant in 1986,[1,2] the Lung Transplantation Program has completed more than 2000 lung transplants. In 2017 alone, about 170 lung transplants were completed at TGH, with only about 3% mortality. Dr Shaf Keshavjee has been part of many firsts of lung transplantation history at TGH. His development of a lung preservation solution that boosted the patient survival for single-lung transplants from 50% to more than 90%—is now a world standard. Another innovation of his team was the technique of ex vivo lung perfusion (EVLP), which allows lungs to be preserved at body temperature for 12 to 18 hours.[3] With the advent of EVLP, marginal donor lungs can be monitored and assessed individually to help transplant surgeons select lungs that are suitable for transplantation.[4] In February, the American Society of Transplantation awarded the Toronto Lung Transplant Program the American Society of Transplantation Innovation Award for 2018 for the clinical translation of EVLP.

I visited Dr Keshavjee in his office when I arrived at Toronto (Figure 1). He asked me many details about what I wanted to learn in TGH and gave me great encouragement and suggestions for my future research plan about lung transplantation. In addition, we talked about the current situation of lung transplantation in China. I felt that Dr Keshavjee was an example of just what my future aspirations are.

FIGURE 1. Shaf Keshavjee, MD, MSc, FRCSC, FACS (*left*), and Dong Tian, MD (*right*).

I started my fellowship when I left Dr Keshavjee's office. Studying in the Lung Transplantation Program, I experienced an unusually busy 2-month period. I shadowed Lung Transplantation Program fellows and observed about 5 lung retrievals (both donor after cardiac death and donor after brain death organs) and 15 single-lung and double lung transplants. I joined 20 meetings and lectures, looked around the medical surgical intensive care unit and general ward, observed EVLP procedures, and so on. I also visited the Latner Thoracic Surgery Research Laboratory, where I was able to observe the Lung Transplantation Program's translational research efforts involving animal lung transplantation. I gained a lot of knowledge of lung transplantation from staff surgeons and fellows during the 2- month experience. More importantly, I am more interested in lung transplantation than ever before. That enthusiasm will be important in my future time at The University of Tokyo Hospital as a PhD researcher.

In conclusion, I am very grateful to the AATS Graham Foundation for Thoracic Surgery Training Fellowship for providing me this great opportunity to visit TGH. I would like to point out that this fellowship really gave me so many wonderful treasures in TGH, and an unforgettable experience. I thank Dr Keshavjee and his lung

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transplantation team for this valuable learning opportunity and for giving me a lot of support during this fellowship.

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References

1. Cooper JD, Pearson FG, Patterson GA, Todd TR, Ginsberg RJ, Goldberg M, et al. Technique of successful lung transplantation in humans. *J Thorac Cardiovasc Surg*. 1987;93:173-81.
2. Patterson GA, Cooper JD, Goldman B, Weisel RD, Pearson FG, Waters PF, et al. Technique of successful clinical double-lung transplantation. *Ann Thorac Surg*. 1988;45:626-33.
3. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic ex vivo lung perfusion. *J Heart Lung Transplant*. 2008;27:1319-25.
4. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med*. 2011;364:1431-40.

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Preface: strategies to achieve long-term success of lung transplantation

Lung transplantation is now an established therapy used in patients suffering from respiratory failure. More than 4,000 lung transplants are reportedly performed every year worldwide (1), but achieving long-term success remains a major obstacle in the field. According to the registry data of the International Society for Heart and Lung Transplantation the median survival after lung transplantation increased from 4.7 years to 6.7 years in the last two decades (1). Although patient survival has improved over the years, most of the difference in survival is derived from the early post-transplant period. Long-term survival curves in different eras are almost parallel (1). This suggests that there have not been substantial improvements in long-term patient management, although there are many new concepts emerging in the field.

To produce this special issue of *Annals of Translational Medicine titled* "Strategies to achieve long-term success of lung transplantation", world experts in the field have generated comprehensive review articles in each specific area. The long-term success of lung transplantation depends primarily on appropriate patient selection, and Ainge-Allen and Granville have summarized all the important aspects of recipient selection in lung transplantation. Immunosuppression strategies play critical roles in protecting transplanted lungs from alloimmune responses after lung transplantation, and Chung and Dilling have contributed an updated appraisal of immunosuppression strategies. Surveillance and treatment of acute cellular rejection are critical components of maintenance, and this is discussed by Greer. Bery and Hachem provide insight into the role of antibody-mediated rejection, which has garnered increased attention recently, and related observations pertaining to immunosuppression increasing the risk of infection are also considered. Prophylaxis and management of bacterial, mycobacterial, and fungal infection in the context of lung transplantation are reviewed by various experts. Increased malignancy and its management are important aspects of post-lung transplantation in patients who subsequently undergo life-long immunosuppression, and this issue is reviewed by Shtraichman and Ahya. Importantly, alloantigen-independent injuries such as primary graft dysfunction and aspiration as well as infection activate innate immunity, which in turn provokes adaptive immune responses. Kawashima and Juvet have summarized the role of innate immunity after lung transplantation. Last but not least, chronic lung allograft dysfunction (CLAD) is a common final pathway of graft failure in the long term. Kotecha *et al*. have contributed an updated review of this important issue. Sato has summarized hypotheses that explain different phenotypes of CLAD, bronchiolitis obliterans syndrome, and restrictive allograft syndrome. We also included an original technical article on orthotopic rat lung transplantation, emphasizing the importance of basic research in the field. As guest editors of this special issue, we felt it was a tremendous opportunity to revisit the multiple complex issues surrounding lung transplantation via a collection of review articles undertaken by world experts.

In general, lung transplant recipients undergo more intensive immunosuppression than patents who receive other organs. This is because transplanted lung allografts are more vulnerable to various types of immune attack associated with innate and adaptive immunity. Notably however, intensive immunosuppression corresponds with increased susceptibility to various types of infection and malignancy. Lastly, multifactorial damage to allografted lungs ultimately drives their functional and mechanical deterioration, in the phenomenon now known as CLAD. Lung transplant recipients are destined to walk a tightrope with respect to the inevitable subtleties involved in balancing the risk of rejection with the risk of infection and other immunosuppression-related complications (*Figure 1A*). An important consideration in this regard is that alloimmune responses and infections or other alloantigen-independent graft injuries are not truly counter-directed, but rather their vector addition is directed toward graft failure or CLAD (*Figure 1B*).

True immunological tolerance has not yet been achieved in lung transplantation, and there is no "ace in the hole" for achieving long-term success in the field. Current best practice incorporates meticulous control of details, and if there is an emerging source of potential graft dysfunction, counteracting it while its magnitude remains small. This special issue of *Annals of Translational Medicine* is intended to cover the diverse array of potential sources of long-term lung allograft dysfunction and/or death. It is also aimed at encouraging lung transplant physicians to react promptly to ominous signs of events associated with lung allograft failure. Meticulous attitudes to patient care minimize the magnitude of such events, helping the graft to remain stable or "silent" in the long term. We believe such proactive patient management to maintain a

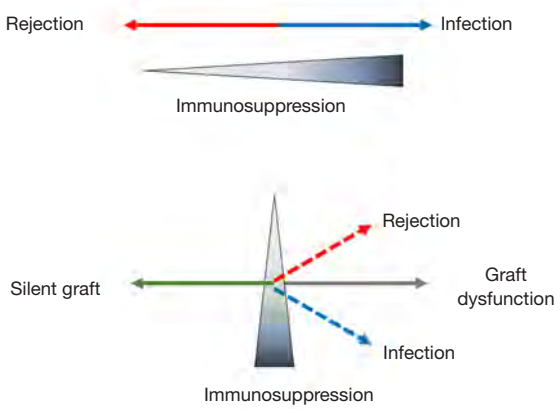

Figure 1 Conventional and new paradigms of lung allograft stability. (A) A depiction of the conventional paradigm involving the inverse relationships between immunosuppression and graft rejection and infection. (B) A new paradigm in which both rejection and infection direct the graft toward failure, but meticulous management by lung transplant physicians may counteract it.

silent graft is the ultimate key to achieving long-term success of lung transplantation.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

References

1. Chambers DC, Cherikh WS, Harhay MO, et al. The International Thoracic Organ Transplant Registry of the International Society for Heart and Lung Transplantation: Thirty-sixth adult lung and heart-lung transplantation Report-2019; Focus theme: Donor and recipient size match. J Heart Lung Transplant 2019;38:1042-55.

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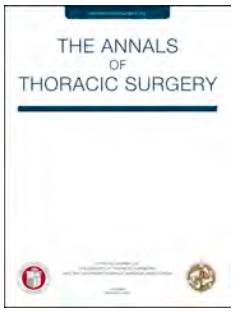

Length of ICU stay: an unneglectable risk factor for postoperative delirium

Dong Tian, MD, Yu Wang, MD, Long-Qi Chen, MD

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Length of ICU stay: an unneglectable risk factor for postoperative delirium

To the Editor:

We read with great interest the article by Dr. Fuchita et al. (1) assessing perioperative risk factors for postoperative delirium among esophagectomy patients by a randomized, double-blind single center clinical trial. This study demonstrated length of ICU stay was the only risk factor for postoperative delirium in patients undergoing esophagectomy.

The following questions are proposed to win an opportunity to discuss with Dr. Fuchita. Firstly, the type of surgery was divided as “Ivor-Lewis” and “other”. However, Jeong et al.(2) evaluated open and minimally invasive approaches and concluded a reduction in the incidence of postoperative delirium by 0.55 times from the use of a minimally invasive approach. We wonder the proportion of minimally invasive approach cases in this study. Secondly, persistent intense thirst was associated with delirium in the ICU(3). If the degree of thirst in patients was considered in this study, it would be a better explanation for the results. Thirdly, previous study demonstrated length of mechanical ventilation, ICU stay and hospital stay were closely related(4). Results from this study showed patients who developed postoperative delirium had longer mechanical ventilation days, ICU days and hospital days in univariate analysis. In multivariate analysis, only ICU length of stay was found to have significant association with postoperative delirium. We hypothesize Dr. Fuchita et al. may include the three variables above together in logistic regression analyses which may affect the statistic results because of closely internal relations among the three variables. In addition, no complete logistic regression analyses result was shown in a Table form. Although the mechanical ventilation days and hospital stays were not the risk factors of postoperative delirium in logistic regression analyses, authors should show all the variables they included.

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References:

1. Fuchita M, Khan SH, Perkins AJ et al. Perioperative risk factors for postoperative delirium in patients undergoing esophagectomy. Ann Thorac Surg 2019.

2. Jeong DM, Kim JA, Ahn HJ, Yang M, Heo BY, Lee SH. Decreased incidence of postoperative delirium in robot-assisted thoracoscopic esophagectomy compared with open transthoracic esophagectomy. Surg Laparosc Endosc Percutan Tech 2016;26(6):516-522.

3. Sato K, Okajima M, Taniguchi T. Association of persistent intense thirst with delirium among critically ill patients: A cross-sectional study. J Pain Symptom Manage 2019.

4. Lanuti M, de Delva PE, Maher A et al. Feasibility and outcomes of an early extubation policy after esophagectomy. Ann Thorac Surg 2006;82(6):2037-2041.

日中笹川医学奨学金制度(学位取得コース)評価書
課程博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　研究者番号：G4005　作成日　：2020年3月9日

氏　名	張 春東	ZHANG CHUNDONG	性別	M	生年月日　1987.04.27
所属機関（役職）	中国医科大学附属第四医院　胃腸外科（主治医師、講師）				
研究先（指導教官）	東京大学大学院医学系研究科　消化管外科学・乳腺内分泌外科学（瀬戸泰之　教授）				
研究テーマ	食道胃接合部がんの起源組織を予測するDNAメチル化マーカーの開発				
専攻種別	□論文博士		☑課程博士		

研究者評価（指導教官記入欄）

成績状況	良	取得単位数 取得単位数22 ／取得すべき単位総数30
学生本人が行った研究の概要	**1) 目的 (Goal)** In this study, we aimed to identify DNA methylation markers to distinguish gastric adenocarcinomas (GACs) and esophageal adenocarcinomas (EACs). **2) 戦略 (Approach)** Identifying tissue origin of adenocarcinomas of esophagogastric junction (AEJ) (especially Siewert type II) is important. DNA methylation is considered to be useful for identifying tissue of origin. **3) 材料と方法 (Materials and methods)** Genome-wide DNA methylation data of 48 GACs and 48 EACs were utilized. Fifteen GACs and 15 EACs were used for first screening, and independent 15 GACs and 15 EACs were used for second screening. The predictive powers of isolated markers were validated using an independent 18 GACs and 18 EACs. We further validated the predictive power of these markers in clinical samples of EACs and GACs. **4) 実験結果 (Results)** In the first screening set, 62 of 485,512 genomic regions were methylated in GACs (≥ 5) but unmethylated in all the 15 EACs. Among them, 42 regions were unmethylated in normal and Barrett's esophagus. In the second screening set, 3 of the 42 regions were differentially methylated and a combination of the latter two loci had good sensitivity and specificity. The predictive power of the combination was validated (sensitivity, 61%; specificity, 100%; accuracy, 81%). On the other hand, two genomic regions were specifically methylated in EAC in the first and second screening. Their combination was validated (sensitivity, 100%; specificity, 94%; accuracy, 97%). Finished works as follows: (1). Designed biotin-primers of the 4 markers for pyrosequencing, and assess the quality; (2). Collected FFPE samples of 7 GACs and 7 EACs from UT hospital; (3). Estimated cancer cell fractions using HE staining samples of 7 GACs and 7 EACs; (4). Isolated and purified DNA from FFPE samples of the 7 GACs and 7 EACs; (5). Bisulfite treated the purified DNA of 7 GACs and 7 EACs. **5) 考察 (Discussion)** These data indicated that these markers are useful identifying tissue origin of AEJ. We continued to collected more FFPE slides of GACs and EACs. These methylation markers are being shifted to pyrosequencing using cancer cell lines and olinical samples.	
総合評価	【良かった点】 本研究は臨床的にも重要な課題であり、解決すべきものであります。よくデザインされた研究計画で、また適格に遂行されていると考えます。	
	【改善すべき点】 食道扁平上皮癌においても、DNA methylation level を測定すべきと考えます。	
	【今後の展望】 マーカーとしてのDNA methylationが多くの臨床検体で評価されることが重要です。	
学位取得見込	張春東君は3年以内に取得できると考えます。	
評価者（指導教官名）　瀬戸　泰之　㊞		

日中笹川医学奨学金制度(学位取得コース)報告書
研究者用

Supported by 日本財団 THE NIPPON FOUNDATION

第40期　　研究者番号：G4005　　作成日：2020年3月5日

氏名	ZHANG　CHUNDONG	張 春東	性別	M	生年月日　1987.04.27
所属機関(役職)	中国医科大学　附属第四医院　胃腸外科　(主治医師 / 講師)				
研究先(指導教官)	東京大学大学院　医学系研究科　消化管外科学・乳腺内分泌外科学　(瀬戸　泰之　教授)				
研究テーマ	食道胃接合部がんの起源組織を予測するDNAメチル化マーカーの開発 Prediction of tissue origin of adenocarcinomas of esophagogastric junction by DNA methylation				
専攻種別	論文博士	☐	課程博士	☑	

1.研究概要

1) 目的 (Goal) In this study, we aimed to identify DNA methylation markers to distinguish gastric adenocarcinomas (GACs) and esophageal adenocarcinomas (EACs).

2) 戦略 (Approach) Identifying tissue origin of adenocarcinomas of esophagogastric junction (AEJ) (especially Siewert type II) is important. DNA methylation is considered to be useful for identifying tissue of origin.

3) 材料と方法 (Materials and methods) Genome-wide DNA methylation data of 48 GACs and 48 EACs were utilized. Fifteen GACs and 15 EACs were used for first screening, and independent 15 GACs and 15 EACs were used for second screening. The predictive powers of isolated markers were validated using an independent 18 GACs and 18 EACs. We further validated the predictive power of these markers in clinical samples of EACs and GACs.

4) 実験結果 (Results) In the first screening set, 62 of 485,512 genomic regions were methylated in GACs (≥ 5) but unmethylated in all the 15 EACs. Among them, 42 regions were unmethylated in normal and Barrett's esophagus. In the second screening set, 3 of the 42 regions were differentially methylated and a combination of the latter two loci had good sensitivity and specificity. The predictive power of the combination was validated (sensitivity, 61%; specificity, 100%; accuracy, 81%). On the other hand, two genomic regions were specifically methylated in EAC in the first and second screening. Their combination was validated (sensitivity, 100%; specificity, 94%; accuracy, 97%).

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(1). Designed biotin-primers of the 4 markers for pyrosequencing, and assess the quality;
(2). Collected FFPE samples of 7 GACs and 7 EACs from UT hospital;
(3). Estimated cancer cell fractions using HE staining samples of 7 GACs and 7 EACs;
(4). Isolated and purified DNA from FFPE samples of the 7 GACs and 7 EACs;
(5). Bisulfite treated the purified DNA of 7 GACs and 7 EACs.

5) 考察 (Discussion) These data indicated that these markers are useful identifying tissue origin of AEJ. We continued to collected more FFPE slides of GACs and EACs. These methylation markers are being shifted to pyrosequencing using cancer cell lines and clinical samples.

6) 参考文献 (References)

[1] Sturm D, Orr BA, Toprak UH, et al. New Brain Tumor Entities Emerge from Molecular Classification of CNS-PNETs. Cell. 2016; 164(5): 1060-1072.
[2] Moran S, Martínez-Cardús A, Sayols S, et al. Epigenetic profiling to classify cancer of unknown primary: a multicentre, retrospective analysis. Lancet Oncol. 2016; (10): 1386-1395.
[3] Yamashita S, Nanjo S, Rehnberg E, et al. Distinct DNA methylation targets by aging and chronic inflammation: a pilot study using gastric mucosa infected with Helicobacter pylori. Clin Epigenetics. 2019; 11(1): 191.
[4] Yoda Y, Takeshima H, Niwa T, et al. Integrated analysis of cancer-related pathways affected by genetic and epigenetic alterations in gastric cancer. Gastric Cancer. 2015; 18(1): 65-76.
[5] Mazer LM, Poultsides GA. What Is the Best Operation for Proximal Gastric Cancer and Distal Esophageal Cancer? Surg Clin North Am. 2019; 99(3): 457-469.
[6] Zong L, Hattori N, Yoda Y, et al. Establishment of a DNA methylation marker to evaluate cancer cell fraction in gastric cancer. Gastric Cancer. 2016; 19(2): 361-369.
[7] Holm K, Hegardt C, Staaf J, et al. Molecular subtypes of breast cancer are associated with characteristic DNA methylation patterns. Breast Cancer Res. 2010; 12(3): R36.

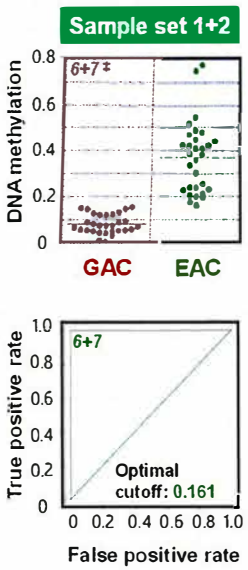
A Combination Marker of EAC-specific Methylation was Screened and Validated

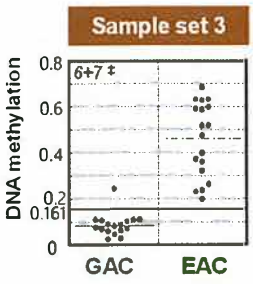
Evaluations of covering ratios by combinations of individual markers

Sample set 1+2

Gene	1	2	3	4	5	6	7	8
1	56.7							
2	73.3	73.3						
3	76.7	76.7	76.7					
4	63.3	76.7	80.8	63.3				
5	73.3	76.7	80.0	73.3	70.0			
6	73.3	80.0	80.0	76.7	83.3	66.7		
7	80.0	83.3	86.7	83.3	83.3	90.0	80.0	
8	56.7	73.3	76.7	73.3	73.3	70.0	80.0	80.0

Combination marker 6+7 has the highest covering ratio of 90% in sample set 1+2

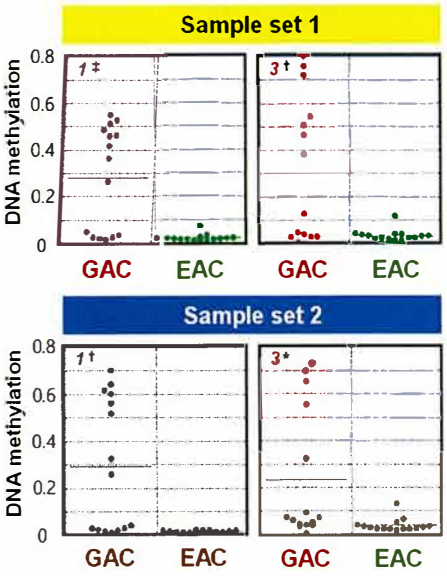


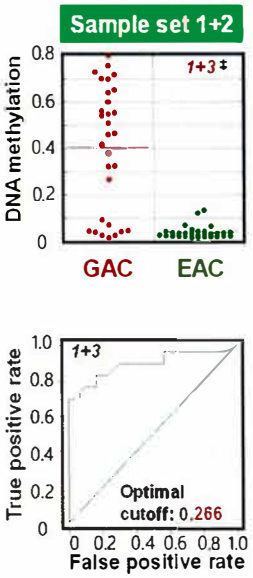


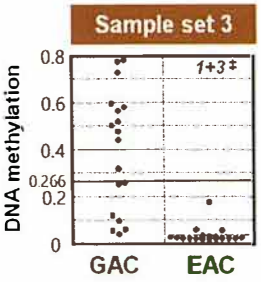
Sensitivity 100%
Specificity 94%
Accuracy 97%

* p <0.05, † p <0.01, ‡ p <0.001

A Combination Marker of GAC-specific Methylation was Screened and Validated



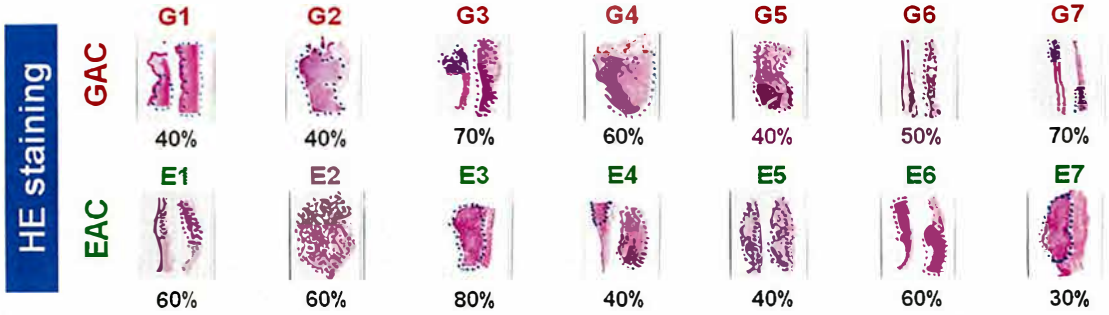




Sensitivity 61%
Specificity 100%
Accuracy 81%

* p <0.05, † p <0.01, ‡ p <0.001

Cancer cell fraction



2. 執筆論文 Publication of thesis ※記載した論文 を添付してください。Attach all of the papers listed below.

論文 名 1 Title	Reevaluation of laparoscopic versus open distal gastrectomy for early gastric cancer in Asia: a meta-analysis of randomized controlled trials					
掲載誌 名 Published journal	*Int J Surg* (IF=3.158) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*					
	2018 年 5 月	56 巻(号)	31 頁 ～ 43 頁	言語 Language	English	
第1著者 名 First author	Chun-Dong Zhang	第2著者 名 Second author	Hiroharu Yamashita	第3著者 名 Third author	Shun Zhang	
その他著者 名 Other authors	Yasuyuki Seto					
論文 名2 Title	Lymphovascular invasion as a predictor for lymph node metastasis and a prognostic factor in gastric cancer patients under 70 years of age: A retrospective analysis					
掲載誌 名 Published journal	*Int J Surg* (IF=3.158)					
	2018 年 5 月	53 巻(号)	214 頁 ～ 220 頁	言語 Language	English	
第1著者 名 First author	Chun-Dong Zhang	第2著者 名 Second author	Fei-Long Ning	第3著者名 Third author	Xian-Tao Zeng	
その他著者 名 Other authors	Dong-Qiu Dai					
論文 名3 Title	Historical Background and Perspective of Gastric Cancer Surgery: West versus Japan					
掲載誌 名 Published journal	*Ann Transl Med* (IF=3.689) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*					
	2019 年 9 月	7(18) 巻(号)	493 頁 ～ 頁	言語 Language	English	
第1著者 名 First author	Chun-Dong Zhang	第2著者 名 Second author	Hiroharu Yamashita	第3著者名 Third author	Yasuyuki Seto	
その他著者 名 Other authors						
論文 名 4 Title	Comparison of Different Lymph Node Staging Schemes in Patients with Resectable Colorectal Cancer					
掲載誌 名 Published journal	*Front Oncol* (# Co-first author) (IF=4.137) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*					
	2019 年 1 月	8 巻(号)	671 頁 ～ 頁	言語 Language	English	
第1著者 名 First author	Jun-Peng Pei #	第2著者 名 Second author	Chun-Dong Zhang #	第3著者名 Third author	Yu-Chen Fan	
その他著者 名 Other authors	Dong-Qiu Dai					
論文 名5 Title	Safety and Oncological Outcomes of Laparoscopic NOSE Surgery Compared with Conventional Laparoscopic Surgery for Colorectal Diseases: A Meta-Analysis					
掲載誌 名 Published journal	*Front Oncol* (# Co-first author) (IF=4.137) *Zhang CD was supported in part by Japan China Sasakawa Medical Fellowship.*					
	2019 年 7 月	9 巻(号)	597 頁 ～ 頁	言語 Language	English	
第1著者 名 First author	Rui-Ji Liu #	第2著者 名 Second author	Chun-Dong Zhang #	第3著者名 Third author	Yu-Chen Fan	
その他著者 名 Other authors	Jun-Peng Pei, Cheng Zhang, Dong-Qiu Dai					

3. 学会発表　Conference presentation　※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載

※Describe your presentation as the principal presenter in major academic meetings including general meetings or

学会名 Conference	The 30th Annual Meeting of the Japanese Society for Gastroenterological Carcinogenesis (JSGC)				
演　題 Topic	Prediction of Tissue Origin of Adenocarcinomas of Esophagogastric Junction by DNA Methylation				
開催日 date	2019　年　11　月　8　日	開催地 venue	Yokohama, Japan		
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter	Hideyuki Takeshima, Yasuyuki Seto, Toshikazu Ushijima				
学会名 Conference	日本消化器癌発生学会(JSGC)特別研究推進，理事長直轄プロジェクト「発癌・進展におけるゲノム・エピゲノムの最新のトピックス（仮）」				
演　題 Topic	食道胃接合部がんの起源細胞を予測するDNAメチル化マーカーの開発				
開催日 date	2020　年　2　月　15　日	開催地 venue	Tokushima, Japan		
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter	Hideyuki Takeshima, Yasuyuki Seto, Toshikazu Ushijima				
学会名 Conference	The 92th Annual Meeting of the Japanese Gastric Cancer Association (JGCA)				
演　題 Topic	Prediction of Tissue of Origin of Adenocarcinomas of Esophagogastric Junction by DNA Methylation				
開催日 date	2020　年　3　月　5　日	開催地 venue	Yokohama, Japan		
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter	Hideyuki Takeshima, Yasuyuki Seto, Toshikazu Ushijima				
学会名 Conference					
演　題 Topic					
開催日 date	年　月　日	開催地 venue			
形式 method	☐ 口頭発表 Oral ☐ ポスター発表 Poste	言語 Language	☐ 日本語	☐ 英語	☐ 中国語
共同演者名 Co-presenter					

4. 受賞（研究業績 Award (Research achievement)

名　称 Award name				
	国名 Country		受賞年 Year of	年　月
名　称 Award name				
	国名 Country		受賞年 Year of	年　月

研究者番号：G4005

5．本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	University of Tokyo
助成金名称 Grant name	University of Tokyo Grants for Ph.D. Research (No. 41197489)
受給期間 Supported period	2019 年 8 月 ～ 2020 年 1 月
受給額 Amount received	50,000/month 円
受給実績 Receipt record	□ 有　□ 無
助成機関名称 Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6．他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	■ 有　□ 無
助成機関名称 Funding agency	China Scholarship Council
奨学金名称 Scholarship name	China Scholarship Council (Chinese Government Scholarship) (No. 201908050148)
受給期間 Supported period	2019 年 7 月 ～ 2022 年 6 月
受給額 Amount received	170,000/month 円

7．研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。 Attach a copy of the article described below

報道発表 Press release	□ 有　□ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞　・雑誌　・Web site　・記者発表　・その他（　　）		
発表タイトル Released title			

8．本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled application	□ 有　□ 無	出願国 Application country	
出願内容（概要） Application contents			

9．その他 Others

指導責任者（署名）

International Journal of Surgery 56 (2018) 31–43

Contents lists available at ScienceDirect

International Journal of Surgery

journal homepage: www.elsevier.com/locate/ijsu

Review

Reevaluation of laparoscopic versus open distal gastrectomy for early gastric cancer in Asia: A meta-analysis of randomized controlled trials

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[b] Department of Gastrointestinal Surgery, The Fourth Affiliated Hospital of China Medical University, Shenyang, Liaoning, China
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ARTICLE INFO

Keywords:
Laparoscopy
Early gastric cancer
Gastrectomy
Meta-analysis

ABSTRACT

Background: The benefits and risks of laparoscopic distal gastrectomy (LADG) are not yet sufficiently clear for acceptance as a standard treatment of early gastric cancer. Previous meta-analyses were not powered to reach definitive conclusions.

Materials and Methods: Randomized controlled trials comparing LADG with open distal gastrectomy (ODG) for early gastric cancer in Asia and published between January 1994 and January 2018 were retrieved from PubMed, Embase, the Cochrane Library, and Google Scholar. Patient characteristics, oncological safety and efficacy, and surgical safety were evaluated following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and Grading of Recommendations Assessment, Development and Evaluation guidelines (GRADE) guidelines. Trial Sequential Analysis (TSA) reduced random error and reinforced the reliability and strength of evidence.

Results: Eight trials including 2666 participants were selected. LADG benefits were an 11.6 cm shorter incision (95% CI: −13.31 to −9.88 cm; $P < 0.0001$), 103.81 ml less blood loss (95% CI: −133.68 to −73.94; $P < 0.0001$), 1.73 times less analgesic use (95% CI: −2.21 to −1.24; $P < 0.0001$), 0.51 days shorter time to first flatus (95% CI: −0.88 to −0.15 days; $P = 0.006$), lower risk of wound dehiscence (RR = 0.24, 95% CI: 0.08–0.78; $P = 0.02$), lower risk of surgical adverse events (RR = 0.69, 95% CI: 0.53–0.91; $P = 0.008$), and lower risk of respiratory complications (RR = 0.40; 95% CI: 0.20–0.79; $P = 0.009$) than ODG. LADG had 2.22 fewer resected lymph nodes (95% CI: −4.33 to −0.12; $P = 0.04$) and 76.61 min longer procedures (76.61 min, 95% CI: 57.74–95.47 min; $P < 0.0001$).

Conclusions: In Asian patients, LADG had similar mortality and oncological safety, better surgical safety, less operative morbidity, less trauma, and faster recovery than ODG. It has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. It is a recommended alternative treatment for experienced surgeons in high-volume centers.

1. Introduction

Gastric cancer is a public health concern worldwide, and especially in Asia [1–3]. Laparoscopic distal gastrectomy (LADG) for gastric cancer was introduced by Kitano et al., in 1994 [4]. Since then, interest in this minimally invasive surgical procedure for the treatment of patients with gastric cancer has been increasing. Its perceived benefits include less trauma, operative blood loss, morbidity, and postoperative pain, and accelerated recovery than open distal gastrectomy (ODG); simultaneously, its perceived risks are related to its complexity and a long learning curve that can prolong the procedure, uncertain surgical safety, inadequate lymph node clearance, and incomplete resection [5–14]. LADG is not yet a standard technique for resection of gastric cancer. Further study is needed before it can be recommended.

LADG is technically complex compared with ODG, and the resulting need for adequate training and experience is one reason that this technique has not yet become accepted worldwide as an alternative gastric cancer treatment. In addition, oncological and surgical safety need to be guaranteed before adoption. It may take longer for LADG to be routinely used to treat patients with advanced than early gastric cancer. LADG is more frequently used to treat patients in Asian countries such as Japan and South Korea, where screening programs have resulted in higher rates of early diagnosis than in other counties [1,15–17]. As early gastric cancer is highly curable, close attention

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should be paid to surgical safety.

The results of randomized controlled trials (RCTs) [5–14] and non-RCTs [18–23] comparing LADG and ODG in patients with early gastric cancer are inconsistent, and previous meta-analyses of RCTs have lacked statistical power [24–32]. Potential bias is likely to be greater for non-RCTs because the quality of evidence is lower than that of RCTs. Consequently, the results of meta-analyses including non-RCTs should be interpreted with caution [33]. The two most recent meta-analyses that included only RCTs included 390 and 732 patients, respectively [31,32]. They lacked adequate power to reach definitive conclusions and may have included false positive errors. Three additional RCTs including 2359 patients have been published and will strengthen the current evidence [34–36]. Before a recommendation for routine clinical use of LADG for patients with early gastric cancer can be made, a high level of evidence is required.

This meta-analysis of the latest available evidence from RCTs re-evaluated the safety and efficacy of LADG compared with ODG. It targeted early gastric cancer because its low probability of lymph node metastasis. The quality of the evidence was evaluated by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool [37,38], and Trial Sequential Analysis (TSA) was used to determine whether the current evidence was sufficient and conclusive [39–43].

2. Materials and methods

The meta-analysis included the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, http://www.prisma-statement.org/) checklist [44]. No registered protocol was applied in the current meta-analysis. The Cochrane Handbook for Systematic Review of Interventions was applied to perform the meta-analysis [33]. The meta-analysis data is available on any reasonable request.

2.1. Search strategies

PubMed, Embase, the Cochrane Library, and Google Scholar were searched for articles published from January 1994 to January 2018 [4], without language restriction. Other non-English language articles were screened using Google Translate (https://translate.google.cn/). The search used Medical Subject Headings (MeSH) and keywords including MeSH “Laparoscopy”, and keywords “laparoscopic”, and “laparoscopy-assisted” and MeSH “Stomach Neoplasms” and keywords “gastric cancer” and “stomach cancer”. Additional searches were performed in the ClinicalTrials.gov registry (www.clinicaltrials.gov) and the reference lists of retrieved studies to identify other potentially eligible articles.

2.2. Selection criteria

RCTs were eligible for inclusion if they included patients with early gastric cancer requiring distal gastrectomy and not suitable for endoscopic mucosal resection (EMR) or endoscopic submucosal dissection (ESD). The eligible interventions were LADG or laparoscopy-assisted distal gastrectomy and comparison with ODG. Eligible studies reported more than one of the following outcomes: procedure-related, postoperative, prognosis, and adverse events.

2.3. Screening and extraction

Two authors independently carried out the initial screening and removed duplicates. References in the included RCTs were screened for eligible articles. All discrepancies were resolved by discussion. If two or more articles were published by the same team from the same institute and contained the same or some of the same participants, only the most detailed article was included. The first author, year of publication, country, number of participants, mean age, treatment, study design, follow-up, reconstruction type, lymph node dissection, surgeon experience, and outcomes data were extracted from each included study. The patient characteristics included age, body mass index (BMI), tumor size, procedure time, length of incision, blood loss, blood transfusion volume, reoperation, operation-related deaths, analgesic use, time to first flatus, time to first water/food intake, postoperative hospital stays, number of lymph nodes retrieved, positive lymph nodes, recurrence, wound infection, wound dehiscence, anastomotic stenosis, postoperative bleeding, delayed gastric emptying, intra-abdominal abscess/fluid collection, pancreatic complications, chyle leakage, overall surgical adverse events, respiratory complications, and surgeon experience. Accordingly, recurrence was recorded until the end of the follow-up periods [5–9,34–36].

2.4. Risk of bias assessment

Two authors independently evaluated the risk of bias for each RCT using the Cochrane Risk of Bias tool [33,45]. The risk of bias in random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and others was scored as high, low or unclear [33,45]. Blinding of participants and personnel was difficult to perform in these RCTs, but the outcomes may be less prone to be influenced by lack of blinding. Any disagreements were resolved by discussion.

2.5. Quality of evidence

Two authors independently assessed the quality of evidence provided by the study outcomes using the GRADE tool (version 3.2, GRADEpro, https://gradepro.org/). The risk of bias, inconsistency, indirectness, imprecision, and other considerations were included in the evaluation, and were scored as very low, low, moderate, and high quality [37,38]. The quality of evidence for operation-related deaths, lymph nodes retrieved, recurrence, reoperation, overall surgical adverse events, and time to first flatus, and other outcomes were evaluated.

2.6. Statistical analysis

The statistical analysis was performed using Review Manager 5.3.5 (Nordic Cochrane Centre), which is recommended by both the PRISMA statement and the Cochrane Library [33,44], and included intention-to-treat populations. Dichotomous variables were assessed by risk ratios (RRs) with 95% confidence intervals (CIs). Continuous variables were assessed by mean differences (MDs) with 95% CIs. A P value < 0.05 was considered statistically significant. A random-effects model was applied to account for methodological or clinical heterogeneity. Methodological heterogeneity among the RCTs was quantified by I^2 and P values, and $I^2 > 50\%$ or $P < 0.10$ indicated significant methodological heterogeneity [33,37]. Publication bias was assessed in funnel plots [44,46]. Sample means and standard deviation (SD) were estimated from sample size, median, range and/or interquartile range, if means and SD were not directly reported in the RCTs [47,48].

2.7.

TSA can reduce false positive (type I) errors by combining the required information size (RIS) and adjusted threshold for statistical significance [39–43]. As early gastric cancer is a highly curable disease; more attention was paid to surgical than to oncological safety in this meta-analysis. Thus, TSA was conducted to estimate the RIS of the overall surgical adverse events using $\alpha = 0.05$, and $\beta = 0.20$ (a power of 80%). The conclusion was sufficient and credible if the cumulative z curve crossed either the trial sequential monitoring boundary or the RIS, with no requirement for further trials [39–43]. TSA software version 0.9.5.10 beta (http://www.ctu.dk/tsa) was used for this analysis.

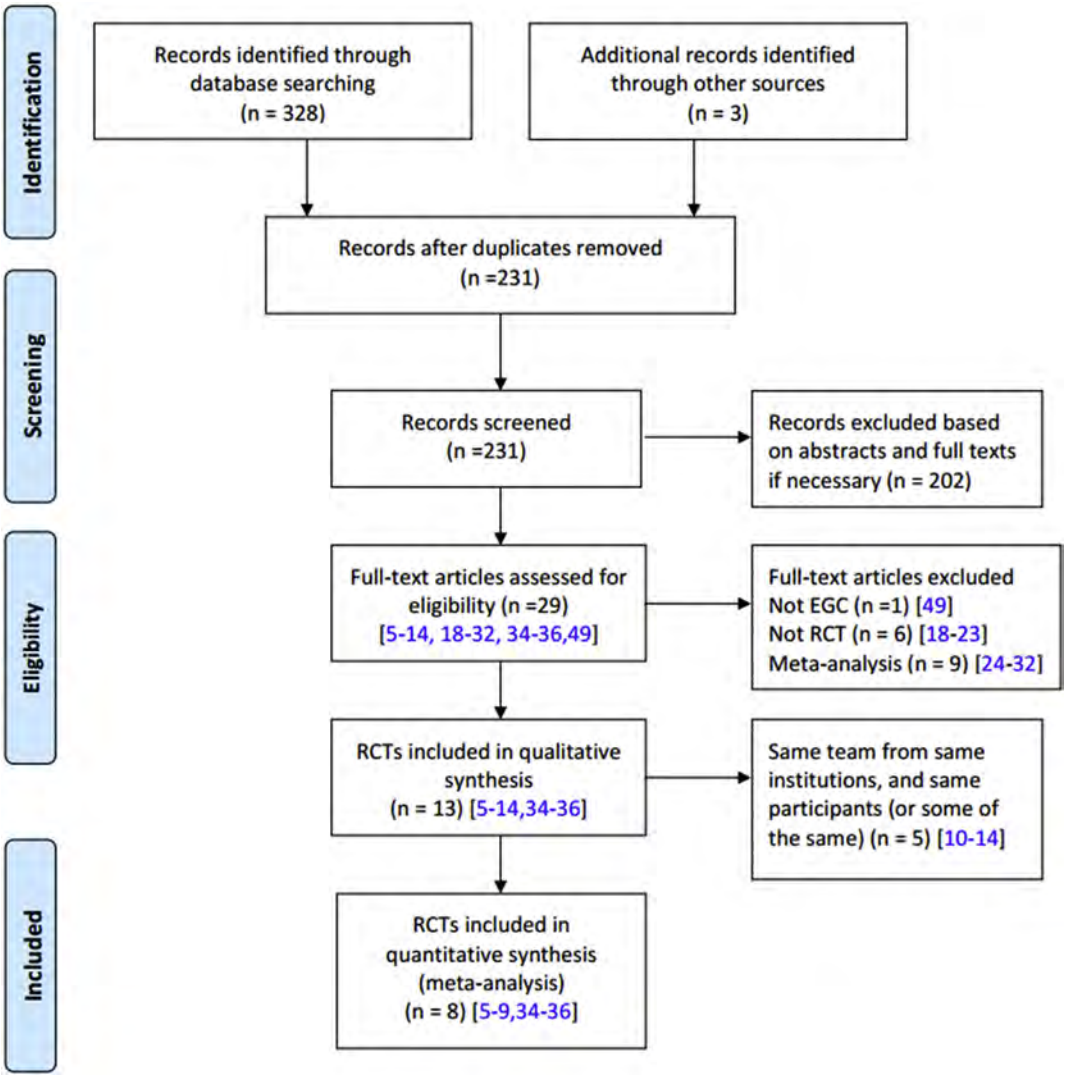

Fig. 1. Flow diagram of study selection.

3. Results

3.1. Trial selection

A flow diagram of the trial selection process and reasons for exclusion is shown (Fig. 1). A total of 331 articles were retrieved, and after removing duplicates and screening the abstract, and full text if necessary, the full text of the 29 remaining articles were screened for eligibility [5–14,18–32,34–36,49]. One article not associated with early gastric cancer [49], six that were not RCTs [18–23], and nine meta-analyses were excluded [24–32]. Of the remaining 13 RCTs [5–14,34–36], five were excluded because they were thought to be published by the same team from the same institute, and contained the same or some of the same, participants [10–14]. Eight RCTs were eventually included in the meta-analysis [5–9,34–36]. Necessary information was still obtained from the five excluded trials [10–14].

3.2. Trial characteristics

Five trials were from Japan and three were from South Korea. They were published between 2002 and 2017, the sample sizes ranged from 28 to 1,384, and a total of 2666 patients were included. The mean age of LADG patients ranged from 56 to 63.2 years; that of ODG patients was 54.5–63.5 years. The median follow-up ranged from 14 to 74.3 months. The reconstruction types in three trials included B-I, B-II, and Roux-en-Y [5,34,35], and B-1 was the only reconstruction performed in four trials [6–9]. The lymph node dissection types included D1, modified D2 lymphadenectomy (D1 +), and D2 lymphadenectomy. The surgeon experience, study design, and outcomes are summarized in Table 1.

3.3. Risk of bias assessment

The risk of bias evaluation of the included RCTs is summarized in Table 2 and Fig. 2. Allocation concealment risk was unclear in five trials [6–9,35], high risk in two [5,34], and low risk in only one trial [36]. Blinding of participants and personnel is difficult to perform in clinical trials, and bias was at high risk in six [5,7,8,34–36], unclear risk in one [9], and at low risk in only one [6]. All trials were at low risk of bias in generation of random number sequences, blinding of outcome assessment, incomplete outcome data, selective reporting and others [5–9,34–36].

3.4. Patient baseline characteristics

The patient baseline characteristics are summarized in Table 3, Fig. 3, and Figs. S1–S3. There were no significant differences in age (MD −0.28 years, 95% CI, −1.75 to 1.19 years, $P = 0.71$; Fig. S1)

Table 1
Characteristics of included randomized controlled trials.

Included Trials	Participants Mean age (years)	Treatments (Number)	Study Design	Follow UP (months)	Reconstruction Type	Lymph Node Dissection	Surgeon Experience	Outcomes
Katai et al., 2017 [34] (Japan)	EGC patients in cT1N0-1, cT2N0 stages. 63.2 vs 63.5	LADG (n = 457) ODG (n = 455)	Multi-center RCT	60.0	B-I B-II Roux-en-Y Gastro-gastro	D1 D1+ D2	LADG: experience of at least 30 of both LADG and ODG operations; ODG: experience of at least 60 ODG operations.	1-9, 11, 14, 17–29.
Kim et al., 2016 [35] (SK)	EGC patients in cT1N0-1, cT2aN0 stages. 56.8 vs 57.6	LADG (n = 686) ODG (n = 698)	Multi-center Prospective RCT	60.0	B-I B-II Roux-en-Y	D1+β D2	Experience of at least 50 each of LADG and ODG. They established a standardized protocol of the procedure.	1-4, 6–9, 13–15, 17–22, 24–29.
Yamashita et al., 2016 [36] (Japan)	EGC patients in cT1 stage. 58.0 vs 61.0	LADG (n = 31) ODG (n = 32)	Single-center RCT	Median: 63.0	Standardized according to the JCGC 2nd English edition.	Standardized according to the JCGC 2nd English edition.	LADG: experience of more than 100 LADG operations; ODG: experience of more than 500 ODG operations.	1-4, 6, 8–11, 13–16, 19, 21, 23, 24, 27–28.
Kim et al., 2013 [5] (SK)	EGC patients in cT1N0-1 stages. 56.7 vs 54.5	LADG (n = 82) ODG (n = 82)	Prospective, RCT	Median: 74.3	B-I B-II Roux-en-Y	D2	A single surgeon with no details of experience.	1-7, 9, 11–14, 16, 17, 21, 23, 24, 27, 29.
Takiguchi et al., 2013 [6] (Japan)	EGC patients in cT1N0-1 stages. 61.5 vs 62.5	LADG (n = 20) ODG (n = 20)	Prospective, RCT	At least 60.0	B-I	D1 D2	A single surgeon well trained in both LADG and ODG operations.	1-7, 9, 14, 29.
Hayashi et al., 2005 [7] (Japan)	EGC patients in cT1 stage. 56.0 vs 62.0	LADG (n = 14) ODG (n = 14)	Prospective, RCT	Median: 39 vs 45	B-I	D2	Both LADG and ODG were performed by a single surgical team that had wide experience with open and laparoscopic procedures.	1, 2, 4, 6, 7, 9–16, 19, 20, 27–29.
Lee et al., 2005 [8] (SK)	EGC patients in cT1 stage. 56.6 vs 59.5	LADG (n = 24) ODG (n = 23)	Prospective, RCT	Median: 14.0	B-I	D2	NM.	1, 4–6, 9–17, 19, 28, 29.
Seigo et al., 2002 [9] (Japan)	EGC patients in cT1 stage. 63.2 vs 60.1	LADG (n = 14) ODG (n = 14)	RCT	Median: 26.0	B-I	NM	An experienced surgeon with the same surgical team.	1, 3–6, 9–16, 23, 25, 27–29.

PICOS, Participants-Interventions-Comparisons-Outcomes-Study design; LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; SK, South Korea; EGC, early gastric cancer; vs, versus; cTNM, clinical TNM staging; NM, not mentioned; JCGC, Japanese Classification of Gastric Cancer; B-I, billroth I; B-II, billroth II.
Outcomes: 1. age; 2. BMI; 3. tumor size; 4. operation time; 5. length of incision; 6. blood loss; 7. blood transfusion volume; 8. reoperation; 9. operation-related deaths; 10. analgesic use; 11. time to first flatus; 12. time to first intake of water/food; 13. duration of postoperative hospital stays; 14. lymph nodes retrieved; 15. positive lymph nodes; 16. recurrence; 17. wound infection; 18. wound dehiscence; 19. anastomotic stenosis; 20. anastomotic stenosis, 21. postoperative bleeding; 22. postoperative bleeding; 23. delayed gastric emptying; 24. intraabdominal abscess/fluid collection; 25. pancreatic complications; 26. chyle leakage; 27. overall surgical adverse events; 28. respiratory complications; 29. surgeon experience.

Table 2
Risk of bias in the included trials assessed with the Cochrane Risk of Bias tool.

Included Trials	Random Sequence Generation[a]	Allocation Concealment[b]	Blinding of Participants and Personnel[c]	Blinding of Outcome Assessment[d]	Incomplete Outcome Data[e,h]	Selective Reporting[f]	Other Bias[g]
Katai 2017 [34]	Low risk	High risk	High risk	Low risk	Low risk	Low risk	Low risk
Kim 2016 [35]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Yamashita 2016 [36]	Low risk	Low risk	High risk	Low risk	Low risk	Low risk	Low risk
Kim 2013 [5]	Low risk	High risk	High risk	Low risk	Low risk	Low risk	Low risk
Takiguchi 2013 [6]	Low risk	Unclear risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hayashi 2005 [7]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Lee 2005 [8]	Low risk	Unclear risk	High risk	Low risk	Low risk	Low risk	Low risk
Seigo 2002 [9]	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Low risk	Low risk

RCTs, randomized controlled trails.
[a] Selection bias.
[b] Selection bias.
[c] Performance bias.
[d] Detection bias.
[e] Attrition bias.
[f] Reporting bias.
[g] Other source of bias.
[h] Blinding of participants and personnel was difficult in these RCTs, but the outcomes may be less prone to be influenced by lack of blinding.

[5–9,34–36], BMI (MD −0.02, −0.23 to 0.20, $P = 0.88$; Fig. S2) [5–7,34–36], or tumor size (MD −0.13 cm, −0.40 to 0.14 cm, $P = 0.33$; Fig. S3) [5,6,9,34–36] in the LADG and ODG groups.

3.5. Procedure-related outcomes

The procedure-related outcomes are summarized in Table 3, Fig. 4, and Figs. S4–S9. The procedure duration was 76.61 min longer (95% CI: 57.74–95.47 min, $P < 0.0001$; Fig. S4) [5–9,34–36], the incision was 11.60 cm shorter (95% CI: −13.31 to −9.88 cm, $P < 0.0001$; Fig. S5) [5,6,8,9,34], and the blood loss was 103.81 ml less (95% CI: −133.68 to −73.94 ml $P < 0.0001$; Fig. S6) [8–12,34–36] with LADG than with ODG. There were no differences between LADG and ODG in blood transfusion volumes (RR 0.90; 95% CI: 0.35–2.33, $P = 0.83$; Fig. S7) [5–7,34,35], reoperation (RR 0.85, (95% CI: 0.38–1.93, $P = 0.70$; Fig. S8) [34–36], or operation-related deaths (RR 2.03, 95% CI: 0.37–11.07; $P = 0.41$; Fig. S9) [5–9,34–36].

3.6. Postoperative outcomes

The postoperative outcomes are summarized in Table 3, Fig. 5, and Figs. S10–S13. Use of analgesics was 1.73 times less (95% CI: −2.21 to −1.24, $P < 0.0001$; Fig. S10) [7–9,36], and time to first flatus was 0.51 days shorter (95% CI −0.88 to −0.15 days $P = 0.006$; Fig. S11) [5,7–9,34,36] with LADG than with ODG. The differences between LADG and ODG in time to first intake water/food (−0.45 days; 95% CI: −1.40 to 0.50 days, $P = 0.35$; Fig. S12) [5,7–9], and duration of postoperative hospital stay (−1.02 days, 95% CI: −2.06 to 0.01 days, $P = 0.05$; Fig. S13) [5,7–9,35,36] were not significant.

3.7. Prognosis outcome

The prognosis outcomes are summarized in Table 3, Fig. 6, and Figs. S14–S16. LADG patients had 2.22 fewer resected lymph nodes than ODG patients (95% CI: −4.33 to −0.12; $P = 0.04$, Fig. S14) [5–9,34–36]. There were no significant differences in positive lymph nodes (RR 0.93, 95% CI: 0.74–1.18; P = 0.57; Fig. S15) [7–9,35,36], or recurrence (RR 0.50, 95% CI: 0.05–5.41; P = 0.57; Fig. S16) [5,7–9,36].

3.8. Adverse event outcomes

The adverse event outcomes are summarized in Table 3, Fig. 7, and Figs. S17–S28. Wound dehiscence (RR 0.24, 95% CI: 0.08–0.78; $P = 0.02$; Fig. S18) [34,35] and risk of respiratory complications (RR 0.40, 95% CI: 0.20–0.79; $P = 0.009$; Fig. S28) were lower with LADG than with ODG [7–9,34–36]. Seven RCTs including 2626 participants reported the overall occurrence of surgical adverse events [5,7–9,34–36]. LADG had a significant lower risk of surgical adverse events than ODG (RR 0.69, 95% CI: 0.53–0.91; $P = 0.008$; Fig. S27) [5,7–9,34–36]. The TSA cumulative z curve crossed the trial sequential monitoring boundary for benefit in LADG, indicating that the evidence is sufficient and conclusive (Fig. 8). TSA thus indicated that early gastric cancer patients would benefit from LADG by having fewer surgical adverse events compared with ODG. Additional RCTs might not be required and might be unlikely to change the current conclusion.

There were no significant differences between LADG and ODG in infection (RR 1.11, 95% CI: 0.47–2.60; $P = 0.81$; Fig. S17) [5,8,34,35], anastomotic stenosis (RR 1.00, 95% CI: 0.28–3.63; $P = 1.00$; Fig. S19) [7,8,34–36], anastomotic leakage (RR 0.63, 95% CI: 0.24–1.65, $P = 0.34$; Fig. S20) [7,34,35], postoperative bleeding (RR 0.69, 95% CI: 0.40–1.19; $P = 0.18$; Fig. S21) [5,34–36], postoperative obstruction/ileus (RR 0.82, 95% CI 0.44–1.55; $P = 0.54$; Fig. S22) [34,35], delayed gastric emptying (RR 0.46, 95% CI: 0.16–1.30; $P = 0.14$; Fig. S23) [5,9,34,36], intra-abdominal abscess/fluid collection (RR 0.71, 95% CI: 0.36–1.39; $P = 0.31$; Fig. S24) [5,34–36], pancreatic complications (RR 1.67, 95% CI: 0.40–6.90; $P = 0.48$; Fig. S25) [9,34,35], or chyle leakage (RR 0.34, 95% CI: 0.01–8.31, $P = 0.51$; Fig. S26) [34,35].

3.9. Learning curve and surgeon experience

Six RCTs reported surgeon experience [6,7,9,34–36], three gave the minimum experience, which ranged from 30 to 100 cases for LADG, and 50 to 500 cases for ODG [34–36]. Three noted that the surgeons or surgical team included “a single surgeon well trained in both LADG and ODG” [6], “a single surgical team that had wide experience with open and laparoscopic procedures” [7], and “an experienced surgeon with the same surgical team” [9].

3.10. GRADE working group scores of evidence and publication bias

GRADE working group evidence scores for the RCT outcomes are summarized in Table 4. The level of evidence was low for operation-related deaths [5–9,34–36], lymph nodes retrieved [5–9,34–36], and overall survival-adverse events [5,7–9,34–36]; and very low for recurrence [5,7–9,36], reoperation [34–36], and time to first flatus [5,7–9,34,36]. The funnel plots showing publication bias are shown in Fig. 9. Publication bias was indicated by funnel plot asymmetry, the

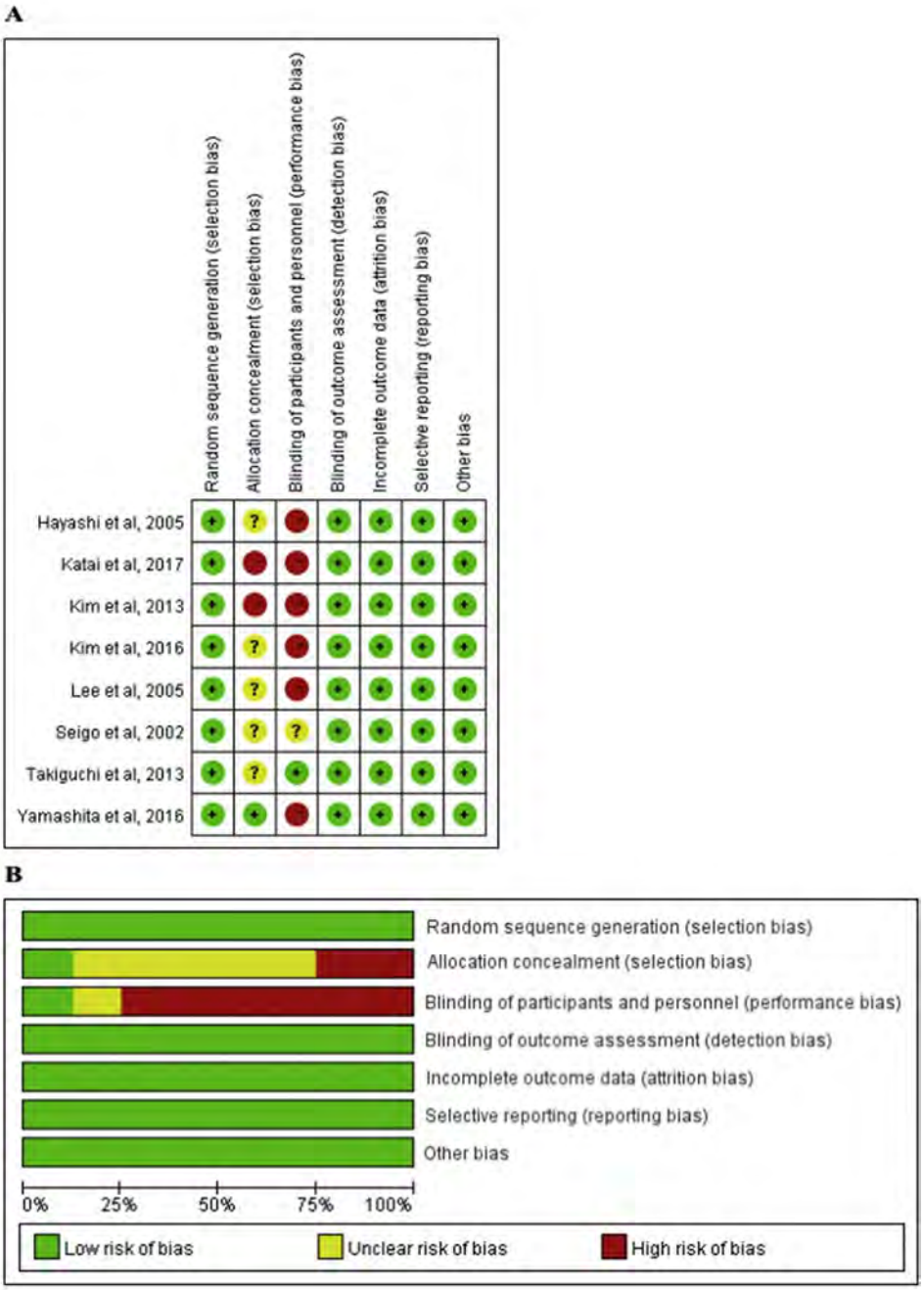

Fig. 2. A Bias risk summary for each element in all included trials; **B** Bias risk for each element as a percentage across all included trials.

absence of RCTs with negative results, and a total of less than nine included RCTs [33,46].

4. Discussion

Early gastric cancer is considered highly curable because of a low probability of lymph node metastasis. LADG cannot be recommended for routine treatment of patients with gastric cancer if its superiority to ODG is not guaranteed. This technically complex and time-consuming procedure should be initially evaluated in early stage disease, and a considerably longer evaluation may be required before LADG is routinely used to advanced gastric cancer. Existing screening programs may account for the higher incidence and diagnosis of early gastric cancer in Japan and South Korea than in western countries [1,15–17]. Furthermore, surgeons in Asian countries, especially Japan and South Korea, might be more experienced in the surgical treatment of gastric cancer than those in western countries. Also, patients in Asia have average lower BMIs than western patients. Previous studies found that a high BMI did not increase the incidence of surgical complications of LADG compared with ODG [50,51]. However, patients with higher BMIs will increase the technical complexity of LADG, with risk of less nodal harvest, prolonged procedure time, and increased postoperative blood loss. Accordingly, this meta-analysis limited the comparison of LADG with ODG in early gastric cancer.

Before evaluation of the oncological safety and effectiveness of LADG, its surgical safety should be guaranteed. This meta-analysis found LADG took longer time than ODG. LADG with lymphadenectomy is a relatively new, time-consuming procedure that is technically complex, but surgeons can overcome those issues through continuous training. Compared with ODG, LADG offers significant benefits of less

Table 3
Main results of meta-analyses including all the outcomes.

Variable	No. of Trials	No. Participants		Effect Estimate RR/MD (95% CI)	*P* Value
		LADG	Total		
Patient baseline characteristics					
Age (years) [5–9,34–36]	8	1328	2666	MD, −0.28 (−1.75, 1.19)	0.71
BMI [5–7,34–36]	6	1290	2591	MD, −0.02 (−0.23, 0.20)	0.88
Tumor size (cm) [5,6,9,34–36]	6	1290	2591	MD, −0.13 (−0.40, 0.14)	0.33
Procedure-related outcomes					
Operation time (mins) [5–9,34–36]	8	1328	2666	MD, 76.61 (57.74, 95.47)	< 0.0001*
Length of incision (cm) [5,6,8,9,34]	5	597	1191	MD, −11.60 (−13.31, −9.88)	< 0.0001*
Blood loss (ml) [5–9,34–36]	8	1328	2666	MD, −103.81 (−133.68, −73.94)	< 0.0001*
Blood transfusion volume [5–7,34,35]	5	1259	2528	RR, 0.90 (0.35, 2.33)	0.83
Reoperation [34–36]	3	1174	2359	RR, 0.85 (0.38, 1.93)	0.70
Operation-related deaths [5–9,34–36]	8	1328	2666	RR, 2.03 (0.37, 11.07)	0.41
Postoperative outcomes					
Analgesic use [7–9,36]	4	83	166	MD, −1.73 (−2.21, −1.24)	< 0.0001*
Time to first flatus (days) [5,7–9,34,36]	6	622	1242	MD, −0.51 (−0.88, −0.15)	0.006*
Time to first intake of water/food (days) [5,7–9]	4	134	267	MD, −0.45 (−1.40, 0.50)	0.35
Duration of postoperative hospital stays (days) [5,7–9,35,36]	6	851	1714	MD, −1.02 (−2.06, 0.01)	0.05
Prognosis outcomes					
Lymph nodes retrieved [5–9,34–36]	8	1328	2666	MD, −2.22 (−4.33, −0.12)	0.04*
Positive lymph nodes [7–9,35,36]	5	769	1550	RR, 0.93 (0.74, 1.18)	0.57
Recurrence [5,7–9,36]	5	165	330	RR, 0.50 (0.05, 5.41)	0.57
Adverse event outcomes					
Wound infection [5,8,34,35]	4	1249	2507	RR, 1.11 (0.47, 2.60)	0.81
Wound dehiscence [34,35]	2	1143	2296	RR, 0.24 (0.08, 0.78)	0.02*
Anastomotic stenosis [7,8,34–36]	5	1212	2434	RR, 1.00 (0.28, 3.63)	1.00
Anastomotic leakage [7,34,35]	3	1157	2324	RR, 0.63 (0.24, 1.65)	0.34
Postoperative bleeding [5,34–36],[a]	4	1256	2523	RR, 0.69 (0.40, 1.19)	0.18
Postoperative obstruction/ileus [34,35]	2	1143	2296	RR, 0.82 (0.44, 1.55)	0.54
Delayed gastric emptying [5,9,34,36]	4	584	1167	RR, 0.46 (0.16, 1.30)	0.14
Intraabdominal abscess/fluid collection [5,34–36]	4	1256	2523	RR, 0.71 (0.36, 1.39)	0.31
Pancreatic complications [9,34,35],[b]	3	1157	2324	RR, 1.67 (0.40, 6.90)	0.48
Chyle leakage [34,35]	2	1143	2296	RR, 0.34 (0.01, 8.31)	0.51
Overall surgical adverse events [5,7–9,34–36],[c]	7	1308	2626	RR, 0.69 (0.53, 0.91)	0.008*
Respiratory complications [7–9,34–36],[d]	6	1226	2462	RR, 0.40 (0.20, 0.79)	0.009*
Learning curve					
Surgeon experience [34–36],[e]	3	1174	2359	–	–

LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; RR, risk ratio; MD, mean difference; 95% CI, 95% confidence interval.

[a] Including: intraabdominal, intraluminal and anastomotic bleeding.

[b] Including: pancreatitis, pancreatic fistula and pancreatic injury.

[c] Including: wound infection, wound dehiscence, anastomotic stenosis, anastomotic leakage, postoperative bleeding, postoperative obstruction/ileus, delayed gastric emptying, intraabdominal abscess/fluid collection, pancreatic complications, and chyle leakage.

[d] Including: pneumonia, bronchiectasis, pulmonary atelectasis and so on.

[e] Trials which have mentioned the details of the least cases experience of the surgeons.

operative blood loss, postoperative pain, overall risk of surgical adverse events, and respiratory complications [5–9,34–36]. Those benefits may be attributed to the less invasive nature of LADG compared with ODG, which contributed to enhanced recovery after surgery. The reason for less operative blood loss in LADG patients may be attributed to the magnified view through the monitor, which permits meticulous dissection to prevent unexpected bleeding, thus preventing interference with surgical vision by blood accumulation. Intra-abdominal bleeding may also lead to reoperation [35]. LADG was also associated with a shorter time to first flatus, and a tendency toward a shorter duration of postoperative hospital stay, although the difference did not reach significance (MD −1.02, 95% CI: −2.06 to 0.01; $P = 0.05$) [5–9,34–36]. No procedure-associated differences in reoperation or operation-related deaths were found, which supported the safety of LADG. The current evidence supports the surgical safety and rapid recovery of LADG.

Oncological safety is of great importance for surgical treatment of

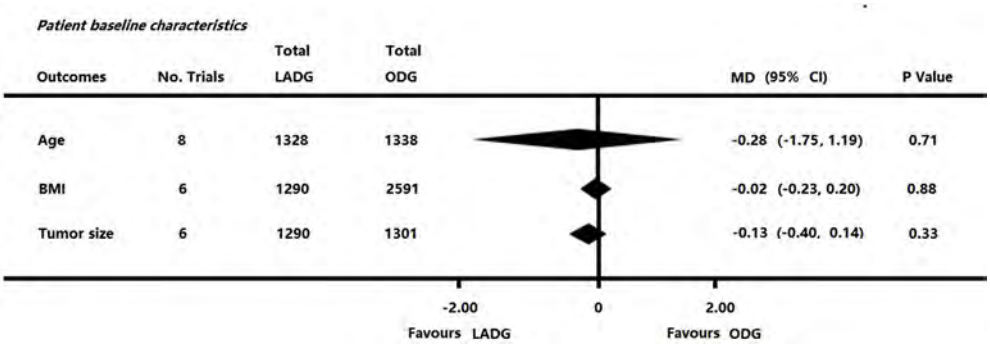

Fig. 3. Patient baseline characteristics.

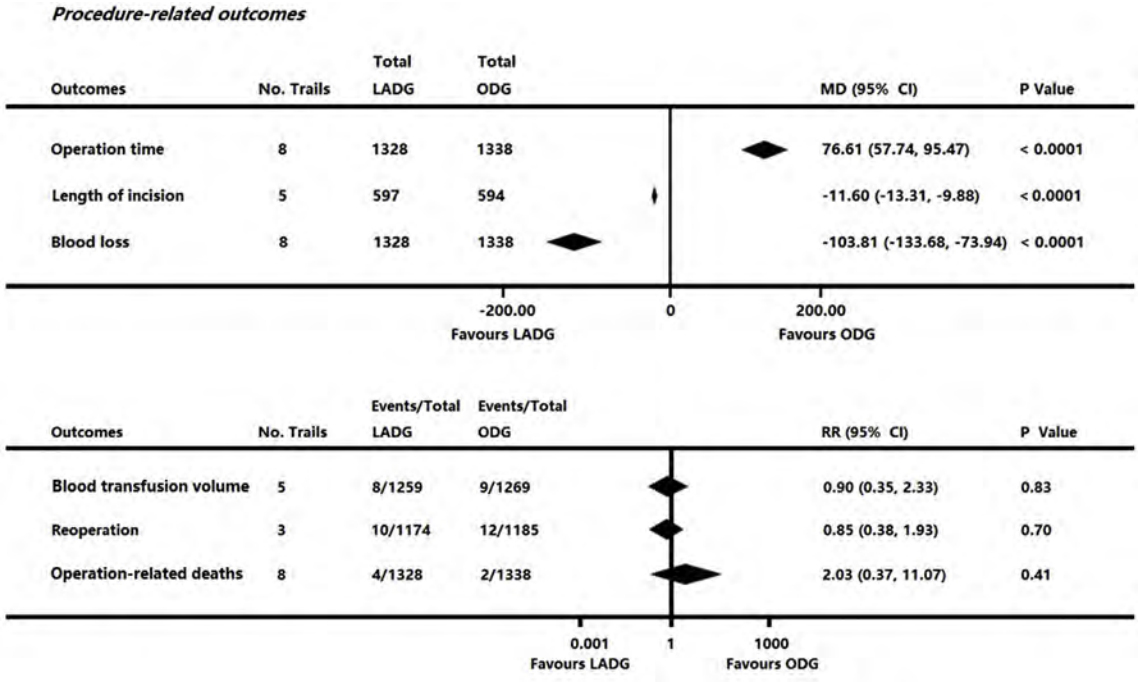

Fig. 4. Procedure-related outcomes.

gastric cancer. Lymph node metastasis is a frequent occurrence in gastric cancer, and adequate nodal harvest is the key step in distal gastrectomy. Lymph nodes dissection is also important for staging, as a larger total lymph node count may have a survival benefit [52]. Compared with ODG, LADG had a mean reduction of 2.2 harvested nodes. The included RCTs reported an average of at least 20.2 harvested nodes with LADG and 24.9 with ODG respectively, both of which are more than the minimum of 15 recommended by the current gastric cancer guidelines of the Union for International Cancer Control/American Joint Cancer Committee, the Japanese Gastric Cancer Association, and the European Society for Medical Oncology [5–9,34–36,53–59]. This meta-analysis found no procedure-related difference in risk of recurrence, or the number of patients with positive lymph nodes i.e., metastasis in one or more regional lymph nodes [5–9,34–36]. Similar results were reported in the included RCTs [5–9,34–36]. LADG and ODG thus had equivalent oncology safety, which supports LADG as an alternative to ODG for early gastric cancer. Importantly, although LADG had a mean reduction of 2.2 harvested nodes compared with ODG, the oncological outcomes were comparable. One plausible explanation might be that most patients included in current study were cT1 cases. Patients with cT1 gastric cancer are less likely to experience lymph node metastasis compared with those with T2 or more advanced tumors and might have better survival after gastrectomy.

This meta-analysis comprehensively and systematically screened the currently available evidence, which supports LADG as an alternative to ODG for early gastric cancer. However, caution still should be exercised in patients with node-positive early gastric cancer, as most patients included in this analysis were node negative; the percentage of node-positive cases in the selected studies ranged from 0% to 15.8%. Consequently, current evidence suggests that LADG has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. LADG can be considered for node-positive cases by experienced surgeons in high-volume centers. The evidence warrants further trials in node-positive early gastric cancer cases, advanced gastric cancer cases, or cases in western countries.

Japan and South Korea have implemented gastric cancer screening programs [1,12–14,60–65]. Because gastric cancer is often diagnosed at an advanced stage, early detection may be the most effective intervention. Patients will benefit from a diagnosis at less advanced stage because of screening programs aiming at early detection. Screening by diagnostic endoscopy, histologic evaluation of biopsies, endoscopic ultrasonography [66], and accurate staging can be used to confirm the presence of abnormal or enlarged lymph nodes likely to harbor cancer. If no suspicion of node-positive cancer is found in an early stage patient who is also not suitable for EMR or ESD [67–69], LADG can be recommended as an alternative to ODG. For early gastric cancer patients

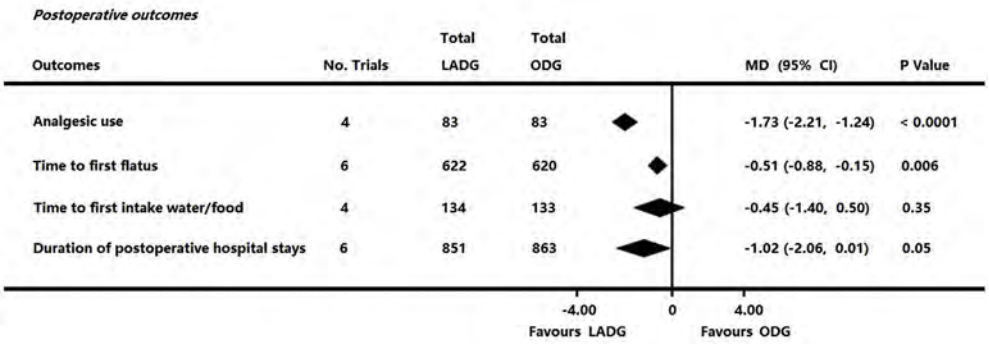

Fig. 5. Postoperative outcomes.

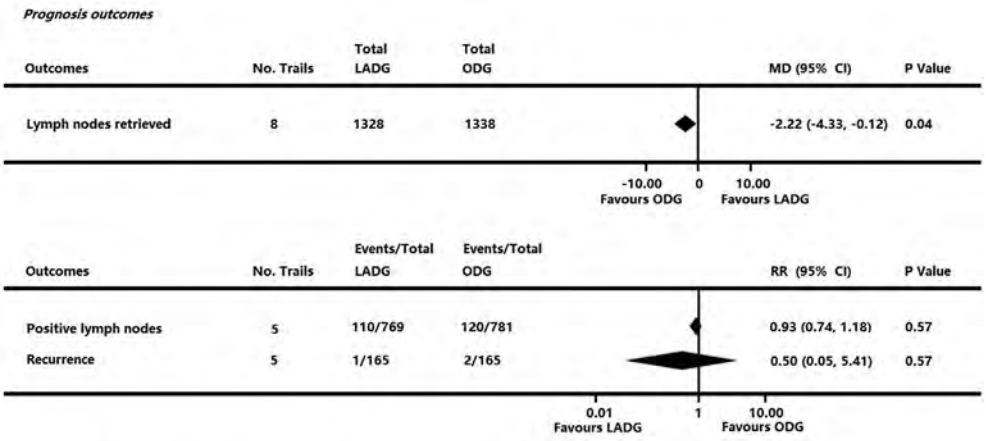

Fig. 6. Prognosis outcomes.

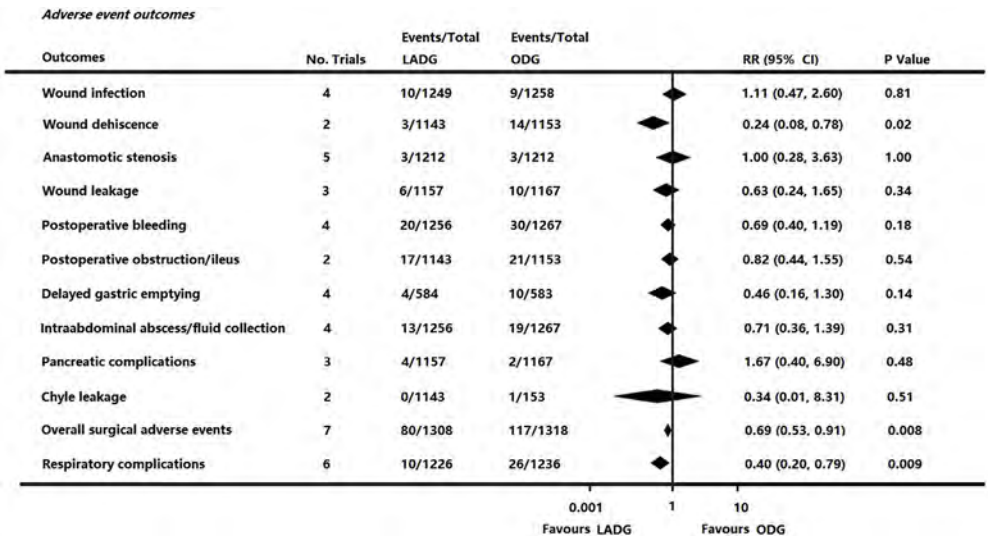

Fig. 7. Adverse event outcomes.

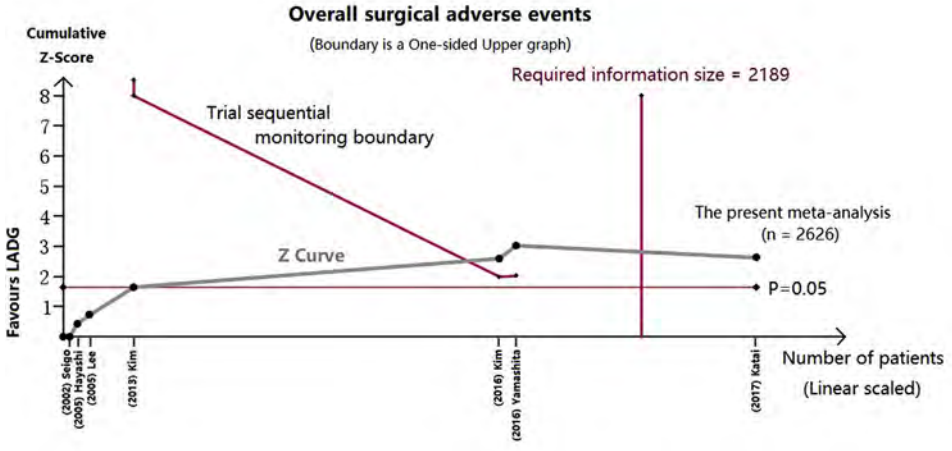

Fig. 8. Trial sequential analysis of overall surgical adverse events in seven RCTs comparing laparoscopic distal gastrectomy with open distal gastrectomy.

with suspected positive nodes, LADG is a choice which should be performed by experienced surgeons in high-volume centers.

Another important issue is how to define an experienced surgeon. The LADG learning curve requires significant training and expertise. Most RCTs included in the meta-analysis described surgeons as "experienced" or "well trained," but only three trials included the number of completed procedures when describing the experience of the surgeons [34–36]. Katai et al. reported "experience of at least 30 of both LADG and ODG operations" for LADG and "experience of at least 60 ODG operations" for ODG [34]. Kim et al. reported "experience of at least 50 each of LADG and ODG operations" for both LADG and ODG [35]. Yamashita et al. reported "experience of more than 100 LADG

Table 4
GRADE evidence profile.

Quality assessment							No of patients		Effect		Quality	Importance
No of studies	Design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	LADG	ODG	Relative (95% CI)	Absolute		
Operation-related deaths [5–9,34–36]												
8 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	None	4/1328 (0.3%)	2/1338 (0.1%) 0%	RR 2.03 (0.37–11.07)	2 more per 1000 (from 1 fewer to 15 more) –	⊕⊕OO LOW	CRITICAL
Lymph nodes retrieved [5–9,34–36] (Better indicated by lower values)												
8 RCTs	Randomised trials	Serious [b]	Serious [d]	No serious indirectness	No serious imprecision	None	1328	1338	–	MD 2.22 lower (4.33–0.12 lower)	⊕⊕OO LOW	CRITICAL
Recurrence [5,7–9,36]												
5 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	Reporting bias [e]	1/165 (0.6%)	2/165 (1.2%) 0%	RR 0.50 (0.05–5.41)	6 fewer per 1000 (from 12 fewer to 53 more) –	⊕OOO VERY LOW	CRITICAL
Reoperation [34–36]												
3 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	Serious [c]	Reporting bias [e]	10/1174 (0.85%)	12/1185 (1%) 1.3%	RR 0.85 (0.38–1.93)	2 fewer per 1000 (from 6 fewer to 9 more) 2 fewer per 1000 (from 8 fewer to 12 more)	⊕OOO VERY LOW	CRITICAL
Overall surgical adverse events [5,7–9,34–36], [a]												
7 RCTs	Randomised trials	Serious [b]	No serious inconsistency	No serious indirectness	No serious imprecision	Reporting bias [e]	80/1308 (6.1%)	117/1318 (8.9%) 8.7%	RR 0.69 (0.53–0.91)	28 fewer per 1000 (from 8 fewer to 42 fewer) 27 fewer per 1000 (from 8 fewer to 41 fewer)	⊕⊕OO LOW	CRITICAL
Time to first flatus [5,7–9,34,36] (Better indicated by lower values)												
6 RCTs	Randomised trials	Serious [b]	Serious [f]	No serious indirectness	No serious imprecision	Reporting bias [e]	622	620	–	MD 0.51 lower (0.88–0.15 lower)	⊕OOO VERY LOW	IMPORTANT

GRADE, Grading of Recommendations Assessment, Development and Evaluation; RCTs, randomized controlled trials; LADG, laparoscopic-assisted distal gastrectomy; ODG, open distal gastrectomy; RR, risk ratio; MD, mean difference; 95% CI, 95% confidence interval; No, number.

GRADE Working Group grades of evidence: High quality: Further research is very unlikely to change our confidence in the estimate of effect; **Moderate quality:** Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate; **Low quality:** Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate; **Very low quality:** We are very uncertain about the estimate.

[a] Including: wound infection, wound dehiscence, anastomotic stenosis, anastomotic leakage, postoperative bleeding, postoperative obstruction/ileus, delayed gastric emptying, intraabdominal abscess/fluid collection, pancreatic complications, and chyle leakage.

[b] Only one RCT included in the present study applied single-blind methods (blind to patients).

[c] The 95% confidence interval (95% CI) for the total effect was too wide.

[d] Heterogeneity ($I^2 = 70\%$, $P = 0.002$) was found.

[e] High risk of publication bias.

[f] Heterogeneity ($I^2 = 92\%$, $P < 0.001$) was found.

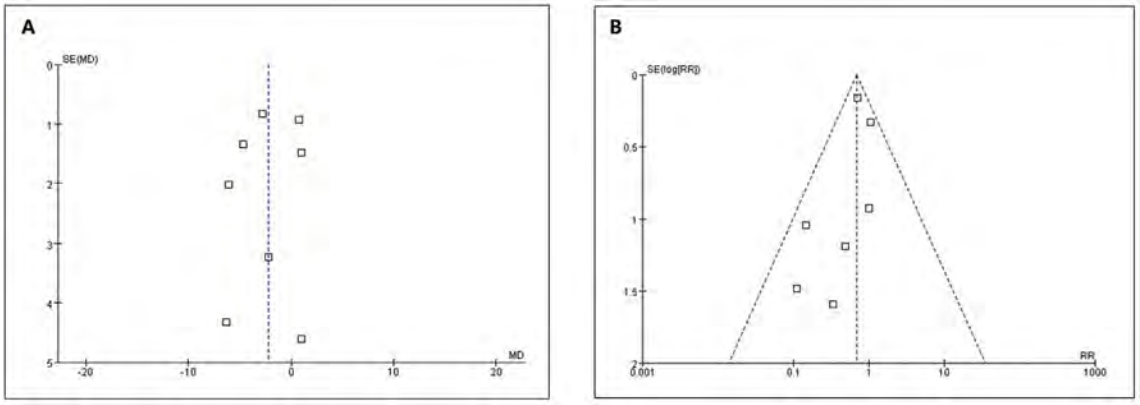

Fig. 9. A Funnel plot for publication bias in lymph nodes retrieved; **B** Funnel plot for publication bias in overall surgical adverse events.

operations" for LADG and "experience of more than 500 ODG operations" for ODG [36]. The fewest procedures performed by experienced surgeons ranged from 30 to 100 cases for LADG, and 50 to 500 cases for ODG [34–36]. Nevertheless, it is still difficult to define the minimum LADG or ODG procedures required as the criterion that defines an experienced LADG surgeon. The LADG learning curve may influence clinical outcomes because it is still relatively new and a technically complex procedure, especially for inexperienced surgeons. The current literature may indicate a minimum experience of no less than 30 LADG and 50 ODG procedures is required [34–36]. Future trials are still needed to confirm the "experienced" requirement.

The number of elderly patients with gastric cancer is increasing because life expectancy is consistently increasing. Compared with younger patients, elderly patients may have an increased surgical risk because of poorer nutritional and functional status, which may result in higher postoperative morbidity and mortality. Importantly, laparoscopy-assisted procedures are associated with less trauma, faster recovery, and similar surgical and oncological safety, compared with open procedures. Therefore, interest in the use of LADG in elderly patients with gastric cancer is increasing. In a meta-analysis of non-RCTs, Zong et al. reported that LADG significantly reduced both operation-related and systemic morbidities and did not increase cardiopulmonary or mental dysfunction compared with ODG in elderly gastric cancer patients [70]. Another meta-analysis of observational studies by Wang et al. demonstrated that compared with ODG, LADG was a feasible and safe approach for elderly patients with gastric cancer. It was associated with less blood loss, faster postoperative recovery, and reduced postoperative morbidity [71]. In the latest meta-analysis of observational studies, Pan et al. reported that the outcomes of LADG in elderly patients were comparable to those in younger patients and that age alone should not preclude LADG in elderly patients with gastric cancer [72].

The strengths of this meta-analysis including adequate power with 2666 participants. All included trials were high quality RCTs. Other strengths were following the PRISMA and GRADE evidence profiles, both of which were recommended by the Cochrane Collaboration [33]. Furthermore, TSA was performed to reduce the influence of random error and confirm whether the evidence was reliable and conclusive. Limitations might exist in this study. Firstly, it included a small percentage of node-positive cancers among the largely node-negative treated population that could have influenced the outcomes. Secondly, all included trials were from Japan and South Korea, none were from China, which has one of the highest incidences of gastric cancer worldwide. Thirdly, bias may have been introduced by differences in the LADG experience and learning status of the surgeons who performed the procedures. Fourthly, none of the trials reported quality of life scores or economic assessments, which are areas of concern. Fifth, data of long-term overall survival (OS) and disease-free survival (DFS) outcomes are not available in the current literature which are important. Finally, the quality of the evidence of the included trials was relatively low, as assessed by the GRADE evidence profile.

In conclusion, the currently available evidence supports LADG an alternative to ODG for Asian patients with early gastric cancer because of similar mortality and oncological safety, better surgical safety, decreased operative morbidity, less trauma, and accelerated recovery. It has a high role to play in node-negative cases due to better short-term outcomes but less nodal harvest. It should be performed by experienced surgeons in high-volume centers, and caution should be exercised with node-positive cases and cases in western countries.

Ethical Approval

There is no need to gain Ethical Approval for this meta-analysis.

Sources of funding

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Author contribution

Zhang C.D., Yamashita H., Zhang S., and Seto Y. are the authors responsible for the study's conception and design. Zhang C.D. and Zhang S., are responsible for acquisition of data, and analysis and interpretation of data. Seto Y. contributed most importantly by giving the final approval.

Conflict-of-interest statement

The authors have declared that there are no conflicts of interest.

Registration unique identifying number

UIN is reviewregistry518.

Guarantor

Yasuyuki Seto and Chun-Dong Zhang.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijsu.2018.05.733.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, et al., Cancer incidence

and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Canc. 136 (2015) E359–E386.
[2] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, et al., Cancer statistics in China, 2015, CA Cancer J. Clin 66 (2016) 115–132.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin 68 (2018) 7–30.
[4] S. Kitano, Y. Iso, M. Moriyama, K. Sugimachi, Laparoscopy-assisted billroth I gastrectomy, Surg. Laparosc. Endosc. 4 (1994) 146–148.
[5] Y.W. Kim, H.M. Yoon, Y.H. Yun, B.H. Nam, B.W. Eom, et al., Long-term outcomes of laparoscopy-assisted distal gastrectomy for early gastric cancer: result of a randomized controlled trial (COACT 0301), Surg. Endosc. 27 (2013) 4267–4276.
[6] S. Takiguchi, Y. Fujiwara, M. Yamasaki, H. Miyata, K. Nakajima, et al., Laparoscopy-assisted distal gastrectomy versus open distal gastrectomy. A prospective randomized single-blind study, World J. Surg. 37 (2013) 2379–2386.
[7] H. Hayashi, T. Ochiai, H. Shimada, Y. Gunji, Prospective randomized study of open versus laparoscopy-assisted distal gastrectomy with extraperigastric lymph node dissection for early gastric cancer, Surg. Endosc. 19 (2005) 1172–1176.
[8] J.H. Lee, H.S. Han, J.H. Lee, A prospective randomized study comparing open vs laparoscopy-assisted distal gastrectomy in early gastric cancer: early results, Surg. Endosc. 19 (2005) 168–173.
[9] S. Kitano, N. Shiraishi, K. Fujii, K. Yasuda, M. Inomata, et al., A randomized controlled trial comparing open vs laparoscopy-assisted distal gastrectomy for the treatment of early gastric cancer: an interim report, Surgery (St Louis) 131 (2002) S306–S311.
[10] K. Nakamura, H. Katai, J. Mizusawa, T. Yoshikawa, M. Ando, et al., A phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric Cancer (JCOG0912), Jpn, J. Clin. Oncol. 43 (2013) 324–327.
[11] S. Sakuramoto, K. Yamashita, S. Kikuchi, N. Futawatari, N. Katada, et al., Laparoscopy versus open distal gastrectomy by expert surgeons for early gastric cancer in Japanese patients: short-term clinical outcomes of a randomized clinical trial, Surg. Endosc. 27 (2013) 1695–1705.
[12] Y.W. Kim, Y.H. Baik, Y.H. Yun, B.H. Nam, D.H. Kim, et al., Improved quality of life outcomes after laparoscopy-assisted distal gastrectomy for early gastric cancer: results of a prospective randomized clinical trial, Ann. Surg. 248 (2008) 721–727.
[13] H.H. Kim, W.J. Hyung, G.S. Cho, M.C. Kim, S.U. Han, et al., Morbidity and mortality of laparoscopic gastrectomy versus open gastrectomy for gastric cancer: an interim report–a phase III multicenter, prospective, randomized Trial (KLASS Trial), Ann. Surg. 251 (2010) 417–420.
[14] K. Fujii, K. Sonoda, K. Izumi, N. Shiraishi, Y. Adachi, et al., T lymphocyte subsets and Th1/Th2 balance after laparoscopy-assisted distal gastrectomy, Surg. Endosc. 17 (2003) 1440–1444.
[15] L. Zong, M. Abe, Y. Seto, J. Ji, The challenge of screening for early gastric cancer in China, Lancet 388 (2016) 2606.
[16] K. Sumiyama K, Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan, Gastric Cancer 20 (2017) 20–27.
[17] C. Hamashima, Cancer screening guidelines and policy making: 15 years of experience in cancer screening guideline development in Japan, Jpn. J. Clin. Oncol. 48 (2018) 278–286.
[18] K. Misawa, M. Fujiwara, M. Ando, S. Ito, Y. Mochizuki, et al., Long-term quality of life after laparoscopic distal gastrectomy for early gastric cancer: results of a prospective multi-institutional comparative trial, Gastric Cancer 18 (2015) 417–425.
[19] J.H. Lee, B.H. Nam, K.W. Ryu, S.Y. Ryu, Y.K. Park, et al., Comparison of outcomes after laparoscopy-assisted and open total gastrectomy for early gastric cancer, Br. J. Surg. 102 (2015) 1500–1505.
[20] T. Yoshikawa, H. Cho, Y. Rino, Y. Yamamoto, M. Kimura, et al., A prospective feasibility and safety study of laparoscopy-assisted distal gastrectomy for clinical stage I gastric cancer initiated by surgeons with much experience of open gastrectomy and laparoscopic surgery, Gastric Cancer 16 (2013) 126–132.
[21] H. Katai, M. Sasako, H. Fukuda, K. Nakamura, N. Hiki, JCOG Gastric Cancer Surgical Study Group, Safety and feasibility of laparoscopy-assisted distal gastrectomy with suprapancreatic nodal dissection for clinical stage I gastric cancer: a multicenter phase II trial (JCOG 0703), Gastric Cancer 13 (2010) 238–244.
[22] Y. Kurokawa, H. Katai, H. Fukuda, M. Sasako, Gastric cancer surgical study group of the Japan clinical oncology group, phase II study of laparoscopy-assisted distal gastrectomy with nodal dissection for clinical stage I gastric cancer: Japan clinical oncology group study JCOG0703, Jpn. J. Clin. Oncol. 38 (2008) 501–503.
[23] J.Y. An, G.U. Heo, J.H. Cheong, W.J. Hyung, S.H. Choi, et al., Assessment of open versus laparoscopy-assisted gastrectomy in lymph node-positive early gastric cancer: a retrospective cohort analysis, J. Surg. Oncol. 102 (2010) 77–81.
[24] X.Z. Chen, J.K. Hu, K. Yang, L. Wang, Q.C. Lu, Short-term evaluation of laparoscopy-assisted distal gastrectomy for predictive early gastric cancer: a meta-analysis of randomized controlled trials, Surg. Laparosc. Endosc. Percutaneous Tech. 19 (2009) 277–284.
[25] J.S. Peng, H. Song, Z.L. Yang, J. Xiang, D.C. Diao, et al., Meta-analysis of laparoscopy-assisted distal gastrectomy and conventional open distal gastrectomy for early gastric cancer, Chin. J. Canc. 29 (2010) 349–354.
[26] H. Ohtani, Y. Tamamori, K. Noguchi, T. Azuma, S. Fujimoto, et al., A meta-analysis of randomized controlled trials that compared laparoscopy-assisted and open distal gastrectomy for early gastric cancer, J. Gastrointest. Surg. 14 (2010) 958–964.
[27] Y. Liang, G. Li, P. Chen, J. Yu, C. Zhang, Laparoscopic versus open gastrectomy for early distal gastric cancer: a meta-analysis, ANZ, J. Surg. 81 (2011) 673–680.
[28] Y.K. Zeng, Z.L. Yang, J.S. Peng, H.S. Lin, L. Cai, Laparoscopy-assisted versus open distal gastrectomy for early gastric cancer: evidence from randomized and non-randomized clinical trials, Ann. Surg. 256 (2012) 39–52.
[29] C.D. Zhang, S.C. Chen, Z.F. Feng, Z.M. Zhao, J.N. Wang, et al., Laparoscopic versus open gastrectomy for early gastric cancer in Asia: a meta-analysis, Surg. Laparosc. Endosc. Percutaneous Tech. 23 (2013) 365–377.
[30] Y. Wang, S. Wang, Z.Q. Huang, W.P. Chou, Meta-analysis of laparoscopy assisted distal gastrectomy and conventional open distal gastrectomy for EGC, Surgeon 12 (2014) 53–58.
[31] Y. Deng, Y. Zhang, T.K. Guo TK, Laparoscopy-assisted versus open distal gastrectomy for early gastric cancer: a meta-analysis based on seven randomized controlled trials, Surg. Oncol 24 (2015) 71–77.
[32] W. Lu, J. Gao, J. Yang, Y. Zhang, W. Lv, et al., Long-term clinical outcomes of laparoscopy-assisted distal gastrectomy versus open distal gastrectomy for early gastric cancer: a comprehensive systematic review and meta-analysis of randomized control trials, Medicine 95 (2016) e3986.
[33] J.P.T. Higgins, S. Green, Cochrane Handbook for systematic Reviews of interventions version 5.1.0. The Cochrane collaboration, Available at: http://handbook.cochrane.org (2011).
[34] H. Katai, J. Mizusawa, H. Katayama, M. Takagi, T. Yoshikawa, et al., Short-term surgical outcomes from a phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastriccancer: Japan Clinical Oncology Group Study JCOG0912, Gastric Cancer 20 (2017) 699–708.
[35] W. Kim, H.H. Kim, S.U. Han, M.C. Kim, W.J. Hyung, Korean laparo-endoscopic gastrointestinal surgery study (KLASS) group, decreased morbidity of laparoscopic distal gastrectomy compared with open distal gastrectomy for stage I gastric cancer: short-term outcomes from a multicenter randomized controlled trial (KLASS-01), Ann. Surg. 263 (2016) 28–35.
[36] K. Yamashita, S. Sakuramoto, S. Kikuchi, N. Futawatari, N. Katada, et al., Laparoscopic versus open distal gastrectomy for early gastric cancer in Japan: long-term clinical outcomes of a randomized clinical trial, Surg. Today 46 (2016) 741–749.
[37] J.P. Higgins, S.G. Thompson, J.J. Deeks, D.G. Altman, Measuring inconsistency in meta-analyses, BMJ 327 (2003) 557–560.
[38] G.H. Guyatt, A.D. Oxman, G.E. Vist, R. Kunz, Y. Falck-Ytter, GRADE Working Group, GRADE: an emerging consensus on rating quality of evidence and strength of recommendations, BMJ 336 (2008) 924–926.
[39] K. Thorlund, J. Engstrøm, J. Wetterslev, J. Brok, G. Imberger, et al., User Manual for Trial Sequential Analysis (TSA), Denmark: Copenhagen Trial Unit, Centre for Clinical Intervention Research, Copenhagen, 2011 1–115.
[40] J. Wetterslev, K. Thorlund, J. Brok, C. Gluud, Trial sequential analysis may establish when firm evidence is reached in cumulative meta-analysis, Journal. of. Clinical. Epidemiology 61 (2008) 64–75.
[41] J. Brok, K. Thorlund, C. Gluud, J. Wetterslev, Trial sequential analysis reveals insufficient information size and potentially false positive results in many meta-analyses, Journal. of. Clinical. Epidemiology 61 (2008) 763–769.
[42] K. Thorlund, P.J. Devereaux, J. Wetterslev, G. Guyatt, J.P. Ioannidis, et al., Can trial sequential monitoring boundaries reduce spurious inferences from meta-analyses? International. Journal. of. Epidemiology 38 (2009) 276–286.
[43] J. Brok, K. Thorlund, J. Wetterslev, C. Gluud, Apparently conclusive meta-analyses may be inconclusive - trial sequential analysis adjustment of random error risk due to repetitive testing of accumulating data in apparently conclusive neonatal meta-analyses, International. Journal. of. Epidemiology 38 (2009) 287–298.
[44] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman DG, P.R.I.S.M.A. Group, Preferred reporting Items for systematic Reviews and meta-analyses: the PRISMA statement, BMJ 339 (2009) b2535.
[45] J.P. Higgins, D.G. Altman, P.C. Gøtzsche, P. Jüni, D. Moher, Cochrane bias methods group; Cochrane statistical methods group, the Cochrane Collaboration's tool for assessing risk of bias in randomised trials, BMJ 343 (2011) d5928.
[46] J.A. Sterne, A.J. Sutton, J.P. Ioannidis, N. Terrin, D.R. Jones, et al., Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials, BMJ 343 (2011) d4002.
[47] D. Luo, X. Wan, J. Liu, T. Tong, Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range, Stat. Meth. Med. Res. 27 (2018) 1785–1805.
[48] X. Wan, W. Wang, J. Liu, T. Tong, Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range, BMC, Med. Res. Methodol 14 (2014) 135.
[49] C.G. Huscher, A. Mingoli, G. Sgarzini, A. Sansonetti, M. Di Paola, et al., Laparoscopic versus open subtotal gastrectomy for distal gastric cancer: five-year results of a randomized prospective trial, Ann. Surg. 241 (2005) 232–237.
[50] H.J. Lee, H.H. Kim, M.C. Kim, S.Y. Ryu, W. Kim, Korean Laparoscopic Gastrointestinal Surgery Study Group, the impact of a high body mass index on laparoscopy assisted gastrectomy for gastric cancer, Surg. Endosc. 23 (2009) 2473–2479.
[51] J.H. Jung, S.Y. Ryu, M.R. Jung, Y.K. Park, O. Jeong, Laparoscopic distal gastrectomy for gastric cancer in morbidly obese patients in South Korea, J. Gastric. Cancer 14 (2014) 187–195.
[52] D.D. Smith, R.R. Schwarz, R.E. Schwarz, Impact of total lymph node count on staging and survival after gastrectomy for gastric cancer: data from a large US-population database, J. Clin. Oncol. 23 (2005) 7114–7124.
[53] Japanese Gastric Cancer Association, Japanese gastric cancer treatment guidelines 2014 (ver. 4), Gastric Cancer 20 (2017) 1–19.
[54] J.A. Ajani, D.J. Bentrem, S. Besh, T.A. D'Amico, P. Das, National Comprehensive Cancer Network, Gastric cancer, version 2.2013: featured updates to the NCCN Guidelines, J. Natl. Compr. Canc. Netw. 11 (2013) 531–546 2013.
[55] J.A. Ajani, T.A. D'Amico, K. Almhanna, D.J. Bentrem, J. Chao, et al., Gastric cancer, version 3. 2016, NCCN clinical practice guidelines in oncology, J. Natl. Compr. Canc. Netw. 14 (2016) 1286–1312.
[56] E.C. Smyth, M. Verheij, W. Allum, D. Cunningham, A. Cervantes, ESMO Guidelines,

Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up, Ann. Oncol. 27 (2016) v38–v49.
[57] T. Waddell, M. Verheij, W. Allum, D. Cunningham, A. Cervantes, et al., European society for medical oncology (ESMO); european society of surgical oncology (ESSO); european society of radiotherapy and oncology (ESTRO), gastric cancer: ESMO-ESSO-ESTRO clinical practice guidelines for diagnosis, treatment and follow-up, Eur. J. Surg. Oncol. 40 (2014) 584–591.
[58] I. Songun, H. Putter, E.M. Kranenbarg, M. Sasako, C.J. van de Velde, Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial, Lancet Oncol. 11 (2010) 439–449.
[59] R.E. Schwarz, D.D. Smith, Clinical impact of lymphadenectomy extent in resetable gastric cancer of advanced stage, Ann. Surg Oncol. 14 (2007) 317–328.
[60] O. Dohi, N. Yagi, A. Majima, Y. Horii, T. Kitaichi, Diagnostic ability of magnifying endoscopy with blue laser imaging for early gastric cancer: a prospective study, Gastric Cancer 20 (2017) 297–303.
[61] Q. Zhang, F. Wang, Z.Y. Chen, Z. Wang, F.C. Zhi, et al., Comparison of the diagnostic efficacy of white light endoscopy and magnifying endoscopy with narrow band imaging for early gastric cancer: a meta-analysis, Gastric Cancer. 19(206) 543–552.
[62] T. Nagahama, K. Yao, N. Uedo, H. Doyama, T. Ueo, et al., Delineation of the extent of early gastric cancer by magnifying narrow-band imaging and chromoendoscopy: a multicenter randomized controlled trial, Endoscopy 50 (2018) 566–576.
[63] K. Sumiyama, Past and current trends in endoscopic diagnosis for early stage gastric cancer in Japan, Gastric Cancer 20 (2017) 20–27.
[64] S. Yamada, H. Doyama, K. Yao, N. Uedo, Y. Ezoe, et al., An efficient diagnostic strategy for small, depressed early gastric cancer with magnifying narrow-band imaging: a post-hoc analysis of a prospective randomized controlled trial, Gastrointest. Endosc. 79 (2014) 55–63.
[65] Y. Ezoe, M. Muto, N. Uedo, H. Doyama, K. Yao, et al., Magnifying narrowband imaging is more accurate than conventional white-light imaging in diagnosis of gastric mucosal cancer, Gastroenterology 141 (2011) 2017–2025.
[66] R.M. Kwee, T.C. Kwee, Imaging in local staging of gastric cancer: a systematic review, J. Clin. Oncol. 25 (2007) 2107–2116.
[67] R. Soetikno, T. Kaltenbach, R. Yeh, T. Gotoda, Endoscopic mucosal resection for early cancers of the upper gastrointestinal tract, J. Clin. Oncol. 23 (2005) 4490–4498.
[68] S. Nakamoto, Y. Sakai, J. Kasanuki, F. Kondo, Y. Ooka, et al., Indications for the use of endoscopic mucosal resection for early gastric cancer in Japan: a comparative study with endoscopic submucosal dissection, Endoscopy 41 (2009) 746–750.
[69] Y.M. Park, E. Cho, H.Y. Kang, J.M. Kim, The effectiveness and safety of endoscopic submucosal dissection compared with endoscopic mucosal resection for early gastric cancer: a systematic review and meta-analysis, Surg. Endosc. 25 (2011) 2666–2677.
[70] L. Zong, A. Wu, W. Wang, J. Deng, S. Aikou, et al., Feasibility of laparoscopic gastrectomy for elderly gastric cancer patients: meta-analysis of non-randomized controlled studies, Oncotarget 8 (2017) 51878–51887.
[71] J.F. Wang, S.Z. Zhang, N.Y. Zhang, Z.Y. Wu, J.Y. Feng, et al., Laparoscopic gastrectomy versus open gastrectomy for elderly patients with gastric cancer: a systematic review and meta-analysis, World J. Surg. Oncol. 14 (2016) 90.
[72] Y. Pan, K. Chen, W.H. Yu, H. Maher, S.H. Wang, et al., Laparoscopic gastrectomy for elderly patients with gastric cancer: a systematic review with meta-analysis, Medicine 97 (2018) e0007.

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Lymphovascular invasion as a predictor for lymph node metastasis and a prognostic factor in gastric cancer patients under 70 years of age: A retrospective analysis

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ABSTRACT

Background: Accumulating evidence has confirmed the potential prognostic value of LVI in patients with cancers. This aim of the current study was to clarify the potential relationship between LVI and lymph node metastasis, establish predictive clinicopathologic prognostic factors for LVI and lymph node metastasis, and determine the prognostic significance of LVI for patients younger than 70 years with resected gastric cancer.

Methods: Overall survival rates were calculated using Kaplan-Meier analysis. Differences in proportions of patients were tested with the χ^2 test. Univariate and multivariate analyses were applied to identify independent prognostic factors. Logistic regression analysis was employed to identify the risk factors predicting the presence of LVI and LN metastasis.

Results: Univariate analysis led to the identification of tumor size, LVI and pN stage as factors significantly correlated with prognosis. Multivariate analysis demonstrated that tumor size, LVI, pN stage, and number of LNs retrieved are independent prognostic factors for the entire population. Logistic regression analysis proved that LVI and pT stage were significantly associated with LN metastasis.

Conclusion: LVI is an independent prognostic factor predicting LN metastasis and a strongly independent predictor of survival for patients with resected gastric cancer. We recommend that LVI should be taken into account as an important adjuvant prognostic factor, specially for pN0 cases with inadequate LNs retrieved. And the maximum number of LNs possible should be retrieved for optimal staging, especially for patients with higher cT stage.

1. Introduction

Gastric cancer has emerged as a major global public health problem [1–4] with the highest incidence in China [5]. Lymphovascular invasion (LVI) is defined as tumor cell spread through the lymphatic vessels [6]. Accumulating evidence has confirmed the potential prognostic value of LVI in patients with cancer of the esophagus [7–9], adenocarcinoma of the esophagogastric junction [6], colon cancer [10], and gastric cancer [11–14].

Importantly, the majority of previous studies have included patients older than 80 or even 85 years [6–14]. However, the average lifespans of men and women in China are 74 and 77 years, respectively. Therefore, the long-term effect of curative gastrectomy for gastric cancer may not be evaluable in such elderly patients [15], and inclusion of patients within this age group may lead to unreliable results.

This aim of the current study was to clarify the potential relationship between LVI and lymph node metastasis, establish predictive clinicopathologic prognostic factors for LVI and lymph node metastasis, and determine the prognostic significance of LVI for patients younger than 70 years with resected gastric cancer.

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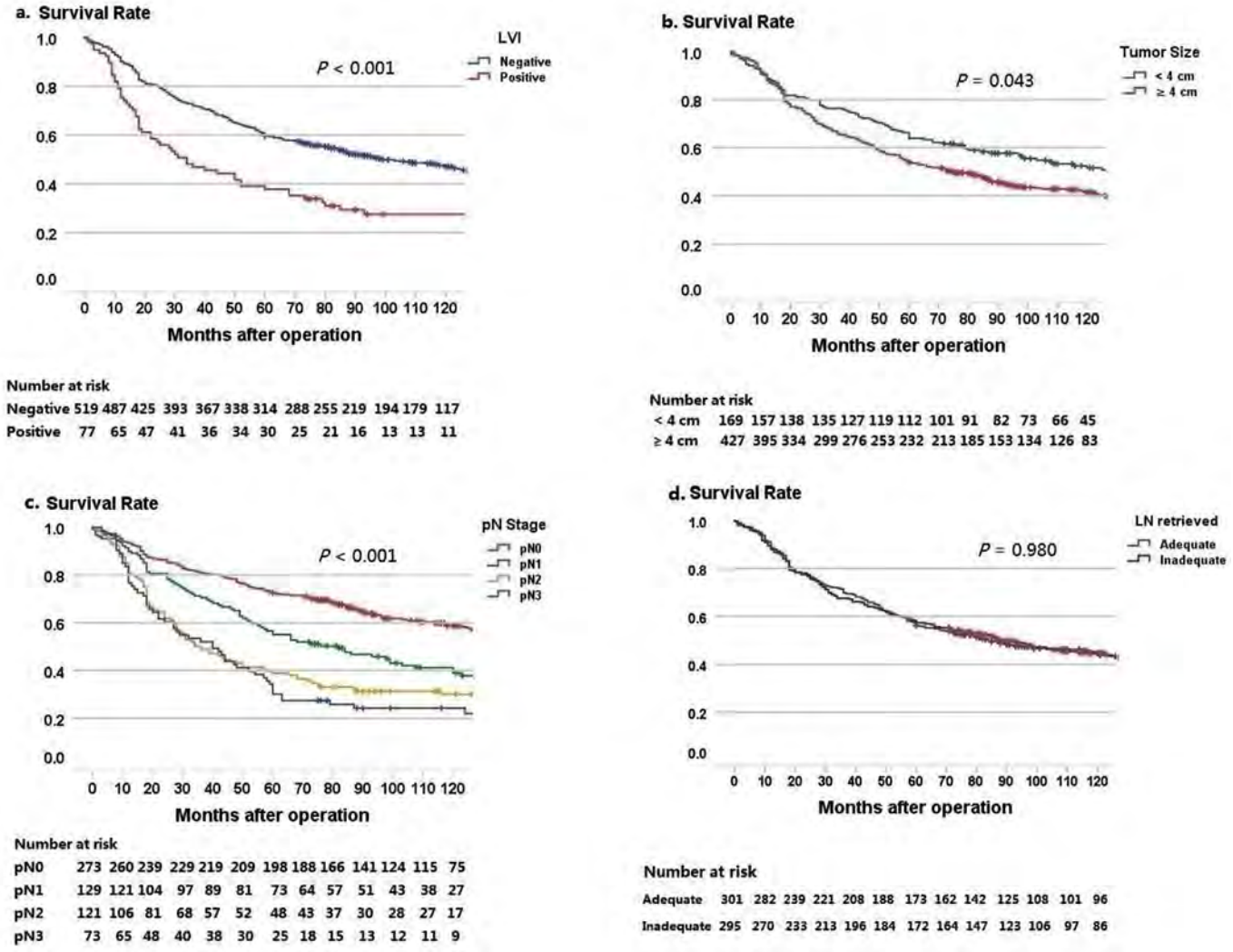

Fig. 1. Kaplan-Meier curves of the entire population according to LVI (Fig 1a), tumor size (Fig 1b), pN stage (Fig 1c), and LNs retrieved (Fig 1d).

2. Methods

Between February 1984 and February 2010, 596 patients with gastric cancer subjected to primary surgical resection in our institution were enrolled into a retrospective database. This study was approved by the Ethics Committee. All patient records and information were anonymized and de-identified prior to analysis. The work has been reported in line with the STROCSS criteria [16].

2.1. Surgical approach

All patients underwent potentially curative resection for histologically proven adenocarcinoma. Patients were subjected to total, proximal subtotal or distal subtotal gastrectomy with standard D2 (D2) or extended D2 (D2+) lymphadenectomy. Following gastrectomy, Billroth I, Billroth II or Roux-Y reconstruction was performed.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: histologically proven stomach adenocarcinoma, curative operation, negative resection margins (R0), complete medical records, D2 or D2+ lymphadenectomy, non-emergent surgery. Exclusion criteria included preoperative adjuvant therapy, laparoscopic-assisted surgery, stage IV cancer, previous or concomitant cancer, and patients over 70 years of age.

The clinicopathologic features investigated for prognostic significance included gender, age, type of anesthesia, blood loss, tumor size, reconstruction type, gastrectomy, histologic grade, depth of invasion (pT stage), number of regional LN metastases (pN stage), LVI, number of lymph nodes (LN) retrieved, recurrence or metastasis, and chemotherapy.

2.3. Pathological assessment

All specimens were analyzed by two independent and experienced pathologists, and different opinions were resolved by discussion to establish the final diagnosis. Carcinoma lesions together with the surrounding gastric wall were fixed in formalin and cut into multiple 5 mm slices in parallel with the lesser curvature. Venous invasion refers to tumor cell lining the venous endothelial surface, and tumor cell thrombi inside the lumen of the vein, which was identified by immunohistochemical staining. The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach was applied to re-stage all patients in this study. According to the current guidelines for gastric cancer, examination of at least 15 LNs is strongly recommended for adequate staging [17,18]. The pathology report mainly included data on tumor size, pT stage, pN stage, LVI, number of LNs retrieved, and histologic grade.

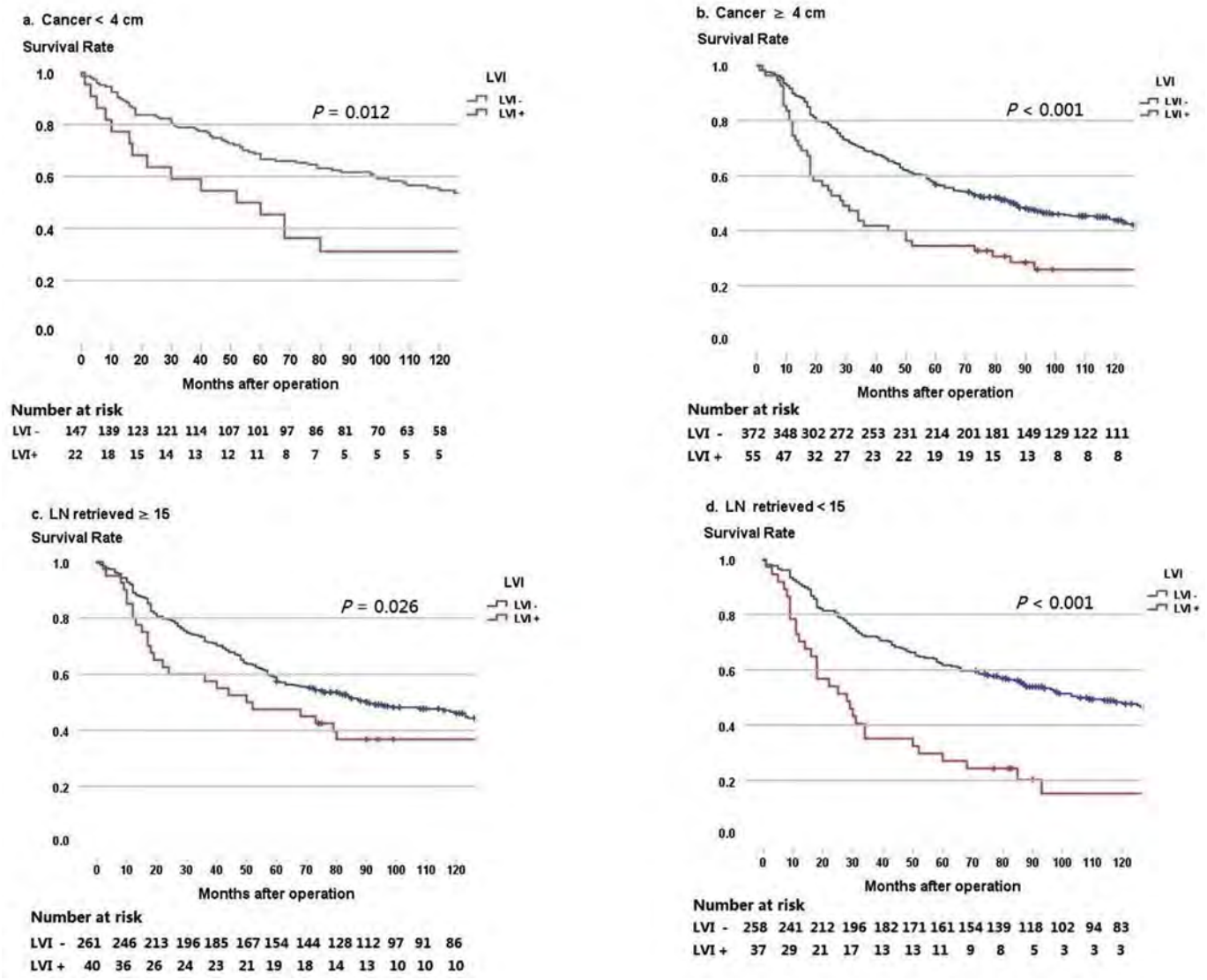

Fig. 2. Kaplan-Meier curves comparing LVI- and LVI+ patient groups with tumor sizes < 4 cm (Fig 2a), tumor sizes ≥ 4 cm (Fig 2b), LNs retrieved ≥ 15 (Fig 2c), and LNs retrieved < 15 (Fig 2d).

2.4. Follow-up

All patients were systematically followed up by personal contact with a phone call until death or the cut-off date (the final follow-up was in October 2014) over a duration of 1–368 months. The follow-up rate was 98.0%, and 12 patients were lost to follow-up in total and were excluded from this study. Complete histories were available for all patients, and physical and chemical profiles were examined every 3 months for 1–2 years, every 6–12 months for 3–5 years and annually thereafter. Overall, 596 patients younger than 70 years with resected gastric cancer were included.

2.5. Statistical analysis

Overall survival rates were calculated using Kaplan-Meier analysis including 95% confidence interval (95% CI), and examined with the log-rank test. The number at risk is shown on all Kaplan-Meier curves (Figs. 1–2). Differences in proportions of patients were tested with the χ^2 test. Univariate analysis with log-rank test and multivariate analysis were applied to identify independent prognostic factors. Logistic regression analysis was employed to identify the risk factors predicting the presence of LVI and LN metastasis. A p value of less than 0.05 was considered significant. All statistical analyses were performed using SPSS Statistical Software (version 22.0) (SPSS, Inc., Chicago, IL, USA).

3. Results

A total of 62 patients older than 70 years (age range: 71–90 years) of age were excluded. Overall, 596 patients with resected gastric cancer were assessed for eligibility. The age range of the entire patient population was between 30 and 70 years. Among the patients examined, absence of LVI (LVI-) was confirmed in 519 and presence of LVI (LVI+) in 77 patients. Within the LVI- group, 134 (25.8%) were female and 385 (74.2%) were male. The LVI+ group comprised 22 (28.6%) female and 55 (71.4%) male patients.

The two groups (LVI- and LVI+) were balanced with respect to gender (p = 0.608), age (p = 0.931), type of anesthesia (p = 0.414), blood loss (p = 0.102), tumor size (p = 0.964), reconstruction type (p = 0.273), gastrectomy (p = 0.511), histologic grade (p = 0.413), pT stage (p = 0.740), number of LNs retrieved (p = 0.786), recurrence or metastasis (p = 0.987), and chemotherapy (p = 0.664). We observed a significant difference only in pN stage (p < 0.001) between LVI- and LVI+ groups in Table 1.

Univariate analysis led to the identification of tumor size

Table 1
Differences in clinicopathologic features in groups of patients with absence and presence of LVI subjected to gastrectomy.

Variables	LVI – *n* (%)	LVI+ *n* (%)	*p* value
Gender			0.608
Female	134 (25.8)	22 (28.6)	
Male	385 (74.2)	55 (71.4)	
Age, years			0.931
< 65	375 (72.3)	56 (72.7)	
≥ 65	144 (27.7)	21 (27.3)	
Type of anesthesia			0.414
General anesthesia	437 (84.2)	62 (80.5)	
Epidural anesthesia	82 (15.8)	15 (19.5)	
Blood loss, ml			
< 500	479 (92.3)	75 (97.4)	0.102
≥ 500	40 (7.7)	2 (2.6)	
Tumor size, cm			0.964
< 4	147 (28.3)	22 (28.6)	
≥ 4	372 (71.7)	55 (71.4)	
Reconstruction type			0.273
Billroth I	428 (82.5)	58 (75.3)	
Billroth II	72 (13.9)	16 (20.8)	
Roux-Y	19 (3.6)	3 (3.9)	
Gastrectomy			0.511
Total	39 (7.5)	5 (6.5)	
Proximal subtotal	54 (10.4)	5 (6.5)	
Distal subtotal	426 (82.1)	67 (87.0)	
Histologic grade			0.413
G1	41 (7.9)	4 (5.2)	
G2	168 (32.4)	20 (26.0)	
G3	278 (53.5)	49 (63.6)	
G4	32 (6.2)	4 (5.2)	
pT stage [a]			0.740
pT1	79 (15.2)	11 (14.3)	
pT2	126 (24.3)	17 (22.1)	
pT3	177 (34.1)	24 (31.2)	
pT4a	137 (26.4)	25 (32.4)	
pN stage [a]			< 0.001
pN0	257 (49.5)	16 (20.8)	
pN1	114 (22.0)	15 (19.5)	
pN2	96 (18.5)	25 (32.4)	
pN3	52 (10.0)	21 (27.3)	
Number of LNs retrieved			0.786
Adequate, n ≥ 15	261 (50.3)	40 (51.9)	
Inadequate, n < 15	258 (49.7)	37 (48.1)	
Recurrence or metastasis			0.987
Absent	350 (67.4)	52 (67.5)	
Present	169 (32.6)	25 (32.5)	
Chemotherapy			0.664
No	449 (86.5)	68 (88.3)	
Yes	70 (13.5)	9 (11.7)	

Two tailed *t*-tests of mean ± standard deviation (SD); *n*, number of patients; LNs, lymph nodes; LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion; G1, well differentiated; G2, moderately differentiated, G3, poorly differentiated, G4, undifferentiated.

[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

(p = 0.043), LVI (p < 0.001) and pN stage (p < 0.001) as factors significantly correlated with prognosis. All clinicopathologic factors were included in the first step of multivariate analysis, which demonstrated that tumor size (RR, 1.332; 95% CI, 1.062–1.671, p = 0.013), LVI (RR, 1.487; 95% CI, 1.122–1.971, p = 0.006), pN stage (RR, 1.413; 95% CI, 1.282–1.558, p < 0.001), and number of LNs retrieved (RR, 1.304; 95% CI, 1.057–1.608, p = 0.013) are independent prognostic factors for the entire population. In the second step of multivariate analysis, histological grade, pT stage, and chemotherapy were added. Notably, tumor size (RR, 1.319; 95% CI, 1.054–1.650, p = 0.015), LVI (RR, 1.489; 95% CI, 1.127–1.968, p = 0.005), pN stage (RR, 1.422; 95% CI, 1.290–1.566, p < 0.001), and number of LNs retrieved (RR, 1.298; 95% CI, 1.054–1.599, p = 0.014) remained independent prognostic factors. The 5-year overall survival rates (5-YSR) are presented in Table 2. Survival curves comparing LVI, tumor size, pN stage, and number of LNs retrieved are shown in Fig. 1.

Prognosis for patients in the LVI- and LVI+ groups stratified by tumor size, pN stage, LVI and number of LNs retrieved was compared. Patients in the LVI+ group had significant poorer 5-YSR than those in the LVI- group. Significant differences in 5-YSR were observed in the entire population (37.7% for LVI+ vs. 59.9% for LVI-, p < 0.001; log-rank test) as well as patients with tumor sizes less than 4 cm (45.5% for LVI+ vs. 66.7% for LVI-, p = 0.012; log-rank test), tumor sizes ≥4 cm (34.5% for LVI+ vs. 56.7% for LVI-, p < 0.001; log-rank test), adequate LNs retrieved (47.5% for LVI+ vs. 57.5% for LVI-, p = 0.026; log-rank test), and inadequate LNs retrieved (27.0% for LVI+ vs. 61.6% for LVI-, p < 0.001; log-rank test). Importantly, patients in the LVI+ group with inadequate LNs retrieved had poorer 5-YSR (27.0%) than those in the LV1- group with lymph node metastasis (73.2% for pN1 and LVI-, 57.9% for pN2 and LVI-, 40.6% for pN3 and LVI-, 30.8% for pN3 and LVI-) in Table 3. Survival curves comparing LVI+ and LVI- groups stratified by tumor size and LNs retrieved are shown in Fig. 2.

Logistic regression analysis was applied to determine the risk factors predictive of LVI+, including tumor size, histologic grade, pT stage. No risk factors were found to be significantly correlated with LVI+ in Table 4. Further logistic regression analysis was applied to determine the risk factors predictive of LN metastasis, including tumor size, histologic grade, pT stage, and LVI. Among the factors examined, LVI (RR, 3.760; 95% CI, 2.087–6.774, p < 0.001) and pT stage (RR, 1.505; 95% CI, 1.272–1.780, p < 0.001) were significantly associated with LN metastasis in Table 5.

4. Discussion

Increasing evidence has confirmed the potential prognostic value of LVI in patients with solid tumors [6–14]. An earlier retrospective study suggested that careful search for vascular invasion in gastric cancer may provide useful information for identifying patients at high risk aged between 23 and 90 years suitable for adjuvant therapy [11]. LVI has been confirmed as an independent prognostic factor in patients aged 32.5–81.1 years with esophageal squamous cell carcinoma [8]. Moreover, LVI has been identified as a strong and independent prognostic factor, and recommended into the TNM staging system for patients aged 17–89 years with primary resected adenocarcinoma of the esophagogastric junction [6].

The majority of previous studies have included patients aged older than 80 or even 85 years. As the average lifespans of men and women in China are 74 and 77 years, respectively, the results would not be as reliable if elderly patients over these age groups are included [15]. If we included patients older than 70 years, they may die because of their own lifespans within 5 years after the surgery, rather than recurrence of metastasis of gastric cancer. Moreover, the 5-YSR of patients is an important index for patients with cancer. Therefore, we only included patients with resected gastric cancer aged younger than 70 years in the present study. However, age selection bias may exist in the current study.

Our two-step multivariate analysis led to the identification of tumor size, LVI, pN stage and adequate or inadequate number of LNs retrieved as independent poor prognostic factors. Tumor sizes ≥4 cm, presence of LVI, higher pN stage, and inadequate number of LNs retrieved were associated with poorer 5-YSR. Considering prognosis, 5-YSR of patients in the LVI+ group was significantly poorer than that of patients without LVI stratified by tumor size and adequate or inadequate number of LNs retrieved. Notably, patients with LVI and inadequate number of LNs retrieved had a 5-YSR of 27.0%, suggesting that both LVI and number of LNs retrieved are prognostic factors for patients with resected gastric cancer.

Considering that pN stage is the most valuable prognostic factor for gastric cancer, we conducted logistic regression analysis of risk factors predictive of LN metastasis [19,20], which revealed a close relationship of metastasis with pT stage and LVI. Importantly, cancers with LVI+

Table 2
Univariate and multivariable analyses of prognostic factors for the entire study population.

Variables	Univariate analysis			Multivariate analysis 1[b]			Multivariate analysis 2[c]		
	n (%)	5-YSR (%)	*p* value	RR	95% CI	*p* value	RR	95% CI	*p* value
Gender			0.121						
Female	156 (26.2)	62.2							
Male	440 (73.8)	54.8							
Age, years			0.627						
< 65	431 (72.3)	56.1							
≥ 65	165 (27.7)	58.2							
Type of anesthesia			0.960						
General anesthesia	499 (83.7)	56.7							
Epidural anesthesia	97 (16.3)	56.7							
Blood loss, ml			0.473						
< 500	554 (93.0)	56.5							
≥ 500	42 (7.0)	59.5							
Tumor size, cm			0.043	1.332	1.062–1.671	0.013	1.319	1.054–1.650	0.015
< 4	169 (28.4)	63.9							
≥ 4	427 (71.6)	53.9							
Reconstruction type			0.176						
Billroth I	486 (81.5)	58.8							
Billroth II	88 (14.8)	46.6							
Roux-Y	22 (3.7)	50.0							
Gastrectomy			0.897						
Total	44 (7.4)	59.1							
Proximal subtotal	59 (9.9)	62.7							
Distal subtotal	493 (82.7)	55.7							
Histologic grade			0.925						
G1	45 (7.6)	62.2							
G2	188 (31.5)	54.3							
G3	327 (54.9)	56.9							
G4	36 (6.0)	61.1							
LVI			< 0.001	1.487	1.122–1.971	0.006	1.489	1.127–1.968	0.005
LVI-	519 (87.1)	59.5							
LVI+	77 (12.9)	37.7							
pT stage [a]			0.372						
pT1	90 (15.1)	61.1							
pT2	143 (24.0)	55.2							
pT3	201 (33.7)	55.7							
pT4a	162 (27.2)	56.8							
pN stage [a]			< 0.001	1.413	1.282–1.558	< 0.001	1.422	1.290–1.566	< 0.001
pN0	273 (45.8)	72.5							
pN1	129 (21.6)	55.0							
pN2	121 (20.3)	38.8							
pN3	73 (12.3)	30.1							
Number of LNs retrieved			0.980	1.304	1.057–1.608	0.013	1.298	1.054–1.599	0.014
Adequate, n ≥ 15	301 (50.5)	56.1							
Inadequate, n < 15	295 (49.5)	57.3							
Recurrence or metastasis			0.208						
Absent	402 (67.4)	58.2							
Present	194 (32.6)	53.6							
Chemotherapy			0.362						
No	517 (86.7)	56.5							
Yes	79 (13.3)	58.2							

n, number of patients; LNs, lymph nodes; RR, relative risk; 95% CI, 95% confidence interval; 5-YSR, five-year overall survival rate (%); LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion; G1, well differentiated; G2, moderately differentiated, G3, poorly differentiated, G4, undifferentiated.

[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

[b] All clinicopathologic factors were included in the first multivariate analysis.

[c] Histological grade, pT stage, and chemotherapy were also included in the second multivariate analysis.

had significant high risk of LN metastasis. Our data highlight the importance of LVI for prognosis and its relationship with LN metastasis. LVI may additionally be an effective predictor of LN metastasis. Moreover, pT may be applied as a predictor of LVI. Thus, cancers with higher pT stage may have higher risk of LN metastasis. Patients with higher clinical T stage (cT stage) should therefore be paid more attention and as many LNs (at least 15 if not more) as possible retrieved for accurate staging [17,18], which may be greatly improved with the availability of effective diagnostic methods, such as endoscopic ultrasound (ESU), CT, PET/CT, MRI, and diagnostic staging laparoscopy (DSL) [21–26].

Similarly, we conducted logistic regression analysis to determine the risk factors of LVI. No risk factors were found to be significantly correlated with LVI+. It is our belief that although the current NCCN guidelines for gastric cancer strongly recommend the examination of at least 15 LNs for adequate staging, some patients still have less than 15 LNs retrieved. For pT1, N0 patients (R0 resection), adjuvant therapy is not recommended by the NCCN guidelines. In addition, for pT2, N0 patients, surveillance is also an option. However, for those pT1-2, N0, and LVI+ patients with inadequate LNs retrieved, and who did not receive adjuvant therapy postoperatively, these patients may have a poor survival rate. Therefore, we believe that LVI should be taken into account as an important adjuvant prognostic factor, especially for pT1-2, N0 patients with inadequate LNs retrieved. A previous study also

Table 3
Comparison of prognosis for patients with gastric cancer in the LVI+ and LVI- groups.

Variables	LVI-		LVI+		*p* value
	n (%)	5-YSR (%)	*n* (%)	5-YSR (%)	
For the entire population	519 (100.0)	59.9	77 (100.0)	37.7	< 0.001
Tumor size, cm					
< 4	147 (28.3)	66.7	22 (28.6)	45.5	0.012
≥ 4	372 (71.7)	56.7	55 (71.4)	34.5	< 0.001
pN stage [a]					
pN0	257 (49.5)	73.2	16 (20.8)	62.5	0.162
pN1	114 (22.0)	57.9	15 (19.5)	33.3	0.057
pN2	96 (18.5)	40.6	25 (32.4)	32.0	0.086
pN3	52 (10.0)	30.8	21 (27.3)	28.6	0.275
Number of LNs retrieved					
Adequate, n ≥ 15	261 (50.3)	57.5	40 (51.9)	47.5	0.026
Inadequate, n < 15	258 (49.7)	61.6	37 (48.1)	27.0	< 0.001

n, number of patients; LNs, lymph nodes; RR, relative risk; 95% CI, 95% confidence interval; 5-YSR, five-year overall survival rate (%); LVI-, absence of lymphovascular invasion; LVI+, presence of lymphovascular invasion.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

Table 4
Logistic regression analysis of risk factors predicting the presence of LVI.

Variables	RR	95% CI	*p* value
Tumor size	0.996	0.585–1.694	0.988
Histologic grade	1.227	0.867–1.735	0.248
pT stage[a]	1.086	0.855–1.379	0.498

RR, relative risk; 95% CI, 95% confidence interval.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

Table 5
Logistic regression analysis of risk factors predicting LN metastasis.

Variables	RR	95% CI	*p* value
Tumor size	1.075	0.740–1.561	0.705
Histologic grade	1.150	0.909–1.455	0.242
pT stage[a]	1.505	1.272–1.780	< 0.001
LVI	3.760	2.087–6.774	< 0.001

n, number; RR, relative risk; 95% CI, 95% confidence interval; LN, lymph node; LVI, lymphovascular invasion.
[a] The 8th Edition of the American Joint Committee on Cancer (AJCC) TNM staging classification for carcinoma of the stomach.

suggested that adjuvant therapy should be considered for LVI+ patients [27]. Therefore, pathology reports in the future should include assessment of LVI. We recommend that LVI should be taken into account as an important adjuvant prognostic factor, especially for pN0 patients with inadequate LNs retrieved. The key for an adequate prognostic assessment of gastric cancer is an adequate lymph node yield. Though the NCCN guidelines for gastric cancer strongly recommend the examination of at least 15 LNs for adequate staging, more lymph node dissection will be better. It is quite obvious that a LVI+ case with inadequate lymph node yield may be misdiagnosed as a N0, being instead a N1, considering the skip metastases issue and the aggressivity of histologic pattern. Therefore, this could lead to undertreatment. A dissection of less than 15 lymph nodes is an inadequate treatment for gastric cancer, which may be not an ideal surgery. Importantly, the LV paten can be an additional help to stratify the risk of recurrence. As far as we are concerned that a retrospective study may be liable for biases; therefore, further multicenter, randomized controlled trials, especially containing postoperative pathology reports as subject, are required.

Overall, our results indicate that LVI is an independent prognostic factor predicting LN metastasis and a strongly independent predictor of survival for patients with resected gastric cancer, specially pN0 cases with inadequate LNs retrieved. In addition, the maximum number of LNs possible should be retrieved for optimal staging, especially for patients with higher cT stage. However, the results of the current study need to be interpreted with caution and further multicenter, randomized controlled trials are required to validate our findings.

Ethical approval

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with Helsinki Declaration of 1964 and later versions.

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Author contribution

CDZ, FLN and DQD conceived of the study. CDZ and FLN collected data. CDZ and FLN provided data analysis. All authors contributed to writing, reviewing, or revising the paper. CDZ, FLN and DQD submitted the final manuscript and all authors read and approved the final manuscript. All authors are grateful to all the previous study authors and study participants.

Conflict-of-interest

The authors declare that they have no conflict of interest.

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References

[1] R.L. Siegel, K.D. Miller, A. Jemal A, Cancer statistics, 2017, CA Cancer J. Clin. 67 (2017) 7–30.
[2] W.B. Goggins, G.K. Wong, Poor survival for US pacific islander cancer patients: evidence from the surveillance, epidemiology, and end results database: 1991 to 2004, J. Clin. Oncol. 25 (2007) 5738–5741.
[3] K. Washington, 7th edition of the AJCC cancer staging manual: stomach, Ann. Surg Oncol. 17 (2010) 3077–3079.
[4] D.M. Parkin, F. Bray, J. Ferlay, P. Pisani, Global cancer statistics, 2002, CA Cancer J. Clin. 55 (2005) 74–108.
[5] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, et al., Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132.
[6] B.H. von Rahden, H.J. Stein, M. Feith, K. Becker, J.R. Siewert, Lymphatic vessel invasion as a prognostic factor in patients with primary resected adenocarcinomas of the esophagogastric junction, J. Clin. Oncol. 23 (2005) 874–879.
[7] M. Sarbia, R. Porschen, F. Borchard, O. Horstmann, R. Willers, et al., Incidence and prognostic significance of vascular and neural invasion in squamous cell carcinomas of the esophagus, Int. J. Canc. 61 (1995) 333–336.
[8] B.L. Brücher, H.J. Stein, M. Werner, J.R. Siewert, Lymphatic vessel invasion is an independent prognostic factor in patients with a primary resected tumor with esophageal squamous cell carcinoma, Cancer 92 (2001) 2228–2233.
[9] S.M. Lagarde, A.W. Phillips, M. Navidi, B. Disep, A. Immanuel, et al., The presence of lymphovascular and perineural infiltration after neoadjuvant therapy and oesophagectomy identifies patients at high risk for recurrence, Br. J. Canc. 113 (2015) 1427–1433.
[10] C.D. Zhang, J.N. Wang, B.Q. Sui, Y.J. Zeng, J.Q. Chen, et al., Prognostic and

predictive model for stage II colon cancer patients with nonemergent surgery: who should receive adjuvant chemotherapy? Medicine (Baltim.) 95 (2016) e2190.
[11] H.E. Gabbert, S. Meier, C.D. Gerharz, G. Hommel, Incidence and prognostic significance of vascular invasion in 529 gastric-cancer patients, Int. J. Canc. 49 (1991) 203–207.
[12] B.J. Dicken, K. Graham, S.M. Hamilton, S. Andrews, R. Lai, et al., Lymphovascular invasion is associated with poor survival in gastric cancer: an application of gene-expression and tissue array techniques, Ann. Surg. 243 (2006) 64–73.
[13] P. Li, H.Q. He, C.M. Zhu, Y.H. Ling, W.M. Hu, et al., The prognostic significance of lymphovascular invasion in patients with resectable gastric cancer: a large retrospective study from Southern China, BMC Canc. 15 (2015) 370.
[14] T. Yamada, H. Sugiyama, D. Ochi, D. Akutsu, H. Suzuki, et al., Risk factors for submucosal and lymphovascular invasion in gastric cancer looking indicative for endoscopic submucosal dissection, Gastric Cancer 17 (2014) 692–696.
[15] L. Zong, M. Abe, Y. Seto, J. Ji, Randomized controlled trial of laparoscopic versus open D2 distal gastrectomy for advanced gastric cancer: how should we define the age of included patents? J. Clin. Oncol. (2016), http://dx.doi.org/10.1200/JCO.2016.68.2500 pii: JCO682500.
[16] R.A. Agha, M.R. Borrelli, M. Vella-Baldacchino, R. Thavayogan, D.P. OrgillSTROCSS Group, The STROCSS statement: strengthening the reporting of cohort studies in surgery, Int. J. Surg. 46 (2017) 198–202.
[17] R.E. Schwarz, D.D. Smith, Clinical impact of lymphadenectomy extent in resectable gastric cancer of advanced stage, Ann. Surg Oncol. 14 (2007) 317–328.
[18] I. Songun, H. Putter, E.M. Kranenbarg, M. Sasako, C.J. van de Velde, Surgical treatment of gastric cancer: 15-year follow-up results of the randomised nationwide Dutch D1D2 trial, Lancet Oncol. 11 (2010) 439–449.
[19] M. Degiuli, M. Sasako, A. Ponti, F. Calvo, Survival results of a multicentre phase II study to evaluate D2 gastrectomy for gastric cancer, Br. J. Canc. 90 (2004) 1727–1732.
[20] G. de Manzoni, G. Verlato, F. Roviello, F. Morgagni, A. Di Leo A, et al., The new TNM classification of lymph node metastasis minimises stage migration problems in gastric cancer patients, Br. J. Canc. 87 (2002) 171–174.
[21] T. Ichikura, S. Tomimatsu, K. Uefuji, M. Kimura, T. Uchida, D. Morita, et al., Evaluation of the New American Joint Committee on Cancer/International Union against cancer classification of lymph node metastasis from gastric carcinoma in comparison with the Japanese classification, Cancer 86 (1999) 553–558.
[22] E.K. Abdalla, P.W. Pisters, Staging and preoperative evaluation of upper gastrointestinal malignancies, Semin. Oncol. 31 (2004) 513–529.
[23] R.M. Kwee, T.C. Kwee, Imaging in local staging of gastric cancer: a systematic review, J. Clin. Oncol. 25 (2007) 2107–2116.
[24] W.A. Weber, K. Ott, Imaging of esophageal and gastric cancer, Semin. Oncol. 31 (2004) 530–541.
[25] D. Bentrem, H. Gerdes, L. Tang, M. Brennan, D. Coit, Clinical correlation of endoscopic ultrasonography with pathologic stage and outcome in patients undergoing curative resection for gastric cancer, Ann. Surg Oncol. 14 (2007) 1853–1859.
[26] T. Irino, T. Sano, N. Hiki, M. Ohashi, S. Nunobe, et al., Diagnostic staging laparoscopy in gastric cancer: a prospective cohort at a cancer institute in Japan, Surg. Endosc. 32 (2018) 268–275.
[27] C. Du, Y. Zhou, K. Huang, G. Zhao, H. Fu, et al., Defining a high-risk subgroup of pathological T2N0 gastric cancer by prognostic risk stratification for adjuvant therapy, J. Gastrointest. Surg. 15 (2011) 2153–2158.

Review Article

Gastric cancer surgery: historical background and perspective in Western countries versus Japan

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Abstract: Gastrectomy plus D2 lymphadenectomy plays a decisive role in the management of resectable gastric cancer in Japan. Before recent advances in chemotherapy, Japanese surgeons considered that extensive surgery involving extended lymphadenectomy with combined resection of neighboring organ(s) was required to eliminate any possible lymphatic cancer spread and improve patient survival. This approach differs radically from that in Western countries, which aim to improve survival outcomes by multidisciplinary approaches including perioperative chemotherapy and/or radiotherapy with limited lymph node dissection. However, a randomized controlled trial conducted in Japan found that more extensive lymphadenectomy including the para-aortic lymph nodes provided no survival benefit over D2 lymphadenectomy. Splenic hilum dissection with splenectomy also failed to show superiority over the procedure without splenectomy in patients with proximal gastric cancer, except in cases with tumor invasion of the greater curvature. Furthermore, bursectomy recently demonstrated similar outcomes to omentectomy alone. Although "D2 lymphadenectomy" as carried out in Japan contributes to low local recurrence rates and good survival outcomes, the results of randomized controlled trials have led to a decreased extent of surgical resection, with no apparent adverse effects on survival outcome. Notably, gastrectomy with D2 dissection has tended to become acceptable for advanced gastric cancer in Western countries, based on the latest results of the Dutch D1D2 trial. Differences in surgical practices between the West and Japan have thus lessened and procedures are becoming more standardized. Japanese D2 lymphadenectomy for advanced gastric cancer is evolving toward more minimally invasive approaches, while consistently striving to achieve the optimal surgical extent, thereby promoting consensus with Western counterparts.

Keywords: Gastric cancer; surgery; lymphadenectomy; Western countries; Japan

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Introduction

Despite a substantial decline in its global incidence, gastric cancer remains the fifth most frequently diagnosed cancer and the third leading cause-related deaths worldwide (1), with an estimated 1,033,701 newly diagnosed cases and 782,685 related deaths in 2018 (2). The incidence rates of gastric cancer in both sexes are highest in Eastern Asia, especially Mongolia, the Republic of Korea, and Japan (2). The first gastrectomy was performed successfully by Billroth in 1881, and radical gastrectomy remains the first choice for achieving a cure in patients with resect able gastric cancer (3-5). Radical gastrectomy involves eradication of the primary lesion with a satisfactory resection margin (R0), together with radical dissection of regional lymph nodes.

However, surgeons have also explored more extensive surgeries aiming to eliminate any possible lymphatic spread by applying extended lymphadenectomy, such as super-extended (D3) lymphadenectomy (6-12) or standardized extended (D2) lymphadenectomy plus para-aortic nodal dissection (PAND) (13-17), together with combined resection, such as prophylactic splenectomy (18-24) or bursectomy (25-30). Radical gastrectomy with D2 dissection has been the standard procedure for locally advanced gastric cancer (AGC) in Japan since 1961 (5,31-36). Gastrectomy with D2 dissection has also recently tended to become acceptable for AGC in Western countries, in light of the latest 15-year follow-up results of the Dutch D1D2 trial, which showed significant survival benefits of D2 over standardized limited (D1) lymphadenectomy (36).

Rapid advances in surgical oncology worldwide have significantly improved the safety of gastrectomy. The 30-day post-gastrectomy mortality rates for patients with gastric cancer in Western countries over the last two decades have ranged from 1.9% to 5.1% (10,37-39), with postoperative in-hospital mortality rates of 5.8% to 9.8% (36,40-42). In contract, the overall operative mortality rates in Japan from 2011 to 2012 were 2.3% after total gastrectomy (43) and 1.07% after distal gastrectomy (44), and the equivalent 30-day mortality rates were 0.9% (43) and 0.45% (44), respectively, which appeared to indicate better outcomes than in Western countries (36-38,40-42,45). However, there remains scope for further global improvements in the safety of gastric cancer surgery. According to the theory of epistemology, involving practice, understanding, re-practice, and re-understanding, the preferred extent of gastric resection and lymph node dissection has experienced a pendulum-like phenomenon, from narrowed to extended, and then narrowed again, gradually rationalized from the original bias. Here, we review and compare the historical backgrounds and perspectives of gastric cancer surgeries in Western countries and Japan.

Epidemiology

Gastric cancer was estimated to account for over a million newly diagnosed cases and nearly 783,000 deaths (equating to 1 in 12 deaths) worldwide in 2018 (2), largely due to population aging and growth (46). One in 27 men and 1 in 68 women will develop gastric cancer before the age of 79 years, with the highest and lowest odds for men in middle (1 in 15) and low-middle sociodemographic index (SDI) countries (1 in 48), respectively, and the highest and lowest odds for women in low (1 in 58) and low-middle (1 in 83) SDI countries, respectively (46). The mortality rates of gastric cancer in men [calculated as age-standardized mortality rate per 100,000 (ASR)] ranged from 4.2 in Switzerland to 24.6 in the Russian Federation among Europe countries, 2.6 in the USA, 25.3 in the Republic of Korea, and 21.0 in Japan during the period 2005–2009 (47). The ASRs for women ranged from 1.9 in France to 10.1 in the Russian Federation, 1.3 in the USA, 9.2 in the Republic of Korea, and 8.0 in Japan, over the same period (47).

Non-cardia gastric cancer (NCGC) is more frequent than cardia gastric cancer (CGC) in most countries, with an estimated 691,000 cases of NCGC and 260,000 cases of CGC worldwide in 2012 (48). Approximately 90% of new NCGC cases were considered to be associated with *Helicobacter pylori* (*H. pylori*) infection (49). However, the incidence of NCGC has been declining worldwide over the last half century, as a result of the decreased prevalence of *H. pylori* and improved food-storage conditions (2). In contract, the incidence of CGC has been steadily increasing, particularly in high income countries, following the distribution characteristics of esophagus cancer in developed countries (50,51), where the incidence rates of Barrett's esophagus are higher than in Eastern countries. The proportion of men with CGC among all gastric cancer cases ranged from 11.6% in Belarus to 72.0% in Finland, and was higher in Northern and Central Europe compared with Southern and Eastern Europe (47). Notably, the incidence of CGC remained unchanged in the USA, according to a recent report (52).

Although the incidence of gastric cancer was expected to follow a decreasing trend owing to a lower incidence of *H. pylori* infection among the younger generation in Japan (53), its incidence has remained the highest of all types of cancers in both males and females (male-to-female ratio >2:1) (54). Considering this high incidence, a cost-effective screening program was initiated to increase the rate of early detection of gastric cancer in Japan. Approximately 48.8% cases were diagnosed with early gastric cancer and 80% of tumors were located in the middle or lower third of the stomach (54-56), with improvements attributed to the screening program (57-62). Notably, the 5-year overall survival rates in Japan were reported to be about 70.0% (54,56), and the good survival outcomes were considered to be least partly attributable to the large proportion of patients diagnosed at an early stage (63).

Table 1 Standards of D1 and D2 lymphadenectomy in Western countries and Japan

Guidelines, year	Country/Region	D1 lymphadenectomy		D2 lymphadenectomy	
		Gastrectomy type	Lymph node stations	Gastrectomy type	Lymph node stations
ESMO, 2016 (4)	Europe	ND	No. 1, 2, 3, 4, 5, 6	ND	No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 11
NCCN, 2017 (3)	USA	ND	No. 1, 2, 3, 4, 5, 6	ND	No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11
JGCA, 2017 (5)	Japan	Total gastrectomy	No. 1, 2, 3, 4, 5, 6, 7	Total gastrectomy	No. 1, 2, 3, 4, 5, 6, 7, 8a, 9, 10, 11p, 11d, 12a
JGCA, 2011 (32)		Distal gastrectomy	No. 1, 3, 4sb, 4d, 5, 6, 7	Distal gastrectomy	No. 1, 3, 4sb, 4d, 5, 6, 7, 8a, 9, 11p, 12a

ESMO, European Society for Medical Oncology; NCCN, National Comprehensive Cancer Network; JGCA, Japanese Gastric Cancer Association; ND, no details; No. 1, right paracardial lymph node; No. 2, left paracardial lymph node; No. 3, perigastric lymph node along lesser curvature; No. 4sb, perigastric lymph node along greater curvature (left group, lymph node along left gastroepiploic artery and short gastric arteries); No. 4d, perigastric lymph node along greater curvature (right group, lymph node along right gastroepiploic artery); No. 4, perigastric lymph node along greater curvature; No. 5, suprapyloric lymph node; No. 6, infrapyloric lymph node; No. 7, lymph node along left gastric artery; No. 8, lymph node along common hepatic artery; No. 8a, lymph node along common hepatic artery (anterosuperior group); No. 9, lymph node around celiac artery; No. 10, lymph node at splenic hilum; No. 11p, lymph node along proximal splenic artery; No. 11d, lymph node along distal splenic artery; No. 11, lymph node along splenic artery; No. 12a, lymph node in hepatoduodenal ligament (along hepatic artery).

D1 and D2 lymphadenectomy

The extent of lymph node dissection with radical gastrectomy has been extensively debated worldwide. According to the recent clinical practice guidelines of European Society for Medical Oncology (ESMO) for gastric cancer, D1 involves perigastric lymph nodes (LNs) of No. 1, 2, 3, 4, 5, 6 and D2 dissection involves LNs of No.1, 2, 3, 4, 5, 6, 7, 8, 9, 11 (4). In addition, based on the latest National Comprehensive Cancer Network (NCCN) guidelines, D1 involves LNs of No. 1, 2, 3, 4, 5, 6 and D2 dissection involves LNs of No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 (3). Notably, the Japanese Gastric Cancer Association has clearly identified the extent of systematic lymph node dissection with gastrectomy type. In total gastrectomy, D1 involves LNs of No.1, 2, 3, 4, 5, 6, 7 and D2 dissection involves LNs of No. 1, 2, 3, 4, 5, 6, 7, 8a, 9, 10, 11p, 11d, 12a (5,32). Whereas, in distal gastrectomy, D1 involves LNs of No. 1, 3, 4sb, 4d, 5, 6, 7 and D2 dissection involves LNs of No. 1, 3, 4sb, 4d, 5, 6, 7, 8a, 9, 11p, 12a (5,32) (*Table 1*).

Both the ESMO and NCCN guidelines did not clarify clear relations between gastrectomy types and extents of systematic lymph node dissection; furthermore, both classified No. 7 lymph node in D2 dissection (3,4). Japanese surgeons, on the other hand, have already changed No. 7 lymph node into D1 dissection for any type of gastrectomy, since the 3rd version of the Japanese gastric cancer treatment guidelines in 2011 (5,31,32). The nodal grouping based on the tumor location was abandoned because that it was too complicated to be accurately understood worldwide; notably, the lymph node stations to be dissected in D1 and D2 dissection have been defined according to gastrectomy type regardless of tumor location in Japan since then (31,32).

Surgery in Western countries

The preferred extent of gastric resection has experienced a pendulum-like phenomenon, switching from narrowed to extended, and then narrowed again, gradually becoming rationalized. The first successful case of distal gastrectomy in the West was performed by Billroth in 1881, though the first patient to undergo distal gastrectomy with Billroth I type reconstruction only survived for 115 days. Schlatter *et al.* performed the first total gastrectomy in 1897, while Mikulicz was reported to be the first to successfully perform cardiectomy (64). Notably, they stressed the importance of studying the pathways of gastric cancer spread, and established the foundation of surgical therapy for gastric cancer as follows: direct infiltration of the submucosa and muscularis (operable), dissemination via the lymphatics (operable), transperitoneal spread with lesions involving the full thickness of the stomach wall (inoperable), and dissemination through the blood stream to distant organs (inoperable) (64). This period represented the dawn of gastric cancer surgery, attributed to Mikulicz's theory of lymphatic drainage of the stomach with removal of all

palpable nodes, along with Billroth's contribution to gastric cancer surgery.

Groves *et al.* reported the first case of omentobursectomy in 1910 (65). They addressed the importance of complete removal of the great omentum by cutting through the peritoneum, which passes from the back of the omentum to the front of the transverse colon, followed by stripping the peritoneum off the upper surface of the transverse mesocolon to the front of the pancreas. Furthermore, they emphasized the need for a more systematic attempt to remove the whole of the associated lymphatic area (65). Although the 3-year survival rate was only 7.6%, possibly due to incomplete lymphadenectomy, his theory nevertheless contributed to later lymphadenectomy practices.

During the period from 1940 to1960, many experts in the West reported extensive surgeries with combined resection of neighboring organs with the aim of improving patient survival (66-68); however, the postoperative morbidity and mortality rates were very high. Cattell *et al.* reported combined resection of the stomach and transverse colon in 1946 (69). In 1947, Pack *et al.* reported total gastrectomy for gastric cancer, with an operative mortality of 20–30% (70), followed later by a series of clinical studies of radical or palliative surgeries for gastric cancer (71-74). Brunschwig *et al.* performed the first gastrectomy with pancreatoduodenectomy (PD) for distal gastric cancer invading the head of pancreas in 1948 (66), and Appleby *et al.* introduced a combined procedure in 1953, including resection of the whole stomach, distal pancreas, spleen, and regional lymph nodes (75). Lawrence *et al.* reported 5-year survival rates before and after the application of extensive surgery of 21.6% from 1931–1950, and 23.3% from 1951–1954 (68); however, no randomized controlled trial (RCTs) were available until 1985 to provide sufficient evidence for any strong recommendations.

Whether or not total gastrectomy could improve the survival of patents with distal gastric cancer thus remained to be validated in the West, and several studies comparing survival rates after total and subtotal gastrectomy for distal gastric cancer were conducted after 1970. McNeer *et al.* reported a better 5-year survival rate following total gastrectomy (43.7%) compared with subtotal gastrectomy (29.8%) (76). A similar result was reported by Lortat-Jacob *et al.*, with total gastrectomy showing a higher 5-year overall survival rate but a higher postoperative mortality than subtotal gastrectomy (77). In contrast, however, Gennari *et al.* in 1986 reported a higher 5-year survival rate after subtotal compared with total gastrectomy in patients with lymph node involvement (78). However, those were all retrospective studies with high risks of bias. Notable, the first global RCT comparing total versus subtotal gastrectomy for gastric antrum cancer was conducted in French in 1989 (79), and demonstrated no survival benefits of total over subtotal gastrectomy. A subsequent RCT by the Italian Gastrointestinal Tumor Study Group in 1999 also found no advantage of total gastrectomy over subtotal gastrectomy (80) (*Table 2*). It is therefore necessary to bear in mind the saying of Confucius, that "excess is just as bad as deficiency".

The issue of whether patients may benefit from D2 dissection remained controversial in Western countries (13,34-36). The United Kingdom Medical Research Council Gastric Cancer Surgical Trial (MRC, ST01) confirmed no survival advantages of D2 over D1 dissection (40,81) (*Table 2*). Similarly, the Dutch D1D2 trial in the Netherlands demonstrated D2 dissection was associated with a higher risk of postoperative morbidity (43% *vs.* 25%; P<0.001) and mortality (10% *vs.* 4%; P=0.004) compared with D1 dissection, with no differences in overall survival rate after the 11-year follow-up period (35% *vs.* 30%; P=0.53) (33). Another RCT conducted by the Italian Gastric Cancer Study Group suggested that D2 dissection may only be a better choice only in patients with nodal metastases (45). However, more recent results of the Dutch D1D2 trial after a 15-year follow-up period showed significant survival benefits of D2 over D1 dissection in terms of cancer-related death rate (48% *vs.* 37%), local recurrence (12% *vs.* 22%) and regional recurrence (13% *vs.* 19%) (36).

In light of those findings and the good survival outcomes after D2 dissection in Japan, gastrectomy with D2 dissection is becoming increasingly acceptable in Western countries. The latest National Comprehensive Cancer Network guidelines for gastric cancer stated that D2 dissection should be considered as a recommended but not a required procedure, nothing that the technical aspects of D2 dissection require a significant degree of training and expertise (3). In addition, the latest European Society for Medical Oncology guidelines for gastric cancer suggested that medically fit patients should undergo D2 dissection in specialized, high-volume centers in Western countries (4,82-84) (*Table 2*). However, further studies are still needed to determine if D2 dissection should become the standard procedure for gastric cancer patients in Western countries.

Table 2 Survival and safety outcomes after gastric cancer surgery in Western countries

References	Country	Study type	Population	pT3-T4	pN (+)	Gastrectomy type	Lymphadenectomy	Adjuvant therapy	Survival rate	Morbidity and mortality
Gouzi, *et al.* 1989 (79)	France 1980–1985	Multicenter, prospective, RCT	169	58%	55.1%	TG: 45%; STG: 55%	ND	None	5-YSR: 48%	Postoperative morbidity: 3.7% (TG: 32%, STG: 34%); postoperative mortality: 2.4% (TG: 1.3%, STG: 3.2%)
Cuschieri, *et al.* 1999 (81)	Italy 1982–1993	Multicenter, RCT	618	48.7%	54.5%	TG: 49.0%; STG: 51.0%	D2: 100%	AC: 1.6%	5-YSR: 64.0% (TG: 62.4%, STG: 65.3%)	Overall mortality: 1.8% (TG: 2.3%, STG: 1.3%)
Cuschieri, *et al.* 1996 (40) Bozzetti, *et al.* 1999 (80)	UK 1986–1994	MRC ST01, prospective, RCT	400	43%	ND	TG: 54.5%; DG: 44.8%	D1: 50%; D2: 50%	None	5-YSR: (D1: 35%, D2: 33%)	Serious morbidity: (D1: 12.5%, D2: 23.5%); postoperative mortality: 9.8% (D1: 6.5%, D2: 13%)
Hartgrink, *et al.* 2004 (33) Songun, *et al.* 2010 (36) Bonenkamp, *et al.* 1999 (41)	Netherlands 1989–1993	Dutch D1D2 (DGCT), RCT	711	26.4%	54.9%	TG: 33.9%; PAG: 66.1%	D1: 53.4%; D2: 46.6%	None	11-Y OS rate: (D1: 30%, D2: 35%); 15-Y OS rate: (D1: 21%, D2: 29%)	Morbidity: (D1: 25%, D2: 43%); postoperative mortality: (D1: 4%, D2: 10%)
Edward, *et al.* 2004 (10)	UK 1996–2002	UK NHS Trust, Prospective	118	69.5%	53.4%	TG: 36.4%; STG: 63.6%	D1: 30.5%; D2: 69.5%	None	5-YSR: (D1: 32%, D2: 59%)	Morbidity: (D1: 25%, D2: 23.2%); 30-day mortality: (D1: 8.3%, D2: 7.3%)
Deqiuli, *et al.* 2014 (45)	Italy 1998–2006	IGCSG-R01, multicenter, RCT	267	28.8%	53.2%	TG: 24.7%; DG: 75.3%	D1: 49.8%; D2: 50.2%	No chemotherapy before or after surgery, until recurrence	5-Y OS rate: (D1: 66.5%, D2: 64.2%)	Morbidity: (D1: 12.0%, D2: 17.9%); operative mortality: (D1: 3.0%, D2: 2.2%)
Selby, *et al.* 2015 (38)	U.S. 2003–2012	Retrospective	238	59%	51%	TG: 92%; MITG: 8%	ND	NAC: 43%; AC: 34%	ND	Major morbidity: 28%; 30-day mortality: 2.5%; 90-day mortality: 2.9%

Table 2 (*continued*)

Table 2 (*continued*)

References	Country	Study type	Population	pT3-T4	pN (+)	Gastrectomy type	Lymphadenectomy	Adjuvant therapy	Survival rate	Morbidity and mortality
Papenfuss, *et al.* 2014 (37)	U.S. 2005–2010	ACS NSQIP, multicenter, prospective	2,580	ND	ND	TG: 38.7%; PAG: 61.3%	ND	NAC: 4.8%; NAR: 2.0%	ND	Serious morbidity: 23.6% (TG: 29.3%, PG: 19.9%); 30-day mortality: 4.1%; (TG: 5.4%, PG: 3.4%)

TG, total gastrectomy; PAG, partial gastrectomy; STG, subtotal gastrectomy; PG, proximal gastrectomy; DG, distal gastrectomy; SG, subtotal gastrectomy; NAR, neoadjuvant radiotherapy; NAC, neoadjuvant chemotherapy; AC, adjuvant chemotherapy; MITG, minimally invasive total gastrectomy; 5-YSR, 5-year survival rate; OS: overall survival; RFS: relapse-free survival; PAND, para-aortic nodal dissection; NCD, National Clinical Database; ACS NSQIP, the American College of Surgeons National Surgical Quality Improvement Program; DGCT, Dutch Gastric Cancer Trial; ACTS-GC, Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer; JCOG, Japan Clinical Oncology Group; pT, pathological T stage; pN, pathological N stage; N (+), lymph node metastasis; RCT, randomized controlled trial; ND, no details.

Surgery in Japan

The first successful case of distal gastrectomy in Japan was performed in 1897 by Kondo, a professor from the First Department of Surgery of Tokyo University Hospital (85), while the first case of total gastrectomy in Japan was reported by Miyake *et al.* in 1918. Based on the fact that nodal metastasis was the most frequent type of cancer spread, surgeons in Japan gradually focused on lymphadenectomy from around 1940, with the aim of eliminating any possible nodal metastasis and thus improving survival. Kuru *et al.* first stressed the use of systematic radical lymphadenectomy in 1935 (86), and Kajitani *et al.* in 1944 emphasized the importance of wide lymphadenectomy to eliminate any possible nodal metastasis (87).

Extended surgeries involving extended lymphadenectomy or combined resection of neighboring organs were subsequently performed to improve patient survival. Extended radical surgery with PD was first reported in Japan by Kajitani *et al.* in 1952, for the treatment of distal gastric cancer involving the head of pancreas (88). Jinnai *et al.* advocated the theory of systematic radical lymphadenectomy and stressed the use of extended lymphadenectomy in 1961 (89). Ohashi *et al.* reported 5-year survivors of gastric cancer treated with PAND in 1976 (90) and Kajitani *et al.* introduced left upper abdominal quadrant evisceration for proximal advanced cancer in 1981 (91). In 1989, Ohta *et al.* stressed the value of total gastrectomy combined with pancreaticosplenectomy for middle gastric cancer (92). However, the lack of evidence from RCTs meant that the role of extended surgery in improving patient survival remained controversial until the past two decades.

D2 dissection plus PAND has not demonstrated any survival benefits over D2 dissection alone. The Japan Clinical Oncology Group (JCOG) conducted a multicenter, RCT (JCOG9501) and showed that D2 dissection plus PAND could be performed safely in patients with low operative risk by specialized surgeons, but no significant improvement in survival was observed (13). Notably, the final results of JCOG 9501 in 2008 confirmed that D2 dissection plus PAND (No.16a2, b1) did not improve 5-year overall survival [hazard ratio (HR), 1.03, 95% confidence interval (CI), 0.77–1.37; P=0.85] or recurrence-free survival (HR, 1.08, 95% CI, 0.83–1.42; P=0.56) in patients with curable gastric cancer, compared with D2 dissection alone (16).

Pancreatic resection frequently resulted in pancreatic-juice leakage, subphrenic abscess, and postoperative diabetes, leading Maruyama *et al.* to develop pancreas-preserving surgery in 1979. They also demonstrated gastric cancer tumors only invaded the pancreas directly, rather than by metastasis to the pancreas. Pancreas-preserving surgery proved superior to pancreas resection in terms of operative mortality, hospital mortality, surgical morbidity, and 5-year survival rate (93). Accordingly, lymphatic channels from the stomach did not flow into the pancreas parenchyma, and surgeons could remove the spleen, splenic artery, fatty connective tissues, and lymph nodes completely without dissecting the pancreas parenchyma or splenic vein (93). The results of an RCT conducted by Furukawa *et al.* in 2000 also supported the superiority of pancreas-preserving surgery (total gastrectomy with dissection of lymph nodes along the splenic artery) over pancreas resection in terms of surgical risk and postoperative glucose tolerance (94).

Splenic hilum nodal dissection with splenectomy showed no benefits over the procedure without splenectomy in patients with proximal gastric cancer (24). A recent, multi-institutional, RCT (JCOG0110) conducted in 505 patients from 36 institutions in Japan (24) confirmed that the addition of splenectomy was associated with higher morbidity and blood loss, but similar operation time. The 5-year survival rates were 75.1% in the splenectomy group and 76.4% in the spleen preservation groups (P=0.025). Splenectomy thus increase operative morbidity without improving survival, and should therefore be avoided in patients undergoing total gastrectomy for proximal gastric cancer, unless it invades the greater curvature.

The role of bursectomy in preventing peritoneal metastasis has long been controversial. One RCT found no survival benefit but a high risk of morbidity for bursectomy in patients with cT3-4a gastric cancer (95). In addition, a recent, phase 3 RCT (JCOG1001) that enrolled 1,204 patients from 57 hospitals in Japan confirmed that bursectomy had no survival advantages over non-bursectomy, indicating that D2 dissection with omentectomy alone should be the recommended surgery for resectable cT3-4a gastric cancer in Japan (96). Furthermore, the Japanese Gastric Cancer Association (JGCA) recommended gastrectomy with D2 dissection as the standard surgical procedure for potentially curable gastric cancer (clinical stage ≥ cT2 and/or cN+) in Japan (5).

Japanese surgeons had long believed that gastric cancer patients should receive extensive surgery, including extended lymphadenectomy or with combined resection of neighboring organs, to eliminate any possible nodal spread and thus improve patient survival. In 1991, 67.6% of Japanese patients with gastric cancer underwent D2 dissection, 9.9% underwent D3 or D4 dissection, 30.7% received total gastrectomy, and 30.3% received combined resection of neighboring organs (1,515 splenectomy, 726 pancreatomy) (56). This situation remained unchanged until the introduction of the new anticancer agent, S-1, for advanced gastrointestinal cancer in Japan in 1999, which proved effective against advanced or recurrent gastrointestinal cancer, with generally mild toxicities and no toxic deaths (97,98) (*Table 3*). Since then, rapid advances in chemotherapy (95,99-111), including targeted therapy (112-114), have led Japanese experts gradually to adopt the Western strategy of improving survival by multidisciplinary approaches, including neoadjuvant or adjuvant chemotherapy. The differences in surgical practice for gastric cancer between the West and Japan have thus gradually lessened, and are becoming increasingly standardized.

Future perspectives

Surgical therapy for gastric cancer originated in Western countries and developed rapidly in Japan. Japanese experience suggests that screening programs should be implemented to improve the early detection of gastric cancer, particularly in high incidence areas. Surgical safety and maximizing the probability of a cure should remain the highest priorities; however, chemotherapy, along with genetic diagnosis and targeted therapy, are gaining importance worldwide. Further studies are needed to consider how best to balance the combinations among neoadjuvant or adjuvant chemotherapy and surgery in patients with gastric cancer. Attempts should also be made to reduce the incidence of gastric cancer, in addition to taking account of quality of life and economic costs. Recent developments and modifications of minimally invasive techniques have also attracted increasing interest (115-118), especially in Japan (119-122). Overall, international cooperation between Western and Eastern countries should be encouraged to establish global standards for the diagnosis and therapy of gastric cancer.

Table 3 Survival and safety outcomes after gastric cancer surgery in Japan

Reference	Country	Study type	Population	pT3-T4	pN (+)	Gastrectomy type	Lymphadenectomy	Adjuvant therapy	Survival rate	Morbidity and mortality
Maruyama, *et al.* 2006 (56)	Japan 1991	JGCA registry, retrospective	7,935	26.6%	40.7%	TG: 30.7%; DG: 66.0%; PG: 3.1%; unknown: 0.2%	D1: 19.1%; D2: 67.6%; D3: 8.4%; Others: 4.9%	ND	5-YSR: 68.2% (stage I: 89.9%, stage II: 69.1%, stage III: 43.5%, stage IV: 9.9%)	Direct mortality: 1.0%
Sasako, *et al.* 2008 (16)	Japan 1995–2001	JCOG9501, multicenter, RCT	523	46.5%	66.5%	TG: 74.6%; DG: 4.0%; others: 21.4%	D2: 50.3%; D2 + PAND: 9.7%	No adjuvant therapy before recurrence	5-Y OS rate: (D2: 69.2%, D2+PAND: 70.3%); 5-Y RFS rate: (D2: 62.6%, D2+PAND: 61.7%)	Surgery-related morbidity: (D2: 20.9%, D2+PAND: 28.1%); major surgery-related morbidity: (D2: 2.3%, D2+PAND: 1.9%); 30-day mortality: (D2: 0.8%, D2+PAND: 0.8%)
Sasako, *et al.* 2011 (95)	Japan 2001–2004	ACTS-GC, RCT	1,059	45.4%	89.2%	ND	D2: 100%	S-1 after surgery: 50%	5-Y OS rate: (surgery alone: 61.1%, S-1 after surgery: 1.7%)	ND
Nashimoto, *et al.* 2013 (54)	Japan 2002	JGCA registry, retrospective	13,002	23.5%	40.2%	TG: 30.5%; DG: 59.6%; Others: 9.9%	D1[a]: 41.6%; D2: 49.2%; others: 9.2%	ND	5-YSR: (stage IA/IB: 92.2%/85.3%, Stage II: 72.1%, stage IIIA/IIIB: 52.8%/31.0%, stage IV: 14.9%)	Direct mortality: 0.48%
Sano, *et al.* 2017 (24)	Japan 2002–2009	Multicenter, RCT	505	26.7%	57.8%	TG + splenectomy: 50.3%; TG +spleen preservation: 49.7%	D2: 100%	No adjuvant therapy in the original protocol; later adjuvant S-1 therapy for stage II/III cases for a year	5-Y OS rate: (splenectomy: 75.1%, spleen preservation: 76.4%); 5-Y RFS rate: (splenectomy: 68.4%, spleen preservation: 70.5%)	Postoperative morbidity: 23.6% (splenectomy: 30.3%, spleen preservation: 16.7%); hospital mortality: 0.6% (splenectomy: 0.4%, spleen preservation: 0.8%)
Watanabe, *et al.* 2014 (43)	Japan 2011	NCD, retrospective	20,011	ND	ND	TG: 100%	ND	ND	ND	Morbidity: 26.2%; 30-day mortality: 0.9%; in hospital mortality: 2.2%; Overall operative mortality: 2.3%

Table 3 (*continued*)

Table 3 (*continued*)

Reference	Country	Study type	Population	pT3-T4	pN (+)	Gastrectomy type	Lymphadenectomy	Adjuvant therapy	Survival rate	Morbidity and mortality
Kunisaki, *et al.* 2017 (44)	Japan 2011–2012	NCD, retrospective	65,906	ND	ND	DG: 100%	ND	ND	ND	30-day mortality: 0.5%; Operative mortality: 1.0%
Kurokawa, *et al.* 2018 (96)	Japan 2010–2015	JCOG1001, RCT	1,204	78.7%	49.1%	TG: 34.6%; DG: 65.4%; bursectomy: 50%; omentectomy: 50%	D2: 100%	AC: 60.6%; bursectomy: 60%; omentectomy: 61%	5-Y OS rate: bursectomy: 76.9%, omentectomy: 76.7%	Serious morbidity: 11.7% (bursectomy: 13%, omentectomy: 11%); hospital mortality: 0.5% (bursectomy: 0.17%, omentectomy: 0.83%)

TG, total gastrectomy; PAG, partial gastrectomy; STG, subtotal gastrectomy; PG, proximal gastrectomy; DG, distal gastrectomy; SG, subtotal gastrectomy; NAR, neoadjuvant radiotherapy; NAC, neoadjuvant chemotherapy; AC, adjuvant chemotherapy; MITG, minimally invasive total gastrectomy; a, including D1, D1+α and D1+β; 5-YSR, 5-year survival rate; OS: overall survival; RFS: relapse-free survival; PAND, para-aortic nodal dissection; NCD, National Clinical Database; ACS NSQIP, the American College of Surgeons National Surgical Quality Improvement Program; DGCT, Dutch Gastric Cancer Trial; ACTS-GC, Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer; JCOG, Japan Clinical Oncology Group; pT, pathological T stage; pN, pathological N stage; N (+), lymph node metastasis; RCT, randomized controlled trial; ND, no details.

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Footnote

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References

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015;136:E359-86.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
3. Ajani JA, D'Amico TA, Almhanna K, et al. Gastric Cancer, Version 3.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2016;14:1286-312.
4. Smyth EC, Verheij M, Allum W, et al. Gastric cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27:v38-49.
5. Japanese Gastric Cancer, A., Japanese gastric cancer treatment guidelines 2014 (ver. 4). Gastric Cancer 2017;20:1-19.
6. Adachi Y, Mimori K, Mori M, et al. Morbidity after D2 and D3 gastrectomy for node-positive gastric carcinoma. J Am Coll Surg 1997;184:240-4.
7. Pacelli F, Doglietto GB, Alfieri S, et al. Avoiding pancreatic necrosis following pancreas-preserving D3 lymphadenectomy for gastric cancer. Br J Surg 1998;85:125-6.
8. Maeta M, Yamashiro H, Saito H, et al. A prospective pilot study of extended (D3) and superextended para-aortic lymphadenectomy (D4) in patients with T3 or T4 gastric cancer managed by total gastrectomy. Surgery 1999;125:325-31.
9. Günther K, Horbach T, Merkel S, et al. D3 lymph

node dissection in gastric cancer: Evaluation of postoperative mortality and complications. Surgery Today 2000;30:700-5.
10. Edwards P, Blackshaw GR, Lewis WG, et al. Prospective comparison of D1 vs modified D2 gastrectomy for carcinoma. Br J Cancer 2004;90:1888-92.
11. Wu CW, Hsiung CA, Lo SS, et al. Nodal dissection for patients with gastric cancer: a randomised controlled trial. Lancet Oncol 2006;7:309-15.
12. Marrelli D, Pedrazzani C, Neri A, et al. Complications after extended (D2) and superextended (D3) lymphadenectomy for gastric cancer: Analysis of potential risk factors. Ann Surg Oncol 2007;14:25-33.
13. Sano T, Sasako M, Yamamoto S, et al. Gastric cancer surgery: morbidity and mortality results from a prospective randomized controlled trial comparing D2 and extended para-aortic lymphadenectomy-Japan clinical oncology group study 9501. J Clin Oncol 2004;22:2767-73.
14. Yoshikawa T, Sasako M, Sano T, et al. Stage migration caused by D2 dissection with para-aortic lymphadenectomy for gastric cancer from the results of a prospective randomized controlled trial. Br J Surg 2006;93:1526-9.
15. Tsujinaka T, Sasako M, Yamamoto S, et al. Influence of overweight on surgical complications for gastric cancer: Results from a randomized control trial comparing D2 and extended para-aortic D3 lymphadenectomy (JCOG9501). Ann Surg Oncol 2007;14:355-61.
16. Sasako M, Sano T, Yamamoto S, et al. D2 lymphadenectomy alone or with para-aortic nodal dissection for gastric cancer. N Engl J Med 2008;359:453-62.
17. Pedrazzani, C. Functional outcomes after extended surgery for gastric cancer. Br J Surg 2011;98:246.
18. Sugimachi K, Kodama Y, Kumashiro R, et al. Critical evaluation of prophylactic splenectomy in total gastrectomy for the stomach cancer. Gan 1980;71:704-9.
19. Sugimachi K, Inokuchi K. Critical-evaluation of prophylactic splenectomy in total gastrectomy for stomach cancer. Gan No Rinsho 1984;30:1057-60.
20. Tamada R, Sugimachi K, Okamura T, et al. Evaluation of splenectomy in total gastrectomy for gastric cancer. Nihon Geka Gakkai Zasshi 1985;86:1124-7.
21. Yu W, Choi GS, Chung HY. Randomized clinical trial of splenectomy versus splenic preservation in patients with proximal gastric cancer. Br J Surg 2006;93:559-63.
22. Ito H, Inoue H, Odaka N, et al. Prognostic impact of prophylactic splenectomy for upper-third gastric cancer: A cohort study. Anticancer Res 2013;33:277-82.
23. Ohkura Y, Haruta S, Shindoh J, et al. Efficacy of prophylactic splenectomy for proximal advanced gastric cancer invading greater curvature. World J Surg Oncol 2017;15:106.
24. Sano T, Sasako M, Mizusawa J, et al. Randomized controlled trial to evaluate splenectomy in total gastrectomy for proximal gastric carcinoma. Ann Surg 2017;265:277-83.
25. Imamura H, Kurokawa Y, Kawada J, et al. Influence of bursectomy on operative morbidity and mortality after radical gastrectomy for gastric cancer: results of a randomized controlled trial. World J Surg 2011;35:625-30.
26. Kayaalp C, Olmez A, Piskin T. Prophylactic bursectomy at radical gastrectomy for gastric cancer. Gastric Cancer 2011;14:399-400.
27. Fujita J, Kurokawa Y, Sugimoto T, et al. Survival benefit of bursectomy in patients with resectable gastric cancer: interim analysis results of a randomized controlled trial. Gastric Cancer 2012;15:42-8.
28. Hundahl, SA. The potential value of bursectomy in operations for trans-serosal gastric adenocarcinoma. Gastric Cancer 2012;15:3-4.
29. Eom BW, Joo J, Kim YW, et al. Role of bursectomy for advanced gastric cancer: Result of a case-control study from a large volume hospital. Eur J Surg Oncol 2013;39:1407-14.
30. Kochi M, Fujii M, Kanamori N, et al. D2 gastrectomy with versus without bursectomy for gastric cancer. Am J Clin Oncol 2014;37:222-6.
31. Sano T, Aiko T. New Japanese classifications and treatment guidelines for gastric cancer: revision concepts and major revised points. Gastric Cancer 2011;14:97-100.
32. Japanese Gastric Cancer Association. Japanese gastric cancer treatment guidelines 2010 (ver. 3). Gastric Cancer 2011;14:113-23.
33. Hartgrink HH, van de Velde CJ, Putter H, et al. Extended lymph node dissection for gastric cancer: Who may benefit? Final results of the Randomized Dutch Gastric Cancer Group Trial. J Clin Oncol 2004;22:2069-77.
34. Smith, DD, Schwarz RR, Schwarz RE. Impact of total lymph node count on staging and survival after gastrectomy for gastric cancer: data from a large US-population database. J Clin Oncol 2005;23:7114-24.
35. Schwarz RE, Smith DD. Clinical impact of lymphadenectomy extent in resectable gastric cancer of advanced stage. Ann Surg Oncol 2007;14:317-28.
36. Songun I, Putter H, Kranenbarg EM, et al. Surgical treatment of gastric cancer: 15-year follow-up results of

the randomised nationwide Dutch D1D2 trial. Lancet Oncol 2010;11:439-49.
37. Papenfuss WA, Kukar M, Oxenberg J, et al., Morbidity and mortality associated with gastrectomy for gastric cancer. Ann Surg Oncol 2014;21:3008-14.
38. Selby LV, Vertosick EA, Sjoberg DD, et al. Morbidity after total gastrectomy: Analysis of 238 patients. J Am Coll Surg 2015;220:863-71.e2.
39. Norero E, Vega EA, Diaz C, et al. Improvement in postoperative mortality in elective gastrectomy for gastric cancer: Analysis of predictive factors in 1066 patients from a single centre. Eur J Surg Oncol 2017;43:1330-6.
40. Cuschieri A, Fayers P, Fielding J, et al. Postoperative morbidity and mortality after D1 and D2 resections for gastric cancer: preliminary results of the MRC randomised controlled surgical trial. Lancet 1996;347:995-9.
41. Bonenkamp JJ, Hermans J, Sasako M, et al. Extended lymph-node dissection for gastric cancer. N Engl J Med 1999;340:908-14.
42. Pasquer A, Renaud F, Hec F, et al. Is centralization needed for esophageal and gastric cancer patients with low operative risk? A nationwide study. Ann Surg 2016;264:823-30.
43. Watanabe M, Miyata H, Gotoh M, et al. Total gastrectomy risk model: data from 20,011 Japanese patients in a nationwide internet-based database. Ann Surg 2014;260:1034-9.
44. Kunisaki C, Miyata H, Konno H, et al. Modeling preoperative risk factors for potentially lethal morbidities using a nationwide Japanese web-based database of patients undergoing distal gastrectomy for gastric cancer. Gastric Cancer 2017;20:496-507.
45. Degiuli M, Sasako M, Ponti A, et al. Randomized clinical trial comparing survival after D1 or D2 gastrectomy for gastric cancer. Br J Surg 2014;101:23-31.
46. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Allen C, Barber RM, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: A systematic analysis for the global burden of disease study. JAMA Oncol 2017;3:524-48.
47. Ferro A, Peleteiro B, Malvezzi M, et al. Worldwide trends in gastric cancer mortality (1980-2011), with predictions to 2015, and incidence by subtype. Eur J Cancer 2014;50:1330-44.
48. Colquhoun A, Arnold M, Ferlay J, et al. Global patterns of cardia and non-cardia gastric cancer incidence in 2012. Gut 2015;64:1881-8.
49. Plummer M, Franceschi S, Vignat J, et al. Global burden of gastric cancer attributable to Helicobacter pylori. Int J Cancer 2015;136:487-90.
50. Powell J, McConkey CC, Gillison EW, Spychal RT. Continuing rising trend in oesophageal adenocarcinoma. Int J Cancer 2002;102:422-7.
51. Crew KD, Neugut AI. Epidemiology of upper gastrointestinal malignancies. Semin Oncol 2004;31:450-64.
52. Wang Z, Graham DY, Khan A, et al. Incidence of gastric cancer in the USA during 1999 to 2013: a 50-state analysis. Int J Epidemiol 2018;47:966-75.
53. Hiroi S, Sugano K, Tanaka S, Kawakami K. Impact of health insurance coverage for Helicobacter pylori gastritis on the trends in eradication therapy in Japan: retrospective observational study and simulation study based on real-world data. BMJ Open 2017;7:e015855.
54. Nashimoto A, Akazawa K, Isobe Y, et al. Gastric cancer treated in 2002 in Japan: 2009 annual report of the JGCA nationwide registry. Gastric Cancer 2013;16:1-27.
55. Maruyama M, Takeshita K, Endo M, et al. Clinicopathological study of gastric carcinoma in high- and low-mortality countries: Comparison between Japan and the United States. Gastric Cancer 1998;1:64-70.
56. Japanese Gastric Cancer Association Registration Committee, Maruyama K, Kaminishi M, et al. Gastric cancer treated in 1991 in Japan: data analysis of nationwide registry. Gastric Cancer 2006;9:51-66.
57. Watabe H, Mitsushima T, Yamaji Y, et al. Predicting the development of gastric cancer from combining Helicobacter pylori antibodies and serum pepsinogen status: a prospective endoscopic cohort study. Gut 2005;54:764-8.
58. Hamashima C, Shibuya D, Yamazaki H, et al. The Japanese guidelines for gastric cancer screening. Jpn J Clin Oncol 2008;38:259-67.
59. Yanaoka K, Oka M, Yoshimura N, et al. Risk of gastric cancer in asymptomatic, middle-aged Japanese subjects based on serum pepsinogen and Helicobacter pylori antibody levels. Int J Cancer 2008;123:917-26.
60. Aikou S, Ohmoto Y, Gunji T, et al. Tests for serum levels of trefoil factor family proteins can improve gastric cancer screening. Gastroenterology 2011;141:837-45.
61. Gotoda T, Ishikawa H, Ohnishi H, et al. Randomized controlled trial comparing gastric cancer screening by gastrointestinal X-ray with serology for Helicobacter pylori and pepsinogens followed by gastrointestinal

endoscopy. Gastric Cancer 2015;18:605-11.e1.
62. Hamashima C, Systematic Review Group and Guideline Development Group for Gastric Cancer Screening Guidelines. Systematic Review, and G. Guideline Development Group for Gastric Cancer Screening, Update version of the Japanese Guidelines for Gastric Cancer Screening. Jpn J Clin Oncol 2018;48:673-83.
63. Inoue M, Tsugane S. Epidemiology of gastric cancer in Japan. Postgrad Med J 2005;81:419-24.
64. Olch PD. Johann von Mikulicz-Radecki. Ann Surg 1960;152:923-6.
65. Groves EW. On the radical operation for cancer of the pylorus: with especial reference to the advantages of the two-stage operation and to the question of removal of the associated lymphatics. Br Med J 1910;1:366-70.
66. Brunschwig A. Pancreato-total gastrectomy and splenectomy for advanced carcinoma of the stomach. Cancer 1948;1:427-30.
67. McNEER G, James A. Resection of stomach and adjacent organs in continuity for advanced cancer. Cancer 1948;1:449-54.
68. Lawrence W Jr, McNeer G. An analysis of the role of radical surgery for gastric cancer. Surg Gynecol Obstet 1960;111:691-6.
69. Cattell RB, Mosely CH. Combined resection of the stomach and transverse colon for carcinoma; report of two cases. Lahey Clin Bull 1946;4:238-41.
70. Pack GT. Radical surgical treatment of gastric cancer. Calif Med 1947;66:120-4.
71. Pack GT. The radical surgical treatment of gastric cancer. J Natl Cancer Inst 1947;7:337-44.
72. Pack GT, Mc NG. End results in the treatment of cancer of the stomach; analysis of 795 cases. Surgery 1948;24:769-78.
73. Pack GT, Mc NG. Surgical treatment of cancers of the gastric cardia. Surgery 1948;23:976-1019.
74. Pack GT, Mc NG. Palliative operations for gastric cancer. Rev Gastroenterol 1949;16:291-321.
75. Appleby LH. The coeliac axis in the expansion of the operation for gastric carcinoma. Cancer 1953;6:704-7.
76. McNeer G, Bowden L, Booner RJ, et al. Elective total gastrectomy for cancer of the stomach: end results. Ann Surg 1974;180:252-6.
77. Lortat-Jacob J, Giuli R, Estenne B, et al. Value of total gastrectomy for treatment of cancers of the stomach. Study of 482 radical operations. Chirurgie 1975;101:59-67.
78. Gennari L, Bozzetti F, Bonfanti G, et al. Subtotal versus total gastrectomy for cancer of the lower two-thirds of the stomach: A new approach to an old problem. Br J Surg 1986;73:534-8.
79. Gouzi JL, Huguier M, Fagniez PL, et al. Total versus subtotal gastrectomy for adenocarcinoma of the gastric antrum. A French prospective controlled study. Ann Surg 1989;209:162-6.
80. Bozzetti F, Marubini E, Bonfanti G, et al. Subtotal versus total gastrectomy for gastric cancer: five-year survival rates in a multicenter randomized Italian trial. Italian Gastrointestinal Tumor Study Group. Ann Surg 1999;230:170-8.
81. Cuschieri A, Weeden S, Fielding J, et al. Patient survival after D1 and D2 resections for gastric cancer: long-term results of the MRC randomized surgical trial. Surgical Co-operative Group. Br J Cancer 1999;79:1522-30.
82. Begg CB, Cramer LD, Hoskins WJ, et al. Impact of hospital volume on operative mortality for major cancer surgery. JAMA 1998;280:1747-51.
83. Birkmeyer JD, Siewers AE, Finlayson EV, et al. Hospital volume and surgical mortality in the United States. N Engl J Med 2002;346:1128-37.
84. Dikken JL, van Sandick JW, Allum WH, et al. Differences in outcomes of oesophageal and gastric cancer surgery across Europe. Br J Surg 2013;100:83-94.
85. Kondo T. An experiment in gastric cancer surgery (in Japanese). Nihon Gekagakkai Zasshi 1899;1:234-52.
86. Kuru M. Statistical consideration of radical surgery for gastric cancer (in Japanese). Rinshou IIgaku 1935;23:390-6.
87. Kajitani T. Lymph node metastasis of gastric cancer (in Japanese). Nihon Gekagakkai Zassi 1944;45:15-6.
88. Kajitani T, Hoshino T. An experience of pancreatoduodenectomy (in Japanese). Rinshougeka 1952;7:231-5.
89. Jinnai D. Extended radical operation of gastric cancer, with special emphasis on the lymph node dissection (in Japanese). Gekashinryou 1961;3:556.
90. Ohashi I, Takagi K, Konishi T, et al. 5-year survivors of gastric cancer patients with para-aortic lymph node metastasis (in Japanese). Nihon Shoukakigeka Gakkaishi 1976;9:507-12.
91. Kajitani T, Takagi K, Ohashi I. Radical surgery for gastric cancer (dissection of the left side) (in Japanese). Gekashinryou 1981;23:412-7.
92. Ohta K, Nishi M, Nakajima T, et al. Indications for total gastrectomy combined with pancreaticosplenectomy in the treatment of middle gastric cancer. Nihon Geka Gakkai Zasshi 1989;90:1326-30.
93. Maruyama K, Sasako M, Kinoshita T, et al. Pancreas-

preserving total gastrectomy for proximal gastric cancer. World J Surg 1995;19:532-6.
94. Furukawa H, Hiratsuka M, Ishikawa O, et al. Total gastrectomy with dissection of lymph nodes along the splenic artery: a pancreas-preserving method. Ann Surg Oncol 2000;7:669-73.
95. Sasako M, Sakuramoto S, Katai H, et al. Five-Year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. J Clin Oncol 2011;29:4387-93.
96. Kurokawa Y, Doki Y, Mizusawa J, et al. Bursectomy versus omentectomy alone for resectable gastric cancer (JCOG1001:a phase 3, open-label, randomised controlled trial. Lancet Gastroenterol Hepatol 2018;3:460-8.
97. Shirasaka T, Aiba K, Araki H, et al. Combination therapy of continuous venous infusion (CVI) of 5-FU and low dose consecutive cisplatin (CDDP), and the new oral anti-cancer drug S-1 for advanced gastro-intestinal cancer. Gan To Kagaku Ryoho 1999;26:456-66.
98. Maehara Y, Sugimachi K, Kurihara M, et al. Clinical evaluation of S-1, a new anticancer agent, in patients with advanced gastrointestinal cancer. Gan To Kagaku Ryoho 1999;26:476-85.
99. Sakuramoto S, Sasako M, Yamaguchi T, et al. Adjuvant chemotherapy for gastric cancer with S-1, an oral fluoropyrimidine. N Engl J Med 2007;357:1810-20.
100. Ychou M, Boige V, Pignon JP, et al. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: An FNCLCC and FFCD multicenter phase III trial. J Clin Oncol 2011;29:1715-21.
101. Noh SH, Park SR, Yang HK, et al. Adjuvant capecitabine plus oxaliplatin for gastric cancer after D2 gastrectomy (CLASSIC:5-year follow-up of an open-label, randomised phase 3 trial. Lancet Oncol 2014;15:1389-96.
102. Tsuburaya A, Yoshida K, Kobayashi M, et al. Sequential paclitaxel followed by tegafur and uracil (UFT) or S-1 versus UFT or S-1 monotherapy as adjuvant chemotherapy for T4a/b gastric cancer (SAMIT:a phase 3 factorial randomised controlled trial. Lancet Oncol 2014;15:886-93.
103. Tanabe K, Fujii M, Nishikawa K, et al. Phase II/III study of second-line chemotherapy comparing irinotecan-alone with S-1 plus irinotecan in advanced gastric cancer refractory to first-line treatment with S-1 (JACCRO GC-05). Ann Oncol 2015;26:1916-22.
104. Ryu MH, Baba E, Lee KH, et al. Comparison of two different S-1 plus cisplatin dosing schedules as first-line chemotherapy for metastatic and/or recurrent gastric cancer: a multicenter, randomized phase III trial (SOS). Ann Oncol 2015;26:2097-101.
105. Fujitani K, Yang HK, Mizusawa J, et al. Gastrectomy plus chemotherapy versus chemotherapy alone for advanced gastric cancer with a single non-curable factor (REGATTA:a phase 3, randomised controlled trial. Lancet Oncol 2016;17:309-18.
106. Yoshikawa T, Morita S, Tanabe K, et al. Survival results of a randomised two-by-two factorial phase II trial comparing neoadjuvant chemotherapy with two and four courses of S-1 plus cisplatin (SC) and paclitaxel plus cisplatin (PC) followed by D2 gastrectomy for resectable advanced gastric cancer. Eur J Cancer 2016;62:103-11.
107. Hironaka S, Sugimoto N, Yamaguchi K, et al. S-1 plus leucovorin versus S-1 plus leucovorin and oxaliplatin versus S-1 plus cisplatin in patients with advanced gastric cancer: a randomised, multicentre, open-label, phase 2 trial. Lancet Oncol 2016;17:99-108.
108. Shitara K, Chin K, Yoshikawa T, et al. Phase II study of adjuvant chemotherapy of S-1 plus oxaliplatin for patients with stage III gastric cancer after D2 gastrectomy. Gastric Cancer 2017;20:175-81.
109. Ito S, Sano T, Mizusawa J, et al. A phase II study of preoperative chemotherapy with docetaxel, cisplatin, and S-1 followed by gastrectomy with D2 plus para-aortic lymph node dissection for gastric cancer with extensive lymph node metastasis: JCOG1002. Gastric Cancer 2017;20:322-31.
110. Kimura Y, Fujii M, Masuishi T, et al. Multicenter phase II study of trastuzumab plus S-1 alone in elderly patients with HER2-positive advanced gastric cancer (JACCRO GC-06). Gastric Cancer 2018;21:421-7.
111. Miura Y, Sukawa Y, Hironaka S, et al. Five-weekly S-1 plus cisplatin therapy combined with trastuzumab therapy in HER2-positive gastric cancer: a phase II trial and biomarker study (WJOG7212G). Gastric Cancer 2018;21:84-95.
112. Bang YJ, Van Cutsem E, Feyereislova A, et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA:a phase 3, open-label, randomised controlled trial. Lancet 2010;376:687-97.
113. Fuchs CS, Tomasek J, Yong CJ, et al. Ramucirumab monotherapy for previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (REGARD:an international, randomised, multicentre,

placebo-controlled, phase 3 trial. Lancet 2014;383:31-9.
114. Wilke H, Muro K, Van Cutsem E, et al. Ramucirumab plus paclitaxel versus placebo plus paclitaxel in patients with previously treated advanced gastric or gastro-oesophageal junction adenocarcinoma (RAINBOW:a double-blind, randomised phase 3 trial. Lancet Oncol 2014;15:1224-35.
115. Hur H, Lee HY, Lee HJ, et al. Efficacy of laparoscopic subtotal gastrectomy with D2 lymphadenectomy for locally advanced gastric cancer: the protocol of the KLASS-02 multicenter randomized controlled clinical trial. BMC Cancer 2015;15:355.
116. Hu Y, Huang C, Sun Y, et al. Morbidity and mortality of laparoscopic versus open D2 distal gastrectomy for advanced gastric cancer: A randomized controlled trial. J Clin Oncol 2016;34:1350-7.
117. Kim W, Kim HH, Han SU, et al. Decreased morbidity of laparoscopic distal gastrectomy compared with open distal gastrectomy for stage I gastric cancer: short-term outcomes from a multicenter randomized controlled trial (KLASS-01). Ann Surg 2016;263:28-35.
118. Zhang CD, Yamashita H, Zhang S, et al. Reevaluation of laparoscopic versus open distal gastrectomy for early gastric cancer in Asia: A meta-analysis of randomized controlled trials. Int J Surg 2018;56:31-43.
119. Fujii K, Sonoda K, Izumi K, et al. T lymphocyte subsets and Th1/Th2 balance after laparoscopy-assisted distal gastrectomy. Surg Endosc 2003;17:1440-4.
120. Yoshikawa T, Cho H, Rino Y, et al. A prospective feasibility and safety study of laparoscopy-assisted distal gastrectomy for clinical stage I gastric cancer initiated by surgeons with much experience of open gastrectomy and laparoscopic surgery. Gastric Cancer 2013;16:126-32.
121. Inaki N, Etoh T, Ohyama T, et al. A multi-institutional, prospective, phase II feasibility study of laparoscopy-assisted distal gastrectomy with D2 lymph node dissection for locally advanced gastric cancer (JLSSG0901). World J Surg 2015;39:2734-41.
122. Nakamura K, Katai H, Mizusawa J, et al. A phase III study of laparoscopy-assisted versus open distal gastrectomy with nodal dissection for clinical stage IA/IB gastric cancer (JCOG0912). Jpn J Clin Oncol 2013;43:324-7.

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BPB Reports

Report

Sensitization of Gastric Cancer Cells to Irinotecan by p53 Activation

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Irinotecan (camptothecin-11 [CPT-11]) is a topoisomerase I inhibitor that has been used in the treatment of a wide spectrum of cancers including gastric cancer. Recent reports suggest that the expression of CES2, a serine hydrolase that converts irinotecan to its active compound SN-38, is regulated by the tumor-suppressor p53. In this study, we investigated whether irinotecan acted synergistically with a p53 activator nutlin-3a in human gastric cancer cells. Nutlin-3a treatment enhanced the expression of CES2 in gastric cancer cell lines with wild-type p53. However, this effect was not observed in cells with non-functional p53. Irinotecan showed synergistic antitumor effects in combination with nutlin-3a in gastric cancer cells with wild-type p53, whereas the survival of cells with non-functional p53 was not significantly affected by the presence of nutlin-3a. These results provide evidence that p53 activation can enhance the antitumor effect of irinotecan or other anticancer prodrugs activated by CES2 in gastric cancer cells through upregulation of CES2 expression.

Key words CES2, gastric cancer, irinotecan, p53, nutlin-3a

INTRODUCTION

Gastric cancer is relatively prevalent malignancy and ranks the fifth most commonly diagnosed malignancy and the third in cancer-related death worldwide.[1)] Gastric cancer is generally asymptomatic in early stages and has progressed to an advanced unresectable stage by the time of presentation.[2)] The prognosis of patients with gastric cancer remains extremely poor.[3)] Even patients with resectable tumor usually have a high rate of local recurrence and distant relapse.[4)] The standard palliative treatment for patients with advanced gastric cancer is chemotherapy, which both controls tumor-related symptoms and improves overall survival. Clinical trials have shown the survival benefit of irinotecan (camptothecin-11 [CPT-11]) in advanced gastric cancer as second-line chemotherapy.[5)]

Irinotecan is an anticancer drug that is used for the treatment of a wide spectrum of cancers including gastrointestinal cancer. Irinotecan is a prodrug and converted to its active compound 7-ethyl-10-hydroxy-camptothecin (SN-38) by the carboxylesterase CES2.[6)] However, the expression of CES2 is frequently downregulated in many types of cancers including gastric cancer,[6)] which may affect the therapeutic efficacy of irinotecan. Recent studies have indicated that CES2 can be transcriptionally activated by p53, a tumor suppressor that controls the transcription of plethora of genes in response to cellular stresses such as DNA damage, oxidative stress, and hypoxia.[7)] Therefore, it is conceivable that activation of p53 could sensitize gastric cancer cells to irinotecan by upregulating CES2 expression.

In this study, using various cell lines of human gastric cancer (Table 1), we provide evidence that irinotecan exerts a synergistic antitumor effect in combination with a p53 activator in human gastric cancer cells. We used nutlin-3a as a p53 activator, which inhibits binding of the E3 ubiquitin ligase MDM2 to p53 and thereby directly activates p53 signaling without genotoxic side effects.[13)] Nutlin-3a treatment enhanced CES2 expression in gastric cancer cells and sensitized these cells to irinotecan in a p53-dependent manner. Our results highlight the importance of *TP53* gene status and the combination use of p53-activating drugs for the efficacy of irinotecan and other antitumor prodrugs that are activated by CES2 in human gastric cancer.

MATERIALS AND METHODS

Cell Lines and Reagents The human gastric cancer cell lines MKN1, MKN7, MKN74 and NUGC4 were obtained from the RIKEN BRC Cell Bank (Tsukuba-shi, Ibaraki, Japan). TMK1 cells were provided by Hiroshima University (Hiroshima-shi, Hiroshima, Japan). NUGC3 cells were provided by the Health Science Research Resources Bank (Sennan-shi, Osaka, Japan). AGS cells were from the American Type Culture Collection (ATCC). The cells were cultured at 37°C and 5% CO_2 in RPMI 1640 medium (Wako) supplemented with 10% fetal bovine serum and penicillin/streptomycin. Nutlin-3a was purchased from AdooQ BioScience. All

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[1] The first two authors equally contributed to this study.

Table 1. Human Gastric Cancer Cell Lines Used in This Study

Name	Histological type[a]	Origin	*TP53* Status	*TP53* Mutation found at				Reference
				cDNA description	Exon	Codon	Amino acid change	
AGS	as	Stomach	wt	-	-	-	-	8
NUGC4	sig	metastasis (LN)	wt	-	-	-	-	8
MKN74	tub2	metastasis (Liver)	wt	-	-	-	-	9
NUGC3	por	metastasis (Brachialis muscle)	mt	c.659A>G	6	220	Tyr to Cys	8
MKN1	por	metastasis (LN)	mt	c.428T>C	5	143	Val to Ala	10
MKN7	tub1	metastasis (LN)	mt	c.832C>T	8	278	Pro to Ser	11
	tub1	metastasis (LN)	mt	c.751A>C	7	251	Ile to Leu	12
TMK1	por	metastasis (Liver)	mt	c.517G>A	5	173	Val to Met	10

[a]According to the Japanese Classification of Gastric Carcinoma. por, poorly differentiated adenocarcinoma; as, adenosquamous carcinoma; tub1, well-differentiated tubular adenocarcinoma; tub2, moderately differentiated tubular adenocarcinoma; sig, signet ring cell carcinoma; LN, lymph node; wt, wild type; mt, mutant.

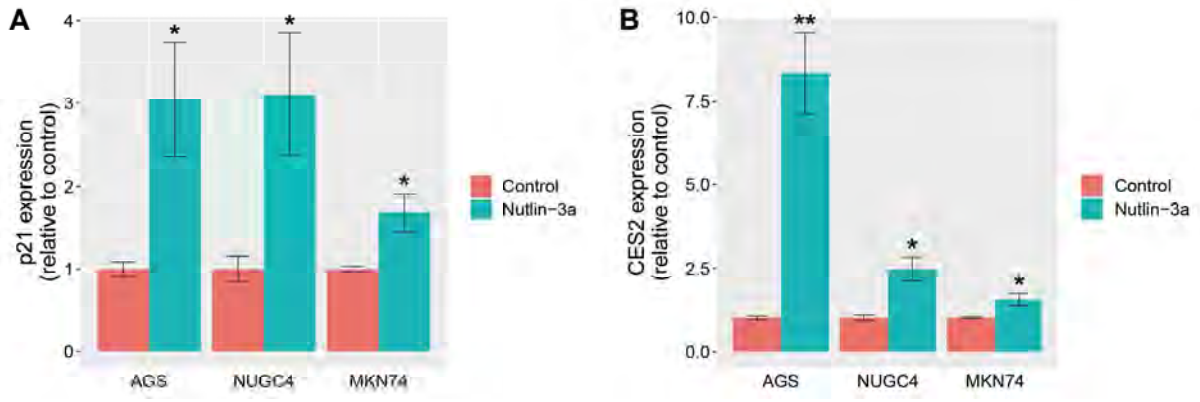

Fig. 1. Upregulation of CES2 Expression by Nutlin-3a in Gastric Cancer Cells with Wild-type p53.

Human gastric cancer cell lines with wild-type p53 (AGS, NUGC4, and MKN74) were treated with 5 μM nutlin-3a for 24 h. The expression of p21 (A) and CES2 (B) was quantified by real-time reverse transcriptase PCR. GAPDH was used as the reference gene. Data represent the mean values ± SEM (three independent experiments). *$p < 0$ 05; **$p < 0$ 01. An unpaired two-tailed *t*-test was used.

reagents were dissolved in sterile dimethyl sulfoxide (DMSO) to make 100 mmol/l (mM) stock. The cells were seeded in 6-well plates at a density of 2.5×10^5 cells/well and incubated for 24 h. The cells were then treated with nutlin-3a for another 24 h. The cells were washed twice with PBS and harvested by scraping.

Real-Time Reverse Transcriptase PCR Total RNA from cultured cell lines was extracted using the FastGene RNA Basic kit (Nippon Genetics Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions. Semiquantitative real-time PCR was performed using the Luna Universal One-Step RT-qPCR Kit (New England BioLabs) on a LightCycler 96 (Roche) in duplicate. The gene expression of target genes was normalized to GAPDH by the differences in Ct values, and then these values were used to calculate the relative mRNA expression levels with the $2^{-\Delta\Delta Ct}$ method. The primer sequences for the genes were as previously described.[14]

Cell Viability Assay Cell viability was determined by XTT (Cell Proliferation Kit II) assay (Roche). Briefly, cells were plated in triplicate into 96-well plates and cultured for 24 h, and then incubated with irinotecan at different concentrations in the presence or absence of 5 μM nutlin-3a for 24 h. The medium was then replaced with fresh medium and the cells were incubated with the XTT reagent for 3 h. The absorbance at 450 nm (reference wavelength at 660 nm) was measured with an iMark Microplate Absorbance Reader (Bio-Rad). Best-fit IC50 values were calculated with Prism 7 (GraphPad Software Inc., San Diego, CA) and compared by an extra sum-of-square *F* test.

RESULTS AND DISCUSSION

CES2 Expression was Upregulated by p53 Activation in Gastric Cancer Cells We first asked whether p53 activation enhanced CES2 expression in gastric cancer cells. Human gastric cancer cell lines with wild-type p53 (AGS, NUGC4, and MKN74) (Table 1) were treated with the p53 activator nutlin-3a. The expression of p21, a major downstream target of p53,[15] was upregulated by nutlin-3a treatment in all p53 wild-type cell lines tested, demonstrating activation of the p53 pathway in these cells (Fig. 1A). We also observed significant upregulation of CES2 expression in these cells (Fig. 1B). In contrast, nutlin-3a treatment did not significantly affect the expression of these genes in gastric cancer cells with p53 mutation (Table 1, Fig. 2). These results indicate that nutlin-3a enhances CES2 expression by activating functional p53 in human gastric cancer cells.

Synergistic Antitumor Effects of Irinotecan and Nutlin-3a in p53 Wild-Type Cells We next investigated whether nutlin-3a treatment sensitized gastric cancer cells to irinotecan, an anticancer prodrug that is converted by CES2 to its active compound SN-38. We treated two p53 wild-type cell lines (AGS and NUGC4) and two cell lines with non-functional p53 (NUGC3 and TMK1) with various concentrations of iri-

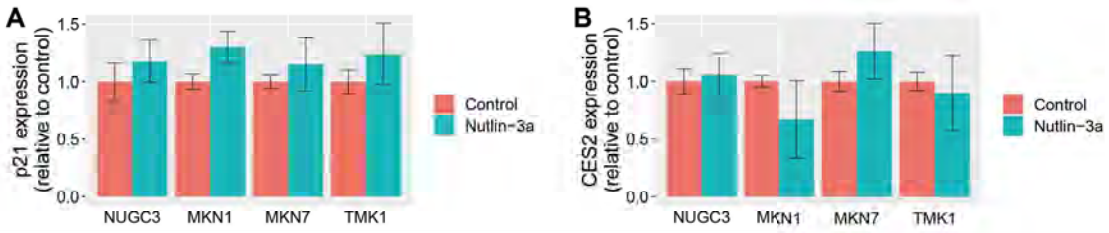

Fig. 2. Effects of Nutlin-3a on CES2 Expression in Gastric Cancer Cells with p53 Mutation.

Human gastric cancer cell lines with p53 mutation (NUGC3, MKN1, MKN7, and TMK1) were treated with 5 μM nutlin-3a for 24 h. The expression of p21 (A) and CES2 (B) was quantified by real-time reverse transcriptase PCR. GAPDH was used as the reference gene. Data represent the mean values ± SEM (three independent experiments). There was no significance between control and nutlin-3a. An unpaired two-tailed *t*-test was used.

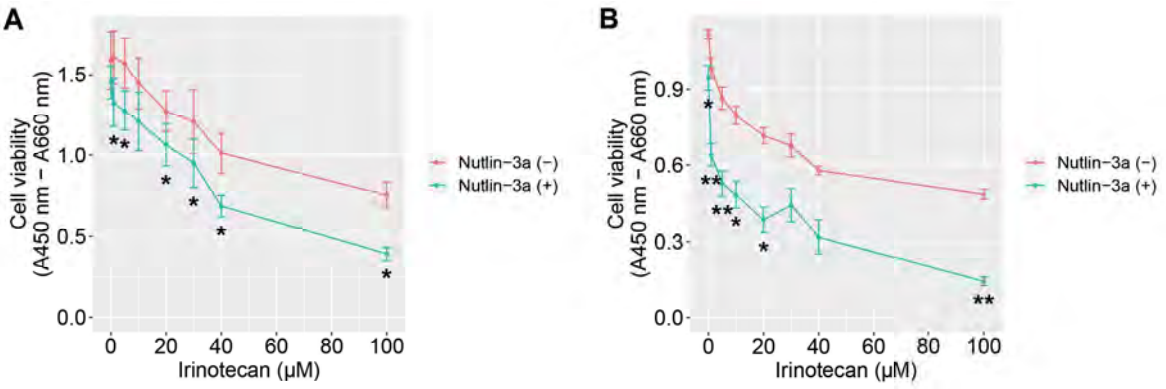

Fig. 3. Synergistic Effects of Nutlin-3a and Irinotecan in Gastric Cancer Cells with Wild-type p53.

AGS (A) and NUGC4 (B) cells were treated with various concentrations of irinotecan in the presence or absence of 5 μM nutlin-3a for 24 h. The cell viability was determined by XTT assay. Data represent the mean values ± SEM (three independent experiments). *$p < 0\ 05$; **$p < 0\ 01$. A paired two-tailed *t*-test was used.

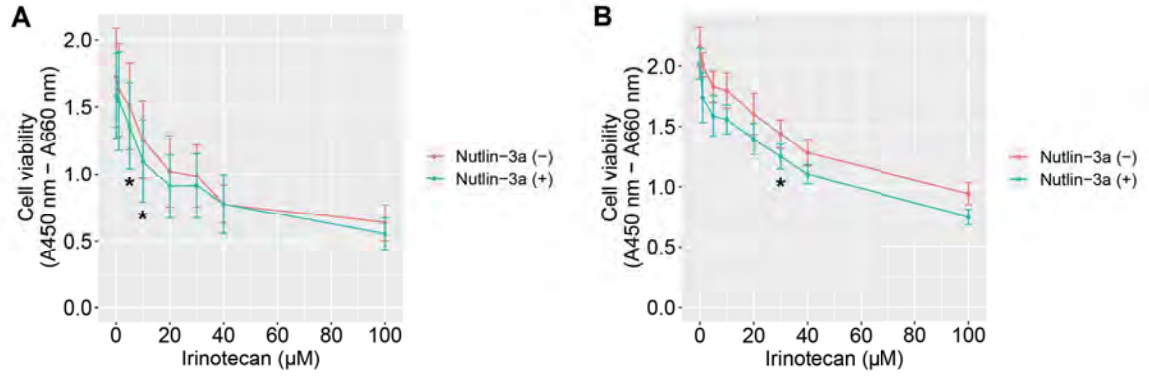

Fig. 4. Effects of Nutlin-3a and Irinotecan in Gastric Cancer Cells with p53 Mutation.

NUGC3 (A) and TMK1 (B) cells were treated with various concentrations of irinotecan in the presence or absence of 5 μM nutlin-3a for 24 h. The cell viability was determined by XTT assay. Data represent the mean values ± SEM (three independent experiments). *$p < 0\ 05$. A paired two-tailed *t*-test was used.

notecan with or without nutlin-3a. In p53 wild-type AGS and NUGC4 cells, the cell viability was not significantly or only slightly affected by single treatment with nutlin-3a, respectively (Fig. 3A and 3B). We observed strong synergistic effects of irinotecan and nutlin-3a in AGS and NUGC4 cells (Fig. 3A and 3B). The IC50 value of irinotecan was decreased by nutlin-3a by 2-fold, from 82.94 μM (95% CI [confidence interval]: 71.47–99.43 μM) to 42.94 μM (95% CI: 35.99–52.86 μM) ($p < 0.0001$) in AGS cells, and 7-fold from 59.59 μM (95% CI: 47.99–78.01 μM) to 8.731 μM (95% CI: 5.058–13.52 μM) ($p < 0.0001$) in NUGC4 cells, respectively. In contrast, nutlin-3a had almost no effect on the sensitivity to irinotecan in cells with non-functional p53 (Fig. 4A and 4B; the IC50 value from 38.69 μM [95% CI: 32.35–47.51 μM] to 35.79 μM [95% CI: 29.19–45.23 μM] in NUGC3 cells [$p = 0.5735$], from 74.25 μM [95% CI: 62.01–93.09 μM] to 56.71 μM [95% CI: 43.49–81.59 μM] in TMK1 cells [$p = 0.1328$]). These results suggest that p53 activation in gastric cancer cells leads to increased conversion of irinotecan to its active compound and thereby enhances the sensitivity to irinotecan.

Although a recent study has shown the survival benefit of irinotecan monotherapy as third-line or later treatment

in advanced gastric cancer,[16] irinotecan has been mostly used in combination with other anticancer drugs such as 5-fluorouracil (5-FU), which also activates p53.[17] Thus, the beneficial effects of these regimens in gastric cancer may be in part attributed to activation of p53 and upregulation of CES2, leading to efficient conversion of irinotecan. In this context, we used nutlin-3a to investigate the role of p53 because it directly activates p53 signaling pathway without untoward genotoxic side effects that may compromise the interpretation of the results. Consequently, we found that nutlin-3a upregulated CES2 expression only in gastric cancer cells with functional p53. Thus, p53 plays an important role in regulating CES2 expression in gastric cancer cells. p53 signaling is frequently dysregulated in many types of cancer including gastric cancer. Indeed, a genomic analysis of gastric adenocarcinomas has found p53 to be the most frequently mutated gene, accounting for 46% of total tumors.[18] In addition, irinotecan exhibits gastrointestinal toxicity and often causes severe diarrhea. Thus, understanding *TP53* gene status of gastric cancer may be useful to predict the efficacy of irinotecan-containing regimens.

In addition to irinotecan, several other anticancer prodrugs are also activated by CES2.[19–21] Capecitabine is an orally administered prodrug of 5-FU, which is effective and well tolerated in the treatment of gastric cancer. Various capecitabine-based chemotherapies have been shown to extend survival in advanced gastric cancer.[22] LY2334737 is an oral prodrug of the clinically efficacious anticancer agent gemcitabine. Gemcitabine is widely used in the treatment of pancreatic cancer[23] and advanced gastric cancer.[24] CES2 also converts Pentyl PABC-Doxaz to the active compound doxazolidine, a formaldehyde conjugate of doxorubicin that exhibits enhanced toxicity against a wide variety of tumor cell lines including cell lines resistant to doxorubicin.[25] Thus, the sensitivity of gastric cancer cells to these prodrugs may also be enhanced by p53 activation.

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Conflict of interest The authors declare no conflict of interest.

REFERENCES

1) Bray F, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.*, **68**, 394–424 (2018).
2) Van Cutsem E, *et al*. Gastric cancer. *Lancet*, **388**, 2654–2664 (2016).
3) Jim MA, *et al*. Stomach cancer survival in the United States by race and stage (2001-2009): findings from the CONCORD-2 study. *Cancer*, **123** (Suppl. 24), 4994–5013 (2017).
4) Ychou M, *et al*. Perioperative chemotherapy compared with surgery alone for resectable gastroesophageal adenocarcinoma: an FNCLCC and FFCD multicenter phase III trial. *J. Clin. Oncol.*, **29**, 1715–1721 (2011).
5) Hironaka S, *et al*. Randomized, open-label, phase III study comparing irinotecan with paclitaxel in patients with advanced gastric cancer without severe peritoneal metastasis after failure of prior combination chemotherapy using fluoropyrimidine plus platinum: WJOG 4007 trial. *J. Clin. Oncol.*, **31**, 4438–4444 (2013).
6) Xu G, *et al*. Human carboxylesterase 2 is commonly expressed in tumor tissue and is correlated with activation of irinotecan. *Clin. Cancer Res.*, **8**, 2605–2611 (2002).
7) Choi W, *et al*. Transcriptional activation of the carboxylesterase 2 gene by the p53 pathway. *Cancer Biol. Ther.*, **5**, 1450–1456 (2006).
8) Matozaki T, *et al*. Missense mutations and a deletion of the p53 gene in human gastric cancer. *Biochem. Biophys. Res. Commun.*, **182**, 215–223 (1992).
9) Yokozaki H. Molecular characteristics of eight gastric cancer cell lines established in Japan. *Pathol. Int.*, **50**, 767–777 (2000).
10) Yamada Y, *et al*. p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. *Cancer Res.*, **51**, 5800–5805 (1991).
11) Tsunemitsu Y, *et al*. Molecular therapy for peritoneal dissemination of xenotransplanted human MKN-45 gastric cancer cells with adenovirus mediated Bax gene transfer. *Gut*, **53**, 554–560 (2004).
12) Mashima T, *et al*. p53-defective tumors with a functional apoptosome-mediated pathway: a new therapeutic target. *J. Natl. Cancer Inst.*, **97**, 765–777 (2005).
13) Vassilev LT, *et al*. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. *Science*, **303**, 844–848 (2004).
14) Ishimine M, *et al*. The Relationship between TP53 Gene Status and Carboxylesterase 2 Expression in Human Colorectal Cancer. *Dis. Markers*, **2018**, 5280736 (2018).
15) Sax JK, El-Deiry WS. p53 downstream targets and chemosensitivity. *Cell Death Differ.*, **10**, 413–417 (2003).
16) Makiyama A, *et al*. Irinotecan monotherapy as third-line or later treatment in advanced gastric cancer. *Gastric Cancer*, **21**, 464–472 (2018).
17) Sun XX, *et al*. 5-fluorouracil activation of p53 involves an MDM2-ribosomal protein interaction. *J. Biol. Chem.*, **282**, 8052–8059 (2007).
18) Cancer Genome Atlas Research N. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*, **513**, 202–209 (2014).
19) Quinney SK, *et al*. Hydrolysis of capecitabine to 5′-deoxy-5-fluorocytidine by human carboxylesterases and inhibition by loperamide. *J. Pharmacol. Exp. Ther.*, **313**, 1011–1016 (2005).
20) Pratt SE, *et al*. Human carboxylesterase-2 hydrolyzes the prodrug of gemcitabine (LY2334737) and confers prodrug sensitivity to cancer cells. *Clin. Cancer Res.*, **19**, 1159–1168 (2013).
21) Barthel BL, *et al*. Identification of human intestinal carboxylesterase as the primary enzyme for activation of a doxazolidine carbamate prodrug. *J. Med. Chem.*, **51**, 298–304 (2008).
22) Okines A, *et al*. Capecitabine in advanced gastric cancer. *Expert Opin. Pharmacother.*, **8**, 2851–2861 (2007).
23) Oettle H, *et al*. Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic cancer: the CONKO-001 randomized trial. *JAMA*, **310**, 1473–1481 (2013).
24) De Lange SM, *et al*. Phase II trial of cisplatin and gemcitabine in patients with advanced gastric cancer. *Ann. Oncol.*, **15**, 484–488 (2004).
25) Barthel BL, *et al*. Preclinical efficacy of a carboxylesterase 2-activated prodrug of doxazolidine. *J. Med. Chem.*, **52**, 7678–7688 (2009).

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Safety and Oncological Outcomes of Laparoscopic NOSE Surgery Compared With Conventional Laparoscopic Surgery for Colorectal Diseases: A Meta-Analysis

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Objective: To evaluate the safety and oncological outcomes of laparoscopic colorectal surgery using natural orifice specimen extraction (NOSE) compared with conventional laparoscopic (CL) colorectal surgery in patients with colorectal diseases.

Methods: We conducted a systematic search of PubMed, EMBASE, and Cochrane databases for randomized controlled trials (RCTs), prospective non-randomized trials and retrospective trials up to September 1, 2018, and used 5-year disease-free survival (DFS), lymph node harvest, surgical site infection (SSI), anastomotic leakage, and intra-abdominal abscess as the main endpoints. Subgroup analyses were conducted according to the different study types [RCT and NRCT (non-randomized controlled trial)]. A sensitivity analysis was carried out to evaluate the reliability of the outcomes. RevMan5.3 software was used for statistical analysis.

Results: Fourteen studies were included (two RCTs, seven retrospective trials and five prospective non-randomized trials) involving a total of 1,435 patients. Compared with CL surgery, the NOSE technique resulted in a shorter hospital stay, shorter time to first flatus, less post-operative pain, and fewer SSIs and total perioperative complications. Anastomotic leakage, blood loss, and intra-abdominal abscess did not differ between the two groups, while operation time was longer in the NOSE group. Oncological outcomes such as proximal margin [weighted mean difference [WMD] = 0.47; 95% confidence interval [CI] −0.49 to 1.42; $P = 0.34$], distal margin (WMD= −0.11; 95% CI −0.66 to 0.45; $P = 0.70$), lymph node harvest (WMD = −0.97; 95% CI −1.97 to 0.03; $P = 0.06$) and 5-year DFS (hazard ratio = 0.84; 95% CI 0.54–1.31; $P = 0.45$) were not different between the NOSE and CL surgery groups.

Conclusions: Compared with CL surgery, NOSE may be a safe procedure, and can achieve similar oncological outcomes. Large multicenter RCTs are needed to provide high-level, evidence-based results in NOSE-treated patients and to determine the risk of local recurrence.

Keywords: natural orifice specimen extraction, colorectal diseases, oncological outcomes, post-operative function, totally intra-abdominal laparoscopic surgery, meta-analysis

INTRODUCTION

Laparoscopic technology has been widely used to treat colorectal cancer (CRC) over the past two decades, and many studies have demonstrated the advantages of laparoscopic surgery and have suggested that it is a less traumatic procedure, with similar oncological outcomes to those of open surgery (1–3). However, current laparoscopic colectomy is considered to be laparoscopically assisted surgery and not a totally intra-abdominal procedure, as it inevitably extends the incision by about 5–8 cm for specimen extraction and intestinal anastomosis (4, 5). Moreover, the laparotomy incision is also a source of post-operative morbidity, such as pain, wound infection, and incisional hernia (6–8).

In an attempt to further reduce surgical trauma, minimally invasive surgery has undergone unprecedented development. Laparoscopic natural orifice specimen extraction (NOSE) surgery is widely regarded as one of the representative new technologies in minimally invasive surgery (4, 9–12). It combines the concept of incisionless surgery and laparoscopy to complete intra-abdominal procedures (including exploration, dissection, and resection of lesions) and uses a natural orifice as a delivery route for specimen extraction without laparotomy incision (13). Compared with other minimally invasive techniques, laparoscopic colorectal surgery with NOSE adopts a transabdominal approach, which is more in line with the surgeon's practice and is easier to operate (5, 14–16). Recently, several studies have reported that laparoscopic NOSE surgery results in significantly fewer perioperative complications and faster recovery of gastrointestinal function (4, 12). However, the safety and oncological outcomes of laparoscopic colorectal surgery with NOSE are unclear. Therefore, we conducted a meta-analysis to determine the safety and oncological outcomes of laparoscopic NOSE surgery compared with conventional laparoscopic (CL) surgery for colorectal diseases.

METHODS

Search Strategy

Two independent researchers systematically searched studies in PubMed, EMBASE, and Cochrane databases from January 1990 to September 1, 2018. The search keywords used were "colorectal diseases," "laparoscopic surgery," "natural orifice specimen extraction," "transvaginal specimen extraction," "transanal specimen extraction," and "transrectal specimen extraction." According to the different requirements of each database, the search strategy was correspondingly changed. Potentially relevant articles were also screened from the references of relevant studies.

Inclusion and Exclusion Criteria

Studies were included if they conformed to the principle of PICO (S) [participants, interventions, comparisons, outcomes, (study design)] (17). Inclusion criteria were as follows: (1) participants: patients were diagnosed with colorectal diseases, either benign or malignant; (2) interventions: totally intra-abdominal laparoscopic colorectal surgery, with the specimen extracted via the rectum, vagina or anus; (3) comparisons: laparoscopic colorectal surgery, with the specimen extracted through the abdominal wall; (4) outcomes: 5-year disease-free survival (DFS), lymph node harvest, proximal margin, distal margin, operation time, hospital stay, total perioperative complications, pain score, time to first flatus, anastomotic leakage, surgical site infection (SSI), blood loss, and intra-abdominal abscess; (5) study design: randomized controlled trials (RCTs), prospective non-randomized trials and retrospective trials based on NOSE. Exclusion criteria were as follows: (1) traditional open surgery; (2) non-colorectal diseases; (3) transanal total mesorectal excision surgery; (4) lack of data, or inability to obtain original data from the author; (5) case reports, letters, reviews, conference abstracts, animal experiments, and expert opinions; (6) studies not written in English or Chinese were excluded.

Data Extraction and Quality Assessment

Two researchers independently extracted data from the studies. For each included study, the following information was extracted: first author, year of publication, country of origin, number of patients, characteristics of the patients [age, gender, body mass index [BMI], clinical TNM stage, diseases type, extent of resection, specimen extraction approach, location of diseases], study type, information on outcome (primary outcomes: 5-year DFS, lymph node harvest, anastomotic leakage, intra-abdominal abscess, and SSI; secondary outcomes: operation time, hospital stay, pain score, time to first flatus, total perioperative complications, blood loss, proximal margin, and distal margin). If there were any doubts or disagreements regarding outcomes, these studies were submitted to a third researcher for arbitration. For retrospective and prospective non-randomized studies, the Newcastle–Ottawa Scale (NOS) was used (18). The assessment of bias in the RCTs was based on the Cochrane Risk of Bias tool (19).

Statistical Analysis

Review Manager Version 5.3 software (Cochrane Collaboration, Oxford, UK) was used for statistical analysis. For continuous

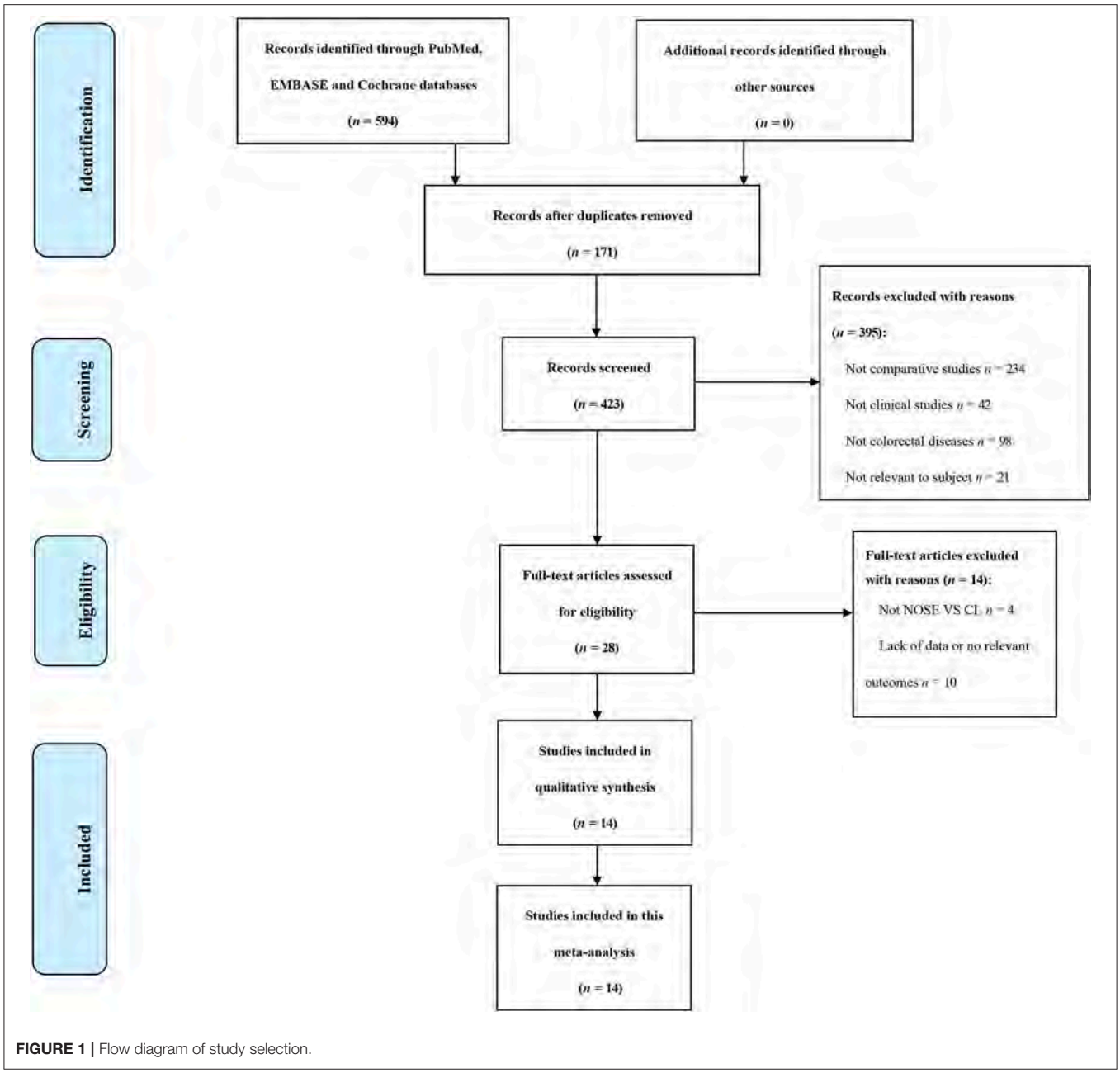

FIGURE 1 | Flow diagram of study selection.

variables, weighted mean difference (WMD) was used. Odds ratio (OR) was used to express dichotomous variables. The hazard ratio (HR) of 5-year DFS was calculated from survival curves using the methods presented by Tierney et al. (20). The confidence interval (CI) was set at 95%, and $P < 0.05$ was considered statistically significant. The Chi-square test or Cochrane Q test was used to calculate heterogeneity, and $I^2 < 50\%$ and $P > 0.10$ were defined as non-significant heterogeneity, and such data were evaluated using the fixed effect model; otherwise, the random effect model was used (21, 22). Subgroup analyses were conducted according to the different study types [RCT and NRCT (non-randomized controlled trial)]. A sensitivity analysis was carried out to evaluate the stability of the outcomes. In addition, publication bias was assessed by Begg's funnel plots and Begg's test (STATA, version 12.0) (23).

RESULTS

Study Selection and Characteristics

A total of 14 studies were included in the meta-analysis [two RCTs (12, 24), seven retrospective studies (4, 5, 10, 11, 25–27), and five prospective non-randomized studies (9, 16, 28–30)]. A flow diagram of study selection is shown in **Figure 1**.

TABLE 1 | Basic characteristics of the included studies.

References	Year	Country/ area	Patients (NOSE/CL)	Gender (NOSE/CL) (M/F)		Age[a] (NOSE/CL)		BMI[a] (NOSE/CL)		Study type	Approach	Disease type	cTNM	Extent of resection (NOSE/CL)	Location
Hisada et al. (4)	2014	Japan	20/50	12/8	NA	63.7 ± 9.0	66.3 ± 11.0	NA	NA	RT	TS	Mal	I-III	NA	Rec
Park et al. (5)	2017	Korea	138/138	32/106	41/97	60.3 ± 10.6	60.4 ± 11.3	23.4 ± 2.9	23.3 ± 3.2	RT	TS /TV	Mal	0-III	NA	Rec
Costantino et al. (9)	2011	France	17/9	6/11	4/5	60.1 ± 9.4	59.5 ± 12.6	25.5 ± 3.0	30.5 ± 4.2	PNT	TS	Ben	NA	NA	SC
Award et al. (10)	2014	USA	20/20	20 (F)	20 (F)	63.6 ± 9.0	66.9 ± 8.9	25.1 ± 6.7	31.6 ± 8.3	RT	TV	Ben/Mal	I-III	24.4 ± 5.9/ 40.1 ± 26.8	RC
Zhang et al. (11)	2014	China	65/132	32/33	57/75	56.1 ± 9.3	55.5 ± 9.5	23.7 ± 2.9	23.1 ± 3.1	RT	TS	Mal	I-III	NA	SC, Rec
Leung et al. (12)	2013	China	35/35	13/22	12/23	62 (51–86)	72 (49–84)	NA	NA	RCT	TS	Mal	NA	NA	LC
Park et al. (16)	2011	Korea	34/34	34 (F)	34(F)	61.0 ± 11.2	63.6 ± 11.6	23.9 ± 3.1	23.1 ± 2.7	PNT	TV	Mal	I-III	NA	RC
Wolthuis et al. (24)	2014	Belgium	20/20	5/15	10/10	54 (31–72)	58 (40–73)	23.5 (18–29)	24 (20–29)	RCT	TR	Ben/Mal	NA	25 (12–44)/18 (12–33)	SC
Denost et al. (25)	2015	France	122/98	70/52	69/29	63 (20–90)	65 (25–85)	24.3 (17.3–33.6)	25.8 (18.8–38.3)	RT	TS	Mal	0-III	NA	Rec
Saurabh et al. (26)	2017	Taiwan	82/106	47/35	65/41	63.3 ± 13.9	64.7 ± 10.9	24.4 ± 4.2	24.4 ± 3.2	RT	TS	Ben/Mal	I-III	16 ± 5.3/ 15.3 ± 5.3	SC, Rec
Xu et al. (27)	2016	China	23/23	13/10	13/10	63.0 ± 9.4	63.5 ± 13.5	22.2 ± 2.7	22.2 ± 3.3	RT	TS	Mal	0-III	NA	LC, Rec
Christoforidis et al. (28)	2012	Switzerland	10/20	3/7	10/10	47 (26–62)	56 (38–81)	27.6 (19.7–30.9)	26.4 (19.4–31.6)	PNT	TR	Ben	NA	NA	LC, Rec
Kim et al. (29)	2014	Korea	58/58	58 (F)	58 (F)	62.8 ± 9.0	63.2 ± 10.7	23.5 ± 2.9	23.2 ± 3.3	PNT	TV	Mal	I-III	NA	LC
Xing et al. (30)	2017	China	16/32	12/4	24/8	61.9 ± 11.8	62.4 ± 12.0	23.1 ± 1.2	23.9 ± 1.7	PNT	TS	Mal	I-III	18.2 ± 4.8/ 19.8 ± 5.7	SC

NOSE, natural orifice specimen extraction; CL, conventional laparoscopic surgery; [a], mean ± SD or median (range); SD, standard deviation; BMI, body mass index; NA, not available; RCT, randomized controlled trial; RT, retrospective trial; PNT, prospective non-randomized trial; cTNM, clinical TNM stage; M, male; F, female; Ben, benign; Mal, malignant; SC, sigmoid colon; LC, left colon; RC, right colon; Rec, rectum; TS, transanal; TV, transvaginal; TR, transrectal; ref, reference.

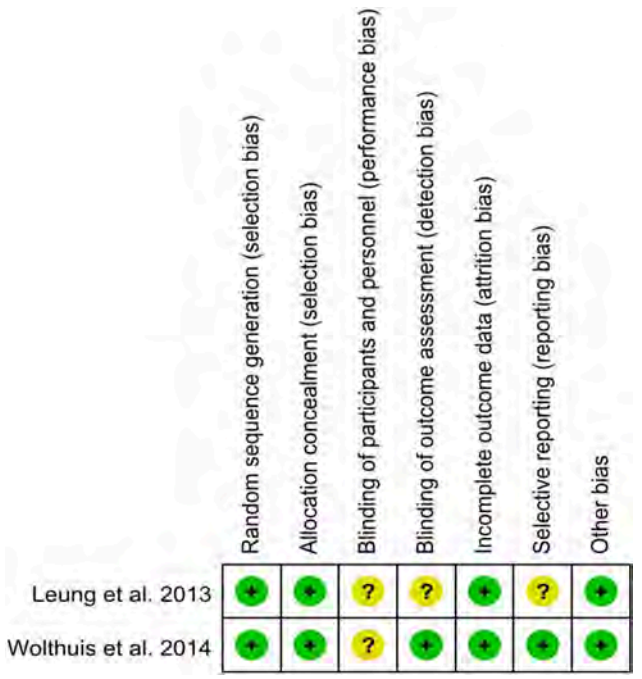

FIGURE 2 | Risk of bias of randomized controlled trials assessed with the Cochrane Risk of Bias tool.

Nine studies were from Asia, four from Europe and one from North America. A total of 1,435 patients were included; 660 in the NOSE group and 775 in the CL surgery group. The two groups were similar in terms of age ($P = 0.12$), body mass index (BMI, $P = 0.15$), and extent of resection ($P = 0.86$). In the included studies, the main steps of NOSE and CL regarding exploration, mobilization, and dissection were the same. NOSE involves a natural orifice for specimen extraction. However, CL surgery involves specimen extraction through the abdominal wall. The basic characteristics of the studies included are summarized in **Table 1**. All non-randomized studies had a NOS score of ≥5, and RCTs had a low risk of bias. Quality assessment results of the included studies are shown in **Table 2**, **Figure 2**, respectively. Forest plots of all the outcomes are shown in **Figures 3–5** and **Supplementary Figures 1–10**. The meta-analysis of the main endpoints is summarized in **Table 3**. The meta-analysis of endpoints for cancers is shown in **Table 4**, **Supplementary Figures 1A, 4A–I**.

Oncological Outcomes

Five-year DFS was reported in two studies (5, 25). Both of which were NRCTs. There was no significant difference in 5-year DFS between the NOSE and CL surgery groups (HR = 0.84; 95% CI 0.54 to 1.31; $P = 0.45$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used (**Figure 3**, **Supplementary Figure 1A**).

A total of seven studies reported lymph node harvest (4, 10, 11, 16, 26, 29, 30). All of which were NRCTs. There was no significant difference in lymph node harvest between the two groups (WMD = −0.97; 95% CI −1.97 to 0.03; $P = 0.06$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used (**Figure 3**, **Supplementary Figure 1B**).

Data on proximal and distal margins were available in three studies (26, 27, 29). All of which were NRCTs. There was no significant difference in proximal margin (WMD = 0.47; 95% CI −0.49 to 1.42; $P = 0.34$) (**Figure 3**, **Supplementary Figure 1C**) and distal margin (WMD = −0.11; 95% CI −0.66 to 0.45; $P = 0.70$) (**Figure 3**, **Supplementary Figure 1D**). No significant heterogeneity in proximal margin ($I^2 = 0\%$) and distal margin ($I^2 = 24\%$) was observed; therefore, the fixed effect model was used.

Safety Outcomes

A total of seven studies reported SSIs (4, 5, 10, 12, 26, 29, 30). There were fewer SSIs in the NOSE group, and the difference was significant (OR = 0.15; 95% CI 0.05 to 0.42; $P < 0.001$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used. The pooled OR for the NRCT subgroup was 0.16 (95% CI 0.05 to 0.48; $P = 0.001$, $I^2 = 0\%$) (**Figure 4**, **Supplementary Figure 2A**).

Anastomotic leakage was reported in six studies (5, 9, 11, 26, 27, 29). All of which were NRCTs. There was no significant difference in anastomotic leakage between the NOSE and CL surgery groups (OR = 0.71; 95% CI 0.36 to 1.38; $P = 0.31$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used (**Figure 4**, **Supplementary Figure 2B**).

A total of seven studies reported blood loss (4, 5, 11, 16, 26, 27, 30). All of which were NRCTs. There was no significant difference between the two groups (WMD = −12.23; 95% CI −29.35 to 4.90; $P = 0.16$). Heterogeneity was observed (P for heterogeneity <0.001, $I^2 = 89\%$); therefore, the random effect model was used (**Figure 4**, **Supplementary Figure 2C**).

Data on intra-abdominal abscess were included in three studies (5, 16, 29). All of which were NRCTs. There was no significant difference between the two groups (OR = 1.34; 95% CI 0.30–6.05; $P = 0.70$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used (**Figure 4**, **Supplementary Figure 2D**).

Data on total perioperative complications (such as wound infection, anastomotic leakage, ischemia, bleeding, ileus, and anal dysfunction) were reported in 12 studies (4, 5, 9–11, 16, 24–29). The results showed that the NOSE group had fewer complications than the CL surgery group (OR = 0.56; 95% CI 0.41 to 0.75; $P < 0.001$). The difference was significant. No obvious heterogeneity was observed ($I^2 = 15\%$); therefore, the fixed effect model was used. The pooled OR for the NRCT subgroup was 0.55 (95% CI 0.41–0.74; $P < 0.001$, $I^2 = 21\%$) (**Figure 4**, **Supplementary Figure 2E**).

Other Outcomes

Data on operation time was available in 12 studies (4, 5, 9–12, 16, 26–30). Compared to the NOSE group, the operation time was shorter in the CL surgery group (WMD = 17.34; 95% CI 6.14–28.54; $P = 0.002$). Significant heterogeneity was observed

TABLE 2 | Newcastle-Ottawa Scale for bias risk assessment of retrospective and prospective non-randomized studies.

References	Case definition	Representativeness	Control selection	Definition of controls	Comparability	Ascertainment of exposure	SMACC	Non-response rate	Total
Hisada et al. (4)	1	1	1	1	1	1	1	0	7
Park et al. (5)	1	1	1	0	2	1	1	1	8
Costantino et al. (9)	1	1	1	1	1	1	1	0	7
Award et al. (10)	1	1	1	1	1	1	1	0	7
Zhang et al. (11)	1	1	1	0	1	1	1	0	6
Park et al. (16)	1	1	1	0	1	1	1	0	6
Denost et al. (25)	1	1	1	0	2	1	1	1	8
Saurabh et al. (26)	1	1	1	0	1	1	1	0	6
Xu et al. (27)	1	0	1	0	1	1	1	0	5
Christoforidis et al. (28)	1	1	1	1	1	1	1	0	7
Kim et al. (29)	1	1	1	0	1	1	1	1	7
Xing et al. (30)	1	0	1	0	1	1	1	0	5

SMACC, same method of ascertainment for cases and controls.

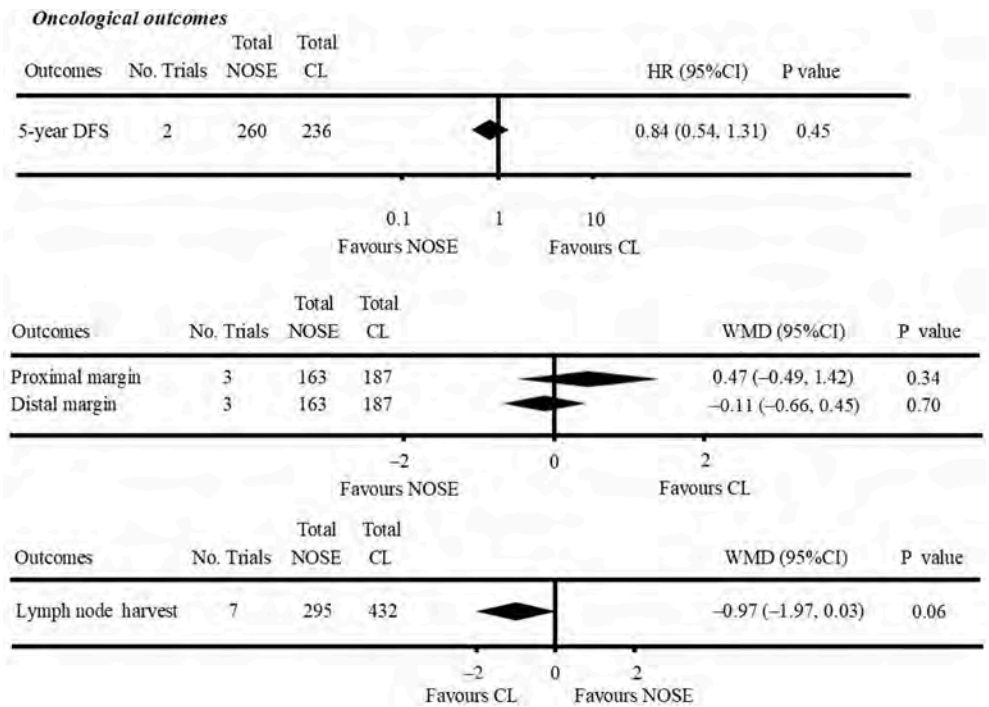

FIGURE 3 | Forest plot of oncological outcomes following natural orifice specimen extraction (NOSE) compared with conventional laparoscopic (CL) surgery.

(P for heterogeneity <0.001, $I^2 = 81\%$); therefore, the random effect model was used. The pooled WMD for the NRCT subgroup was 18.83 (95% CI 6.48–31.17; $P = 0.003$, $I^2 = 82\%$) (**Figure 5**, **Supplementary Figure 3A**).

A total of 12 studies reported data on hospital stay (4, 5, 9–12, 16, 26–30). Patients in the NOSE group had a reduced hospital stay compared with patients in the CL surgery group. The difference was significant (WMD = −0.56; 95% CI −1.09 to −0.04; $P = 0.03$). Significant heterogeneity was observed (P for heterogeneity = 0.01, $I^2 = 54\%$); therefore, the random effect model was used. The pooled WMD for the NRCT subgroup was −0.66 (95% CI −1.22 to −0.10; $P = 0.02$, $I^2 = 50\%$) (**Figure 5**, **Supplementary Figure 3B**).

Five studies reported pain score using the visual analog scale (VAS) on post-operative day 1 (9, 11, 16, 29, 30). All of which were NRCTs. The NOSE group had a lower VAS score than the CL surgery group. The difference was significant (WMD = −1.42; 95% CI −1.94 to −0.90; $P < 0.001$). Significant

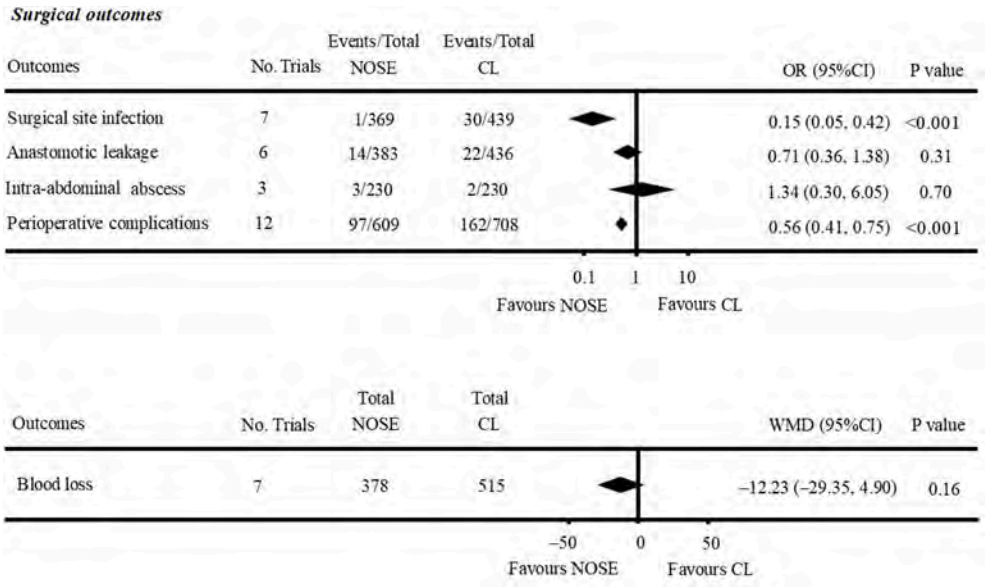

FIGURE 4 | Forest plot of surgical outcomes following NOSE compared with CL.

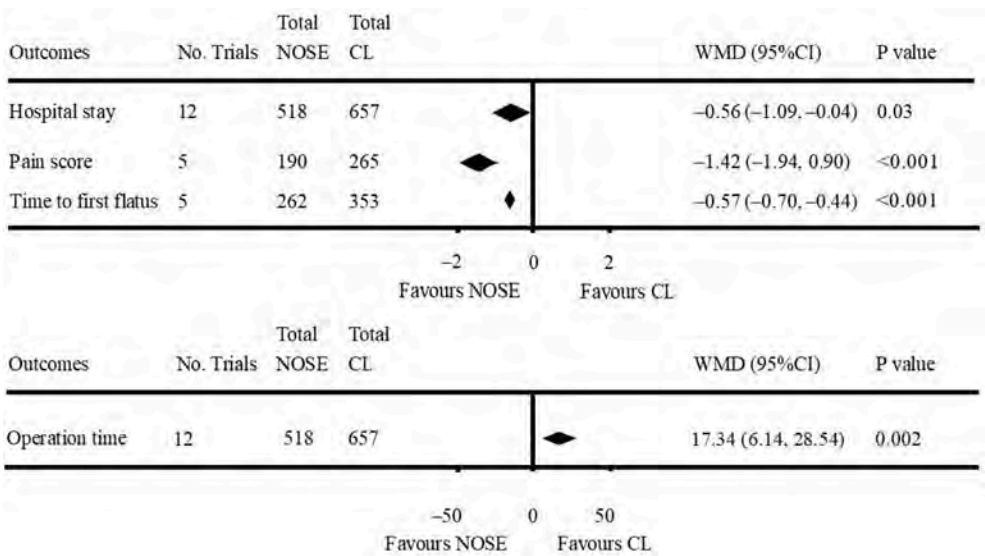

FIGURE 5 | Forest plot of other outcomes following NOSE compared with CL.

heterogeneity was observed (P for heterogeneity < 0.001, $I^2 = 85\%$); therefore, the random effect model was used (**Figure 5**, **Supplementary Figure 3C**).

Data on time to first flatus was included in five studies (11, 16, 26, 27, 29). All of which were NRCTs. Compared with the CL surgery group, time to first flatus was shorter in the NOSE group. The difference was significant (WMD $= -0.57$; 95% CI -0.70 to -0.44; $P < 0.001$). No significant heterogeneity was observed ($I^2 = 0\%$); therefore, the fixed effect model was used (**Figure 5**, **Supplementary Figure 3D**).

Publication Bias

We performed a funnel plot of the studies included to assess publication bias. No obvious asymmetry was noted and none of the studies were outside the limits of the 95% CI (**Figure 6**). No significant publication bias among these studies was observed using Begg's test ($P = 0.373$). In addition, a sensitivity analysis was performed using six outcomes (lymph node harvest, SSI, anastomotic leakage, total perioperative complications, operation time, hospital stay) and the results are shown in **Table 5**. Forest plots based on exclusion criteria in the sensitivity analysis are

TABLE 3 | Meta-analysis of main endpoints.

Endpoints	No. of patients	No. of trials (No. of RCTs)	NOSE	CL	HR/OR/WMD (95%CI)	*P*-value	I^2 (%)	*P*-value for heterogeneity
ONCOLOGICAL OUTCOMES (REFERENCES)								
Five-year DFS (5, 25)	496	2 (0)	260	236	0.84 (0.54 to 1.31)	0.45	0	0.83
Lymph node harvest (4, 10, 11, 16, 26, 29, 30)	727	7 (0)	295	432	−0.97 (−1.97 to 0.03)	0.06	0	0.76
Proximal margin, cm (26, 27, 29)	350	3 (0)	163	187	0.47 (−0.49 to 1.42)	0.34	0	0.72
Distal margin, cm (26, 27, 29)	350	3 (0)	163	187	−0.11 (−0.66 to 0.45)	0.70	24	0.27
SAFETY OUTCOMES								
Surgical site infection (4, 5, 10, 12, 26, 29, 30)	808	7 (1)	369	439	0.15 (0.05 to 0.42)	< 0.001	0	0.99
Anastomotic leakage (5, 9, 11, 26, 27, 29)	819	6 (0)	383	436	0.71 (0.36 to 1.38)	0.31	0	0.96
Blood loss, ml (4, 5, 11, 16, 26, 27, 30)	893	7 (0)	378	515	−12.23 (−29.35 to 4.90)	0.16	89	< 0.001
Intra-abdominal abscess (5, 16, 29)	460	3 (0)	230	230	1.34 (0.30 to 6.05)	0.70	0	0.47
Total perioperative complications (4, 5, 9–11, 16, 24–29)	1,317	12 (1)	609	708	0.56 (0.41 to 0.75)	< 0.001	15	0.30
OTHER OUTCOMES								
Operation time, min (4, 5, 9–12, 16, 26–30)	1,175	12 (1)	518	657	17.34 (6.14 to 28.54)	0.002	81	< 0.001
Hospital stay, day (4, 5, 9–12, 16, 26–30)	1,175	12 (1)	518	657	−0.56 (−1.09 to −0.04)	0.03	54	0.01
Pain score (9, 11, 16, 29, 30)	455	5 (0)	190	265	−1.42 (−1.94 to 0.90)	< 0.001	85	< 0.001
Time to first flatus, day (11, 16, 26, 27, 29)	615	5 (0)	262	353	−0.57 (−0.70 to −0.44)	< 0.001	0	0.48

NOSE, natural orifice specimen extraction; CL, conventional laparoscopic surgery; HR, hazard ratio; OR, odds ratio; WMD, weighted mean difference; 95%CI; 95% confidence interval; DFS, diseases-free survival.

shown in **Supplementary Figures 5–10**. Finally, exclusion of any single study and sensitivity analysis based on various exclusion criteria did not affect the pooled results, except hospital stay based on prospective studies.

DISCUSSION

This meta-analysis mainly focused on the oncological and safety outcomes of laparoscopic colorectal surgery using NOSE. We found that oncological outcomes and safety outcomes of NOSE were not significantly different to those of CL surgery.

Surgical safety is always an important concern for surgeons. Severe post-operative complications may even lead to failure of the entire operation (31–34). An enterotomy within the peritoneal cavity and insertion of an anvil into the abdominal cavity through a natural orifice are necessary in some approaches of NOSE, which can cause bacteriological concerns (13, 28, 35). Costantino et al. and Wolthuis et al. studied the bacterial positive rate in peritoneal fluid culture and demonstrated that, although NOSE had a higher risk of peritoneal contamination, there were no significant differences in clinical outcomes between the two groups (9, 24). Recently, a multicenter study of 718 cases from China further showed that the incidence of intraperitoneal infection after NOSE was only 0.8% (36). To reduce the risk of peritoneal bacterial contamination, pre-operative administration of prophylactic antibiotics, pre-operative bowel preparation, intraoperative peritoneal irrigation and intraoperative transanal lavage are considered routine procedures in NOSE (15). From our pooled data, SSI was reduced in the NOSE group and the incidence of intra-abdominal abscess was not significantly different between the two groups. Post-operative anastomotic leakage is another severe complication in colorectal surgery, and must be avoided. Several factors are considered to increase the incidence of anastomotic leakage, such as excessive tension in the reconstructed bowel, anastomotic ischemia, and anastomotic technique (4, 33). A circular stapler device and end-to-end anastomosis are commonly used in both groups. However, anastomosis in CL colectomy is performed extracorporeally and differs from that in totally laparoscopic surgery with intracorporeal anastomosis (IA). During laparoscopic left colectomy with extracorporeal anastomosis (EA), exteriorization of the bowel requires greater mobilization of colonic segments and the mesentery, which may result in mesenteric laceration and bleeding, further endangering the blood supply of the anastomotic stoma. However, IA requires less mobilization than EA, and therefore facilitates the achievement of tension-free anastomosis. Recent studies have demonstrated that compared to

TABLE 4 | Meta-analysis of endpoints for cancers.

Endpoints (references)	No. of patients	No. of trials (No. of RCTs)	NOSE	CL	HR/OR/WMD (95%CI)	*P*-value	I^2 (%)	*P*-value for heterogeneity
Five-year DFS (5, 25)	496	2 (0)	260	236	0.84 (0.54 to 1.31)	0.45	0	0.83
Lymph node harvest (4, 11, 16, 29, 30)	499	5 (0)	193	306	−1.15 (−2.40 to 0.11)	0.07	0	0.75
Proximal margin, cm (27, 29)	162	2 (0)	81	81	1.24 (−1.03 to 3.50)	0.28	0	0.74
Distal margin, cm (27, 29)	162	2 (0)	81	81	−0.48 (−1.21 to 0.25)	0.20	0	0.59
Surgical site infection (4, 5, 12, 29, 30)	580	5 (1)	267	313	0.14 (0.04 to 0.46)	0.001	0	0.98
Anastomotic leakage (5, 11, 27, 29)	605	4 (0)	284	321	0.65 (0.31 to 1.37)	0.25	0	0.88
Blood loss, ml (4, 5, 11, 16, 27, 30)	705	6 (0)	296	409	−12.35 (−32.62 to 7.93)	0.23	91	< 0.001
Total perioperative complications (4, 5, 11, 16, 26, 27, 29)	993	7 (0)	460	533	0.52 (0.37 to 0.73)	< 0.001	0	0.46
Operation time, min (4, 5, 11, 12, 16, 27, 29, 30)	891	8 (1)	389	502	13.70 (0.97 to 26.43)	0.03	83	< 0.001
Hospital stay, day (4, 5, 11, 12, 16, 27, 29, 30)	891	8 (1)	389	502	−0.64 (−1.19 to −0.09)	0.02	55	0.03

NOSE, natural orifice specimen extraction; CL, conventional laparoscopic surgery; OR, odds ratio; WMD, weighted mean difference; 95%CI; 95% confidence interval; Unite; min, minute.

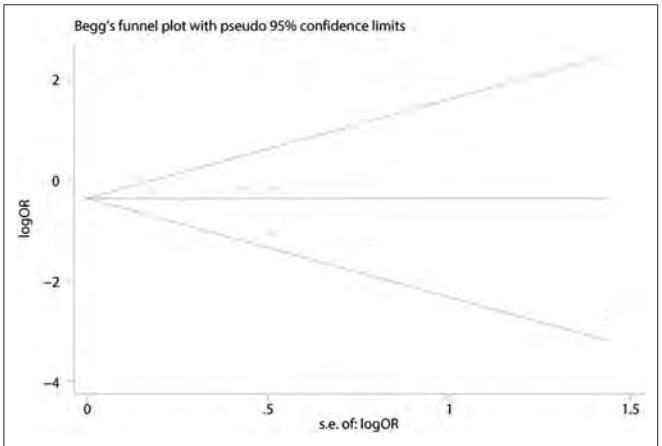

FIGURE 6 | Funnel plot of publication bias based on total perioperative complications (Begg's test, $P = 0.373$).

EA, IA is not associated with a greater incidence of anastomotic leakage (37–39). NOSE has a significant advantage in that it reduces anastomotic leakage in ultra-low rectal anus-preserving surgery (40). Unlike CL surgery in a narrow pelvic cavity, NOSE can evaginate the rectal specimen through the anus to the external, and easily close the distal rectum end under direct vision, further reducing the incidence of anastomotic leakage (41). The incidence of anastomotic leakage across the included studies in the NOSE group was 3.6% and was 5% in the CL group (**Figure 4**). From the pooled data in the present study, the incidence of anastomotic leakage was not significantly different between the two groups. In summary, we consider that laparoscopic colorectal surgery with NOSE is surgically safe.

Lymph node metastasis, local recurrence (LR) and positive margin are life-threatening conditions in colorectal cancer surgery, often associated with worse overall survival (OS) and DFS (42–47). In this study, we used 5-year DFS to evaluate the long-term oncological safety of the NOSE technique. Anatomically, the distribution of lymphatic vessels is in parallel with the colonic mesenteric vessels. When the pre-resection margin of the bowel is determined, the corresponding mesenteric vessels are ligated, and the adherent lymph nodes are removed accordingly. The exploration, mobilization and dissection steps in NOSE and CL are almost the same, which indicates a similar lymph node harvest in both groups. In our meta-analysis, the number of lymph nodes harvested was not significantly different between the two groups. In addition, the 2017 National Comprehensive Cancer Network guidelines recommend removal of at least 12 lymph nodes during lymphadenectomy for cancer surgery. In all the studies included, more than 12 lymph nodes were removed in each group. Therefore, we suggest that laparoscopic NOSE can achieve adequate lymph node harvest similar to CL surgery. Complying with the principle of tumor-free during surgery is another challenge for NOSE. This concern arises from an incision at the colorectal stump (or vagina) in the abdominopelvic cavity and specimen extraction through a narrow natural orifice which may cause cancer cell implantation, and is a significant issue regarding LR and DFS (48). However, in clinical practice, the following steps are taken to reduce the risk of tumor seeding and peritoneal contamination: distal cytocidal rectal lavage; and a specimen extraction bag or a professional platform [transanal endoscopic operation [TEO] device or transanal endoscopic microsurgery [TEM] device] is inserted during the retrieval phase (15). Moreover, previous studies have confirmed that LR after NOSE is comparable to CL (5, 25). A tumor can achieve distant invasion by intramural spread; therefore, inadequate surgical resection may lead to a positive margin which is an independent factor of DFS (49). Three of the included studies reported surgical margin status

TABLE 5 | Sensitivity analysis of endpoints of interests.

Endpoints (references)	No. of patients	No. of trials	NOSE	CL	OR or WMD (95%CI)	*P*-value	I^2 (%)	*P* value for heterogeneity
LYMPH NODE HARVEST								
All included trails (4, 10, 11, 16, 26, 29, 30)	727	7	295	432	−0.97 (−1.97 to 0.03)	0.06	0	0.76
BMI ≤ 30 (kg/m^2) (4, 11, 16, 26, 29, 30)	687	6	275	412	−0.87 (−1.89 to 0.16)	0.10	0	0.77
Sample number >30 (11, 16, 26, 29)	569	4	239	330	−0.59 (−1.99 to 0.80)	0.41	0	0.64
Non-RCT (NOS ≥ 6) (4, 10, 11, 16, 26, 29)	679	6	279	400	−0.71 (−1.98 to 0.56)	0.27	0	0.71
Prospective trials (16, 29, 30)	232	3	108	124	−1.11 (−2.59 to 0.38)	0.14	0	0.53
TOTAL PERIOPERATIVE COMPLICATIONS								
All included trails (4, 5, 9–11, 16, 24–29)	1,317	12	609	708	0.56 (0.41 to 0.75)	<0.001	15	0.30
BMI ≤ 30 (kg/m^2) (4, 5, 11, 16, 24, 26, 27, 29)	1,001	8	440	561	0.50 (0.34 to 0.74)	< 0.001	0	0.52
Sample number > 30 (5, 11, 16, 25, 26, 29)	1,065	6	499	566	0.57 (0.41 to 0.79)	<0.001	0	0.59
Non-RCT (NOS ≥ 6) (4, 5, 9–11, 16, 25, 26, 28, 29)	1,231	10	566	665	0.57 (0.42 to 0.78)	<0.001	11	0.34
Prospective trials (5, 9, 16, 24, 29)	526	5	267	259	0.58 (0.36 to 0.93)	0.03	0	0.41
ANASTOMOTIC LEAKAGE								
All included trails (5, 9, 11, 26, 27, 29)	819	6	383	436	0.71 (0.36 to 1.38)	0.31	0	0.96
BMI ≤ 30 (kg/m^2) (5, 11, 26, 27, 29)	793	5	366	427	0.68 (0.34 to 1.34)	0.26	0	0.95
Sample number > 30 (5, 11, 26, 29)	747	4	343	404	0.70 (0.35 to 1.42)	0.33	0	0.92
Non-RCT (NOS ≥ 6) (5, 9, 11, 26, 29)	773	5	360	413	0.73 (0.37 to 1.46)	0.38	0	0.94
Prospective trials (5, 29)	392	2	196	196	0.75 (0.31 to 1.78)	0.51	0	0.60
SURGICAL SITE INFECTION								
All included trails (4, 5, 10, 12, 26, 29, 30)	808	7	369	439	0.15 (0.05 to 0.42)	< 0.001	0	0.99
BMI ≤ 30 (kg/m^2) (4, 5, 12, 26, 29, 30)	768	6	349	419	0.14 (0.04 to 0.42)	< 0.001	0	0.99
Sample number > 30 (5, 12, 26, 29)	650	4	313	337	0.12 (0.03 to 0.45)	0.002	0	1.00
Non-RCT (NOS ≥ 6) (4, 5, 10, 26, 29)	690	5	318	372	0.14 (0.04 to 0.47)	0.001	0	0.99
Prospective trials (5, 12, 29, 30)	510	4	247	263	0.14 (0.04 to 0.53)	0.004	0	0.93
OPERATION TIME								
All included trails (4, 5, 9–12, 16, 26–30)	1,175	12	518	657	17.34 (6.14 to 28.54)	0.002	81	<0.001
BMI ≤ 30 (kg/m^2) (4, 5, 11, 12, 16, 26, 27, 29, 30)	1,079	9	471	608	13.17 (1.86 to 24.47)	0.02	80	<0.001
Sample number > 30 (5, 11, 12, 16, 26, 29)	915	6	412	503	11.08 (0.46 to 21.70)	0.04	72	0.003
Non-RCT (NOS ≥ 6) (4, 5, 9–11, 16, 26, 28, 29)	1,011	9	444	567	14.84 (1.04 to 28.63)	0.03	81	<0.001
Prospective trials (5, 9, 12, 16, 29, 30)	604	6	298	306	17.36 (11.43 to 23.28)	<0.001	0	0.65
HOSPITAL STAY								
All included trails (4, 5, 9–12, 16, 26–30)	1,175	12	518	657	−0.56 (−1.09 to −0.04)	0.03	54	0.01
BMI ≤ 30 (kg/m^2) (4, 5, 11, 12, 16, 26, 27, 29, 30)	1,079	9	471	608	−0.71 (−1.19 to −0.23)	0.004	51	0.04
Sample number > 30 (5, 11, 12, 16, 26, 29)	915	6	412	503	−0.84 (−1.16 to −0.53)	<0.001	43	0.12
Non-RCT (NOS ≥ 6) (4, 5, 9–11, 16, 26, 28, 29)	1,011	9	444	567	−0.88 (−1.21 to −0.56)	<0.001	41	0.09
Prospective trials (5, 9, 12, 16, 29, 30)	604	6	298	306	−0.57 (−1.35 to 0.20)	0.15	62	0.02

NOSE, natural orifice specimen extraction; CL, conventional laparoscopic surgery; OR, odds ratio; WMD, weighted mean difference; 95%CI; 95% confidence interval; BMI, body mass index.

(26, 27, 29). All of which showed no positive surgical margin in the NOSE procedures, and the margin was the recommended distance from the center of the tumor (50). From our pooled data, the proximal margin and distal margin in the NOSE group were not significantly different compared to the CL group. We also conclude from this meta-analysis that 5-year DFS in the two treatment groups was not significantly different. Based on the above findings, we suggest that laparoscopic colorectal surgery with NOSE meets the expectations concerning oncological safety.

Previous studies have reported faster gastrointestinal recovery, less post-operative pain and shorter hospital stay following laparoscopic colorectal surgery with NOSE (51–54). The results of our meta-analysis also suggested that the NOSE group had less post-operative pain, shorter hospital stay and shorter time to first flatus. Possible reasons for these advantages are as follows: Laparotomy incision which traumatizes the abdominal wall, is more likely to cause vessel and nerve injury, and lead to increasing post-operative somatic pain (16). Reduction of pain is constructive for post-operative stress which consists

of inflammatory cascades, which once activated, may have an adverse influence on recovery and hospital stay (55). Several studies have also reported a decrease in post-operative analgesia requirement, which may be beneficial for faster recovery (24, 26, 29). The NOSE technique is conducted totally intraperitoneal; therefore, avoids intraabdominal organs contacting the external environment, and disturbance in the abdominal cavity is slight (56). In addition, patients in the NOSE group had early ambulation which also led to faster gastrointestinal recovery (11, 57). However, the operation time in the CL surgery group was shorter than that in the NOSE group, probably due to the time needed for purse-string suturing and anastomosis of the colorectal stump (10, 16). One study reported a decreasing trend in operation time, indicating the existence of a learning curve in NOSE (4). Therefore, we are convinced that an experienced surgeon may not need more time to complete this procedure. In conclusion, as an incisionless operation, the NOSE technique can aid early post-operative recovery of gastrointestinal function.

However, there were some limitations in our meta-analysis. Firstly, only two RCTs were included in our study, which may influence the power of pooled results. Secondly, differences in surgical proficiency in NOSE technology, T stage and tumor location may lead to heterogeneity of some results. For instance, operation time ranged from ∼105–240 min and hospital stay from about 4.8–12.9 d in the NOSE group. Thirdly, long-term outcomes such as LR and OS are still lacking which could provide further support of oncological safety.

Ma et al. conducted a meta-analysis on NOSE in 2015 (58). The analysis included nine studies and a total of 837 patients, and concluded that laparoscopic colorectal surgery with NOSE can reduce the duration of hospital stay, accelerate post-operative recovery with better cosmetic results, and result in less post-operative pain. However, there are still concerns regarding the surgical and oncological safety of this technique. Therefore, we conducted a meta-analysis of 14 studies including a total of 1,435 patients. Moreover, we analyzed studies only involving malignancies and concluded that the results were consistent with our conclusions. The results of the sensitivity analysis and subgroup analyses also support our conclusions and further provide robust evidence on the reliability of our results. All statistical methods mentioned above add credibility to the pooled results of our meta-analysis. In summary, 5-year DFS, lymph node harvest and surgical margin in the NOSE group were comparable to those in the CL group. Moreover, the NOSE group had similar blood loss and anastomotic leakage to the CL group, and a reduced incidence of SSI and total perioperative complications than the CL group.

In conclusion, laparoscopic colorectal surgery with NOSE can achieve comparable oncological and surgical safety to CL surgery. In addition, the NOSE technique has clear advantages in terms of early recovery of gastrointestinal function. However, large multicenter RCTs are needed in the future to provide high-level, evidence-based results regarding functional outcomes assessing anal or vaginal dysfunction and long-term oncological outcomes to further evaluate the feasibility of the NOSE technique.

DATA AVAILABILITY

Publicly available datasets were analyzed in this study. These data can be found at the following address: doi. http://doi.org/10.6084/m9.figshare.7856828.

AUTHOR CONTRIBUTIONS

R-JL and C-DZ contributed to study design, data extraction, data analysis, and manuscript writing. They also reviewed and revised the paper and approved and submitted the final manuscript. Y-CF reviewed and revised the paper. D-QD wrote, reviewed, and revised the paper and submitted the final manuscript. All authors approved the final manuscript and its submission.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00597/full#supplementary-material

REFERENCES

1. Braga M, Frasson M, Zuliani W, Vignali A, Pecorelli N, Di Carlo V. Randomized clinical trial of laparoscopic vs. open left colonic resection. *Br J Surg*. (2010) 97:1180–6. doi: 10.1002/bjs.7094
2. Lacy AM, García-Valdecasas JC, Delgado S, Castells A, Taurá P, Piqué JM, et al. Laparoscopy-assisted colectomy vs. open colectomy for treatment of non-metastatic colon cancer: a randomised trial. *Lancet*. (2002) 359:2224–9. doi: 10.1016/S0140-6736(02)09290-5
3. Wang CL, Qu G, Xu HW. The short- and long-term outcomes of laparoscopic vs. open surgery for colorectal cancer: a meta-analysis. *Int J Colorectal Dis*. (2014) 29:309–20. doi: 10.1007/s00384-013-1827-1
4. Hisada M, Katsumata K, Ishizaki T, Enomoto M, Matsudo T, Kasuya K, et al. Complete laparoscopic resection of the rectum using natural orifice specimen extraction. *World J Gastroenterol*. (2014) 20:16707–13. doi: 10.3748/wjg.v20.i44.16707
5. Park JS, Kang H, Park SY, Kim HJ, Lee IT, Choi GS. Long-term outcomes after Natural Orifice Specimen Extraction vs. conventional laparoscopy-assisted surgery for rectal cancer: a matched case-control study. *Ann Surg Treat Res*. (2018) 94:26–35. doi: 10.4174/astr.2018.94.1.26
6. Romy S, Eisenring MC, Bettschart V, Petignat C, Francioli P, Troillet N. Laparoscope use and surgical site infections in digestive surgery. *Ann Surg*. (2008) 247:627–32. doi: 10.1097/SLA.0b013e3181638609

7. Singh R, Omiccioli A, Hegge S, McKinley C. Does the extraction-site location in laparoscopic colorectal surgery have an impact on incisional hernia rates? *Surg Endosc*. (2008) 22:2596–600. doi: 10.1007/s00464-008-9845-8
8. Winslow ER, Fleshman JW, Birnbaum EH, Brunt LM. Wound complications of laparoscopic vs. open colectomy. *Surg Endosc*. (2002) 16:1420–5. doi: 10.1007/s00464-002-8837-3
9. Costantino FA, Diana M, Wall J, Leroy J, Mutter D, Marescaux J. Prospective evaluation of peritoneal fluid contamination following transabdominal vs. *transanal specimen extraction in laparoscopic left-sided colorectal resections. Surg Endosc*. (2012) 26:1495–500. doi: 10.1007/s00464-011-2066-6
10. Awad ZT, Griffin R. Laparoscopic right hemicolectomy: a comparison of natural orifice vs. transabdominal specimen extraction. *Surg Endosc*. (2014) 28:2871–6. doi: 10.1007/s00464-014-3540-8
11. Xingmao Z, Haitao Z, Jianwei L, Huirong H, Junjie H, Zhixiang Z. Totally laparoscopic resection with natural orifice specimen extraction (NOSE) has more advantages comparing with laparoscopic-assisted resection for selected patients with sigmoid colon or rectal cancer. *Int J Colorectal Dis*. (2014) 29:1119–24. doi: 10.1007/s00384-014-1950-7
12. Leung AL, Cheung HY, Fok BK, Chung CC, Li MK, Tang CN. Prospective randomized trial of hybrid NOTES colectomy vs. conventional laparoscopic colectomy for left-sided colonic tumors. *World J Surg*. (2013) 37:2678–82. doi: 10.1007/s00268-013-2163-x
13. Wolthuis AM, de Buck van Overstraeten A, D'Hoore A. Laparoscopic natural orifice specimen extraction-colectomy: a systematic review. *World J Gastroenterol*. (2014) 20:12981–92. doi: 10.3748/wjg.v20.i36.12981
14. Chen C, Chen H, Yang M, Wu X, Yuan X, Zhu C, et al. Laparoscopy-assisted natural orifice specimen extraction to treat tumors of the sigmoid colon and rectum: the short- and long-term outcomes of a retrospective study. *J Laparoendosc Adv Surg Tech A*. (2019) 29:801–8. doi: 10.1089/lap.2018.0601
15. Guan X, Liu Z, Longo A, Cai JC, Chen LC, International Alliance of NOSES. International consensus on natural orifice specimen extraction surgery (NOSES) for colorectal cancer. *Gastroenterol Rep*. (2019) 7:24–31. doi: 10.1093/gastro/goy055
16. Park JS, Choi GS, Kim HJ, Park SY, Jun SH. Natural orifice specimen extraction vs. conventional laparoscopically assisted right hemicolectomy. *Br J Surg*. (2011) 98:710–5. doi: 10.1002/bjs.7419
17. Moher D, Liberati A, Tetzlaff J, Altman DG, Prisma Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol*. (2009) 62:1006–12. doi: 10.1016/j.jclinepi.2009.06.005
18. Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol*. (2010) 25:603–5. doi: 10.1007/s10654-010-9491-z
19. Higgins JP, Altman DG, Gøtzsche PC, Jüni P, Moher D, Oxman AD, et al. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ*. (2011) 343:d5928. doi: 10.1136/bmj.d5928
20. Tierney JF, Stewart LA, Ghersi D, Burdett S, Sydes MR. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials*. 8:16. doi: 10.1186/1745-6215-8-16
21. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557
22. Petitti DB. Approaches to heterogeneity in meta-analysis. *Stat Med*. (2001) 20:3625–33. doi: 10.1002/sim.1091
23. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. (1994) 50:1088–101. doi: 10.2307/2533446
24. Wolthuis AM, Fieuws S, Van Den Bosch A, de Buck van Overstraeten A, D'Hoore A. Randomized clinical trial of laparoscopic colectomy with or without natural-orifice specimen extraction. *Br J Surg*. (2014) 102:630–7. doi: 10.1002/bjs.9757
25. Denost Q, Adam JP, Pontallier A, Celerier B, Laurent C, Rullier E. Laparoscopic total mesorectal excision with coloanal anastomosis for rectal cancer. *Ann Surg*. (2015) 261:138–43. doi: 10.1097/SLA.0000000000000855
26. Saurabh B, Chang SC, Ke TW, Huang YC, Kato T, Wang HM, et al. Natural orifice specimen extraction with single stapling colorectal anastomosis for laparoscopic anterior resection: feasibility, outcomes, and technical considerations. *Dis Colon Rectum*. (2017) 60:43–50. doi: 10.1097/DCR.0000000000000739
27. Xu SZ, Ding ZJ, Zhang SF, Qiu XF, Yan F, Yuan SS, et al. Propensity score matching-based analysis of short-term outcomes of Laparoscopic-assisted Natural Orifice Specimen Extraction for left colorectal cancer radical resection. *Zhonghua Yi Xue Za Zhi*. (2016) 96:1578–81. doi: 10.3760/cma.j.issn.0376-2491.2016.20.007
28. Christoforidis D, Clerc D, Demartines N. Transrectal specimen extraction after laparoscopic left colectomy: a case-matched study. *Colorectal Dis*. (2012) 15:347–53. doi: 10.1111/codi.12006
29. Kim HJ, Choi GS, Park JS, Park SY, Ryuk JP, Yoon SH. Transvaginal specimen extraction vs. conventional minilaparotomy after laparoscopic anterior resection for colorectal cancer: mid-term results of a case-matched study. *Surg Endosc*. (2014) 28:2342–8. doi: 10.1007/s00464-014-3466-1
30. Xing J, Zhang C, Yang X, Wang H, Wang H, Yu E, et al. [Comparison of short-term outcomes of transrectal specimen extraction during laparoscopic sigmoid radical resection vs. conventional laparoscopically assisted procedure]. *Zhonghua Wei Chang Wai Ke Za Zhi*. (2017) 20:665–70. doi: 10.3760/cma.j.issn.1671-0274.2017.06.015
31. Frye J, Bokey EL, Chapuis PH, Sinclair G, Dent OF. Anastomotic leakage after resection of colorectal cancer generates prodigious use of hospital resources. *Colorectal Dis*. (2009) 11:917–20. doi: 10.1111/j.1463-1318.2008.01728.x
32. Boccola MA, Lin J, Rozen WM, Ho YH. Reducing anastomotic leakage in oncologic colorectal surgery: an evidence-based review. *Anticancer Res*. (2010) 30:601–7.
33. Frasson M, Granero-Castro P, Ramos Rodriguez JL, Flor-Lorente B, Braithwaite M, Marti Martinez E, et al. Risk factors for anastomotic leak and postoperative morbidity and mortality after elective right colectomy for cancer: results from a prospective, multicentric study of 1102 patients. *Int J Colorectal Dis*. (2016) 31:105–14. doi: 10.1007/s00384-015-2376-6
34. Nikolian VC, Kamdar NS, Regenbogen SE, Morris AM, Byrn JC, Suwanabol PA, et al. Anastomotic leak after colorectal resection: a population-based study of risk factors and hospital variation. *Surgery*. (2017) 161:1619–27. doi: 10.1016/j.surg.2016.12.033
35. Bucher P, Wutrich P, Pugin F, Gonzales M, Gervaz P, Morel P. Totally intracorporeal laparoscopic colorectal anastomosis using circular stapler. *Surg Endosc*. (2008) 22:1278–82. doi: 10.1007/s00464-007-9607-z
36. Guan X, Wang GY, Zhou ZQ, Zhou HT, Chen YG. Retrospective study of 718 colorectal neoplasms treated by natural orifice specimen extraction surgery in 79 hospitals. *Chin J Colorec Dis*. (2017) 6:469–77. doi: 10.3877/cma.j.issn.2095-3224.2017.06.006
37. Carlini M, Spoletini D, Castaldi F, Giovannini C, Passaro U. Laparoscopic resection of splenic flexure tumors. *Updates Surg*. (2016) 68:77–83. doi: 10.1007/s13304-016-0357-0
38. Milone M, Angelini P, Berardi G, Burati M, Corcione F, Delrio P. Intracorporeal vs. extracorporeal anastomosis after laparoscopic left colectomy for splenic flexure cancer: results from a multi-institutional audit on 181 consecutive patients. *Surg Endosc*. (2018) 32:3467–73. doi: 10.1007/s00464-018-6065-8
39. Swaid F, Sroka G, Madi H, Shteinberg D, Somri M, Matter I. Totally laparoscopic vs. laparoscopic-assisted left colectomy for cancer: a retrospective review. *Surg Endosc*. (2016) 30:2481–8. doi: 10.1007/s00464-015-4502-5
40. Wang X. [Prospects and challenges of natural orifice specimen extraction surgery, natural orifice transluminal endoscopic surgery and transanal total mesorectal excision]. *Zhonghua Wei Chang Wai Ke Za Zhi*. (2018) 21:856–61. doi: 10.3760/cma.j.issn.1671-0274.2018.08.003
41. Han FH, Hua LX, Zhao Z, Wu JH, Zhan WH. Transanal natural orifice specimen extraction for laparoscopic anterior resection in rectal cancer. *World J Gastroenterol*. (2013) 19:7751–7. doi: 10.3748/wjg.v19.i43.7751
42. Ishihara S, Nishikawa T, Tanaka T, Tanaka J, Kiyomatsu T, Kawai K, et al. Prognostic impact of tumor location in stage IV colon cancer: a propensity score analysis in a multicenter study. *Int J Surg*. (2014) 12:925–30. doi: 10.1016/j.ijsu.2014.07.270
43. Iversen H, Martling A, Johansson H, Nilsson PJ, Holm T. Pelvic local recurrence from colorectal cancer: surgical challenge with changing preconditions. *Colorectal Dis*. (2018) 20:399–406. doi: 10.1111/codi.13966
44. Le Voyer TE, Sigurdson ER, Hanlon AL, Mayer RJ, Macdonald JS, Catalano PJ, et al. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. *J Clin Oncol*. (2003) 21:2912–9. doi: 10.1200/JCO.2003.05.062
45. Storli KE, Sondenaa K, Bukholm IR, Nesvik I, Bru T, Furnes B, et al. Overall survival after resection for colon cancer in a national cohort study was adversely affected by TNM stage, lymph node ratio, gender, and old age. *Int J Colorectal Dis*. (2011) 26:1299–307. doi: 10.1007/s00384-011-1244-2

46. Sudo M, Furuya S, Shimizu H, Nakata Y, Iino H, Shiraishi K, et al. Long-term outcomes after surgical resection in patients with stage IV colorectal cancer: a retrospective study of 129 patients at a single institution. *World J Surg Oncol*. (2019) 17:56. doi: 10.1186/s12957-019-1599-3
47. Swanson RS, Compton CC, Stewart AK, Bland KI. The prognosis of T3N0 colon cancer Is dependent on the number of lymph nodes examined. *Ann Surg Oncol*. (2003) 10:65–71. doi: 10.1245/ASO.2003.03.058
48. Ngu J, Wong AS. Transanal natural orifice specimen extraction in colorectal surgery: bacteriological and oncological concerns. *ANZ J Surg*. (2016) 86:299–302. doi: 10.1111/ans.13383
49. Birbeck KF, Macklin CP, Tiffin NJ Parsons W, Dixon MF, Mapstone NP, Abbott CR, et al. Rates of circumferential resection margin involvement vary between surgeons and predict outcomes in rectal cancer surgery. *Ann Surg*. (2002) 235:449–57. doi: 10.1097/00000658-200204000-00001
50. Morlote DM, Alexis JB. Is the routine microscopic examination of proximal and distal resection margins in colorectal cancer surgery justified? *Ann Diagn Pathol*. (2016) 23:35–7. doi: 10.1016/j.anndiagpath.2016.05.001
51. Flora ED, Wilson TG, Martin IJ, O'Rourke NA, Maddern GJ. A review of natural orifice translumenal endoscopic surgery (NOTES) for intra-abdominal surgery: experimental models, techniques, and applicability to the clinical setting. *Ann Surg*. (2008) 247:583–602. doi: 10.1097/SLA.0b013e3181656ce9
52. Weibel S, Jokinen J, Pace NL, Schnabel A, Hollmann MW, Hahnenkamp K, et al. Efficacy and safety of intravenous lidocaine for postoperative analgesia and recovery after surgery: a systematic review with trial sequential analysis. *Br J Anaesth*. (2016) 116:770–83. doi: 10.1093/bja/aew101
53. Willingham FF, Brugge WR. Taking NOTES: translumenal flexible endoscopy and endoscopic surgery. *Curr Opin Gastroenterol*. (2007) 23:550–5. doi: 10.1097/MOG.0b013e32828621b3
54. Wolthuis AM, Meuleman C, Tomassetti C, D'Hooghe T, Fieuws S, Penninckx F, et al. Laparoscopic sigmoid resection with transrectal specimen extraction: a novel technique for the treatment of bowel endometriosis. *Hum Reprod*. (2011) 26:1348–55. doi: 10.1093/humrep/der072
55. Leung KL, Lai PB, Ho RL, Meng WC, Yiu RY, Lee JF, et al. Systemic cytokine response after laparoscopic-assisted resection of rectosigmoid carcinoma: a prospective randomized trial. *Ann Surg*. (2000) 231:506–11. doi: 10.1097/00000658-200004000-00008
56. Akiyoshi T, Kuroyanagi H, Oya M, Konishi T, Fukuda M, Fujimoto Y, et al. Short-term outcomes of laparoscopic rectal surgery for primary rectal cancer in elderly patients: is it safe and beneficial? *J Gastrointest Surg*. (2009) 13:1614–8. doi: 10.1007/s11605-009-0961-0
57. Nishimura A, Kawahara M, Suda K, Makino S, Kawachi Y, Nikkuni K. Totally laparoscopic sigmoid colectomy with transanal specimen extraction. *Surg Endosc*. (2011) 25:3459–63. doi: 10.1007/s00464-011-1716-z
58. Ma B, Huang XZ, Gao P, Zhao JH, Song YX, Sun JX, et al. Laparoscopic resection with natural orifice specimen extraction vs. conventional laparoscopy for colorectal disease: a meta-analysis. *Int J Colorectal Dis*. (2015) 30:1479–88. doi: 10.1007/s00384-015-2337-0

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

日中笹川医学奨学金制度（学位取得コース）評価書
論文博士：指導教官用

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　研究者番号：G4006　作成日：2020 年 3 月 日

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研究テーマ	家族性片麻痺性片頭痛 2 型モデルマウスを用いた片頭痛病態の解明				
専攻種別	☑ 論文博士		☐ 課程博士		

研究者評価（指導教官記入欄）

成績状況	優 良 可 不可	取得単位数 / 取得単位数／取得すべき単位総数
学生本人が行った研究の概要	片頭痛の病態解明を目的とし、その前兆と関連が深いと考えられている大脳皮質性拡延性抑制（CSD）に着目した研究を展開している。唐君は、家族性片麻痺性片頭痛 2 型モデルマウスは野生型マウスに比して、CSD 誘発の感受性が高い一方で、同回復が遅いことを立証した。さらに CSD による神経活動亢進は脳の広い範囲で認められ、当該モデルマウスでは特に扁桃体でその傾向を認めることを確認した。また、ヒト片頭痛の前兆時に認められる光過敏行動を CSD 誘発マウスにおいて評価する実験系の確立にも成功し、既存の片頭痛治療薬であるスマトリプタンや新規治療薬候補（抗 CGRP 薬）であるオルセゲパントが当該事象を緩和することをマウスにおいても実証した。これら結果は片頭痛の病態解明の一助となることが期待できる。	
総合評価	【良かった点】 マウスを用いた実験手技（手術、免疫組織化学、行動実験等）を確実に習得し、自立した実験を遂行することが可能になった。多くのプロセスが必要な実験においても地道に取り組み、自ら創意工夫し効率的な進め方が実践できる状態にある。関連する多くの学術論文より自学自習し、自身の研究活動に反映するほか、指導者や共同研究者と質の高い討議も可能である。高い協調性もあり、研究室のスタッフより厚い信頼を獲得している。	
	【改善すべき点】 特記事項なし。	
	【今後の展望】 学術論文を纏めるために必要な実験は終了しており、専門誌への投稿中である。今後、査読者より指摘された事項に関する追加実験や反駁を行う。時間的余裕があれば、唐君の将来の研究活動の一助となるべく、当研究室で遂行中の実験的脳虚血モデルの作成や二光子顕微鏡を用いた組織の撮像などの実験手技習得に時間を割きたいと考えている。	
学位取得見込	家族性片麻痺性片頭痛 2 型モデルマウスにおける CSD 感受性に関する研究については、2019 年 11 月に専門学術誌へ投稿を済ませ、査読中である。光過敏行動に対する片頭痛薬剤の効果に関する研究についても近日中に専門学術誌への投稿を予定している。これら論文が受理され、その他諸規定をクリアすれば、学位論文として受理され、論文博士の受審が可能になると考える。	

評価者（指導教官名）中原 仁 ㊞

日中笹川医学奨学金制度（学位取得コース）報告書
（研究者用）

Supported by 日本財団 THE NIPPON FOUNDATION

第 40 期　　研究者番号：G4006　　作成日：2020 年 03 月 01 日

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研究先（指導教官）	慶應義塾大学　医学部　内科学教室（神経内科）　（中原　仁　教授　）				
研究テーマ	1. Characteristics of cortical spreading depression and c-Fos expression in a mouse model having the same mutation as the familial hemiplegic migraine 2 2. Cortical spreading depolarization-induced photophobic behavior and hypomotility can be reversed by sumatriptan and olcegepant.				
専攻種別	論文博士 ☑		課程博士 ☐		

実験一：1.研究概要

1）Purpose(目的)

To clarify the pathogenesis of FHM2 by comparing the characteristics of cortical spreading depression (CSD) and the expression of c-Fos in the brain after CSD in adult mice.

2）Approach (戦略)

CSD is thought to be the underlying mechanism of migraine aura. Three relatives defined as familial hemiplegic migraine 2 (FHM2) characterized by complicated forms of aura had the point mutation E700K in ATP1A2 exon 15[1].

3）Materials and methods (材料と方法)

CSD was induced by applying stepwise increases of KCl concentration to C57BL/6J-Tg(Atp1a2*E700K)9151Kwk mice (Tg, both sexes) and corresponding wild-type animals (WT). Under urethane anesthesia, the responsiveness and threshold to CSD were examined[2], and the distribution of expression of c-Fos, a neuronal activity marker, was immunohistochemically determined.

4）Results (実験結果)

Overall, Tg mice showed both faster propagation velocity and longer full-width-at-half-maximum than WT, representing a slower recovery from DC deflection. The CSD threshold tended to be lower in Tg, especially in females. c-Fos-positive cells were markedly enhanced in the ipsilateral somatosensory cortex, piriform cortex, and amygdala, and weakly enhanced in striatum. Numbers of c-Fos positive cells were greater in the ipsilateral amygdala of Tg, as compared with WT.

5）Discussion (考察)

The effect of CSD may be greater in E700K transgenic mice than that in WT, while threshold for CSD shows little change, but the. Higher c-Fos expression in the amygdala may indicate alterations of the limbic system in Tg, suggesting an enhanced linkage between CSD and amygdala connectivity in FHM2 patients.

6）Reference(参考文献)

[1] Pierelli F, Grieco GS, Pauri F, et al. A novel ATP1A2 mutation in a family with FHM type II. Cephalalgia. 2006,26(3):324-328.

[2] Unekawa M, Ikeda K, Tomita Y, et al. Enhanced susceptibility to cortical spreading

depression in two types of Na+,K+-ATPase α2 subunit-deficient mice as a model of familial hemiplegic migraine 2. Cephalalgia. 2018, 38(9):1515-1524

実験二：2.研究概要

1）Purpose(目的)

To elucidate whether cortical spreading depolarization(CSD) induction caused photophobic behavior in mice and whether this phenomenon can be reversed by sumatriptan (SUM; 5-HT1B/1D agonist) or olcegepant (OLC; calcitonin generelated peptide receptor antagonist).

2）Approach (戦略)

CSD is regarded as the neural mechanism underlying migraine aura [1,2]. Photophobia is not only a most bothersome accompanying symptom during migraine attacks but also a persistent complaint in the interictal period in some patients, which leads to significantly lowered quality of life. Photophobia is known to be more prevalent in patients with migraine with aura than in those with migraine without aura [3].

3）Materials and methods (材料と方法)

Adult male C57BL/6 mice were divided into 5 experimental groups (Sham-Vehicle, CSD-Vehicle, CSD-SUM 0.6 mg/kg, CSD-OLC 0.25 mg/kg, and CSD-OLC 1.0 mg/kg; each N=8). Five times of CSD were elicited by the application of KCl over the occipital cortical surface. Locomotive behavior was monitored for 30min in the free-moving light/dark chamber at 24 h before and 24 h after CSD or sham operation. The drugs and vehicle were intraperitoneally injected just before the last behavior test.

4）Results (実験結果)

The mice subjected to CSD spent significantly less time in the light compartment than sham-operated mice at 24 h after surgical procedures (121 ± 39 sec vs. 412 ± 103 sec, P=0.0021), indicating development of photophobia. Locomotive activity significantly reduced in CSD-affected mice than in sham-operated mice in both zones. Both SUM and OLC prolonged the time spent in light and reversed CSD-induced reductions in ambulatory time and distance.

5）Discussion (考察)

CSD is causative of photophobia, which can be alleviated by sumatriptan and olcegepant. Further, these agents are capable of improving locomotive activity irrespective of the presence of light stimulation.

6）Reference(参考文献)

[1] Leao AAP. Spreading depression of activity in the cerebral cortex. J Neurophysiol. 1944;7:359-90.

[2] Ayata C. Cortical spreading depression triggers migraine attack: pro. Headache. 2010; 50(4):725-30.

[3] Noseda R, Copenhagen D, Burstein R. Current understanding of photophobia, visual networks and headaches. Cephalalgia. 2018:333102418784750.

2. 執筆論文 Publication of thesis ※記載した論文を添付してください。Attach all of the papers listed below.

論文名 1 Title	Characteristics of cortical spreading depression and c-Fos expression in a mouse model having the same mutation as the familial hemiplegic migraine 2（投稿）					
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～	頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 2 Title	Cortical spreading depolarization-induced photophobic behavior and hypomotility can be reversed by sumatriptan and olcegepant（投稿）					
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～	頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 3 Title	Transient receptor potential cation channels and migraine disease mechanisms（投稿）					
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～	頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 4 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～	頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						
論文名 5 Title						
掲載誌名 Published journal						
	年 月	巻(号)	頁 ～	頁	言語 Language	
第1著者名 First author		第2著者名 Second author		第3著者名 Third author		
その他著者名 Other authors						

3. 学会発表 Conference presentation ※筆頭演者として総会・国際学会を含む主な学会で発表したものを記載してください。

※Describe your presentation as the principal presenter in major academic meetings including general meetings or international meetings

学会名 Conference	第61回日本脳循環代謝学会学術集会				
演 題 Topic	Property of behavior after cortical spreading depression in Na⁺,K ⁺-ATPase α2 subunit defective mice				
開催日 date	2018 年 10 月 19 日	開催地 venue	盛岡		
形式 method	☐ 口頭発表 Oral ☑ ポスター発表 Poster	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共 同演者名 Co-presenter					
学会名 Conference	International Conference on Spreading Depolarizations				
演 題 Topic	Responsiveness to potassium-induced cortical spreading depression and subsequent c-Fos expression in a mouse model of familial hemiplegic migraine 2				
開催日 date	2019 年 7 月 1 日	開催地 venue	横浜		
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter					
学会名 Conference	BRAIN & BRAIN PET				
演 題 Topic	Effects of spreading depolarization on development of cerebral infarction after middle cerebral artery occlusion in Na+,K+-ATPase α2 subunit-deficient mice				
開催日 date	2019 年 7 月 6 日	開催地 venue	横浜		
形式 method	☐ 口頭発表 Oral ☑ ポスター発表 Poster	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter					
学会名 Conference	第62回日本脳循環代謝学会学術集会				
演 題 Topic	Effects of K^+-induced cortical spreading depression in a mouse model of familial hemiplegic migraine 2				
開催日 date	2019 年 11 月 29 日	開催地 venue	仙台		
形式 method	☑ 口頭発表 Oral ☐ ポスター発表 Poster	言語 Language	☐ 日本語	☑ 英語	☐ 中国語
共同演者名 Co-presenter					

4. 受賞（研究業績） Award (Research achievement)

名 称 Award name				
	国名 Country		受賞年 Year of award	年 月
名 称 Award name				
	国名 Country		受賞年 Year of award	年 月

5. 本研究テーマに関わる他の研究助成金受給 Other research grants concerned with your resarch theme

受給実績 Receipt record	□ 有 □ 無
助成機関名称 Funding agency	
~~助成金名称~~ Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円
受給実績 Receipt record	□ 有 □ 無
~~助成機関名称~~ Funding agency	
助成金名称 Grant name	
受給期間 Supported period	年 月 ～ 年 月
受給額 Amount received	円

6. 他の奨学金受給 Another awarded scholarship

受給実績 Receipt record	■ 有 □ 無
助成機関名称 Funding agency	china scholarship council
~~奨学金名称~~ Scholarship name	National scholarship for studying abroad
受給期間 Supported period	2019 年 9 月 ～ 2020 年 9 月
受給額 Amount received	204万 円

7. 研究活動に関する報道発表 Press release concerned with your research activities

※記載した記事を添付してください。Attach a copy of the article described below

報道発表 Press release	□ 有 □ 無	発表年月日 Date of release	
発表機関 Released medium			
発表形式 Release method	・新聞 ・雑誌 ・Web site ・記者発表 ・その他（ ）		
発表タイトル Released title			

8. 本研究テーマに関する特許出願予定 Patent application concerned with your research theme

出願予定 Scheduled	□ 有 □ 無	出願国 Application	
出願内容（概要） Application contents			

9. その他 Others

指導責任者（署名） 中原 仁 ㊞