Research Article

Prognosis and Efficacy of Laparoscopic Surgery on Patients with Endometrial Carcinoma: Systematic Evaluation and Meta-Analysis

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Objective. The prognosis and efficacy of laparoscopic surgery (LPS) and open surgery or robotic surgery (RS) on endometrial carcinoma (EC) patients were compared.

Methods. Data as of May 2021 were retrieved from databases like PubMed, Embase, Cochrane Library, and Web of Science. The study involved randomized controlled trials (RCTs), cohort studies, or case-control studies for comparing the effects of LPS and open surgery or robotic surgery (RS) on EC treatment. The primary outcomes included duration of operation, blood loss, length of stay (LOS), postoperative complications, and recurrence rate. Secondary outcomes included 3-year progression-free survival (PFS) rate/disease-free survival (DFS) rate and 3-year overall survival (OS) rate.

Results. A total of 24 studies were involved, and all of them were cohort studies except 1 RCT and 1 case-control study. There was no significant difference in duration of operation between LPS and open surgery (MD = -0.06, 95% CI: -0.37 to 0.25) or RS (MD = -0.15, 95% CI: -1.27 to 0.96). In comparison with the open surgery, LPS remarkably reduced blood loss (MD = -0.43, 95% CI: -0.58 to -0.29), LOS (MD = -0.71, 95% CI: -0.92 to -0.50), and the complication occurrence rate (RR = 0.83, 95% CI: 0.73 to 0.95). However, LPS and RS saw no difference in blood loss (MD = 0.01, 95% CI: -0.77 to 0.79). Besides, in comparison with RS, LPS prominently shortened the LOS (MD = 0.26, 95% CI: 0.12 to 0.40) but increased the complication occurrence rate (RR = 1.74, 95% CI: 1.57 to 1.92). In contrast to open surgery or RS, LPS saw no difference in occurrence rate (RR = 0.75, 95% CI: 0.56 to 1.01; RR = 0.97, 95% CI: 0.62 to 1.53), 3-year PFS/DFS (RR = 0.99, 95% CI: 0.90 to 1.09; RR = 1.30, 95% CI: 0.87 to 1.96), and 3-year OS (RR = 0.97, 95% CI: 0.91 to 1.04; RR = 1.21, 95% CI: 0.91 to 1.60). Conclusion. In sum, LPS was better than open surgery, which manifested in the aspects of less blood loss, shorter LOS, and fewer complications. LPS, therefore, was the most suitable option for EC patients. Nevertheless, LPS had no advantage over RS, and sufficient prospective RCTs are needed to further confirm its strengths.

1. Introduction

Endometrial carcinoma (EC) is the most commonly diagnosed gynecologic malignant tumor, especially in some developed countries [1], whose 5-year survival rate was 34.7% (445805 cases) [2]. The risk factors of EC include early menarche, delay menopause, diabetes, polycystic ovarian syndrome (PCOS), metabolic syndrome, current treatments with tamoxifen, and obesity [3–5]. With the increase of risk factors such as aging of population and obesity, the morbidity of EC will continue to rise. Most EC patients who are diagnosed in the early stages (Federation Internationale de Gynecologie et Obstetrique (FIGO) stage I or II) have better prognoses [6].

Surgery remains the major treatment for early EC, which mainly include vaginal surgery, laparotomy (LT), or open surgery, laparoscopic surgery (LPS), and robotic surgery (RS). Clinical practice guidelines and multiple clinical trials indicate that minimally invasive surgery (MIS) is recommended as the preferred surgical approach for EC patients [7, 8]. In the past, LT has always been the first choice for early EC patients. Since the first report of LPS on EC in 1993 [9], LPS, as a MIS, has become increasingly popular in the treatment of EC [10, 11]. In contrast to open
surgery, LPS is characterized by less blood loss, less rena-
scent adhesion and lower morbidity [12]. RS is a new MIS
developed on the basis of LPS. Previous studies show that
RS for EC treatment results in a shorter length of stay
(LOS), less blood loss, lower conversion rate of open surgery,
and lower occurrence rate of intraoperative damage to sur-
rounding organs, compared to LPS [13, 14]. However, in
contrast to LPS, RS prolongs operation and recovery time
[15, 16]. Recently, a multicenter retrospective study com-
pared the therapeutic efficacy of LPS and radical abdominal
hysterectomy on early EC patients. The result indicated a
decrease in the disease-free survival (DFS) of patients
who underwent LPS [17]. However, a prospective study
validated that LPS could dramatically improve the short-
and long-term quality of life (QOL) of EC patients [18].
Hence, this study systematically evaluated the prognosis
and efficacy of LPS.

Consequently, we conducted a meta-analysis to elucidate
the prognosis and effect of LPS on EC by comparison with
open surgery or RS from the perspective of perioperative
results, postoperative complications, recurrence rate, and
survival time. This effort will bring insight into the surgical
treatment of EC patients.

2. Methods

2.1. Literature Retrieval. The study was performed in adher-
ence to the Preferred Reporting Items for Systematic reviews
and Meta-Analyses (PRISMA) statement [19]. All relevant
literature included in the databases like PubMed, Embase,
Cochrane Library, and Web of Science were retrieved from
the construction of the databases to May 2021. Keywords used
for searching included “endometrial carcinoma,” “hyste-
rotoscopic surgery,” “minimally invasive surgery,” “laparoscopic
surgery,” “robotic surgery,” and “open surgery.” The detailed
strategy of literature retrieval was as follows: ((((Endometrial
Neoplasm“[MeSH Terms]) OR (Endometrial Carcinoma“
[MeSH Terms])) OR (Endometrial Cancer“[MeSH Terms])
AND (((Laparoscopy[Title/Abstract]) OR (HysteroScope[Ti-
tle/Abstract])) OR (Minimally invasive[Title/Abstract]))
OR (Open[Title/Abstract]) OR (robotic[Title/Abstract]))
AND ((operation[Title/Abstract]) OR (surgery[Title/Abstract])).

2.2. Selection of Studies. Inclusion criteria of the literature
were as follows: (1) patients diagnosed with EC; (2) compar-
ison between effects of LPS and open surgery or RS on EC
treatment; (3) at least one of the results such as duration of
operation, blood loss, LOS, postoperative complications,
recurrence rate, 3-year progression-free survival (PFS) rate/
DFS rate, and 3-year overall survival (OS) rate was reported;
and (4) study was designed as RCT, cohort study, or case-
control study. The following were the exclusion criteria of
the literature: (1) repeated publication, case series, case
report, comments, meeting abstract, review, editorial, letter,
and so on; (2) data were not sufficient to obtain the result
of our study; (3) article replications; and (4) studies lack of
efficacy-related data.

2.3. Data Extraction and Quality Assessment. The informa-
tion obtained from the literature included the information
of the authors, publication year, country, study design, the
year the samples were collected, the number of samples,
and intervening measures. Data of the patients included
age, body mass index (BMI), FIGO stage, pathological
grading, and outcome indicator. Primary outcomes
included duration of operation, blood loss, LOS, and post-
operative complications. Secondary outcomes involved
postoperative recurrence rate, 3-year PFS rate/DFS rate,
and 3-year OS rate.

Cochrane risk of bias assessment tool was employed to
assess the quality of RCT which was graded as “low risk,”
“high risk,” and “uncertain risk.” Besides, the Newcastle-
Ottawa Scale (NOS) was utilized to evaluate the risk of pub-
lication bias in observational studies. Aggregate points of
NOS were 9, and literature with the points greater than or
equal to 6 was considered of good quality.

The search and selection of articles and the extraction
and quality evaluation of data were independently finished
by two investigators. Disputes were solved by the third
investigator through consultation.

2.4. Statistical Analysis. Meta-analysis was performed using
the Stata 16.0 software. Continuous data were expressed as
mean ± standard deviation (SD). Mean difference (MD)
was measured via continuous results, and 95% confidence
intervals (CIs) were used to assess the concrete therapeutic
effect. If CI included 0, it denoted no statistical difference
between two groups. Besides, two-category data were
merged and analyzed utilizing relative risk (RR) and their
95% CI. If CI included 1, it indicated no statistical difference
between two groups. I^2 statistic was applied to assess
the statistical heterogeneity of studies involved. A random
effect model was used if p < 0.1 or I^2 > 50%, suggesting
a remarkable heterogeneity. Otherwise, a fixed effect model
was used.

3. Results

3.1. Screening and Selection of Reports. A total of 1666
reports were retrieved based on the established searching
strategies, among which 224 reports were excluded. Further,
1400 reports were excluded by scanning their title and
abstract. After reading the full text of the remaining 42
reports, 7 reports of them reported unrelated data while 11
of them lacked sufficient data for obtaining the result of
our study. Finally, 24 reports were selected for our study
[13, 20–42]. The procedures of report screening are shown
in Figure 1.

3.2. Characteristics of the Studies and Quality Evaluation. A
total of 24 reports were involved in the study, in which 6814
patients underwent LPS and 5315 patients underwent open
surgery. Besides, 6121 patients underwent RS. Except for 1
RCT and 1 case-control study, the other reports were cohort
studies. All characteristics of reports involved and the results
of quality assessment are displayed in Table 1. Papers with
the points greater than or equal to 6 were of high quality.
3.3. Results of Meta-Analysis

3.3.1. Duration of Operation and Blood Loss. In respect of the operation time, 9 studies compared that in LPS and open surgery ($I^2 = 95.2\%$), and 2 studies compared that in LPS and RS ($I^2 = 98.0\%$). Due to huge heterogeneity, a random effect model was introduced for analysis. The outcome of meta-analysis suggested that there was no significant difference in duration of operation between LPS and open surgery ($MD = -0.06, 95\% CI: -0.37 to 0.25$) or RS ($MD = -0.15, 95\% CI: -1.27 to 0.96$) (Figures 2(a) and 2(b)).

In respect of blood loss, 6 studies compared that in LPS and open surgery ($I^2 = 47.1\%$), and 2 studies compared that in LPS and RS ($I^2 = 96.0\%$). Based on the results of heterogeneity analysis, a fixed effect model and a random effect model were employed, respectively. The result of meta-analysis demonstrated that the blood loss of LPS was dramatically decreased in contrast to open surgery ($MD = -0.43, 95\% CI: -0.58 to -0.29$), but it had no remarkable difference when compared with that of RS ($MD = 0.01, 95\% CI: -0.77 to 0.79$) (Figures 3(a) and 3(b)).

3.3.2. Postoperative LOS, Complications, and Recurrence Rate. In respect of LOS, 5 studies compared that in LPS and open surgery ($I^2 = 72.1\%$), and 2 studies compared that in LPS and RS ($I^2 = 0.0\%$). Based on the results of heterogeneity analysis, a random effect model and a fixed effect model were employed, respectively. It was exhibited in meta-analysis that the LOS of LPS was shorter than that of open surgery ($MD = -0.71, 95\% CI: -0.92 to -0.50$) (Figure 4(a)). Meanwhile, the LOS of RS was shorter by comparison with that of LPS (Figure 4(b)).

In respect of postoperative complications, 15 studies compared that in LPS and open surgery ($I^2 = 83.3\%$), and 8 studies compared that in LPS and RS ($I^2 = 76.6\%$). Due to huge heterogeneity, a random effect model was employed. As demonstrated in the result of meta-analysis, LPS resulted in a decrease in the occurrence rate of complications relative to open surgery ($RR = 0.83, 95\% CI: 0.73 to 0.95$) (Figure 5(a)) but an increase in that compared to RS ($RR = 1.74, 95\% CI: 1.57 to 1.92$) (Figure 5(b)).

In respect of recurrence rate, 7 studies compared that in LPS and open surgery ($I^2 = 0.0\%$), and 3 studies compared that in LPS and RS ($I^2 = 5.3\%$). Because of small heterogeneity, a fixed effect model was employed. According to the result of meta-analysis, there was no notable difference in recurrence rate between LPS and open surgery ($RR = 0.75, 95\% CI: 0.56 to 1.01$) or RS ($RR = 0.97, 95\% CI: 0.62 to 1.53$) (Figures 6(a) and 6(b)).

3.3.3. The 3-Year PFS/DFS and OS. In respect of 3-year PFS/DFS, 2 studies compared that in LPS and open surgery ($I^2 = 0.0\%$), and 3 studies compared that in LPS and RS ($I^2 = 90.2\%$). Based on the heterogeneity analysis, a fixed effect model and a random effect model were employed,
<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Center</th>
<th>Study design</th>
<th>Year of sample collection</th>
<th>FIGO stage</th>
<th>Grade</th>
<th>Age, mean (SD), y</th>
<th>BMI, mean (SD), kg/m²</th>
<th>Sample size (n)</th>
<th>Interventions</th>
<th>NOS</th>
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<tbody>
<tr>
<td>Escobar et al.</td>
<td>2012</td>
<td>USA</td>
<td>Multi</td>
<td>Retrospective cohort study</td>
<td>2009-2010</td>
<td>I-II</td>
<td>I-III</td>
<td>60.9 (12.1)</td>
<td>59.7 (9.2)</td>
<td>619 (11.4)</td>
<td>31.2 (6.7)</td>
<td>31.4 (6.6)</td>
</tr>
<tr>
<td>Wright et al.</td>
<td>2012</td>
<td>USA</td>
<td>Multi</td>
<td>Retrospective cohort study</td>
<td>2008-10-2010</td>
<td>I-IV-V</td>
<td>I-III</td>
<td>61</td>
<td>29.3</td>
<td>29.2</td>
<td>232 183</td>
<td>1027</td>
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<tr>
<td>Obermaire et al.</td>
<td>2012</td>
<td>Australia, New Zealand</td>
<td>Multi</td>
<td>RCT</td>
<td>2005-10-2010-06</td>
<td>I-IV-V</td>
<td>I-III</td>
<td>63.2 (11.0)</td>
<td>66.7 (11.3)</td>
<td>29.8 (7.1)</td>
<td>29.7 (8.2)</td>
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<td>2013</td>
<td>USA</td>
<td>Multi</td>
<td>Retrospective cohort study</td>
<td>2008-2012</td>
<td>I-III</td>
<td>I-III</td>
<td>55.3</td>
<td>53.6 (11.1)</td>
<td>536 (11.3)</td>
<td>25.6 (5.6)</td>
<td>26.0 (5.2)</td>
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<td>Bogani et al.</td>
<td>2014</td>
<td>Italy</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2005-2013</td>
<td>I-III</td>
<td>I-III</td>
<td>61</td>
<td>29.9</td>
<td>30.4</td>
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<td>Chien et al.</td>
<td>2014</td>
<td>China</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2005-2013</td>
<td>I-III</td>
<td>I-III</td>
<td>51.4</td>
<td>53.6 (11.1)</td>
<td>536 (11.3)</td>
<td>25.6 (5.6)</td>
<td>26.0 (5.2)</td>
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<tr>
<td>Jin et al.</td>
<td>2015</td>
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<td>Single</td>
<td>Retrospective cohort study</td>
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<td>I-III</td>
<td>63</td>
<td>30.4</td>
<td>30.7</td>
<td>400 1687 1625</td>
<td>6</td>
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<tr>
<td>Deur et al.</td>
<td>2018</td>
<td>Italy</td>
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<td>Retrospective cohort study</td>
<td>2005-2014</td>
<td>I-III</td>
<td>I-III</td>
<td>35.0</td>
<td>35.8 (7.4)</td>
<td>35.8 (7.4)</td>
<td>286 515</td>
<td>9</td>
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<td>Vardar et al.</td>
<td>2018</td>
<td>Denmark</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2005-2014</td>
<td>I-III</td>
<td>I-III</td>
<td>65</td>
<td>25.8</td>
<td>33.1</td>
<td>243 35.3</td>
<td>569 1282 712</td>
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<tr>
<td>Ghanali et al.</td>
<td>2018</td>
<td>Malaysia</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2010-2014</td>
<td>I-III</td>
<td>I-III</td>
<td>55.62 (12.75)</td>
<td>57.79 (9.63)</td>
<td>32.57 (8.89)</td>
<td>29.24 (3.71)</td>
<td>26 14</td>
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<tr>
<td>Dieterich et al.</td>
<td>2019</td>
<td>Germany</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2005-2014</td>
<td>I-III</td>
<td>I-III</td>
<td>55.3</td>
<td>56.0 (10.3)</td>
<td>23.4 (4.4)</td>
<td>24.4 (4.9)</td>
<td>226 252</td>
</tr>
<tr>
<td>Papalia et al.</td>
<td>2020</td>
<td>Switzerland</td>
<td>Single</td>
<td>Retrospective cohort study</td>
<td>2000-2014</td>
<td>I-III</td>
<td>I-III</td>
<td>55.3</td>
<td>56.0 (10.3)</td>
<td>23.4 (4.4)</td>
<td>24.4 (4.9)</td>
<td>226 252</td>
</tr>
</tbody>
</table>
respectively. The outcome of meta-analysis indicated an insignificant difference in 3-year PFS/DFS between LPS and open surgery (RR = 0.99, 95% CI: 0.90 to 1.09) or RS (RR = 1.30, 95% CI: 0.87 to 1.96) (Figures 7(a) and 7(b)).

In respect of 3-year OS, 3 studies compared that in LPS and open surgery ($I^2 = 0\%$, $p = 0.000$), and 4 studies compared that in LPS and RS ($I^2 = 91.2\%$). Based on the results of heterogeneity analysis, a fixed effect model and a random effect model were employed, respectively. As shown in the result of meta-analysis, there was no prominent difference in 3-year OS between LPS and open surgery (RR = 0.97, 95% CI: 0.91 to 1.04) or RS (RR = 1.21, 95% CI: 0.91 to 1.60) (Figures 8(a) and 8(b)).

4. Discussion

This study found that LPS did not improve the survival time of patients. A retrospective study compared the clinical effect of LPS and open surgery on the treatment of low risk EC patients (grade 1 or 2 EC and mesometrium invasion < 1/2). The result suggested that the 5-year recurrence-free survival (RFS) and OS of LPS were similar to those of open surgery [43]. Besides, another study reported the 5-year survival rate of EC patients who underwent LPS, open surgery, or RS, suggesting that there was no significant difference in 5-year DFS and OS of patients [44]. In addition, a multicenter database study verified that the long-term prognosis of MIS on treatment of high-risk EC was no worse than that of LT [45].

The above results were in agreement with the outcome of our study, which indicated that LPS did not dramatically improve the 3-year PFS/DFS and OS of patients.

Generally, LPS takes longer time on operation [36]. However, our study manifested that there was no significant difference in duration of operation between LPS and open surgery or RS. Importantly, since the duration of operation would be subjected to the skill of the operator, we could not figure out which method was the most potential to reduce the duration of operation among these surgeries. Meanwhile, the blood loss of LPS was obviously lower than that of open surgery, but it had no remarkable difference in comparison with that of RS.

The LOS of LPS was notably shorter than that of open surgery and LPS also resulted in fewer postoperative complications. However, RS was overwhelmingly better than LPS in the aspect of duration of time and postoperative complications. This study suggested that no obvious difference in recurrence rate was found between LPS and open surgery or RS.

A previous meta-analysis has proved that uterine manipulator is irrelevant to an increase in occurrence rate of positive peritoneal cytology, lymphovascular space invasion, or recurrence in EC patients [46]. Our study only investigated...
Overall, DL ($I^2 = 72.1\%, \ p = 0.006$) –0.95 (–1.21, –0.70) 19.67

Effect (95% CI) Weight %

Chiou (2014) –0.52 (–0.75, –0.28) 23.45
Ruan (2018) –0.45 (–0.66, –0.24) 22.05
Vardar (2018) –0.81 (–0.96, –0.66) 28.53
Ghazali (2018) –1.05 (–1.75, –0.36) 4.10
Dieterich (2019) –0.32 (–0.55, –0.09) 20.9
Papadia (2019) –0.98 (–1.58, –0.38) 5.26
Overall, DL ($I^2 = 47.1\%, \ p = 0.092$) –0.43 (–0.58, –0.29) 100.00

Note: Weights are from random-effects model

Figure 3: Forest plot about the comparison on blood loss. (a) LPS vs. open surgery; (b) LPS vs. RS.

Author (year) Effect (95% CI) Weight %

Chiou (2014) 0.42 (0.15, 0.69) 49.05
Corrado (2018) –0.38 (–0.54, –0.22) 50.95
Overall, DL ($I^2 = 96.0\%, \ p = 0.000$) 0.01 (–0.77, 0.79) 100.00

Figure 4: Forest plot about comparison on LOS. (a) LPS vs. open surgery; (b) LPS vs. RS.
conventional LPS. A recent meta-analysis involved 4 RCTs that compared the clinical effect of laparoendoscopic single-site surgery (LESS) and conventional LPS on the treatment of EC patients, which suggested an insignificant difference between the two surgeries. Meanwhile, LESS only has advantage on reducing trauma [47]. Additionally, another meta-analysis that was similar to our study compared the differences between RS and LPS or open surgery. The analysis suggested that RS was characterized by less blood loss and blood transfusion, fewer postoperative complications, and less conversion to LT plus shorter LOS compared with the other two surgeries. However, RS took a longer time on operation in surgical staging of EC [48]. Interestingly, these results were consistent with the outcomes of our study. Moreover, we also analyzed the oncological outcome of EC patients.

Figure 5: Forest plot comparing complications. (a) LPS vs. open surgery; (b) LPS vs. RS.
### Table 1: Risk Ratio and Weight Percentage for Overall DL

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Risk ratio (95% CI)</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, DL ((I^2 = 0.0%, p = 0.898))</td>
<td>0.75 (0.56, 1.01)</td>
<td>100.00</td>
</tr>
</tbody>
</table>

### Figure 6: Forest plot of comparison on recurrence rate. (a) LPS vs. open surgery; (b) LPS vs. RS.

### Table 2: Risk Ratio and Weight Percentage for Overall DL

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Risk ratio (95% CI)</th>
<th>Weight %</th>
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</thead>
<tbody>
<tr>
<td>Overall, DL ((I^2 = 0.0%, p = 0.348))</td>
<td>0.97 (0.62, 1.53)</td>
<td>100.00</td>
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</tbody>
</table>

### Figure 7: Forest plot about comparison on 3-year PFS/DFS. (a) LPS vs. open surgery; (b) LPS vs. RS.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Risk ratio (95% CI)</th>
<th>Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, DL ((I^2 = 90.2%, p = 0.825))</td>
<td>0.99 (0.90, 1.09)</td>
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</table>

<table>
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<tr>
<th>Author (year)</th>
<th>Risk ratio (95% CI)</th>
<th>Weight %</th>
</tr>
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<tbody>
<tr>
<td>Overall, DL ((I^2 = 90.2%, p = 0.000))</td>
<td>1.30 (0.87, 1.96)</td>
<td>100.00</td>
</tr>
</tbody>
</table>
number of samples. Furthermore, we also analyzed the survival time of patients. But few samples were involved in the analysis, which was a limitation of our study.

Inevitably, there were limitations in this study. First of all, most of the studies involved in our study were retrospective cohort studies which are inherently subjected to the risk of selection bias. Secondly, the speculation about whether various risk factors affect the prognosis of EC patients who underwent LPS is needed to be further verified. In addition, Cusimano et al. [49] performed a meta-analysis on EC patients with obesity who underwent LPS or robotic hysterectomy. The result of the analysis revealed that LPS and robotic hysterectomy had similar incidence of perioperative complications. However, robotic hysterectomy may reduce conversions due to the positional intolerance of patients suffering from morbid obesity. Finally, because of the lack of related reports on 5-year survival time of patients, we only analyzed the 3-year PFS/DFS and OS of the patients.

In summary, our study revealed that LPS was a safe and effective treatment for EC patients, which was better than open surgery. Nevertheless, LPS was at a disadvantage in the comparison with RS on duration of operation and postoperative complications. In the future, more randomized trials with complete data are needed to verify our conclusion.

Data Availability
The data used to support the findings of this study are included within the article. The data and materials in the current study are available from the corresponding author on reasonable request.

Conflicts of Interest
The authors declare no conflicts of interest.

Authors’ Contributions
Jiong Ma and Xuejun Chen wrote the main manuscript text, and Chunxia Zhou and Jinyan Chen prepared Figures 1–8. All authors reviewed the manuscript.

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