Ex-vivo lung perfusion versus standard protocol lung transplantation—mid-term survival and meta-analysis

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Background: While extended criteria lung donation has helped expand the lung donor pool, utilization of lungs from donors of at least one other solid organ is still limited to around 15–30%. Ex-vivo lung perfusion (EVLP) offers the ability to expand the number of useable lung grafts through assessment and reconditioning of explanted lungs, particularly those not initially meeting criteria for transplantation. This meta-analysis aimed to examine the mid- to long-term survival and other short-term outcomes of patients transplanted with EVLP-treated lungs versus standard/cold-storage protocol lungs.

Methods: Literature search of ten medical databases was conducted for original studies involving "ex-vivo lung perfusion" and "EVLP". Included articles were assessed by two independent researchers, survival data from Kaplan-Meier curves digitized, and individual patient data imputed to conduct aggregated survival analysis. Meta-analyses of suitably reported outcomes were conducted using a random-effects model.

Results: Thirteen studies met inclusion criteria, with a total of 407 EVLP lung transplants and 1,765 as per standard/cold storage protocol. One study was a randomized controlled trial while the remainder were single-institution cohort series of varying design. The majority of donor lungs were from brain death donors, with EVLP lungs having significantly worse PaO_2/FiO_2 ratio and significantly greater rate of abnormal chest X-ray. Aggregated survival analysis of all included studies revealed no significant survival difference for EVLP or standard protocol lungs (hazard ratio 1.00; 95% confidence interval: 0.79–1.27, P=0.981). Survival at 12, 24, and 36 months for the EVLP cohort was 84%, 79%, and 74%, respectively. Survival at 12, 24, and 36 months for the standard protocol cohort was 85%, 79%, and 73%, respectively. Meta-analysis did not find a significant difference in risk of 30-day mortality or primary graft dysfunction grade 3 at 72 hours between cohorts.

Conclusions: There was no significant difference in mid- to long-term survival of EVLP lung transplant patients when compared to standard protocol donor lungs. The incidence of 30-day mortality and primary graft dysfunction grade 3 at 72 hours did not differ significantly between groups. EVLP offers the potential to increase lung donor utilization while providing similar short-term outcomes and mid- to long-term survival.

Keywords: Ex-vivo lung perfusion (EVLP); lung transplantation; meta-analysis; survival

Introduction

While lung transplantation (LTx) for patients with end-stage pulmonary disease can be a life-saving measure, the scarcity of suitable donor lungs results in up to 30% of these patients dying while on the waiting list (1,2). Obtaining donor organs for LTx is particularly problematic, with donors of at least one other solid organ only having suitable lungs in 15–30% of cases according to current assessment criteria (3-5). End-of-life care, be it in the intensive care unit or otherwise, often results in damage to potential grafts and even under optimal donor circumstances, brain death still has the deleterious effect of causing neurogenic edema and a cytokine storm-induced inflammatory response (6).

Standard transplant protocols developed and refined since the 1960's involve infusion of the donor lung with a specially formulated perfusate before inflation of the lung, stapling of the trachea, and cold preservation until transplantation (7). In contrast, ex-vivo lung perfusion (EVLP) involves continuous ventilation and perfusion of donor lungs, offering the potential to both extend the ability to functionally assess grafts, as well as recondition them to a transplant-suitable standard, thereby expanding the available pool of grafts (8). EVLP can be administered via several different established protocols which vary perfusate composition, flow, temperature, pressure, and ventilation, with off-the-shelf EVLP equipment such as the "XVIVO Perfusion System (XPS)", "Organ Care System (OCS)", and "Vivoline LS-1" now on the market (1).

While extended-criteria lungs have been shown to have comparable follow-up survival outcomes to standard criteria lungs using standard lung transplant methods, the use of EVLP to further expand the donor pool raises the question of EVLP recipient long-term outcomes (3,8,9). The objective of this meta-analysis was to aggregate mid- to long-term survival data and available post-operative outcomes from studies comparing LTx recipients who received EVLP treated grafts to those receiving standard protocol (cold-preservation) grafts.

Methods

Literature search

Ten medical literature databases were queried from their dates of inception to August 2019. These included Medline, Embase, PubMed, and the Ovid "Evidence-Based Medicine Reviews" collection, which includes the Cochrane databases, as well as national college and government repositories. A broad search strategy was deliberately used, using terms "ex-vivo lung perfusion" (as a whole term and individually) and "EVLP".

Two independent researchers (P.F. and B.M.) screened reference list results and full texts, with inclusion at each stage determined by consensus with the senior researcher (A.C.). Studies were included if they were comparative studies reporting primary mid- to long-term outcome data for recipients after lung transplant using standard protocol or EVLP. Studies needed to include at least five transplant recipients per arm. Non-comparative studies, animal studies, case reports, conference abstracts, reviews, and editorials were excluded. Where duplicate series exist, the study containing the most complete and up-to-date data was retained. The reference list of all included studies was examined to identify further articles meeting the inclusion criteria.

The primary endpoint was overall Kaplan-Meier survival reported to at least 6-months. Secondary endpoints were determined as those reported in at least half of included studies and included 30-day mortality, post-operative graft dysfunction grade 3 at 72 h, intensive care unit length of stay (LOS), and hospital LOS.

Quality analysis

A 19-point metric adopted from the Canadian Institute of Health Economics was used to assess included studies' quality. This tool was designed with a Delphi methodology of health research stakeholders to evaluate the quality of case series, with examined domains including the number of centers data was collected from, study design, completeness of recipient and donor baseline data reporting, completeness of intraoperative and post-operative outcome reporting, and potential indicators of bias or conflict (*Table S1*). Total scores for each study were tallied to determine quality strata, with studies scoring below 12 deemed standard quality, 12 to 14 moderate quality, and 14 to 19 high quality.

Statistical analysis

Data extraction was performed by two independent researchers (P.F. and B.M.) with data checking and validation by the senior researcher (A.C.). Where data was expressed as median and range or interquartile range, it was converted to mean and standard deviation using statistical methods to facilitate pooling (10,11). Furthermore, where no standard deviation or range was provided, a sample value was imputed as the mean of other provided values (12,13).

Pooling was performed using meta-analysis of proportions or means. Differences in baseline data and outcomes were summarized as relative risk (RR) and mean difference (MD) for proportion and continuous data, respectively, with 95% confidence intervals (95% CI) provided. A random effects model was applied for all analyses to account for between-study variance due to recipient and donor selection, procedural, and care differences not accounted for in institutional series. Studies with zero-event outcomes in both arms were not weighted in meta-analysis.

Survival data was aggregated using the method for secondary survival analysis developed by Guyot and colleagues (14). This approach imputes individual patient time-to-event data, taking digitized Kaplan-Meier survival curves (Engauge Digitizer, Mark Mitchell, GitHub) and patient number-at-risk data as inputs. The imputed individual patient data is then pooled as an overall cohort and aggregated survival curves generated. Hazard ratio (HR) between EVLP and standard treatment protocol is calculated from Kaplan-Meier data using a Cox proportional hazard model (15). Proportionality was tested with a Schoenfeld residual test.

Publication bias was examined with funnel plots and also by Egger's test for study endpoints. Heterogeneity amongst studies were assessed using the I^2 statistic, with consideration of I^2 confidence intervals (16). I^2 thresholds of 0–49%, 50–74%, and ≥75% were considered as low, moderate, and high heterogeneity, respectively (17). Potential sources of heterogeneity and inconsistency of treatment effect were identified and explored with the aid of leave-one-out sensitivity analyses.

Two-tailed P values less than 0.05 were deemed as significant. All statistics were performed with R (R foundation for statistical computing, Vienna, Austria).

Results

Literature search identified 2,252 references with 174 full-text articles screened for inclusion. Reference list search of initially included articles led to the identification of one additional study. In all, 13 studies were included for analysis (PRISMA diagram provided in *Figure S1*) (4,5,8,18-27). One randomized controlled trial (RCT) was included (27), while four studies were prospective, non-randomized trials (18,20-22), six were retrospective series (4,5,8,23,25,26), and two were series with their design not fully described (19,24). One study was rated as standard quality, four as moderate quality, and eight as high quality (*Table S2*). The majority of

studies had small EVLP cohorts, with a median cohort size of 14 patients, and the largest EVLP cohort of 151 patients contained in the RCT by Warnecke *et al.* All but one study were from European centres.

The thirteen comparative studies contained in-total 2,172 lung transplant recipients, with 1,765 transplanted using standard/cold storage protocol and 407 transplanted with EVLP lungs. Where reported, the mean follow-up ranged from 0.7–10 years, with a median of 1-year follow-up.

The mean age of EVLP lung transplant recipients was 51.3 years (95% CI: 49.7–52.9; n=385), with 53.3% males (95% CI: 50.0–56.5; n=884/1,640), while standard protocol lung transplant recipients had a mean age of 48.6 years (95% CI: 46.6–50.6, n=1,765), with 54.0% males (95% CI: 50.0–58.0, n=722/1,321). The pooled recipient cohorts differed only in that COPD was more prevalent in the EVLP recipients (40.4% *vs.* 32.8%, P=0.046). Recipient baseline details and risk are summarized in *Table 1* and further detailed in *Table S3*.

Despite high heterogeneity for some patient/donor baseline parameters, baseline data sensitivity analysis showed these were often due to factors such as inter-study variation in the number of DCD donors or donor PaO_2/FiO_2 ratio. Reasons for study exclusion were not identified and sensitivity analysis of study endpoints did not demonstrate individual studies changing overall findings of significance (with the exception of 30-day mortality).

Overall there were 2,178 lung donors, with 413 EVLP and 1,765 standard protocol donors. While not detailed across all studies, the majority of all grafts were from brain-dead donors (DBD, 88.0%; 95% CI: 80.4–93.0%). Mean age of EVLP donors was 47.3 years (95% CI: 44.5–50.1; n=359) and standard donors, 45.6 years (95% CI: 43.4–47.8; n=1,360). As expected with EVLP's application for graft reconditioning, EVLP donors had significantly greater rates of abnormal chest X-ray [62.0%; (95% CI: 48.2–74.1%), versus 36.6% (95% CI: 25.6–49.2%), P=0.011], and poorer PaO_2/FiO_2 ratio [287 mmHg (95% CI: 217–358) versus 439 mmHg (95% CI: 427–451), P<0.001]. This difference in PaO_2/FiO_2 was further accentuated when the pooled PaO_2/FiO_2 included only studies that provided pre-EVLP values of PaO_2/FiO_2 (excluding Warnecke *et al.*): EVLP 272 mmHg (95% CI: 218–326, n=190), versus, standard 440 mmHg (95% CI: 426–454, n=1,126), MD –154 mmHg (95% CI: –214 – –94.3], I^2=95%, P<0.001). Pooled donor baseline details are provided in *Table 2* and per-study data in *Table S4*.

The mean EVLP time was 234 min (95% CI: 215–253,

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Table 1 Recipient baseline characteristics

Characteristic	EVLP, % (95% CI)	n reported	Standard, % (95% CI)	n reported	RR, MD (95% CI)	I^2	P
Patients	407	–	1,765	–	–	–	–
Males	50.6 (45.1–56.2)	162/319	54.0 (50.0–58.0)	722/1,321	0.96 (0.83–1.10)	16	0.541
Age (years)	51.3 (49.7–52.9)	385	48.6 (46.6–50.6)	1,765	0.99 (−0.79–2.78)	17	0.275
IPF/ILD	26.2 (20.2–33.2)	109/407	19.9 (14.4–26.8)	390/1,765	1.03 (0.79–1.35)	23	0.8
COPD*	40.4 (28.4–53.6)	118/400	32.8 (25.4–41.3)	525/1,737	1.23 (1.00–1.50)	35	0.046
Cystic fibrosis	24.6 (19.0–31.1)	94/407	21.8 (15.4–29.9)	388/1,765	1.12 (0.85–1.49)	31	0.411
PA hypertension	6.9 (4.6–10.3)	20/351	4.9 (3.8–6.4)	69/1,478	1.38 (0.76–2.50)	0	0.289
Re-transplant	2.8 (0.8–9.2)	2/99	5.5 (2.8–10.7)	28/526	0.50 (0.14–1.81)	0	0.289

*, COPD patients include those listed as alpha-1 antitrypsin deficient. CI, confidence interval; RR, relative risk; MD, mean difference; EVLP, ex-vivo lung perfusion; IPF/ILD, idiopathic pulmonary fibrosis/interstitial lung disease; COPD, chronic obstructive pulmonary disease; PA, pulmonary artery.

Table 2 Pooled donor baseline characteristics

Characteristic	EVLP, % (95% CI)	n reported	Standard, % (95% CI)	n reported	RR, MD (95% CI)	I^2	P
Patients	413		1,765		–	–	–
Males	53.3 (47.4–59.2)	147/275	47.1 (40.7–53.6)	515/1,106	1.16 (0.94–1.43)	48	0.176
Age (years)	47.3 (44.5–50.1)	359	45.6 (43.4–47.8)	1,360	2.08 (−1.35–5.5)	65	0.234
DBD	82.1 (67.0–91.2)	173/213	91.8 (83.4–96.1)	1,154/1,271	0.90 (0.74–1.10)	97	0.303
DCD	20.6 (9.8–38.3)	38/176	9.7 (4.6–19.2)	116/1,190	1.99 (0.61–6.45)	88	0.251
Smoking history	37.1 (23.3–53.5)	96/319	34.2 (24.0–46.1)	384/985	1.17 (0.97–1.40)	0	0.095
Abnormal CXR	62.0 (48.2–74.1)	165/294	36.6 (25.6–49.2)	366/959	1.63 (1.12–2.36)	82	0.011
PaO_2/FiO_2 (mmHg)*	287 [217–358], n=341	341	439 [427–451]	1,295	−138 (−208−−67.4)	98	<0.001

Number of EVLP donors is greater than number of recipients due to studies where discarded graft characteristics could not be distinguished from retained grafts. *, pooled values for all included studies. Note: pre-EVLP PaO_2/FiO_2 was provided for all studies except Warnecke *et al.*, which provided post-EVLP values. PaO_2/FiO_2 excluding Warnecke (i.e., pre-EVLP values only): EVLP 272 mmHg (95% CI: 218; 326, n=190), standard 440 mmHg (95% CI: 426–454; n=1,126), MD −154 mmHg (95% CI: −214 − −94.3, I^2=95%; P<0.001). CI, confidence interval; RR, relative risk; MD, mean difference; EVLP, ex-vivo lung perfusion; DBD, donation after brain death; DCD, donation after circulatory death; CXR, chest X-ray; PaO_2/FiO_2, gradient partial pressure arterial oxygen over fraction of inspired oxygen.

n=294). EVLP was performed using the proprietary XVIVO system (XVIVO, Denver, CO, USA) in five studies, Vivoline LS-1 (Vivoline Medical AB, Lund, Sweden) in three studies, Organ Care System (OCS-TransMedics Inc., Boston, MA, USA) in two studies, and not-fully described or administered with custom circuits in four studies (*Table S5*). The majority of patients received double-lung transplants. Intraoperative parameters were similar between EVLP and standard

protocol groups (*Table 3*).

The primary outcome of Kaplan-Meier survival was reported from 12 to 120 months. Survival data in the single RCT was only available for the per-protocol, rather than the intention-to-treat, population, hence, the number at risk is reduced from the total number of patients reported for baseline and intraoperative data (27). Aggregated survival for individual patient data demonstrated almost identical

Table 3 Pooled intraoperative data

Characteristic	EVLP, % (95% CI)	n reported	Standard, % (95% CI)	n reported	RR, MD (95% CI)	I^2	P
Patients	407		1,765		–	–	–
Ventilator bridged	7.1 (4.7–10.7)	20/308	5.1 (3.9–6.7)	49/968	1.43 (0.86–2.38)	0	0.166
ECMO bridged	7.2 (3.1–15.8)	16/317	6.3 (4.3–9.1)	54/968	1.12 (0.60–2.10)	12	0.718
Single lung transplant	11.0 (6.4–18.2)	31/398	10.1 (5.8–16.9)	152/1,628	1.11 (0.65–1.90)	40	0.708
Double lung transplant	88.3 (80.8–93.1)	366/398	89.2 (82.2–93.7)	1,468/1,628	1.00 (0.95–1.04)	50	0.918
Intraoperative ECMO	40.9 (25.0–59.1)	63/147	28.0 (12.1–52.2)	240/995	1.63 (0.94–2.83)	89	0.080
EVLP time (minutes)	234 (215–253)	292	–	–	–	–	–

CI, confidence interval; RR, relative risk; MD, mean difference; EVLP, ex-vivo lung perfusion; ECMO, extra-corporeal membrane oxygenation.
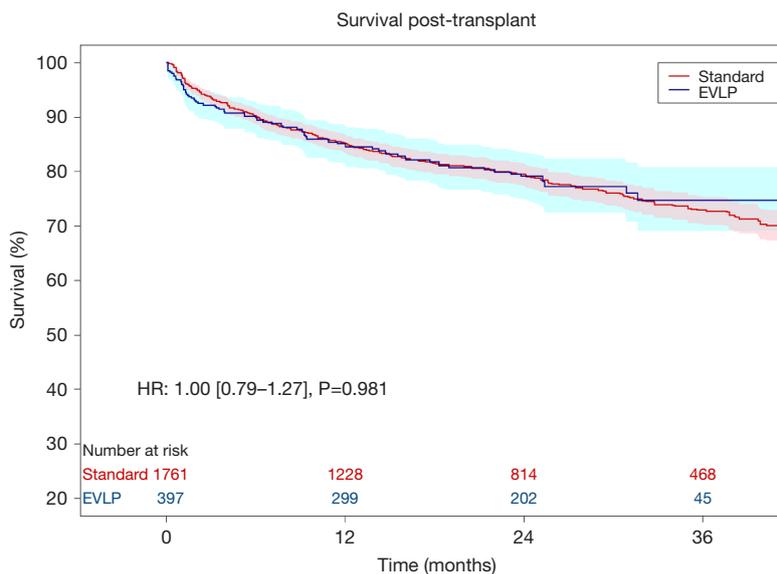

Figure 1 Aggregated survival for all included studies. HR, hazard ratio (Cox proportional model); EVLP, ex-vivo lung perfusion.

survival profiles for both EVLP and standard/cold storage protocol lung transplant recipients. Survival at 12, 24, and 36 months for the EVLP cohort was 84%, 79%, and 74%, respectively, compared to 85%, 79%, and 73%, for the same time periods in the standard protocol cohort. Cox proportional hazard analysis demonstrated a hazard ratio of 1.00 (95% CI: 0.79–1.27; P=0.981; *Figure 1*).

Leave-one-out analysis of Kaplan-Meier data was conducted to examine the sensitivity of Kaplan-Meier and hazard ratio results to individual patient data from each study. No single study was found to significantly influence survival outcomes. The aggregated survival curves from the non-randomized studies (and no matching between arm— excluding Warnecke *et al.*) is provided in *Figure S2* (HR 1.16; 95% CI: 0.89–1.51; P=0.276).

Operative outcomes were inconsistently reported across included studies, with only 30-day mortality, primary graft dysfunction grade 3 at 72 h (PGD3), extubation time, ICU LOS, and hospital LOS reported for the majority of studies. The 30-day mortality was similar between EVLP and standard cohorts, RR 2.04 (95% CI: 0.88–4.72, I^2=0%, P=0.095), however, this result was obtained after exclusion of one particular study (27), which detailed each early death as non-EVLP related (due to iatrogenic surgical

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Table 4 Pooled post-operative outcomes (where reported)

Characteristic	EVLP, % (95% CI)	n reported	Standard, % (95% CI)	n reported	RR, MD (95% CI)	I^2	P
Patients*	397		1,761		–	–	–
30-day mortality^	5.7 (3.4–9.5)	11/253	3.5 (2.5–4.9)	29/1,005	2.04 (0.88–4.72)	0	0.095
PGD grade 3 at 72 h	9.7 (4.5–19.8)	15/247	10.5 (5.9–18.0)	82/829	1.15 (0.69–1.89)	0	0.592

*, total number of patients for reported outcomes reduced since Warnecke *et al.* only provided Kaplan-Meier follow up for the per-protocol population, not the intention-to-treat population. ^, Warnecke *et al.* excluded following sensitivity analysis; deaths not directly attributable to EVLP. CI, confidence interval; RR, relative risk; MD, mean difference; EVLP, ex-vivo lung perfusion; PGD, primary graft dysfunction.
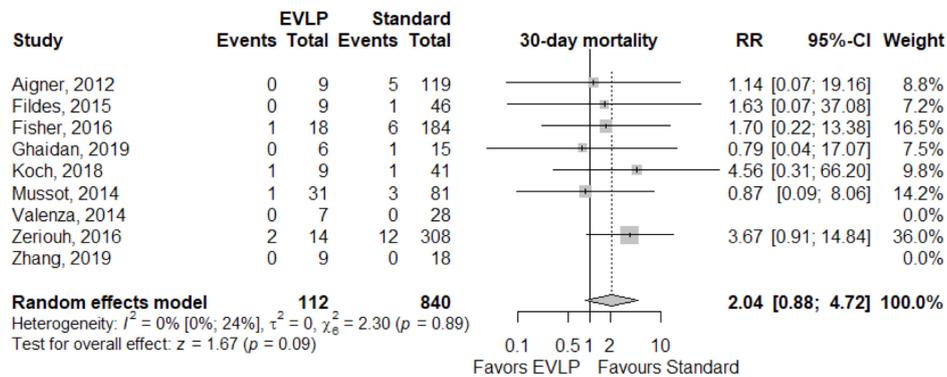
Figure 2 The 30-day mortality post-transplant. EVLP, ex-vivo lung perfusion; RR, relative risk; CI, confidence interval.
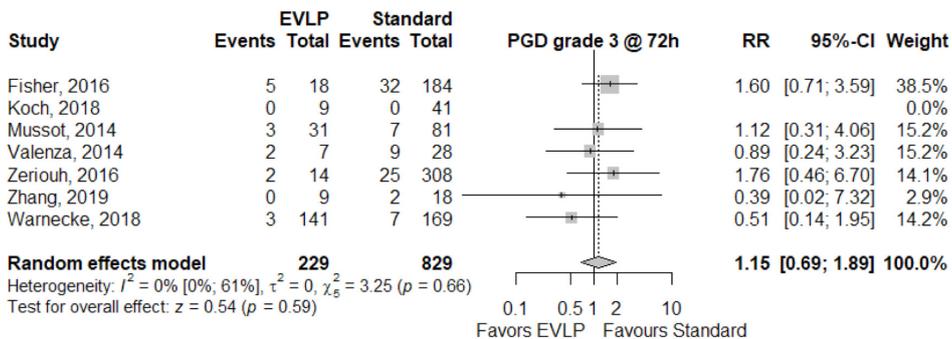
Figure 3 Primary graft dysfunction grade 3 at 72 h post-transplant. EVLP, ex-vivo lung perfusion; PGD, primary graft dysfunction; RR, relative risk; CI, confidence interval.

complications, patients' compliance with medications, and patients' cardiac risk factors). Including all studies gave a significant 30-day mortality result for EVLP: RR 2.39 (95% CI: 1.07–5.35, I^2=0%, P=0.034). Primary graft dysfunction grade 3 at 72 hours did not reach significance (RR 1.15; 95% CI: 0.69–1.89, I^2=0%, P=0.592). While continuous outcomes such as extubation time, intensive care unit,

and hospital LOS were widely reported, heterogeneity of reporting and non-availability of standard deviation data meant the majority of data required imputation and multiple assumptions to allow pooling, and hence, it was not conducted. Per-study outcome data are detailed in *Table S6*, and pooled secondary outcomes in *Table 4* and *Figures 2,3*.

Publication bias in meta-analysed endpoints was not identified from visual inspection of funnel plots or application of Egger's test (*Figures S3,S4*). Point values of heterogeneity for secondary outcomes remained at zero (I^2=0%) for the sensitivity analysis of secondary outcomes.

Discussion

This meta-analysis combined results from 13 different studies detailing mid- to long-term survival data to demonstrate that there was no significant survival difference for transplant recipients receiving donor lungs treated with EVLP versus standard/cold storage protocol lungs. Neither 30-day mortality or grade 3 primary graft dysfunction at 72 h post-transplant (PGD3 at 72 h) were found to differ significantly between EVLP and standard cohorts.

The direction of primary and secondary outcomes of this meta-analysis were found to be concordant with all included studies, with the exception of the INSPIRE RCT, which identified a significantly greater 30-day mortality in the EVLP group but also a significant reduction in PGD3 at 72 h for EVLP patients. The authors of that study accounted for the significant 30-day mortality signal as non-EVLP related (due to iatrogenic surgical complications, patients' compliance with medications, and patients' cardiac risk factors) and this led to that outcome's exclusion from this meta-analysis (27).

The PaO_2/FiO_2 threshold for acceptance of donor grafts varies between centres (22), as well as how this value is measured (for example, variations in time points, positive end expiratory pressure). However, this was not shown to significantly affect overall survival outcomes between EVLP and standard protocol lungs at a meta-analysis level, nor within included studies, which included donor lungs with pre-EVLP pooled mean PaO_2/FiO_2 value of 272 mmHg [and as low as 150 mmHg (4)]. Collectively, this information could be used to shape EVLP donor acceptance criteria and potentially further increase the number of donor lungs accepted for EVLP, for example from DCD donors, who typically have worse PaO_2 values (4,23,25). The divergent but statistically non-significant survival curves evidenced with the removal of the RCT (*Figure S2*) likely reflect the fact that the INSPIRE RCT made up a large proportion of the pooled EVLP cohort and required both EVLP and standard donor groups to have similarly high PaO_2/FiO_2 ratios for acceptance. This was in contrast to the other included studies, where EVLP was used for reconditioning grafts with poorer pre-EVLP PaO_2/FiO_2 ratios.

PGD3 at 72 hours has been found to correlate with increased 30-day, 90-day, and 1-year mortality (26,28), as well as chronic lung allograft dysfunction (27). While the reduction in PGD3 at 72 h was only significant in the INSPIRE RCT, a reduced incidence of PGD3 at 72 h in EVLP transplant recipients was a finding shared by several other included studies (5,18,26). Though these studies' findings on PGD3 did not reach significance and had smaller cohorts than the RCT, they all noted an absence of PGD3 events in their EVLP recipients, and other non-zero event studies similarly noted non-significant lower incidences. This was the case despite a worse PaO_2/FiO_2 in the meta-analysis EVLP donor group and in individual studies, where poorer PaO_2/FiO_2 donor lungs were accepted for EVLP treatment. Unlike other included studies, the INSPIRE RCT was exceptional in its exclusion of donors with PaO_2/FiO_2 <300 mmHg for both EVLP and standard groups. Although PGD3 was a secondary outcome in this meta-analysis, it's non-significant difference between EVLP and standard groups in combination with the non-significant survival difference at follow-up is potentially valuable, as it adds weight to the restorative ability of EVLP and its potential for good long-term outcomes.

Other secondary outcomes examined and commented upon in many studies were post-transplant extubation time and ICU length of stay, with concerns that using high-risk lungs for EVLP may lead to an increase in these parameters and their associated risks (8,23). Pooling of these continuous outcomes was precluded by heterogeneity in reporting and a need to impute data for the majority of included studies. While the limitations of vote counting in meta-analysis are acknowledged (29), only one of thirteen included studies found significantly longer extubation times and ICU length of stay for EVLP (22).

While the constituent studies of this meta-analysis applied varying EVLP protocols and methodologies, it is clear that EVLP provides the ability to expand the pool of available donor lungs by reconditioning and reassessing lungs not considered suitable under standard criteria lung transplant. Using marginal lungs under extended-criteria for standard protocol lung transplant has been reported as doubling utilization rates to around 30–40% (30,31). Using EVLP however, studies in the present analysis reported conversion rates from EVLP evaluated lungs to transplant ranging from 34% to 97% (8,20,21,23,24,26). This allowed increased donor utilization, ranging from 33% to 50% (8,22,23). Along with the non-significant differences in outcomes found in this meta-analysis, this demonstrates

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the real possibility of EVLP to reduce the number of lung transplant wait-list deaths. Additionally, it adds weight to the argument that satisfactory results can be obtained with more than one EVLP protocol (22).

This meta-analysis is potentially limited by it being largely comprised of institutional series, however, RCT in this area are difficult since transplanting initially rejected or marginal (beyond extended criteria) donor lungs as a control arm would be ethically difficult to justify (21). Although statistical heterogeneity was low in meta-analysis endpoints, it remained moderate to high for some baseline and intraoperative characteristics. This heterogeneity is likely due to differences in centers' donor criteria and operative methods (for example, single versus double lung), and sensitivity analysis did not demonstrate an effect on significance of outcomes. This does not however, mean that all sources of heterogeneity were able to be accounted for and the authors acknowledge that even where I^2 point values were low, I^2 confidence intervals often still included at least moderate heterogeneity values (16). Additionally, many studies were retrospective and included a range of recruitment years and recruitment period lengths. As EVLP methodology and knowledge (and lung transplant more broadly) is a rapidly-evolving field, this may have introduced some learning-curve type confounding effect (25).

Conclusions

Aggregated patient survival data analysis of EVLP and standard/cold-storage lung transplant recipients demonstrated no significant difference in survival at mid- to long-term follow-up. Meta-analysis demonstrated lungs accepted for EVLP had significantly lower PaO_2/FiO_2 ratio and a greater incidence of radiographic abnormality, however, this did not translate to a significant difference in overall survival, 30-day mortality, or primary graft dysfunction grade 3 at 72 h between EVLP and standard cohorts. EVLP offers the ability to expand the lung donor pool with acceptable mid- to long-term survival outcomes.

Acknowledgments

None.

Footnote

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

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| Table S1 Canadian Institute of Health Economics quality appraisal checklist (modified)	
Domain	Description
1	Was the hypothesis/aim/objective of the study clearly stated (e.g., PICO)?
2	Was the study conducted prospectively (stated as such)?
3	Were the cases collected in more than one centre?
4	Were patients recruited consecutively?
5	Were the characteristics of the patients included in the study described?
6	Were the eligibility criteria (i.e., inclusion and exclusion criteria) for entry into the study clearly stated?
7	Did patients enter the study at a similar point in the disease?
8	Was the intervention of interest clearly described?
9	Were additional interventions (co–interventions) clearly described?
10	Were relevant outcome measures established a priori?
11	Were the relevant outcomes measured using appropriate objective/subjective methods?
12	Were the relevant outcome measures made before and after the intervention?
13	Were the statistical tests used to assess the relevant outcomes appropriate?
14	Was follow–up long enough for important events and outcomes to occur?
15	Were losses to follow–up reported?
16	Did the study provided estimates of random variability in the data analysis of relevant outcomes?
17	Were the adverse events reported?
18	Were the conclusions of the study supported by results?
19	Were conflicts of interest reported?
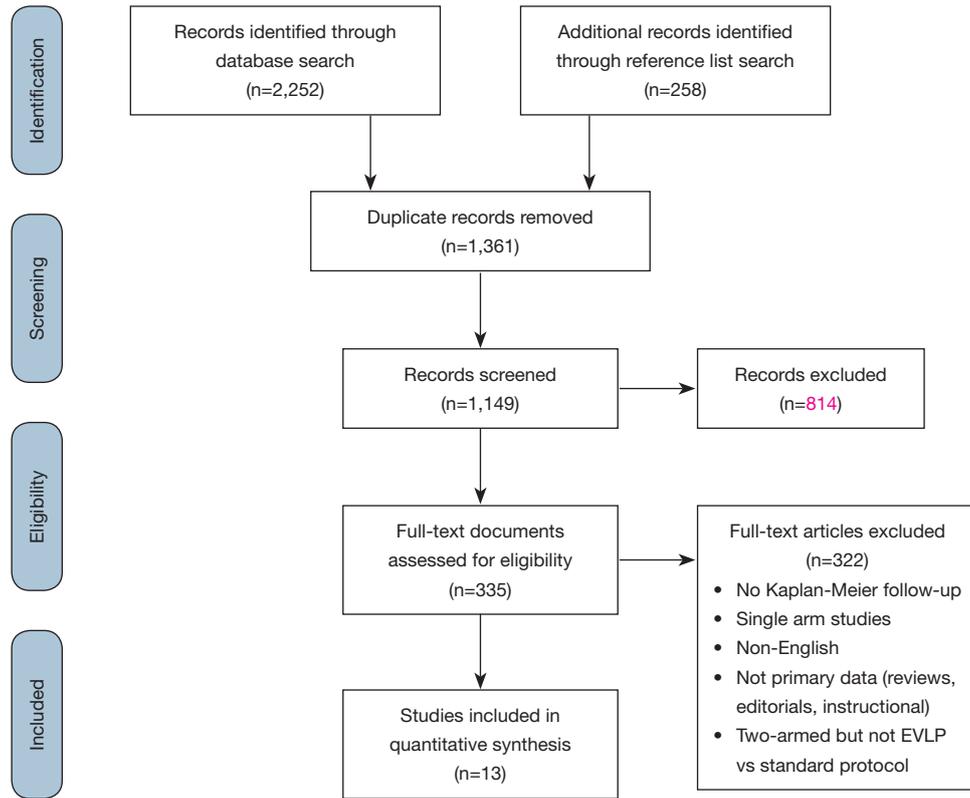
Figure S1 PRIMSA flow chart detailing the literature search process for EVLP versus standard protocol lung transplantation.

Table S2 Study details

Author, year	Country	Recruitment years	Study design — Retro-/prospective	Study design — Single/multi-centre	Type	Quality (NIH)	Mean F/U (y)	Demographic — Group	Demographic — Patients (n)
Aigner, 2012	Austria	2010–2011	P	S	Series	MQ	0.77	EVLP	9
			–				0.77	Std	119
Fildes, 2015		2014	–	MC	Series	SQ	–	EVLP	9
							–	Std	46
Fisher, 2016	England	2012–2014	P	MC	Series	HQ	1	EVLP	18
							1	Std	184
Ghaidan, 2019	Sweden	2006–2007	R	S	Series	MQ	10	EVLP	6
							10	Std	15
Koch, 2018	Germany	2016–2017	R	S	Series	MQ	1	EVLP	9
							1	Std	41
Mussot, 2014	France	2011–2013	P	S	Series	MQ	1	EVLP	31
							1	Std	81
Nilsson, 2019	Sweden, Denmark	2011–2015	P	MC	Series	HQ	1	EVLP	54
							1	Std	271
Tikkanen, 2015	Canada	2008–2012	R	S	Series	HQ	–	EVLP	63
							–	Std	340
Valenza, 2014	Italy	2011–2013	–	S	Series	HQ	0.71	EVLP	7
							0.71	Std	28
Wallinder, 2016	Sweden	2011–2015	R	S	Series	HQ	1.63	EVLP	27
							1.35	Std	145
Warneke, 2018	Germany	2011–1014	P	MC	RCT	HQ	2	EVLP	151
							2	Std	169
Zeriouh, 2016	UK	2007–2014	R	–	Series	HQ	0.5±0.5*	EVLP	14
							2±2.3*	Std	308
Zhang, 2019	Netherlands	2012–2016	R	S	Series	HQ	3	EVLP	9
							3	Std	18

*, converted from median (interquartile range) or median (range) for pooling. R, retrospective; P, prospective; S, single-centre; MC, multi-centre; SQ, standard quality; MQ, moderate quality; HQ, high quality; EVLP, ex-vivo lung perfusion; Std, standard protocol (cold storage).

Table S3 Recipient baseline data

Author, year	Group	Patients (n)	Males (n)	Age (years)	Indication IPF/ILD	COPD + α1	CF	Pulm A HTN	Re–xplant	Other
Aigner, 2012	EVLP	9	6	50.0±16.1*	4	3	2	1		
	Std	119	58	42.8±10.4*		0				
Fildes, 2015	EVLP	9	5	53±9.4	1	6	1	1		
	Std	46	22	49±12	3	24	9	3		7
Fisher, 2016	EVLP	18	13	49.0±13.3*	7	5	4	1	0	1
	Std	184	106	51.0±3.3*	47	40	47	3	0	47
Ghaidan, 2019	EVLP	6	3	54.1±10.4	1	4	1	0	0	
	Std	15	6	42.6±14.8	1	5	7	1	0	1
Koch, 2018	EVLP	9	6	55±7	2	8	0	0	0	1
	Std	41	24	55±6	10	22	3	0	1	5
Mussot, 2014	EVLP	31	11	40.0±9.8*	3	9	15			4
	Std	81	39	41.0±13.9*	12	16	40			13
Nilsson, 2019	EVLP	54		52±12	13	21	11	1	0	8
	Std	271		51±13	68	111	33	16	0	43
Tikkanen, 2015	EVLP	63	31	50.3±14.6	22	20	14	3	1	3
	Std	340	199	52.3±14.2	121	90	67	14	14	34
Valenza, 2014	EVLP	7		38±15	0	0	4			3
	Std	28		49±14	11	0	10			7
Wallinder, 2016	EVLP	27		55±13	6	11	5		1	4
	Std	145		52±14	35	54	10	11	13	22
Warnecke, 2018	EVLP	151	77	50.4±13.1	49	46	31	13		12
	Std	169	106	50.0±13.6	57	52	40	6		14
Zeriouh, 2016	EVLP	14	6	44.7±23.2*	0	7	4	0		3
	Std	308	154	43.7±18.7*	23	127	118	15		25
Zhang, 2019	EVLP	9	4	53±13.3	1	6	2			
	Std	18	8	50±9.5	2	12	4			

*, converted from median (interquartile range) or median (range) for pooling. IPF/ILD, interstitial pulmonary fibrosis/interstitial lung disease; COPD + α1, chronic obstructive pulmonary disease (including alpha-1 antitrypsin deficiency); Pulm A HTN, pulmonary artery hypertension; EVLP, ex-vivo lung perfusion; Std, standard protocol (cold storage).

Table S4 Donor baseline data

Author, year	Group	n patients	Males (n)	Age	DBD (n)	DCD (n)	Smoking Hx (n)	Abnormal CXR (n)	PaO$_2$/FIO$_2$ (mmHg)	Ventilator time (days)	Cause of death Intracranial bleed (n)	CVA/ stroke (n)	Cardiac (n)	Trauma (n)	Suicide (n)
Aigner, 2012	EVLP	13		42.1±12.5*					208.5±40.0*	7.6±4.7*	5	2	–	6	–
	Std	119							477.0±114.2*						
Fildes, 2015	EVLP	9	5	54±10.1					229±73	1.69±0.68					
	Std	46	16	45±13.1					450±87	2.08±1.73					
Fisher, 2016	EVLP	18	10	50.5	13	5			307.0±97.1*						
	Std	184	86	44	152	31									
Ghaidan, 2019	EVLP	6		50.0±7.1*			3		154.2±37.9*		–	–	–	–	–
	Std	15			–				–		–	–	–	–	–
Koch, 2018	EVLP	11	8	54±14	11	0	6	10	270±74		4	1	0	2	1
	Std	41	21	54±16	41	0	18	18	413±96		19	8	0	9	0
Mussot, 2014	EVLP	31		45.9±13.3*	31	0	9	22	278.6±69.9*						
	Std	81		46.4±16.4*	81	0	17	16	397.6±105.1*						
Nilsson, 2019	EVLP	54			54	0									
	Std	271			271	0									
Tikkanen, 2015	EVLP	63	32	43.1±14.9	36	27	34	39	384±102						
	Std	340	160	45.8±17.6	322	18	163	170	456±76						
Valenza, 2014	EVLP	7		54±9	7	0	4	6	264±78	3.9	–	6	–	0	–
	Std	28		40±15	28	0	8	11	453±119		–	17	–	9	–
Wallinder, 2016	EVLP	27		47±18			2	20	218±86	2.3					3
	Std	145		50±17					426±82						
Warnecke, 2018	EVLP	151	79	42.2±14.4			27	65/141	438.5±80.0^						
	Std	169	102	40.2±13.7			27	76/161	431.7±73.8^						
Zeriouh, 2016	EVLP	14	9	48.7±14.1*	11	3	7	4	428	2.7±0.8*	12	0	5	1	–
	Std	308	121	43.3±13.4	248	60	143	75	435	2.0±1.5*	202	23	69	31	–
Zhang, 2019	EVLP	9	4	41±12.7	6	3	4		286±100	4.0±0.9*	3	2	–	1	–
	Std	18	9	52±16.3	11	7	8		452±59	4.3±1.5*	3	6	–	3	–

*, converted from median (interquartile range) or median (range) for pooling. ^, final PaO$_2$/FiO$_2$– Warnecke *et al.* excluded lungs with PaO$_2$/FiO$_2$ <300 mmHg (initial values not provided). DBD, donation after brain death; DCD, donation after circulatory death; Hx, history; CXR, chest X-ray; PaO$_2$/FiO$_2$, arterial partial oxygen pressure to fraction inspired oxygen ratio; CVA, cerebrovascular accident; EVLP, ex-vivo lung perfusion; Std, standard protocol (cold storage).

Table S5 Operative data

Author, year	Group	Ventilator-bridged (n)	ECMO-bridged (n)	Single lung (n)	Double lung (n)	Ischaemic time (min)	Ischaemic time second lung (min)	EVLP time (min)	CPB use (n)	Intra-op ECMO (n)	EVLP system
Aigner, 2012	EVLP	–	1	0	9	579.8±55.4*	–	216.5±35.5*	–	4	Custom/NS
	Std							–			
Fildes, 2015	EVLP	–	–	0	9						Custom/NS
	Std			0	46						
Fisher, 2016	EVLP			2	16				16	7	Vivoline LS–1
	Std			24	152				116	6	
Ghaidan, 2019	EVLP	0	0	0	6						Custom/NS
	Std	1	1	0	15						
Koch, 2018	EVLP		0	1	7^	434±94	506±96			9	XVIVO
	Std		0	0	41	291±72	374±84			39	
Mussot, 2014	EVLP		–	0	31	447	447	243			XVIVO
	Std		–	0	81	360	360	–			
Nilsson, 2019	EVLP	5	1	7	47			200±94		29	Vivoline LS–1
	Std	12	16	37	234			–		125	
Tikkanen, 2015	EVLP	6	4	15	48						XVIVO
	Std	19	13	45	295						
Valenza, 2014	EVLP	1	3	1	6	–	700	268±104		5	Custom/NS
	Std	1	5	14	14	–	446±140	–		8	
Wallinder, 2016	EVLP	1	0	5	22	–	–	275.7±142.8*		4	Vivoline LS–1 AND XVIVO
	Std	7	10	32	113	–	–	–		36	
Wernecke, 2018	EVLP	7	7	0	151			220.8±91.7			OCS
	Std	9	9	0	169						
Zeriouh, 2016	EVLP			0	14	–	–	342±149		2	OCS
	Std			0	308	–	–	–		20	
Zhang, 2019	EVLP						720±84	231.0±44.6*		3	XVIVO
	Std						474±162	–		6	

ECMO, exta-corporeal membrane oxygenation; CPB, cardiopulmonary bypass; Intra-op, intraoperative; EVLP, ex-vivo lung perfusion; Std, standard protocol (cold storage). *, converted from median (interquartile range) or median (range) for pooling. ^, Koch *et al.* included 1 bilobar transplant in the EVLP group.
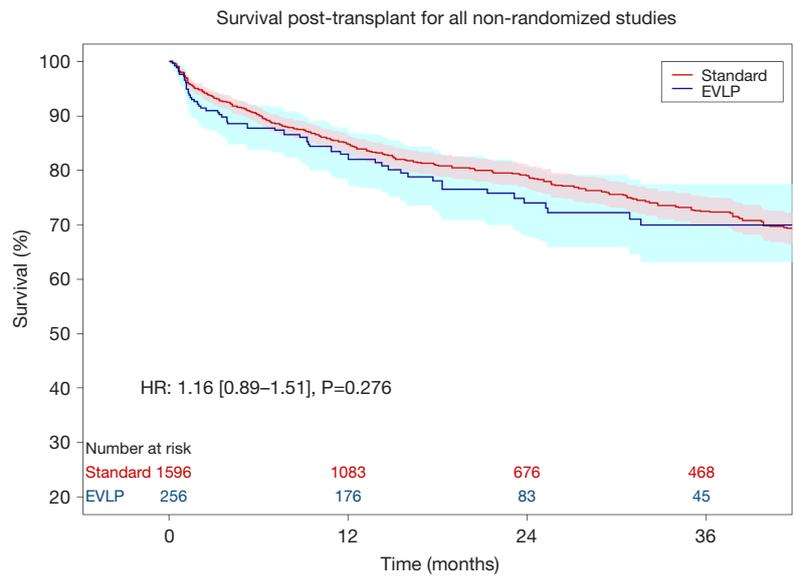
Figure S2 Kaplan-Meier survival post-transplant for all non-randomized series (ie: excluding the RCT by Warnecke *et al.*). EVLP, ex-vivo lung perfusion; HR, hazard ratio.

Table S6 Recipient outcomes

Author, year	Group	Patients (n)	In-hospital mortality (n)	30-day mortality (n)	Post-op ECMO (n)	Extubation time (h)	Pneumonia (n)	Primary graft dysfunction (n)	ICU LOS (d)	Hospital LOS (d)
Aigner, 2012	EVLP	9	1	0	1	62.4±45.6		0	5.6±1.6	28.9±16.9
	Std	119	–	5		110.4±196.8			9.7±9.4	27.8±19
Fildes, 2015	EVLP	9	0	0			2		19	54
	Std	46	1	1			8		10	39
Fisher, 2016	EVLP	18		1		72		5	14.5 [1.7–98]	28 [16–100]
	Std	184		6		38		32	4.3 [0.4–100.6]	28 [2–99]
Ghaidan, 2019	EVLP	6	0	0						
	Std	15	0	1						
Koch, 2018	EVLP	9		1	1	221±307		0	12.5±13.4	26±16
	Std	41	0	1	0	124±249		0	18.9±57	26±16
Mussot, 2014	EVLP	31		1	–	24 [0–43]		3	9 [2–45]	37
	Std	81		3	–	24 [0–34]		7	6 [2–28]	28
Nilsson, 2019	EVLP	54	1			18 [2–912]			4 [2–65]	30
	Std	271	4			7 [0–2,280]			3 [1–156]	28
Tikkanen, 2015	EVLP	63								
	Std	340								
Valenza, 2014	EVLP	7	0	0	1	72		2	10 [5–18]	
	Std	28	0	0	2	36		9	5.5 [4–21.5]	
Wallinder, 2016	EVLP	27	1		2	79±44		3	8±9	
	Std	145	8		6	120±26		17	8±13	
Wernecke, 2018	EVLP	141^	9	6			15	3		
	Std	165^	11	0			26	7		
Zeriouh, 2016	EVLP	14		2		74 [13–924]		2	5 [3–39]	23 [18–76]
	Std	308		12		34 [19–942]		25	6 [3–21]	32 [22–51]
Zhang, 2019	EVLP	9	0	0				0	11 [4–26]	31 [27–46]
	Std	18	0	0				2	5.2 [3–13]	42 [25–50]

*, converted from median (interquartile range) or median [range] for pooling. ^, Wernecke et al. reported both intention-to-treat and per-protocol data. Per-protocol data listed. ECMO, extra-corporeal membrane oxygenation; CPB, cardiopulmonary bypass; Intra-op, intraoperative; EVLP, ex-vivo lung perfusion; Std, standard protocol (cold storage).
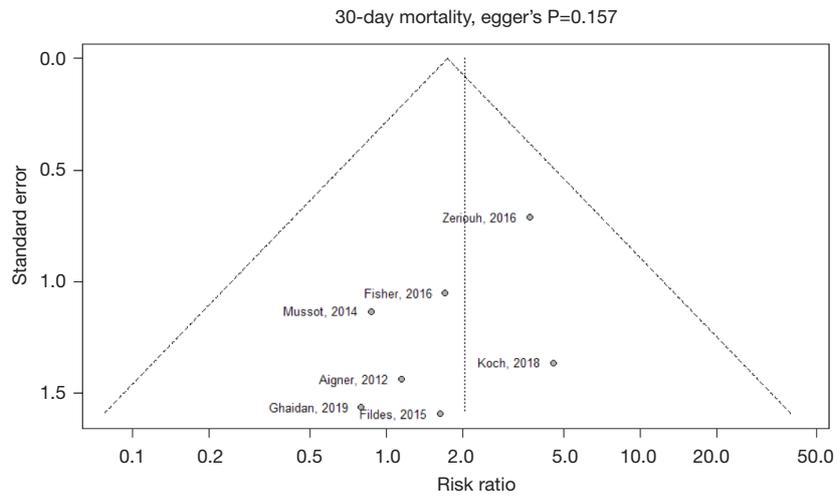
Figure S3 Funnel plot for secondary outcome: 30–day mortality.
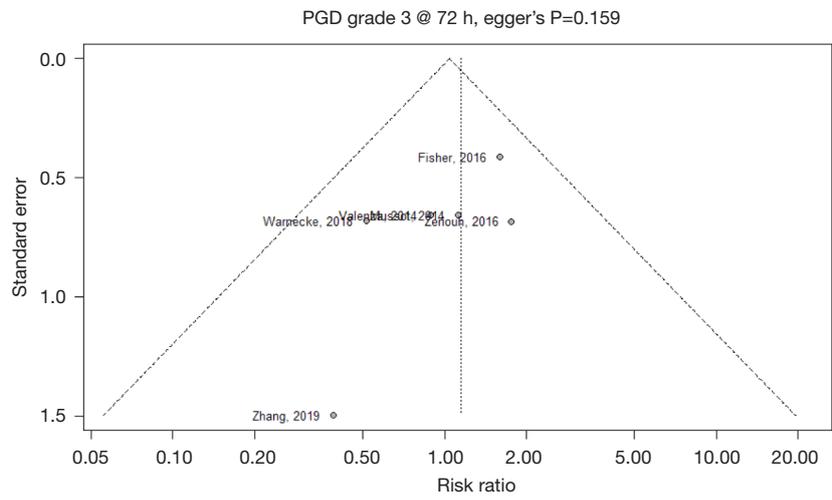

Figure S4 Funnel plot for secondary outcome: primary graft dysfunction (PGD) at 72 hours post-operatively.