BMJ Open

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-038385
Article Type:	Original research
Date Submitted by the Author:	09-Mar-2020
Complete List of Authors:	Zhang, Liyan; School of Basic Medicine, Gansu University of Chinese Medicine, Pei Jing, Yan Yang, Kehu; evidence based meidicine center, lanzhou university Wu, Shanlian Zhu, Xin-Yu; Lanzhou University, Institute of Epidemiology and Health Statistics Chen, Xiaojie Li, Li Cao, Yunshan Zhang, Min
Keywords:	Respiratory physiology < THORACIC MEDICINE, Adult surgery < SURGERY, Adult thoracic medicine < THORACIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE PAGE

Title

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Running Title: Association between splenectomy and chronic thromboembolic pulmonary

Authors' names and affiliations

Liyan Zhang[1,2,3,4+], BS, Peijing Yan[3+], MS, Kehu Yang[3,4,6], MD , Shanlian Wu[1,2], BS, Xinyu Zhu[7], MS, Xiaojie Chen[7], MS, Li Li[7], MS, Min Zhang[2*], MD, PhD, Yunshan Cao[5*], MD, PhD

(1) School of Basic Medicine, Gansu University of Chinese Medicine, Lanzhou, 730000, China

(2) Department of Pathology, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(3) Institute of Clinical Research and Evidence Based Medicine, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(4) Evidence Based Medicine Center, School of Basic Medical Sciences, Lanzhou University, Lanzhou, 730000, China

(5) Department of Cardiology ,Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(6) Key Laboratory of Evidence-based Medicine and Knowledge Translation of Gansu Province, Lanzhou, 730000, China

(7) Department of Science and Research, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

+: Contributed equally

*: Corresponding author

Corresponding author

Min Zhang, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail:sallyzhangmin@126.com

Yunshan Cao, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail: yunshancao@126.com

Acknowledgement of grant support

This study was supported by the National Natural Science Foundation of China (grant no. 81860059), the International Cooperation Exchange Project of Gansu province (grant no. 18YF1WA046) and CAS 'Light of West China' Program granted to MZ.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Data sharing statement

No additional data available.

Abstract

Objectives: Whether undergone splenectomy will increase the risk of chronic thromboembolic pulmonary hypertension (CTEPH) remain unclear. We hold a systematic review and meta-analysis to explore the association between splenectomy and CTEPH.

Methods: The PubMed, Embase, Cochrane Library databases were systematically searched for records of splenectomy and CTEPH. Newcastle-Ottawa scale and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used to assess the quality of the included studies and each quality item was grade as low risk or high risk. Random-effects model was used to calculate different effective values.

Results: In total, 8 trials involving 6190 participants fulfilled the inclusion criteria. The prevalence estimates of splenectomy reported by 8 trials with a crude summary prevalence of 4.6% (122/2635 individuals; 95%CI: 0.03, 0.06, I^2=71.5%, p<0.01). Subgroup analysis showed statistically significant association of splenectomy in CTEPH patients (OR: 3.04, 95%CI: 1.51 to 6.14, I^2=0.0%) compared with idiopathic pulmonary arterial hypertension (IPAH) patients. And there showed significant association of splenectomy in CTEPH patients (OR: 5.10, 95%CI: 1.66 to 15.68, I^2=0.0%) compared with pulmonary thromboembolism (PE) patients.

Conclusion: Prevalence of splenectomy in CTEPH was 4.6%, and CTEPH was associated with splenectomy. But high-quality prospective trials were needed.

Keywords: Pulmonary hypertension, chronic thromboembolic pulmonary

hypertension, splenectomy, systematic review, meta-analysis

Strengths and limitations of this study

This is the first systematic review and meta-analysis suggested chronic thromboembolic pulmonary hypertension was associated with splenectomy.

However, the trials we included were not random control trials and had small sample size.

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a disease of obstructive pulmonary artery remodelling as a consequence of major vessel thromboembolism. CTEPH is defined as an increase in mean pulmonary arterial pressure(mPAP) ⩾ 25 mmHg and the presence of at least one segmental perfusion defect despite 3 months of anticoagulation therapy[1]. CTEPH is a series disease with high mortality. There was a study reported the CTEPH patients only treated with anticoagulants died within three years of follow-up up to 90%[2].

CTEPH is considered to be caused by single or recurrent pulmonary thromboembolism (PE) caused by venous thrombosis[3]. Thrombocytopenia and increased platelet reactivity after splenectomy may promote thrombosis[4, 5]. This may be related to the loss of spleen filtration. It has been previously reported that splenectomy can increase the incidence of venous thromboembolism[6]. But as a study reported, the incidence of splenectomy in CTEPH patients was similar to IPAH[7].

2018 Cologne Consensus Conference memtioned the interplay between splenectomy and several factors promoted the transformed of pulmonary embolism

into CTEPH[8]. 2015 ESC/ERS guidelines showed 3.4% of patients with CTEPH have undergone splenectomy[9]. Based on these findings, it is difficult to determine the relationship between splenectomy and CTEPH. In addition, high quality meta-analysis has been increasingly regarded as one of the key tools for achieving evidence[10, 11]. But there was no meta-analysis about this topic. So we conduct this systematic review and meta-analysis to confirm whether splenectomy can increase the risk of CTEPH.

Methods

This meta-analysis was performed according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA)[12-14]. A MeaSurement Tool to Assess systematic Reviews (AMSTAR 2) was used to assess methodological quality of this systematic review and meta-analysis[15, 16].

Search strategy

We searched the PubMed, Cochrane library and EMBASE database from databases inception to April 7, 2019, using the keywords splenectomy, splenectomies, hypertension, pulmonary, pulmonary hypertension and chronic thromboembolic pulmonary hypertension to identify all potential eligible trials. We did not have language restrictions. Reference lists of those articles relevant to the topic were hand-searched for the identifcation of potentially relevant articles. Specific search strategies are reported in the Supplementary Appendix 1.

Study selection

We selected trials based on the following inclusion criteria: (1) trials enrolling

patients diagnosed with CTEPH and reported any splenectomy profile; (2) trials only reported the prevalence of splenectomy in CTEPH or comparing the prevalence of splenectomy in CTEPH with control group. Exclusion criteria were (1) conference abstracts, reviews, case reports, animal trials, letter and other unrelated topics; (2) trials contain duplicate data.

Quality assessment

Two authors independently assessed the risk of bias of these nonrandomized studies using the Newcastle-Ottawa scale, which assesses sample representativeness and size, assesses representativeness of the cases compared with control group, comparability between CTEPH and control group, ascertainment of splenectomy, and thoroughness of descriptive statistics reporting. Studies were judged as high risk of bias when lower than 3 points, and judged as low risk of bias when higher than 3 points. Observational studies was assessed the risk of bias using an adapted version of the STROBE guidelines[17]. We evaluated 22 items to reveal the strengths and weaknesses of the trial to facilitate rational interpretation and application of trial results. The third author resolved disagreements.

Data extraction

Two authors independently extracted the following information from each trial: lead author; publication year; country of origin; study type; sample size; patients characteristics; the patients type in control group; odds ratio(OR) of splenectomy; and the prevalence of splenectomy. Disagreements were resolved by the third author.

The primary outcome was ORs of splenectomy. The secondary outcome was the

prevalence estimates of splenectomy. All the eight trials reported the prevalence of splenectomy, and five trials reported the ORs of splenectomy.

Statistical analysis

We performed meta-analysis to calculate ORs and 95%confidence intervals (CIs) of dichotomous outcome data. We also calculated the estimated effects of the prevalence of splenectomy. Forest plots showed individual studies and meta-analysis estimates[18]. We used random-effects model to pool the data and evaluate statistical heterogeneity between summary data using I^2 statistic. In this meta-analysis, I^2>50% indicates a significant heterogeneity between studies[19].

Sensitivity analysis was performed to determine the influence of each trial on the association of splenectomy with CTEPH when compared with control group.

To evaluate whether the association between splenectomy and CTEPH was changed when compared with different control group, we performed subgroup based on different control group. We performed subgroup analysis to assess whether the difference was statistically significant. Publication bias was assessed by examining funnel plots, and Egger test was performed to investigate publication bias of included trials[20, 21].

We used Review Manager 5.3 (Nordic Cochrane Centre, The Cochrane Collaboration) and Stata 13.0 (StataCorp, TX) to analyze. P<0.05 indicated a statistical significant difference.

Patient and public involvement

Patients and the public were not involved in this review.

Results

Study identification and selection

By the search strategy, 422 potentially eligible records were identified when excluded duplicate trials. We screened titles and abstracts of these records for inclusion. After excluding conference abstracts, reviews, case reports, animal trials, letter and other unrelated topics, 21 trials were reviewed full-text. Finally, eight trials[7, 22-28] involving a total of 6190 patients were included in this meta-analysis (Fig 1).

Patients characteristic of the included trials were reported in Table 1. The included trials mostly were retrospective study. The majority of patients included were from Europe and equal distributed between genders. All the trials reported the prevalence of splenectomy in CTEPH but three observational studies[26-28] did not report the association of splenectomy between CTEPH group and control group, so we only included the prevalence of splenectomy in CTEPH.

Newcastle-Ottawa score components for five individual trials appear in Table 1. One trial[25] was low quality, and four[7, 22-24] were high quality. The STROBE scores of three individual trials ranged from 17[26, 28] to 18[27](Table 1), but all trials were not described any efforts to address potential sources of bias. Furthermore, two trials did not clearly define variables[26, 27], two trials failed to report other analyses as subgroup analyses or sensitivity analyses[26, 28] and two trials did not report the source of funding[26, 28].

Splenectomy and CTEPH Risk

Five trials compared the risk of splenectomy in CTEPH patients with IPAH patients or PE patients. As shown in Fig 2, there was significant association of splenectomy with CTEPH (OR: 3.52, 95%CI: 1.49 to 6.38, I^2=0.0%). Subgroup analysis showed statistically significant association of splenectomy in CTEPH patients (OR: 3.04, 95%CI: 1.51 to 6.14, I^2=0.0%) compared with IPAH patients. And there was significant association of splenectomy in CTEPH patients (OR: 5.10, 95%CI: 1.66 to 15.68, I^2=0.0%) compared with PE patients.

We performed a sensitivity analysis to assess the weight of each trial. Sensitivity analysis in this meta-analysis was excluded each trial serially repeated, showed that no individual trial affected the overall association estimate significantly in eTable 1(Online Resource 2).

Prevalence of splenectomy in CTEPH

We performed this meta-analysis pooling of the prevalence estimates of splenectomy reported by 8 trials with a crude summary prevalence of 4.6% (122/2635 individuals; 95%CI: 0.03 to 0.06, I^2=71.5%, p<0.01) (Fig 3). The prevalence estimates reported by the individual trials ranged from 0.0% to 8.6%.

Sensitivity analysis of this study excluded each trial serially repeated, showed that no individual trial affected the overall association estimate significantly in eTable 2.

Publication bias

There was not revealed significant asymmetry through visual inspection of the funnel plot of studies reporting on splenectomy (Fig 4). Egger test did not show the significant publication bias (P=0.24).

Discussion

Results of this systematic review and meta-analysis showed statistically significant association of splenectomy in CTEPH patients compared with IPAH or PE patients. And the prevalence estimates showed that 4.6% of CTEPH patients with splenectomy. Sensitivity analysis showed that no individual trial affected the overall association estimate significantly.

The prerequisite of CTEPH may not embolism, and CTEPH probably be a consequence of thrombosis rather than embolism[29]. Splenectomy may promote venous thromboembolic disease in some speculation. After splenectomy transient thrombocytosis will appear immediately but it is not usually associated with thrombotic events[30, 31]. And it has also been reported that erythrocyte membrane components have an effect on venous thromboembolic diseases [32, 33]. In fact, anionic phospholipids of the erythrocyte membrane, including phosphatidylserine, which is known to promote the coagulation process, are localized in the intima of the cell membrane of a normal individual[33]. Abnormally exposed phosphatidylserine will promote activation of the coagulation process by immobilizing the enzyme complex in the outer erythrocyte membrane. It has been reported that the number of red blood cells with altered phosphatidylserine expression is increased by 20-fold after splenectomy in thalassemia patients[32]. These cells are also obtained as procoagulant phenotypic markers that accelerate thrombin formation. Loss of spleen filtration will result in the retention of abnormal red blood cells in the peripheral circulation after splenectomy, leading to activation of the coagulation cascade even in the absence of

chronic hemolysis.

As the results showed, 4.6% of patients with CTEPH underwent splenectomy. The prevalence of splenectomy varies widely, from 0 to 8.6%. As the 2015 ESC/ERS guidelines reported, the prevalence of splenectomy in CTEPH patients was 3.4%. The median number of prevalence we calculated was greater than guidelines memtioned. Meanwhile, the association between the CTEPH group and the control group for splenectomy was statistically significant. This is consistent with the results mentioned in the guidelines, further indicating that splenectomy is a risk factor for increasing CTEPH.

To our knowledge, this is the first systematic review and meta-analysis to explore the association between splenectomy and CTEPH. However, this study has some limitations. Firstly, the trials we included were not random control trials and had small sample size, it might cause bias. Secondly, trauma is the main indication for splenectomy and the surgery after traumatic abdominal may be a relevant factor of thromboembolism. Therefore, trauma and the surgery may cause substantial heterogeneity in this study, but we did not have enough information to further explore.

In conclusion, our study found that the prevalence of splenectomy in CTEPH was 4.6%, and CTEPH was associated with splenectomy. High-quality prospective trials were needed to further explore the (causal) relationship between CTEPH and splenectomy.

Authors' contributions

LZ and PY analyzed the patient data and were the major contributors in the preparation of the manuscript. SW and KY analyzed part of the patient data. XZ performed the literature search and extracted the data. XC was responsible for the statistical analysis. LL made substantial contributions to the conception of the study. MZ and YC drafted the manuscript. All the authors have read and approved the final version of this manuscript.

References

1. Kim NH, Delcroix M, Jenkins DP, et al. Chronic thromboembolic pulmonary hypertension. *J Am Coll Cardiol*. 2013;62:D92-99.
2. Lewczuk J, Piszko P, Jagas J, et al. Prognostic factors in medically treated patients with chronic pulmonary embolism. *Chest*. 2001;119:818-823.
3. Lang IM. Chronic thromboembolic pulmonary hypertension--not so rare after all. *The New England journal of medicine*. 2004;350:2236-2238.
4. Singer ST, Kuypers F, Fineman J, et al. Elevated tricuspid regurgitant jet velocity in subgroups of thalassemia patients: insight into pathophysiology and the effect of splenectomy. *Annals of hematology*. 2014;93:1139-1148.
5. Villagra J, Shiva S, Hunter LA, et al. Platelet activation in patients with sickle disease, hemolysis-associated pulmonary hypertension, and nitric oxide scavenging by cell-free hemoglobin. *Blood*. 2007;110:2166-2172.
6. Boyle S, White RH, Brunson A, et al. Splenectomy and the incidence of venous thromboembolism and sepsis in patients with immune thrombocytopenia. *Blood*. 2013;121:4782-4790.
7. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.
8. Wilkens H, Konstantinides S, Lang IM, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): Updated Recommendations from the Cologne Consensus Conference 2018. *International journal of cardiology*. 2018;272s:69-78.
9. Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2016;37:67-119.
10. Yao L, Sun R, Chen YL, et al. The quality of evidence in Chinese meta-analyses needs to be improved. *Journal of clinical epidemiology*. 2016;74:73-79.
11. Tian J, Zhang J, Ge L, et al. The methodological and reporting quality of systematic reviews from China and the USA are similar. *Journal of clinical epidemiology*. 2017;85:50-58.
12. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration.

BMJ (Clinical research ed). 2009;339:b2700.

13. Ge L, Tian JH, Li YN, et al. Association between prospective registration and overall reporting and methodological quality of systematic reviews: a meta-epidemiological study. *Journal of clinical epidemiology*. 2018;93:45-55.

14. Wang X, Chen Y, Yao L, et al. Reporting of declarations and conflicts of interest in WHO guidelines can be further improved. *Journal of clinical epidemiology*. 2018;98:1-8.

15. Pieper D, Buechter RB, Li L, et al. Systematic review found AMSTAR, but not R(evised)-AMSTAR, to have good measurement properties. *Journal of clinical epidemiology*. 2015;68:574-583.

16. Yan P, Yao L, Li H, et al. The methodological quality of robotic surgical meta-analyses needed to be improved: a cross-sectional study. *Journal of clinical epidemiology*. 2019;109:20-29.

17. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of clinical epidemiology*. 2008;61:344-349.

18. Xu R, Ding S, Zhao Y, et al. Autologous transplantation of bone marrow/blood-derived cells for chronic ischemic heart disease: a systematic review and meta-analysis. *The Canadian journal of cardiology*. 2014;30:1370-1377.

19. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed)*. 2003;327:557-560.

20. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed)*. 1997;315:629-634.

21. Sterne JA and Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal of clinical epidemiology*. 2001;54:1046-1055.

22. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.

23. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.

24. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.

25. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.

26. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.

27. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.

28. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.

29. Frey MK, Alias S, Winter MP, et al. Splenectomy is modifying the vascular remodeling of thrombosis. *Journal of the American Heart Association*. 2014;3:e000772.

30. Boxer MA, Braun J and Ellman L. Thromboembolic risk of postsplenectomy thrombocytosis. *Archives of surgery (Chicago, Ill : 1960)*. 1978;113:808-809.

31. Visudhiphan S, Ketsa-Ard K, Piankijagum A, et al. Blood coagulation and platelet profiles in

persistent post-splenectomy thrombocytosis. The relationship to thromboembolism. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*. 1985;39:264-271.

32. Kuypers FA, Yuan J, Lewis RA, et al. Membrane phospholipid asymmetry in human thalassemia. *Blood*. 1998;91:3044-3051.

33. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/beta-thalassaemic patients. *British journal of haematology*. 2002;118:893-898.

Table1: Characteristics of Included Studies

No.	Author	Year	Location	Study type	Number of patients	Age	Proportion of females(%)	BMI	Control	NOS scores	STROBE scores
					CTEPH/Control	CTEPH/Control	CTEPH/Control	CTEPH/Control			
1	X Jais[22]	2005	France	Case-control study	257/276	51/46	47.4/60	NA	IPAH	6	NA
2	D. Bonderman[23]	2009	Europe	Retrospective cohort study	433/254	58/50.5	52.4/65.8	1.8/1.81	IPAH	7	NA
3	Martinez C[24]	2018	England	Case-control study	283/2356	NA	54.1/51.7	NA	PE	6	NA
4	Irene M. Lang[7]	2013	Europe	Case-control study	436/158	65/59	49.3/66.5	NA	IPAH	6	NA
5	Nicolas Coquoz[25]	2018	Switzerland	Cohort study	4/504	47/61.3	75/46.4	33/28	PE	3	NA
6	Joanna Pepke-Zaba[27]	2015	Europe and Canada	Observational study	679/NA	63/NA	49.9/NA	NA	NA	NA	18
7	Bohacekova M[28]	2016	Slovakia	Observational study	81/NA	60.5/NA	62.9/NA	27.8/NA	NA	NA	17
8	R. Condliffe[26]	2009	UK	Observational study	469/NA	NA/NA	NA	NA	NA	NA	17

No.: number; BMI: body mass index; CTEPH: chronic thromboembolic pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; PE: pulmonary thromboembolism; CTEPH/Control: CTEPH group/control group

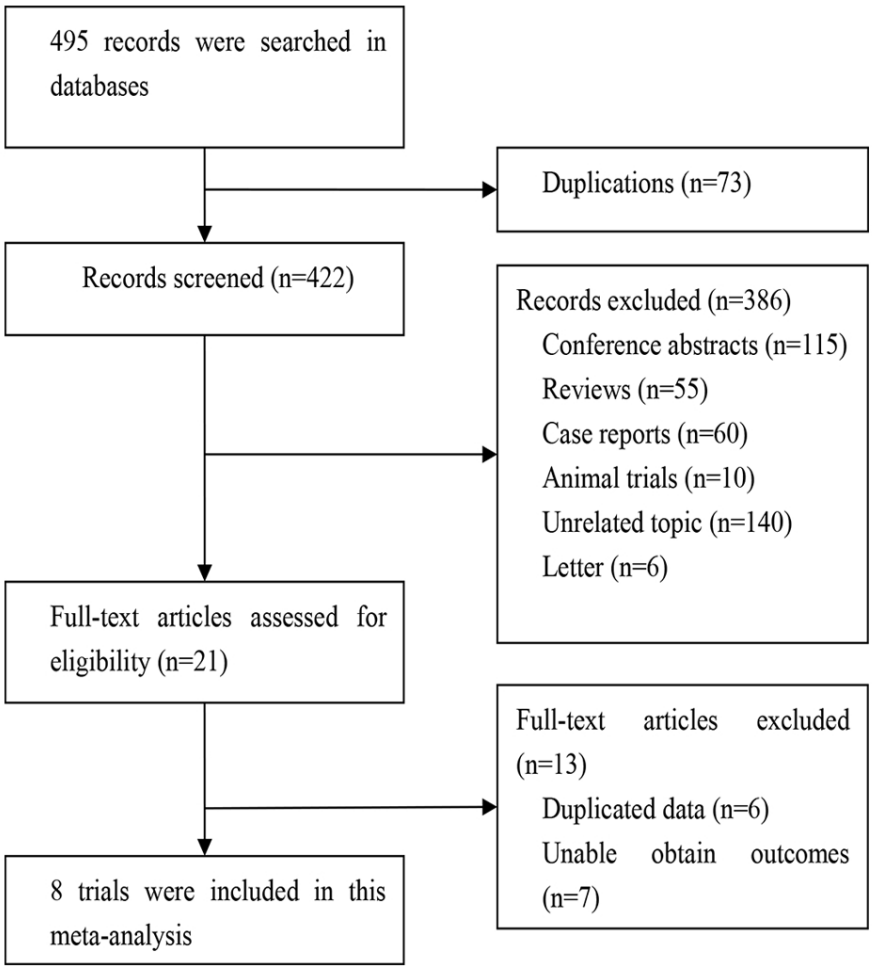
Figure legends

Fig 1 Flow chart of study search and selection process

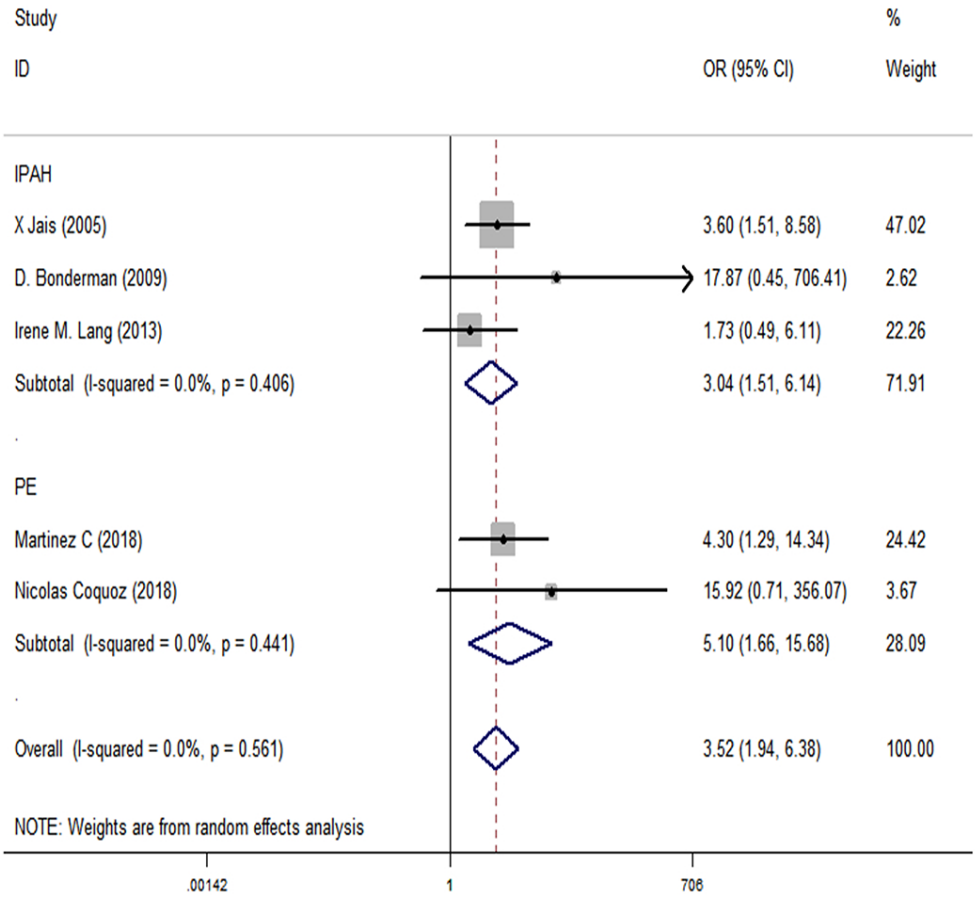
Fig 2 Forest plot with meta-analysis of the association of splenectomy between chronic thromboembolic pulmonary hypertension, idiopathic pulmonary arterial hypertension and pulmonary thromboembolism

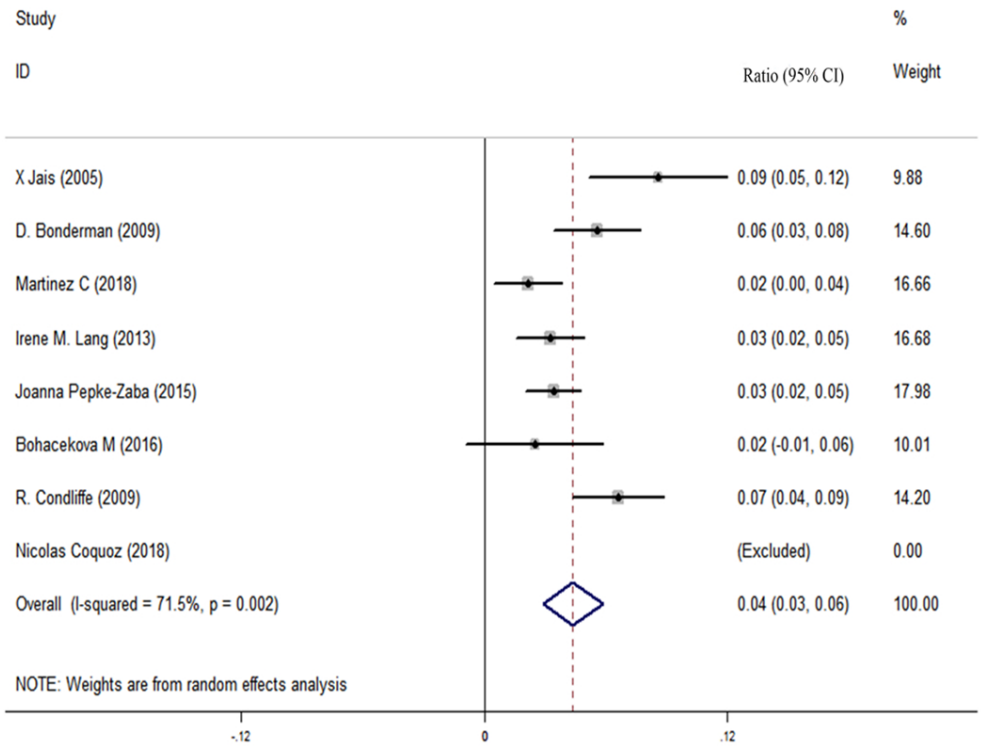
Fig 3 Forest plot with meta-analysis of the prevalence and 95% CI of splenectomy in patients with chronic thromboembolic pulmonary hypertension in the assessed studies

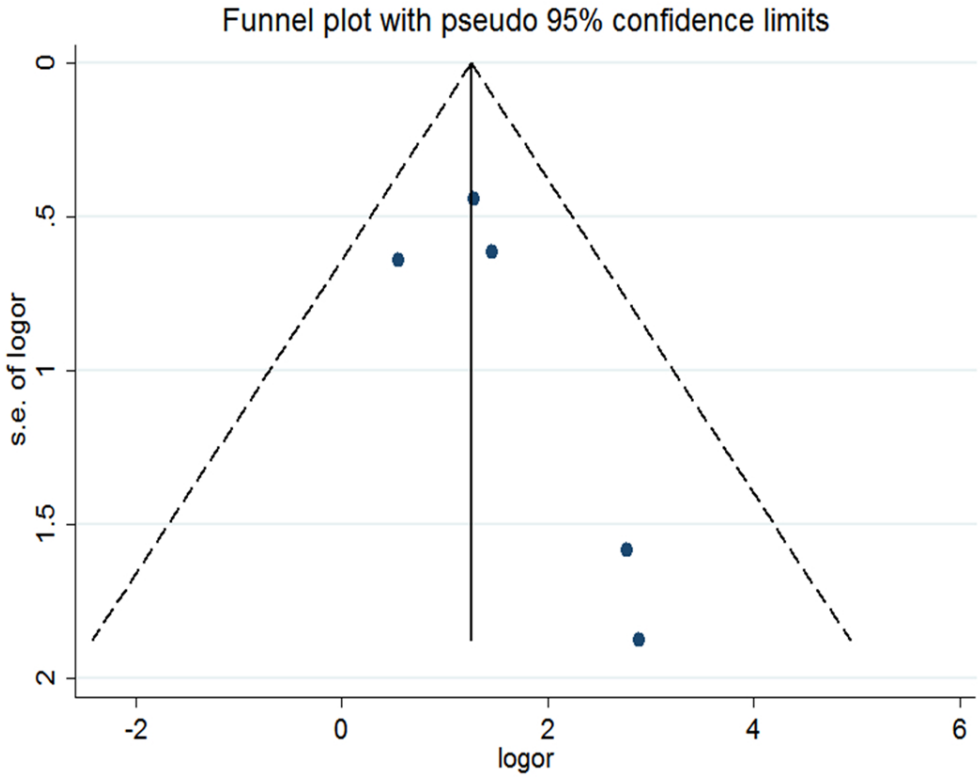
Fig 4 Funnel plot to assess publication bias



90x90mm (300 x 300 DPI)







90x79mm (300 x 300 DPI)

Search strategy

Pubmed

(((splenectomies[MeSH Terms]) OR splenectomy)) AND (((Hypertension, Pulmonary[MeSH Terms]) OR pulmonary hypertension) OR chronic thromboembolic pulmonary hypertension)

The Cochrane library

#1 MeSH descriptor: [Hypertension, Pulmonary] explode all trees

#2 (Chronic thromboembolic pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#3 (pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#4 MeSH descriptor: [Splenectomy] explode all trees

#5 (splenectomies):ti,ab,kw (Word variations have been searched)

#6 #1 OR #2 OR #3

#7 #4 OR #5

#8 #6 AND #7

Embase

#1. 'chronic thromboembolic pulmonary hypertension'/exp

#2. 'pulmonary hypertension':ab,ti,kw

#3. 'splenectomy'/exp

#4. 'splenectomies':ab,ti,kw

#5. #1 OR #2

#6. #3 OR #4

#7. #5 AND #6

eTable 1. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No of studies	No of participants	OR[95%CI]
X Jais[1]	4	1151	3.45[1.52,7.81]
D. Bonderman[2]	4	975	3.37[1.84,6.15]
Martinez C[3]	4	1125	3.30[1.66,6.54]
Irene M. Lang[4]	4	977	4.31[2.19,8.47]
Nicolas Coquoz[5]	4	1404	3.32[1.81,6.09]

eTable 2. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No of studies	No of participants	Ratio[95%CI]
X Jais[1]	6	2374	0.39[0.26,0.52]
D. Bonderman[2]	6	2198	0.42[0.25,0.58]
Martinez C[3]	6	2348	0.48[0.32,0.63]
Irene M. Lang[4]	6	2200	0.46[0.28,0.64]
Nicolas Coquoz[5]	7	2631	0.43[0.29,0.58]
Joanna Pepke-Zaba[6]	6	1954	0.46[0.27,0.65]
Bohacekova M[7]	6	2550	0.46[0.30,0.62]
R. Condliffe[8]	6	2162	0.39[0.25,0.54]

No.: number; OR: odds ratio; 95%CI: 95%confidence intervals

References

1. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.
2. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.
3. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.
4. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.

5. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.

6. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.

7. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.

8. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

BMJ Open

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-038385.R1
Article Type:	Original research
Date Submitted by the Author:	03-Aug-2020
Complete List of Authors:	Zhang, Liyan; School of Basic Medicine, Gansu University of Chinese Medicine, Pei Jing, Yan; West China School of Public Health and West China Fourth Hospital, Sichuan University, Department of Epidemiology and Health Statistics Yang, Kehu; evidence based meidicine center, lanzhou university Wu, Shanlian; School of Basic Medicine, Gansu University of Chinese Medicine, School of Basic Medicine Zhu, Xin-Yu; Gansu Provincial Hospital, Department of Science and Research Chen, Xiaojie; Gansu Provincial Hospital, Department of Science and Research Li, Li; Gansu Provincial Hospital, Department of Science and Research Cao, Yunshan; Gansu Provincial Hospital, Department of Cardiology Zhang, Min; Gansu Provincial Hospital, Department of Pathology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine, Respiratory medicine
Keywords:	Respiratory physiology < THORACIC MEDICINE, Adult surgery < SURGERY, Adult thoracic medicine < THORACIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE PAGE

Title

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Running Title: Splenectomy and CTEPH

Authors' names and affiliations

Liyan Zhang[1,2,3,4+], BS, Peijing Yan[5+], MS, Kehu Yang[3,4,6], MD, Shanlian Wu[1,2], MS, Xinyu Zhu[7], MS, Xiaojie Chen[7], MS, Li Li[7], MS, Yunshan Cao[8*], MD, PhD, Min Zhang[2*], MD, PhD

(1) School of Basic Medicine, Gansu University of Chinese Medicine, Lanzhou, 730000, China

(2) Department of Pathology, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(3) Institute of Clinical Research and Evidence Based Medicine, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(4) Evidence Based Medicine Center, School of Basic Medical Sciences, Lanzhou University, Lanzhou, 730000, China

(5) Department of Epidemiology and Health Statistics, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, Sichuan, 610044, China

(6) Key Laboratory of Evidence-based Medicine and Knowledge Translation of Gansu Province, Lanzhou, 730000, China

(7) Department of Science and Research, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

(8) Department of Cardiology, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

[+]: Contributed equally

[*]: Corresponding author

Corresponding author

Min Zhang, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail: sallyzhangmin@126.com

Yunshan Cao, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail: yunshancao@126.com

Acknowledgement of grant support

This study was supported by the National Natural Science Foundation of China (grant no. 81860059), the International Cooperation Exchange Project of Gansu province (grant no. 18YF1WA046) and CAS 'Light of West China' Program granted to MZ and YC.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Data sharing statement

No additional data available.

Abstract

Objectives: Whether splenectomy increases the risk of chronic thromboembolic pulmonary hypertension (CTEPH) remains unclear. We conducted a systematic review and meta-analysis to explore the association between splenectomy and CTEPH.

Methods: The PubMed, Embase, and Cochrane Library databases were systematically searched for records of splenectomy and CTEPH. The Newcastle-Ottawa scale and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used to assess the quality of the included studies and each quality item was graded as low risk or high risk. The random-effects model was used to calculate different effective values.

Results: In total, eight trials involving 6190 participants fulfilled the inclusion criteria. The overall pooled crude prevalence of splenectomy was 4.0% (95%CI: 0.03 to 0.06, I^2=71.5%, p<0.01) in the CTEPH patients. Subgroup analysis showed a statistically significant high incidence of splenectomy in the CTEPH patients (OR=3.04, 95%CI: 1.51 to 6.14, I^2=0.0%) compared to that in idiopathic pulmonary arterial hypertension (IPAH) patients. There was a significantly high incidence of splenectomy in the CTEPH patients (OR=5.10, 95%CI: 1.66 to 15.68, I^2=0.0%) compared to that in pulmonary thromboembolism (PE) patients.

Conclusion: The prevalence of splenectomy in CTEPH patients was 4.0%, and CTEPH might be associated with splenectomy. However, high-quality prospective trials are needed.

PROSPERO registration number: CRD42020137591.

Keywords: Pulmonary hypertension, chronic thromboembolic pulmonary hypertension, splenectomy, systematic review, meta-analysis

Strengths and limitations of this study

This systematic review focuses on the prevalence of splenectomy, and also evaluates the association of splenectomy in the CTEPH patients compare to that in PAH or PE patients.

Absence of evident publication bias increases the reliability of our findings.

However, the trials included were not randomized controlled trials and sample size was small.

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a disease of pulmonary artery obstruction and non-obstructive pulmonary artery remodeling as a consequence of pulmonary artery thromboembolism, which eventually leads to right heart failure and death[1]. CTEPH, a well-known long-lasting complication of acute pulmonary thromboembolism (PE) associated with poor thrombus resolution and altered pulmonary artery hemodynamics[2] is considered post-pulmonary embolism syndrome[3]. In long-term follow-up, the mortality of CTEPH was high, and with increases in pulmonary artery pressure, the mortality rate gradually increased[4]. Lupus

anticoagulant and antiphospholipid antibodies and coagulation factor FVIII have been associated with CTEPH[5, 6].

Splenectomy can also increase the incidence of venous thromboembolism[7]. The 2018Cologne Consensus Conference mentioned interplay splenectomy and several factors were shown to promote the transformation of a pulmonary embolism into a fibrotic vascular occlusion[8]. Previous studies reported that 2.1% to 8.6% of the patients with CTEPH had undergone splenectomy[9, 10]. Another study showed that the incidence of splenectomy in CTEPH patients was similar to that in idiopathic pulmonary arterial hypertension (IPAH) patients[11]. Based on these findings, it is difficult to determine the relationship between splenectomy and CTEPH. Therefore, this systematic review and meta-analysis were conducted to confirm whether splenectomy increased the risk of CTEPH.

Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA)[12-14]. The MeaSurement Tool to Assess systematic Reviews (AMSTAR 2) was used to assess the methodological quality of this systematic review and meta-analysis[15, 16], and this study was registered in PROSPERO (registration number: CRD42020137591).

Search strategy

PubMed, the Cochrane library, and the EMBASE database were searched from the database inceptions to April 7, 2019, using the keywords splenectomy, splenectomies, hypertension, pulmonary, pulmonary hypertension, and chronic

thromboembolic pulmonary hypertension to identify all potentially eligible trials. No language restrictions were imposed. The reference lists of articles relevant to the topic were hand-searched to identify other potentially relevant articles. The specific search strategies are reported in Supplementary Appendix 1.

Study selection

Trials were selected that enrolled patients diagnosed with CTEPH and reported any splenectomy profile, and trials that reported the prevalence of splenectomy in CTEPH patients. The exclusion criteria were conference abstracts, reviews, case reports, animal trials, letters, and other unrelated topics, and trials that contained duplicate data.

Quality assessment

Two authors independently assessed the risk of bias in the nonrandomized studies using the Newcastle-Ottawa Scale, which assesses sample representativeness and size, and assessed the representativeness of the cases compared with IPAH or PE, the comparability between CTEPH and IPAH or PE, ascertainment of splenectomy, and the thoroughness of descriptive statistics reporting. Studies with scores of less than 3 points were judged as having a high risk of bias, and as a low risk of bias with more than 3 points. The risk of bias in the observational studies was assessed using an adapted version of the STROBE guidelines[17]. Twenty-two items were evaluated to reveal the strengths and weaknesses of the trials to facilitate rational interpretation and the application of the trial results. The third author resolved disagreements.

Data extraction

Two authors independently extracted the following information from each trial: lead author, publication year, country of origin, study type, sample size, patient characteristics, the patient type in the control group, the odds ratio (OR) of splenectomy, and the prevalence of splenectomy. Disagreements were resolved by the third author.

The primary outcome was the ORs of splenectomy. The secondary outcome was the prevalence estimates of splenectomy. All eight trials reported the prevalence of splenectomy and five trials reported the ORs of splenectomy.

Statistical analysis

A meta-analysis was performed to calculate the ORs and 95%confidence intervals (CIs) of the dichotomous outcome data. The prevalence of splenectomy was also calculated. Forest plots showed the individual studies and the meta-analysis estimates[18]. A random-effects model was used to pool the data and evaluate the statistical heterogeneity between the summary data using the I^2 statistic. In this meta-analysis, an I^2>50% indicated a significant heterogeneity between studies[19].

Sensitivity analysis was performed to determine the influence of each trial on the association of splenectomy with CTEPH compared to the control group.

To evaluate whether the association between splenectomy and CTEPH was changed when compared with different control groups, subgroup analyses were performed based on different control groups. Publication bias was assessed by examining funnel plots and by the Egger test[20, 21].

Review Manager 5.3 (Nordic Cochrane Centre, The Cochrane Collaboration) and

Stata 13.0 (StataCorp, TX) were used to analyze the data. $P<0.05$ indicated a statistically significant difference.

Patient and public involvement

Patients and the public were not involved in this review.

Results

Study identification and selection

By the search strategy, 422 potentially eligible records were identified when duplicate trials were excluded. The titles and abstracts of the identified records were screened for inclusion. After excluding conference abstracts, reviews, case reports, animal trials, letters, and other unrelated topics, the full text of 21 trials was reviewed. Finally, eight trials[6, 9-11, 22-25] involving a total of 6190 patients were included in the meta-analysis (Fig. 1).

The patient characteristics in the included trials are reported in Table 1. The included trials were mostly retrospective studies. The majority of patients included were from Europe and equally distributed between genders. All the trials reported the prevalence of splenectomy in CTEPH patients but three observational studies[23-25]did not report the incidence of splenectomy between the CTEPH group and the IPAH or PE group, so only the prevalence of splenectomy in the CTEPH patients was included. And only two trials reported the causes of the splenectomies[9, 23].

The Newcastle-Ottawa score components for the five individual trials appear in Table 1. One trial[22] was low quality, and four[6, 9-11] were of high quality. The STROBE scores of the three individual trials ranged from 17[23, 25] to 18[24] (Table 1), but no trials

described any efforts to address potential sources of bias. Furthermore, two trials did not clearly define the variables[23, 24], two trials failed to report other analyses such as subgroup or sensitivity analyses[23, 25] and two trials did not report the source of funding[23, 25].

Prevalence of splenectomy in CTEPH

The pooled crude prevalence of splenectomy in CTEPH patients from eight trials was 4.0% (95%CI: 0.03 to 0.06, I^2=71.5%, P<0.01) (Fig. 2).The prevalence reported by the individual trials ranged from 2.0% to 9.0%.

Sensitivity analysis of this study excluded each serially repeated trials and showed that no individual trial significantly affected the overall prevalence of splenectomy in the CTEPH patients (eTable 1).

Comparisons of incidence of splenectomy among CTEPH, IPAH, and PE patients

Five trials compared the incidence of splenectomy in CTEPH patients with that in IPAH patients or PE patients. As shown in Fig. 3, subgroup analysis showed a statistically significant high incidence of splenectomy in the CTEPH patients (OR=3.04, 95%CI: 1.51 to 6.14, I^2=0.0%) compared to that in the IPAH patients. There was also a significantly high incidence of splenectomy in the CTEPH patients (OR =5.10, 95%CI: 1.66 to 15.68, I^2=0.0%) compared to that in the PE patients.

A sensitivity analysis was performed to assess the weight of each trial. Sensitivity analysis in this meta-analysis excluded each serially repeated trial and showed that no individual trial significantly affected the overall incidence of splenectomy in the CTEPH patients and IPAH or PE patients (eTable 2).

Publication bias

No significant asymmetry was apparent by visual inspection of the funnel plot of studies reporting on splenectomies (Fig. 4). The Egger test did not show significant publication bias (P=0.24).

Discussion

The results of this systematic review and meta-analysis showed a statistically significant high incidence of splenectomies in CTEPH patients compared to IPAH or PE patients. It showed that splenectomy could be significantly associated with CTEPH. The pooled prevalence of CTEPH patients with splenectomies was 4.0%. Sensitivity analysis showed that no individual trial significantly affected the overall incidence.

The prerequisite for CTEPH may be both *in situ* thrombosis and embolism[26]. Patients undergoing splenectomy may have significant enrichment of anion phospholipids[27] and platelet-derived microparticles (MP)[28]. These MPs contribute to thrombus formation by acting as pro-coagulants by providing a negatively charged surface for the assembly of coagulation proteases[29]. Erythrocyte membrane components have been reported to have an effect on venous thromboembolic diseases [30, 31]. The number of red blood cells with altered phosphatidylserine expression was increased 20-fold after splenectomies in thalassemia patients[30]. These cells are also procoagulant phenotypic markers that accelerate thrombin formation. Also, the loss of splenic filtration will result in the retention of abnormal red blood cells in the peripheral circulation after splenectomy, leading to the activation of the coagulation

cascade, even in the absence of chronic hemolysis.

Therefore, we suggest that the development of thrombotic complications in patients undergoing splenectomy should be monitored closely by routine electrocardiogram (ECG) and/or echocardiography[32]. Splenectomized patients who present with exertional dyspnea, ECG with right ventricular overload (RVO), and right heart enlargement and/or elevated pulmonary arterial pressure by echocardiography should be referred to the center of pulmonary hypertension for further assessment[32].

In conclusion, this study found that the prevalence of splenectomy in CTEPH was 4.0%, and CTEPH was associated with splenectomy. High-quality prospective trials are needed to further explore the (causal) relationship between CTEPH and splenectomy.

Limitations

Firstly, the trials included were not randomized controlled trials and had small sample sizes, which might cause bias. Secondly, trauma is the main indication for splenectomy, and surgery after a traumatic abdominal injury may be a relevant factor in thromboembolism. Therefore, trauma and surgery may have caused substantial heterogeneity in this study. However, there was not enough information to explore these factors further. Thirdly, many hematological disorders responsible for splenectomy are confounding factors for CTEPH, but there was insufficient information for subgroup analysis.

Authors' contributions

LZ and PY analyzed the patient data and were the major contributors in the

preparation of the manuscript. SW and KY analyzed part of the patient data. XZ performed the literature search and extracted the data. XC was responsible for the statistical analysis. LL made substantial contributions to the conception of the study. MZ and YC drafted the manuscript. All the authors have read and approved the final version of this manuscript.

References

1. Kim NH, Delcroix M, Jais X, et al. Chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2019;53.
2. Lang IM, Pesavento R, Bonderman D, et al. Risk factors and basic mechanisms of chronic thromboembolic pulmonary hypertension: a current understanding. *The European respiratory journal*. 2013;41:462-468.
3. Klok FA, van der Hulle T, den Exter PL, et al. The post-PE syndrome: a new concept for chronic complications of pulmonary embolism. *Blood reviews*. 2014;28:221-226.
4. Riedel M, Stanek V, Widimsky J, et al. Longterm follow-up of patients with pulmonary thromboembolism. Late prognosis and evolution of hemodynamic and respiratory data. *Chest*. 1982;81:151-158.
5. Wolf M, Boyer-Neumann C, Parent F, et al. Thrombotic risk factors in pulmonary hypertension. *The European respiratory journal*. 2000;15:395-399.
6. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.
7. Boyle S, White RH, Brunson A, et al. Splenectomy and the incidence of venous thromboembolism and sepsis in patients with immune thrombocytopenia. *Blood*. 2013;121:4782-4790.
8. Wilkens H, Konstantinides S, Lang IM, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): Updated Recommendations from the Cologne Consensus Conference 2018. *International journal of cardiology*. 2018;272s:69-78.
9. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.
10. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.
11. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.
12. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ (Clinical research ed)*. 2009;339:b2700.
13. Ge L, Tian JH, Li YN, et al. Association between prospective registration and overall reporting and methodological quality of systematic reviews: a meta-epidemiological study. *Journal of clinical epidemiology*. 2018;93:45-55.

14. Wang X, Chen Y, Yao L, et al. Reporting of declarations and conflicts of interest in WHO guidelines can be further improved. *Journal of clinical epidemiology*. 2018;98:1-8.
15. Pieper D, Buechter RB, Li L, et al. Systematic review found AMSTAR, but not R(evised)-AMSTAR, to have good measurement properties. *Journal of clinical epidemiology*. 2015;68:574-583.
16. Yan P, Yao L, Li H, et al. The methodological quality of robotic surgical meta-analyses needed to be improved: a cross-sectional study. *Journal of clinical epidemiology*. 2019;109:20-29.
17. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of clinical epidemiology*. 2008;61:344-349.
18. Xu R, Ding S, Zhao Y, et al. Autologous transplantation of bone marrow/blood-derived cells for chronic ischemic heart disease: a systematic review and meta-analysis. *The Canadian journal of cardiology*. 2014;30:1370-1377.
19. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed)*. 2003;327:557-560.
20. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed)*. 1997;315:629-634.
21. Sterne JA and Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal of clinical epidemiology*. 2001;54:1046-1055.
22. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.
23. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.
24. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.
25. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.
26. Frey MK, Alias S, Winter MP, et al. Splenectomy is modifying the vascular remodeling of thrombosis. *Journal of the American Heart Association*. 2014;3:e000772.
27. Frey MK, Alias S, Winter MP, et al. Splenectomy is modifying the vascular remodeling of thrombosis. *Journal of the American Heart Association*. 2014;3:e000772.
28. Fontana V, Jy W, Ahn ER, et al. Increased procoagulant cell-derived microparticles (C-MP) in splenectomized patients with ITP. *Thrombosis research*. 2008;122:599-603.
29. Peacock AJ. Pulmonary hypertension after splenectomy: a consequence of loss of the splenic filter or is there something more? *Thorax*. 2005;60:983-984.
30. Kuypers FA, Yuan J, Lewis RA, et al. Membrane phospholipid asymmetry in human thalassemia. *Blood*. 1998;91:3044-3051.
31. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/beta-thalassaemic patients. *British journal of haematology*. 2002;118:893-898.
32. Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS):

Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2016;37:67-119.

Table1: Characteristics of Included Studies

No.	Author	Year	Location	Study type	Number of patients	Age (years)	Proportion of females (%)	BMI (kg·m^{-2})	Control	NOS scores	STROBE scores
					CTEPH/Control	CTEPH/Control	CTEPH/Control	CTEPH/Control			
1	X Jaïs[9]	2005	France	Case-control study	257/276	51.0/46.0	47.4/60.0	—	IPAH	6	—
2	Diana Bonderman[6]	2009	Europe	Retrospective cohort study	433/254	58.0/50.5	52.4/65.8	26.2/25.2	IPAH	7	—
3	Carlos Martinez[10]	2018	England	Case-control study	283/2356	—	54.1/51.7	—	PE	6	—
4	Irene M. Lang[11]	2013	Europe	Case-control study	436/158	65.0/59.0	49.3/66.5	—	IPAH	6	—
5	Nicolas Coquoz[22]	2018	Switzerland	Cohort study	4/504	47.0/61.3	75.0/46.4	33.0/28.0	PE	3	—
6	Joanna Pepke-Zaba[24]	2015	Europe and Canada	Observational study	679/—	63.0/—	49.9/—	—	—	—	18
7	M.Bohacekova[25]	2016	Slovakia	Observational study	81/—	60.5/—	62.9/—	27.4/—	—	—	17
8	R. Condliffe[23]	2009	UK	Observational study	469/—	—	—	—	—	—	17

BMI: body mass index; CTEPH: chronic thromboembolic pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; PE: pulmonary thromboembolism; CTEPH/Control: CTEPH group/control group

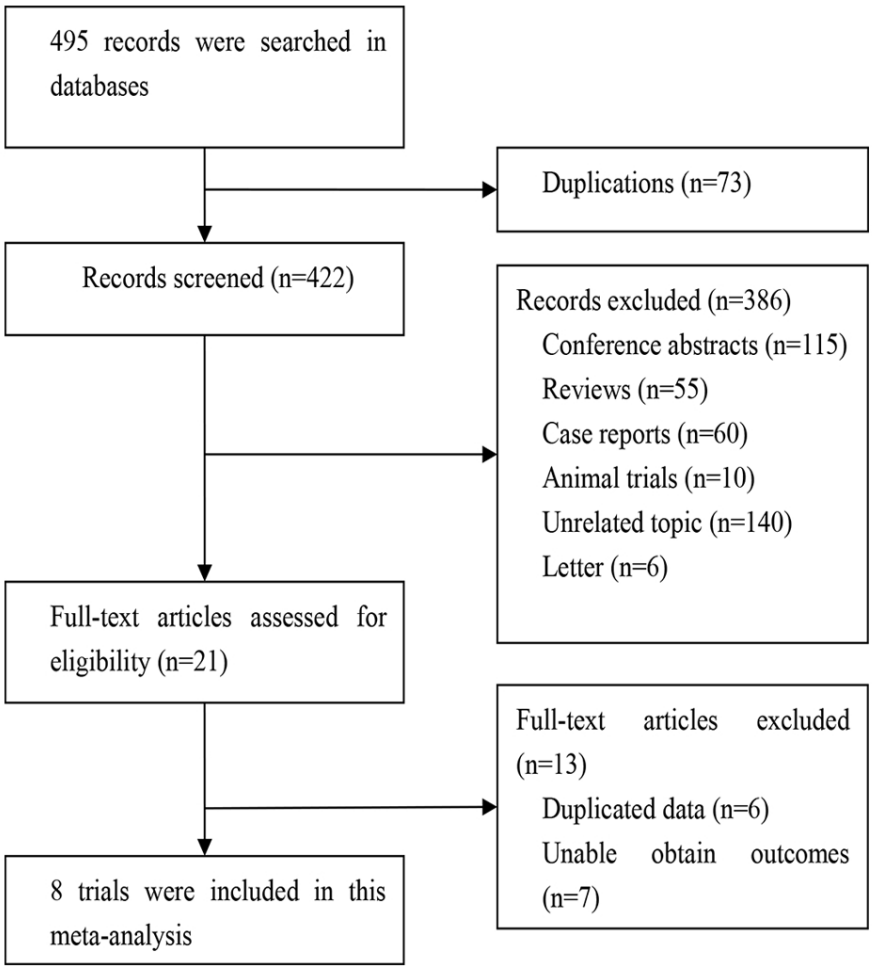
Figure legends

Fig. 1 Flow chart of study search and selection process

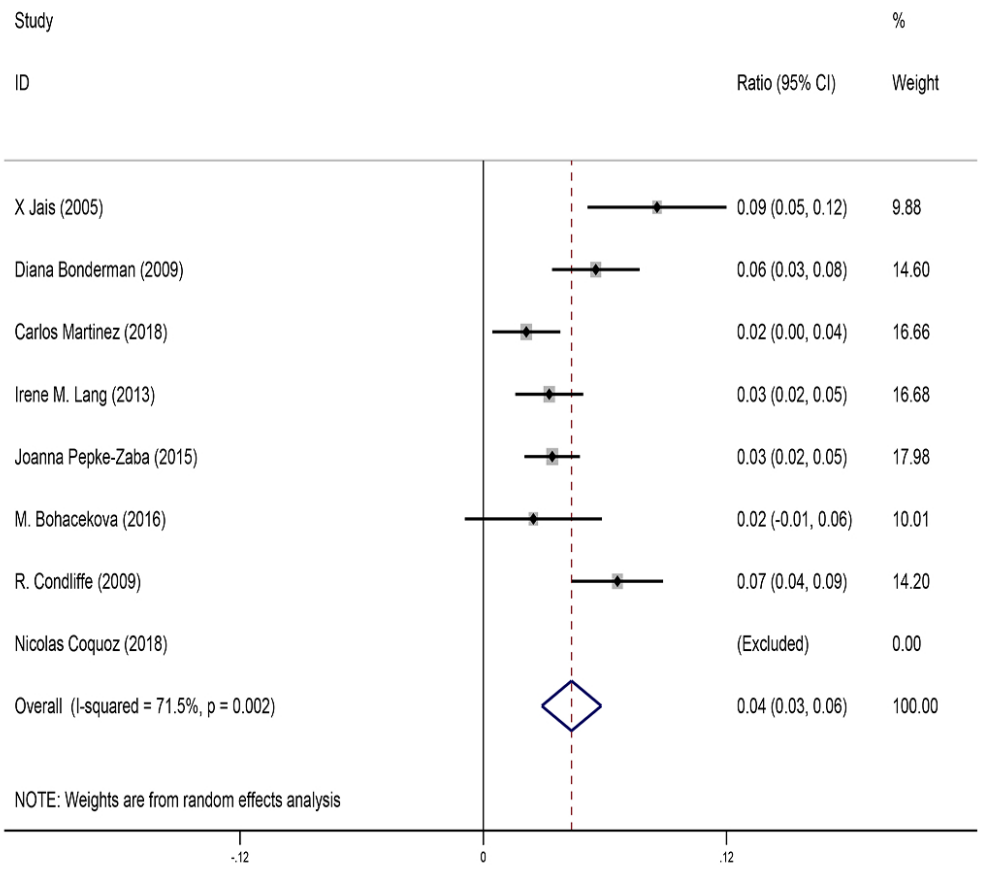
Fig. 2 Forest plot with meta-analysis of the prevalence and 95% CI of splenectomy in patients with chronic thromboembolic pulmonary hypertension in the assessed studies

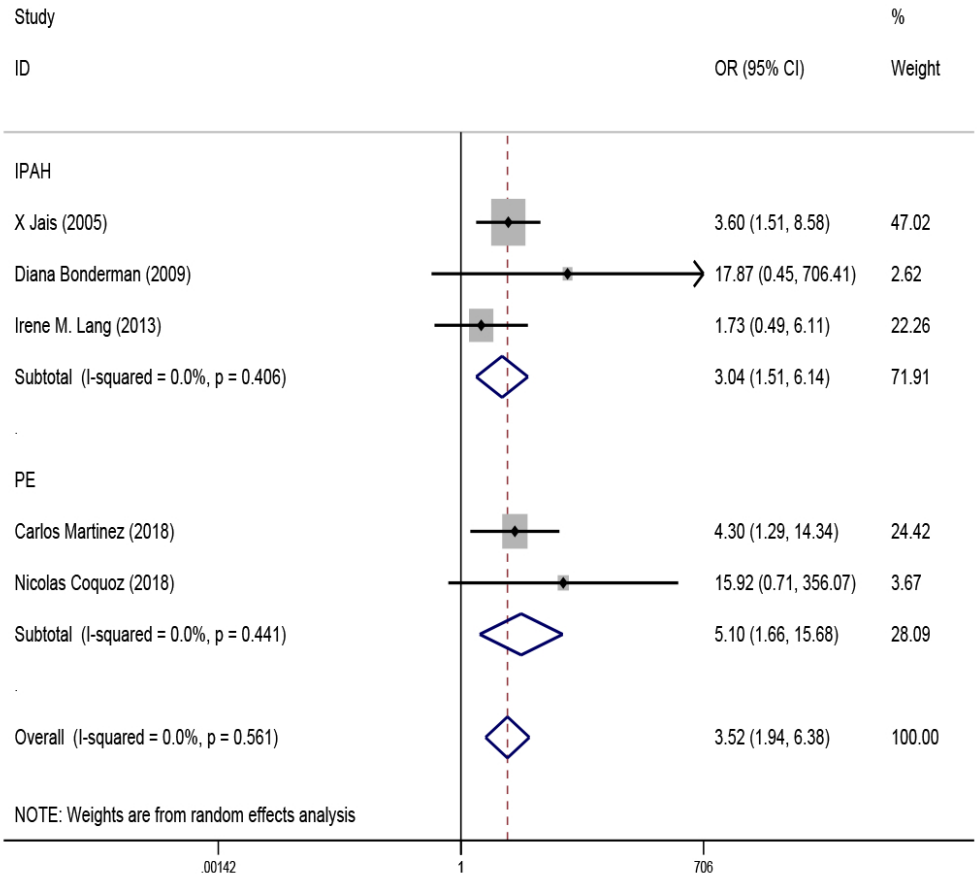
Fig. 3 Forest plot with meta-analysis of the association of splenectomy between chronic thromboembolic pulmonary hypertension, idiopathic pulmonary arterial hypertension and pulmonary thromboembolism

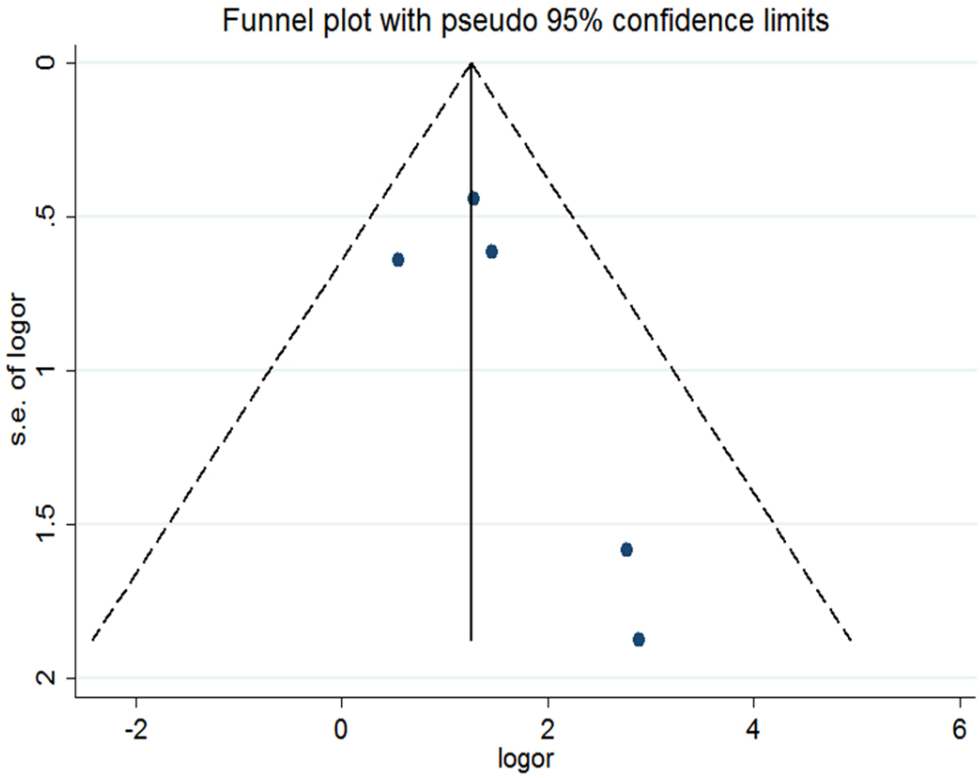
Fig. 4 Funnel plot to assess publication bias



90x90mm (300 x 300 DPI)







90x79mm (300 x 300 DPI)

Search strategy

Pubmed

(((splenectomies[MeSH Terms]) OR splenectomy)) AND (((Hypertension, Pulmonary[MeSH Terms]) OR pulmonary hypertension) OR chronic thromboembolic pulmonary hypertension)

The Cochrane library

#1 MeSH descriptor: [Hypertension, Pulmonary] explode all trees

#2 (Chronic thromboembolic pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#3 (pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#4 MeSH descriptor: [Splenectomy] explode all trees

#5 (splenectomies):ti,ab,kw (Word variations have been searched)

#6 #1 OR #2 OR #3

#7 #4 OR #5

#8 #6 AND #7

Embase

#1. 'chronic thromboembolic pulmonary hypertension'/exp

#2. 'pulmonary hypertension':ab,ti,kw

#3. 'splenectomy'/exp

#4. 'splenectomies':ab,ti,kw

#5. #1 OR #2

#6. #3 OR #4

#7. #5 AND #6

eTable 1. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No. of studies	No. of participants	Ratio[95%CI]
X Jaïs[1]	6	2374	0.39[0.26,0.52]
Diana Bonderman[2]	6	2198	0.42[0.25,0.58]
Carlos Martinez [3]	6	2348	0.48[0.32,0.63]
Irene M. Lang[4]	6	2200	0.46[0.28,0.64]
Nicolas Coquoz[5]	7	2631	0.43[0.29,0.58]
Joanna Pepke-Zaba[6]	6	1954	0.46[0.27,0.65]
M. Bohacekova[7]	6	2550	0.46[0.30,0.62]
R. Condliffe[8]	6	2162	0.39[0.25,0.54]

eTable 2. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No. of studies	No. of participants	OR[95%CI]
X Jaïs[1]	4	1151	3.45[1.52,7.81]
Diana Bonderman[2]	4	975	3.37[1.84,6.15]
Carlos Martinez [3]	4	1125	3.30[1.66,6.54]
Irene M. Lang[4]	4	977	4.31[2.19,8.47]
Nicolas Coquoz[5]	4	1404	3.32[1.81,6.09]

OR: odds ratio, 95%CI: 95%confidence intervals

References

1. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.
2. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.
3. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.
4. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.
5. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.
6. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.

7. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.

8. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.

BMJ Open

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-038385.R2
Article Type:	Original research
Date Submitted by the Author:	06-Nov-2020
Complete List of Authors:	Zhang, Liyan; School of Basic Medicine, Gansu University of Chinese Medicine, Pei Jing, Yan; West China School of Public Health and West China Fourth Hospital, Sichuan University, Department of Epidemiology and Health Statistics Yang, Kehu; evidence based meidicine center, lanzhou university Wu, Shanlian; Ganzhou People's Hospital, Department of Pathology Bai, Yuping Zhu, Xin-Yu; Gansu Provincial Hospital, Department of Science and Research Chen, Xiaojie; Gansu Provincial Hospital, Department of Science and Research Li, Li; Gansu Provincial Hospital, Department of Science and Research Cao, Yunshan; Gansu Provincial Hospital, Department of Cardiology Zhang, Min; Gansu Provincial Hospital, Department of Pathology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Cardiovascular medicine, Respiratory medicine
Keywords:	Respiratory physiology < THORACIC MEDICINE, Adult surgery < SURGERY, Adult thoracic medicine < THORACIC MEDICINE

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

TITLE PAGE

Title

Association between splenectomy and chronic thromboembolic pulmonary hypertension: A systematic review and meta-analysis

Running Title: Splenectomy and CTEPH

Authors' names and affiliations

Liyan Zhang[1,2,3,4+], BS, Peijing Yan[5+], MS, Kehu Yang[3,4,6], MD, Shanlian Wu[7], MS, Yuping Bai[1,2], BS, Xinyu Zhu[8], MS, Xiaojie Chen[8], MS, Li Li[8], MS, Yunshan Cao[9*], MD, PhD, Min Zhang[2*], MD, PhD

[1]School of Basic Medicine, Gansu University of Chinese Medicine, Lanzhou, 730000, China

[2]Department of Pathology, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

[3]Institute of Clinical Research and Evidence Based Medicine, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

[4]Evidence Based Medicine Center, School of Basic Medical Sciences, Lanzhou University, Lanzhou, 730000, China

[5]Department of Epidemiology and Health Statistics, West China School of Public Health and West China Fourth Hospital, Sichuan University, Chengdu, Sichuan, 610044, China

[6]Key Laboratory of Evidence-based Medicine and Knowledge Translation of Gansu Province, Lanzhou, 730000, China

[7]Department of Pathology, Ganzhou People's Hospital, Ganzhou, 341000, China

[8]Department of Science and Research, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

[9]Department of Cardiology, Gansu Provincial Hospital, Lanzhou University, Lanzhou, 730000, China

[+]: Contributed equally

[*]: Corresponding author

Corresponding author

Min Zhang, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail: sallyzhangmin@126.com

Yunshan Cao, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China

E-mail: yunshancao@126.com

Abstract

Objectives: Whether splenectomy increases the risk of chronic thromboembolic pulmonary hypertension (CTEPH) remains unclear. We conducted a systematic review and meta-analysis to explore the association between splenectomy and CTEPH.

Design: Systematic review and meta-analysis.

Data sources: PubMed, Embase, and Cochrane Library databases.

Methods: Two authors independently searched and extracted the data. The Newcastle-Ottawa scale and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were used to assess the quality of the included studies and each quality item was graded as low risk or high risk. The random-effects model was used to calculate different effective values.

Results: In total, eight trials involving 6183 participants fulfilled the inclusion criteria. The overall pooled crude prevalence of splenectomy was 4.0% (95%CI: 0.03 to 0.06, I^2=71.5%, P<0.01) in the CTEPH patients. Subgroup analysis showed a statistically significant high incidence of splenectomy in the CTEPH patients (OR=2.94, 95%CI: 1.62 to 5.33, I^2=0.0%) compared to that in pulmonary arterial hypertension (PAH) patients. There was a significantly high incidence of splenectomy in the CTEPH patients (OR=5.59, 95%CI: 2.12 to 14.74, I^2=0.0%) compared to that in thromboembolism disease (venous thromboembolism or pulmonary embolism) patients.

Conclusion: The prevalence of splenectomy in CTEPH patients was 4.0%, and CTEPH might be associated with splenectomy. However, high-quality prospective trials are needed.

PROSPERO registration number: CRD42020137591.

Keywords: Pulmonary hypertension, chronic thromboembolic pulmonary hypertension, splenectomy, systematic review, meta-analysis

Strengths and limitations of this study

This systematic review focuses on the prevalence of splenectomy, and also evaluates

the association of splenectomy in the CTEPH patients compare to that in PAH or thromboembolism disease patients.

Absence of evident publication bias increases the reliability of our findings.

However, the trials included were not randomized controlled trials and sample size was small.

Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a disease of pulmonary artery obstruction and non-obstructive pulmonary artery remodeling as a consequence of pulmonary artery thromboembolism, which eventually leads to right heart failure and death[1]. CTEPH, a well-known long-lasting complication of acute pulmonary thromboembolism associated with poor thrombus resolution and altered pulmonary artery hemodynamics[2] is considered post-pulmonary embolism syndrome[3]. In long-term follow-up, the mortality of CTEPH was high, and with increases in pulmonary artery pressure, the mortality rate gradually increased[4]. Lupus anticoagulant and antiphospholipid antibodies and coagulation factor FVIII have been associated with CTEPH[5, 6].

Splenectomy can also increase the incidence of venous thromboembolism[7]. The 2018Cologne Consensus Conference mentioned interplay splenectomy and several factors were shown to promote the transformation of a pulmonary embolism into a fibrotic vascular occlusion[8]. Previous studies reported that 2.1% to 8.6% of the patients with CTEPH had undergone splenectomy[9, 10]. Another study showed that the incidence of splenectomy in CTEPH patients was similar to that in idiopathic

pulmonary arterial hypertension (IPAH) patients[11]. Based on these findings, it is difficult to determine the relationship between splenectomy and CTEPH. Therefore, this systematic review and meta-analysis were conducted to confirm whether splenectomy increased the risk of CTEPH.

Methods

This meta-analysis was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA)[12-14]. The MeaSurement Tool to Assess systematic Reviews (AMSTAR 2) was used to assess the methodological quality of this systematic review and meta-analysis[15, 16], and this study was registered in PROSPERO (registration number: CRD42020137591).

Search strategy

PubMed, the Cochrane library, and the EMBASE database were searched from the database inceptions to April 7, 2019, using the keywords splenectomy, splenectomies, hypertension, pulmonary, pulmonary hypertension, and chronic thromboembolic pulmonary hypertension to identify all potentially eligible trials. No language restrictions were imposed. The reference lists of articles relevant to the topic were hand-searched to identify other potentially relevant articles. The specific search strategies are reported in Supplementary Appendix 1.

Study selection

Trials were selected that enrolled patients diagnosed with CTEPH and reported any splenectomy profile, and trials that reported the prevalence of splenectomy in CTEPH patients. The exclusion criteria were conference abstracts, reviews, case

reports, animal trials, letters, and other unrelated topics, and trials that contained duplicate data.

Quality assessment

Two authors independently assessed the risk of bias in the nonrandomized studies using the Newcastle-Ottawa Scale, which assesses sample representativeness and size, and assessed the representativeness of the cases compared with control group, the comparability between CTEPH and control group, ascertainment of splenectomy, and the thoroughness of descriptive statistics reporting. Studies with scores of less than 3 points were judged as having a high risk of bias, and as a low risk of bias with more than 3 points. The risk of bias in the observational studies was assessed using an adapted version of the STROBE guidelines[17]. Twenty-two items were evaluated to reveal the strengths and weaknesses of the trials to facilitate rational interpretation and the application of the trial results. The third author resolved disagreements.

Data extraction

Two authors independently extracted the following information from each trial: lead author, publication year, country of origin, study type, sample size, patient characteristics, the patient type in the control group, the odds ratio (OR) of splenectomy, and the prevalence of splenectomy. Disagreements were resolved by the third author.

The primary outcome was the prevalence estimates of splenectomy. The secondary outcome was the ORs of splenectomy. All eight trials reported the prevalence of splenectomy and five trials reported the ORs of splenectomy.

Statistical analysis

A meta-analysis was performed to calculate the ORs and 95%confidence intervals (CIs) of the dichotomous outcome data. The prevalence of splenectomy was also calculated. Forest plots showed the individual studies and the meta-analysis estimates[18]. A random-effects model was used to pool the data and evaluate the statistical heterogeneity between the summary data using the I^2 statistic. In this meta-analysis, an I^2>50% indicated a significant heterogeneity between studies[19].

Sensitivity analysis was performed to determine the influence of each trial on the association of splenectomy with CTEPH compared to the control group.

To evaluate whether the association between splenectomy and CTEPH was changed when compared with different control groups, subgroup analyses were performed based on different control groups. The control groups were PAH or thromboembolism disease (venous thromboembolism or pulmonary embolism). Publication bias was assessed by examining funnel plots and by the Egger test[20, 21].

Review Manager 5.3 (Nordic Cochrane Centre, The Cochrane Collaboration) and Stata 13.0 (StataCorp, TX) were used to analyze the data. P<0.05 indicated a statistically significant difference.

Patient and public involvement

Patients and the public were not involved in this review.

Results

Study identification and selection

By the search strategy, 422 potentially eligible records were identified when

duplicate trials were excluded. The titles and abstracts of the identified records were screened for inclusion. After excluding conference abstracts, reviews, case reports, animal trials, letters, and other unrelated topics, the full text of 21 trials was reviewed. Finally, eight trials[6, 9-11, 22-25] involving a total of 6183 patients were included in the meta-analysis (Fig. 1).

The patient characteristics in the included trials are reported in Table 1. The included trials were mostly retrospective studies. The majority of patients included were from Europe and equally distributed between genders. All the trials reported the prevalence of splenectomy in CTEPH patients but three observational studies[23-25]did not report the incidence of splenectomy between the CTEPH group and the PAH or thromboembolism disease (venous thromboembolism or pulmonary embolism) group, so only the prevalence of splenectomy in the CTEPH patients was included. And only two trials reported the causes of the splenectomies[9, 23].

The Newcastle-Ottawa score components for the five individual trials appear in Table 1. One trial[22] was low quality, and four[6, 9-11] were of high quality. The STROBE scores of the three individual trials ranged from 17[23, 25] to 18[24] (Table 1), but no trials described any efforts to address potential sources of bias. Furthermore, two trials did not clearly define the variables[23, 24], two trials failed to report other analyses such as subgroup or sensitivity analyses[23, 25] and two trials did not report the source of funding[23, 25].

Prevalence of splenectomy in CTEPH

The pooled crude prevalence of splenectomy in CTEPH patients from eight trials

was 4.0% (95%CI: 0.03 to 0.06, I^2=71.5%, P<0.01) (Fig. 2).The prevalence reported by the individual trials ranged from 2.0% to 9.0%.

Sensitivity analysis of this study excluded each serially repeated trials and showed that no individual trial significantly affected the overall prevalence of splenectomy in the CTEPH patients (eTable 1).

Comparisons of incidence of splenectomy among CTEPH, PAH, and thromboembolism disease patients

Five trials compared the incidence of splenectomy in CTEPH patients with that in PAH patients or thromboembolism disease patients. As shown in Fig. 3, subgroup analysis showed a statistically significant high incidence of splenectomy in the CTEPH patients (OR=2.94, 95%CI: 1.62 to 5.33, I^2=0.0%) compared to that in the PAH patients. There was also a significantly high incidence of splenectomy in the CTEPH patients (OR=5.59, 95%CI: 2.12 to 14.74, I^2=0.0%) compared to that in the thromboembolism disease patients.

A sensitivity analysis was performed to assess the weight of each trial. Sensitivity analysis in this meta-analysis excluded each serially repeated trial and showed that no individual trial significantly affected the overall incidence of splenectomy in the CTEPH patients and PAH or thromboembolism disease patients (eTable 2).

Publication bias

No significant asymmetry was apparent by visual inspection of the funnel plot of studies reporting on splenectomies (Fig. 4). The Egger test did not show significant publication bias (P=0.52).

Discussion

The results of this systematic review and meta-analysis showed a statistically significant high incidence of splenectomies in CTEPH patients compared to PAH or thromboembolism disease patients. It showed that splenectomy could be significantly associated with CTEPH. The pooled prevalence of CTEPH patients with splenectomies was 4.0%. Sensitivity analysis showed that no individual trial significantly affected the overall incidence.

The prerequisite for CTEPH may be both *in situ* thrombosis and embolism[26]. Patients undergoing splenectomy may have significant enrichment of anion phospholipids[27] and platelet-derived microparticles (MP)[28]. These MPs contribute to thrombus formation by acting as pro-coagulants by providing a negatively charged surface for the assembly of coagulation proteases[29]. Erythrocyte membrane components have been reported to have an effect on venous thromboembolic diseases [30, 31]. The number of red blood cells with altered phosphatidylserine expression was increased 20-fold after splenectomies in thalassemia patients[30]. These cells are also procoagulant phenotypic markers that accelerate thrombin formation. Also, the loss of splenic filtration will result in the retention of abnormal red blood cells in the peripheral circulation after splenectomy, leading to the activation of the coagulation cascade, even in the absence of chronic hemolysis.

Therefore, we suggest that the development of thrombotic complications in patients undergoing splenectomy should be monitored closely by routine electrocardiogram (ECG) and/or echocardiography[32]. Splenectomized patients who present with

exertional dyspnea, ECG with right ventricular overload (RVO), and right heart enlargement and/or elevated pulmonary arterial pressure by echocardiography should be referred to the center of pulmonary hypertension for further assessment[32].

In conclusion, this study found that the prevalence of splenectomy in CTEPH was 4.0%, and CTEPH was associated with splenectomy. High-quality prospective trials are needed to further explore the (causal) relationship between CTEPH and splenectomy.

Limitations

Firstly, the trials included were not randomized controlled trials and had small sample sizes, which might cause bias. Secondly, trauma is the main indication for splenectomy, and surgery after a traumatic abdominal injury may be a relevant factor in thromboembolism. Therefore, trauma and surgery may have caused substantial heterogeneity in this study. However, there was not enough information to explore these factors further. Thirdly, many hematological disorders responsible for splenectomy are confounding factors for CTEPH, but there was insufficient information for subgroup analysis.

Authors' contributions

LZ and PY analyzed the patient data and were the major contributors in the preparation of the manuscript. SW and KY analyzed part of the patient data. XZ and YB performed the literature search and extracted the data. XC was responsible for the statistical analysis. LL made substantial contributions to the conception of the study. MZ and YC drafted the manuscript. All the authors have read and approved the final

version of this manuscript.

Data sharing statement

No additional data available.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethics approval

Not applicable.

Funding

This study was supported by the National Natural Science Foundation of China (grant no. 81860059), the International Cooperation Exchange Project of Gansu province (grant no. 18YF1WA046) and CAS 'Light of West China' Program granted to MZ and YC.

References

1. Kim NH, Delcroix M, Jais X, et al. Chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2019;53.
2. Lang IM, Pesavento R, Bonderman D, et al. Risk factors and basic mechanisms of chronic thromboembolic pulmonary hypertension: a current understanding. *The European respiratory journal*. 2013;41:462-468.
3. Klok FA, van der Hulle T, den Exter PL, et al. The post-PE syndrome: a new concept for chronic complications of pulmonary embolism. *Blood reviews*. 2014;28:221-226.
4. Riedel M, Stanek V, Widimsky J, et al. Longterm follow-up of patients with pulmonary thromboembolism. Late prognosis and evolution of hemodynamic and respiratory data. *Chest*. 1982;81:151-158.
5. Wolf M, Boyer-Neumann C, Parent F, et al. Thrombotic risk factors in pulmonary hypertension. *The European respiratory journal*. 2000;15:395-399.
6. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.
7. Boyle S, White RH, Brunson A, et al. Splenectomy and the incidence of venous thromboembolism and sepsis in patients with immune thrombocytopenia. *Blood*. 2013;121:4782-4790.
8. Wilkens H, Konstantinides S, Lang IM, et al. Chronic thromboembolic pulmonary hypertension

(CTEPH): Updated Recommendations from the Cologne Consensus Conference 2018. *International journal of cardiology*. 2018;272s:69-78.

9. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.

10. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.

11. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.

12. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ (Clinical research ed)*. 2009;339:b2700.

13. Ge L, Tian JH, Li YN, et al. Association between prospective registration and overall reporting and methodological quality of systematic reviews: a meta-epidemiological study. *Journal of clinical epidemiology*. 2018;93:45-55.

14. Wang X, Chen Y, Yao L, et al. Reporting of declarations and conflicts of interest in WHO guidelines can be further improved. *Journal of clinical epidemiology*. 2018;98:1-8.

15. Pieper D, Buechter RB, Li L, et al. Systematic review found AMSTAR, but not R(evised)-AMSTAR, to have good measurement properties. *Journal of clinical epidemiology*. 2015;68:574-583.

16. Yan P, Yao L, Li H, et al. The methodological quality of robotic surgical meta-analyses needed to be improved: a cross-sectional study. *Journal of clinical epidemiology*. 2019;109:20-29.

17. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Journal of clinical epidemiology*. 2008;61:344-349.

18. Xu R, Ding S, Zhao Y, et al. Autologous transplantation of bone marrow/blood-derived cells for chronic ischemic heart disease: a systematic review and meta-analysis. *The Canadian journal of cardiology*. 2014;30:1370-1377.

19. Higgins JP, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. *BMJ (Clinical research ed)*. 2003;327:557-560.

20. Egger M, Davey Smith G, Schneider M, et al. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed)*. 1997;315:629-634.

21. Sterne JA and Egger M. Funnel plots for detecting bias in meta-analysis: guidelines on choice of axis. *Journal of clinical epidemiology*. 2001;54:1046-1055.

22. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.

23. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.

24. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.

25. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.

26. Frey MK, Alias S, Winter MP, et al. Splenectomy is modifying the vascular remodeling of thrombosis. *Journal of the American Heart Association*. 2014;3:e000772.
27. Frey MK, Alias S, Winter MP, et al. Splenectomy is modifying the vascular remodeling of thrombosis. *Journal of the American Heart Association*. 2014;3:e000772.
28. Fontana V, Jy W, Ahn ER, et al. Increased procoagulant cell-derived microparticles (C-MP) in splenectomized patients with ITP. *Thrombosis research*. 2008;122:599-603.
29. Peacock AJ. Pulmonary hypertension after splenectomy: a consequence of loss of the splenic filter or is there something more? *Thorax*. 2005;60:983-984.
30. Kuypers FA, Yuan J, Lewis RA, et al. Membrane phospholipid asymmetry in human thalassemia. *Blood*. 1998;91:3044-3051.
31. Atichartakarn V, Angchaisuksiri P, Aryurachai K, et al. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/beta-thalassaemic patients. *British journal of haematology*. 2002;118:893-898.
32. Galie N, Humbert M, Vachiery JL, et al. 2015 ESC/ERS Guidelines for the diagnosis and treatment of pulmonary hypertension: The Joint Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS): Endorsed by: Association for European Paediatric and Congenital Cardiology (AEPC), International Society for Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2016;37:67-119.

Table1: Characteristics of Included Studies

No.	Author	Year	Location	Study type	Number of patients	Age (years)	Proportion of females (%)	BMI (kg·m^{-2})	Control	NOS scores	STROBE scores
					CTEPH/Control	CTEPH/Control	CTEPH/Control	CTEPH/Control			
1	X Jaïs[9]	2005	France	Case-control study	257/276	51.0/46.0	47.4/60.0	—	IPAH	6	—
2	Diana Bonderman[6]	2009	Europe	Retrospective cohort study	433/254	58.0/50.5	52.4/65.8	26.2/25.2	PAH	7	—
3	Carlos Martinez[10]	2018	England	Cohort study	283/2356	—	54.1/51.7	—	VTE	6	—
4	Irene M. Lang[11]	2013	Europe	Case-control study	436/158	65.0/59.0	49.3/66.5	—	IPAH	6	—
5	Nicolas Coquoz[22]	2018	Switzerland	Observational study	4/504	47.0/61.3	75.0/46.4	33.0/28.0	PE	3	—
6	Joanna Pepke-Zaba[24]	2011	Europe and Canada	Cohort study	679/—	63.0/—	49.9/—	—	—	—	18
7	M.Bohacekova[25]	2016	Slovakia	Observational study	81/—	60.5/—	37.0/—	27.4/—	—	—	17
8	R. Condliffe[23]	2009	UK	Observational study	469/—	—	—	—	—	—	17

BMI: body mass index; CTEPH: chronic thromboembolic pulmonary hypertension; IPAH: idiopathic pulmonary arterial hypertension; PE: pulmonary embolism; VTE: venous thromboembolism; CTEPH/Control: CTEPH group/control group

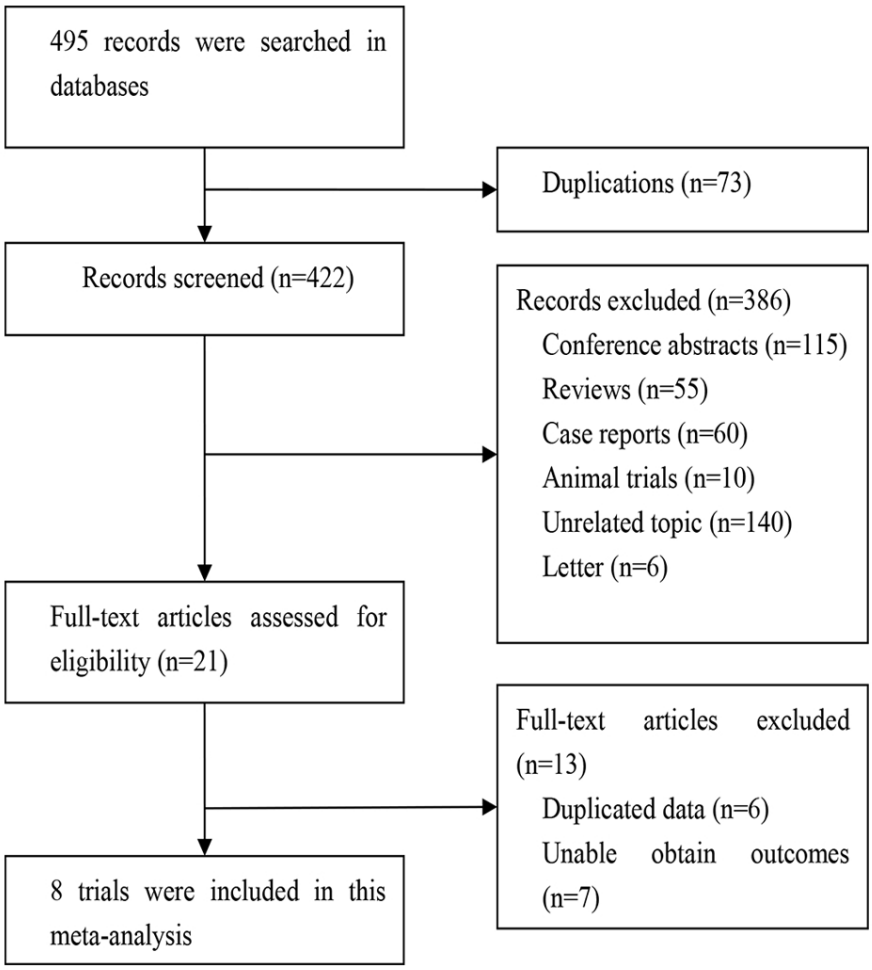
Figure legends

Fig. 1 Flow chart of study search and selection process

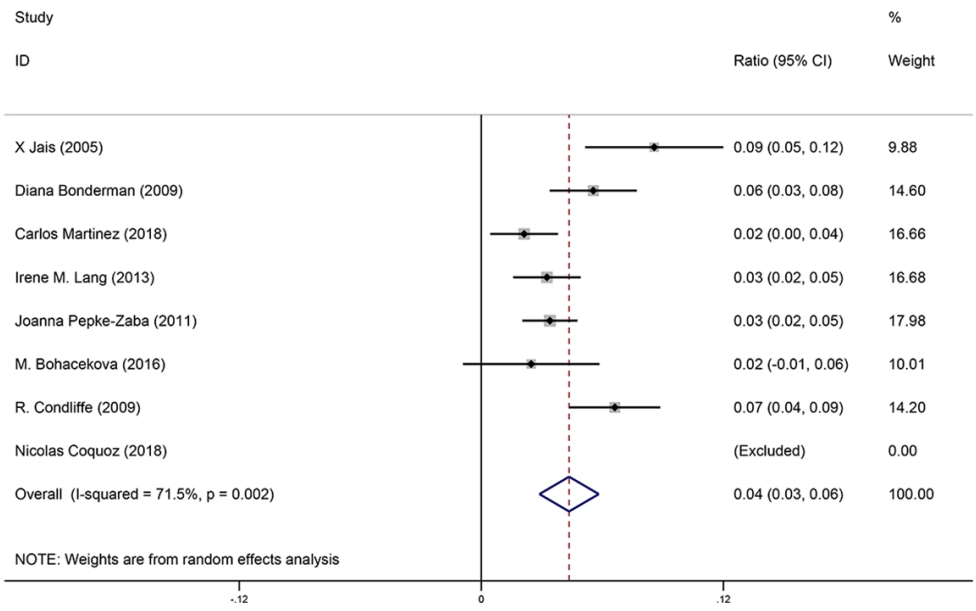
Fig. 2 Forest plot with meta-analysis of the prevalence and 95% CI of splenectomy in patients with chronic thromboembolic pulmonary hypertension in the assessed studies

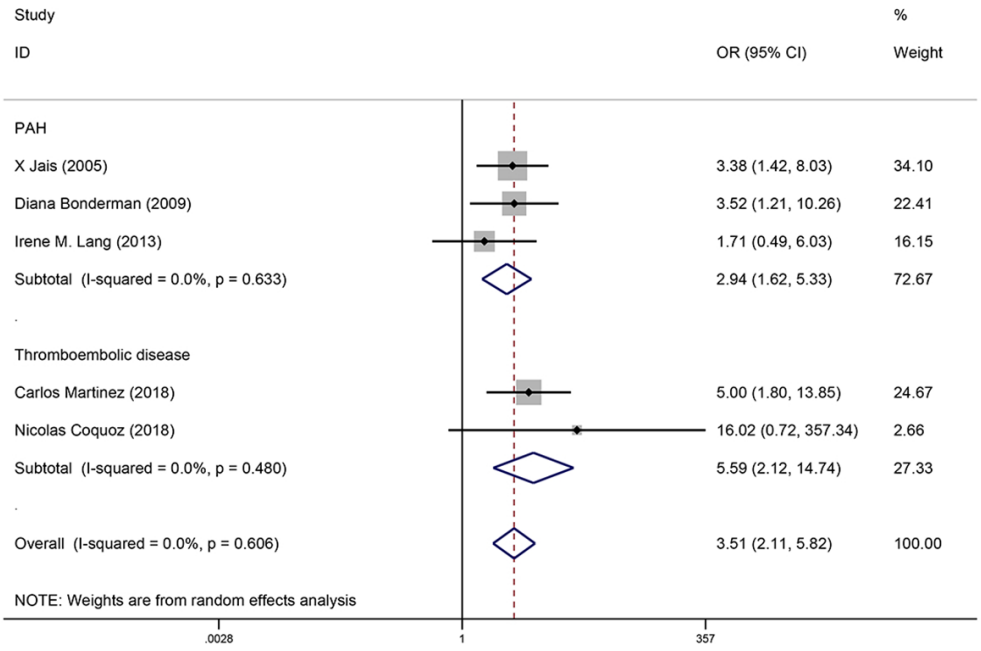
Fig. 3 Forest plot with meta-analysis of the association of splenectomy between chronic thromboembolic pulmonary hypertension, pulmonary arterial hypertension and thromboembolism disease

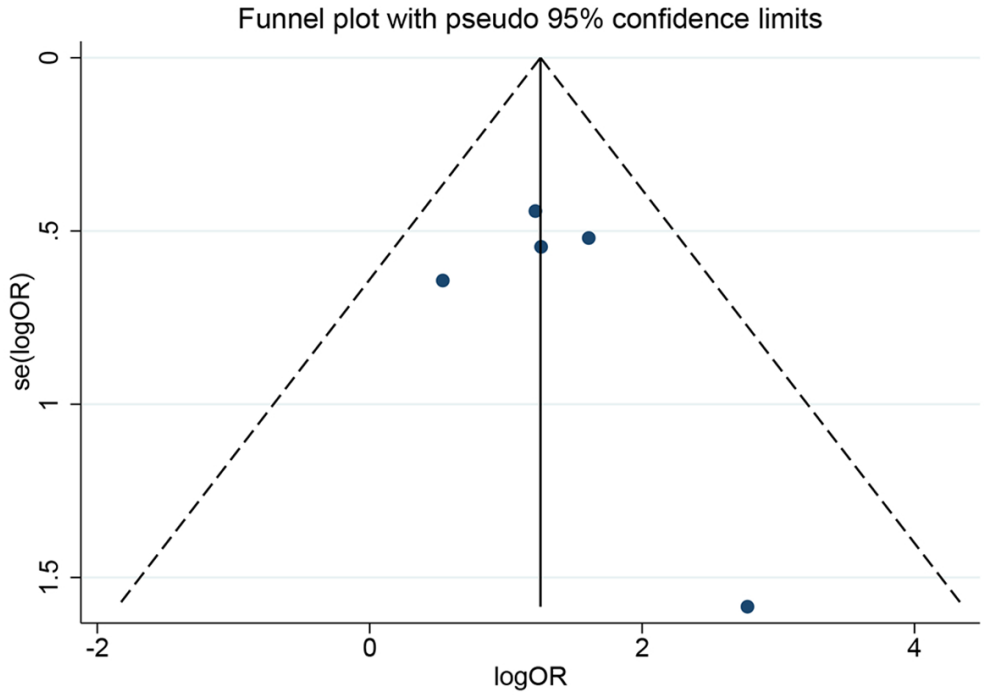
Fig. 4 Funnel plot to assess publication bias



90x90mm (300 x 300 DPI)







Search strategy

Pubmed

(((splenectomies[MeSH Terms]) OR splenectomy)) AND (((Hypertension, Pulmonary[MeSH Terms]) OR pulmonary hypertension) OR chronic thromboembolic pulmonary hypertension)

The Cochrane library

#1 MeSH descriptor: [Hypertension, Pulmonary] explode all trees

#2 (Chronic thromboembolic pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#3 (pulmonary hypertension):ti,ab,kw (Word variations have been searched)

#4 MeSH descriptor: [Splenectomy] explode all trees

#5 (splenectomies):ti,ab,kw (Word variations have been searched)

#6 #1 OR #2 OR #3

#7 #4 OR #5

#8 #6 AND #7

Embase

#1. 'chronic thromboembolic pulmonary hypertension'/exp

#2. 'pulmonary hypertension':ab,ti,kw

#3. 'splenectomy'/exp

#4. 'splenectomies':ab,ti,kw

#5. #1 OR #2

#6. #3 OR #4

#7. #5 AND #6

eTable 1. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No. studies	No. participants	Ratio[95%CI]
X Jaïs[1]	6	2374	0.39[0.26,0.52]
Diana Bonderman[2]	6	2198	0.42[0.25,0.58]
Carlos Martinez [3]	6	2348	0.48[0.32,0.63]
Irene M. Lang[4]	6	2200	0.46[0.28,0.64]
Nicolas Coquoz[5]	7	2631	0.43[0.29,0.58]
Joanna Pepke-Zaba[6]	6	1954	0.46[0.27,0.65]
M. Bohacekova[7]	6	2550	0.46[0.30,0.62]
R. Condliffe[8]	6	2162	0.39[0.25,0.54]

eTable 2. Results of Sensitivity Analyses With Exclusion of the Listed Trials

Removed trials	No. studies	No. participants	OR[95%CI]
X Jaïs[1]	4	4423	3.57[1.92,6.67]
Diana Bonderman[2]	4	4269	3.50[1.97,6.22]
Carlos Martinez [3]	4	2317	3.12[1.74,5.59]
Irene M. Lang[4]	4	4367	4.02[2.31,7.00]
Nicolas Coquoz[5]	4	4448	3.36[2.01,5.62]

OR: odds ratio, 95%CI: 95%confidence intervals

References

1. Jais X, Ioos V, Jardim C, et al. Splenectomy and chronic thromboembolic pulmonary hypertension. *Thorax*. 2005;60:1031-1034.
2. Bonderman D, Wilkens H, Wakounig S, et al. Risk factors for chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:325-331.
3. Martinez C, Wallenhorst C, Teal S, et al. Incidence and risk factors of chronic thromboembolic pulmonary hypertension following venous thromboembolism, a population-based cohort study in England. *Pulm Circ*. 2018;8:2045894018791358.
4. Lang IM, Simonneau G, Pepke-Zaba JW, et al. Factors associated with diagnosis and operability of chronic thromboembolic pulmonary hypertension. A case-control study. *Thrombosis and haemostasis*. 2013;110:83-91.
5. Coquoz N, Weilenmann D, Stolz D, et al. Multicentre observational screening survey for the detection of CTEPH following pulmonary embolism. *The European respiratory journal*. 2018;51:1702505.
6. Pepke-Zaba J, Delcroix M, Lang I, et al. Chronic thromboembolic pulmonary hypertension (CTEPH): results from an international prospective registry. *Circulation*. 2011;124:1973-1981.

7. Bohacekova M, Kaldararova M, Valkovicova T, et al. Risk factors detection in chronic thromboembolic pulmonary hypertension, a tool for risk quantification? *Bratislavske lekarske listy*. 2016;117:577-582.

8. Condliffe R, Kiely DG, Gibbs JS, et al. Prognostic and aetiological factors in chronic thromboembolic pulmonary hypertension. *The European respiratory journal*. 2009;33:332-338.

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	8
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	9
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: www.prisma-statement.org.