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Splenectomy correlates with increased risk of empyema: a population-based cohort study in Taiwan

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ABSTRACT

Objectives. Little is known about the risk of empyema in patients with splenectomy. We investigate the relationship between splenectomy and empyema in Taiwan.

Methods. We conducted a population-based cohort analysis using the hospitalization dataset of the Taiwan National Health Insurance Program. There were 13193 subjects aged 20 to 84 years who were newly diagnosed with splenectomy in 2000 to 2010 as the splenectomy group and 52464 randomly selected subjects without splenectomy as the non-splenectomy group. Both groups were matched by sex, age, comorbidities, and hospitalization year of performing splenectomy. The incidence of empyema at the end of 2011 was calculated. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for empyema associated with splenectomy and other comorbidities. **Results**. The overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). After adjusted for confounders, the adjusted HR of empyema was 2.89 in subjects with splenectomy (95%CI 2.60, 3.22), compared with subjects without splenectomy. In further analysis, even in the absence of comorbidities studied, the HR was 4.52 for those with splenectomy alone (95% CI=3.80, 5.37). **Conclusions**. Patients with splenectomy are associated with 2.89-fold increased risk of empyema. Even in the absence of comorbidities, the risk remains high.

Keywords: empyema; splenectomy; Taiwan National Health Insurance Program

Strengths and limitations of this study.

1. This is the first original holistic study on the association between splenectomy and empyema.
2. We used a hospitalization dataset with a large sample size and great statistical power.
3. Some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database.
4. The underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database.
5. Such a study design does not permit to conclude a substantial causality.

INTRODUCTION

Empyema is a suppurative infection of the pleural space. The creation of empyema can be divided into two distinct mechanisms. Empyema occurs most commonly after pneumonia, with direct spread of organisms into the pleural space. This occurs in approximately 1% to 5% of pneumonias.[1,2] The other mechanism occurs after surgery, most commonly of the thorax, esophagus, lung or heart. Empyema is an ancient disease that continues to be an important clinical problem nowadays. Despite the centuries of learned experience, the appearance of antibiotics and the use of different pneumococcal vaccines, empyema remains the most common complication of pneumonia and an important cause of morbidity worldwide.[3]

The human spleen mainly serves an immune function against invading microorganisms.[4,5] The immunologic and hematologic functions of the spleen in humans are well known. Specifically, the spleen protects against infections mediated by innate and adaptive immunity.[6,7] Patients with splenectomy are more likely than those without splenectomy to suffer severe life-threatening infections. Splenectomy is associated with increased risk of some diseases, including pulmonary tuberculosis, type 2 diabetes mellitus, pyogenic liver abscess, renal and perinephric abscesses, and acute pancreatitis, [8-12] but empyema has not yet been studied.

We rationally hypothesize an association between splenectomy and empyema due to the immunocompromised condition caused by splenectomy, which can further increase the risk of microorganism invasion of the pleural space. However, published literature from epidemiological studies on this issue is scarce. Given that splenectomy is associated with overwhelming postsplenectomy infections and empyema carries a potential fatality, exploring the risk of empyema in patients with splenectomy may have significant clinical and public health implications. Therefore, to explore whether there is an association between splenectomy and empyema, we conducted a

nationwide cohort study using the hospitalization dataset of the Taiwan National Health Insurance Program.

METHODS

Study design and data source

Taiwan is an independent country with more than 23 million people. We conducted a population-based cohort study using insurance claim data from the Taiwan National Health Insurance Program which covers 99% of the whole Taiwan population since 1995.[13] The details of the insurance program have been well written in previous studies.[14-17] The study was approved by the Institutional Review Board of China Medical University and Hospital in Taiwan (CMUH-104-REC2-115).

Sampled Participants

Using the hospitalization dataset of the Taiwan National Health Insurance Program, all hospitalized subjects aged 20 to 84 who performed splenectomy (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9 procedure code 41.5) between January 1, 2000 and December 31, 2010, were identified as the splenectomy group. The date for performing splenectomy was defined as the index date. For each subject with splenectomy, 4 subjects without splenectomy were randomly selected from the same database as the non-splenectomy group. Both groups were matched by sex, age (every 5-year span), comorbidities, and the hospitalization year of performing splenectomy. To reduce the biased results, subjects with empyema diagnosis (ICD-9 codes 510, 511.1, 511.8, and 511.9) within one month after performing splenectomy were excluded from the study.

Outcome and Comorbidities

The main outcome was a new diagnosis of empyema based on hospital discharge registries during the follow-up period. Each subject was monitored from the index date until being diagnosed with empyema, or being censored because of loss to

follow-up, death, or withdrawal from insurance, or to the end of December 31, 2011. Comorbidities investigated in the study were included as follows: alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases (including cirrhosis, alcoholic liver damage, hepatitis B, hepatitis C, and other chronic hepatitis), chronic obstructive pulmonary diseases, and diabetes mellitus. All comorbidities were diagnosed with ICD-9 codes, which have been well assessed in previous studies.[18-21]

Statistical analysis

The differences between sex, age, and comorbidities between the splenectomy group and the non-splenectomy group were compared by using the Chi-square test for categorical variables, and *t* test for continuous variables. Follow-up time (person-years) was used to estimate the incidence rate and incidence rate ratio (IRR) with 95% confidence interval (CI) of splenectomy group to non-splenectomy group using Poisson regression, by sex, age, and follow-up period. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) of empyema associated with splenectomy and other comorbidities after simultaneously adjusted for variables found to be significant in the univariable Cox proportion hazard regression model. The proportional hazard model assumption was examined by using a test of scaled Schoenfeld residuals. In the model evaluating empyema risk throughout the overall follow-up period, results of the test revealed a significant relationship between Schoenfeld residuals for splenectomy and follow-up time, suggesting the proportionality assumption was violated (*P* value < 0.001). In the subsequent analysis, we stratified the follow-up period to deal with the violation of proportional hazard assumption (Please see Table 2). All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Baseline information of the study population

Table 1 discloses the baseline information of the study population. There were 13193 subjects with splenectomy and 52464 subjects without splenectomy during the study period, with similar distributions in sex and age. The mean ages (mean ± standard deviation) were 52.8 ± 17.2 years in the splenectomy group and 52.5 ± 17.2 years in the non-splenectomy group (*t* test, $P = 0.05$). The mean follow-up periods (mean ± standard deviation) were 4.37± 3.44 years in the splenectomy group and 5.75 ± 3.32 years in the non-splenectomy group (*t* test, $P < 0.001$). There was no significant difference in the prevalence of comorbidities studied between the splenectomy group and the non-splenectomy group (Chi-square test, $P > 0.05$ for all).

Incidence of empyema stratified by sex, age, and follow-up period

Table 2 discloses the incidence rates of empyema**.** At the end of the cohort study, the overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The incidence rates of empyema, as stratified by sex, age, and follow-up period, were all higher in the splenectomy group than those in the non-splenectomy group. The incidence rates of empyema increased with age in both groups, with the highest in the splenectomy group aged 65 to 84 years (19.2 per 1,000 person-years). Stratified analysis by follow-up period disclosed that the incidence rates of empyema decreased with the follow-up time in both groups. The risk of empyema in the splenectomy group was significantly higher in the first 5 years of follow-up (incidence rate ratio 2.87, 95% CI 2.73, 3.01). However, the risk of empyema still existed in the splenectomy group even after 5 years (incidence rate ratio 1.73, 95% CI 1.60, 1.88).

Hazard ratio of empyema associated with splenectomy and other comorbidities

Table 3 discloses the HR of empyema associated with splenectomy and other comorbidities. Only those found significantly in the univariable analysis were further examined in the multivariable analysis. After adjusted for age, sex, alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary disease, and diabetes mellitus, the multivariable Cox proportional hazards regression model disclosed that the adjusted HR of empyema was 2.89 in subjects with splenectomy (95% CI 2.60, 3.22), compared with subjects without splenectomy. Male (HR 1.51, 95 % CI 1.35, 1.68), alcohol-related diseases (HR 2.25, 95 % CI 1.76, 2.86), cancers (HR 1.92, 95 % CI 1.71, 2.17), chronic kidney disease (HR 2.13, 95 % CI 1.67, 2.70), chronic liver diseases (HR 1.90, 95 % CI 1.68, 2.14), chronic obstructive pulmonary diseases (HR 1.75, 95 % CI 1.48, 2.07), and diabetes mellitus (HR 1.85, 95 % CI 1.65, 2.07) were also associated with empyema. Every one-year increase in age was associated with a 1.05-fold increased risk of empyema (95% CI 1.05, 1.06).

Interaction effect on risk of empyema between splenectomy and other comorbidities

Table 4 discloses interaction effect on risk of empyema between splenectomy and other comorbidities including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. As a reference of subjects without splenectomy and without any comorbidity, the adjusted HR of empyema was 4.52 for subjects with splenectomy alone and without any comorbidity (95% CI 3.80, 5.37). The HR markedly increased to 8.23 for those with splenectomy and comorbid with any comorbidity (95% CI 6.98, 9.70). It means that there is an interaction effect on risk of empyema between splenectomy and other comorbidities.

DISCUSSION

A review by Sinwar found that the duration between splenectomy and onset of overwhelming post-splenectomy infection could range from less than 1 week to more than 20 years.[22] To diminish biased results, we excluded patients who underwent splenectomy within 1 month of empyema diagnosis to ensure that splenectomy truly preceded the onset of empyema. In this population-based cohort study, we noticed that splenectomy was associated with increased hazard ratio of empyema (adjusted HR 2.89). To the best of our knowledge, this is the first population-based cohort study to examine this issue. Some studies have found that splenectomy is associated with increased risk of some diseases. Lai et al. found that the adjusted odds ratio of acute pancreatitis was 2.90 for subjects with splenectomy (95% CI, 1.39–6.05) compared with subjects without splenectomy,[12] the adjusted hazard ratio of renal and perinephric abscesses was 2.24 for the splenectomy group (95% CI, 1.30–3.88) when compared with the non-splenectomy group,[11] the adjusted hazard ratio of pyogenic liver abscess was 3.89 in subjects with splenectomy (95% confidence interval, 3.20–4.72) when compared with subjects without splenectomy,[10] and the odds of pulmonary tuberculosis were 1.91 in patients with splenectomy (95% CI 1.06–3.44) compared with the participants without splenectomy.[8]

Extensive evidence has revealed that the human spleen mainly serves a protective role against invading microorganisms on the basis of bactericidal capacity of lymphoid cells and macrophages and the humoral immune response.[6,23-25] Upon removal of the spleen, normal immune functions, such as phagocytic activities and the humoral immune response, may be significantly changed. Therefore, impaired immune functions after splenectomy may increase the risk of life-threatening infection and empyema.

In our study, the risk of empyema in the splenectomy group was higher in the first

5 years of follow-up than after 5 years (incidence rate ratio 2.87 vs 1.72). However, the risk of empyema still existed in the splenectomy group even after 5 years. These findings are compatible with previous studies showing that the majority of severe infections occur within the first 3 years after splenectomy, and, although the risk declines over time, the risk might last for more than 5 years after splenectomy.[26-28] The mechanism remains unknown. We speculate that with time, the immune system could develop ways to compensate or overcome these immune deficits. So the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up. Future studies are needed to confirm this hypothesis.

In this study, after controlling for potential confounding factors, we also observed that patients with splenectomy were at increased risk of empyema (adjusted HR 2.89). The HR seems to be higher than that observed for other comorbidities. The HR was not confounded by other studied comorbidities because there was no significant difference in the prevalence of comorbidities between the splenectomy group and the non-splenectomy group, so the increased risk of empyema in patients with splenectomy cannot be totally attributable to the effect of comorbidities. In further analysis, even in the absence of any comorbidity, patients with splenectomy still had a higher risk of empyema than those without splenectomy (adjusted HR 4.52). This indicates splenectomy may have a unique role on risk of empyema independent of other comorbidities. These findings are compatible with the literature showing that patients with splenectomy are not only more prone than those without splenectomy to suffer severe life-threatening infection due to the immunocompromised condition caused by splenectomy[29,30] but are also at an increased risk of developing empyema.

Some limitations in the present study deserve discussion. First, some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database. We used

alcohol-related diseases instead of alcohol consumption and chronic obstructive pulmonary disease instead of cigarette smoking. Second, the underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database. Splenectomy is commonly performed in certain disorders and diseases, such as hematological disorders, gastric cancer, or trauma, so these background conditions might confound the results. Whether the reasons for splenectomy are associated with empyema cannot be clarified in this study. Third, such a study design does not permit to conclude a substantial causality. Further prospective studies are needed to confirm our findings.

The strength of this study is that this is the first original holistic study on the association between splenectomy and empyema. Although the underlying mechanism linking splenectomy and empyema cannot be completely determined, our findings are clinically important. In addition, we used a hospitalization dataset with a large sample size and great statistical power. The diagnosis codes of included comorbidities have been documented in previous studies.[31,32] The study design and statistical methods are described in detail. Our results were relatively convincing because the splenectomy group and the non-splenectomy group had similar distributions of studied comorbidities. Therefore, the confounding effects of these comorbidities on risk of empyema should be minimal.

We conclude that patients with splenectomy are associated with 2.89-fold increased risk of empyema, particularly comorbid with other conditions, including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. Even in the absence of comorbidities, the risk remains high.

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Specific author contributions

Hsien-Feng Lin and Kuan-Fu Liao substantially planned and conducted this study, participated in the data interpretation, and also critically revised the article.

Ching-Mei Chang and Cheng-Li Lin conducted the data analysis and critically revised the article.

Shih-Wei Lai substantially planned and conducted this study, contributed to the conception of the article, initiated the draft of the article, and critically revised the article.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

REFERENCES

1. Ahmed RA, Marrie TJ, Huang JQ. Thoracic empyema in patients with community-acquired pneumonia. Am J Med 2006;119:877-83.
2. Shields T, Locicero J, Ponn R, Rusch V, eds. General Thoracic Surgery, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2005:823.
3. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennet JE, Mandell RD, editors. Douglas and Bennett's principles and practice of infectious diseases. Edinburgh: Churchill Livingstone; 2009. pp. 917-24.
4. Spencer RP, Pearson HA. The spleen as a hematological organ. Semin Nucl Med. 1975;5:95–102.
5. Trigg ME. Immune function of the spleen. South Med J. 1979;72:593–9.
6. Jirillo E, Mastronardi ML, Altamura M, Munno I, Miniello S, Urgesi G, et al. The immunocompromised host: immune alterations in splenectomized patients and clinical implications. Curr Pharm Des. 2003;9:1918–23.
7. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97.
8. Lai SW, Wang IK, Lin CL, Chen HJ, Liao KF. Splenectomy correlates with increased risk of pulmonary tuberculosis: a case-control study in Taiwan. Clin Microbiol Infect 2014;20:764-7.
9. Wu SC, Fu CY, Muo CH, Chang YJ. Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 2014;208:811-6.
10. Lai SW, Lai HC, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Pyogenic Liver Abscess: A Nationwide Cohort Study in Taiwan. J Epidemiol 2015;25:561-6.
11. Lai SW, Lin HF, Lin CL, Liao KF. Splenectomy and risk of renal and perinephric abscesses: A population-based cohort study in Taiwan. Medicine 2016;95:31(e4438).
12. Lai SW, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Acute Pancreatitis: A Case-Control Study in Taiwan. J Epidemiol 2016;26:488-92.
13. National Health Insurance Research Database. Taiwan. http://nhird.nhri.org.tw/en/index.html [cited in 2016 September 1, English version].
14. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine 2010;89:295-9.
15. Chen HY, Lai SW, Muo CH, Chen PC, Wang IJ. Ethambutol-induced optic

neuropathy: a nationwide population-based study from Taiwan. Br J Ophthalmol 2012;96:1368-71.
16. Yang SP, Muo CH, Wang IK, et al. Risk of type 2 diabetes mellitus in female breast cancer patients treated with morphine: A retrospective population-based time-dependent cohort study. Diabetes Res Clin Pract 2015;110:285-90.
17. Tsai TY, Lin CC, Peng CY, et al. The association between biliary tract inflammation and risk of digestive system cancers: A population-based cohort study. Medicine 2016;95:31(e4427).
18. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 2012;107:46-52.
19. Liao KF, Lin CL, Lai SW, Chen WC. Zolpidem Use Associated With Increased Risk of Pyogenic Liver Abscess: A Case-Control Study in Taiwan. Medicine 2015;94:32(e1302).
20. Wong TS, Liao KF, Lin CM, Lin CL, Chen WC, Lai SW. Chronic Pancreatitis Correlates With Increased Risk of Cerebrovascular Disease: A Retrospective Population-Based Cohort Study in Taiwan. Medicine 2016;95:15(e3266).
21. Liao KF, Lai SW, Lin CL, Chien SH. Appendectomy correlates with increased risk of pyogenic liver abscess: A population-based cohort study in Taiwan. Medicine 2016;95:26(e4015).
22. Sinwar PD. Overwhelming post splenectomy infection syndrome—review study. Int J Surg. 2014;12:1314–6.
23. Eibl M. Immunological consequences of splenectomy. Prog Pediatr Surg. 1985;18:139–45.
24. Van Rooijen N. The humoral immune response in the spleen. Res Immunol. 1991;142:328–30.
25. Altamura M, Caradonna L, Amati L, Pellegrino NM, Urgesi G, Miniello S. Splenectomy and sepsis: the role of the spleen in the immune-mediated bacterial clearance. Immunopharmacol Immunotoxicol. 2001;23:153–61.
26. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post-splenectomy patients. J Infect. 2001;43:182–6.
27. Kyaw MH, Holmes EM, Toolis F, Wayne B, Chalmers J, Jones IG, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. 2006;119:276 e1–7.
28. Dendle C, Sundararajan V, Spelman T, Jolley D, Woolley I. Splenectomy sequelae: an analysis of infectious outcomes among adults in Victoria. Med J Aust. 2012;196:582–6.
29. Di Cataldo A, Puleo S, Li Destri G, Racalbuto A, Trombatore G, Latteri F, et al.

Splenic trauma and overwhelming postsplenectomy infection. Br J Surg. 1987;74:343–5.

30. Lynch AM, Kapila R. Overwhelming postsplenectomy infection. Infect Dis Clin North Am. 1996;10:693–707.
31. Lai SW, Muo CH, Liao KF, Sung FC, Chen PC. Risk of acute pancreatitis in type 2 diabetes and risk reduction on anti-diabetic drugs: a population-based cohort study in Taiwan. Am J Gastroenterol. 2011;106:1697–704.
32. Lai HC, Lin CC, Cheng KS, Kao JT, Chou JW, Peng CY, et al. Increased incidence of gastrointestinal cancers among patients with pyogenic liver abscess: a population-based cohort study. Gastroenterology. 2014;146:129–37 e1.

Table 1. Baseline characteristics between splenectomy group and non-splenectomy group

	Splenectomy				
	No N=52464		Yes N=13193		
	N	%	n	%	*P* value*
Sex					0.88
Female	20431	38.9	5128	38.9	
Male	32033	61.1	8065	61.1	
Age group (years)					0.98
20–39	13415	25.6	3364	25.5	
40–64	24112	46.0	6062	46.0	
65-84	14937	28.5	3767	28.6	
Age (years), mean (standard deviation) †	52.5	17.2	52.8	17.2	0.05
Follow-up period (years), mean (standard deviation) †	5.75	3.32	4.37	3.44	< 0.001
Baseline comorbidities					
Alcohol-related diseases	1787	3.41	452	3.43	0.91
Cancers	7677	14.6	1962	14.9	0.49
Chronic kidney diseases	1068	2.04	270	2.05	0.94
Chronic liver diseases	7791	14.9	1971	14.9	0.80
Chronic obstructive pulmonary diseases	2002	3.82	507	3.84	0.89
Diabetes mellitus	7856	15.0	1983	15.0	0.87

Data are presented as the number of subjects in each group, with percentages given in parentheses, or mean with standard deviation given in parentheses.

*Chi-square test, and †t-test comparing subjects with and without splenectomy.

Table 2. Incidence density of empyema estimated by sex, age, and follow-up period between splenectomy group and non-splenectomy group

	Non-splenectomy				Splenectomy					
	N	Cases	Person-years	Incidence[†]	N	Cases	Person-years	Incidence [†]	IRR[#]	(95% CI)
All	52464	1042	301484	3.46	13193	510	57622	8.85	2.56	(2.44,2.69)
Sex										
Female	20431	317	119441	2.65	5128	159	23004	6.91	2.60	(2.40, 2.82)
Male	32033	725	182042	3.98	8065	351	34618	10.1	2.55	(2.39, 2.71)
Age group (years)										
20–39	13415	57	84004	0.68	3364	65	19700	3.30	4.86	(4.39, 5.39)
40–64	24112	354	140554	2.52	6062	224	26423	8.48	3.37	(3.13, 3.62)
65-84	14937	631	76925	8.20	3767	221	11499	19.2	2.34	(2.14, 2.56)
Follow-up period (years)										
< 5	52464	720	206010	3.49	13193	416	41545	10.0	2.87	(2.73, 3.01)
≥ 5	28456	322	95474	3.37	5052	94	16077	5.85	1.73	(1.60, 1.88)

[†] Incidence rate: per 1,000 person-years.

[#]IRR (incidence rate ratio): splenectomy vs. non-splenectomy (95% CI)

Table 3. Adjusted hazard ratio and 95% confidence interval of empyema associated with splenectomy and other comorbidities

	Crude	Adjusted†
Variable	HR (95%CI)	HR (95%CI)
Sex (male vs. female)	1.48 (1.33, 1.65)	1.51 (1.35, 1.68)
Age (per one year)	1.05 (1.04, 1.05)	1.05 (1.05, 1.06)
Baseline comorbidities (yes vs. no)		
Splenectomy	**2.52 (2.26, 2.80)**	**2.89 (2.60, 3.22)**
Alcohol-related diseases	1.61 (1.28, 2.03)	2.25 (1.76, 2.86)
Cancers	2.60 (2.31, 2.92)	1.92 (1.71, 2.17)
Chronic kidney diseases	3.04 (2.40, 3.85)	2.13 (1.67, 2.70)
Chronic liver diseases	2.07 (1.84, 2.34)	1.90 (1.68, 2.14)
Chronic obstructive pulmonary diseases	3.95 (3.37, 4.64)	1.75 (1.48, 2.07)
Diabetes mellitus	2.80 (2.51, 3.13)	1.85 (1.65, 2.07)

Only those found to be significant in the univariable analysis were further examined in the multivariable analysis.

†Additionally adjusted for age, sex, alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus

Table 4. Cox proportional hazard regression analysis for risk of empyema stratified by splenectomy and comorbidities

Variable		Event	Incidence†	Adjusted HR# (95% CI)
Splenectomy	Any comorbidity*			
No	No	299	1.49	1(Reference)
No	Yes	743	7.34	3.64(3.18, 4.17)
Yes	No	230	5.75	4.52(3.80, 5.37)
Yes	Yes	280	15.9	8.23(6.98, 9.70)

† Incidence rate: per 1,000 person-years

#Adjusted for sex and age

*Comorbidities including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus

95% CI: 95% confidence interval

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ABSTRACT

Objectives. Little is known about the risk of empyema in patients with splenectomy. We investigate the relationship between splenectomy and empyema in Taiwan.

Methods. We conducted a population-based cohort analysis using the hospitalization dataset of the Taiwan National Health Insurance Program. There were 13193 subjects aged 20 to 84 years who were newly diagnosed with splenectomy in 2000 to 2010 as the splenectomy group and 52464 randomly selected subjects without splenectomy as the non-splenectomy group. Both groups were matched by sex, age, comorbidities, and hospitalization year of performing splenectomy. The incidence of empyema at the end of 2011 was calculated. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for empyema associated with splenectomy and other comorbidities. **Results**. The overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001). After adjusted for confounders, the adjusted HR of empyema was 2.89 in subjects with splenectomy (95%CI 2.60, 3.22), compared with subjects without splenectomy. In further analysis, in the absence of comorbidities studied, the HR was 4.52 for those with splenectomy alone (95% CI=3.80, 5.37). **Conclusions**. Patients with splenectomy are associated with 2.89-fold increased risk of empyema. In the absence of comorbidities, the risk remains high.

Keywords: empyema; splenectomy; Taiwan National Health Insurance Program

Strengths and limitations of this study.

1. This is the first original study on the association between splenectomy and empyema.
2. We used a hospitalization dataset with a large sample size and great statistical power.
3. Some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database.
4. The underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database.
5. Such a study design does not permit to conclude a substantial causality.

INTRODUCTION

Empyema is a suppurative infection of the pleural space. The creation of empyema can be divided into two distinct mechanisms. Empyema occurs most commonly after pneumonia, with direct spread of organisms into the pleural space. This occurs in approximately 1% to 5% of pneumonias.[1,2] The other mechanism occurs after surgery, most commonly of the thorax, esophagus, lung or heart. Empyema is an ancient disease that continues to be an important clinical problem nowadays. Despite the centuries of learned experience, the appearance of antibiotics and the use of different pneumococcal vaccines, empyema remains the most common complication of pneumonia and an important cause of morbidity worldwide.[3] The incidence of pleural infections diminished significantly during the first half of the 20th century because of the development of antibiotics. However, this trend changed at the end of the 20th century and, since the decade of the 1990s the incidence of empyema has tended to be increasing worldwide.

The human spleen mainly serves an immune function against invading microorganisms.[4,5] The immunologic and hematologic functions of the spleen in humans are well known. Specifically, the spleen protects against infections mediated by innate and adaptive immunity.[6,7] Patients with splenectomy are more likely than those without splenectomy to suffer severe life-threatening infections. Splenectomy is associated with increased risk of some diseases, including pulmonary tuberculosis, type 2 diabetes mellitus, pyogenic liver abscess, renal and perinephric abscesses, and acute pancreatitis, [8-12] but empyema has not yet been studied.

Despite the incidence of empyema has tended to be increasing worldwide, no study has evaluated the association between splenectomy and empyema. We rationally hypothesize an association between splenectomy and empyema due to the immunocompromised condition caused by splenectomy, which can further increase

the risk of microorganism invasion of the pleural space. However, published literature from epidemiological studies on this issue is scarce. Given that splenectomy is associated with overwhelming postsplenectomy infections and empyema carries a potential fatality, exploring the risk of empyema in patients with splenectomy may have significant clinical and public health implications. Therefore, to explore whether there is an association between splenectomy and empyema, we conducted a nationwide cohort study using the hospitalization dataset of the Taiwan National Health Insurance Program.

METHODS

Study design and data source

Taiwan is an independent country with more than 23 million people. We conducted a population-based cohort study using insurance claim data from the Taiwan National Health Insurance Program which covers 99% of the whole Taiwan population since 1995.[13] The details of the insurance program have been well written in previous studies.[14-17] The study was approved by the Institutional Review Board of China Medical University and Hospital in Taiwan (CMUH-104-REC2-115).

Sampled Participants

Using the hospitalization dataset of the Taiwan National Health Insurance Program, all hospitalized subjects aged 20 to 84 who performed splenectomy (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9 procedure code 41.5) between January 1, 2000 and December 31, 2010, were identified as the splenectomy group. The date for undergoing splenectomy was defined as the index date. For each subject with splenectomy, 4 subjects without splenectomy were randomly selected from the same database as the non-splenectomy group. Both groups were matched by sex, age (every 5-year span), comorbidities, and the hospitalization year of performing splenectomy. To reduce the biased results, subjects

who had an empyema diagnosis (ICD-9 codes 510, 511.1, 511.8, and 511.9) within one month after performing splenectomy were excluded from the study.

Outcome and Comorbidities

The main outcome was a new diagnosis of empyema based on hospital discharge registries during the follow-up period. Each subject was monitored from the index date until being diagnosed with empyema, or being censored because of loss to follow-up, death, or withdrawal from insurance, or to the end of December 31, 2011. Comorbidities investigated in the study were included as follows: alcohol-related disease, cancers, chronic kidney disease, chronic liver disease (including cirrhosis, alcoholic liver damage, hepatitis B, hepatitis C, and other chronic hepatitis), chronic obstructive pulmonary disease, and diabetes mellitus. All comorbidities were diagnosed with ICD-9 codes, which have been well assessed in previous studies.[18-28]

Statistical analysis

The differences between sex, age, and comorbidities between the splenectomy group and the non-splenectomy group were compared by using the Chi-square test for categorical variables, and *t*-test for continuous variables. Follow-up time (person-years) was used to estimate the incidence rate and incidence rate ratio (IRR) with 95% confidence interval (CI) of splenectomy group to non-splenectomy group using Poisson regression, by sex, age, and follow-up period. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) of empyema associated with splenectomy and other comorbidities after simultaneously adjusted for variables found to be significant in the univariable Cox proportion hazard regression model. The proportional hazard model assumption was examined by using a test of scaled Schoenfeld residuals. In the model evaluating empyema risk throughout the overall follow-up period, results of the test revealed a significant relationship between Schoenfeld residuals for splenectomy

and follow-up time, suggesting the proportionality assumption was violated (*P* value < 0.001). In the subsequent analysis, we stratified the follow-up period to deal with the violation of proportional hazard assumption (Please see Table 2). All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Baseline information of the study population

Table 1 discloses the baseline information of the study population. There were 13193 subjects with splenectomy and 52464 subjects without splenectomy during the study period, with similar distributions in sex and age. The mean ages (mean ± standard deviation) were 52.8 ± 17.2 years in the splenectomy group and 52.5 ± 17.2 years in the non-splenectomy group (*t* test, $P = 0.05$). The mean follow-up periods (mean ± standard deviation) were 4.37± 3.44 years in the splenectomy group and 5.75 ± 3.32 years in the non-splenectomy group (*t* test, $P < 0.001$). There was no significant difference in the prevalence of comorbidities studied between the splenectomy group and the non-splenectomy group (Chi-square test, $P > 0.05$ for all).

Incidence of empyema stratified by sex, age, and follow-up period

Table 2 discloses the incidence rates of empyema**.** At the end of the cohort study, the overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The incidence rates of empyema, as stratified by sex, age, and follow-up period, were all higher in the splenectomy group than those in the non-splenectomy group. The incidence rates of empyema increased with age in both groups, with the highest in the splenectomy group aged 65 to 84 years (19.2 per 1,000 person-years). Stratified analysis by follow-up period disclosed that the incidence rates of empyema decreased with the follow-up time in both groups. The risk of empyema in the splenectomy group was significantly higher in the first 5 years of follow-up (incidence rate ratio 2.87, 95% CI 2.73, 3.01). However, the risk of empyema still existed in the splenectomy group even after 5 years (incidence rate ratio 1.73, 95% CI 1.60, 1.88).

In Figure 1, the Kaplan-Meier model revealed that the splenectomy group had a

higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001).

Hazard ratio of empyema associated with splenectomy and other comorbidities

Table 3 discloses the HR of empyema associated with splenectomy and other comorbidities. Variable found to be statistically significant in the univariable model were further examined in the multivariable model. After adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus, the multivariable Cox proportional hazards regression model disclosed that the adjusted HR of empyema was 2.89 in subjects with splenectomy (95% CI 2.60, 3.22), compared with subjects without splenectomy.

Interaction effect on risk of empyema between splenectomy and other comorbidities

Table 4 discloses interaction effect on risk of empyema between splenectomy and other comorbidities including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. As a reference of subjects without splenectomy and without any comorbidity, the adjusted HR of empyema was 4.52 for subjects with splenectomy alone and without any comorbidity (95% CI 3.80, 5.37). The HR markedly increased to 8.23 for those with splenectomy and comorbid with any comorbidity (95% CI 6.98, 9.70). It means that there is an interaction effect on risk of empyema between splenectomy and other comorbidities.

DISCUSSION

A review by Sinwar found that the duration between splenectomy and onset of overwhelming post-splenectomy infection could range from less than 1 week to more than 20 years.[29] To diminish biased results, we excluded patients who underwent splenectomy within 1 month of empyema diagnosis to ensure that splenectomy truly preceded the onset of empyema. In this population-based cohort study, we noticed that splenectomy was associated with increased hazard ratio of empyema (adjusted HR 2.89). To the best of our knowledge, this is the first population-based cohort study to examine this issue. Some studies have found that splenectomy is associated with increased risk of some diseases. Lai et al. found that the adjusted odds ratio of acute pancreatitis was 2.90 for subjects with splenectomy (95% CI, 1.39–6.05) compared with subjects without splenectomy,[12] the adjusted hazard ratio of renal and perinephric abscesses was 2.24 for the splenectomy group (95% CI, 1.30–3.88) when compared with the non-splenectomy group,[11] the adjusted hazard ratio of pyogenic liver abscess was 3.89 in subjects with splenectomy (95% confidence interval, 3.20–4.72) when compared with subjects without splenectomy,[10] and the odds of pulmonary tuberculosis were 1.91 in patients with splenectomy (95% CI 1.06–3.44) compared with the participants without splenectomy.[8]

Extensive evidence has revealed that the human spleen mainly serves a protective role against invading microorganisms on the basis of bactericidal capacity of lymphoid cells and macrophages and the humoral immune response.[6,30-32] Upon removal of the spleen, normal immune functions, such as phagocytic activities and the humoral immune response, may be significantly changed. Therefore, impaired immune functions after splenectomy may increase the risk of life-threatening infection and empyema.

In our study, the risk of empyema in the splenectomy group was higher in the first

5 years of follow-up than after 5 years (incidence rate ratio 2.87 vs 1.72). However, the risk of empyema still existed in the splenectomy group even after 5 years. These findings are compatible with previous studies showing that the majority of severe infections occur within the first 3 years after splenectomy, and, although the risk declines over time, the risk might last for more than 5 years after splenectomy.[33-35] The mechanism remains unknown. We speculate that with time, the immune system could develop ways to compensate or overcome these immune deficits. So the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up. Future studies are needed to confirm this hypothesis.

In this study, after controlling for potential confounding factors, we also observed that patients with splenectomy were at increased risk of empyema (adjusted HR 2.89). The HR seems to be higher than that observed for comorbidities. The HR was not confounded by comorbidities studied because there was no significant difference in the prevalence of comorbidities between the splenectomy group and the non-splenectomy group. It means the increased hazard of empyema in patients with splenectomy cannot be totally attributable to the effect of comorbidities. Though these comorbidities were found to be associated with empyema, to minimize their confounding effects, we made a further analysis, even in absence of any comorbidity, patients with splenectomy still had a higher hazard of empyema (HR 4.52). These results indicate that not requiring the presence of comorbidity, splenectomy may have a unique role on risk of empyema. These findings are compatible with the literature that patients with splenectomy are not only more prone to suffer severe life-threatening infection due to the immunocompromised condition caused by splenectomy[36,37] but also at an increased hazard of developing empyema.

Some limitations in the present study deserve discussion. First, some traditional behavior risk factors including alcohol consumption and cigarette smoking were not

recorded due to the inherent limitation of this insurance database. We used alcohol-related diseases instead of alcohol consumption and chronic obstructive pulmonary disease instead of cigarette smoking. Second, the underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database. Splenectomy is commonly performed in certain disorders and diseases, such as hematological disorders, gastric cancer, or trauma, so these background conditions might confound the results. Whether the reasons for splenectomy are associated with empyema cannot be clarified in this study. Third, Due to the inherent limitation of this insurance database, the underlying causes for splenectomy were not recorded. The cause of splenectomy could be the cause of the empyema, for example, splenic abscess. From a view of the good quality of the Taiwan medical system, it does not need to spend one month to confirm a diagnosis of empyema from the onset of empyema prodrome. In order to reduce the biased results, subjects who had an empyema diagnosis within one month after performing splenectomy were excluded from the study. Therefore, it is less possible that splenectomy could be the cause of the empyema. Fourth, empyema could correlate very well with open surgery. However, due to the same limitation, the splenectomized type was not recorded. We did not know how the patients were splenectomized, open surgery or laparoscopic surgery. Similarly, we did not know that patients underwent total splenectomy or partial splenectomy. Fifth, lack of vaccination could correlate very well with empyema. However, due to the same limitation, we did not know how many of the splenectomized patients were vaccinated against encapsulated bacteria (especially Streptococcus pneumoniae). We could not investigate whether pneumococcal vaccination might decrease the risk of empyema among patients with splenectomy in Taiwan. Sixth, due to the same limitation, causative pathogens were not recorded. We could not investigate what kind of bacteria would cause the

empyema among patients with splenectomy. Lack of such information does not permit the present study to conclude a substantial causality. Further prospective studies are needed to confirm our findings.

The strength of this study is that this is the first original study on the association between splenectomy and empyema. Although the underlying mechanism linking splenectomy and empyema cannot be completely determined, our findings are clinically important. In addition, we used a hospitalization dataset with a large sample size and great statistical power. The diagnosis codes of included comorbidities have been documented in previous studies. [18-28] The study design and statistical methods are described in detail. Our results were relatively convincing because the splenectomy group and the non-splenectomy group had similar distributions of studied comorbidities. Therefore, the confounding effects of these comorbidities on risk of empyema should be minimal.

We conclude that patients with splenectomy are associated with 2.89-fold increased risk of empyema, particularly comorbid with other conditions, including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. Even in the absence of comorbidities, the risk remains high.

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Specific author contributions

Hsien-Feng Lin and Kuan-Fu Liao substantially planned and conducted this study, participated in the data interpretation, and also critically revised the article.

Ching-Mei Chang and Cheng-Li Lin conducted the data analysis and critically revised the article.

Shih-Wei Lai substantially planned and conducted this study, contributed to the conception of the article, initiated the draft of the article, and critically revised the article.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

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REFERENCES

1. Ahmed RA, Marrie TJ, Huang JQ. Thoracic empyema in patients with community-acquired pneumonia. Am J Med 2006;119:877-83.
2. Shields T, Locicero J, Ponn R, Rusch V, eds. General Thoracic Surgery, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2005:823.
3. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennet JE, Mandell RD, editors. Douglas and Bennett's principles and practice of infectious diseases. Edinburgh: Churchill Livingstone; 2009. pp. 917-24.
4. Spencer RP, Pearson HA. The spleen as a hematological organ. Semin Nucl Med. 1975;5:95–102.
5. Trigg ME. Immune function of the spleen. South Med J. 1979;72:593–9.
6. Jirillo E, Mastronardi ML, Altamura M, Munno I, Miniello S, Urgesi G, et al. The immunocompromised host: immune alterations in splenectomized patients and clinical implications. Curr Pharm Des. 2003;9:1918–23.
7. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97.
8. Lai SW, Wang IK, Lin CL, Chen HJ, Liao KF. Splenectomy correlates with increased risk of pulmonary tuberculosis: a case-control study in Taiwan. Clin Microbiol Infect 2014;20:764-7.
9. Wu SC, Fu CY, Muo CH, Chang YJ. Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 2014;208:811-6.
10. Lai SW, Lai HC, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Pyogenic Liver Abscess: A Nationwide Cohort Study in Taiwan. J Epidemiol 2015;25:561-6.
11. Lai SW, Lin HF, Lin CL, Liao KF. Splenectomy and risk of renal and perinephric abscesses: A population-based cohort study in Taiwan. Medicine 2016;95:31(e4438).
12. Lai SW, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Acute Pancreatitis: A Case-Control Study in Taiwan. J Epidemiol 2016;26:488-92.
13. National Health Insurance Research Database. Taiwan. http://nhird.nhri.org.tw/en/index.html [cited in March 1, 2017, English version].
14. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine 2010;89:295-9.
15. Chen HY, Lai SW, Muo CH, Chen PC, Wang IJ. Ethambutol-induced optic neuropathy: a nationwide population-based study from Taiwan. Br J Ophthalmol

2012;96:1368-71.
16. Yang SP, Muo CH, Wang IK, et al. Risk of type 2 diabetes mellitus in female breast cancer patients treated with morphine: A retrospective population-based time-dependent cohort study. Diabetes Res Clin Pract 2015;110:285-90.
17. Tsai TY, Lin CC, Peng CY, et al. The association between biliary tract inflammation and risk of digestive system cancers: A population-based cohort study. Medicine 2016;95:31(e4427).
18. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 2012;107:46-52.
19. Liao KF, Lin CL, Lai SW, Chen WC. Zolpidem Use Associated With Increased Risk of Pyogenic Liver Abscess: A Case-Control Study in Taiwan. Medicine 2015;94:32(e1302).
20. Wong TS, Liao KF, Lin CM, Lin CL, Chen WC, Lai SW. Chronic Pancreatitis Correlates With Increased Risk of Cerebrovascular Disease: A Retrospective Population-Based Cohort Study in Taiwan. Medicine 2016;95:15(e3266).
21. Liao KF, Lai SW, Lin CL, Chien SH. Appendectomy correlates with increased risk of pyogenic liver abscess: A population-based cohort study in Taiwan. Medicine 2016;95:26(e4015).
22. Cheng KC, Lin WY, Liu CS, Lin CC, Lai HC, Lai SW. Association of different types of liver disease with demographic and clinical factors. BioMedicine-Taiwan 2016;6:16-22.
23. Liao KF, Cheng KC, Lin CL, Lai SW. Etodolac and the risk of acute pancreatitis. BioMedicine-Taiwan 2017;7:25-9.
24. Shen ML, Liao KF, Tsai SM, Lin CL, Lai SW. Herpes zoster correlates with pyogenic liver abscesses in Taiwan. BioMedicine-Taiwan 2016;6:24-9.
25. Lai SW. Risks and benefits of zolpidem use in Taiwan: a narrative review. BioMedicine-Taiwan 2016;6:9-11.
26. Lin HF, Lai SW, Lin WY, Liu CS, Lin CC, Chang CM. Prevalence and factors of elevated alanine aminotransferase in central Taiwan - a retrospective study. BioMedicine-Taiwan 2016;6:25-30.
27. Lai SW, Muo CH, Liao KF, Sung FC, Chen PC. Risk of acute pancreatitis in type 2 diabetes and risk reduction on anti-diabetic drugs: a population-based cohort study in Taiwan. Am J Gastroenterol. 2011;106:1697–704.
28. Lai HC, Lin CC, Cheng KS, Kao JT, Chou JW, Peng CY, et al. Increased incidence of gastrointestinal cancers among patients with pyogenic liver abscess: a population-based cohort study. Gastroenterology. 2014;146:129–37 e1.
29. Sinwar PD. Overwhelming post splenectomy infection syndrome—review study.

Int J Surg. 2014;12:1314–6.
30. Eibl M. Immunological consequences of splenectomy. Prog Pediatr Surg. 1985;18:139–45.
31. Van Rooijen N. The humoral immune response in the spleen. Res Immunol. 1991;142:328–30.
32. Altamura M, Caradonna L, Amati L, Pellegrino NM, Urgesi G, Miniello S. Splenectomy and sepsis: the role of the spleen in the immune-mediated bacterial clearance. Immunopharmacol Immunotoxicol. 2001;23:153–61.
33. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post-splenectomy patients. J Infect. 2001;43:182–6.
34. Kyaw MH, Holmes EM, Toolis F, Wayne B, Chalmers J, Jones IG, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. 2006;119:276 e1–7.
35. Dendle C, Sundararajan V, Spelman T, Jolley D, Woolley I. Splenectomy sequelae: an analysis of infectious outcomes among adults in Victoria. Med J Aust. 2012;196:582–6.
36. Di Cataldo A, Puleo S, Li Destri G, Racalbuto A, Trombatore G, Latteri F, et al. Splenic trauma and overwhelming postsplenectomy infection. Br J Surg. 1987;74:343–5.
37. Lynch AM, Kapila R. Overwhelming postsplenectomy infection. Infect Dis Clin North Am. 1996;10:693–707.

Table 1. Baseline characteristics between splenectomy group and non-splenectomy group

	Splenectomy				
	No N=52464		Yes N=13193		
Variable	N	%	n	%	*P* value*
Sex					0.88
Female	20431	38.9	5128	38.9	
Male	32033	61.1	8065	61.1	
Age group (years)					0.98
20–39	13415	25.6	3364	25.5	
40–64	24112	46.0	6062	46.0	
65-84	14937	28.5	3767	28.6	
Age (years), mean (standard deviation) †	52.5	(17.2)	52.8	(17.2)	0.05
Follow-up period (years), mean (standard deviation) †	5.75	(3.32)	4.37	(3.44)	< 0.001
Baseline comorbidities					
Alcohol-related disease	1787	3.41	452	3.43	0.91
Cancers	7677	14.6	1962	14.9	0.49
Chronic kidney disease	1068	2.04	270	2.05	0.94
Chronic liver disease	7791	14.9	1971	14.9	0.80
Chronic obstructive pulmonary disease	2002	3.82	507	3.84	0.89
Diabetes mellitus	7856	15.0	1983	15.0	0.87

Data are presented as the number of subjects in each group, with percentages given in parentheses, or mean with standard deviation given in parentheses.

*Chi-square test, and †*t*-test comparing subjects with and without splenectomy.

Table 2. Incidence density of empyema estimated by sex, age, and follow-up period between splenectomy group and non-splenectomy group

Variable	Non-splenectomy				Splenectomy				IRR[#]	(95% CI)
	N	Cases	Person-years	Incidence[†]	N	Cases	Person-years	Incidence[†]		
All	52464	1042	301484	3.46	13193	510	57622	8.85	2.56	(2.44,2.69)
Sex										
Female	20431	317	119441	2.65	5128	159	23004	6.91	2.60	(2.40, 2.82)
Male	32033	725	182042	3.98	8065	351	34618	10.1	2.55	(2.39, 2.71)
Age group (years)										
20–39	13415	57	84004	0.68	3364	65	19700	3.30	4.86	(4.39, 5.39)
40–64	24112	354	140554	2.52	6062	224	26423	8.48	3.37	(3.13, 3.62)
65-84	14937	631	76925	8.20	3767	221	11499	19.2	2.34	(2.14, 2.56)
Follow-up period (years)										
< 5	52464	720	206010	3.49	13193	416	41545	10.0	2.87	(2.73, 3.01)
≥ 5	28456	322	95474	3.37	5052	94	16077	5.85	1.73	(1.60, 1.88)

[†] Incidence rate: per 1,000 person-years.

[#]IRR (incidence rate ratio): splenectomy vs. non-splenectomy (95% CI)

Table 3. Adjusted hazard ratio and 95% confidence interval of empyema associated with splenectomy and other comorbidities

	Crude	Adjusted[†]
Variable	HR (95%CI)	HR (95%CI)
Sex (male vs. female)	1.48 (1.33, 1.65)	1.51 (1.35, 1.68)
Age (per one year)	1.05 (1.04, 1.05)	1.05 (1.05, 1.06)
Baseline comorbidities (yes vs. no)		
Splenectomy	**2.52 (2.26, 2.80)**	**2.89 (2.60, 3.22)**
Alcohol-related disease	1.61 (1.28, 2.03)	2.25 (1.76, 2.86)
Cancers	2.60 (2.31, 2.92)	1.92 (1.71, 2.17)
Chronic kidney disease	3.04 (2.40, 3.85)	2.13 (1.67, 2.70)
Chronic liver disease	2.07 (1.84, 2.34)	1.90 (1.68, 2.14)
Chronic obstructive pulmonary disease	3.95 (3.37, 4.64)	1.75 (1.48, 2.07)
Diabetes mellitus	2.80 (2.51, 3.13)	1.85 (1.65, 2.07)

[†]Variable found to be statistically significant in the univariable model were further examined in the multivariable model.

Adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

Table 4. Cox proportional hazard regression analysis for risk of empyema stratified by splenectomy and comorbidities

Variable		Event	Incidence†	Adjusted HR# (95% CI)
Splenectomy	Any comorbidity*			
No	No	299	1.49	1(Reference)
No	Yes	743	7.34	3.64(3.18, 4.17)
Yes	No	230	5.75	4.52(3.80, 5.37)
Yes	Yes	280	15.9	8.23(6.98, 9.70)

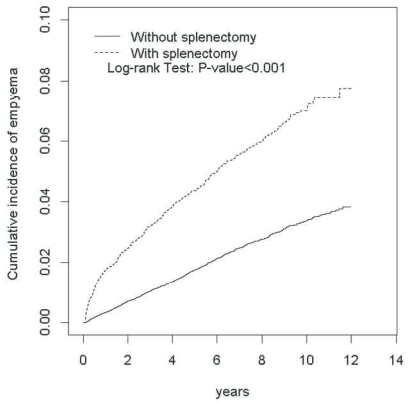
† Incidence rate: per 1,000 person-years

#Adjusted for sex and age

*Comorbidities including alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

95% CI: 95% confidence interval

Fig 1: Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001)



190x107mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses	3,4	
Methods				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*	
Bias	9	Describe any efforts to address potential sources of bias	4,5	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7,8*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Population-based cohort study examining the association between splenectomy and empyema in adults in Taiwan

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ABSTRACT

Objectives. Little is known about the risk of empyema in patients with splenectomy. We investigate the relationship between splenectomy and empyema in Taiwan.

Methods. We conducted a population-based cohort analysis using the hospitalization dataset of the Taiwan National Health Insurance Program. There were 13193 subjects aged 20 to 84 years who were newly diagnosed with splenectomy in 2000 to 2010 as the splenectomy group and 52464 randomly selected subjects without splenectomy as the non-splenectomy group. Both groups were matched by sex, age, comorbidities, and hospitalization year of undergoing splenectomy. The incidence of empyema at the end of 2011 was calculated. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for empyema associated with splenectomy and other comorbidities. **Results**. The overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up). After adjusted for confounders, the adjusted HR of empyema was 2.89 in subjects with splenectomy (95%CI 2.60, 3.22), compared with subjects without splenectomy. In further analysis, in the absence of comorbidities studied, the HR was 4.52 for those with splenectomy alone (95% CI=3.80, 5.37). **Conclusions**. Patients with splenectomy are associated with 2.89-fold increased risk of empyema. In the absence of comorbidities, the risk remains high. The incidence rate ratio between splenectomy and non-splenectomy from 2.87 reduced to 1.73 after 5 years, but the risk of empyema still existed in the splenectomy group. Future studies are needed to confirm that if the study had an even longer follow up, this average would have gone down further.

Keywords: empyema; splenectomy; Taiwan National Health Insurance Program

Strengths and limitations of this study.

1. This is the first original study on the association between splenectomy and empyema.
2. We used a hospitalization dataset with a large sample size and great statistical power.
3. Some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database.
4. The underlying causes for splenectomy in this present study were not recorded

due to the inherent limitation of this insurance database.

5. Such a study design does not permit to conclude a substantial causality.

INTRODUCTION

Empyema is a suppurative infection of the pleural space. The creation of empyema can be divided into two distinct mechanisms. Empyema occurs most commonly after pneumonia, with direct spread of organisms into the pleural space. This occurs in approximately 1% to 5% of pneumonias.[1,2] The other mechanism occurs after surgery, most commonly of the thorax, esophagus, lung or heart. Empyema is an ancient disease that continues to be an important clinical problem nowadays. Despite the centuries of learned experience, the appearance of antibiotics and the use of different pneumococcal vaccines, empyema remains the most common complication of pneumonia and an important cause of morbidity worldwide.[3] The incidence of pleural infections diminished significantly during the first half of the 20th century because of the development of antibiotics. However, this trend changed at the end of the 20th century and, since the decade of the 1990s the incidence of empyema has tended to be increasing worldwide.

The human spleen mainly serves an immune function against invading microorganisms.[4,5] The immunologic and hematologic functions of the spleen in humans are well known. Specifically, the spleen protects against infections mediated by innate and adaptive immunity.[6,7] Patients with splenectomy are more likely than those without splenectomy to suffer severe life-threatening infections. Splenectomy is associated with increased risk of some diseases, including pulmonary tuberculosis, type 2 diabetes mellitus, pyogenic liver abscess, renal and perinephric abscesses, and acute pancreatitis, [8-12] but empyema has not yet been studied.

Despite the incidence of empyema has tended to be increasing worldwide, no study has evaluated the association between splenectomy and empyema. We rationally hypothesize an association between splenectomy and empyema due to the immunocompromised condition caused by splenectomy, which can further increase

the risk of microorganism invasion of the pleural space. However, published literature from epidemiological studies on this issue is scarce. Given that splenectomy is associated with overwhelming postsplenectomy infections and empyema carries a potential fatality, exploring the risk of empyema in patients with splenectomy may have significant clinical and public health implications. Therefore, to explore whether there is an association between splenectomy and empyema, we conducted a nationwide cohort study using the hospitalization dataset of the Taiwan National Health Insurance Program.

METHODS

Study design and data source

Taiwan is an independent country with more than 23 million people. We conducted a population-based cohort study using insurance claim data from the Taiwan National Health Insurance Program which covers 99% of the whole Taiwan population since 1995.[13] The details of the insurance program have been well written in previous studies.[14-17] The study was approved by the Institutional Review Board of China Medical University and Hospital in Taiwan (CMUH-104-REC2-115).

Sampled Participants

Using the hospitalization dataset of the Taiwan National Health Insurance Program, all hospitalized subjects aged 20 to 84 who underwent splenectomy (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9 procedure code 41.5) between January 1, 2000 and December 31, 2010, were identified as the splenectomy group. The date for undergoing splenectomy was defined as the index date. For each subject with splenectomy, 4 subjects without splenectomy were randomly selected from the same database as the non-splenectomy group. Both groups were matched by sex, age (every 5-year span), comorbidities, and the hospitalization year of undergoing splenectomy. To reduce the biased results, subjects

who had an empyema diagnosis (ICD-9 codes 510, 511.1, 511.8, and 511.9) within one month after undergoing splenectomy were excluded from the study.

Outcome and Comorbidities

The main outcome was a new diagnosis of empyema based on hospital discharge registries during the follow-up period. Each subject was monitored from the index date until being diagnosed with empyema, or being censored because of loss to follow-up, death, or withdrawal from insurance, or to the end of December 31, 2011. Comorbidities investigated in the study were included as follows: alcohol-related disease, cancers, chronic kidney disease, chronic liver disease (including cirrhosis, alcoholic liver damage, hepatitis B, hepatitis C, and other chronic hepatitis), chronic obstructive pulmonary disease, and diabetes mellitus. All comorbidities were diagnosed with ICD-9 codes, which have been well assessed in previous studies.[18-28]

Statistical analysis

The differences between sex, age, and comorbidities between the splenectomy group and the non-splenectomy group were compared by using the Chi-square test for categorical variables, and *t*-test for continuous variables. Follow-up time (person-years) was used to estimate the incidence rate and incidence rate ratio (IRR) with 95% confidence interval (CI) of splenectomy group to non-splenectomy group using Poisson regression, by sex, age, and follow-up period. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) of empyema associated with splenectomy and other comorbidities after simultaneously adjusted for variables found to be significant in the univariable Cox proportion hazard regression model. The proportional hazard model assumption was examined by using a test of scaled Schoenfeld residuals. In the model evaluating empyema risk throughout the overall follow-up period, results of the test revealed a significant relationship between Schoenfeld residuals for splenectomy

and follow-up time, suggesting the proportionality assumption was violated (*P* value < 0.001). In the subsequent analysis, we stratified the follow-up period to deal with the violation of proportional hazard assumption (Please see Table 2). All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Baseline information of the study population

Table 1 discloses the baseline information of the study population. There were 13193 subjects with splenectomy and 52464 subjects without splenectomy during the study period, with similar distributions in sex and age. The mean ages (mean ± standard deviation) were 52.8 ± 17.2 years in the splenectomy group and 52.5 ± 17.2 years in the non-splenectomy group (*t* test, $P = 0.05$). The mean follow-up periods (mean ± standard deviation) were 4.37± 3.44 years in the splenectomy group and 5.75 ± 3.32 years in the non-splenectomy group (*t* test, $P < 0.001$). There was no significant difference in the prevalence of comorbidities studied between the splenectomy group and the non-splenectomy group (Chi-square test, $P > 0.05$ for all).

Incidence of empyema stratified by sex, age, and follow-up period

Table 2 discloses the incidence rates of empyema**.** At the end of the cohort study, the overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The incidence rates of empyema, as stratified by sex, age, and follow-up period, were all higher in the splenectomy group than those in the non-splenectomy group. The incidence rates of empyema increased with age in both groups, with the highest in the splenectomy group aged 65 to 84 years (19.2 per 1,000 person-years). Stratified analysis by follow-up period disclosed that the incidence rates of empyema decreased with the follow-up time in both groups. The risk of empyema in the splenectomy group was significantly higher in the first 5 years of follow-up (incidence rate ratio 2.87, 95% CI 2.73, 3.01). However, the risk of empyema still existed in the splenectomy group even after 5 years (incidence rate ratio 1.73, 95% CI 1.60, 1.88).

In Figure 1, the Kaplan-Meier model revealed that the splenectomy group had a

higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001).

Hazard ratio of empyema associated with splenectomy and other comorbidities

Table 3 discloses the HR of empyema associated with splenectomy and other comorbidities. Variable found to be statistically significant in the univariable model were further examined in the multivariable model. After adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus, the multivariable Cox proportional hazards regression model disclosed that the adjusted HR of empyema was 2.89 in subjects with splenectomy (95% CI 2.60, 3.22), compared with subjects without splenectomy.

Interaction effect on risk of empyema between splenectomy and other comorbidities

Table 4 discloses interaction effect on risk of empyema between splenectomy and other comorbidities including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. As a reference of subjects without splenectomy and without any comorbidity, the adjusted HR of empyema was 4.52 for subjects with splenectomy alone and without any comorbidity (95% CI 3.80, 5.37). The HR markedly increased to 8.23 for those with splenectomy and comorbid with any comorbidity (95% CI 6.98, 9.70). It means that there is an interaction effect on risk of empyema between splenectomy and other comorbidities.

DISCUSSION

A review by Sinwar found that the duration between splenectomy and onset of overwhelming post-splenectomy infection could range from less than 1 week to more than 20 years.[29] To diminish biased results, we excluded patients who underwent splenectomy within 1 month of empyema diagnosis to ensure that splenectomy truly preceded the onset of empyema. In this population-based cohort study, we noticed that splenectomy was associated with increased hazard ratio of empyema (adjusted HR 2.89). To the best of our knowledge, this is the first population-based cohort study to examine this issue. Some studies have found that splenectomy is associated with increased risk of some diseases. Lai et al. found that the adjusted odds ratio of acute pancreatitis was 2.90 for subjects with splenectomy (95% CI, 1.39–6.05) compared with subjects without splenectomy,[12] the adjusted hazard ratio of renal and perinephric abscesses was 2.24 for the splenectomy group (95% CI, 1.30–3.88) when compared with the non-splenectomy group,[11] the adjusted hazard ratio of pyogenic liver abscess was 3.89 in subjects with splenectomy (95% confidence interval, 3.20–4.72) when compared with subjects without splenectomy,[10] and the odds of pulmonary tuberculosis were 1.91 in patients with splenectomy (95% CI 1.06–3.44) compared with the participants without splenectomy.[8]

Extensive evidence has revealed that the human spleen mainly serves a protective role against invading microorganisms on the basis of bactericidal capacity of lymphoid cells and macrophages and the humoral immune response.[6,30-32] Upon removal of the spleen, normal immune functions, such as phagocytic activities and the humoral immune response, may be significantly changed. Therefore, impaired immune functions after splenectomy may increase the risk of life-threatening infection and empyema.

In our study, the risk of empyema in the splenectomy group was higher in the first

5 years of follow-up than after 5 years (incidence rate ratio 2.87 vs 1.73). However, the risk of empyema still existed in the splenectomy group even after 5 years. These findings are compatible with previous studies showing that the majority of severe infections occur within the first 3 years after splenectomy, and, although the risk declines over time, the risk might last for more than 5 years after splenectomy.[33-35] The mechanism remains unknown. We speculate that with time, the immune system could develop ways to compensate or overcome these immune deficits. So the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up. Future studies are needed to confirm this hypothesis.

In this study, after controlling for potential confounding factors, we also observed that patients with splenectomy were at increased risk of empyema (adjusted HR 2.89). The phenomenon that would normally be regarded as counter-intuitive and the reason were unclear. It may be due to some comorbidities which could be potentially related to empyema should be included in the study, but we lose them. Future studies are needed to explain this phenomenon. The HR seems to be higher than that observed for comorbidities. The HR was not confounded by comorbidities studied because there was no significant difference in the prevalence of comorbidities between the splenectomy group and the non-splenectomy group. It means the increased hazard of empyema in patients with splenectomy cannot be totally attributable to the effect of comorbidities. Though these comorbidities were found to be associated with empyema, to minimize their confounding effects, we made a further analysis, in absence of any comorbidity, patients with splenectomy still had a higher hazard of empyema (HR 4.52). These results indicate that not requiring the presence of comorbidity, splenectomy may have a unique role on risk of empyema. These findings are compatible with the literature that patients with splenectomy are not only more prone to suffer severe life-threatening infection due to the immunocompromised

condition caused by splenectomy[36,37] but also at an increased hazard of developing empyema.

Some limitations in the present study deserve discussion. First, some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database. We used alcohol-related diseases instead of alcohol consumption and chronic obstructive pulmonary disease instead of cigarette smoking. Second, the underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database. Splenectomy is commonly underwent in certain disorders and diseases, such as hematological disorders, gastric cancer, or trauma, so these background conditions might confound the results. Whether the reasons for splenectomy are associated with empyema cannot be clarified in this study. Third, Due to the inherent limitation of this insurance database, the underlying causes for splenectomy were not recorded. The cause of splenectomy could be the cause of the empyema, for example, splenic abscess. From a view of the good quality of the Taiwan medical system, it does not need to spend one month to confirm a diagnosis of empyema from the onset of empyema prodrome. In order to reduce the biased results, subjects who had an empyema diagnosis within one month after undergoing splenectomy were excluded from the study. Therefore, it is less possible that splenectomy could be the cause of the empyema. Fourth, empyema could correlate very well with open surgery. However, due to the same limitation, the splenectomized type was not recorded. We did not know how the patients were splenectomized, open surgery or laparoscopic surgery. Similarly, we did not know that patients underwent total splenectomy or partial splenectomy. Fifth, lack of vaccination could correlate very well with empyema. However, due to the same limitation, we did not know how many of the splenectomized patients were vaccinated against encapsulated bacteria

(especially Streptococcus pneumoniae). We could not investigate whether pneumococcal vaccination might decrease the risk of empyema among patients with splenectomy in Taiwan. Sixth, due to the same limitation, causative pathogens were not recorded. We could not investigate what kind of bacteria would cause the empyema among patients with splenectomy. Lack of such information does not permit the present study to conclude a substantial causality. Further prospective studies are needed to confirm our findings.

The strength of this study is that this is the first original study on the association between splenectomy and empyema. Although the underlying mechanism linking splenectomy and empyema cannot be completely determined, our findings are clinically important. In addition, we used a hospitalization dataset with a large sample size and great statistical power. The diagnosis codes of included comorbidities have been documented in previous studies.[18-28] The study design and statistical methods are described in detail. Our results were relatively convincing because the splenectomy group and the non-splenectomy group had similar distributions of studied comorbidities. Therefore, the confounding effects of these comorbidities on risk of empyema should be minimal.

We conclude that patients with splenectomy are associated with 2.89-fold increased risk of empyema, particularly comorbid with other conditions, including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. In the absence of comorbidities, the risk remains high. The incidence rate ratio (IRR) between splenectomy and non-splenectomy from 2.87 reduced to 1.73.after 5 years, but the risk of empyema still existed in the splenectomy group even after 5 years. Future studies are needed to confirm that if the study had an even longer follow up, this average would have gone down further.

Data sharing statement: no additional data available

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Specific author contributions

Hsien-Feng Lin and Kuan-Fu Liao substantially planned and conducted this study, participated in the data interpretation, and also critically revised the article.

Ching-Mei Chang and Cheng-Li Lin conducted the data analysis and critically revised the article.

Shih-Wei Lai substantially planned and conducted this study, contributed to the conception of the article, initiated the draft of the article, and critically revised the article.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

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REFERENCES

1. Ahmed RA, Marrie TJ, Huang JQ. Thoracic empyema in patients with community-acquired pneumonia. Am J Med 2006;119:877-83.
2. Shields T, Locicero J, Ponn R, Rusch V, eds. General Thoracic Surgery, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2005:823.
3. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennet JE, Mandell RD, editors. Douglas and Bennett's principles and practice of infectious diseases. Edinburgh: Churchill Livingstone; 2009. pp. 917-24.
4. Spencer RP, Pearson HA. The spleen as a hematological organ. Semin Nucl Med. 1975;5:95–102.
5. Trigg ME. Immune function of the spleen. South Med J. 1979;72:593–9.
6. Jirillo E, Mastronardi ML, Altamura M, Munno I, Miniello S, Urgesi G, et al. The immunocompromised host: immune alterations in splenectomized patients and clinical implications. Curr Pharm Des. 2003;9:1918–23.
7. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97.
8. Lai SW, Wang IK, Lin CL, Chen HJ, Liao KF. Splenectomy correlates with increased risk of pulmonary tuberculosis: a case-control study in Taiwan. Clin Microbiol Infect 2014;20:764-7.
9. Wu SC, Fu CY, Muo CH, Chang YJ. Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 2014;208:811-6.
10. Lai SW, Lai HC, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Pyogenic Liver Abscess: A Nationwide Cohort Study in Taiwan. J Epidemiol 2015;25:561-6.
11. Lai SW, Lin HF, Lin CL, Liao KF. Splenectomy and risk of renal and perinephric abscesses: A population-based cohort study in Taiwan. Medicine 2016;95:31(e4438).
12. Lai SW, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Acute Pancreatitis: A Case-Control Study in Taiwan. J Epidemiol 2016;26:488-92.
13. National Health Insurance Research Database. Taiwan. http://nhird.nhri.org.tw/en/index.html [cited in March 1, 2017, English version].
14. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine 2010;89:295-9.
15. Chen HY, Lai SW, Muo CH, Chen PC, Wang IJ. Ethambutol-induced optic neuropathy: a nationwide population-based study from Taiwan. Br J Ophthalmol

2012;96:1368-71.
16. Yang SP, Muo CH, Wang IK, et al. Risk of type 2 diabetes mellitus in female breast cancer patients treated with morphine: A retrospective population-based time-dependent cohort study. Diabetes Res Clin Pract 2015;110:285-90.
17. Tsai TY, Lin CC, Peng CY, et al. The association between biliary tract inflammation and risk of digestive system cancers: A population-based cohort study. Medicine 2016;95:31(e4427).
18. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 2012;107:46-52.
19. Liao KF, Lin CL, Lai SW, Chen WC. Zolpidem Use Associated With Increased Risk of Pyogenic Liver Abscess: A Case-Control Study in Taiwan. Medicine 2015;94:32(e1302).
20. Wong TS, Liao KF, Lin CM, Lin CL, Chen WC, Lai SW. Chronic Pancreatitis Correlates With Increased Risk of Cerebrovascular Disease: A Retrospective Population-Based Cohort Study in Taiwan. Medicine 2016;95:15(e3266).
21. Liao KF, Lai SW, Lin CL, Chien SH. Appendectomy correlates with increased risk of pyogenic liver abscess: A population-based cohort study in Taiwan. Medicine 2016;95:26(e4015).
22. Cheng KC, Lin WY, Liu CS, Lin CC, Lai HC, Lai SW. Association of different types of liver disease with demographic and clinical factors. BioMedicine-Taiwan 2016;6:16-22.
23. Liao KF, Cheng KC, Lin CL, Lai SW. Etodolac and the risk of acute pancreatitis. BioMedicine-Taiwan 2017;7:25-9.
24. Shen ML, Liao KF, Tsai SM, Lin CL, Lai SW. Herpes zoster correlates with pyogenic liver abscesses in Taiwan. BioMedicine-Taiwan 2016;6:24-9.
25. Lai SW. Risks and benefits of zolpidem use in Taiwan: a narrative review. BioMedicine-Taiwan 2016;6:9-11.
26. Lin HF, Lai SW, Lin WY, Liu CS, Lin CC, Chang CM. Prevalence and factors of elevated alanine aminotransferase in central Taiwan - a retrospective study. BioMedicine-Taiwan 2016;6:25-30.
27. Lai SW, Muo CH, Liao KF, Sung FC, Chen PC. Risk of acute pancreatitis in type 2 diabetes and risk reduction on anti-diabetic drugs: a population-based cohort study in Taiwan. Am J Gastroenterol. 2011;106:1697–704.
28. Lai HC, Lin CC, Cheng KS, Kao JT, Chou JW, Peng CY, et al. Increased incidence of gastrointestinal cancers among patients with pyogenic liver abscess: a population-based cohort study. Gastroenterology. 2014;146:129–37 e1.
29. Sinwar PD. Overwhelming post splenectomy infection syndrome—review study.

Int J Surg. 2014;12:1314–6.
30. Eibl M. Immunological consequences of splenectomy. Prog Pediatr Surg. 1985;18:139–45.
31. Van Rooijen N. The humoral immune response in the spleen. Res Immunol. 1991;142:328–30.
32. Altamura M, Caradonna L, Amati L, Pellegrino NM, Urgesi G, Miniello S. Splenectomy and sepsis: the role of the spleen in the immune-mediated bacterial clearance. Immunopharmacol Immunotoxicol. 2001;23:153–61.
33. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post-splenectomy patients. J Infect. 2001;43:182–6.
34. Kyaw MH, Holmes EM, Toolis F, Wayne B, Chalmers J, Jones IG, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. 2006;119:276 e1–7.
35. Dendle C, Sundararajan V, Spelman T, Jolley D, Woolley I. Splenectomy sequelae: an analysis of infectious outcomes among adults in Victoria. Med J Aust. 2012;196:582–6.
36. Di Cataldo A, Puleo S, Li Destri G, Racalbuto A, Trombatore G, Latteri F, et al. Splenic trauma and overwhelming postsplenectomy infection. Br J Surg. 1987;74:343–5.
37. Lynch AM, Kapila R. Overwhelming postsplenectomy infection. Infect Dis Clin North Am. 1996;10:693–707.

Table 1. Baseline characteristics between splenectomy group and non-splenectomy group

	Splenectomy				
	No		Yes		
	N=52464		N=13193		
Characteristic	N	%	n	%	*P* value*
Sex					0.88
Female	20431	38.9	5128	38.9	
Male	32033	61.1	8065	61.1	
Age group (years)					0.98
20–39	13415	25.6	3364	25.5	
40–64	24112	46.0	6062	46.0	
65-84	14937	28.5	3767	28.6	
Age (years), mean (standard deviation) †	52.5	(17.2)	52.8	(17.2)	0.05
Follow-up period (years), mean (standard deviation) †	5.75	(3.32)	4.37	(3.44)	< 0.001
Baseline comorbidities					
Alcohol-related disease	1787	3.41	452	3.43	0.91
Cancers	7677	14.6	1962	14.9	0.49
Chronic kidney disease	1068	2.04	270	2.05	0.94
Chronic liver disease	7791	14.9	1971	14.9	0.80
Chronic obstructive pulmonary disease	2002	3.82	507	3.84	0.89
Diabetes mellitus	7856	15.0	1983	15.0	0.87

Data are presented as the number of subjects in each group, with percentages given in parentheses, or mean with standard deviation given in parentheses.

*Chi-square test, and †*t*-test comparing subjects with and without splenectomy.

Table 2. Incidence density of empyema estimated by sex, age, and follow-up period between splenectomy group and non-splenectomy group

Variable	Non-splenectomy				Splenectomy				IRR[#]	(95% CI)
	N	Cases	Person-years	Incidence[†]	N	Cases	Person-years	Incidence[†]		
All	52464	1042	301484	3.46	13193	510	57622	8.85	2.56	(2.44,2.69)
Sex										
Female	20431	317	119441	2.65	5128	159	23004	6.91	2.60	(2.40, 2.82)
Male	32033	725	182042	3.98	8065	351	34618	10.1	2.55	(2.39, 2.71)
Age group (years)										
20–39	13415	57	84004	0.68	3364	65	19700	3.30	4.86	(4.39, 5.39)
40–64	24112	354	140554	2.52	6062	224	26423	8.48	3.37	(3.13, 3.62)
65-84	14937	631	76925	8.20	3767	221	11499	19.2	2.34	(2.14, 2.56)
Follow-up period (years)										
< 5	52464	720	206010	3.49	13193	416	41545	10.0	2.87	(2.73, 3.01)
≥ 5	28456	322	95474	3.37	5052	94	16077	5.85	1.73	(1.60, 1.88)

[†] Incidence rate: per 1,000 person-years.

[#]IRR (incidence rate ratio): splenectomy vs. non-splenectomy (95% CI)

Table 3. Adjusted hazard ratio and 95% confidence interval of empyema associated with splenectomy and other comorbidities

	Crude	Adjusted[†]
Variable	HR (95%CI)	HR (95%CI)
Sex (male vs. female)	1.48 (1.33, 1.65)	1.51 (1.35, 1.68)
Age (per one year)	1.05 (1.04, 1.05)	1.05 (1.05, 1.06)
Baseline comorbidities (yes vs. no)		
Splenectomy	**2.52 (2.26, 2.80)**	**2.89 (2.60, 3.22)**
Alcohol-related disease	1.61 (1.28, 2.03)	2.25 (1.76, 2.86)
Cancers	2.60 (2.31, 2.92)	1.92 (1.71, 2.17)
Chronic kidney disease	3.04 (2.40, 3.85)	2.13 (1.67, 2.70)
Chronic liver disease	2.07 (1.84, 2.34)	1.90 (1.68, 2.14)
Chronic obstructive pulmonary disease	3.95 (3.37, 4.64)	1.75 (1.48, 2.07)
Diabetes mellitus	2.80 (2.51, 3.13)	1.85 (1.65, 2.07)

[†]Variables found to be statistically significant in the univariable model were further examined in the multivariable model. Adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

Table 4. Cox proportional hazard regression analysis for risk of empyema stratified by splenectomy and comorbidities

Variable		Event	Incidence†	Adjusted HR# (95% CI)
Splenectomy	Any comorbidity*			
No	No	299	1.49	1(Reference)
No	Yes	743	7.34	3.64(3.18, 4.17)
Yes	No	230	5.75	4.52(3.80, 5.37)
Yes	Yes	280	15.9	8.23(6.98, 9.70)

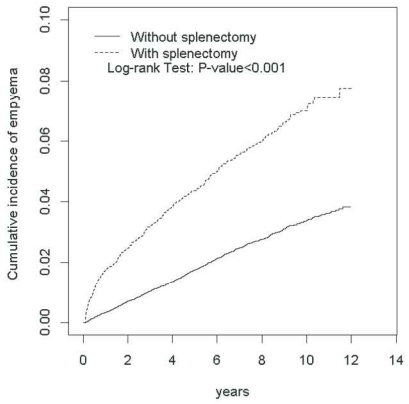
† Incidence rate: per 1,000 person-years

#Adjusted for sex and age

*Comorbidities including alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

95% CI: 95% confidence interval

Fig 1: Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001)



190x107mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses	3,4	
Methods				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*	
Bias	9	Describe any efforts to address potential sources of bias	4,5	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7,8*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Population-based cohort study examining the association between splenectomy and empyema in adults in Taiwan

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ABSTRACT

Objectives. Little is known about the risk of empyema in patients with splenectomy. We investigate the relationship between splenectomy and empyema in Taiwan.

Methods. We conducted a population-based cohort analysis using the hospitalization dataset of the Taiwan National Health Insurance Program. There were 13193 subjects aged 20 to 84 years who were newly diagnosed with splenectomy in 2000 to 2010 as the splenectomy group and 52464 randomly selected subjects without splenectomy as the non-splenectomy group. Both groups were matched by sex, age, comorbidities, and hospitalization year of undergoing splenectomy. The incidence of empyema at the end of 2011 was calculated. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for empyema associated with splenectomy and other comorbidities. **Results**. The overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up). After adjusted for confounders, the adjusted HR of empyema was 2.89 in subjects with splenectomy (95%CI 2.60, 3.22), compared with subjects without splenectomy. In further analysis, in the absence of comorbidities studied, the HR was 4.52 for those with splenectomy alone (95% CI=3.80, 5.37). **Conclusions**. The incidence rate ratio between splenectomy and non-splenectomy from 2.87 reduced to 1.73.after 5 years, but the risk of empyema still existed in the splenectomy group. Patients with splenectomy are associated with 2.89-fold increased risk of empyema. In the absence of comorbidities, the risk remains high. Future studies are needed to confirm that if the study had an even longer follow up, this average would have gone down further.

Keywords: empyema; splenectomy; Taiwan National Health Insurance Program

Strengths and limitations of this study.

1. This is the first original study on the association between splenectomy and empyema.
2. We used a hospitalization dataset with a large sample size and great statistical power.
3. Some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database.
4. The underlying causes for splenectomy in this present study were not recorded

due to the inherent limitation of this insurance database.

5. Such a study design does not permit to conclude a substantial causality.

INTRODUCTION

Empyema is a suppurative infection of the pleural space. The creation of empyema can be divided into two distinct mechanisms. Empyema occurs most commonly after pneumonia, with direct spread of organisms into the pleural space. This occurs in approximately 1% to 5% of pneumonias.[1,2] The other mechanism occurs after surgery, most commonly of the thorax, esophagus, lung or heart. Empyema is an ancient disease that continues to be an important clinical problem nowadays. Despite the centuries of learned experience, the appearance of antibiotics and the use of different pneumococcal vaccines, empyema remains the most common complication of pneumonia and an important cause of morbidity worldwide.[3] The incidence of pleural infections diminished significantly during the first half of the 20th century because of the development of antibiotics. However, this trend changed at the end of the 20th century and, since the decade of the 1990s the incidence of empyema has tended to be increasing worldwide.

The human spleen mainly serves an immune function against invading microorganisms.[4,5] The immunologic and hematologic functions of the spleen in humans are well known. Specifically, the spleen protects against infections mediated by innate and adaptive immunity.[6,7] Patients with splenectomy are more likely than those without splenectomy to suffer severe life-threatening infections. Splenectomy is associated with increased risk of some diseases, including pulmonary tuberculosis, type 2 diabetes mellitus, pyogenic liver abscess, renal and perinephric abscesses, and acute pancreatitis, [8-12] but empyema has not yet been studied.

Despite the incidence of empyema has tended to be increasing worldwide, no study has evaluated the association between splenectomy and empyema. We rationally hypothesize an association between splenectomy and empyema due to the immunocompromised condition caused by splenectomy, which can further increase

the risk of microorganism invasion of the pleural space. However, published literature from epidemiological studies on this issue is scarce. Given that splenectomy is associated with overwhelming postsplenectomy infections and empyema carries a potential fatality, exploring the risk of empyema in patients with splenectomy may have significant clinical and public health implications. Therefore, to explore whether there is an association between splenectomy and empyema, we conducted a nationwide cohort study using the hospitalization dataset of the Taiwan National Health Insurance Program.

METHODS

Study design and data source

Taiwan is an independent country with more than 23 million people. We conducted a population-based cohort study using insurance claim data from the Taiwan National Health Insurance Program which covers 99% of the whole Taiwan population since 1995.[13] The details of the insurance program have been well written in previous studies.[14-17] The study was approved by the Research Ethics Committee of China Medical University and Hospital in Taiwan (CMUH-104-REC2-115).

Sampled Participants

Using the hospitalization dataset of the Taiwan National Health Insurance Program, all hospitalized subjects aged 20 to 84 who underwent splenectomy (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9 procedure code 41.5) between January 1, 2000 and December 31, 2010, were identified as the splenectomy group. The date for undergoing splenectomy was defined as the index date. For each subject with splenectomy, 4 subjects without splenectomy were randomly selected from the same database as the non-splenectomy group. Both groups were matched by sex, age (every 5-year span), comorbidities, and the hospitalization year of undergoing splenectomy. To reduce the biased results, subjects

who had an empyema diagnosis (ICD-9 codes 510, 511.1, 511.8, and 511.9) within one month after undergoing splenectomy were excluded from the study.

Outcome and Comorbidities

The main outcome was a new diagnosis of empyema based on hospital discharge registries during the follow-up period. Each subject was monitored from the index date until being diagnosed with empyema, or being censored because of loss to follow-up, death, or withdrawal from insurance, or to the end of December 31, 2011. Comorbidities investigated in the study were included as follows: alcohol-related disease, cancers, chronic kidney disease, chronic liver disease (including cirrhosis, alcoholic liver damage, hepatitis B, hepatitis C, and other chronic hepatitis), chronic obstructive pulmonary disease, and diabetes mellitus. All comorbidities were diagnosed with ICD-9 codes, which have been well assessed in previous studies.[18-28]

Statistical analysis

The differences between sex, age, and comorbidities between the splenectomy group and the non-splenectomy group were compared by using the Chi-square test for categorical variables, and *t*-test for continuous variables. Follow-up time (person-years) was used to estimate the incidence rate and incidence rate ratio (IRR) with 95% confidence interval (CI) of splenectomy group to non-splenectomy group using Poisson regression, by sex, age, and follow-up period. The multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) of empyema associated with splenectomy and other comorbidities after simultaneously adjusted for variables found to be significant in the univariable Cox proportion hazard regression model. The proportional hazard model assumption was examined by using a test of scaled Schoenfeld residuals. In the model evaluating empyema risk throughout the overall follow-up period, results of the test revealed a significant relationship between Schoenfeld residuals for splenectomy

and follow-up time, suggesting the proportionality assumption was violated (*P* value < 0.001). In the subsequent analysis, we stratified the follow-up period to deal with the violation of proportional hazard assumption. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Two-tailed $P < 0.05$ was considered statistically significant.

RESULTS

Baseline information of the study population

Table 1 discloses the baseline information of the study population. There were 13193 subjects with splenectomy and 52464 subjects without splenectomy during the study period, with similar distributions in sex and age. The mean ages (mean ± standard deviation) were 52.8 ± 17.2 years in the splenectomy group and 52.5 ± 17.2 years in the non-splenectomy group (*t* test, $P = 0.05$). The mean follow-up periods (mean ± standard deviation) were 4.37± 3.44 years in the splenectomy group and 5.75 ± 3.32 years in the non-splenectomy group (*t* test, $P < 0.001$). There was no significant difference in the prevalence of comorbidities studied between the splenectomy group and the non-splenectomy group (Chi-square test, $P > 0.05$ for all).

Incidence of empyema stratified by sex, age, and follow-up period

Table 2 discloses the incidence rates of empyema**.** At the end of the cohort study, the overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than that in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years, 95% CI 2.44, 2.69). The incidence rates of empyema, as stratified by sex, age, and follow-up period, were all higher in the splenectomy group than those in the non-splenectomy group. The incidence rates of empyema increased with age in both groups, with the highest in the splenectomy group aged 65 to 84 years (19.2 per 1,000 person-years). Stratified analysis by follow-up period disclosed that the incidence rates of empyema decreased with the follow-up time in both groups. The risk of empyema in the splenectomy group was significantly higher in the first 5 years of follow-up (incidence rate ratio 2.87, 95% CI 2.73, 3.01). However, the risk of empyema still existed in the splenectomy group even after 5 years (incidence rate ratio 1.73, 95% CI 1.60, 1.88).

In Figure 1, the Kaplan-Meier model revealed that the splenectomy group had a

higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; P <0.001).

Hazard ratio of empyema associated with splenectomy and other comorbidities

Table 3 discloses the HR of empyema associated with splenectomy and other comorbidities. Variable found to be statistically significant in the univariable model were further examined in the multivariable model. After adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus, the multivariable Cox proportional hazards regression model disclosed that the adjusted HR of empyema was 2.89 in subjects with splenectomy (95% CI 2.60, 3.22), compared with subjects without splenectomy.

Interaction effect on risk of empyema between splenectomy and other comorbidities

Table 4 discloses interaction effect on risk of empyema between splenectomy and other comorbidities including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. As a reference of subjects without splenectomy and without any comorbidity, the adjusted HR of empyema was 4.52 for subjects with splenectomy alone and without any comorbidity (95% CI 3.80, 5.37). The HR markedly increased to 8.23 for those with splenectomy and comorbid with any comorbidity (95% CI 6.98, 9.70). It means that there is an interaction effect on risk of empyema between splenectomy and other comorbidities.

DISCUSSION

A review by Sinwar found that the duration between splenectomy and onset of overwhelming post-splenectomy infection could range from less than 1 week to more than 20 years.[29] To diminish biased results, we excluded patients who underwent splenectomy within 1 month of empyema diagnosis to ensure that splenectomy truly preceded the onset of empyema.

Extensive evidence has revealed that the human spleen mainly serves a protective role against invading microorganisms on the basis of bactericidal capacity of lymphoid cells and macrophages and the humoral immune response.[6,30-32] Upon removal of the spleen, normal immune functions, such as phagocytic activities and the humoral immune response, may be significantly changed. Therefore, impaired immune functions after splenectomy may increase the risk of life-threatening infection and empyema. In our study, the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up than after 5 years (incidence rate ratio 2.87 vs 1.73). However, the risk of empyema still existed in the splenectomy group even after 5 years. These findings are compatible with previous studies showing that the majority of severe infections occur within the first 3 years after splenectomy, and, although the risk declines over time, the risk might last for more than 5 years after splenectomy.[33-35] The mechanism remains unknown. We speculate that with time, the immune system could develop ways to compensate or overcome these immune deficits. So the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up. Future studies are needed to confirm this hypothesis.

In this population-based cohort study, we noticed that splenectomy was associated with increased hazard ratio of empyema (adjusted HR 2.89). To the best of our knowledge, this is the first population-based cohort study to examine this issue. Some studies have found that splenectomy is associated with increased risk of some diseases.

Lai et al. found that the adjusted odds ratio of acute pancreatitis was 2.90 for subjects with splenectomy (95% CI, 1.39–6.05) compared with subjects without splenectomy,[12] the adjusted hazard ratio of renal and perinephric abscesses was 2.24 for the splenectomy group (95% CI, 1.30–3.88) when compared with the non-splenectomy group,[11] the adjusted hazard ratio of pyogenic liver abscess was 3.89 in subjects with splenectomy (95% confidence interval, 3.20–4.72) when compared with subjects without splenectomy,[10] and the odds of pulmonary tuberculosis were 1.91 in patients with splenectomy (95% CI 1.06–3.44) compared with the participants without splenectomy.[8]

In this study, after controlling for potential confounding factors, we also observed that patients with splenectomy were at increased risk of empyema (adjusted HR 2.89). The phenomenon that would normally be regarded as counter-intuitive and the reason were unclear. It may be due to some comorbidities which could be potentially related to empyema should be included in the study, but we lose them. Future studies are needed to explain this phenomenon. The HR seems to be higher than that observed for comorbidities. The HR was not confounded by comorbidities studied because there was no significant difference in the prevalence of comorbidities between the splenectomy group and the non-splenectomy group. It means the increased hazard of empyema in patients with splenectomy cannot be totally attributable to the effect of comorbidities. Though these comorbidities were found to be associated with empyema, to minimize their confounding effects, we made a further analysis, in absence of any comorbidity, patients with splenectomy still had a higher hazard of empyema (HR 4.52). These results indicate that not requiring the presence of comorbidity, splenectomy may have a unique role on risk of empyema. These findings are compatible with the literature that patients with splenectomy are not only more prone to suffer severe life-threatening infection due to the immunocompromised

condition caused by splenectomy[36,37] but also at an increased hazard of developing empyema.

Some limitations in the present study deserve discussion. First, some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database. We used alcohol-related diseases instead of alcohol consumption and chronic obstructive pulmonary disease instead of cigarette smoking. Second, the underlying causes for splenectomy in this present study were not recorded due to the inherent limitation of this insurance database. Splenectomy is commonly underwent in certain disorders and diseases, such as hematological disorders, gastric cancer, or trauma, so these background conditions might confound the results. Whether the reasons for splenectomy are associated with empyema cannot be clarified in this study. Third, Due to the inherent limitation of this insurance database, the underlying causes for splenectomy were not recorded. The cause of splenectomy could be the cause of the empyema, for example, splenic abscess. From a view of the good quality of the Taiwan medical system, it does not need to spend one month to confirm a diagnosis of empyema from the onset of empyema prodrome. In order to reduce the biased results, subjects who had an empyema diagnosis within one month after undergoing splenectomy were excluded from the study. Therefore, it is less possible that splenectomy could be the cause of the empyema. Fourth, empyema could correlate very well with open surgery. However, due to the same limitation, the splenectomized type was not recorded. We did not know how the patients were splenectomized, open surgery or laparoscopic surgery. Similarly, we did not know that patients underwent total splenectomy or partial splenectomy. Fifth, lack of vaccination could correlate very well with empyema. However, due to the same limitation, we did not know how many of the splenectomized patients were vaccinated against encapsulated bacteria

(especially Streptococcus pneumoniae). We could not investigate whether pneumococcal vaccination might decrease the risk of empyema among patients with splenectomy in Taiwan. Sixth, due to the same limitation, causative pathogens were not recorded. We could not investigate what kind of bacteria would cause the empyema among patients with splenectomy. Lack of such information does not permit the present study to conclude a substantial causality. Further prospective studies are needed to confirm our findings.

The strength of this study is that this is the first original study on the association between splenectomy and empyema. Although the underlying mechanism linking splenectomy and empyema cannot be completely determined, our findings are clinically important. In addition, we used a hospitalization dataset with a large sample size and great statistical power. The diagnosis codes of included comorbidities have been documented in previous studies.[18-28] The study design and statistical methods are described in detail. Our results were relatively convincing because the splenectomy group and the non-splenectomy group had similar distributions of studied comorbidities. Therefore, the confounding effects of these comorbidities on risk of empyema should be minimal.

We conclude that the incidence rate ratio between splenectomy and non-splenectomy from 2.87 reduced to 1.73 after 5 years, but the risk of empyema still existed in the splenectomy group even after 5 years. Patients with splenectomy are associated with 2.89-fold increased risk of empyema, particularly comorbid with other conditions, including alcohol-related diseases, cancers, chronic kidney diseases, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus. In the absence of comorbidities, the risk remains high. Future studies are needed to confirm that if the study had an even longer follow up, this average would have gone down further.

Data sharing statement: no additional data available

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Specific author contributions

Hsien-Feng Lin and Kuan-Fu Liao substantially planned and conducted this study, participated in the data interpretation, and also critically revised the article.

Ching-Mei Chang and Cheng-Li Lin conducted the data analysis and critically revised the article.

Shih-Wei Lai substantially planned and conducted this study, contributed to the conception of the article, initiated the draft of the article, and critically revised the article.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

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REFERENCES

1. Ahmed RA, Marrie TJ, Huang JQ. Thoracic empyema in patients with community-acquired pneumonia. Am J Med 2006;119:877-83.
2. Shields T, Locicero J, Ponn R, Rusch V, eds. General Thoracic Surgery, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2005:823.
3. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennet JE, Mandell RD, editors. Douglas and Bennett's principles and practice of infectious diseases. Edinburgh: Churchill Livingstone; 2009. pp. 917-24.
4. Spencer RP, Pearson HA. The spleen as a hematological organ. Semin Nucl Med. 1975;5:95–102.
5. Trigg ME. Immune function of the spleen. South Med J. 1979;72:593–9.
6. Jirillo E, Mastronardi ML, Altamura M, Munno I, Miniello S, Urgesi G, et al. The immunocompromised host: immune alterations in splenectomized patients and clinical implications. Curr Pharm Des. 2003;9:1918–23.
7. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97.
8. Lai SW, Wang IK, Lin CL, Chen HJ, Liao KF. Splenectomy correlates with increased risk of pulmonary tuberculosis: a case-control study in Taiwan. Clin Microbiol Infect 2014;20:764-7.
9. Wu SC, Fu CY, Muo CH, Chang YJ. Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 2014;208:811-6.
10. Lai SW, Lai HC, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Pyogenic Liver Abscess: A Nationwide Cohort Study in Taiwan. J Epidemiol 2015;25:561-6.
11. Lai SW, Lin HF, Lin CL, Liao KF. Splenectomy and risk of renal and perinephric abscesses: A population-based cohort study in Taiwan. Medicine 2016;95:31(e4438).
12. Lai SW, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Acute Pancreatitis: A Case-Control Study in Taiwan. J Epidemiol 2016;26:488-92.
13. National Health Insurance Research Database. Taiwan. http://nhird.nhri.org.tw/en/index.html [cited in March 1, 2017, English version].
14. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine 2010;89:295-9.
15. Chen HY, Lai SW, Muo CH, Chen PC, Wang IJ. Ethambutol-induced optic neuropathy: a nationwide population-based study from Taiwan. Br J Ophthalmol

2012;96:1368-71.
16. Yang SP, Muo CH, Wang IK, et al. Risk of type 2 diabetes mellitus in female breast cancer patients treated with morphine: A retrospective population-based time-dependent cohort study. Diabetes Res Clin Pract 2015;110:285-90.
17. Tsai TY, Lin CC, Peng CY, et al. The association between biliary tract inflammation and risk of digestive system cancers: A population-based cohort study. Medicine 2016;95:31(e4427).
18. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 2012;107:46-52.
19. Liao KF, Lin CL, Lai SW, Chen WC. Zolpidem Use Associated With Increased Risk of Pyogenic Liver Abscess: A Case-Control Study in Taiwan. Medicine 2015;94:32(e1302).
20. Wong TS, Liao KF, Lin CM, Lin CL, Chen WC, Lai SW. Chronic Pancreatitis Correlates With Increased Risk of Cerebrovascular Disease: A Retrospective Population-Based Cohort Study in Taiwan. Medicine 2016;95:15(e3266).
21. Liao KF, Lai SW, Lin CL, Chien SH. Appendectomy correlates with increased risk of pyogenic liver abscess: A population-based cohort study in Taiwan. Medicine 2016;95:26(e4015).
22. Cheng KC, Lin WY, Liu CS, Lin CC, Lai HC, Lai SW. Association of different types of liver disease with demographic and clinical factors. BioMedicine-Taiwan 2016;6:16-22.
23. Liao KF, Cheng KC, Lin CL, Lai SW. Etodolac and the risk of acute pancreatitis. BioMedicine-Taiwan 2017;7:25-9.
24. Shen ML, Liao KF, Tsai SM, Lin CL, Lai SW. Herpes zoster correlates with pyogenic liver abscesses in Taiwan. BioMedicine-Taiwan 2016;6:24-9.
25. Lai SW. Risks and benefits of zolpidem use in Taiwan: a narrative review. BioMedicine-Taiwan 2016;6:9-11.
26. Lin HF, Lai SW, Lin WY, Liu CS, Lin CC, Chang CM. Prevalence and factors of elevated alanine aminotransferase in central Taiwan - a retrospective study. BioMedicine-Taiwan 2016;6:25-30.
27. Lai SW, Muo CH, Liao KF, Sung FC, Chen PC. Risk of acute pancreatitis in type 2 diabetes and risk reduction on anti-diabetic drugs: a population-based cohort study in Taiwan. Am J Gastroenterol. 2011;106:1697–704.
28. Liao KF, Huang PT, Lin CC, Lin CL, Lai SW. Fluvastatin use and risk of acute pancreatitis:a population-based case-control study in Taiwan. BioMedicine-Taiwan 2017;7:1-5.
29. Sinwar PD. Overwhelming post splenectomy infection syndrome—review study.

Int J Surg. 2014;12:1314–6.
30. Eibl M. Immunological consequences of splenectomy. Prog Pediatr Surg. 1985;18:139–45.
31. Van Rooijen N. The humoral immune response in the spleen. Res Immunol. 1991;142:328–30.
32. Altamura M, Caradonna L, Amati L, Pellegrino NM, Urgesi G, Miniello S. Splenectomy and sepsis: the role of the spleen in the immune-mediated bacterial clearance. Immunopharmacol Immunotoxicol. 2001;23:153–61.
33. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post-splenectomy patients. J Infect. 2001;43:182–6.
34. Kyaw MH, Holmes EM, Toolis F, Wayne B, Chalmers J, Jones IG, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. 2006;119:276 e1–7.
35. Dendle C, Sundararajan V, Spelman T, Jolley D, Woolley I. Splenectomy sequelae: an analysis of infectious outcomes among adults in Victoria. Med J Aust. 2012;196:582–6.
36. Di Cataldo A, Puleo S, Li Destri G, Racalbuto A, Trombatore G, Latteri F, et al. Splenic trauma and overwhelming postsplenectomy infection. Br J Surg. 1987;74:343–5.
37. Lynch AM, Kapila R. Overwhelming postsplenectomy infection. Infect Dis Clin North Am. 1996;10:693–707.

Table 1. Baseline characteristics between splenectomy group and non-splenectomy group

	Splenectomy				
	No N=52464		Yes N=13193		
Characteristic	N	%	n	%	*P* value*
Sex					0.88
Female	20431	38.9	5128	38.9	
Male	32033	61.1	8065	61.1	
Age group (years)					0.98
20–39	13415	25.6	3364	25.5	
40–64	24112	46.0	6062	46.0	
65-84	14937	28.5	3767	28.6	
Age (years), mean (standard deviation) †	52.5	(17.2)	52.8	(17.2)	0.05
Follow-up period (years), mean (standard deviation) †	5.75	(3.32)	4.37	(3.44)	< 0.001
Baseline comorbidities					
Alcohol-related disease	1787	3.41	452	3.43	0.91
Cancers	7677	14.6	1962	14.9	0.49
Chronic kidney disease	1068	2.04	270	2.05	0.94
Chronic liver disease	7791	14.9	1971	14.9	0.80
Chronic obstructive pulmonary disease	2002	3.82	507	3.84	0.89
Diabetes mellitus	7856	15.0	1983	15.0	0.87

Data are presented as the number of subjects in each group, with percentages given in parentheses, or mean with standard deviation given in parentheses.

*Chi-square test, and †*t*-test comparing subjects with and without splenectomy.

Table 2. Incidence density of empyema estimated by sex, age, and follow-up period between splenectomy group and non-splenectomy group

Variable	Non-splenectomy				Splenectomy				IRR[#]	(95% CI)
	N	Cases	Person-years	Incidence[†]	N	Cases	Person-years	Incidence[†]		
All	52464	1042	301484	3.46	13193	510	57622	8.85	2.56	(2.44,2.69)
Sex										
Female	20431	317	119441	2.65	5128	159	23004	6.91	2.60	(2.40, 2.82)
Male	32033	725	182042	3.98	8065	351	34618	10.1	2.55	(2.39, 2.71)
Age group (years)										
20–39	13415	57	84004	0.68	3364	65	19700	3.30	4.86	(4.39, 5.39)
40–64	24112	354	140554	2.52	6062	224	26423	8.48	3.37	(3.13, 3.62)
65-84	14937	631	76925	8.20	3767	221	11499	19.2	2.34	(2.14, 2.56)
Follow-up period (years)										
< 5	52464	720	206010	3.49	13193	416	41545	10.0	2.87	(2.73, 3.01)
≥ 5	28456	322	95474	3.37	5052	94	16077	5.85	1.73	(1.60, 1.88)

[†] Incidence rate: per 1,000 person-years.

[#]IRR (incidence rate ratio): splenectomy vs. non-splenectomy (95% CI)

Table 3. Adjusted hazard ratio and 95% confidence interval of empyema associated with splenectomy and other comorbidities

	Crude	Adjusted†
Variable	HR (95%CI)	HR (95%CI)
Sex (male vs. female)	1.48 (1.33, 1.65)	1.51 (1.35, 1.68)
Age (per one year)	1.05 (1.04, 1.05)	1.05 (1.05, 1.06)
Baseline comorbidities (yes vs. no)		
Splenectomy	**2.52 (2.26, 2.80)**	**2.89 (2.60, 3.22)**
Alcohol-related disease	1.61 (1.28, 2.03)	2.25 (1.76, 2.86)
Cancers	2.60 (2.31, 2.92)	1.92 (1.71, 2.17)
Chronic kidney disease	3.04 (2.40, 3.85)	2.13 (1.67, 2.70)
Chronic liver disease	2.07 (1.84, 2.34)	1.90 (1.68, 2.14)
Chronic obstructive pulmonary disease	3.95 (3.37, 4.64)	1.75 (1.48, 2.07)
Diabetes mellitus	2.80 (2.51, 3.13)	1.85 (1.65, 2.07)

†Variables found to be statistically significant in the univariable model were further examined in the multivariable model. Adjusted for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

Table 4. Cox proportional hazard regression analysis for risk of empyema stratified by splenectomy and comorbidities

Variable		Event	Incidence†	Adjusted HR# (95% CI)
Splenectomy	Any comorbidity*			
No	No	299	1.49	1(Reference)
No	Yes	743	7.34	3.64(3.18, 4.17)
Yes	No	230	5.75	4.52(3.80, 5.37)
Yes	Yes	280	15.9	8.23(6.98, 9.70)

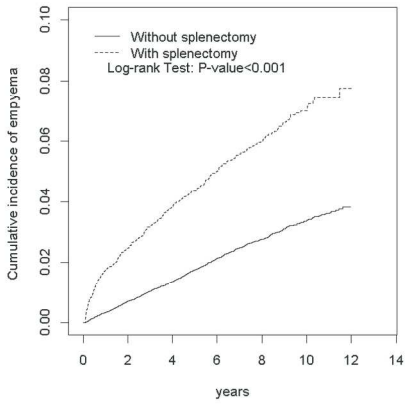
† Incidence rate: per 1,000 person-years

#Adjusted for sex and age

*Comorbidities including alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

95% CI: 95% confidence interval

Fig 1: Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; *P* <0.001)



190x107mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses	3,4	
Methods				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*	
Bias	9	Describe any efforts to address potential sources of bias	4,5	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7,8*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Population-based cohort study examining the association between splenectomy and empyema in adults in Taiwan

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Keywords:	empyema, splenectomy, Taiwan National Health Insurance Program

Population-based cohort study examining the association between splenectomy and empyema in adults in Taiwan

Running head: splenectomy and empyema

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ABSTRACT

Objective. This study aimed to investigate the association between splenectomy and empyema in Taiwan. **Methods**. A population-based cohort study was conducted using the hospitalization dataset of the Taiwan National Health Insurance Program. A total of 13193 subjects aged 20–84 years who were newly diagnosed with splenectomy from 2000 to 2010 were enrolled in the splenectomy group and 52464 randomly selected subjects without splenectomy were enrolled in the non-splenectomy group. Both groups were matched by sex, age, comorbidities, and the index year of undergoing splenectomy. The incidence of empyema at the end of 2011 was calculated. A multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% confidence interval (CI) of empyema associated with splenectomy and other comorbidities. **Results**. The overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years). The Kaplan–Meier analysis revealed a higher cumulative incidence of empyema in the splenectomy group than in the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up). After adjusting for confounding variables, the adjusted HR of empyema was 2.89 for the splenectomy group compared with that for the non-splenectomy group. Further analysis revealed that HR of empyema was 4.52 for subjects with splenectomy alone. **Conclusion**. The incidence rate ratio between the splenectomy and non-splenectomy groups reduced from 2.87 in the first 5 years of follow-up to 1.73 in the period following the 5 years. Future studies are required to confirm whether a longer follow-up period would further reduce this average ratio. For the splenectomy group, the overall HR of developing empyema was 2.89 after adjusting for age, sex, and comorbidities, which was identified from previous literature. The risk of empyema following splenectomy remains high despite the absence of these comorbidities.

Keywords: empyema; splenectomy; Taiwan National Health Insurance Program

Strengths and limitations of this study.

1. This is the first original study on the association between splenectomy and empyema.
2. We used a hospitalization dataset with a large sample size and great statistical power.
3. Some traditional behavior risk factors including alcohol consumption and cigarette smoking were not recorded due to the inherent limitation of this insurance database.
4. This case-control study included only patients with splenectomy, which may limit

the generalisability of the study results to the general population.

5. Such a study design does not permit to conclude a substantial causality.

INTRODUCTION

Pleural empyema is a suppurative infection of the pleural cavity. The etiology of empyema is classified as two distinct mechanisms. Empyema most commonly occurs following pneumonia as microorganisms spread directly into the pleural cavity. This occurs in approximately 1%–5% of pneumonia cases.[1,2] The second mechanism occurs following surgery, most commonly of the thorax, esophagus, lung, or heart. Although empyema is an ancient disease with centuries of learned experience, it continues to be an important clinical problem. Despite the use of antibiotics and different pneumococcal vaccines, empyema remains the most common complication of pneumonia and is an important cause of morbidity worldwide.[3] The development of antibiotics in the first half of the 20th century significantly contributed in decreasing the incidence of pleural infection. However, this trend shifted at the end of the 20th century, and the incidence of empyema has tended to increase worldwide.

The human spleen mainly serves as an immune responder against invading microorganisms.[4,5] Immunologic responses and hematologic functions of the human spleen is well-known. The spleen, mediated by the innate and adaptive immunity, protects the body against infections.[6,7] Therefore, patients with splenectomy are more likely than those without splenectomy to develop severe life-threatening infections. Splenectomy is associated with an increased risk of some diseases, including pulmonary tuberculosis, type 2 diabetes mellitus, pyogenic liver abscess, renal and perinephric abscesses, and acute pancreatitis; [8-12] however, to our knowledge, post-splenectomy empyema has not yet been studied.

Despite the trend of increasing incidence of empyema worldwide, no study has evaluated the association between splenectomy and empyema. Here we rationally hypothesize an association between splenectomy and empyema owing to the immunocompromized condition induced by splenectomy, which can further increase

the risk of microorganism invasion of the pleural cavity. However, there is limited published literature regarding epidemiological studies on this issue. As splenectomy is associated with overwhelming post-splenectomy infections and empyema carries potential fatality, exploring the risk of empyema in patients with splenectomy may have significant clinical and public health implications. Therefore, to explore whether an association between splenectomy and empyema exists, we conducted a nationwide cohort study using the hospitalization dataset of the Taiwan National Health Insurance Program.

METHODS

Study design and data source

Taiwan is an independent country with over 23 million people.[13-17] We conducted a population-based cohort study using insurance claim data from the Taiwan National Health Insurance Program, which has covered 99% of the Taiwan population since 1995 and thus is a thorough representative sample of the population.[18] The details of the insurance program have been well-documented in previous studies.[19-22] This study was approved by the Research Ethics Committee of China Medical University and Hospital in Taiwan (CMUH-104-REC2-115).

Study subjects

Using the hospitalization dataset of the Taiwan National Health Insurance Program, all hospitalized subjects aged 20–84 years who underwent splenectomy (International Classification of Diseases, Ninth Revision, Clinical Modification, ICD-9 procedure code 41.5) between January 1, 2000 and December 31, 2010 were categorized in the splenectomy group. The year of undergoing splenectomy was defined as the index year. For each subject in the splenectomy group, four subjects who did not undergo splenectomy were randomly selected from the same database and were categorized in the non-splenectomy group. Both groups were matched with regard to sex, age (every

5-year span), comorbidities, and the index year of undergoing splenectomy. To reduce potentially biased results, subjects with an empyema diagnosis (ICD-9 codes 510, 511.1, 511.8, and 511.9) within 1 month following splenectomy were excluded.

Outcome and Comorbidities

The main outcome was a new diagnosis of empyema on the basis of hospital discharge registries during the follow-up period. Each subject was monitored from the index year until being diagnosed with empyema; being censored because of the loss to follow-up, death, or withdrawal from insurance; or at the end of December 31, 2011, namely the end of the study. The following comorbidities were investigated: alcohol-related disease, cancer, chronic kidney disease, chronic liver disease (including cirrhosis, alcoholic liver damage, hepatitis B, hepatitis C, and other chronic hepatitis), chronic obstructive pulmonary disease, and diabetes mellitus. All comorbidities were diagnosed according to the ICD-9 codes, which have been well assessed in previous studies.[23-33]

Statistical analysis

The differences between the splenectomy and non-splenectomy groups with respect to sex, age, and comorbidities were compared using chi-square test for categorical variables and t-test for continuous variables. The subject's sex, age, and follow-up period (in person-years) were used to estimate incidence rate and incidence rate ratio (IRR) of the splenectomy group to the non-splenectomy group with 95% confidence interval (CI) using Poisson regression. Multivariable Cox proportional hazards regression model was used to estimate the hazard ratio (HR) with 95% CI of empyema associated with splenectomy and other comorbidities, after simultaneously adjusting for confounding variables in the univariable Cox proportional hazard regression model. The proportional hazard model assumption was examined using a test of scaled Schoenfeld residuals. The results of the model that evaluated the risk of

empyema throughout the follow-up period revealed a significant association between Schoenfeld residuals for splenectomy and follow-up period, suggesting that the proportionality assumption was violated ($P < 0.001$). In the subsequent analysis, we stratified the follow-up period to avoid violation of the proportional hazard assumption. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, North Carolina, USA). Two-tailed P values of <0.05 were considered to be statistically significant.

RESULTS

Baseline data of the study subjects

Table 1 shows the baseline data of the study subjects. A total of 13193 subjects with splenectomy and 52464 subjects without splenectomy were included, with similar distributions in sex and age. Mean ages (mean ± standard deviation) were 52.8 ± 17.2 years in the splenectomy group and 52.5 ± 17.2 years in the non-splenectomy group (t-test; $P = 0.05$). Mean follow-up periods (mean ± standard deviation) were 4.37 ± 3.44 person-years in the splenectomy group and 5.75 ± 3.32 person-years in the non-splenectomy group (t-test; $P < 0.001$). There was no significant difference in the prevalence of comorbidities between the splenectomy and non-splenectomy groups (chi-square test; $P > 0.05$ for all).

Incidence of empyema stratified by sex, age, and follow-up period

Table 2 shows the incidence rates of empyema. At the end of the cohort study, the overall incidence rate of empyema was 2.56-fold higher in the splenectomy group than in the non-splenectomy group (8.85 vs. 3.46 per 1,000 person-years; 95% CI, 2.44–2.69). The incidence rate of empyema, stratified by sex, age, and follow-up period, was higher in the splenectomy group than in the non-splenectomy group. The incidence rate of empyema increased with age in both the groups, with the highest rate reported in the splenectomy group with subjects aged 65–84 years (19.2 per 1,000 person-years). Stratified analysis by follow-up period revealed that the incidence rate of empyema decreased with the follow-up period in both the groups. The risk of empyema in the splenectomy group was significantly higher in the first 5 years of follow-up (IRR, 2.87; 95% CI, 2.73–3.01). However, risk of empyema continued to exist in the splenectomy group even after 5 years (IRR, 1.73; 95% CI, 1.60–1.88).

The Kaplan–Meier model revealed a higher cumulative incidence of pleural empyema in the splenectomy group than in the non-splenectomy group (6.99% vs.

3.37% at the end of follow-up; P < 0.001; Figure 1).

HR of empyema associated with splenectomy and other comorbidities

Table 3 displays HR of empyema associated with splenectomy and other comorbidities. Variables that were found to be statistically significant in the univariable model were further examined in the multivariable model. After adjusting for age, sex, alcohol-related disease, cancers, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus, the multivariable Cox proportional hazards regression model revealed that the adjusted HR of empyema was 2.89 in the splenectomy group (95% CI, 2.60–3.22) compared with that in the non-splenectomy group.

Interaction effect between splenectomy and other comorbidities on the risk of empyema

Table 4 displays the interaction effect between splenectomy and other comorbidities, including alcohol-related diseases, cancers, chronic kidney disease, chronic liver diseases, chronic obstructive pulmonary diseases, and diabetes mellitus, on the risk of empyema. The adjusted HR of empyema was 4.52 for subjects with splenectomy alone and without any comorbidity (95% CI, 3.80–5.37). HR markedly increased to 8.23 for subjects with splenectomy and with any comorbidity (95% CI, 6.98–9.70), demonstrating an interaction effect between splenectomy and other comorbidities on the risk of empyema.

DISCUSSION

Sinwar[34] found that the duration between splenectomy and the onset of overwhelming post-splenectomy infections could range from <1 week to >20 years. To reduce biased results, patients who underwent splenectomy within 1 month of empyema diagnosis were excluded to ensure that splenectomy truly preceded the onset of empyema.

Extensive evidence has supported the protective role of the human spleen against invading microorganisms on the basis of the bactericidal capacity of lymphoid cells and macrophages, as well as humoral immune response.[6,35-37] Following splenectomy, normal immune functions such as phagocytic activity and humoral immune response may be significantly changed. Therefore, impaired post-splenectomy immune functions may increase the risk of a life-threatening infection and empyema. In our study, the risk of empyema in the splenectomy group was higher in the first 5 years of follow-up than after the first 5 years (IRR, 2.87 vs 1.73). However, the risk of empyema continued to exist in the splenectomy group, even after the first 5 years. These findings are compatible with previously reported findings that revealed that the majority of severe infections occur within the first 3 years following splenectomy; although the risk declines over time, it may last for >5 years following splenectomy.[38-40] However, the exact mechanism underlying this risk remains unknown. We speculate that with time, the immune system may develop compensatory mechanisms that may overcome these immune deficits. Future studies are required to confirm this hypothesis.

To the best of our knowledge, this population-based cohort study is the first to reveal that splenectomy is associated with an increased HR of empyema (adjusted HR, 2.89). Some studies have reported that splenectomy is associated with an increased risk of diseases. Lai et al. compared between splenectomy patients and

non-splenectomy patients and found that for post-splenectomy patients, the adjusted odds ratio of acute pancreatitis was 2.90 (95% CI, 1.39–6.05), [12] the adjusted HR of renal and perinephric abscesses was 2.24 (95% CI, 1.30–3.88),[11] the adjusted HR of pyogenic liver abscess was 3.89 (95% CI, 3.20–4.72),[10] and the odds ratio of pulmonary tuberculosis were 1.91 (95% CI, 1.06–3.44).[8]

In this study, after adjusting for potential confounding variables, we also observed that the splenectomy group was at an increased risk of empyema (adjusted HR, 2.89). This phenomenon would typically be regarded as counterintuitive, but the exact reason remains unclear. A possible reason can be that some comorbidities that are potentially associated with empyema should have been included in the study; however, further studies are required to explain this phenomenon. The HR was higher than that observed for comorbidities. HR was not confounded by comorbidities because there was no significant difference in the prevalence of comorbidities between the splenectomy and non-splenectomy groups. This indicates that the increased HR of empyema in patients with splenectomy cannot be completely attributed to the prevalence of comorbidities. Although these comorbidities were found to be associated with empyema, to minimize their confounding effects, a further analysis was conducted. It was noted that in absence of any comorbidity, patients with splenectomy continued to have a higher HR of empyema (HR, 4.52). These results indicate that even in the absence of comorbidities, splenectomy may have a unique role in the risk of developing empyema. These findings are compatible with previously reported findings in the literature, in which patients with splenectomy are more prone to have severe life-threatening infections owing to an immunocompromized condition following splenectomy[41,42] and are at an increased risk of developing empyema.

The limitations of this study as follows. First, some traditional behavior risk factors,

including alcohol consumption and cigarette smoking, were not considered owing to inherent limitations of the insurance database. We used alcohol-related diseases instead of alcohol consumption and chronic obstructive pulmonary disease instead of cigarette smoking. Second, the underlying causes for splenectomy were also not recorded owing to limitation of the database. Splenectomy is common in certain disorders and diseases such as hematological disorders, gastric cancer, or trauma; thus, these background conditions may confound the results. We could not clarify the association of the cause of splenectomy with the development of empyema in this study. Third, owing to the limitation of the database, the underlying causes for splenectomy were not recorded. The cause of splenectomy could be the cause of empyema, for example, splenic abscess. Considering the high quality of the Taiwan medical system, 1 month is not required to confirm empyema diagnosis from the onset of empyema prodrome. To reduce biased results, subjects with an empyema diagnosis within 1 month following splenectomy were excluded. Therefore, it is less likely that splenectomy is the cause of empyema. Fourth, empyema could very well correlate with open surgery. However, owing to the previously mentioned limitations, the splenectomy type was not recorded. It is not known if splenectomy was performed via open or laparoscopic surgery or if it was a total or partial splenectomy. Fifth, the lack of vaccination could very well correlate with empyema. However, the number of subjects with splenectomy who were vaccinated against encapsulated bacteria (particularly *Streptococcus pneumoniae*) was unknown; thus, we could not investigate whether pneumococcal vaccination decreased the risk of empyema. Sixth, as causative pathogens were not recorded, the types of bacteria that contributed to empyema development could not be investigated. The lack of such data did not permit us to conclude a substantial causality. This case-control study included only patients with splenectomy, which may limit the generalisability of the study results to the

general population. Such a study design does not permit to conclude a substantial causality. Further prospective studies are required to confirm the findings of our study.

To the best of our knowledge, this is the first original study to describe the association between splenectomy and empyema. Although the underlying mechanisms that associate splenectomy and empyema could not be completely determined, our findings are novel and clinically important. In addition, we used a hospitalization dataset that had a large sample size and substantial statistical power. The diagnosis codes of the included comorbidities have been previously documented.[23-33] The study design and statistical methodology are described in detail, and our results are relatively promising. Because the splenectomy and non-splenectomy groups had similar distributions of the studied comorbidities, the confounding effects of the comorbidities on the risk of empyema appear to be minimal.

The incidence rate ratio between the splenectomy and non-splenectomy groups reduced from 2.87 in the first 5 years of follow-up to 1.73 in the period following the 5 years. Future studies are required to confirm whether a longer follow-up period would further reduce this average ratio. For the splenectomy group, the overall HR of developing empyema was 2.89 after adjusting for age, sex, and comorbidities, which were identified from previous literature (including alcohol-related disease, cancer, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus). The risk of empyema following splenectomy remains high despite the absence of these comorbidities.

Data sharing statement: no additional data available

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Specific author contributions

Hsien-Feng Lin and Kuan-Fu Liao planned and conducted this study, participated in the data interpretation, and revised the article.

Ching-Mei Chang and Cheng-Li Lin conducted the data analysis and revised the article.

Shih-Wei Lai planned and conducted this study, contributed to the conception of the article, initiated the draft of the article, and revised the article.

Conflict of Interest Statement

The authors disclose no conflicts of interest.

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REFERENCES

1. Ahmed RA, Marrie TJ, Huang JQ. Thoracic empyema in patients with community-acquired pneumonia. Am J Med 2006;119:877-83.
2. Shields T, Locicero J, Ponn R, Rusch V, eds. General Thoracic Surgery, 6th edn. Philadelphia: Lippincott Williams & Wilkins, 2005:823.
3. Septimus EJ. Pleural effusion and empyema. In: Mandell GL, Bennet JE, Mandell RD, editors. Douglas and Bennett's principles and practice of infectious diseases. Edinburgh: Churchill Livingstone; 2009. pp. 917-24.
4. Spencer RP, Pearson HA. The spleen as a hematological organ. Semin Nucl Med. 1975;5:95–102.
5. Trigg ME. Immune function of the spleen. South Med J. 1979;72:593–9.
6. Jirillo E, Mastronardi ML, Altamura M, Munno I, Miniello S, Urgesi G, et al. The immunocompromised host: immune alterations in splenectomized patients and clinical implications. Curr Pharm Des. 2003;9:1918–23.
7. Di Sabatino A, Carsetti R, Corazza GR. Post-splenectomy and hyposplenic states. Lancet. 2011;378:86–97.
8. Lai SW, Wang IK, Lin CL, Chen HJ, Liao KF. Splenectomy correlates with increased risk of pulmonary tuberculosis: a case-control study in Taiwan. Clin Microbiol Infect 2014;20:764-7.
9. Wu SC, Fu CY, Muo CH, Chang YJ. Splenectomy in trauma patients is associated with an increased risk of postoperative type II diabetes: a nationwide population-based study. Am J Surg 2014;208:811-6.
10. Lai SW, Lai HC, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Pyogenic Liver Abscess: A Nationwide Cohort Study in Taiwan. J Epidemiol 2015;25:561-6.
11. Lai SW, Lin HF, Lin CL, Liao KF. Splenectomy and risk of renal and perinephric abscesses: A population-based cohort study in Taiwan. Medicine 2016;95:31(e4438).
12. Lai SW, Lin CL, Liao KF. Splenectomy Correlates With Increased Risk of Acute Pancreatitis: A Case-Control Study in Taiwan. J Epidemiol 2016;26:488-92.
13. Liu WH, Liu TC, Mong MC. Antibacterial effects and action modes of asiatic acid. Biomedicine-Taiwan. 2015;5:22-9.
14. Lin CJ, Lai CK, Kao MC, Wu LT, Lo UG, Lin LC, et al. Impact of cholesterol on disease progression. Biomedicine-Taiwan. 2015;5:1-7.
15. Lin CH, Li TC, Tsai PP, Lin WC. The relationships of the pulmonary arteries to lung lesions aid in differential diagnosis using computed tomography. Biomedicine-Taiwan. 2015;5:31-8.

16. Li TC, Li CI, Liao LN, Liu CS, Yang CW, Lin CH, et al. Associations of EDNRA and EDN1 polymorphisms with carotid intima media thickness through interactions with gender, regular exercise, and obesity in subjects in Taiwan: Taichung Community Health Study (TCHS). Biomedicine-Taiwan. 2015;5:8-14.
17. Chen HX, Lai CH, Hsu HY, Huang JC, Wu HS, Ho MW, et al. The bacterial interactions in the nasopharynx of children receiving adenoidectomy. Biomedicine-Taiwan. 2015;5:39-43.
18. National Health Insurance Research Database. Taiwan. http://nhird.nhri.org.tw/en/index.html [cited in March 1, 2017, English version].
19. Lai SW, Liao KF, Liao CC, Muo CH, Liu CS, Sung FC. Polypharmacy correlates with increased risk for hip fracture in the elderly: a population-based study. Medicine 2010;89:295-9.
20. Chen HY, Lai SW, Muo CH, Chen PC, Wang IJ. Ethambutol-induced optic neuropathy: a nationwide population-based study from Taiwan. Br J Ophthalmol 2012;96:1368-71.
21. Yang SP, Muo CH, Wang IK, et al. Risk of type 2 diabetes mellitus in female breast cancer patients treated with morphine: A retrospective population-based time-dependent cohort study. Diabetes Res Clin Pract 2015;110:285-90.
22. Tsai TY, Lin CC, Peng CY, et al. The association between biliary tract inflammation and risk of digestive system cancers: A population-based cohort study. Medicine 2016;95: e4427.
23. Lai SW, Chen PC, Liao KF, Muo CH, Lin CC, Sung FC. Risk of hepatocellular carcinoma in diabetic patients and risk reduction associated with anti-diabetic therapy: a population-based cohort study. Am J Gastroenterol 2012;107:46-52.
24. Liao KF, Lin CL, Lai SW, Chen WC. Zolpidem Use Associated With Increased Risk of Pyogenic Liver Abscess: A Case-Control Study in Taiwan. Medicine 2015;94:e1302.
25. Wong TS, Liao KF, Lin CM, Lin CL, Chen WC, Lai SW. Chronic Pancreatitis Correlates With Increased Risk of Cerebrovascular Disease: A Retrospective Population-Based Cohort Study in Taiwan. Medicine 2016;95:e3266.
26. Liao KF, Lai SW, Lin CL, Chien SH. Appendectomy correlates with increased risk of pyogenic liver abscess: A population-based cohort study in Taiwan. Medicine 2016;95:e4015.
27. Cheng KC, Lin WY, Liu CS, Lin CC, Lai HC, Lai SW. Association of different types of liver disease with demographic and clinical factors. BioMedicine-Taiwan 2016;6:16-22.
28. Liao KF, Cheng KC, Lin CL, Lai SW. Etodolac and the risk of acute pancreatitis. BioMedicine-Taiwan 2017;7:25-9.

29. Shen ML, Liao KF, Tsai SM, Lin CL, Lai SW. Herpes zoster correlates with pyogenic liver abscesses in Taiwan. BioMedicine-Taiwan 2016;6:24-9.
30. Lai SW. Risks and benefits of zolpidem use in Taiwan: a narrative review. BioMedicine-Taiwan 2016;6:9-11.
31. Cheng KC, Lee TL, Lin YJ et al. Facility evaluation of resigned hospital physicians:managerial implications for hospital physician manpower. Biomedicine 2016; 6: 30-9.
32. Lai SW, Lin CL, Liao KF. Risk of contracting pneumonia among patients with predialysis chronic kidney disease: a population-based cohort study in Taiwan. BioMedicine-Taiwan 2017; 7: 42-47.
33. Liao KF, Huang PT, Lin CC, Lin CL, Lai SW. Fluvastatin use and risk of acute pancreatitis:a population-based case-control study in Taiwan. BioMedicine-Taiwan 2017;7: 24-28.
34. Sinwar PD. Overwhelming post splenectomy infection syndrome—review study. Int J Surg. 2014;12:1314–6.
35. Eibl M. Immunological consequences of splenectomy. Prog Pediatr Surg. 1985;18:139–45.
36. Van Rooijen N. The humoral immune response in the spleen. Res Immunol. 1991;142:328–30.
37. Altamura M, Caradonna L, Amati L, Pellegrino NM, Urgesi G, Miniello S. Splenectomy and sepsis: the role of the spleen in the immune-mediated bacterial clearance. Immunopharmacol Immunotoxicol. 2001;23:153–61.
38. Bisharat N, Omari H, Lavi I, Raz R. Risk of infection and death among post-splenectomy patients. J Infect. 2001;43:182–6.
39. Kyaw MH, Holmes EM, Toolis F, Wayne B, Chalmers J, Jones IG, et al. Evaluation of severe infection and survival after splenectomy. Am J Med. 2006;119:276 e1–7.
40. Dendle C, Sundararajan V, Spelman T, Jolley D, Woolley I. Splenectomy sequelae: an analysis of infectious outcomes among adults in Victoria. Med J Aust. 2012;196:582–6.
41. Di Cataldo A, Puleo S, Li Destri G, Racalbuto A, Trombatore G, Latteri F, et al. Splenic trauma and overwhelming postsplenectomy infection. Br J Surg. 1987;74:343–5.
42. Lynch AM, Kapila R. Overwhelming postsplenectomy infection. Infect Dis Clin North Am. 1996;10:693–707.

Table 1. Baseline characteristics between splenectomy group and non-splenectomy group

	Splenectomy				
	No N=52464		Yes N=13193		
Variable	N	%	n	%	*P* value*
Sex					0.88
Female	20431	38.9	5128	38.9	
Male	32033	61.1	8065	61.1	
Age group (years)					0.98
20–39	13415	25.6	3364	25.5	
40–64	24112	46.0	6062	46.0	
65-84	14937	28.5	3767	28.6	
Age (years), mean (standard deviation) †	52.5	(17.2)	52.8	(17.2)	0.05
Follow-up period (years), mean (standard deviation) †	5.75	(3.32)	4.37	(3.44)	< 0.001
Baseline comorbidities					
Alcohol-related disease	1787	3.41	452	3.43	0.91
Cancer	7677	14.6	1962	14.9	0.49
Chronic kidney disease	1068	2.04	270	2.05	0.94
Chronic liver disease	7791	14.9	1971	14.9	0.80
Chronic obstructive pulmonary disease	2002	3.82	507	3.84	0.89
Diabetes mellitus	7856	15.0	1983	15.0	0.87

Data are presented as the number of subjects in each group, with percentages given in parentheses, or mean with standard deviation given in parentheses.

*Chi-square test, and †*t*-test comparing subjects with and without splenectomy.

Table 2. Incidence density of empyema estimated by sex, age, and follow-up period between splenectomy group and non-splenectomy group

Variable	Non-splenectomy				Splenectomy				IRR[#]	(95% CI)
	N	Cases	Person-years	Incidence[†]	N	Cases	Person-years	Incidence[†]		
All	52464	1042	301484	3.46	13193	510	57622	8.85	2.56	(2.44,2.69)
Sex										
Female	20431	317	119441	2.65	5128	159	23004	6.91	2.60	(2.40, 2.82)
Male	32033	725	182042	3.98	8065	351	34618	10.1	2.55	(2.39, 2.71)
Age group (years)										
20–39	13415	57	84004	0.68	3364	65	19700	3.30	4.86	(4.39, 5.39)
40–64	24112	354	140554	2.52	6062	224	26423	8.48	3.37	(3.13, 3.62)
65-84	14937	631	76925	8.20	3767	221	11499	19.2	2.34	(2.14, 2.56)
Follow-up period (years)										
< 5	52464	720	206010	3.49	13193	416	41545	10.0	2.87	(2.73, 3.01)
≥ 5	28456	322	95474	3.37	5052	94	16077	5.85	1.73	(1.60, 1.88)

[†] Incidence rate: per 1,000 person-years.

[#]IRR (incidence rate ratio): splenectomy vs. non-splenectomy (95% confidence interval)

Table 3. Adjusted hazard ratio and 95% confidence interval of empyema associated with splenectomy and other comorbidities

	Crude	Adjusted†
Variable	HR (95%CI)	HR (95%CI)
Sex (male vs. female)	1.48 (1.33, 1.65)	1.51 (1.35, 1.68)
Age (per one year)	1.05 (1.04, 1.05)	1.05 (1.05, 1.06)
Baseline comorbidities (yes vs. no)		
Splenectomy	**2.52 (2.26, 2.80)**	**2.89 (2.60, 3.22)**
Alcohol-related disease	1.61 (1.28, 2.03)	2.25 (1.76, 2.86)
Cancer	2.60 (2.31, 2.92)	1.92 (1.71, 2.17)
Chronic kidney disease	3.04 (2.40, 3.85)	2.13 (1.67, 2.70)
Chronic liver disease	2.07 (1.84, 2.34)	1.90 (1.68, 2.14)
Chronic obstructive pulmonary disease	3.95 (3.37, 4.64)	1.75 (1.48, 2.07)
Diabetes mellitus	2.80 (2.51, 3.13)	1.85 (1.65, 2.07)

†Variables found to be statistically significant in the univariable model were further examined in the multivariable model. Adjusted for age, sex, alcohol-related disease, cancer, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

Table 4. Cox proportional hazard regression analysis for risk of empyema stratified by splenectomy and comorbidities

Variable		Event	Incidence†	Adjusted HR# (95% CI)
Splenectomy	Any comorbidity*			
No	No	299	1.49	1(Reference)
No	Yes	743	7.34	3.64(3.18, 4.17)
Yes	No	230	5.75	4.52(3.80, 5.37)
Yes	Yes	280	15.9	8.23(6.98, 9.70)

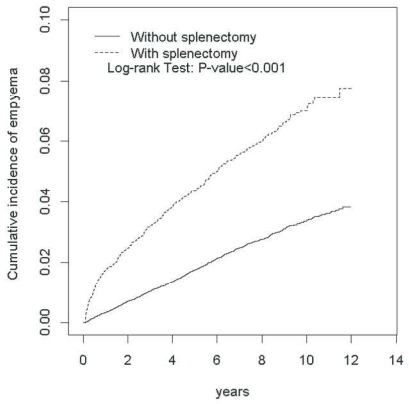
† Incidence rate: per 1,000 person-years

#Adjusted for sex and age

*Comorbidities including alcohol-related disease, cancer, chronic kidney disease, chronic liver disease, chronic obstructive pulmonary disease, and diabetes mellitus

95% CI: 95% confidence interval

Fig 1: Kaplan-Meier model revealed that the splenectomy group had a higher cumulative incidence of pleural empyema than the non-splenectomy group (6.99% vs. 3.37% at the end of follow-up; *P* <0.001)



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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3	
Objectives	3	State specific objectives, including any prespecified hypotheses	3,4	
Methods				
Study design	4	Present key elements of study design early in the paper	4	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*	
Bias	9	Describe any efforts to address potential sources of bias	4,5	
Study size	10	Explain how the study size was arrived at	4	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	5
		(*e*) Describe any sensitivity analyses	6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	7
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*7,8*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	8
		(*b*) Report category boundaries when continuous variables were categorized	8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	8

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Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8
Discussion			
Key results	18	Summarise key results with reference to study objectives	9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	10-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	12
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	13

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.