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Gallbladder Cancer and Dysplasia in Cholecystectomy Specimens: A Large Study in High-Incidence Regions of South America

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BACKGROUND AND AIMS: **Gallstone disease has been causally linked to gallbladder cancer (GBC) via the carcinogenesis model of gallstones and inflammation leading to gallbladder dysplasia then GBC. Efficient GBC prevention through cholecystectomy requires accurate prediction of individual GBC risk, especially in low- and middle-income regions, where studies tend to be small and of low quality, and where financial and surgical capacity are limited.**

METHODS: **In a collaborative study from high GBC incidence regions of Argentina, Bolivia, Chile, and Peru, we collected and validated clinical information from 10,561 patients with gallstone disease who underwent cholecystectomy. After checking data reliability, we used multiple logistic regression to identify the main factors associated with GBC and dysplasia risk.**

RESULTS: **The highest GBC and dysplasia risk was found in patients with clinical suspicion of GBC, followed by planned open cholecystectomy, female sex, gallstones over 3 cm, hypercholesterolemia, smoking, and age at cholecystectomy. Clinical suspicion of GBC and age at cholecystectomy showed heterogeneous odds ratios depending on the recruitment site. The identified risk**

Abbreviations used in this paper: CI, confidence interval; DYS, gallbladder dysplasia; GBC, gallbladder cancer; OR, odds ratio; PAF, population attributable fraction.

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factors, and the magnitude of their effects, were different for GBC and dysplasia. The mean age at cholecystectomy was 47 years, compared with 50 years for low-grade dysplasia, 62 years for high-grade dysplasia, and 64 years for GBC.

CONCLUSIONS: **These recruitment site–specific risk factors may help refine current prevention strategies by prioritizing prophylactic cholecystectomy in high-risk patients. The approach used in this study may guide future investigations on GBC prevention in high-incidence, low-income regions.**

Keywords: Gallbladder Cancer; Cholecystectomy; Gallstones; Gallbladder Dysplasia; South America; Risk Factors.

Gallbladder cancer (GBC) (International Classification of Diseases–Tenth Revision diagnosis code C23) has remained relatively understudied possibly because most deaths from GBC occur in low- and middle-income countries, including several South American regions in or near the Andes with high incidence and mortality rates.[1–3] GBC has one of the poorest prognoses of all gastrointestinal cancers, as it develops asymptomatically in its early stages, and treatment options are often very limited at the time of diagnosis.[4,5] Current strategies to prevent GBC, in particular the Chilean program "Essential Health Care Guarantee number 26" (Garantía Explícita en Salud no. 26), aim to reduce GBC mortality by surgically removing the gallbladder in patients with gallstones before GBC develops, a procedure referred to as prophylactic cholecystectomy.[6] Considering that most GBC cases in South America originate from gallstone disease that progresses to cholecystitis, dysplasia and carcinoma over a period that has been reported to take 15–25 years, prophylactic cholecystectomy is considered promising for primary GBC prevention.[7–9] However, the associated costs, and short- and long-term cholecystectomy complications (eg, bile duct injury, fatty food intolerance, and increased risk of other tumors) need to be carefully weighed, especially in low-income regions with limited financial and surgical capacity.[10,11]

Understanding GBC risk factors in patients with gallstone disease undergoing cholecystectomy is essential to develop effective prevention strategies in high-incidence, low- to middle-income regions.[12] Some nongenetic and genetic factors associated with the development of GBC have been identified. However, only a few mostly small and low quality studies have investigated their combined contribution to GBC risk.[13–21] Furthermore, the few existing large studies have examined patients of European origin, and ethnicity likely affects GBC predisposition. For example, a recent study found a direct causal effect of body mass index on GBC risk in Chileans, but only an indirect effect mediated by gallstones in Europeans.[22] The association between genetic ancestry and GBC risk in Chile appears to be limited to a specific type of indigenous Native American ancestry, namely the Mapuche ancestry (the largest ethnic group in Chile).[23] Therefore, GBC risk factors may vary depending on the population studied, and individualized predictions of GBC risk may not be transferable from one population to another.

To improve the accuracy of GBC risk prediction by adequately accounting for geographical, environmental, lifestyle, ethnic, and molecular differences, the European Union is funding a European-Latin American research consortium toward eradication of preventable GBC.[24] One of the consortium's initial research activities was the capture and analysis of epidemiological and perioperative clinical information from patients undergoing cholecystectomy in participating hospitals from Argentina, Bolivia, Peru, and Chile. We report here the methodology used and the main results of this effort to identify GBC risk factors and risk profiles that may help improve current prevention strategies in high-incidence, low- to middle-income regions of South America.

Materials and Methods

This retrospective observational study included 10,561 patients with gallstone disease who underwent cholecystectomy in 2018 at 11 recruitment sites (14 hospitals) in Peru, Bolivia, Argentina, and Chile.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the responsible local ethics committees. After a comparison of the clinical information available at each participating hospital, extensive discussions with participating clinicians and 3 rounds of revisions with all participants (first draft, updated version, final version), a list of 20 potential risk factors for GBC and gallbladder dysplasia after cholecystectomy was compiled, and a standardized case report form with predefined fields was created. Please see the Supplementary Material for detailed information on the investigated study population, ethical approvals, investigated variables, and data curation.

Variables with missing data proportions above 30% were excluded from further analysis, and observations with no information were aggregated into a separate "missing" category. Multiple logistic regression was applied to investigate the association between the list of remaining epidemiological and perioperative clinical variables, and GBC or gallbladder dysplasia risk. We also (1) estimated associations for each recruitment site separately and combined them in a random-effects meta-

analysis, reporting the percentage of variation across recruitment sites I^2 and the probability value for heterogeneity based on Cochran's Q statistic, (2) investigated the associations with GBC risk and gallbladder dysplasia risk separately, and (3) explored risk profile differences between recruitment sites by stratified, principal component, and cluster analysis (see the Supplementary Material and Supplementary Table 1 for detailed information). Linear regression was applied to estimate the mean time of progression from gallstone disease to low- and high-grade dysplasia, and to GBC.

Data preparation and statistical analyses were conducted with SAS version 9.4 (SAS Institute). The R software environment for statistical computing and graphics (version 4.3.3 for Windows; R Foundation for Statistical Computing) was used to create the figures and perform the cluster analyses. The program code for reproducing all calculations is available at www.biometrie.uni-heidelberg.de/StatisticalGenetics/Software_and_Data.

What You Need to Know

Background

South American regions near the Andes have high incidence of gallstone-related gallbladder cancer (GBC).

Findings

The prevalence of GBC and dysplasia in the participating recruitment sites ranged from 1% to 4%. The age at cholecystectomy was 57 years for patients with GBC or dysplasia, compared with 47 years in unaffected patients. The risk factors identified varied by recruitment site, and were different for GBC and dysplasia.

Implications for patient care

Region-specific GBC prevention strategies that take into account specific risk factors and the optimal age for prophylactic cholecystectomy are needed.

Results

Among the 10,561 patients with gallstone disease who underwent cholecystectomy, 322 (3%) had significant findings on pathological examination: 126 presented with GBC, 53 had high-grade dysplasia, and 143 had low-grade dysplasia (Table 1). The overall prevalence of GBC or gallbladder dysplasia was 3%, but large differences were present between recruitment sites: compared with Talca (Chile) as reference, patients who underwent cholecystectomy at Dr. Jose Joaquin Aguirre Hospital in Santiago (Chile) had an increased risk of GBC or gallbladder dysplasia (odds ratio [OR], 1.75), whereas patients who had cholecystectomy in Rancagua (Chile) had a decreased risk (OR, 0.31).

In addition to recruitment site, the multiple logistic regression model showed probability values (P) of <.10 for clinical suspicion of GBC (OR, 7.15), planned open cholecystectomy (OR, 1.92), sex (for females: OR, 1.78), gallstones over 3 cm (OR, 1.63), hypercholesterolemia (OR, 1.57), smoking (OR, 1.33), age at cholecystectomy (4% increased risk per additional year at surgery), and alcohol consumption (OR, 0.65). On average, gallstone patients without GBC or dysplasia underwent cholecystectomy at the age of 47 years, compared with 50 years for low-grade dysplasia, 62 years for high-grade dysplasia, and 64 years for GBC. The corresponding estimates for the progression time were 3 years (95% confidence interval [CI], 1 to 6 years) from symptomatic gallstone disease to low-grade gallbladder dysplasia, 12 years (95% CI, 7 to 17 years) from low-grade to high-grade gallbladder dysplasia, and 2 years (95% CI, −2 to 6 years) from high-grade dysplasia to GBC.

Figure 1 shows the estimated age- and sex-specific risk of GBC and gallbladder dysplasia based on the logistic regression model. The median cumulative risks at 80 years of age of GBC and dysplasia in patients with cholecystectomy were 7.1% in females and 4.0% in males. The cumulative risks at 80 years of age of GBC and dysplasia in female patients with cholecystectomy in the highest and lowest 10% risk percentiles were 12.0% and 4.1%, respectively, compared with 7.0% and 2.3% in male patients, respectively.

The right-hand columns in Table 1 show the results of the random-effects meta-analysis. The summary ORs from the meta-analysis and the ORs from the multiple logistic regression model, which included recruitment site as a covariate, were consistent (overlapping 95% CIs). Two risk factors showed heterogenous ORs depending on the recruitment site: Clinical suspicion of GBC ($I^2 = 81\%$) and age at cholecystectomy ($I^2 = 66\%$). The forest plots in Figure 2 show the estimated ORs for each recruitment site and variables with a $P < .10$ in the logistic regression model. Some risk factors had to be excluded from the model due to convergence problems (no event for some recruitment sites by risk factor combinations [eg, sex for Cochabamba, Bolivia]). Leave-one-out sensitivity analyses are depicted in Supplementary Figure 1.

Because clinical suspicion of GBC and planned open cholecystectomy are subjective in nature and difficult for clinicians to duplicate, we also fitted a multiple logistic regression model without these 2 factors. In addition to recruitment site, the updated regression model showed $P < .10$ for the variables sex (for females: OR, 1.73), Indigenous American surname/s (OR, 1.53), smoking (OR, 1.44), age at cholecystectomy (4% increased risk per additional year at surgery), and alcohol consumption (OR, 0.56) (Supplementary Table 2).

To group the recruitment sites according to risk profiles for GBC and gallbladder dysplasia, we performed principal component and cluster analyses based on the estimated population attributable fractions (PAFs). The first 3 principal components explained a PAF variance higher than 80%, and we decided to categorize the

Table 1. Main Characteristics of the Study Population and Results From Multiple Logistic Regression Analysis and Random-Effects Meta-Analysis

			Multiple Logistic Regression		Random Effects		
Variable	No GBC or DYS (n = 10,239)	GBC or DYS (n = 322)	OR (95% CI)	*P* Value	OR (95% CI)	I^2 (%)	Heterogeneity *P* Value
Recruitment site				.0002			
Arequipa, Peru	625	26 (4%)	1.01 (0.54–1.92)				
Cochabamba, Bolivia	606	11 (2%)	0.58 (0.06–5.44)				
Jujuy, Argentina	917	13 (1%)	1.01 (0.32–3.22)				
Arica, Chile	335	9 (3%)	1.03 (0.50–2.12)				
Santiago 1, Chile	1285	49 (4%)	1.75 (1.11–2.78)[a]				
Santiago 2, Chile	663	29 (4%)	1.20 (0.72–1.99)				
Rancagua, Chile	1016	16 (2%)	0.31 (0.17-0.59)[a]				
Talca, Chile	2219	89 (4%)	Ref.				
Concepción, Chile	740	31 (4%)	1.23 (0.72–2.08)				
Temuco, Chile	722	22 (3%)	0.60 (0.34–1.05)				
Puerto Montt, Chile	1111	27 (2%)	0.78 (0.37–1.64)				
Age at cholecystectomy (years)	47 ± 17	57 ± 16	1.04 (1.03–1.04)[a]	<.0001	1.04 (1.02–1.06)[a]	66	.003
Sex				<.0001		0	.73
Male	3054	75 (2%)	Ref.				
Female	7185	247 (3%)	1.78 (1.34–2.37)[a]		1.69 (1.25–2.28)[a]		
Indigenous American surname/s				.18			
No	8378	264 (3%)	Ref.				
Yes	1233	46 (4%)	1.45 (0.96–2.16)				
Missing	628	12 (2%)	1.78 (0.22–14.4)				
Body mass index				.89			
<25 kg/m^2	1714	58 (3%)	Ref.				
25 kg/m^2 or more	5436	168 (3%)	0.93 (0.67–1.29)				
Missing	3089	96 (3%)	0.92 (0.61–1.37)				
Type of health insurance				.35			
Public	8579	289 (3%)	Ref.				
Private	1512	30 (2%)	0.72 (0.45–1.15)				
Missing	148	3 (2%)	0.73 (0.20–2.59)				
Smoking				.07		49	.07
No	6033	205 (3%)	Ref.				
Yes	1705	62 (4%)	1.33 (0.96–1.86)		1.46 (0.82–2.59)		
Missing	2501	55 (2%)	0.73 (0.41–1.28)				
Alcohol consumption				.06		31	.22
No	5593	219 (4%)	Ref.				
Yes	2074	43 (2%)	0.65 (0.45-0.93)[a]		0.72 (0.43–1.21)		
Missing	2572	60 (2%)	0.89 (0.51–1.56)				
Diabetes				.28			
No	9279	280 (3%)	Ref.				
Yes	960	42 (4%)	0.81 (0.55–1.19)				
Hypertension				.95			
No	7961	205 (3%)	Ref.				
Yes	2278	117 (5%)	0.99 (0.74–1.32)				
Hypercholesterolemia				.05		0	.46
No	9767	293 (3%)	Ref.				
Yes	472	29 (6%)	1.57 (1.01–2.45)[a]		1.68 (1.07–2.64)[a]		
Previous hospitalizations for gallstones				.13			
No	6845	226 (3%)	Ref.				
Yes	1316	45 (3%)	1.45 (1.00–2.12)				
Missing	2078	51 (2%)	1.29 (0.78–2.12)				

Table 1. Continued

Variable	No GBC or DYS (n = 10,239)	GBC or DYS (n = 322)	Multiple Logistic Regression		Random Effects		
			OR (95% CI)	*P* Value	OR (95% CI)	I^2 (%)	Heterogeneity *P* Value
Gallstones over 3 cm				.005		0	.52
No	7436	228 (3%)	Ref.				
Yes	370	23 (6%)	1.63 (1.01–2.63)[a]		1.61 (0.91–2.85)		
Missing	2433	71 (3%)	1.74 (1.18–2.57)[a]				
Ultrasound findings				.48			
Multiple stones	4019	125 (3%)	Ref.				
Cholecystitis	2096	62 (3%)	0.97 (0.69–1.39)				
Single stone	1972	65 (3%)	0.97 (0.69–1.35)				
Other	611	24 (4%)	0.82 (0.50–1.34)				
Missing	1541	46 (3%)	0.69 (0.45–1.06)				
Clinical suspicion of GBC				<.0001		81	<.0001
No	8847	230 (3%)	Ref.				
Yes	446	85 (16%)	7.15 (5.21–9.81)[a]		15.03 (5.63–40.12)[a]		
Missing	946	7 (1%)	0.17 (0.05-0.61)[a]				
Planned open cholecystectomy				<.0001		0	.28
No	8992	240 (3%)	Ref.				
Yes	1176	71 (6%)	1.92 (1.34–2.76)[a]		2.17 (1.49–3.17)[a]		
Missing	71	11 (13%)	4.93 (2.31–10.5)[a]				

Values are n, n (%), or mean ± SD, unless otherwise indicated.
CI, confidence interval; DYS, gallbladder dysplasia; GBC, gallbladder cancer; OR, odds ratio.
[a]95% CIs that do not include 1.00.

recruitment sites into 4 clusters. Figure 3 shows the geographic location, cluster group, and spider charts for each recruitment site, with the distance from the center of the spider chart representing the estimated PAF. The first cluster (in yellow) included Cochabamba (Bolivia), Jujuy (Argentina), and Temuco (Chile), with higher PAFs for planned open cholecystectomy, hypercholesterolemia, and gallstones over 3 cm than for the other recruitment sites (Supplementary Table 3). The second cluster included 3 sites in central Chile (Dr. Sótero del Río hospital in Santiago, Rancagua, and Concepción) and was characterized by large PAF for clinical suspicion of GBC. The third cluster included only the Dr. Jose Joaquin Aguirre Hospital in Santiago and showed a similar risk profile to the second cluster, except for the elevated PAFs for smoking. The fourth cluster included Arequipa (Peru), Arica (Chile), Talca (Chile), and Puerto Montt (Chile), with relatively high PAFs for sex and alcohol consumption.

Table 2 shows separate OR estimates for gallbladder dysplasia and GBC. Identified risk factors ($P < .05$) for dysplasia included recruitment site, age at cholecystectomy, sex, hypercholesterolemia, and clinical suspicion of GBC. In contrast, GBC risk was associated ($P < .05$) with recruitment site, age at cholecystectomy, alcohol consumption, clinical suspicion of GBC, and planned open cholecystectomy. Age at cholecystectomy had a greater impact on GBC (6% risk increase per year at surgery) than on dysplasia (3% risk increase). Clinical suspicion of GBC was a strong risk factor for both GBC and dysplasia; the estimated risk for dysplasia (OR, 2.27) was significantly lower than for GBC (OR, 29.1).

Discussion

The aim of the present study was to (1) identify risk factors associated with GBC and gallbladder dysplasia in South American regions with high incidence and low to middle income and (2) examine regional differences in risk profiles. The identified risk factors and risk profile differences may guide the design and refinement of current GBC prevention strategies, and also contribute to a better understanding of the development of GBC.

We observed large differences in the prevalence of GBC or gallbladder dysplasia between recruitment sites, ranging from 1% in Jujuy, Argentina, to 4% in centers such us the Hospital Dr. José Joaquín Aguirre in Santiago de Chile. These differences could be attributed to different clinical practice (eg, pathological evaluation), general healthcare, average age at cholecystectomy, socioeconomic and income levels, genetic factors, diet, and lifestyle. Chile began implementing a cholecystectomy program in 2006 (Garantía Explícita en Salud no. 26) that translates into about 50,000 gallbladder surgeries per year.[25,26] In Argentina, Bolivia, and Peru, there is no such program, and we were expecting homogeneous risk profiles within each country that explain the differences in prevalence, but this was not the case. For example, we

Figure 1. Estimated age- and sex-specific risk of GBC or dysplasia based on the multiple logistic regression model for different risk percentile categories.

identified 3 distinct risk profiles in southern Chile (Concepción, Temuco and Puerto Montt) (Figure 3).

Clinical suspicion of GBC was the strongest identified binary risk factor, with a higher relative risk for GBC than for dysplasia. We found notable differences in the estimated relative risks for this factor between recruitment sites, with similar ORs in the 2 hospitals in Santiago (Chile) and in Concepción (Chile). Other risk factors identified included age at cholecystectomy (6% risk increase per year for GBC, and 3% for dysplasia), planned

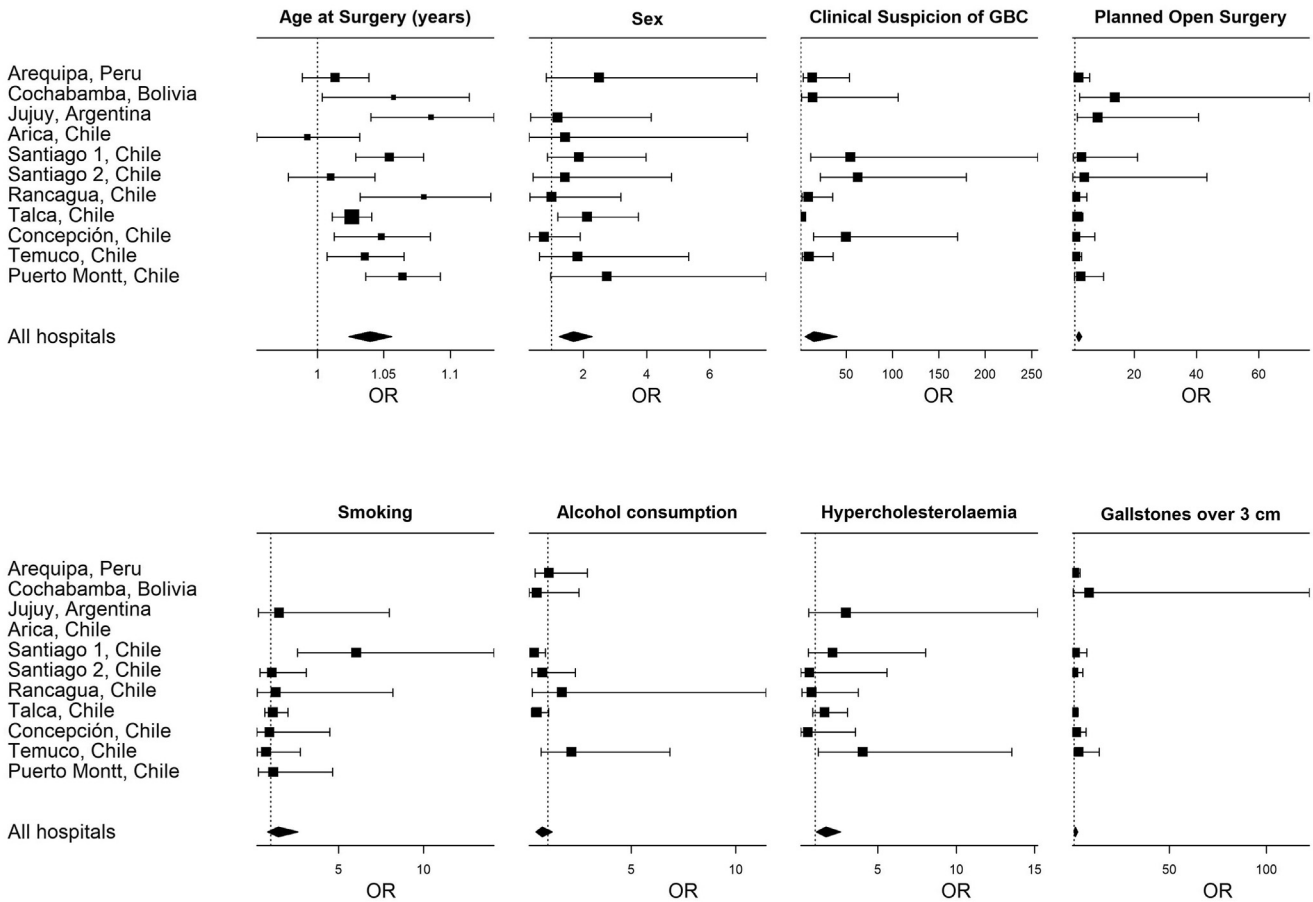

Figure 2. Forest plots of estimated ORs for GBC or dysplasia for each recruitment site and the risk factors with a *P* value <.10 in the multiple logistic regression model. OR, odds ratio.

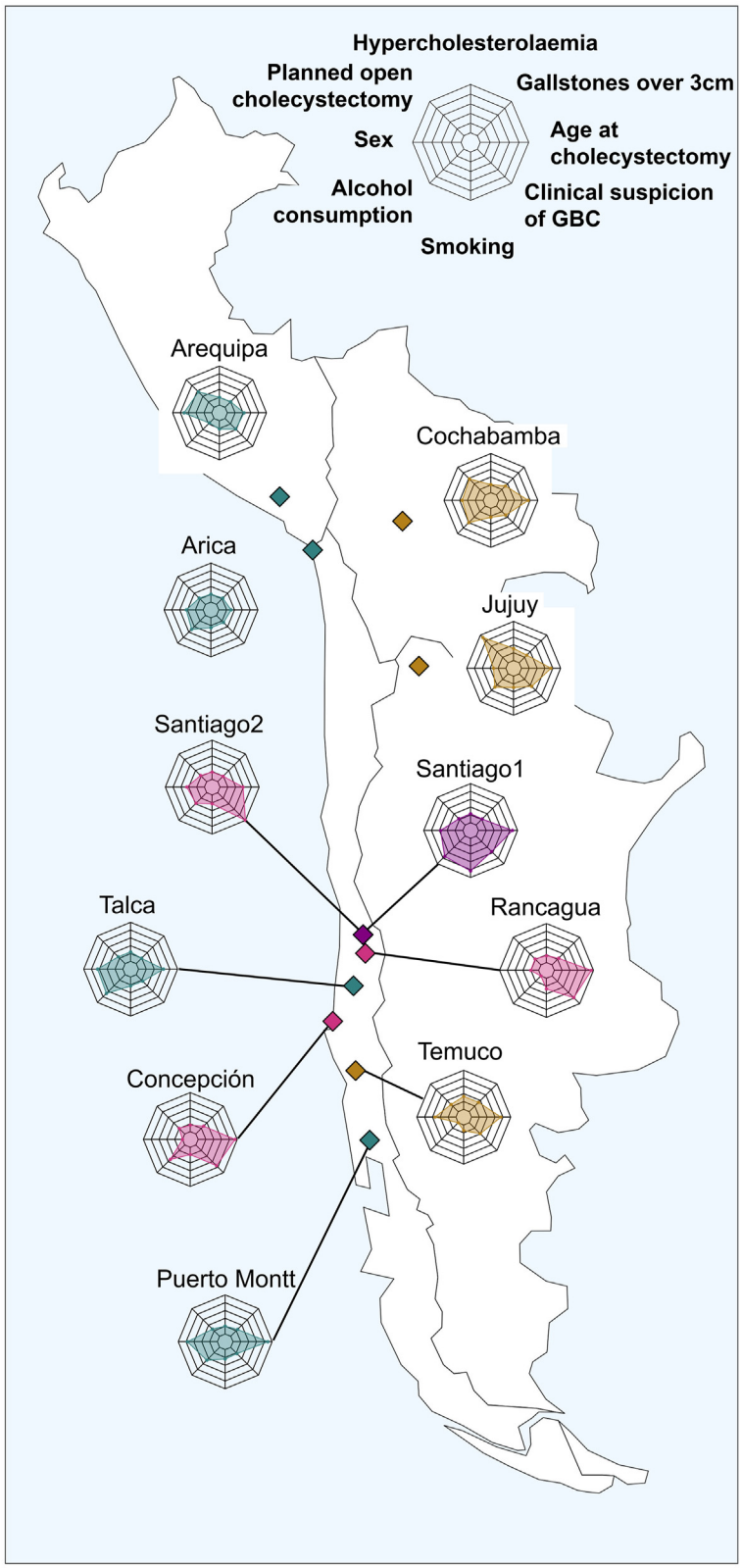

Figure 3. Geographic location of the recruitment sites, along with spider charts depicting site-specific PAFs of GBC and dysplasia, and color-coded clusters of recruitment sites with similar risk profiles. The first cluster (yellow) had higher PAFs for planned open cholecystectomy, hypercholesterolemia, and gallstones over 3 cm than for the other recruitment sites. The second cluster (pink) was characterized by large PAF for clinical suspicion of GBC. The third cluster (purple) showed a similar risk profile to the second cluster, except for the elevated PAFs for smoking. The fourth cluster (blue) had relatively high PAFs for sex and alcohol consumption. The lines of the spider chart from the center to the edge correspond to PAFs of –0.2, 0, 0.2, 0.4, 0.6, and 0.8. The exact PAF values are shown in Supplementary Table 3.

open cholecystectomy (for GBC only), female sex (for dysplasia only), and hypercholesterolemia (for dysplasia only). Because no association between alcohol consumption and GBC has been previously described,[27] we hypothesized reverse causality: the reason for the lower risk of GBC observed in alcohol consumers was that GBC patients tended to consume less alcohol than patients affected only by gallstone disease.

The predominant pathway of GBC development in most regions of the world is associated with gallstones and gallbladder inflammation, while a less common mechanism, especially frequent in Japan, is linked to a

Table 2. Main Characteristics of the Study Population, and Separate Results From Multiple Logistic Regression Analysis Stratified by GBC and DYS

Variable	No GBC or DYS (n = 10,239)	DYS (n = 196)	OR (95% CI)	*P* Value	GBC (n = 126)	OR (95% CI)	*P* Value
Recruitment site				<.0001			<.0001
Arequipa, Peru	625	17 (3)	1.16 (0.63–2.14)		9 (1)	2.38 (0.88–6.41)	
Cochabamba, Bolivia	606	3 (0)	0.23 (0.07-0.75)[a]		8 (1)	7.80 (2.95–20.62)[a]	
Jujuy, Argentina	917	12 (1)	7.00 (2.14–22.9)[a]		1 (0)	0.21 (0.02–2.57)	
Arica, Chile	335	6 (2)	0.64 (0.27–1.49)		3 (1)	4.05 (1.08–15.11)[a]	
Santiago 1, Chile	1285	36 (3)	1.05 (0.66–1.66)		13 (1)	4.81 (2.04–11.37)[a]	
Santiago 2, Chile	663	15 (2)	0.78 (0.43–1.41)		14 (2)	5.76 (2.53–13.11)[a]	
Rancagua, Chile	1016	11 (1)	0.32 (0.16-0.64)[a]		5 (0)	0.64 (0.21–1.92)	
Talca, Chile	2219	69 (3)	Ref.		20 (1)	Ref.	
Concepción, Chile	740	2 (0)	0.09 (0.02-0.36)[a]		29 (4)	12.9 (5.84–28.4)[a]	
Temuco, Chile	722	10 (1)	0.45 (0.23-0.91)[a]		12 (2)	2.08 (0.87–4.97)	
Puerto Montt, Chile	1111	15 (1)	0.51 (0.25–1.06)		12 (1)	5.86 (2.12–16.2)[a]	
Age at cholecystectomy (years)				<.0001			<.0001
mean (SD)	47 (17)	53 (16)	1.03 (1.02–1.04)[a]		64 (13)	1.06 (1.04–1.07)[a]	
Sex				.0002			.14
Male	3054	41 (1)	Ref.		34 (1)	Ref.	
Female	7185	155 (2)	2.00 (1.38–2.89)[a]		92 (1)	1.40 (0.89–2.20)	
Smoking				.07			.75
No	6033	123 (2)	Ref.		82 (1)	Ref.	
Yes	1705	44 (3)	1.43 (0.97–2.11)		18 (1)	1.17 (0.64–2.16)	
Missing	2501	29 (1)	0.70 (0.34–1.48)		26 (1)	0.83 (0.34–2.01)	
Alcohol consumption				.53			.02
No	5593	130 (2)	Ref.		89 (2)	Ref.	
Yes	2074	34 (2)	0.79 (0.52–1.20)		9 (0)	0.34 (0.15-0.73)[a]	
Missing	2572	32 (1)	1.04 (0.50–2.15)		28 (1)	0.63 (0.27–1.50)	
Hypercholesterolemia				.04			.56
No	9767	178 (2)	Ref.		115 (1)	Ref.	
Yes	472	18 (4)	1.71 (1.02–2.89)[a]		11 (2)	1.25 (0.60–2.62)	
Gallstones over 3 cm				.10			.08
No	7436	142 (2)	Ref.		86 (1)	Ref.	
Yes	370	12 (3)	1.71 (0.93–3.16)		11 (3)	1.43 (0.65–3.14)	
Missing	2433	42 (2)	1.41 (0.88–2.25)		29 (1)	1.92 (1.06–3.50)[a]	
Clinical suspicion of GBC				<.0001			<.0001
No	8847	164 (2)	Ref.		66 (1)	Ref.	
Yes	446	26 (6)	2.27 (1.39–3.70)		59 (12)	29.1 (18.04–46.9)[a]	
Missing	946	6 (1)	0.04 (0.01-0.14)[a]		1 (0)	1.16 (0.10–12.74)	
Planned open cholecystectomy				.12			<.0001
No	8992	165 (2)	Ref.		75 (1)	Ref.	
Yes	1176	28 (2)	1.00 (0.60–1.66)		43 (4)	4.14 (2.41–7.12)[a]	
Missing	71	3 (4)	3.56 (1.06–12.0)[a]		8 (10)	4.33 (1.59–11.8)[a]	

Values are n or n (%).
CI, confidence interval; DYS, gallbladder dysplasia; GBC, Gallbladder cancer; OR, odds ratio.
[a]95% Cis that do not include 1.00.

congenital abnormality of the pancreatic bile-duct junction.[8] The sequence of flat-epithelial changes leading to GBC is well established, with time frames reported in the literature of 5 years between cholecystitis and dysplasia and 15 years between dysplasia and GBC.[7] The present results allow refinement of progression time estimates for high-incidence regions of South America: we found mean age differences of 3 years from cholecystectomy to low-grade dysplasia, 12 years from low- to high-grade dysplasia, and 2 years from high-grade dysplasia to GBC. This information could be used to optimize primary and secondary GBC prevention. For example, the current Chilean cholecystectomy program funds prophylactic surgeries for gallstone disease patients between 35 and 49 years of age,[25,26] and this study suggests that the age of initiation of this program could be increased.

Pooling all recruitment sites, each additional year at cholecystectomy translated into a 4% increased risk of

GBC or gallbladder dysplasia, but we detected heterogeneity in the effect of age between centers, with nonsignificant OR estimates for Arequipa (Peru), Arica (Chile), and the Dr. Sótero del Río hospital in Santiago de Chile (Figure 2). As Figure 2 also shows, female sex was especially relevant in Talca (Chile) and Puerto Montt (Chile), and the risk factor "planned open cholecystectomy" was characteristic of Cochabamba (Bolivia) and Jujuy (Argentina). The participating hospitals in these recruitment sites serve patients with similar socioeconomic, age, genetic, and lifestyle characteristics, which was reflected in similar risk profiles (Figure 3). It should be noted that the association between hypercholesterolemia and gallbladder dysplasia was specific to Temuco (Chile). The specific risk factors identified for each recruitment site may inform clinical practice. For example, prophylactic cholecystectomy might be prioritized according to age and sex in Talca (Chile), while age and suspected GBC appear to be particularly relevant in Concepción (Chile).

The study has some limitations. Factors predictive of GBC risk may have been overlooked because people exposed to these factors were not referred for surgery. Some promising risk factors (eg, number of children in women, family history of GBC) could not be considered due to the high proportion of missing data, and other previously identified risk associations (eg, with individual proportions of indigenous South American Mapuche ancestry, GBC susceptibility variants, low socioeconomic status, and dietary factors) could not be investigated because the information was not available. The ORs for GBC reported in the literature (9.2–10.1 for gallstones larger than 3 cm, vs 2.4 for gallstones 2.0–2.9 cm in diameter) led us to use a cutoff point of 3 cm, but the predictive value of gallstone size could potentially be optimized.[28] On the other hand, the risk factors identified and the methodology proposed, with the consensual creation of a standardized case report form with predefined fields, extensive training and supervision of staff responsible for data collection, and blinded data validation, reflect the real constraints and opportunities of research in low-income regions. It should be noted here that, although precise information on socioeconomic status and proportion of indigenous American ancestry was not available, the type of health insurance and indigenous American surnames, respectively, provided some related information for adjustment in the multiple logistic regression analyses.

To our knowledge, this is the largest study to date examining the combined contribution of multiple factors to GBC and gallbladder dysplasia risk in high-incidence regions of South America. Few other studies with a similar design have included more than 100 GBC cases and/or more than 1 hospital (Supplementary Table 4)[13–21,29–33]: our team recently developed and internally validated multifactorial risk prediction models for the Chilean population relying on established demographic risk factors gallstones, body mass index, education, Mapuche surnames, number of children, and family history of GBC, and genetic risk factors indigenous American Mapuche ancestry and GBC susceptibility variant rs17209837 for 473 GBC patients from 16 Chilean hospitals.[29] The global model included all risk factors except Mapuche surnames, which became redundant once Mapuche ancestry was taken into account. Zhu et al[30] analyzed risk factors to construct prediction models with external validation using 288 GBC patients with gallstones from 2 hospitals in China. Age, size of gallstones, course of gallstones, and the 2 antigens CEA and CA19-9 were identified as independent risk factors. Based on the Swedish Register for Gallstone Surgery, Muszynska et al[14] found 215 GBC cases among 36,140 patients with cholecystectomy (crude incidence rate 0.6%). Along with age and female sex, jaundice, acute cholecystitis, and ultrasound findings were identified as GBC risk factors. Zhang et al[13] explored the predictive ability of tumor markers in 144 Chinese GBC (with and without gallstones) and 116 gallstone disease patients. After accounting for age, sex, and gallstones, GBC patients presented with elevated levels of total bilirubin, alkaline phosphatase, and carcinoembryonic antigen 125. Serra et al[18] investigated 114 Chilean GBC patients and 114 matched control subjects at the Universidad de Chile in Santiago and found associations between red chili pepper consumption and low socioeconomic status with GBC risk. Although none of the previous studies considered clinical suspicion of GBC, other studies have previously reported thickening of the gallbladder wall as a strong GBC risk factor (OR $\geq$3.5).[15,20,21] We are confident that ongoing and future studies, such as the GECKO (Global Evaluation of Cholecystectomy Knowledge and Outcomes) study (gecko@globalsurg.org) may use the methodology of the present investigation to refine our results, and ultimately improve GBC prevention, especially in low-income regions.

In conclusion, the prevalence of dysplasia and GBC in the participating recruitment sites varied from 1% to 4%. The mean age at cholecystectomy was 47 years for patients without GBC or dysplasia, compared with 57 years on average for GBC or dysplasia patients. Specific risk factors were identified in different hospitals that may guide the improvement of current clinical practice. On the path to precision GBC prevention, large collaborative studies are urgently needed at regional and continental levels, considering the different financial and surgical capacities as well as modifiable and genetic-molecular risk factors.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2024.12.027.

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Conflicts of interest
The authors disclose no conflicts.

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Data Availability
The data that support the findings of this study are available on request from the corresponding author. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. To gain access, data requestors will need to sign a data access agreement.

Supplementary Material

Study Population

The sites where patients underwent cholecystectomy were as follows: Hospital Regional Honorio Delgado and Hospital Goyeneche for Arequipa (Peru); Caja Nacional de la Salud and Seguro Social Universitario for Bolivia; Hospital Pablo Soria for Argentina; and Hospital Regional de Arica, Hospital Clínico Universidad de Chile in Santiago, Hospital Dr. Sótero del Río in Santiago, Hospital Regional de Rancagua, Hospital Regional de Talca, Hospital Regional de Concepción, Hospital Dr. Hernán Henríquez Aravena in Temuco, and Hospital Regional de Puerto Montt for Chile. The 11 recruitment sites were located in low- to middle-income regions with a high incidence of gallstone disease and gallbladder cancer (GBC).[e1] The case group for the primary analysis included gallstone disease patients who presented with GBC or gallbladder dysplasia on pathological examination after cholecystectomy, and the unaffected control group included patients without pre- or neoplastic pathological findings. Separate comparative analyses were also performed for GBC cases alone and for gallbladder dysplasia cases alone.

Ethical Approvals

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethics committees of the Instituto Nacional de Enfermedades Neoplásicas in Lima (Peru), Facultad de Medicina, Universidad Mayor de San Simón in Cochabamba (Bolivia), Comité Provincial de Ética de Investigación in Jujuy (Argentina), Comisión Provincial de Investigaciones Biomédicas in Salta (Argentina), Servicio de Salud Metropolitano Oriente and Servicio de Salud Metropolitano Sur Oriente in Santiago de Chile, Servicio de Salud Maule and Universidad Católica del Maule in Talca (Chile), Servicio de Salud Concepción in Concepción (Chile), Servicio de Salud Araucanía Sur in Temuco (Chile), and the medical faculties of Universidad de Chile and Pontificia Universidad Católica de Chile as part of the project "Establishment and Exploitation of a European-Latin American Research Consortium towards Eradication of Preventable Cancer – EULAT Eradicate GBC" funded by the European Union's Horizon 2020 programme. The study was based on pseudo-anonymized archival data, so it was not necessary to contact patients or obtain written informed consent prior to participation. Ethical approvals for data retrieval are available upon request.

Selected Variables Investigated in the Study

We expected a minimum of 2% of patients with cholecystectomy to have GBC or dysplasia, resulting in at least $10{,}000 \times 0.02 = 200$ GBC or dysplasia diagnoses. This led us to limit the number of variables investigated to 20 (10 events per variable). The 20 potential risk factors investigated in the study included established ones (eg, age and sex) and potential ones (eg, diabetes and hypercholesterolemia), and epidemiological and perioperative clinical factors. Simplicity was prioritized, taking into account the constraints of hospitals in low-income regions that still used handwritten clinical data forms and did not have centralized pathology services (the patient pays and decides whether and where to have their resected gallbladder pathologically examined). Perioperative variables included previous hospitalizations for gallstones (yes/no), ultrasound findings (single stone, multiple stones, cholecystitis, other), size of gallstone/s (smaller or larger than 3 cm), type of planned surgery (laparoscopic or open), and clinical suspicion of GBC (yes/no). The latter was based on (1) identification of a gallbladder mass >2.5 cm or focal/irregular thickening of the gallbladder wall on either ultrasound, computed tomography or magnetic resonance imaging regardless of additional symptoms; and/or (2) signs such as jaundice, right upper quadrant pain, palpable gallbladder mass, or impairment of general condition (anorexia, asthenia, and weight loss); and/or (3) altered laboratory tests (eg, CA 19-9 >100 U/mL, bilirubin >7 mg/dL). The standardized form used for data retrieval (available upon request) included the following variables:

1. Age at cholecystectomy (years)
2. Female sex (yes/no)
3. Number of children (for women)
4. Indigenous American surname(s) (yes/no)
5. Weight (kilograms)
6. Height (centimeters)
7. Type of health insurance (public/private)
8. Smoking (yes/no)
9. Alcohol consumption (yes/no)
10. Diabetes (yes/no)
11. Hypertension (yes/no)
12. Hypercholesterolemia (yes/no)
13. Previous hospitalizations for gallstones (yes/no)
14. Gallstones over 3 cm (yes/no)
15. Ultrasound findings (cholecystitis, multiple stones, single stone, other)
16. Clinical suspicion of GBC
17. Planned open cholecystectomy
18. Individual history of typhoid fever

19. Family history of gallbladder cancer
20. Family history of gallstones

Data Curation

Staff responsible for retrieving archival information at each recruitment site were trained in data collection, including the completion of standardized case report forms, and the generation, secure storage, and disposal of anonymous patient codes. Only staff responsible for data retrieval had access to patients' identities. After the epidemiological and clinical information was collected for the first 20 patients at each recruitment site, the case report forms were reviewed by the statistical analysis team, and feedback was provided to the data retrieval staff.

Once the complete pseudo-anonymized data were sent to the statistical analysis team, 5% of patients were randomly selected from 8 recruitment sites that represented 82% of the study population, and the patient lists were sent to participating hospitals for blinded data validation (initial and validation data were collected by different staff). The reliability of the epidemiological and clinical data collected was quantified by the percentage of completely identical initial and validation values.

Statistical Analysis

Variables with missing data proportions above 30% were excluded from further analysis, and observations with no information were aggregated into a separate "missing" category. Multiple logistic regression was applied to investigate the association between the remaining epidemiological and perioperative clinical variables, and GBC or gallbladder dysplasia risk. The regression model included a recruitment site identifier, thus accounting for potential intersite heterogeneity. The description of results and subsequent analyses focused on variables with an association probability value below 0.1.

We conducted a random-effects meta-analysis to combine the recruitment site–specific estimates of relative risks and examine their heterogeneity between recruitment sites. Briefly, multiple logistic regression analyses stratified by recruitment site yielded site-specific effect estimates and standard errors. If the inclusion of a particular variable led to convergence problems for a particular recruitment site (eg, due to few observations for a variable level), the variable was not considered for the recruitment site. Effect estimates were pooled using the restricted maximum-likelihood method and the percentage of variation across recruitment sites I^2 and the probability value for heterogeneity based on Cochran's Q statistic were reported. In addition, a leave-one-out sensitivity analysis was carried out to examine the influence of each recruitment site on the pooled effect estimates.

In addition to the main analysis, separate analyses of association with GBC risk and gallbladder dysplasia risk were conducted. Because 2 identified risk factors, "clinical suspicion of GBC" and "planned open cholecystectomy," are subjective in nature and difficult to replicate, we also performed sensitivity analyses excluding them from the multiple logistic regression model.

Assessment of Risk Profile Differences Between Recruitment Sites

Principal component and cluster analyses were based on the proportion of GBC and gallbladder dysplasia cases attributable to each risk factor for each recruitment site (population attributable fraction [PAF]), estimated using Miettinen's formula:

$$\mathrm{PAF} = \frac{(OR-1)}{OR} \times \frac{P_e \times OR}{[(P_e \times OR) + (1 - P_e)]},$$

where OR represented the risk factor and recruitment site-specific odds ratio, and Pe was the prevalence of exposure to the risk factor at the recruitment site. Missing ORs were replaced with overall OR estimates from the main analysis, and median values were used to estimate PAFs for age at cholecystectomy (so that $P_e = 0.5$). To avoid sensitivity toward outliers, all variable-specific PAFs were standardized. The optimal number of clusters of recruitment sites was determined by considering an explained variance of at least 80% in the principal component analysis, and recruitment sites were grouped using the k-means algorithm for cluster partitioning. Because the k-means algorithm randomly selects the centers of the clusters, the clustering algorithm was run 100 times and the best cluster model was selected by minimizing the sum of squares within the clusters.

Data Reliability and Missing Data Proportions

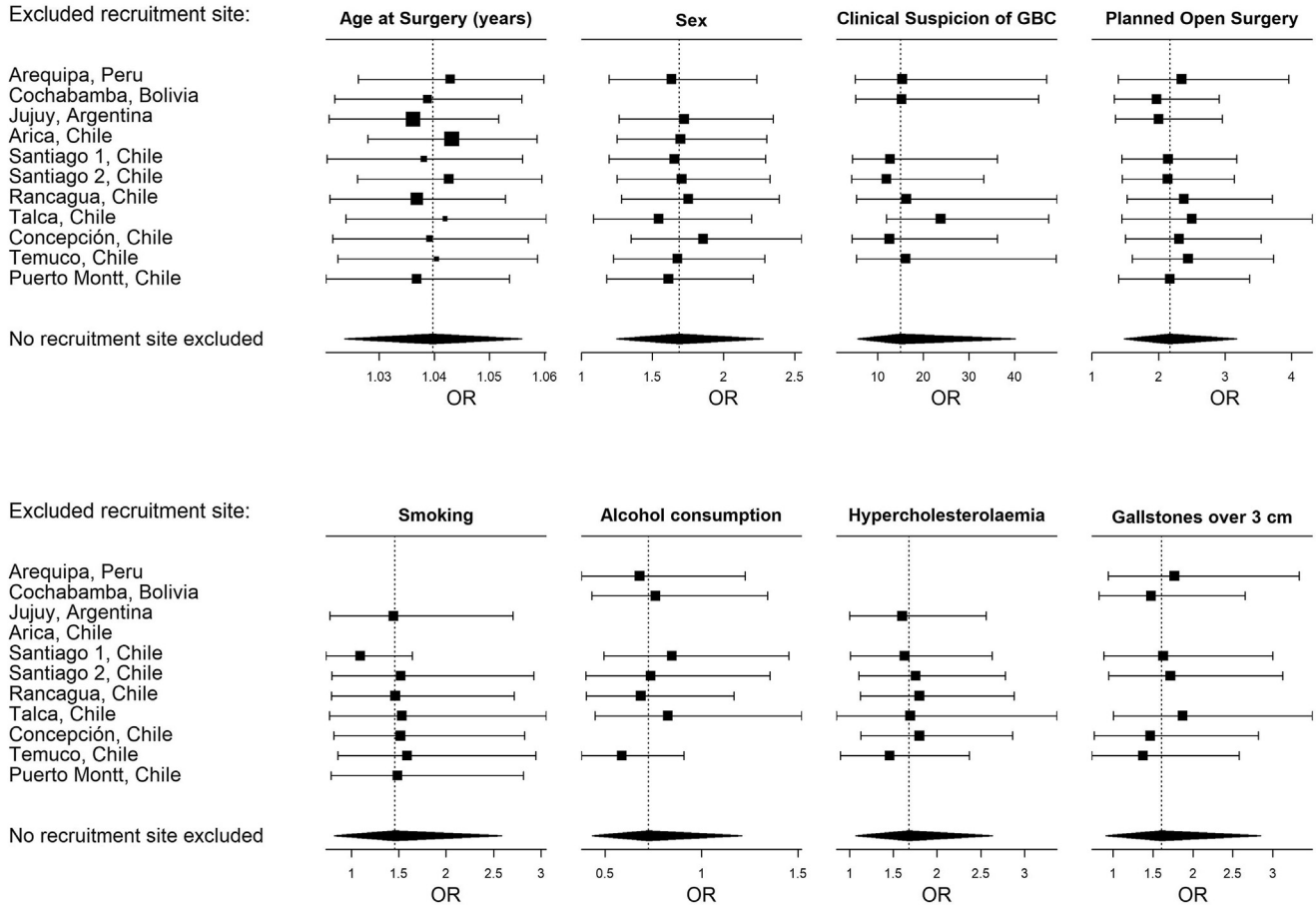
As described in the Materials and Methods, 5% of study participants from 8 recruitment sites (82% of the study population) were randomly selected, and their data were validated blindly (Supplementary Table 1). The average reliability was high: the lowest percentage of identical values for the first and second data retrieval (93%) was found for the variable "ultrasound findings." When stratified by recruitment site, the lowest reliabilities were found for "age at cholecystectomy" (54%) and "hypertension" (74%) in Concepción (Chile), and for "ultrasound findings" (71%) in Temuco (Chile).

Missing data proportions higher than 30% led to the exclusion of number of children in women (45%), typhoid fever (58%), family history of GBC (87%), and family history of gallstones (95%) from further analyses. Among the 4077 female study participants with available

information on the number of children, patients with GBC or dysplasia had more children (mean 2.68; 95% confidence interval, 2.34–3.02) than unaffected control subjects (mean 2.15; 95% confidence interval, 2.09–2.20; $P = .0009$). Of the 4446 patients with cholecystectomy and available information on "typhoid fever history," no patients with a history of typhoid fever presented with GBC or gallbladder dysplasia, compared with 117 (3%) patients without a typhoid fever history (Fisher's exact test, $P = .53$). Among the 1417 patients with available information on "family history of GBC," the incidence of GBC or gallbladder dysplasia was 10% in patients with a family history of GBC, compared with 5% in patients without a family history ($P = .10$). Of the 527 patients with available information on "family history of gallstones," 6% of patients with a family history presented with GBC or gallbladder dysplasia, compared with 3% of patients without a family history ($P = .12$). Among the remaining variables, the highest proportions of missing data were found for body mass index (30%), alcohol consumption (25%), and smoking (24%).

Supplementary Reference

e1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209–249.



Supplementary Figure 1. Forest plots of estimated ORs for GBC or dysplasia for each risk factor, after excluding one recruitment site at a time. GBC, gallbladder cancer; OR, odds ratio.

Supplementary Table 1. Reliability (Percentage of Times Initial and Validation Values Were Identical) for 5% of Randomly Selected Study Participants From 8 Recruitment Sites

Variable	Average	Arequipa	Santiago1	Santiago2	Rancagua	Talca	Concepción	Temuco	Puerto Montt
Age at cholecystectomy	93%	100%	100%	100%	100%	100%	54%	88%	100%
Female sex	98%	100%	100%	100%	100%	100%	85%	100%	100%
Number of children (for women)	97%	100%	96%	100%	100%	100%	97%	85%	100%
Clinical suspicion of GBC	100%	100%	100%	100%	100%	100%	100%	100%	100%
Weight	98%	100%	96%	100%	100%	100%	92%	98%	96%
Height	98%	100%	100%	100%	100%	100%	95%	93%	98%
Type of health insurance	100%	100%	99%	100%	100%	100%	100%	100%	100%
Indigenous American surname(s)	97%	100%	99%	100%	100%	100%	90%	90%	100%
Family history of GBC	100%	100%	100%	100%	100%	100%	100%	100%	100%
Smoking	98%	100%	97%	100%	100%	100%	95%	93%	100%
Alcohol consumption	98%	100%	100%	100%	100%	100%	100%	85%	100%
Diabetes	98%	100%	100%	100%	100%	100%	92%	90%	100%
Hypertension	96%	100%	100%	100%	100%	100%	74%	98%	100%
Hypercholesterolemia	97%	100%	100%	100%	100%	100%	87%	93%	100%
Typhoid fever history	100%	100%	100%	100%	100%	100%	100%	98%	100%
Gallstones over 3 cm	98%	100%	100%	100%	100%	100%	87%	95%	100%
Previous hospitalizations for gallstones	97%	100%	100%	100%	100%	100%	90%	90%	100%
Open cholecystectomy	99%	100%	100%	100%	100%	100%	97%	98%	100%
Ultrasound findings	93%	100%	100%	97%	100%	100%	79%	71%	94%
Family history of gallstones	100%	100%	100%	100%	100%	100%	100%	100%	100%
Investigated outcome (GBC, gallbladder dysplasia, or other)	99%	100%	97%	100%	100%	100%	97%	100%	98%

GBC, gallbladder cancer.

Supplementary Table 2. Main Characteristics of the Study Population and Results From Multiple Logistic Regression Analysis and Random-Effects Meta-Analysis, After Excluding the Risk Factors Clinical Suspicion of GBC and Planned Open Cholecystectomy

Variable	No GBC or DYS (n = 10239)	GBC or DYS (n = 322)	Multiple logistic regression	
			OR (95% CI)	*P* Value
Recruitment site				.0002
Arequipa, Peru	625	26 (4)	1.09 (0.61–1.96)	
Cochabamba, Bolivia	606	11 (2)	0.60 (0.06–5.65)	
Jujuy, Argentina	917	13 (1)	0.27 (0.13–0.57)[a]	
Arica, Chile	335	9 (3)	0.59 (0.29–1.21)	
Santiago 1, Chile	1285	49 (4)	1.17 (0.76–1.81)	
Santiago 2, Chile	663	29 (4)	0.97 (0.60–1.59)	
Rancagua, Chile	1016	16 (2)	0.34 (0.18–0.63)[a]	
Talca, Chile	2219	89 (4)	Ref.	
Concepción, Chile	740	31 (4)	1.01 (0.62–1.66)	
Temuco, Chile	722	22 (3)	0.51 (0.30–0.86)[a]	
Puerto Montt, Chile	1111	27 (2)	0.53 (0.26–1.09)	
Age at cholecystectomy, y	47 ± 17	57 ± 16	1.04 (1.03–1.05)[a]	<.0001
Sex				.0001
Male	3054	75 (2)	Ref.	
Female	7185	247 (3)	1.73 (1.31–2.29)	
Indigenous American surname(s)				.09
No	8378	264 (3)	Ref.	
Yes	1233	46 (4)	1.53 (1.04–2.26)[a]	
Missing	628	12 (2)	1.39 (0.17–11.3)	
Body mass index				.60
<25 kg/m^2	1714	58 (3)	Ref.	
25 kg/m^2 or more	5436	168 (3)	0.88 (0.65–1.21)	
Missing	3089	96 (3)	0.82 (0.56–1.22)	
Type of health insurance				.20
Public	8579	289 (3)	Ref.	
Privat	1512	30 (2)	0.67 (0.42–1.06)	
Missing	148	3 (2)	0.71 (0.21–2.37)	
Smoking				.01
No	6033	205 (3)	Ref.	
Yes	1705	62 (4)	1.44 (1.05–1.98)[a]	
Missing	2501	55 (2)	0.70 (0.40–1.20)	
Alcohol consumption				.01
No	5593	219 (4)	Ref.	
Yes	2074	43 (2)	0.58 (0.41–0.83)[a]	
Missing	2572	60 (2)	0.87 (0.50–1.49)	

Values are n, n (%), or mean ± SD.
CI, confidence interval; DYS, gallbladder dysplasia; GBC, gallbladder cancer; OR, odds ratio.
[a]95% Cis that do not include 1.00.

Supplementary Table 3. Population Attributable Fractions for Each Recruitment Site and Risk Factor Selected in the Final Regression Model

		Cluster 1			Cluster 2			Cluster 3				Cluster 4
Variable	Level	Cochabamba (Bolivia)	Jujuy (Argentina)	Temuco	Santiago2	Rancagua	Concepción	Arequipa (Peru)	Arica	Talca	Puerto Montt	Santiago1
Hypercholesterolemia	Yes	0.006	0.106	0.131	–0.014	–0.026	–0.034	0.000	0.003	0.041	0.001	0.029
Planned open cholecystectomy	Yes	0.379	0.712	0.058	0.021	0.013	0.003	0.364	0.026	0.047	0.061	0.029
Sex	Female	0.337	0.116	0.336	0.250	0.002	–0.205	0.509	0.233	0.444	0.566	0.370
Alcohol consumption	No	0.401	0.277	–0.787	0.188	–0.429	0.321	–0.037	0.302	0.475	0.266	0.538
Smoking	Yes	0.028	0.09	–0.057	0.028	0.076	–0.015	0.014	0.057	0.031	0.041	0.632
Clinical Suspicion of GBC	Yes	0.130	0.254	0.197	0.810	0.586	0.548	0.186	0.051	0.037	0.026	0.361
Age at cholecystectomy	$\geq$ Site-specific median	0.555	0.555	0.549	0.386	0.746	0.747	0.229	0.114	0.436	0.692	0.669
Gallstones over 3 cm	Yes	0.125	0.074	0.134	–0005	0.036	0.083	0.004	0.027	–0.008	0.048	0.017

Note that negative values imply a protective effect of this variable level for the corresponding recruitment site. The following levels were used for population attributable fraction estimation: age at cholecystectomy (higher than the median age) and alcohol consumption (no).
GBC, gallbladder cancer.