



# Gallbladder cancer in South America: epidemiology and prevention

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**Abstract:** The incidence and the mortality of gallbladder cancer (GBC) show significant variation worldwide, with high age-standardized rates in western South America (SA). Due to the lack of effective measures for prevention, the late diagnosis and the small benefit of systemic treatment, GBC has an ominous prognosis and became an important public health problem in this part of the continent, where the most important risk factors are gallstone disease, female gender, age, ethnic groups, and low socioeconomic status. Many genetic abnormalities have been described in series from SA, some of them similar and others unique in comparison to gene alterations in GBC from other regions of the world. Prophylactic cholecystectomy (PC) is one of the strategies to decrease the mortality but its cost-effectiveness is questionable. A way to improve the performance of PC is to identify molecular risk factors that in combination with currently known ones detect patients with very high risk for developing GBC. Also, more research studies are required to better understand the epidemiology and molecular biology in order to improve the prevention and treatment of this lethal disease.

**Keywords:** Gallbladder cancer (GBC); South America (SA); epidemiology; prophylactic cholecystectomy (PC); molecular biology; risk factors

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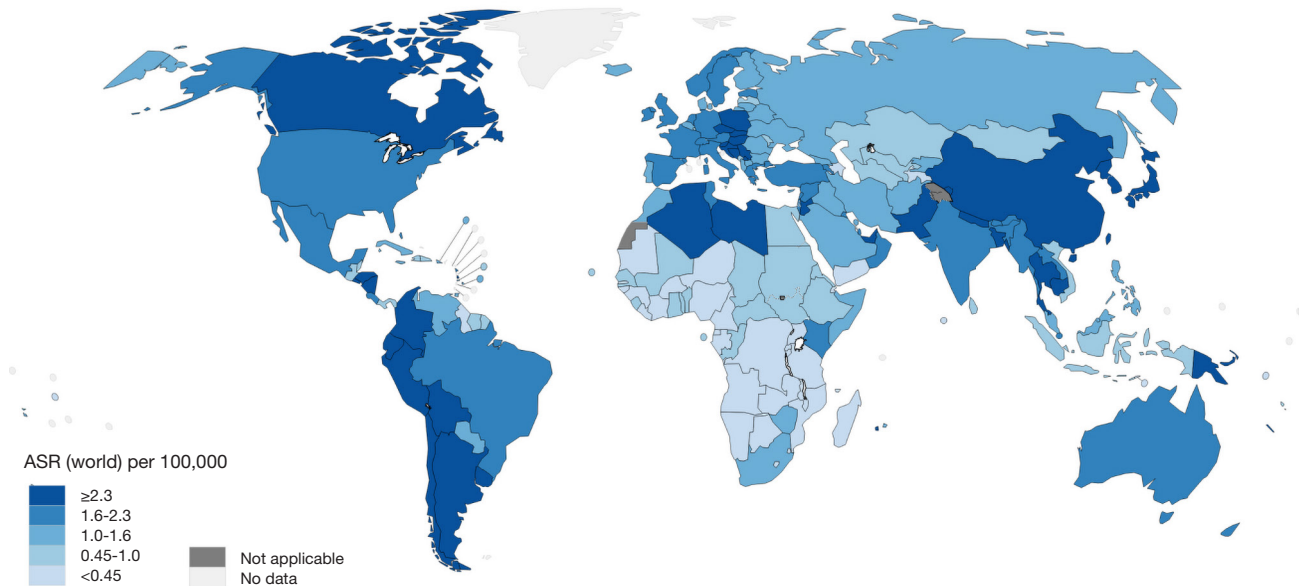
## Introduction

Gallbladder cancer (GBC) is a biliary tract malignancy with a high incidence in specific regions of some developing countries (1), where its late diagnosis and ominous prognosis make GBC an important public health problem. Carcinogenesis is poorly understood due to the scarce knowledge of genetic and environmental factors. The present review provides an update of the epidemiology, the molecular genetics, the risk factors, the screening and the early diagnosis of GBC in South America (SA), focusing on high-risk areas. The goal is to improve our knowledge of this dismal disease, for which immediate public health decisions are required.

## Epidemiology

Based on current estimates GBC occupied the 20th place in incidence and the 17th place in mortality worldwide in 2018 [International Classification of Diseases 10<sup>o</sup> Revision (ICD10): C23-24] (*Figure 1*), representing 1.3% of new cancer cases and 1.7% of cancer-related deaths (excluding non-melanoma skin cancer) (1). Although C23-24 deaths are due to GBC and extrahepatic bile duct cancer, the majority correspond to GBC. GBC represents 4% of gastrointestinal cancers in both sexes; 6% in women and 3% in men. In Japan, based on the number of cases and not on rates, GBC accounts for 8% and 5% of gastrointestinal cancers in women and men, respectively. Indeed, incidence

Estimated age-standardized incidence rates (world) in 2018, gallbladder, both sexes, all ages



**Figure 1** Gallbladder cancer incidence in both sexes, worldwide, 2018. Estimated age-standardized rates (World) per 100,000 per year. Data source: GLOBOCAN 2018. WHO, IARC: <https://gco.iarc.fr/today/>.

and mortality age-standardized rates (ASR) are higher in females worldwide (2.4 and 1.8 per 100,000 females per year, and 2.2 and 1.6 per 100,000 men per year, respectively), except in some Asian countries like Republic of Korea and Japan where the highest ASR values were in men (1).

Due to demographic changes, the burden of GBC is expected to increase all over the world by more than 75% (165,600 new cases and 130,400 new deaths) by the year 2040.

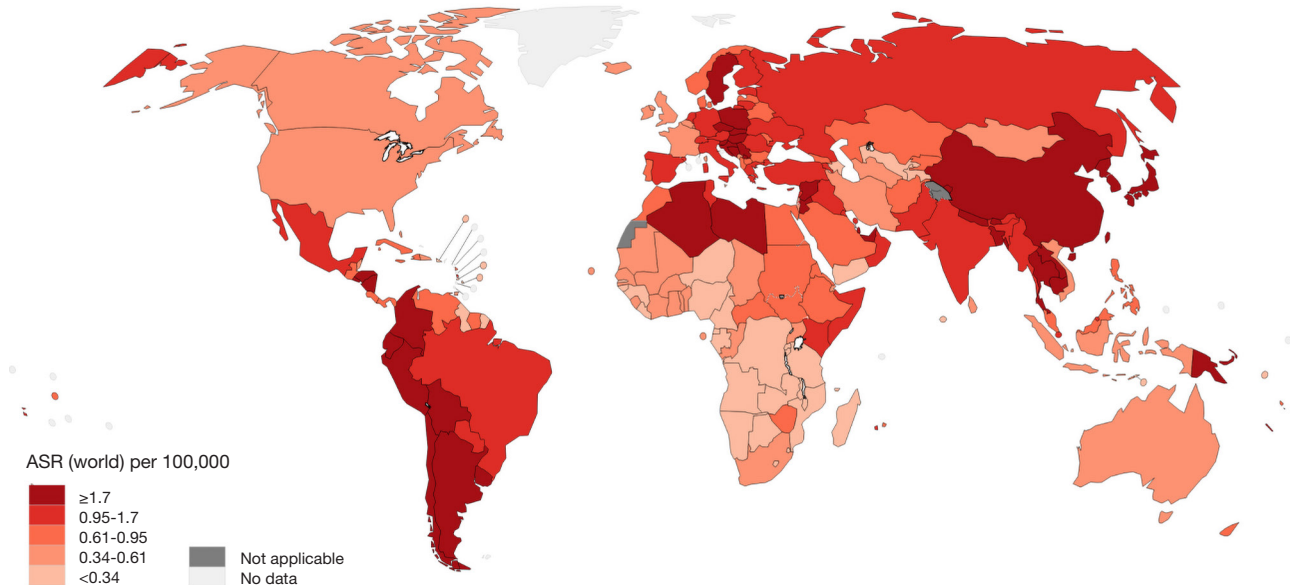
Incidence and mortality of GBC show significant variation worldwide (1,2). The highest estimated ASR is over 2 per 100,000 per year in countries from Eastern Asia (over 2.7) and the west part of SA. While, the regions of North America, Northern Europe, Australia, and New Zealand show incidence and mortality ASRs around 1 and even lower (Figures 1,2).

In SA, 15,114 new GBC cases and 11,097 GBC-related deaths were estimated for the year 2018. Incidence and mortality ASR exhibit striking geographic variation, reaching epidemic levels particularly in the Andean Area (3,4). The highest incidence and mortality ASRs have been reported in Bolivia (14.0 and 10.6, respectively), Chile (9.3 and 5.4) where GBC is the second leading cause of death from cancer in women, and Peru (4.8 and 3.1). In these

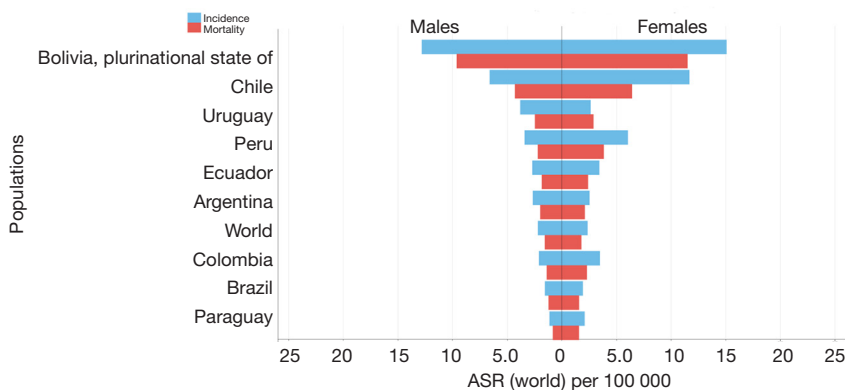
countries, GBC mortality showed an unequal distribution. The areas with the highest values were: the south-inland region in Chile; the region near Lake Titicaca in Bolivia and the area of Trujillo city in Peru (5). GBC is more frequent among female indigenous people (Mapuche) and in people living in areas with high poverty and insufficient access to health services (Figure 3) (6-10).

The International Agency for Research on Cancer (IARC) estimates GBC incidence in Argentina from population-based cancer registries (PBCR) that have reached the standards for data quality. In 2018, the incidence ASR was 2.7 in males and 2.5 in females for the whole country (1). However, these rates are based on five registries, those with high-quality data, and they do not take into account the heterogeneous distribution of GBC inside the country. The geographic variation is shown with the analysis of GBC mortality, instead of incidence because it is much more comprehensive encompassing the twenty-three provinces and the federal district. The latest official information comes from the 2017 DEIS (Department of Statistics and Health Information, for its acronym in Spanish) mortality database. In this year a total of 1,285 deaths were registered as GBC (ICD 10: C23-24) all over the country, with a mean age of 69 (95% confidence interval: 68–70) in both sexes. The ASRs were 1.9 in

Estimated age-standardized mortality rates (world) in 2018, gallbladder, both sexes, all ages



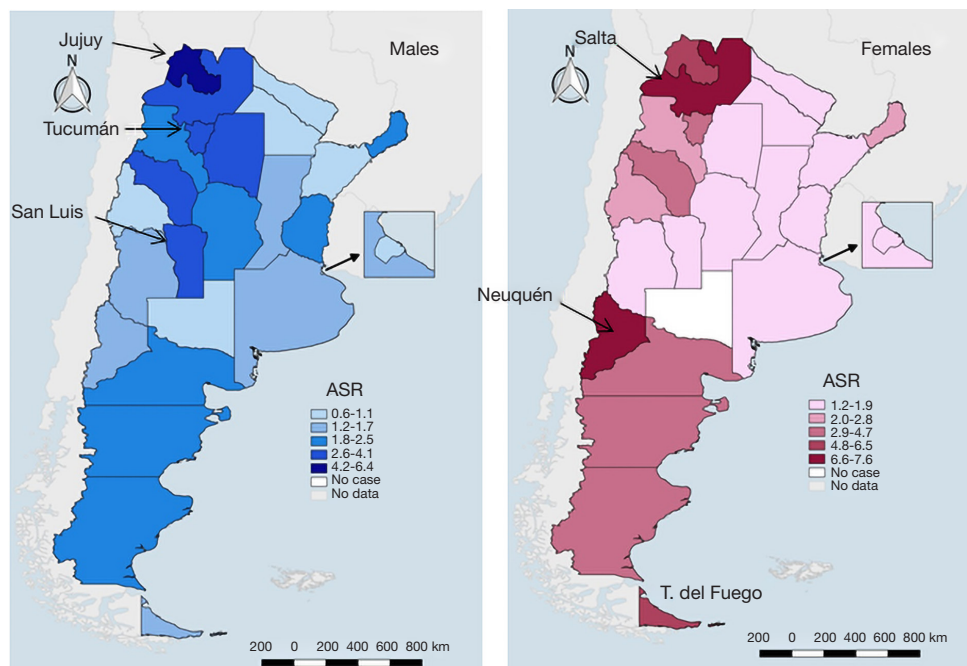
**Figure 2** Gallbladder cancer mortality in both sexes, worldwide, 2018. Estimated age-standardized rates (World) per 100,000 per year. Data source: GLOBOCAN 2018. WHO, IARC: <https://gco.iarc.fr/today/>.



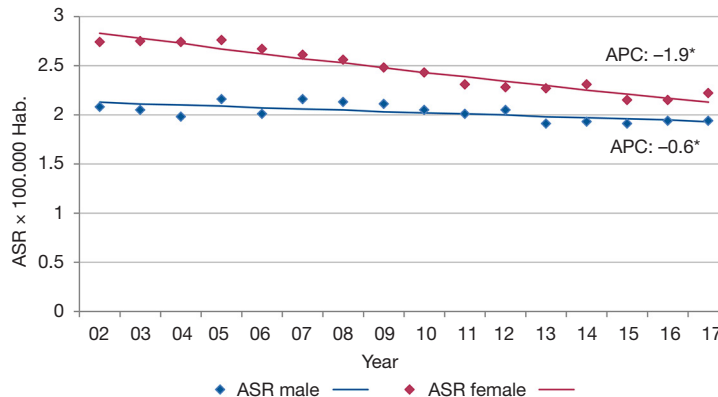
**Figure 3** Gallbladder cancer incidence and mortality in males and females of all ages in the world and countries of South America, 2018. Estimated age-standardized rates (World) per 100,000 per year. Data source: GLOBOCAN 2018. WHO, IARC: <https://gco.iarc.fr/today/>.

men and 2.2 in women, with no statistically significant difference. However, there was a high geographic variation. In both sexes the highest mortality ASRs (quintile with the highest value range), were observed in the northwestern provinces of Jujuy (6.6 in females and 6.4 in males) and Salta (7.6 in females and 4.2 in males); in both regions GBC is the third leading cause of death from cancer in women. Age-standardized rates were followed in men by the western provinces of San Luis and Tucuman (3.3 in both), and in women by the western province of Neuquén (6.9) and the

southern province of Tierra del Fuego (5.9) (Figure 4). In contrast, the provinces at the east of the country showed mortality ASR from 1.2 to 1.9 per 100,000 women per year and from 0.6 to 1.7 per 100,000 men per year (quintile with the lowest value range). GBC mortality trends decreased over the period 2002–2017 in both sexes. The estimated annual percentage of change (APC) at any age was -1.9% in women and -0.6% in men, both reaching statistical significance (Figure 5). In the provinces with the highest mortality ASR (Jujuy, Salta, and Neuquén) the decrease



**Figure 4** Gallbladder cancer mortality in males and females by provinces of Argentina, 2017. Age-standardized rates (World) per 100,000 per year. Data source: own production from the DEIS data.

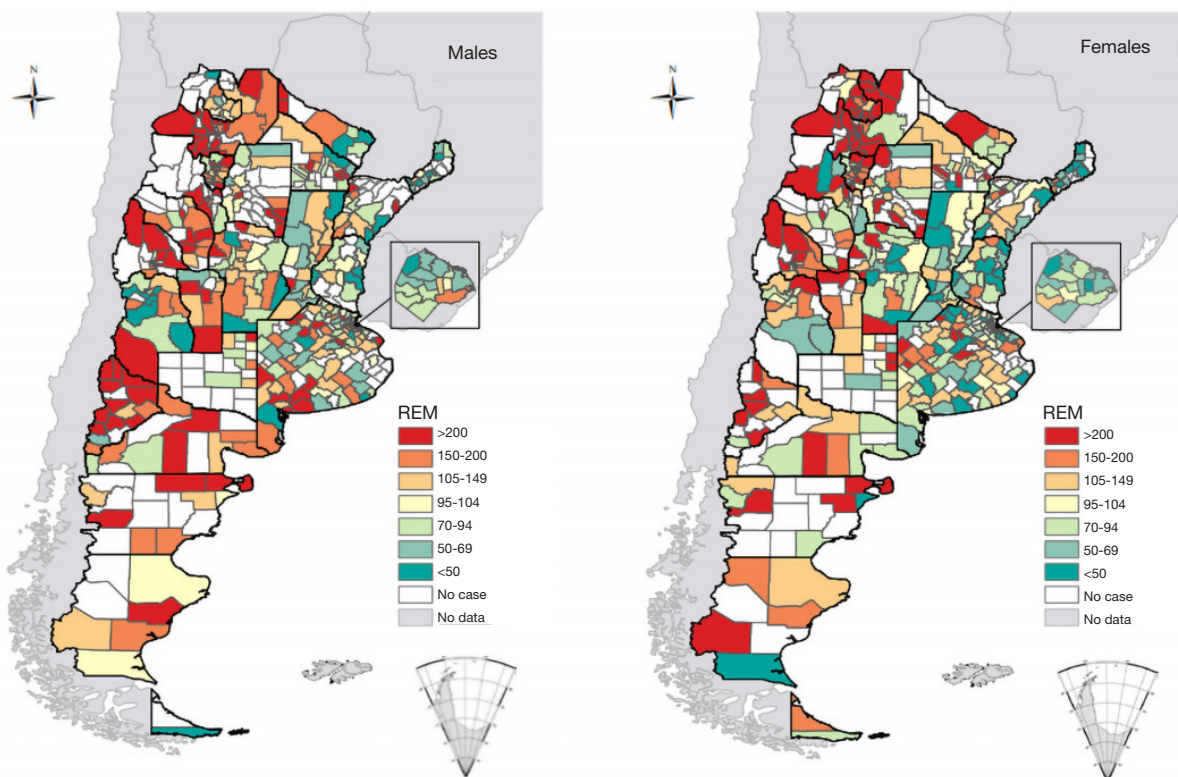


**Figure 5** Gallbladder cancer mortality trends by sex in Argentina, 2002–2017. Age-standardized rates by world population, per 100,000 males and females per year and estimated annual percentage of change (APC). \*, trend value statistically significant ( $P < 0.05$ ). Data source: own production from the DEIS data.

was observed in both sexes, with the exception of Salta where the mortality ASR increased in men of any age, and in men and women over 50 years old (Atlas 2002–2016 not published). Actually, a consistent increase in the age-specific mortality rate has been observed in men and women older than 50 years all over the country (Atlas trends 2002–2016, not published).

The distribution of GBC mortality ASR by departments in Argentina (provinces are politically divided in departments), shows a pattern similar to provinces, with values two hundred percent higher than the national average in poor areas at the north of the country as well as in the Andean regions (11) (Figure 6).

GBC is a highly lethal disease. Approximately 30% of



**Figure 6** Gallbladder cancer standardized mortality ratios by sex and department of residence in Argentina, 2011–2015. Data source: Atlas de mortalidad por cáncer: Argentina, 2011-2015. [https://www.researchgate.net/publication/325108328\\_Atlas\\_de\\_mortalidad\\_por\\_cancer\\_Argentina\\_2011-2015](https://www.researchgate.net/publication/325108328_Atlas_de_mortalidad_por_cancer_Argentina_2011-2015).

**Table 1** Gallbladder cancer estimated age-standardized incidence, mortality and survival rates by country income level in 2018

Countries by income	Female				Male			
	Incidence	Mortality	Ratio	5-year survival	Incidence	Mortality	Ratio	5-year survival
High	2.4	1.5	1.6	38%	2.9	1.7	1.7	41%
Upper middles	2.4	2	1.2	17%	2.3	1.8	1.3	22%
Low middle	2.2	1.6	1.4	27%	1.4	1.1	1.3	21%
Low	1.4	1	1.4	29%	1	0.7	1.4	28%

Incidence & mortality are expressed as ASR by world population. Ratio: ASR incidence/ASR Mortality. 5-year survival estimated by  $1 - (\text{ASR Mortality} / \text{ASR Incidence})$ . Data source: own production whit Globocan data. ASR, age-standardized rate.

patients with lesions confined to the gallbladder wall survive 5 years and only 10% with more advanced stages survive 1-year (12,13). The majority of cases are diagnosed at later stages, and this explains the high lethality.

The mortality/incidence (MI) ratio is another way to estimate the survival of cancer patients. Interestingly, there is a great disparity in the GBC MI ratio worldwide. Based on this ratio the survival is around forty and twenty percent

in high-income and middle-income countries, respectively. The intermediate range is observed in low-income countries (Table 1). This disparity could be due to differences in socioeconomic status, education level, and life expectancy. Also, the MI ratio varies according to sex. With the exception of Chile, survival rates worldwide (based on GBC MI ratios) were higher in men, which suggests that GBC in women is not only more prevalent but also more lethal.



**Table 2** Main risk factors to GBC

Inflammatory
Gallstones
Porcelain gallbladder
Primary sclerosing cholangitis
Anomalous pancreaticobiliary duct junction
Chronic infection
Non inflammatory
Gender
Age
Socioeconomic status
Genetic
Aflatoxin
GBC, gallbladder cancer.

## Risk factors

Several risk factors for GBC have been identified, many of which share a common feature of chronic inflammation of the gallbladder (14-16). Other risk factors are age, female gender, genetic characteristics, socioeconomic status, and dietary patterns. The main risk factors are summarized in *Table 2*.

### Gallstone disease

Gallstone disease or gallbladder lithiasis is present in 70 to 90 percent of patients with GBC (16-18). A history of gallstones appears to be one of the strongest risk factors for the development of GBC (18-20). A case-control study from Shanghai showed a 34-fold increase in the risk of GBC in patients with gallbladder lithiasis (16). One autopsy data-based study from Chile suggested that the risk of GBC in patients with gallstones is seven times greater compared to patients without it.

The overall incidence of GBC in patients with gallstone disease is only 0.5 to 4 percent. The risk increases with the duration of lithiasis and the size of gallstones (21,22). Indeed, the relative risk of GBC in patients with gallstones is 2.4 if the diameter is 2.0–2.9 cm, but increases to 10.1 if larger than 3.0 cm (16).

Since 10% to 30% of GBC cases do not have gallstone disease and only a small proportion (0.5–4%) of patients with gallbladder lithiasis develops GBC (23-26), other

factors may be important in the development of GBC. For example, some ethnic and racial groups show an incidence of GBC higher than expected for their frequency of gallstone disease, suggesting that other environmental or genetic factors may also play a role in the course of carcinogenesis (27).

### Porcelain gallbladder

Porcelain gallbladder is detected in 0.06 to 0.08 percent of cholecystectomy specimens (28). It has a female predominance and the incidence of GBC in patients with porcelain gallbladder is between 2 and 3 percent (29).

Patients with selective mucosal calcification of the gallbladder wall appear to be at higher risk for GBC compared with those in whom the gallbladder wall is completely calcified (complete type) (28-31).

### Primary sclerosing cholangitis (PSC)

PSC is a chronic fibro-inflammatory syndrome which has a close association with the chronic inflammation and the carcinogenesis. There is an increased frequency of GBC in patients with PSC, which in all likelihood correlates with the ongoing inflammation, facilitating a metaplasia-dysplasia-carcinoma sequence (32,33).

### Anomalous pancreaticobiliary duct junction

Several studies have suggested that an anomalous pancreaticobiliary duct junction (APBDJ) is associated with the development of GBC (34-36). Patients with GBC and APBDJ are generally young, have a low incidence of gallstones, and their tumors show a higher frequency of *K-ras* mutations (37,38). The anomalous ductal union is seen in about 17% of patients with GBC compared with less than 3% in patients with other hepatobiliary disorders. In the APBDJ the pancreatic duct ends into the common bile duct outside the duodenal wall and not under the control of the sphincter; therefore, pancreatic juice can freely flow back into the gallbladder causing bile stasis, which leads to precancerous changes in the gallbladder mucosa. In APBDJ cases without dilatation of the bile duct, the risk for GBC is higher compared with those with bile-duct dilatation.

### Chronic infection

In endemic areas, 1–4% of acutely infected individuals

become chronic asymptomatic carriers of Salmonella Typhi (*S. Typhi*). One meta-analysis of 17 case-control and cohort studies suggested an association between chronic *S. Typhi* carriage and elevated risk of GBC (39). A study performed in Mexico and Bolivia concluded that patients diagnosed for typhoid disease had a twelve fold higher risk of developing GBC (40).

### **Geography/ethnicity**

GBC has a wide geographic variation, as previously described (1,41). GBC particularly afflicts some indigenous populations (27). We will describe this topic in more detail later, in “Genetics” item.

### **Socioeconomic status**

In a case-control study of 228 people from Chile by Serra *et al.*, very low socioeconomic status was associated with GBC, with an odds ratio (OR) of 6.3 (95% CI: 1.7–23.0) (22). It is conceivable that people with very low socioeconomic status have less access to the health care system and, therefore they are more likely to have longstanding gallstone disease.

### **Sex**

As described above, GBC predominates in women over men worldwide. Females having early menarche, late menopause, multiple pregnancies, and childbirths appear to have an increased risk for developing GBC according to a case-control study from India (42). Interestingly, estrogens increase the formation of gallstones, mainly by elevating biliary cholesterol (43).

### **Obesity**

Overweight has been consistently associated with an increased risk for GBC. For each 5-point increment in body mass index, the relative risk increases by 1.59 in women and 1.09 in men (44).

### **Genetics**

GBC ensues from an interaction between an innate genetic predisposition and exposure to environmental risk factors. Genetic factors undoubtedly govern some of the widely variable frequencies of GBC worldwide.

The contribution of the genetic background in the development of GBC emerges from the hypothesis about the migratory phenomenon from high incidence areas in Chile and Bolivia to Argentina. The northwestern region in Argentina shares borders with the Chilean region of Antofagasta and GBC mortality rates are similar on both sides of the Andes Mountains. Similarly, in the province of Neuquen in the western south of Argentina, mortality rates due to GBC are among the highest in the country. In this area the ancestral Pewenches were invaded by the Mapuches, coming from a southern region of Chile with the highest mortality rates of GBC, establishing a bidirectional population flow that still happens nowadays. Consequently, it is tempting to speculate that the high mortality rates of GBC in Neuquen are partially due to the migration of Mapuches from Chile (45). Also, the northern provinces of Salta and Jujuy where GBC is the third leading cause of death from cancer in women, have been receiving migrating people from Bolivia for decades, where GBC mortality is very high.

Lorenzo Bermejo and others investigated the relationship between the type of Native American ancestry and the main causes of death. Based on the genotypic genome data of 1805 Chileans, they showed an association between Mapuche ancestry and GBC; every 1 percent increase in Mapuche ancestry represented a 3.7 percent increase in the risk of death from GBC (95% CI: 3.1% to 4.3%,  $P=6\times 10^{-27}$ ) (45). For the first time, a strong association between ancestry and risk of GBC is shown and supports the migratory phenomenon described above as the main reason for the increased risk of GBC in the western province of Neuquen in Argentina.

### **Aflatoxin**

Several investigations have shown an elevated concentration of aflatoxin in red chili peppers which are consumed in Andean areas from Chile, Bolivia Peru with the highest GBC incidence ASR (46,47). Nogueira *et al.* (48) performed a case-control study with 112 Chilean men and women, and found that patients with GBC had higher levels of aflatoxin B1-DNA adducts in peripheral blood compared with healthy control individuals (OR 13.2; 95% CI: 4.3–47.9) and with control individuals with gallstones (OR 9.4; 95% CI: 2.8–37.2). Koshiol *et al.* also studied this association in a case-control study from Shanghai, China (49). The plasma level of aflatoxin B1 (AFB1-lysine adduct) was found three more times in patients with GBC compared with controls (OR, 2.71; 95% CI: 1.70–4.33). The population attributable

fraction for cancer-related to aflatoxin was 20% (95% CI: 15–25%).

In summary, there is evidence to consider red chili pepper, a frequently consumed food in some regions of SA, as a risk factor for GBC. This, in turn, could be mediated by the aflatoxin mutagen, a contaminant of red chili pepper.

### Age

GBC rates tend to increase with advancing age (50). In the 2017 DEIS mortality database from the entire country of Argentina, age-specific GBC mortality rates increased consistently with age, the peak age of death was 80 and over (289/1,285), and 7 percent of deaths (88/1,285) were in patients younger than 50 years old. An analysis of sixty cases from provinces at high risk in Argentina (ILOGI database, data not published) also revealed an increment of GBC mortality with increasing age. However, the peak age of death was 54–59, and 17 percent of deaths occurred in people under 50 years old.

### Tobacco and alcohol

Smoking and alcohol (40) have been described as risk factors for GBC, although with a low level of evidence. Recently McGee reported a meta-analysis from 26 prospective studies to evaluate associations of cigarette smoking and alcohol consumption with biliary tract cancer (51). Although current smoking was associated with intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC) and ampulla of Vater cancer (AVC), no convincing association was observed between smoking and GBC. Alcohol consumption was only associated with ICC. Therefore, the evidence is not sufficient to state that alcohol or tobacco consumption is a risk factor for GBC.

### Molecular biology

The molecular biology of biliary cancer, including GBC, is poorly understood (52). The information available is very limited (53) even more in SA, is based in retrospective data with a low number of samples, and is not representative of majorly affected racial-ethnic groups.

While there is consensus regarding chronic epithelial inflammation as an initial pathogenic event (54), the mechanism of tumor progression is not clearly defined (55). Two models have been postulated: metaplasia to dysplasia,

and adenoma to dysplasia. The first shows alterations in tumor suppressor genes (*CDKN2A*, *p53*, *p57*, *KIP2*), adhesion molecules (*CEA*, *CD99*), oncogenes (*C-erb-B2*, *KRAS*) and others genes (*bTert*, *VEGF*, *Muc-1*, *iNOS*, *COX2*, *MGMT*) (56,57). In the adenoma-carcinoma model, alterations in cell adhesion molecules (*CD54* and *CD56*) and mutations of the *CTNNB1* gene have been reported (58,59). The model of metaplasia-dysplasia has greater acceptance because these histologic alterations are more frequent in GBC (60% and 90% respectively) (60) compared to adenomatous polyps in less than 3% of specimens obtained from early GBC (61).

As in other tumors, GBC carcinogenesis involves multiple genetic alterations. Tumor suppressor genes, oncogenes, DNA repair gene mutations, microsatellite instability (MSI) and epigenetic alterations are reported with greater frequency and wide variation (62). Genetic abnormalities in GBC worldwide are depicted in *Table 3*. We will describe the main molecular abnormalities in GBC reported from SA (*Table 4*).

### Genetic alterations

#### Tumor suppressor genes

In 42 cases of GBC from Chile (20/42, 48%) and Japan (22/42, 52%), mutations in exons 5 to 8 which deregulate the *p53* gene were reported (63). In the Chilean patients, they found at least 1 mutation in 55.5% (11/20) of the cases and described 13 different mutations. There was geographical variation: C:G to A:T transition and C:G pair mutation mainly in Chilean patients, and A:T pair mutation predominantly in patients from Japan.

Wistuba *et al.* studied the *p53* protein overexpression by immunohistochemistry in normal epithelium and premalignant lesions in patients from Temuco, a southern area in Chile with a very high incidence of GBC (64). Normal and metaplastic epithelium did not overexpressed *p53*. On the contrary, the protein was overexpressed in 34 of 52 (65.4%) GBC, 21 of 47 (44.7%) carcinomas in situ, and 11 of 34 (32.4%) dysplastic tissues. This suggests that *p53* inactivation is an early event in the pathogenesis of GBC.

Asay *et al.* (65) reported overexpression of *p53* in 18/36 (50%) GBC patients from Bolivia, a frequency similar to other world areas with high GBC incidence (66).

Roa *et al.* found inactivation of the *CDKN2A* gene in 13/38 (34%) cases of GBC from Chile. In 9/38 (24%) samples it was produced by methylation in promoter areas, while in 4/38 (11%) by allelic imbalance. Although not



**Table 3** Most frequently altered genes in GBC carcinogenesis, worldwide (prevalence in parenthesis) (55-62)

Type	Chronic inflammation	Preneoplastic lesions	GBC
Oncogenes	<i>KRAS</i> (15–58%)	–	<i>KRAS</i> (10–67%)
	–	<i>EGFR</i> (15–71%)	<i>EGFR</i> (63.4%)
	–	–	<i>p21</i> (28%)
	–	–	<i>Her2/neu</i> (10–64%)
Tumor suppressor genes	<i>CDKN2A</i> (100%)	<i>CDKN2A</i> (50–100%)	<i>CDKN2A</i> (10–50%)
	<i>p53</i> (16%)	<i>p53</i> (25–47%)	<i>p53</i> (58–100%)
	<i>FHIT</i> (9%)	<i>FHIT</i> (45–58%)	<i>FHIT</i> (55–79%)
	<i>Rb</i> (100%)	<i>Rb</i> (100%)	<i>Rb</i> (58%)
	<i>VHL</i> (88%)	<i>VHL</i> (80%)	<i>VHL</i> (48%)
–	–	<i>p57</i> (19%)	
Adhesion molecules	–	<i>A Catenin</i> (26–88%)	<i>A Catenin</i> (16%)
	–	<i>B Catenin</i> (40–100%)	<i>B Catenin</i> (41%)
	–	<i>Y Catenin</i> (66–77%)	<i>Y Catenin</i> (43%)
	–	–	<i>E Catenin</i> (70–89%)
	<i>MUC1</i> (4%)	<i>MUC1</i> (78%)	<i>MUC1</i> (78–89%)
	–	<i>CEA</i> (11–70%)	<i>CD44y6</i> (39–76%)
	–	<i>CD99</i> (67–87%)	<i>CD54</i> (39%)
–	–	<i>CD56</i> (10%)	
Microsatellite instability	–	<i>MSI</i> (33–83%)	<i>MSI</i> (10%)
Angiogenesis	–	<i>Cox2</i> (45–87%)	<i>Cox2</i> (59–71%)
	<i>VEGF</i> (16%)	<i>VEGF</i> (5–42%)	<i>VEGF A</i> (75–81%)
	–	–	<i>Thrombospondin 1</i> (74%)
Cell cycle regulators	<i>Ciclin D 1</i> (7%)	<i>Ciclin D 1</i> (57–67%)	<i>Ciclin D 1</i> (41–68%)
	–	<i>Cyclin E</i> (12%)	<i>Cyclin E</i> (33%)
	–	–	<i>P27Kip1</i> (43–65%)
Apoptosis	–	–	<i>Caspase</i> (77–95%)
	–	–	<i>Bcl-2</i> (34%)
Other genes	<i>iNOS</i> (100%)	<i>iNOS</i> (70%)	–
	<i>hTERT</i> (4%)	<i>hTERT</i> (25–82%)	<i>hTERT</i> (66–93%)
	–	–	<i>MGMT</i> (59%)

GBC, gallbladder cancer.

statistically significant, the inactivation of p16 (the main product of *CDKN2*) was more frequent in the Mapuche ethnic group and males. It was associated with ominous prognosis (P=0.09) (67).

ILOGI (68) (for its acronym in Spanish, Latin-American

Intergroup of Gastrointestinal Oncology) analyzed the mRNA levels of the *FBXW7* gene (also known as *bCdc4* tumor suppressor gene) in 64 BC samples, the majority with GBC (27 GBC, 26 CC, 6 VBT, and 5 BC not specified) from Argentina (69). A correlation with survival

**Table 4** Most frequently altered genes in GBC carcinogenesis, SA (prevalence in parenthesis)

Type	Preneoplastic lesions	GBC
Oncogenes	–	<i>KRAS</i> (0–30%)
	–	<i>Her2/neu</i> (12.8%)
	–	<i>C-MET</i> (48%)
Tumor suppressor genes	–	<i>CDKN2A</i> (34%)
	<i>p53</i> (32–44%)	<i>p53</i> (55%)
	–	<i>FBXW7</i>
Genomic imbalance	–	<i>Aneuploidy</i> (26%)
Microsatellite instability	<i>MSI</i> (33–83%)	<i>MSI</i> (10%)
Other molecular: genes alterations	–	<i>KIT</i> (6%)
	–	<i>MRP4</i> (66%)

GBC, gallbladder cancer; SA, South America.

was reported. Median progression-free survival was 4.9, 7.6 and 26.9 months for low, medium and high *FBXW7* expression tertiles, respectively. OS was 6.2 months in the lowest tercile, 8 in the intermediate tercile, and not reached in the highest tercile. No *FBXW7* hotspot mutations were detected.

### Oncogenes

Activation of the *RAS* family is one of the most commonly reported events in GBC (66–70). A study of 25 GBC cases from Chile (71) described genetic alterations related to tumor progression *RAS* gene point mutations were reported in 2 cases (2/21, 10%), 1 *K-ras* and 1 *N-ras* in codon 12 and 61 respectively. All of them were found in invasive and poorly-differentiated tumors.

Discordant results of *K-ras* mutations were reported in neighboring regions of SA. These abnormalities were found in GBC in 29–30% (10/33, 10/35) of cases in Chile (72,73), 17% (10/58) in Argentina (54), 2.8% (1/36) in Bolivia (65) and 0% (0/30) in Peru (74). For comparison outside SA, in India, Korea, and Europe *K-ras* mutations were described in 41% (16/39), 20% (3/15) and 5% (1/20) of cases, respectively (75,76). It would be interesting to study the prevalence of biliopancreatic duct malformations in these populations since published series suggest a relationship of *K-ras* mutations to this congenital abnormality, stronger than the association of *K-ras* mutations with biliary lithiasis (77).

In Chile, Roa *et al.* reported the largest study of *Her2/neu* in GBC in SA (78). Utilizing the most accepted IHC criteria for breast cancer they found expression of *Her2/neu*

in 24/187 (12.8%) cases. Patients with *HER2/neu* overexpression showed a lower 5-year overall survival, in comparison to those without overexpression (34% versus 41%, not statistically significant). Overexpression was more frequent in advanced cancers (13.8%) and well-differentiated tumors (17.4%), although this difference was not statistically significant.

Arroyo *et al.* (from ILOGI) reported over-expression of the *c-MET* protein in 25/52 (48%) GBC samples from Argentina (79). Patients with this molecular abnormality had a median overall survival of 11.3 months compared to 18.2 months in patients without it (P=0.068). Over-expression correlated with tumor grade (P=0.015), showing an inverse association. In EEUU, the *MET* amplification was reported in low frequency (1%) in a series of 85 GBC cases studied with a next-generation sequencing platform (80).

### Other molecular-genetic alterations

Arroyo *et al.* analyzed the expression of the *KIT* protein in GBC cases from northwestern Argentina (81). Immunohistochemistry revealed a 3+ score in only 3/50 samples (6%), all being poorly differentiated tumors. Went *et al.* reported similar results, with no expression in 27 GBC samples from European patients (82). In contrast, weak and moderate *KIT* expression was described in 7/12 (58%) biliary tract cases from the EEUU (83), with no discrimination between GBC and cholangiocarcinoma.

ILOGI studied the expression of multidrug resistance-associated protein 4 (*MRP4*) in 15 GBC samples from Salta and Jujuy, the provinces with the highest mortality

rates of GBC in Argentina (Figure 4) (11). MRP4 overexpression increases cell proliferation, tumor growth and chemoresistance in pancreatic cancer (84). Ten out of fifteen (66%) samples showed high expression, and this was associated, although not statistically significant, with shorter overall survival (85). These findings led to an expansion study which is in progress.

### Genomic imbalances

Roa *et al.* studied the content of DNA in 112 samples of GBC from Chile, by flow cytometry (86). They found aneuploidy in 26% of cases (29/112) and this abnormality was associated with lymphatic tumor involvement: 73%, 16/22, in aneuploid tumors versus 48%, 22/46, in diploid tumors ( $P=0.05$ ).

### Epigenetic alterations

Although less frequently studied, epigenetics has a relevant role in gallbladder carcinogenesis (87). DNA methylation is the most commonly reported event of epigenetics in GBC (59). García *et al.* in Chile published a series of 102 samples with distinct evolutionary stages (cholecystitis, metaplasia, GBC) (88). They found a significant relationship between survival and methylation of *MGMT* and *DCL1* genes, with better survival for the first case ( $P=0.006$ ) and poor prognosis for the second ( $P=0.04$ ). They also showed a progressive methylation index from cholecystitis to advanced GBC in *DAPK1*, *DLC1*, *TIMP3*, and *RAR beta2* genes ( $P\leq 0.05$ ) indicating that aberrant hypermethylation of promoter regions is an early, progressive and cumulative event in gallbladder carcinogenesis. The methylation status of some individual genes could be utilized as a biomarker with potential clinical applications in diagnosis or prognosis of GBC if they are validated in a greater number of samples.

### MSI

High MSI (MSI-H) has been reported in 6/59 (10%) GBC samples from Temuco, Chile, with equal distribution between early and late stages (89). It was also found in 2/6 (33%) samples with metaplasia and 5/6 (83%) with dysplasia. Similarly, one study from Japan found MSI in 9/30 (30%) samples with cholecystitis, 7/17 (41%) with carcinoma, 5/15 (33%) adjacent non-neoplastic mucosa (90). It seems that MSI is an early event in GBC carcinogenesis. This requires confirmation in larger series.

### Screening and prophylactic cholecystectomy (PC)

Therapy for metastatic GBC is minimally effective. Prevention (primary and early detection) is the ideal strategy to decrease the rising death rates from this fatal tumor. Prevention requires a thorough evaluation of risk factors and identification of high-risk asymptomatic subgroups of patients in whom PC could be beneficial.

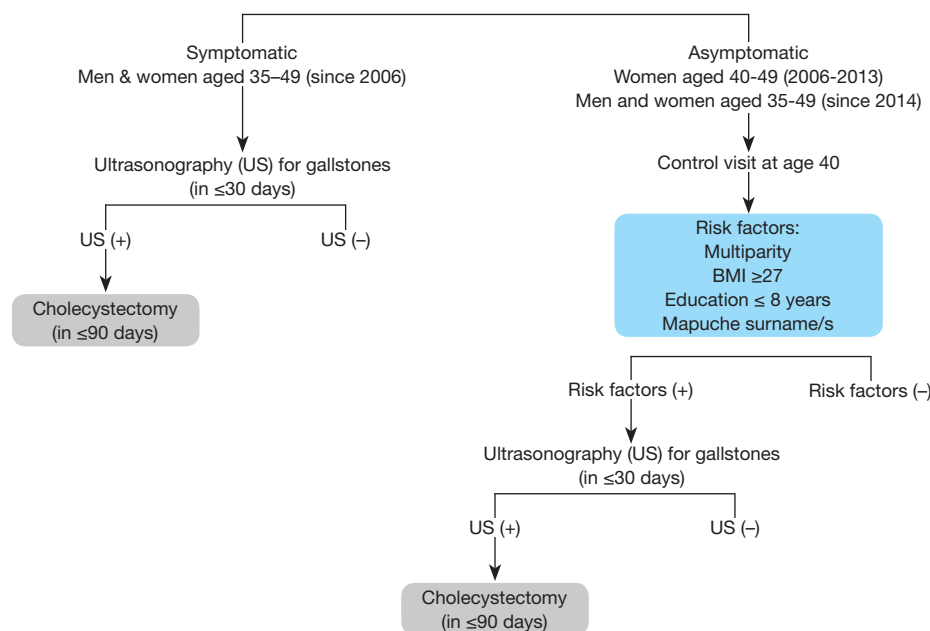
The serum markers CD34, Ca15-3, MIB-1, CEA, and Ca19-9 have been tested for the early diagnosis of GBC (91-93). Although some of them can contribute to the diagnosis of suspicious cases and the prognosis of GBC, the very low specificity precludes their utilization for early diagnosis. Even more, none of these serum markers has been validated for this purpose in a clinical setting.

Cholecystectomy avoids the development of GBC (primary prevention) and treats GBC at the early stages when it is found incidentally (secondary prevention). Indeed, five-year survival rates for mucosal (T1a) and submucosal (T1b) invasive GBC are 94% and 89%, respectively (94,95). Also, two series from SA described five-year survival rates of 50% and 53% for incidental GBC overall (96,97). Therefore, cholecystectomy in patients at risk is a potential strategy for prevention.

Several studies have shown a correlation between rates of cholecystectomy (the majority for treating symptomatic gallstone disease) and GBC incidence or mortality (98-100). Chianale *et al.* (99) studied a possible correlation between the GBC mortality and the cholecystectomy rates during a decade in Chile. The results suggested that changes in the number of cholecystectomies performed in a specific geographic area would reciprocally change the death rate from GBC. They estimated that an increase in the number of cholecystectomies by 12,500 per year would decrease the death rate from this cancer by approximately 1.0 per 100,000 people in Chile.

Alexander *et al.* (100) studied all patients diagnosed with GBC in the area of the Dutch Eindhoven Cancer Registry between 1975 and 2008 ( $n=659$ ). They observed that the incidence ASR of GBC in the south of the Netherlands has declined drastically along these three decades, and concluded that this is probably associated with a rise in the number of cholecystectomies during those years.

Diehl *et al.* (101) analyzed GBC mortality and its relationship with cholecystectomy rates. The data was taken from the national health statistics of England and Wales, Scotland, EEUU, Sweden, Canada, and Australia during 1967-1977. Trends in GBC mortality were inversely



**Figure 7** Screening with ultrasonography and cholecystectomy in Chile (EHG program) (109). Data source: adapted from the Chilean guidelines for GBC prevention.

associated with trends in cholecystectomy rates, the only exception being women in Canada.

All these studies have been performed in low-risk populations and do not have cost-effectiveness analyses. They did not compare GBC mortality in patients with asymptomatic gallbladder stone disease who underwent cholecystectomy versus those who did not. Also, although gallstone disease is the main risk factor for GBC many others can determine variations in the incidence and the mortality. Therefore, the evidence that these studies generate is weak to establish cholecystectomy as a standard procedure for the prevention of GBC. Even more, studies based on decision analysis models concluded that the role of cholecystectomy in asymptomatic patients for the prevention of GBC (PC) was not better than the current recommendation for expectant management of gallstone disease (102-105).

A way to improve the results of PC for the prevention of GBC is to select a population with other risk factors that in addition to gallstone disease or because of their intrinsic role, confer a higher risk of GBC. Some of these factors are polyps larger than 10 millimeters (106), porcelain gallbladder (29), stones larger than three centimeters (21), ethnic groups like Mapuches (45), and people living in areas at high risk for GBC (33-45). However, the evidence to

support the use of PC in these subgroups of patients is not sufficient.

In Chile, where GBC is the second leading cause of death from cancer in women, there is a national program, named explicit health guarantees (EHG) which utilizes PC intending to decrease mortality from GBC (107). The rationale is to remove the target organ at an age when the prevalence of lithiasis is high and considering that the latency period between acquisition of gallstones and cancer is estimated in 7 to 8 years (108). Screening with ultrasonography is performed in the following groups in the general population (Figure 7):

- ❖ Men and women, age 35 to 49 years, with symptomatic lithiasis.
- ❖ Men and women, age 35 to 49 years, asymptomatic, with any of the following risk factors: multiparity, body mass index equal to or greater than 27, less than 8 years of formal education, or Mapuche ethnicity that includes at least one surname corresponding to this genealogical origin.

If the ultrasonography reveals gallstone disease the patient undergoes PC. If there is no lithiasis, the patient is not operated.

Since the implementation of the EHG program in 2006, the number of cholecystectomies has progressively increased

(55,665 hospital discharges due to cholecystectomy in 2006; 75,882 in 2012). However, the age-adjusted mortality rate of GBC, including the age group targeted by the intervention, decreased before the plan started, from 12.57 per 100,000 inhabitants in the year 2000 to 8.17 in 2012 (109). This trend may be due to other factors like improvements in environmental and socio-economic sanitation. Problems observed with the EHG program are the following: it has not yet achieved an acceptable level of cholecystectomies, has not included women over 50 years old (a subgroup with very high risk), and has not prioritized patients from regions with the highest prevalence of GBC, which are in the South of Chile (109,110).

In spite of this, some cost-effectiveness studies and statistical modeling suggest that the strategy of programs like EHG would be effective (decrease in GBC mortality) at an acceptable incremental cost (111). PC has the additional benefit of preventing the inflammatory and infectious complications of gallstone disease (acute and chronic cholecystitis, acute pancreatitis, obstructive jaundice) with the consequent reduction in the associated morbidity and mortality and the socio-economic impact they have (112). However, other cost-effectiveness analyses reached different conclusions. Kapoor *et al.* (111) performed this type of study in the state of Uttar Pradesh in India, which has a population of two hundred million and a prevalence of gallstone disease of four percent (eight million patients). If one center has to perform five PC daily over two hundred working days per year, eight hundred such centers working for ten years would be required to treat every patient in the country. With a 0.5% risk of bile duct injury, this could result in forty thousand bile duct injuries.

## Discussion

The patterns of distribution of risk factors to GBC are different worldwide and by regions. This is the case for Argentina wherein GBC mortality ASR for the entire country in women and men was 2.2 and 1.9 respectively in 2017 constituting the tenth and the fourteen leading cause of death from cancer in women and men, respectively. But in the northwestern provinces of Jujuy and Salta, it was much higher (6.6 and 7.6 in women and men, respectively) with GBC reaching the third place in women and the fourth in men in the leading causes of death from cancer. Argentina is a very extensive country with important differences in ethnicity, environment, cultures and socioeconomic status among its distinct regions. This, in turn, produces an

important geographic variation in the prevalence of risk factors. For instance, Mapuche ancestry, a risk factor for GBC, is much more prevalent in the province of Neuquen where the GBC mortality ASR in women is 6.9. Although not proven, it is possible that migration from Bolivia (the country with the highest GBC mortality in SA) to the northwestern provinces of Salta and Jujuy in Argentina has been introducing a genetic risk factor. Also important, Salta and Jujuy are among the poorest provinces in Argentina. Indeed low socioeconomic status is a risk factor for GBC probably because the low access to health services contributes to longstanding gallstone disease, a well-known risk factor for GBC. Another risk factor that is distributed not homogeneously is the consumption of red chili pepper, which is more prevalent in regions from the western countries of Chile, Bolivia, and Peru, where mortality due to GBC is the highest in SA. The wide geographic variation in the incidence and mortality of GBC in SA requires that epidemiological analyses be carried out by regions and not by countries. This would allow the identification of areas at high risk for GBC where public health programs and resources must be applied. Examples of these regions are the western provinces of Argentina, some southern regions of Chile and the area near Trujillo city in Peru.

As cancer is a multifactorial disease further research is required to find new risk factors that explain the particular distribution of GBC in SA, and contribute to the implementation of preventive measures. Also as GBC has particular risk factors that are not shared by other biliary cancers (ICC, ECC, and AVC), the anatomic origin of biliary tract cancer has to be taken into account in registries as well as in molecular and clinical studies, where GBC should be analyzed in a separate way

In Argentina, GBC mortality ASR has been decreasing from 2002 to 2017, more in women. This could be related to the increment in the number of cholecystectomies due to gallstone disease along those years since 1 to 3 percent of these operations find an incidental GBC (23-25). However, a decrease in other risk factors may have contributed to the observed decrement in GBC mortality as well.

Aging is a risk factor for GBC. Also, analysis of the data mentioned above, suggests that people with GBC in high-risk regions are younger in comparison to patients with GBC in other areas. If this is confirmed in larger series it would suggest a genetic predisposition in areas at high risk, since it is known from other tumor models that cancer cases with genetic predisposition affect people at a younger age (112).

Gallstone disease is the main risk factor for GBC (70



to 90 percent of the cases in high-incidence regions from SA). Fortunately, only 0.5 to 4 percent of the patients with lithiasis develop GBC and other risk factors also contribute to the susceptibility to this disease. The carcinogenesis of GBC is a multistep, multifactorial process (113), which has to be explored in-depth in order to identify novel risk factors that permit to stratify individuals according to their GBC risk and improve the efficiency of prevention strategies. The current prevention programs rely on the PC offered to patients with gallbladder lithiasis. However, even if additional risk factors like sex, obesity, Native American ancestry, multiparity, and educational level are considered, the cost-effectiveness of PC for the general population is questionable. To optimize the prevention of GBC in regions of high and low incidence, a team of European-Latin American researchers and clinicians from Argentina, Bolivia, Chile, Peru, France, Norway and the UK led by Prof. Dr. Lorenzo Bermejo in Germany aim to conduct an ambitious research project. The final objective is to investigate the potential of circulating biomarkers (small non-coding RNAs among others) through the model of GBC carcinogenesis represented by the sequence of normal gallbladders with stones and inflammation, low-grade dysplasia, high-grade dysplasia, in situ carcinoma and invasive carcinoma. The underlying hypothesis is that each step of GBC development is reflected in unique patterns of circulating biomarkers, leading to more efficient strategies for GBC prevention. Patients with a high individual risk of developing GBC should be prioritized for undergoing PC.

The heterogeneity of molecular biology reports of GBC probably is due to the different evolutionary steps of carcinogenesis at which studies were performed, the low number of cases, the different methods for analysis and the mixing of biliary tumors from the different anatomic locations. Also, GBC, ICC, ECC, and AVC have different molecular abnormalities (57). In order to comprehensively study the molecular alterations of GBC in SA, ILOGI and Phoenix Mayo Clinic are analyzing 58 samples from high-risk areas in Argentina in more than 150 genes. The findings will be correlated with clinical characteristics and survival. Since the prevalence of risk factors is distinctive in regions of SA with high-risk for GBC it is possible that molecular abnormalities are different in these areas in comparison to other regions of the world.

In conclusion, GBC is a significant problem for public health in western regions of SA. The treatment of GBC is not effective and GBC prevention constitutes the most efficient strategy to decrease mortality. PC is a promising

preventive measure but its cost-effectiveness has to be optimized. The future identification of novel biomarkers of GBC risk combined with established risk factors will permit effective implementation of GBC prevention programs to decrease the high mortality of GBC observed in western SA.

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## Footnote

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

*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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