Review Article

Gallbladder cancer: South American experience

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Abstract: Large differences in terms of incidence and mortality due to gallbladder cancer (GBC) have been reported worldwide. Moreover, it seems that GBC has unique characteristics in South America. We surveyed the literature looking for information about the epidemiology, basic and translational research, and clinical trials performed in South America in order to critically analyze the magnitude of this health problem in the region. Compared to other geographic areas, age-standardized mortality rates (ASMR) for GBC in women are very high, particularly in many western areas of South America. Genetic, as well as dietary and environmental factors likely contribute to the pathogenesis of this disease in the area. Compared to other regions the profile of abnormalities of key genes such as *KRAS* and *TP53* in GBC seems to slightly differ in South America, while the clinical behavior appears to be similar with a median overall survival (OS) of 6.5 to 8 months in advanced GBC. In contrast to Europe and USA, prophylactic cholecystectomy is a common practice in western areas of South America. GBC particularly affects women in South America, and represents a significant public health problem. It appears to have peculiarities that pose an urgent need for additional research aimed to discover risk factors, molecular events associated with its development and new treatment options for this lethal disease.

Keywords: Gallbladder cancer (GBC); South America; epidemiology; pathogenesis; genetic abnormalities

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Introduction

Gallbladder cancer (GBC) is a common disease in South America (1). It is possible that risk factors, molecular abnormalities and clinical behavior are different in comparison to GBC diagnosed in other regions. We present an overview of the situation of this disease in the area where we reside. Clinical characteristics, risk factors, genetic abnormalities and treatment approaches are described in order to picture the magnitude of this problem.

Epidemiology

Biliary tract cancers (BTC) include GBC, intrahepatic-cholangiocarcinoma (CC), extrahepatic-cholangiocarcinoma (EHCC) and some forms of ampullary cancer. Among them, GBC is the most prevalent cancer type in South America. Its incidence and mortality rates vary among areas; even within the same country remarkable differences in terms of mortality rates have been observed (1,2). Cancer registries are not well developed in some countries of South America, and in these cases mortality rates constitute an alternative to indirectly obtain information about the magnitude of this health problem. Furthermore, utilizing this approach is reliable because GBC is a highly lethal disease; therefore mortality rates can equalize incidence. This notwithstanding, caution is warranted because mortality

Table 1 GBC and extrahepatic cholangiocarcinoma ASMR and ASIR among women in South American countries[§]

Country	Region	Geographic location in South America	ASMR ($\times 10^5$ women/year)	ASIR ($\times 10^5$ women/year)
Argentina	Jujuy	West	8.9	—
Argentina	Misiones	East	1.5	—
Brazil	Country	East	1.7	—
Uruguay	Country	East	4.1	—
Peru	Trujillo	West	—	7.5
Ecuador	Quito	West	—	7.4

[§], incidence rates equalize mortality rates; the majority of cases correspond to gallbladder cancer; ASMR, age-standardized mortality rates; ASIR, age-standardized incidence rates; GBC, gallbladder cancer.

rates due to GBC may be underestimated as well. In fact, in many South American countries the death certificates, which are the source for obtaining mortality rates, do not show that cancer (any type) was the cause of death. An example of such situation is reflected by the data on age-standardized mortality rates (ASMR) from Argentina *vs.* USA. In the former the ASMR due to cancer in general was 88 and 131/10^5/year for women and men, respectively (2). While in the later the ASMR were 145 and 208/10^5/year for women and men, respectively (3). This big difference in terms of ASMR can be explained, at least in part, by sub-registration of death from cancer.

Hepatobiliary tumors fall into two categories of the International Agency for Research on Cancer (IARC) report. Intrahepatic-CC and liver cancer are included in the ICD-10 C22 category, whereas GBC and EHCC in the ICD-10 C23-24. Intriguingly, ampullary cancer still remains unclassified in this report. In South America, the ASMR for tumors included within the category ICD-10 C22 are similar or below to that reported for other parts of the world (1). On the contrary, the geographic distribution of ASMR data for tumors belonging to the ICD-10 C23-24 category, in which GBC accounts for the majority of deaths, is substantially different. Furthermore, important variations in terms of ASMR in women have been found among South American countries (*Table 1*).

In Chile, and according to official statistics (4), a total of 23,716 deaths caused by GBC and EHCC have been reported for the period 2000–2012; among them 72.3% were women. Median ASMR was 15.04/10^5 women/year [interquartile range (IQR) 4.56)]. The highest ASMR were registered in the southern regions of Los Ríos (24.26; IQR 5.64), Los Lagos (23.69; IQR 4.14) and Aysén (18.29; IQR 7.78); while the lowest ASMR were described in the metropolitan region of Santiago de Chile (11.40; IQR 3.52) at the center, and Tarapacá at the north of the country (11.42; IQR 8.37). GBC and EHCC were the second leading cause of death from cancer in women in the whole country. On the other hand, the median ASMR for Chilean men was 5.52 (IQR 1.73), and the region of Aysen showed the highest ASMR (8.83; IQR 6.91).

In Brazil (5), GBC and EHCC accounted for 16,808 deaths in the period 2007–2012, and the majority corresponded to women (66.8%). The median national ASMR was 1.79/10^5 women/year (IQR 0.19). The highest ASMR were registered in the southern region of Rio Grande do Sul (2.08; IQR 0.16). Despite of this GBC and EHCC do not fall within the ten most common type of cancer causing deaths in every regions of the country, in both genders.

In Uruguay (6), at the southeast of South America, there were 1,058 deaths due to GBC and EHCC during the period 2006–2010, and 63.1% of them were women. The national ASMR for that period was 4.1/10^5 women/year.

Peru and Ecuador located at the central-western area of SA are two countries in which incidence rates are available. Age-standardized incidence rates (ASIR) were 7.5/10^5 women/year in Trujillo (Peru) and 7.4 in Quito (Ecuador) (7).

In Argentina, GBC and EHCC accounted for 5,262 (1.5%) of all cancer deaths in the period 2007–2011 (2). Out of them 3,806 (72.3%) occurred in women. The country overall ASMR was 2.5/10^5 women/year [95% confidence interval (CI), 2.4–2.5)] and 2.1/10^5 men/year (95% CI, 2.0–2.1). GBC and EHCC are among the ten most frequent (8^{th}) cause of death from cancer in women, but not for men.

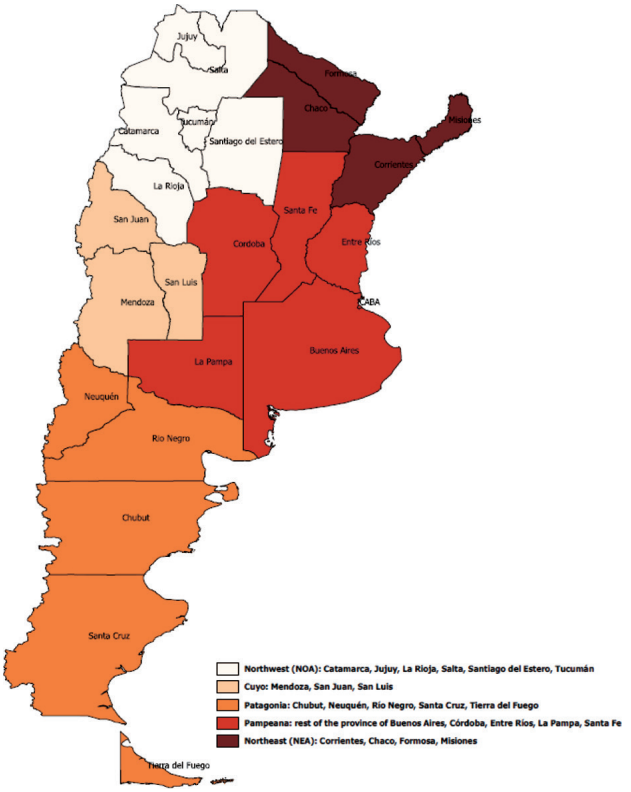

Figure 1 Regions and territories of Argentina.

The territory of Argentina can be divided in five geographic regions (*Figure 1*) which exhibit particular ethnical, political, historical, socio-economical characteristics, as well as differences in term of access to health care (8). Interestingly, the ASMR because GBC and EHCC varies between these regions (*Table 2*, *Figure 2*). The highest ASMR have been determined for the Northwest (NW) and the Patagonia regions, while the lowest values were estimated for the Northeast (NE) and Pampeana regions on the east side of the country.

In the NW region, Jujuy showed an ASMR for women of 8.9 (95% CI, 7.4–10.3). Similarly, in the province of Salta the ASMR for women was 7.0 (95% CI, 6.0–8.0), occupying the third place among the leading causes of death from cancer in women in both provinces. In the province of La Rioja ASMR for women was 4.4 (95% CI, 3.1–6.0) being the fifth leading cause of cancer death in women.

In the province of Neuquén (Patagonia region) the ASMR for GBC and EHCC was 4.6 (95% CI, 3.5–5.8), placing these tumor types at the 8th leading cause of cancer death in women. In Rio Negro, belonging to the same region, women ASMR was 4.1 (95% CI, 3.2–5.1), reaching the 7th place in the leading causes of death from cancer in women.

The lowest ASMRs for GBC and EHCC in women (1.4) were registered in the provinces of Entre Rios and Buenos Aires, and the Autonomous City of Buenos Aires itself (CABA), all of which belong to the eastern Pampeana region.

In summary, the data about mortality rates due to GBC indicate that the incidence of this tumor type in South America is distinct in different geographic areas. While in the eastern countries, like Uruguay and Brazil and the NE region of Argentina the data on ASMR due to GBC are similar to those of the United States of America and Western Europe (9), in Chile and western areas of Argentina the same measurements yielded higher values. In western countries such as Peru and Ecuador ASIR (which equalizes ASMR) were high. These data suggest that there is a widely extended territory near the Andes Mountains in which people are at high risk of developing GBC.

Etiology and risk factors for GBC in South America

The pathogenesis of GBC is not completely elucidated; however it is known that genetics and environmental factors play important roles in the initiation, promotion and progression of the disease (*Table 3*).

Non-manageable risk factors

GBC mortality increases with aging. In Argentina, in the period 2007–2011 the number of deaths in women was 70, 340, 456 and 547 in the 40–44, 55–59, 64–69 and 75–79 year groups respectively; in men deaths due to GBC were 31, 232, 367 and 415 in the same age groups, respectively (2). In the USA, in the period 2008–2012, a similar distribution of deaths due to GBC has been observed in both sexes (3). We could speculate that this epidemiological characteristic responds to the accumulation of hits which are required by the process of carcinogenesis.

As mentioned before this tumor type is more prevalent among women, and it has been proposed a link between this feature and the estrogen levels. Females having early menarche, late menopause, multiple pregnancies and childbirths appear to have an increased risk of developing GBC according to a case-control study from India (19). Interestingly, estrogens increase the formation of gallstones, mainly by elevating biliary cholesterol (20). This could

Table 2 Women ASMR from gallbladder cancer and extrahepatic cholangiocarcinoma in Argentina during the period 2007–2011

Territory	Cases	ASMR ($\times 10^5$ women/year)	95% CI	Regional median ASMR
NW				
Jujuy	161	8.9	7.4–10.3	4.4
Salta	206	7.0	6.0–8.0	
Catamarca	41	2.8	2.4–4.7	
La Rioja	41	4.4	3.1–6.0	
Tucumán	169	3.8	3.2– 4.4	
Santiago del Estero	86	3.9	3.1–4.8	
Cuyo				
San Juan	90	4.0	3.2–5.0	4
San Luis	52	4.0	3.0–5.3	
Mendoza	184	2.8	2.4–3.3	
Patagonia				
Neuquén	65	4.6	3.5–5.8	3.8
Río Negro	75	4.1	3.2–5.1	
Chubut	45	3.4	2.4–4.6	
Santa Cruz	19	3.6	2.1–5.7	
Tierra del Fuego	9	4.0	1.8–7.7	
Pampeana				
Buenos Aires	1,258	1.4	1.9–2.1	1.6
CABA	303	1.4	1.2–1.6	
La Pampa	26	1.8	1.1–2.7	
Córdoba	390	2.7	2.4–3.0	
Santa Fe	278	1.9	2.1–5.7	
Entre Ríos	78	1.4	1.1–1.8	
NE				
Corrientes	73	2.5	1.9–2.5	2.3
Chaco	85	3.2	2.5–4.0	
Misiones	38	1.5	1.1–2.1	
Formosa	26	2.1	1.3–3.0	
Country total	3,798	2.5	2.4–2.5	2.5

ASMR, age-standardized mortality rates; CI, confidence interval; CABA, Autonomous City of Buenos Aires; GBC, gallbladder cancer; NW, Northwest; NE, Northeast.

explain, at least partially, why GBC is more prevalent in women.

The contribution of the genetic background as predisposing factor for GBC emerges from the analysis of migratory phenomenon from high incidence areas in Chile and Bolivia to Argentina. The NW region in Argentina shares

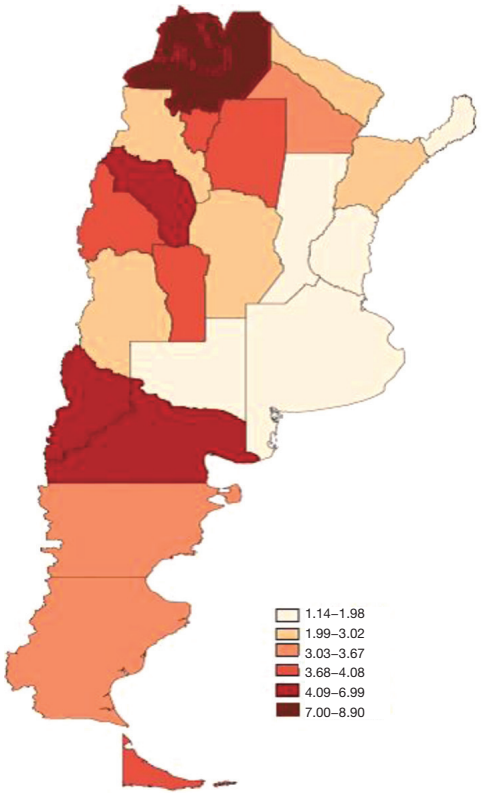

Figure 2 Age-standardized mortality rates (ASMR) from gallbladder cancer (GBC) and extrahepatic cholangiocarcinoma among women (aged 1–77 years) in different regions of Argentina during the period 2007–2011.

borders with the Chilean region of Antofagasta, and GBC mortality rates are similar. The original natives belonged to the Omaguacas, Atacamas and Diaguitas tribes, who have particular cultural, social, dietary habits and obviously specific genomic characteristics. Some of them changed dramatically after the Spanish conquest, but resilience processes in valleys, however, could preserve them to certain extent. Furthermore, this ethnic reality has been maintained until today by permanent migrations from Bolivia to Argentina, to the point that today about 350,000 Bolivians and their natural descendants (Argentinean) live in Argentina, mostly in Jujuy, Salta and Buenos Aires (21). Similar situation is observed in Patagonia, another high incidence region in Argentina, where the ancestral Pewenches were invaded by Mapuches, coming from the south of Chile, establishing a bidirectional population flow that persists nowadays (22). Therefore, and given the historic and demographic evidences connecting populations

Table 3 Risk factors for GBC in South America

Non-manageable
Age (2,3)
Female Gender (2,4,7)
Genetic background (10-13)
Manageable
Gallstone disease (14)
Red chili pepper consumption and aflatoxin (14-17)
Very low socioeconomic status (14)
Typhoid disease (11,12,18)

GBC, gallbladder cancer.

at both sides of the Andes, it is tempting to speculate that genetic background common to those persons predispose them to GBC development. Of course, thorough investigations are needed to verify this hypothesis, but a recent study on the genetic variants of the Arsenic^{+++} Methyl Transferase gene (*AS3MT*) provided the first evidence of human adaptation to a toxic chemical (23). It is well known that chronic arsenic poisoning may lead to skin cancer, and this study revealed that there are, among the population residing in the Andean area of Province of Salta, women carrying certain haplotype of the *AS3MT* gene, involved in arsenic metabolism, that protect them from the toxic effects of arsenic. Additional evidence for the notion that genetic factors may contribute to GBC development comes from a case-control study in a Chilean population about lipid metabolism-related genes. In fact, the rs693 polymorphism of the Apolipoprotein B gene and the rs708272 polymorphism of the Cholesteryl-Ester-Transfer-Protein gene were associated with an increased risk of GBC (OR 5.04; 95% CI, 1.43–17.8) (10).

Manageable risk factors

Cholelithiasis which leads to chronic cholecystitis remains as the most important risk factor for the disease worldwide. Gallstones are present in 45–100% of the cases (24), and mucosal changes from cholecystitis, hyperplasia, metaplasia, dysplasia, to carcinoma correlate positively with the weight, and size of the stones (25). The association between gallstone disease and GBC is even stronger if this has a longstanding history as shown by Serra *et al.* in a case-control study of 228 persons from Chile. They reported

Table 4 Frequent genetic abnormalities in GBC

Chromosomal (31,32)
Gains: 7p, 7q, 8q and 17q
Loses: 3p, 9p, 10q, 11p, 17p and 21p
Structural aberrations: del(3)(p13), i(5)(p10), del(6)(q13), del(9)(p13), del(16)(q22), del(17)(p11), i(17)(q10), del(19)(p13) and i(21)(q10)
Molecular
Oncogenes: *K-RAS* (33-35)
Tumor suppressor genes:p16 (36), FHIT (37), TP53 (34,36,38,39)

GBC, gallbladder cancer.

that 15% of cancer cases had a history of gallstone disease longer than 24 years compared to only 4% among control cases (OR 11; 95% CI, 1.4–85.2) (14).

In the case-control study mentioned above Serra *et al.* also found that red chili pepper consumption was associated with GBC (together with very low socioeconomic status and longstanding gallstone disease). The association resisted the statistic challenge by multivariate analysis with ORs of 3.2 (95% CI, 1.7–5.9). Since this is a retrospective analysis it is subjected to biases, including recall bias, a common limitation when using food frequency questionnaires. In support of that finding other studies reported an association of chili pepper consumption with stomach, liver and esophageal cancer (26-28). Furthermore, in the search for the potential etiological factor related to this dietary habit, several investigations have shown elevated concentration of aflatoxin in red chili peppers consumed by populations having high GBC incidence in the Andean areas of Chile, Bolivia and Peru (15,16). Nogueira *et al.* (17) also analyzed this association by performing a case-control study including 112 Chilean men and women (not distinguishing gender), and they found that patients with GBC had higher levels of aflatoxin B1-DNA adducts in peripheral blood than healthy control individuals (OR 13.2; 95% CI, 4.3–47.9) and control individuals with gallstones (OR 9.4; 95% CI, 2.8–37.2). In summary, there is evidence to consider red chili pepper, a frequently consumed food in some regions of South America, as a risk factor for GBC. This, in turn, could be mediated by the mutagen aflatoxin, a contaminant of red chili pepper. However, due to the limitations of the studies outlined before, additional research is required to confirm the association of red chili pepper consumption and GBC development.

Very low socioeconomic status was found associated to GBC in the study by Serra *et al.* described above (14), with a ORs of 6.3 (95% CI, 1.7–23.0) after multivariable analysis. It is conceivable that people with very low socioeconomic status have less access to the health care system and, therefore is more likely to have longstanding gallstone disease.

A study performed in Mexico and Bolivia showed that patients diagnosed for typhoid disease had a twelvefold higher risk of developing GBC (11) which is in agreement with studies carried out in USA and Asia (12,18).

Other possible risk factors are chronic biliary tract infection, diet, elevated body mass index, smoking or chewing tobacco and genetic factors (11,13,19,29).

In summary, age, gender (female) and the genetic make-up appear as important non modifiable risk factors for GBC, whereas cholelithiasis, typhoid disease, as well as consumption of red chili pepper contaminated with aflatoxin and very low socioeconomic status emerge as risk factors on which we may be able to intervene.

Molecular pathology of GBC

Castillo *et al.* (30) proposed that the malignant transformation of the gallbladder may occur through two alternative pathways (models): the sequential change from dysplasia to carcinoma and the adenoma-carcinoma model. The first postulates that the gallbladder epithelium undergoes metaplasia as an adaptive response to chronic irritation and inflammation. In fact, such change is found in approximately 50% of the patients with cholecystitis, and, eventually, on top of the metaplasia, the dysplasia may develop, which progress to carcinoma. The second model postulates that GBC originates through the malignant transformation of glandular tumors, such as benign adenomas.

Regardless the course of the disease, it is accepted that, like in the vast majority of neoplastic processes, genetic changes play a pivotal role in the development of GBC (31). The most frequent genetic abnormalities in GBC are depicted in *Table 4*. Classical cytogenetic studies performed on tumor samples of patients from low endemic areas, more than a decade ago, showed that the chromosome profile of GBC is complex. The tumors contain multiple clones, and the chromosome profile was characterized by gains of the chromosome arms 7p, 7q, 8q and 17q, and loses of 3p, 9p, 10q, 11p, 17p and 21p. Furthermore, several

recurrent chromosome aberrations were detected, including del(3)(p13), i(5)(p10), del(6)(q13), del(9)(p13), del(16)(q22), del(17)(p11), i(17)(q10), del(19)(p13) and i(21)(q10), suggesting that these regions contain genes involved in the development of GBC (32).

Roa *et al.* (40), performed studies aimed to detect genomic imbalances in GBC from Chile, and to find whether there is any association with the prognosis of the patients. Flow cytometry analysis showed that 24% of the tumors (29/120) were aneuploidy. However five years survival was not significantly worse among these patients compared to those with diploid tumors. Molecular studies has allowed to identify, among others, tumor suppressor genes, oncogenes, genes involved in DNA repair which play important roles in the pathogenesis of GBC. Furthermore, and based on the analysis of genetic abnormalities of different lesions in patients from Chile, it has been possible to propose a sequence of molecular events implicated in GBC development (30). Activation of the members of the RAS family of genes (*H-RAS*, *N-RAS* y *K-RAS*) is perhaps one of the most frequent events in human neoplasia. However the data on activating mutation of *K-RAS* in this tumor type are quite variable. For example, Pai *et al.* (33) reported that only two out 29 (7%) analyzed tumors of patients from USA exhibited *K-RAS* codon 12 mutations, while the frequency of mutation in tissue samples of patients from India and Korea was near to 50% and 20%, respectively (36,41). Interestingly, differences in the frequency of *K-RAS* mutation were also detected between patients from the NW of Argentina and from Bolivia; despite both populations subjected to genetic analysis belonged to the same geographic area. The study performed to identify the status of *K-RAS* in GBC patients from Bolivia showed that one out of 36 (2.8%) had activating mutations of the gene (34), while patients from the NW of Argentina exhibited higher frequency of this gene abnormalities. In fact, in a study including 58 patients of BTC, with a majority of GBC, 23 patients (39%) had amplification and 8 (17%) had mutation of the *K-RAS* gene (unpublished data). Even more, in 21 invasive GBC samples from Chile *RAS* mutations were found in only 2 (10%) (35). This variation among high risk areas suggests that environmental factors and the genetic background of the people may be related to such differences.

Tumor suppressor genes *FHIT* mapping on chromosome 3p14, *p16* on 9p21 and *TP53* on 17p13 are among the most frequently found altered in GBC, and this is consistent with the data about imbalances of their genomic regions. Kim *et al.* (36) have detected deletions of the chromosome region 9p21 and methylation of the promoter region of the *p16* gene in 48% of GBC. This correlated with loss of the protein expression in 50% of tumors, determined by immuno-histochemistry. Methylation in the promoter region of the *FHIT* and *p16* genes has been also examined by Roa *et al.* (37) in GBC samples of patients from Chile. Methyl-specific PCR showed that 30% of the tumors had abnormal methylation of the promoter region of the FHIT gene and 20% of *p16* gene promoter, and this was in agreement with an altered immune-histochemical pattern of their protein product. Considering the number of scientific papers available in the literature, *TP53* is the tumor suppressor gene most thoroughly investigated in this tumor type (38). The authors mentioned above demonstrated that the percentages of tumors with deletions of the locus (81%) and mutations of exons 6–8 (67%) are in agreement with the high incidence (66%) of overexpression of the mutated protein in tumor cells (36). The *TP53* gene status has been also the focus of genetic studies performed on samples from patients from Chile and Bolivia. Unlike to what happens to the *K-RAS* gene, the status of abnormalities of this gene is similar among high risk GBC regions. Mutations of TP53 were found in more than 50% of tumor samples from Bolivia and Chile. However, differences in the type of mutations were found, and despite of the fact that exons 5 and 8 were affected, only a few mutations occurred in hot-spot codons (34,39). In another study from Chile, Wistuba *et al.* (35), found that deletion of the *TP53* locus was an early event present in 58%, 85% and 91% of dysplastic tissue, carcinoma *in situ* and invasive carcinoma, respectively.

We have analyzed the expression of the KIT protein in GBC samples from our area (42). Contrary to the data obtained from a series of patients from USA (43), which showed expression of the protein in the majority of tumors, only three out of the 50 tumor samples we subjected to standard protein immuno-detection, exhibited positive reaction. Interestingly all three positive were poorly differentiated or undifferentiated tumors, raising the hypothesis that this metabolic pathway could be utilized by a subset of aggressive GBC.

Roa *et al.* (44) analyzed the presence of microsatellite instability (MSI) in 59 samples from patients with GBC and 22 with chronic cholecystitis from Temuco, a high risk area for GBC in Chile, and 6 out of the 59 (10%) samples showed high MSI. Interestingly none of chronic

Table 5 Data from surgical series of resectable GBC from South America

Reference	Country	N	T1[ǂ] (%)	Extended cholecystectomy (%)	CHT	Radiaton therapy	OS (%)
Manterola *et al.* (49)	Chile	40	20	100	Yes	Not	50
Lendoire *et al.* (50)	Argentina	24	4	100	Yes[£]	Not	53
González *et al.* (51)	Chile	95	28	52	Yes	Yes	42

[ǂ], TNM classification system of malignant tumors; [£], only for residual disease; CHT, chemotherapy; N, number of patients; OS, overall survival; GBC, gallbladder cancer.

cholecystitis cases exhibited MSI, whereas it was present in 83% (5/6) of surrounding dysplasia, in 33% (2/6) of surrounding intestinal metaplasia, and with equal proportion in early and advanced stages GBC samples, suggesting the hypothesis that mismatch repair deficiency is an early event in gallbladder carcinogenesis. In contrast MSI has been rarely found in samples from European GBC patients (45). Analysis of MSI as a sign of mismatch repair deficiency is even more interesting because this molecular abnormality has recently been found to be a predictor of impressive responses to anti PD-1 therapy, including patients with BTC (46).

The Gastrointestinal Oncology Latin-American Intergroup (ILOGI) analyzed the transcriptional level of different genes as predictive or prognostic factors in 54 BTC samples (including GBC, CC and ampullary cancer). Among others the mRNA level of FBXW7 a gene that encodes an ubiquitin ligase that interacts with oncoproteins, correlated with progression free survival (PFS) and overall survival (OS) in 19 patients (47). Median PFS was 4.9, 7.6 and 26.9 months for tumors in the lowest, middle and upper tercile of expression level. The corresponding median OS were 6.2, 8.8 and not reached. Mutational analysis of the *FBXW7* gene on 30 samples did not reveal structural anomalies (unpublished data). This is in contrast with a finding by Akhoondi *et al.* (48) who reported inactivating mutations in 7/20 (35%) non-South-American CCs. It would be interesting to expand these analyses to a higher number of cases in order to confirm *FBXW7* mRNA level as a prognostic factor in BTC, and to confirm if FBXW7 inactivating mutations have different prevalence in CCs from South America respect to tumors from other regions.

Given the much higher incidence of GBC in the Western area of South America, our results in tumors from this area reinforce the notion that particular environmental or genetic factors are leading to specific genetic alterations which may result in different molecular pathways.

Clinical experience and clinical trials in South America

In South America, as expected, the management of the patients with GBC has been also carried out by surgery, chemotherapy (CHT), radiotherapy or the combination of them. Consequently a number of studies, including clinical series as well as clinical trials have been performed aimed to gain insights into the different treatment options for GBC in South America (*Tables 5*,*6*).

Before describing the studies on early stage GBC one should keep in mind that the majority of early stage GBC cases are diagnosed as an incidental finding after a simple laparoscopic cholecystectomy. Under this scenario, it is recommended performing an extended cholecystectomy. This surgical procedure starts with an initial examination of the peritoneal cavity and the retroperitoneal lymph nodes. If no tumor is found it continues with a regional lymphadenectomy, a wedge resection of the gallbladder bed (usually including segments IVb and V of the liver), and resection of port sites. Supporting evidence for such aggressive approach comes from the facts that in tumors staged T2 (invasion without penetration of serosa) lymph node spread occurs in 48% of patients (56,57) and for stage T3 lesions (perforation of serosa or direct invasion to liver and/or one adjacent organ) the spread to lymph nodes occurs in 72% of patients (57).

Manterola *et al.* (49) treated 40 patients from Chile with extended cholecystectomy followed by CHT with 5-fluorouracil/leucovorin. According to the TNM Classification of Malignant Tumors (58), the depth of penetration of their tumors wereT1/T2/T3/T4 in 8/12/12/8 patients, respectively, and the 5 year OS was 50%. Similarly, Lendoire *et al.* (50) reported 53% 5 year OS among 24 GBC patients from Argentina, including 1 T1, 12 T2 and 11 T3, who were treated with extended cholecystectomy and CHT. A large retrospective study was

Table 6 Clinical trials and series on advanced BTC from South America

Reference	Country	Design	CHT	N (GBC/CC)	ORR (%)	mOS (months)
Gallardo *et al.* (52)	Chile	Prospective phase 2	Gemcitabine	25 (25/0)	36	7.0
Reyes-Vidal *et al.* (53)	Chile	Prospective phase 2	Gemcitabine; cisplatin	42 (42/0)	48	6.5
Carraro *et al.* (54)	Argentina	Prospective phase 2	Gemcitabine; cisplatin	8 (5/3)	62	NR
Arroyo *et al.* (55)	Argentina	Retrospective	Various	69 (54/15)	27	8[#]

NR, not reported; [#], only GBC patients; CHT, chemotherapy; N, number of patients; ORR, overall response rate; GBC, gallbladder cancer; CC, cholangiocarcinoma; OS, overall survival; BTC, biliary tract cancer.

recently presented by González *et al.* at the 2015 Latin-American Symposium of Oncologic Gastroenterology (SLAGO) in Viña del Mar (Chile) (51). These authors analyzed the outcome of 95 patients with GBC, including mostly very early stage tumors (27 T1, 34 T2 and 34 T3). Among them, only 49 patients (52%) were subjected to extended cholecystectomy and the remaining received simple cholecystectomy. Adjuvant CHT and 3D radiation therapy were utilized. Of the total, 42% of the patients were alive after 5 years. However, clear differences in term of OS were achieved by patients who received either extended or simple cholecystectomy, 55% of those who underwent the extended procedure were alive after 5 years, whereas only 29% of those who received simple cholecystectomy were alive after 5 years. Moreover, in this study the performance of the patients who underwent extended cholecystectomy was also evaluated in relationship with the number of lymph nodes examined. Five year OS was 67% for those patients who had four or more lymph nodes examined, whereas it descended to 47% among patients with less than four lymph nodes subjected to histopathology exam. Although the data were obtained retrospectively, they show the importance of extended cholecystectomy as the best treatment alternative for those cases with early stage GBC. Another important point that can be deduced from the analysis of the surgical series presented here is that adjuvant CHT after a complete resection of GBC is indicated in the majority of cases in South America. This is in agreement with the literature reporting that adjuvant CHT is utilized by 70% of the centers worldwide for the treatment of this type of cancer (59). However, since this decision is based on low level evidence it must be confirmed by the ongoing phase 3 trials (in Japan, France and the United Kingdom) which are testing the hypothesis that surgery and adjuvant CHT cure more patients than surgery alone.

Finally, it is important to mention that reports from the United States of America and other countries from Asia and Europe showed that the 5 year OS rates varied between 54–100% for T2, 16–63% for T3, and 8–25% for T4 disease (60), which are to certain extent comparable with the OS data from patients with early stage GBCs from South America. Therefore, we can assume that the treatment strategies established in our patients are well supported by the clinical evidence.

As regard to advanced GBC, several attempts to treat the patients with CHT have been performed. Gallardo *et al.* (52) carried out a phase 2 trial with first line gemcitabine in 26 patients with advanced GBC from Chile, and reported that an overall response rate (ORR, the sum of partial and complete responses) was obtained in 9/25 (36%) and that stable disease was achieved by 6/25 (25%) patients, while the median OS was 7 months. More than a decade ago, Reyes-Vidal *et al.* (53) presented at the 2004 ASCO Gastrointestinal (GI) Meeting results of a phase 2 trial with gemcitabine combined with cisplatin in the first line setting in 42 patients with advanced GBC from Chile. ORR was achieved by 20 out of the 42 (48%) patients, and median survival was 6.5 months. Similar treatment protocol was administrated to a group of 8 patients with locally advanced and metastatic GBC and CC from Argentina, and response was obtained in 5 of them (62%); however data about the OS was not reported (54). We have surveyed the clinical data from 173 patients with GBC and CC from the NW region of Argentina, attended by physicians' members of the ILOGI group. There were clear gender differences between the two clinical entities, GBC was more prevalent among women and CC was evenly distributed. Furthermore, GBCs were almost always detected at earlier stages than CC, which could be due to the fact that GBC is diagnosed as an incidental finding. More important, 69 patients (54 GBC and 15 CC) were assessed for their response to gemcitabine or cisplatin-based CHT, and overall response was obtained

in 16 out of 54 (30%) GBC patients and 3 out 15 (20%) CC patients. The median OS for the GBC patients was 8 months (55).

In these three trials and the retrospective series the ORR ranged from 27% to 62%. However, and despite the good response rates, the OS observed among these patients was dismal. Similarly, the OS in advanced GBC patients from other regions of the world is poor with no evidence of cure in any subgroup of patients (61,62). Therefore, new treatment strategies for advanced GBC are required. This could be accomplished by the identification of new targets and the appropriate therapy. In fact recent analyses in tissue samples from GBC in 100 patients found between 1 and 2 potentially targetable genomic alterations per tumor, among them *ERBB2*, *PIK3CA*, *CDKN2A/B* and *KRAS* abnormalities (63,64).

Since no single test for the early diagnosis has been shown to decrease mortality, it is reasonable to think that prophylactic simple cholecystectomy in a population at high risk for GBC in endemic areas could be beneficial, in particular for patients with gallstone disease who live in western South America. In fact, and in agreement with this notion, the Ministry of Health of Chile published a clinical guideline for the management of this type of patients, in which the use of prophylactic cholecystectomy in the population with gallstone disease, regardless of the presence of symptoms is highly recommended (65). In contrast, prophylactic cholecystectomy is not recommended in the majority of patients with asymptomatic gallstone disease except for select groups (66).

In summary, GBC is a frequent disease in many western regions of South America, with a dismal prognosis in the majority of patients. There is an urgent need of developing high quality cancer registries in these regions, to perform basic research and clinical trials, which can contribute to the better management of the patients.

Conclusions

GBC affects more frequently women in many western regions of South America. It represents a significant challenge to the public health system because this tumor type is among the leading causes of death due to cancer in these territories. It is likely that genetic, dietary habits and environmental factors contribute to the pathogenesis of this disease. Some differences in term of *RAS*, *TP53*, *FHIT* and *p16* genes abnormalities have been found in GBC from South America compared to tumors from other regions. However, and similarly to other countries, extended cholecystectomy, and eventually adjuvant CHT, is the standard procedure for the treatment of resectable tumors, while the combination of gemcitabine and cisplatin is frequently administrated as the first line CHT for the treatment of advanced GBC in South America. In contrast to other regions, prophylactic cholecystectomy is a common practice in many western areas of South America. Due to the high incidence and the dismal outcome of the patients with GBC comprehensive and high quality cancer registries and more translational research should be carried out in order to minimize the negative impact that this disease has among South Americans.

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Footnote

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