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A revuew of the histopathological disease profile of gallbladder specimens after cholecystectomy

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UNIVERSITY OF THE WITWATERSRAND

HISTOLOGICAL REVIEW OF GALLBLADDER SPECIMENS AFTER CHOLECYSTECTOMY

Is routine submission of all gallbladder specimens justified?

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A Dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfilment of the requirements for the Degree of Masters of Medicine in the branch of General Surgery

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DECLARATION

I, Dr T.T. Mahlobo declare that this dissertation is my own, unaided work. It is submitted in fulfilment of the requirements for the degree of Masters of Medicine in the branch of General Surgery to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. This research paper has not been submitted for any degree or examination at any other university.

Dr T.T. Mahlobo

_____

(Signature of candidate)

On this Wednesday, 21 February 2018, in Johannesburg, South Africa.

Master Neo K. Mahlobo: I hope you will one day understand why I did this and all our time it took away from us.

To my parents and family: thank you for the lessons on perseverance and determination.

Rebecca: thank you. And Pula.

ABSTRACT

Gallbladder cancer (GBCa) has a dismal prognosis, with poor short-term and long-term outcomes, even following surgery and all current adjuvant therapies. Routine submission of all post-cholecystectomy gallbladder specimens (GBS) for histopathology to detect cancer is standard practice at all University of the Witwatersrand (Wits) hospitals, as at many institutions globally. The cost-*in*effectiveness associated with the results adding no value to overall patient care is debated. The low reported rate of GBCa – between 0.27% and 3.6% of all GBS –prompted advocacy for selective GBS submission based on demographic, clinical, and macroscopic features as indications for evaluation, considered logical from a practical and cost-effective perspective, especially in resource-constrained healthcare systems.

Retrospective analysis of histopathology reports of 1194 adult GBS was performed. The histopathology findings of GBS submitted to the National Health Laboratory Service (NHLS) between January 1, 2010 and December 31, 2012 from three Wits hospitals were entered into spreadsheets, categorised into malignant, premalignant, and benign, and analysed, allowing determination of the profile of gallbladder disease. The frequency of GBCa determined, multivariate analysis of demographic and diagnostic subtypes was used to identify associations or risk factors for GBCa.

The mean age of adult patients was 46.62 years (standard deviation, 17.81; range, 34-87); 925 (77.5%) female and 269 (22.5%) male. Benign diseases were documented in 1159 (97.1%) adult GBS with acute and chronic cholecystitis, in 705 (59.04%) and 401 (33.58%) specimens, respectively, representing 92.6% of total GBS. Forty-five (4.43%) and 33 (2.7%) specimens were 'normal' and benign tumours, respectively. GBCa and premalignant diseases composed 20 (1.67%) and 8 (0.7%) specimens, respectively with incidental GBCa found in 7 (0.59%) of 20 GBCa cases. Surgeon's macroscopic appearance assessments were inadequately documented, so the value of this practice could not be determined. A small number (48) of GBS were obtained from paediatric patients <18 years of age where-in acute cholecystitis was most commonly diagnosed, no malignancies but one case of cytological atypia detected.

The GBS disease profile and incidence of GBCa in this study were consistent with reports from international literature. No single demographic or clinical factor was identified to guide the surgeon in being more selective in submitting GBS. However, with only 7 cases of incidental GBCa in 1194 adult specimens, the routine submission of all GBS specimens to rule out malignancy cannot be justified and is not cost-effective.

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NOMENCLATURE: LIST OF ACRONYMS AND ABBREVIATIONS

5-FU	5-fluorouracil
AIDS	Acquired immunodeficiency syndrome
AJCC	American Joint Committee on Cancer
BIN	Biliary intra-epithelial neoplasia
CHBAH	Chris Hani Baragwanath Academic Hospital
CIS	Carcinoma in situ
CT	Computed tomography
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
GBS	Gallbladder specimen(s)
GBCa	Gallbladder cancer(s)
HIV	Human immunodeficiency virus
HJH	Helen Joseph Hospital
MD-CT	Multidetector computed tomography
NCCN	National Comprehensive Cancer Network
NHLS	National Health Laboratory Service
RSA	Republic of South Africa
SD	Standard deviation
TNM	Tumour Node Metastasis Cancer staging system according to the American Joint Committee on Cancer
Wits	University of the Witwatersrand

1 INTRODUCTION

Cholecystectomy, generally considered to be optimal treatment for symptomatic gallbladder disease, is a common operation globally [(1)]. It is standard practice at the University of the Witwatersrand (Wits) academic hospitals to submit all post-cholecystectomy gallbladder specimens (GBS) for histopathologic examination. In practice, this means that all post-cholecystectomy GBS are examined by pathologists—regardless of the indication for the operation. Henderson *et al.* [(2)] estimated that 20 000 000 people in the United States of America (USA) have gallstones, and up to 40% of these individuals will require prophylactic or therapeutic cholecystectomy in their lifetime. This represents an extremely large number of cholecystectomies and an equally large number of GBS examined 'routinely', with no specific consideration by clinicians regarding the necessity for this investigation. To place this in context, the costs of over 700 000 cholecystectomies performed in the USA was estimated at $6.5 billion in 2005–2006 [(3)]. Further, improved imaging and detection of gallstones in recent years has increased the number of referrals for cholecystectomy, which at least partly accounts for the increased number of GBS submitted for histologic examination [(2)].

While histopathologic examination is the gold standard for diagnosing gallbladder disease and gallbladder cancer (GBCa), it rarely influences further treatment—surgical, chemotherapy, or radiotherapy—for these disorders. The utility and cost-effectiveness of the practice of routinely submitting all post-cholecystectomy GBS has been questioned [(4)]. The controversy centres on whether all GBS should be routinely submitted for histopathologic analysis, or whether a more selective submission approach should be employed [(5,6,7)]. If a selective approach is to be used, then the question arises regarding which criteria - demographic or clinical features - should be used to establish a submission protocol for surgeons.

Histopathologically, gallbladder diseases are categorised as benign, pre-malignant, or malignant. The diseases are listed below [3,4,8]:

i. Gallstone diseases
    - Asymptomatic (incidental findings)
    - Biliary colic (symptomatic gallstone diseases)

ii. Acute and chronic cholecystitis
    - Calculous or acalculous cholecystitis
    - Complicated cholecystitis
    - Xanthogranulomatous cholecystitis
    - Steatocholecystitis
    - Eosinophilic cholecystitis
    - Gallbladder opportunistic infections
    - Gallbladder diseases secondary to liver flukes and other parasitic infestations

iii. Cholesterolosis

iv. Gallbladder dysmotility disorders

v. Gallbladder polyps

vi. Gallbladder disease secondary to systemic diseases (e.g., vasculitis)

vii. Gallbladder malignancies

To understand the rationale that drives the practice of submitting all specimens for histopathologic examination, one must understand the spectrum of gallbladder diseases, including their prevalence, treatment options, and outcomes. This particularly applies to GBCa. GBS are submitted to avoid missing this malignancy. However, numerous reviews have continued to highlight the poor prognosis of GBCa, even with extended surgical resection and advances in adjuvant and neoadjuvant chemo- and radiation therapies. Various tools used for investigating GBCa, along with

its management options and associated outcomes, are discussed below based on a comprehensive review of literature.

Most patients with GBCa have inoperable disease at presentation, even when it is diagnosed intra-operatively as incidental GBCa. Hence, very few patients with GBCa are suitable for any attempt at curative surgery and/or curative adjuvant therapies [4]. Gallbladder malignancy has been reported in 0.5% to 1.7% of all GBS sent for histopathologic examination [4]. The estimated mean overall survival for patients with GBCa is between 6 and 12 months from the time of diagnosis, and the overall 5-year survival rate is 5% [8-13]. Recent studies on the incidence of GBCa and their recommendations regarding submission of GBS for histology are summarised in Table 1.1.

Table 1-1 Incidence of gallbladder cancer in previous publications

Study	Study Type	Study duration, years	No. of Specimens	No. (%) of GBCa*	Age, years**	Recommendation***
Barcia *et al*. (2004) [15]	Retrospective	5	802	5 (0.62%)	55.8	Selective submission
Darmas *et al.* (2007) [16]	Retrospective	5	1452	4 (0.27%)	68 (74 M, 63 F)	Selective submission
Oommen *et al.* (2007) [7]	Retrospective	5	976	1	NA	Selective submission
De Zoysa *et al.* (2010) [9]	Retrospective	1	477	4 (0.8%)	60 (68 M, 57 F)	Selective submission
Memon *et al.* (2011) [4]	Retrospective	3	282	4 (1.4%)	45	Selective submission
Chin *et al*. (2012) [17]	Retrospective	12	1375	7 (0.50%)	12–85 (52)	Selective submission
Soomro *et al.* (2013) [18]	Retrospective	2	521	19 (3.64%)	35–70 (55 M, 54 F)	Send all specimens
Total			11 249	44 (0.391%)		

* Number and percentage of gallbladder specimens with primary gallbladder cancer
** Mean or range (mean)
*** Some authors have recommended the selective submission of gallbladder specimens based on patient characteristics (age of at least 60 years and female sex), surgeons' assessment of intra-operative findings (suspicious gallbladder), and the specimens' macroscopic appearance (assessed by the surgeon intra-operatively). Some authors also consider pre-operative imaging features. Intra-operative frozen sectioning and biopsy are recommended in some instances.
Abbreviations: F, female; GBCa, gallbladder cancer; M, male; NA, not applicable; No., number
[4,7,9,15,16,17].

To avoid confusion and earlier noted ambiguity in the literature, the recommended nomenclature for clinicopathologic diagnostic categories of GBCa is shown in Table 1-2. This is based on clinical examination, imaging modalities, operative and post-operative findings, and/or histologic examination of GBS. The five GBCa diagnostic categories are obvious, suspected, unsuspected, incidental, and missed [14].

Table 1-2 Gallbladder cancer nomenclature

Recommended Terminology [14]	**Description**
Obvious	Clinical and radiologic diagnosis of GBCa is clear
Suspected	Ultrasound and/or CT shows focal irregular gallbladder wall thickening, single large sessile polyp, or gallbladder wall calcification (suspicious lesions)
Unsuspected (unexpected)	No clinical or radiologic suspicion of GBCa; GBCa is suspected during operation for presumed gallstone disease
Incidental	GBCa is not suspected at operation or on gross examination of the GBS; GBCa is diagnosed for first time on histopathologic examination of the GBS
Missed	GBCa (early-stage disease) is not diagnosed on routine histopathologic assessment but recurrence occurs within a few months
Abbreviations: CT, computed tomography; GBCa, gallbladder cancer; GBS, gallbladder specimen	

The consequences of the increased use of laparoscopic cholecystectomy, especially in relation to incidental GBCa, have been investigated. Behari and colleagues [14] found the rate of incidental GBCa to be 1% of all cholecystectomies (open and laparoscopic procedures combined), ranging from 0.5% to 2.1% for laparoscopic cholecystectomy. However, this area requires further research, as more complex surgery may result in an increase in the conversion rate from laparoscopic to open cholecystectomy in the setting of suspected or incidental GBCa.

The detection of GBCa and other rare, albeit treatable, gallbladder disorders, such as tuberculosis and neuroendocrine tumours, as an acceptable reason for routinely submitting all GBS for

histopathology in South Africa is at best speculative, as the gallbladder disease spectrum (disease profile) and relative incidence of these conditions in this country are unclear. Extrapolations are made from studies conducted in other parts of the world.

A number of GBS are submitted from the paediatric population globally and likewise in the research endeavour described later in this dissertation, thus a discussion of the disease profile in this group is necessary. The indications for cholecystectomy in the childhood population differ from those in adults. There is a negligible risk of malignant disease in the paediatric population; however, histology results are often included in literature based on specimen registry databases. According to Stinton *et al.* [8], paediatric gallbladder disease, which was previously thought to be rare, is being diagnosed with increasing frequency, with some risk factors similar to those of adults. In their discussion of various childhood gallbladder diseases, Svensson *et al.* [19] note their increasing prevalence and, in particular, the significant role played by obesity in this age group.

This dissertation presents a review of the literature on GBS in which GBCa was detected, followed by an analysis of GBS histopathology report data from Wits University affiliated academic hospitals. The histopathologic disease profile was determined for all GBS obtained at three Wits hospitals in Johannesburg, South Africa, between January 1, 2010 and December 31, 2012. This included both paediatric and adult age groups. The aim of the research was to identify any significant demographic or clinical factors and GBS macroscopic characteristics that suggest or correlate with a finding of malignancy. Such features, if identified, may assist surgeons to more selectively submit specimens for histopathologic analysis [5,6]. As only GBS with higher malignancy risk features would be submitted, this would translate into cost savings. The cost-effectiveness of this approach is discussed and counterarguments are presented.

Clinicians have a responsibility to eliminate unnecessary investigations and therefore associated costs without lowering standards of care or missing treatable pathology. The costs associated with

GBS assessment are not insignificant, and these costs are reviewed and discussed in relation to the results obtained from the analysis of data from Wits hospitals. Whitehead and colleagues state that allocation of resources for competing healthcare interventions requires thorough evaluation, and each intervention's impact on costs and health outcomes determines its utility and cost-effectiveness [20]. These resources include both financial and human resources (and time), the latter being somewhat challenging to quantify.

In this context, this paper seeks to answer the overarching question of whether the practice of routine histopathologic analysis of all GBS is justified. This required an analysis and overview of the profile of diseases diagnosed through histological analyses of these specimens, their relative frequency, available treatment options, and clinical outcomes. A review of GBCa and costs of GBS histopathologic analysis was also conducted. The implications of all these aspects are discussed. Three research questions (stated in the Research methods section) arising from the controversial practice of routinely submitting all GBS for histopathology are answered and discussed.

2 LITERATURE REVIEW

Symptomatic cholelithiasis is the leading indication for cholecystectomy [4]. The routine submission of GBS for histology is an effort to identify GBCa and other rare diseases, such as tuberculosis and neuroendocrine tumours, with the assumption that patients might benefit from further treatment modalities once diagnosed [3]. Firm diagnoses are often only possible through tissue histology, despite significant improvements in diagnostic imaging in recent years.

2.1 Adult Population

Symptomatic cholelithiasis is the leading indication for cholecystectomy in adults. Other indications, such as gallbladder polyps and tumours (benign or malignant), have been associated with biliary colic [4]. It has been argued that some benign gallbladder conditions have malignant potential and, thus, all specimens should undergo histopathologic analysis [1]. Moreover, it has been reported that cholecystectomy fails to relieve symptoms in 10% to 33% of patients [8]. This has led to the publication of multiple consensus papers recommending against unnecessary cholecystectomy [8]. Diagnostic imaging plays a pivotal role in increasing the accuracy of diagnosis. Some differential diagnoses rely substantially on histopathologic analysis of tissue, requiring operative procedures to obtain samples. Most pathologies, however, can now be accurately diagnosed using various advanced imaging techniques without the need for histopathologic examination.

An overview follows of the various adult population gallbladder diseases (benign, premalignant, and malignant), including their pathobiology, diagnostic work-up (in the setting of suspected GBCa), and treatment options.

2.1.1 Benign gallbladder diseases

Benign gallbladder diseases include the following [2]:

i) Cholelithiasis

ii) Acute cholecystitis

- Calculous and acalculous
- Complications of acute cholecystitis

iii) Chronic cholecystitis (calculous and acalculous)

- Chronic cholecystitis subtypes, such as xanthogranulomatous, eosinophilic, chronic follicular, and immune-mediated injury cholecystitis

iv) Complications of cholelithiasis and cholecystitis, including hydrops, mucocele, bile peritonitis, and gallstone spillage

v) Cholecystitis afflicting human immunodeficiency virus (HIV) - positive patients with acquired immunodeficiency syndrome (AIDS)

vi) Cholesterolosis

vii) Various other conditions, including parasitic infections, ischaemic cholecystitis, and chemical cholecystitis

2.1.1.1 Cholelithiasis

Gallstone formation depends on an intricate interplay between multiple factors; some factors are modifiable, whereas others are not. Modifiable risk factors include sedentary lifestyle, obesity, metabolic syndrome, smoking, and rapid weight loss. Non-modifiable factors include advanced age, female sex, ethnicity, insulin resistance, and family history/genetics [2,3,8]. Other risk factors include pregnancy, drugs (such as oral contraceptives and certain lipid-lowering drugs), total parenteral nutrition, and prolonged fasting or starvation states. Diseases such as Crohn's disease, cirrhosis, and haemolysis predispose to pigmented gallstone formation [8,21].

The aetiology of cholesterol gallstones is at least partly attributed to genetic/familial factors in 30% of patients [3]. Over 75% of gallstones are cholesterol stones, but there is no conclusive evidence of their association with high blood cholesterol levels [2, 3]. Non-cholesterol gallstones are associated with other conditions, including advanced age, chronic haemolysis, alcoholism, cirrhosis, pancreatitis, total parenteral nutrition, and Crohn's disease [2].

Stinton *et al*. [8] reported that gallstones will develop in 10% to 20% of the USA adult population, but will remain silent or asymptomatic in 80% of these patients. The risk of symptoms or complications is approximately 2% to 3% per annum (with a 1% to 2% per year risk of major complications) and approximately 10% by the fifth year [8]. While it is generally accepted practice to expectantly manage most patients with asymptomatic gallstones, some patients will benefit from prophylactic cholecystectomy [1]. Indications for prophylactic cholecystectomy include anomalous junction of the pancreatico-biliary duct with or without choledochal cyst, porcelain gallbladder, polyps with a diameter over 1 cm (especially if they are single and/or sessile), gallstones over 3 cm in diameter, and *Salmonella typhi* carriers; this last indication, however, is controversial [1]. See Table 2-1 for a list of these and other possible indications.

Table 2-1 Indications for prophylactic cholecystectomy

High-risk situation [1]	**Comment**
Large (>3 cm) gallstones or multiple stones (packed gallbladder)	Higher risk of developing gallbladder cancer
Sickle cell disease (pigmented gallstones)	Stone complications are often difficult to distinguish from sickle cell crises Complications are frequent and may be life-threatening
Solid organ transplantation (heart, lung, kidney, or pancreas)	Complicated gallbladder symptoms may develop, especially in the first 2 years after transplantation
Abdominal surgery (other reasons) or bariatric surgery for morbid obesity	Controversial in some centres

While cholelithiasis is common, it is common practice to remove gallstones from the GBS prior to submitting the specimen for histopathology. This can lead to underestimation of the frequency of stones reported in histopathologic specimens.

2.1.1.2 Acute Cholecystitis.

Acute calculous cholecystitis occurs in 20% of patients with symptomatic cholelithiasis, whereas acalculous cholecystitis is found in 10% of patients with acute cholecystitis [(2)]. Acalculous cholecystitis occurs most commonly in patients with significant co-morbid diseases who are admitted to the intensive care unit and in HIV-infected individuals [(2)]. Acute emphysematous cholecystitis (commonly found in diabetics) represents an advanced form of acute gangrenous cholecystitis and various other forms of acute cholecystitis [(2)].

2.1.1.3 Chronic cholecystitis

Chronic cholecystitis represents the final stage of recurrent episodes of acute cholecystitis. It is usually secondary to gallstones and has a risk factor profile and epidemiology similar to those of gallstone diseases [(2)]. Gallstones are not found in 12% to 20% of chronic cholecystitis specimens and factors thought to play a pathogenetic role in these situations include the following [(2)]:

i. Bile-induced mucosal irritation
ii. Gallbladder dysmotility
iii. Post-inflammatory stenosis
iv. Aberrant cystic duct anatomy
v. Immune-mediated injury
vi. Steatocholecystitis
vii. Chronic infection

Xanthogranulomatous cholecystitis is a subtype of chronic cholecystitis associated with gallstones, which has been found in 1.8% to 8.9% of specimens obtained during cholecystectomy [(2)]. It is characterised by multiple firm ill-defined yellow nodules that may appear concerning for malignancy but have not been firmly associated with an increased risk of malignancy [(2)]. A diagnosis of eosinophilic cholecystitis should prompt a search for parasitic infections, vasculitis, drug reactions, eosinophilic gastroenteritis, or Churge-Strauss syndrome [(2)].

2.1.1.4 Cholecystitis in HIV-positive patients

In a review of 107 gallbladder specimens from HIV-infected patients with AIDS, Henderson *et al.* [(2)] found the following:

- Inflammation was present in 99 specimens (92%)
- Acalculous cholecystitis was present in 72 specimens (73%)
- 46 specimens (43%) were associated with an opportunistic infection
- 22 specimens (21%) had one pathogen
    - Cryptosporidium: commonest infection (in bile duct or stool), noted in 12 patients (11.2%)
    - Cytomegalovirus: second most common pathogen, noted in 10 patients (10%)

Other factors contributing to GB disease in HIV-infected individuals include opportunistic infections complicating AIDS, such as *Mycobacterium avium-intracellulare* complex and *Mycobacterium tuberculosis, Pneumocystis jirovecii, Isospora belli, Cryptococcus neoformans, Cyclospora cayetanensis, Giardia lamblia,* and cytomegalovirus. These organisms cause severe gallbladder mucosal injury and ulceration [(2)]. Epstein-Barr virus has been implicated in tumour transformation of gallbladder wall smooth muscle cells in isolated reports [(22)].

2.1.1.5 Cholesterolosis and other conditions

Cholesterolosis has been detected in 9% to 26% of GBS and reflects an abnormal accumulation of lipid-laden macrophages in the lamina propria of the gallbladder [(2)]. It has no discernible relationship to cholelithiasis [(2)].t is thought that when pain is experienced by individuals with cholesterolosis, the pain is secondary to gallstone disease, not the cholesterolosis [(2)].

Liver flukes (namely *Clonorchis sinensis* and *Opisthorchis viverrini*) typically cause GBD through heavy biliary tract infestation [(2)].

2.1.2 Benign gallbladder tumours

Various benign gallbladder tumours are described in the literature. The potential for the malignant transformation of these lesions remains controversial. They are categorised as follows [(23)]:

- Epithelial tumours
    - Adenomas (gastric, intestinal, or biliary cell types)
    - Cystadenomas
- Mesenchymal tumours (less frequently encountered)
    - Correspond to their soft tissue cell origin
    - Neurogenic tumours are the most common
- Tumour-like lesions of various types stemming from any of the following processes:
    - Metaplasia
    - Hyperplasia
    - Heterotopia
    - Chronic cholecystitis

Benign and tumour-like lesions may resemble malignancy in their clinical presentation, radiologic findings, and even histologic appearance, as stated by Van Patten *et al*. [(23)]. The neoplastic potential of benign gallbladder lesions remains unclear. In their study of 1605 cholecystectomy specimens,

Kozuka *et al.*[24] found 11 benign adenomas and 79 invasive carcinomas. Seven adenomas exhibited malignant changes, and adenomatous residue was found in 15 of 79 patients (19.0%) with invasive carcinoma [24]. Roa *et al.* [25] reported that gallbladder adenomas were found in only 0.14% of cholecystectomies, and adenomatous remnants in the mucosa adjacent to early carcinomas were present in less than 3% of malignancies, suggesting that this carcinogenic pathway is of limited importance. Golding *et al.*'s [26] literature review supports these findings, with the incidence of transformation from adenoma to gallbladder adenocarcinoma being low: between 0.14% and 1.1% of all GBS submitted for histopathologic analysis. According to Roa *et al.* [25], 1% of GBS demonstrate isolated epithelial dysplasia. Mucosal 'pre-neoplastic' lesions, including metaplasia, dysplasia, and CIS, are found adjacent to GBCa in 66%, 81.3%, and 69%, of specimens, respectively [25].

The results of multiple studies support the notion that gallbladder carcinogenesis from benign tumours is a rare occurrence, according to Goldin *et al.* [26]. These authors stated that while gallbladder adenoma to adenocarcinoma transformation has been demonstrated, the incidence is low, varying from 0.14% to 1.1% in different studies [26]. Epidemiologic and molecular studies have not described a pathophysiologic association between gallbladder adenomas and GBCa or progression from adenomas to GBCa, although studies have revealed the following [25, 26]:

- Activation by mutation of the *K-ras* gene: some authors have demonstrated no mutations in this gene, while others have reported mutations in 40% to 50% of patients with GBCa.
- Expression of the cyclin-dependent kinase inhibitor p21 has been detected in 28% of GBCa (although p21 alone has no known effect on survival in patients with GBCa),
- *TP53* gene mutations, with associated accumulation of p53, have been reported in between 27% and 70% of patients with GBCa.

Other molecules or molecular pathways that may be involved in the pathogenesis of GBCa are still under investigation. They are beyond the scope of this paper but include gene *p16* inactivation, cyclooxygenase-2, microsatellite instability, vascular endothelial growth factor, fragile histidine triad gene, and hTERT/telomerase. The reader is referred to the Imperial College of London's update for a concise review of this subject [26].

2.1.3 Gallbladder cancer

2.1.3.1 Gallbladder cancer epidemiology

As previously mentioned, understanding GBCa, including its management and prognosis, is important to enable an understanding of the significance of histological findings of GBS for this malignancy. GBCa is an aggressive tumour with a poor prognosis [27]. It is the most common cancer of the biliary tract and the fifth most common gastrointestinal malignancy [10, 28]. A very rare malignancy, it has a screening prevalence of approximately 0.011% in the general population [15, 29]. According to D'hondt *et al.* [30], GBCa accounts for 4% of all gastrointestinal malignancies.

A 2013 American Cancer Society study found new GBCa cases represented 0.62% of all new cancer cases in the USA [31]. Chile has the highest incidence of GBCa globally: 12.3 per 100 000 men and 27.3 per 100 000 women [17]. The average incidence in other countries is 3 to 4 per 100 000 population [17, 32]. GBCa is more common among older women, with a 3:1 female to male prevalence ratio in the general population [3,32 - 34]. A Malaysian 12-year study that evaluated 1375 GBS found GBCa in 7 specimens (0.005%) [17, 18]. Soomro *et al.* [18], in a 2-year study, found GBCa in 19 (3.64%) of 521 specimens.

2.1.3.2 Gallbladder cancer prognosis

The estimated mean overall survival from the time of diagnosis of patients with GBCa is between 6 to 12 months, and the overall 5-year survival rate is 5% [8 - 13]. A group from India reported a 68% 5-year survival rate for patients with early mucosal tumours and those infiltrating the gallbladder

muscularis layers [28]. This is higher than the results of a French study, which reported actuarial 5-year survival rates of 44% for stage I, 22% for stage II, and 0% for stage III cancers, respectively [35]. Ogura *et al*. [36] reported cumulative 5-year survival rates between 72.5% and 82.6% in the early stages of gallbladder malignancies managed by simple cholecystectomy. Aloia *et al.* [37], in their expert consensus statement from North America, report that most patients, however, present with advanced-stage GBCa and have less than 10% survival at 5 years.

The estimated 1-year mortality rate for all stages combined is very high: 88% [38]. In a long-term (1968–1998) retrospective analysis of Scotland cancer registries [34], the 1-, 3-, and 5-year survival rates of patients diagnosed with GBCa, were 20%, 12%, and 6%, respectively. This survival pattern remained unchanged over the 30-year study period [34].

The above observations underline the extremely poor prognosis of GBCa in different parts of the world. This occurs despite advances in knowledge of GBCa pathology, peri-operative imaging, surgical techniques (including laparoscopic techniques), and adjuvant therapies, as discussed further in the sections below.

2.1.3.3 Gallbladder cancer risk factors and pathology

The association between GBCa and gallstones has been reported to be as high as 90% [15]. Gallstones and chronic gallbladder inflammation are thought to play a role in the development of GBCa, although the exact pathophysiologic mechanisms remain unknown [4]. Only a minority of individuals with gallstones develop GBCa, however, with a reported incidence of GBCa in this patient population of between 0.3% and 3% [4, 39 - 40]. It is now understood that sustained chronic inflammation promotes gallbladder carcinogenesis, in which an interplay of risk factors produce the necessary and persistent inflammatory process for the development of GBCa [41].

Risk factors for developing GBCa include gallstones, ethnicity (for example, a noted high prevalence in American Indians), genetic predilection, lifestyle factors (such as multiparity and

obesity), chronic inflammation, and chronic infections [33]. Factors contributing to poor outcomes in patients with GBCa include delayed presentation, delayed diagnosis, and poor response to surgical, neoadjuvant, and adjuvant therapies [33,42]. The use of any risk factors as the basis for submitting specimens for histopathology will yield very low GBCa positive results [33,42. No single risk factor has been documented as the common trigger for the development of GBCa [33].

Bizama *et al.* [43] provides a thorough review of the currently understood role of genetic changes and molecular pathways in GBCa, which have stimulated the development of targeted therapies used in recent GBCa clinical trials.

Distinguishing GBCa from similar diseases poses difficulties in diagnosis and management [23,27]. Xanthogranulomatous cholecystitis mimics GBCa, as it is characterised by tumour-like lesions or focal areas of wall thickening with evidence of extension into surrounding structures [23,27]. Gallbladder polyps are easily confused with GBCa [8]. A 9-year single-centre study from St. James Hospital at Leeds (United Kingdom [UK]) of patients with gallbladder polyps reported that the prevalence of GBCa ranged from 0.08% in the Caucasian population to 5.5% in patients of Indian ancestry [44]. Studies from Denmark and Taiwan of patients with gallbladder polyps have found GBCa prevalence rates of 4.3% and 6.9%, respectively [29]. Cholesterol polyps, which account for 60% to 90% of polypoid gallbladder lesions, are rarely associated with dysplasia [4,23,27]. Inflammatory polyps, which are also rare, are associated with acute and chronic cholecystitis, leading to their perceived association with GBCa [27]. Large polyps, with a diameter of more than 1 cm, have a higher risk of malignant transformation and are associated with GBCa in 8% of cases [45]. Multiple other polypoid lesions described in the literature, although exceedingly rare in clinical practice, require histological evaluation to exclude cancer [27].

Porcelain gallbladders are diagnosed in 1% to 5% of GBS [8,38,39]. The patchy intramural calcification variant, postulated to confer a higher risk of malignant transformation than complete

calcification, is a known risk factor for GBCa [8,39]. Recently, however, this clinical association seems to be less common and less important than previously thought [39].

Therefore, gallbladder lesions previously thought to confer a significant risk of GBCa have been found to be equal or even less frequent than GBCa. While further histological investigation of these lesions is warranted, their rarity suggests that their pathophysiologic importance in the development of GBCa is at most trivial.

2.1.3.4 Gallbladder cancer histopathology

Macroscopic examination of GBS has been reported as a sensitive method for identifying GBCa, leading some authors to propose selective approaches for determining which specimens should be submitted for histopathology examination [7,9,16] . Nevertheless, other researchers reported no significant macroscopic features suggestive of malignancy in 37% to 55% cases of histologically-diagnosed GBCa, leading to recommendations for routine submission of all GBS [46, 47] .

Giang *et al.* [46] found post-operative macroscopic abnormalities in 12 (52%) of 23 GBCa specimens, while pre-operatively, GBCa was only suspected in 5 patients (22%). According to Hayes *et al.* [48], although the negative predictive value of macroscopic lesions for GBCa in their study was high (99.03%), this was a function of the rarity of the disease, and it is worth noting that 50% of the invasive cancers had no macroscopic findings. These results of these two studies are important, since a selective approach to GBS submission would depend on macroscopic examination of the GBS by the surgeon at the time of cholecystectomy. If 50% of specimens found to have GBCa on histopathologic examination by a pathologist had no significant macroscopic features suggesting malignancy, a selective approach would be inappropriate. Romero-González and colleagues [49] compared detailed macroscopic gallbladder analysis performed by a surgeon versus that performed by a pathologist. The surgeon used a combination of patient risk factor profile assessment and protocol-driven assessment of the GBS in the operating theatre, looking for

such abnormalities as masses, indurations, calcifications, or ulcers. GBS assessment was completed in less than 5 minutes [49]. The surgeon indicated whether the GBS seemed to represent a malignancy; this was later compared to the pathologist's findings [49]. Three of the 150 GBS evaluated contained GBCa, which was detected by both the surgeon and pathologist. The other 147 cases were documented as negative by both parties, suggesting that simple assessment by surgeons yielded 100% specificity and sensitivity [49]. The positive and negative predictive values were 85.7% and 99.6%, respectively [49]. These results refuted earlier data showing poor association between abnormal macroscopic GBS features and GBCa findings on histology. However, the number of positive GBCa specimens in this latter study was low (only 3), in contrast to the 23 GBCa specimens in the study by Giang *et al.* [46], which leads to the question of the comparative statistical significance of the results from Romero-González and colleagues.

Adenocarcinoma is the most common histological subtype of GBCa; infrequent subtypes include squamous carcinoma, adenosquamous carcinoma, sarcoma, adenosarcoma, and unspecified carcinoma [50]. Diagnostic work-ups, including preoperative imaging, have become essential for diagnosing and staging GBCa, which some authors have suggested are a more cost-effective means of diagnosis than routine histopathological assessment of all GBS, as discussed below [51, 52].

2.1.3.5 Gallbladder cancer imaging

Ultrasound examinations are particularly inaccurate for detecting GBCa, and reliance on their guidance is the 'Achilles heel' when evaluating patients presenting with vague right upper quadrant symptoms and/or suspicion of GBCa [53].

Improvements and the increased ubiquity of computed tomography (CT) scanners has improved pre-operative diagnostic yield and accuracy in medicine in general. CT has become an indispensable part of the armamentarium of methods for evaluating patients presenting with abdominal pain of various aetiologies. Further, CT scanners now use multiple detectors for reduced

scanning time and improved image quality with less artefacts, when compared to earlier-generation single-detector scanners.

A meta-analysis regarding the assessment of GBCa resectability using multidetector CT (MD-CT) by Li *et al.* [54] reported a sensitivity of 99% and specificity of 76%. Although MD-CT is more sensitive in detecting and staging GBCa than ultrasonography, it has limitations [53-55]. MD-CT increases the accuracy of GBCa tumour stage detection from 72% (using trans-abdominal ultrasound imaging) to 85%, but its sensitivity for differentiating T1 from T2 tumours is only 65% [53]. Locoregional lymphadenopathy associated with acute and chronic inflammation tends to upstage cancers on MD-CT imaging, which negatively affects their utility [53].

Radiology expertise and detailed pre-operative reviews of reconstructed images are essential for accurate diagnosis, staging, and operative planning in patients with GBCa [13,53, 54,56]. According to Kim *et al.* [57], three main findings are suggestive of GBCa on imaging:

1. Gallbladder wall thickening, which may be focal or diffuse and, in some cases, have associated wall irregularity
2. Intraluminal gallbladder mass, which may be in the form of a polyp
3. An extensive mass (involving the liver in advanced disease), which may obscure or replace the gallbladder

The role of magnetic resonance imaging (MRI) to diagnose and stage gallbladder malignancy has been described. Diffusion-weighted imaging and quantitative analyses of these images are useful tools not only for differentiating GBCa from benign gallbladder pathology, but also for disease staging [58-59]. MRI scanners are not readily available because of their high capital and user costs, which is their main limitation, but in future, MRI is likely to play a more important role in the diagnostic work-up of GBCa [58] . At the time of writing this dissertation, MRI scans, although

available at the Wits academic hospitals, were not an integral component of the diagnostic strategy of gallbladder pathologies; the work-up was limited to transabdominal ultrasonography and CT.

2.1.3.6 Gallbladder cancer staging

The stage of presentation of patients with GBCa determines the likelihood of successful attempted curative surgery, adjuvant therapies, and neoadjuvant therapies, as well as patient survival [55, 60-62]. GBCa spreads directly to adjacent organs, such as the liver, but also spreads haematogenously and via lymphatics to locoregional nodal basins, the peritoneum, and distant organs [27,50,60]. Radiological and, in some cases histological, evidence of spread forms the basis for both the choice of therapy and estimation of prognosis [45,60,62]. The American Joint Committee on Cancer (AJCC 2010) [60] system stages all cancers according to the primary tumour (T), lymph nodes (N), and distant/metastatic (M) spread (Table 2-2).

Table 2-2: Gallbladder cancer TNM staging (AJCC 2010)

Primary Tumour (T) [60]	Tx	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	Tis	Carcinoma in situ
	T1	Lamina propria or muscular layer breached
	T1a	Lamina propria breached
	T1b	Muscular layer breached
	T2	Perimuscular connective tissue invaded; no extension beyond serosa or into liver
	T3	Serosa breached and/or liver and/or one adjacent organ/structure (stomach, duodenum, colon, pancreas, omentum, or extrahepatic bile ducts)
	T4	Main portal vein or hepatic artery or into one or more extrahepatic organs or structures invaded
Regional Lymph Nodes (N) [60]	Nx	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Local nodes (cystic duct, common bile duct, hepatic artery, and/or periportal vein nodes)
	N2	Distant nodes (periaortic, pericaval, superior mesenteric artery, and/or celiac artery)
Distant Metastasis (M) [60]	M0	Distant metastasis absent
	M1	Evidence or confirmation of distant metastasis

Patients with T1 tumours have a better prognosis than those with T4 tumours, with 5-year mortality rates of up to 15% and over 80%, respectively. Surgery is generally curative for early-stage disease [63]. Patients with T1a GBCa have 5-year survival rates above 90% after simple cholecystectomy if there is no residual disease (R0 resection), but in the presence of residual tumour, repeat or re-attempted resection (re-operation) provides no discernible benefit [14].

In their study, D'hondt *et al.* [30] reported liver involvement in 0%, 20.8%, 58.3%, and 100% of primary T1 (pT1), pT2, pT3, and pT4 GBCa, respectively. With T1b tumours, 15% to 20% had lymph node involvement, 13% exhibited liver involvement, and 60% recurred following simple cholecystectomy [30]. Because of the reported higher incidence of locoregional failure after simple cholecystectomy for T1b lesions, extended resection of the gallbladder bed, including lymph node dissection, has been recommended [14]. Some researchers have reported improved survival with extended cholecystectomy for these tumours [14], but a systemic review by Lee *et al.* [64] found no evidence supporting the use of extended cholecystectomy instead of simple cholecystectomy for managing T1b GBCa. Together, these studies emphasise the much poorer outcomes for advanced GBCa, which generally can be diagnosed with high accuracy using MD-CT and MRI, as mentioned above. The use of histopathological analysis of GBS to determine and direct further resections has had poor results.

In their mortality registry review of autopsy findings in patients with GBCa, Kingham *et al.* [27] estimated the proportions of regional invasive and metastatic disease:

- Lymphatic involvement in over 90% of patients
- Liver involvement (segments IV, V and VIII) in 60% of patients
- Blood and intraperitoneal metastatic disease in 60% to 80% of patients

The extent of disease directly affects prognosis and should be used more stringently to determine treatment options offered to patients, as outcomes differ significantly with disease stage (Table 2-3). Use of pre-operative imaging for GBCa staging can reduce most attempts at curative surgery for advanced GBCa disease, for which the results are dismal.

Table 2-3: Gallbladder cancer anatomic/pathologic stages (AJCC 2010)

Anatomic Stage/Prognostic Group [60]	
Stage 0	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2 N0 M0
Stage IIIA	T3 N0 M0
Stage IIIB	T1-3 N1 M0
Stage IIIC	T4 N0 M0
Stage IVA	T4 N0-1 M0
Stage IVB	Any T N2 M0 Any T Any N M1

2.1.3.7 Management of gallbladder cancer

Current challenges in the management of GBCa are summarised in Table 2-4.

Table 2-4 Gallbladder cancer management challenges

Imaging modalities	Widespread use of ultrasound for staging despite its significant diagnostic limitations
	Systematic diagnostic delays and failure to detect early GBCa
	Capital costs associated with advanced MD-CT and MRI scans and maintenance plus specialised training
	Shortage of expert MD-CT and MRI radiologists for accurate early diagnosis and staging
Surgery	Poor surgical outcomes associated with advanced disease, even with extensive resections
	Persistently poor outcomes associated with managing of patients with GBCa
	High post-operative morbidity and mortality for extensive/radical GBCa surgery
	High post-operative recurrence rates in 'resectable' GBCa despite curative intent
Neoadjuvant and adjuvant therapies	GBCa is one of the most chemoresistant tumours
	Available chemotherapy and combination regimens are of limited efficacy
	Limited number of randomised clinical trials
Radiation therapy	Currently applied on an 'all comers' basis, with little evidence of clinical efficacy
Abbreviations: GBCa, gallbladder cancer; MD-CT, multidetector computed tomography; MRI, magnetic resonance imaging (28) (13) (69) (45) (63) (65) (73) (74) (75) (76) (56)	

2.1.3.7.1 Treatment of gallbladder cancer

Discussion of the treatment of GBCa highlights the generally poor outcomes associated with most therapies currently available. These therapies are offered to patients diagnosed with GBCa based on both imaging and histopathology findings, but despite durative intent, the prognosis of the more advanced tumours, with or without treatment, remains poor, as discussed above.

The optimal treatment of GBCa depends on the disease stage at presentation. Locoregional spread detected on pre-operative imaging or during surgery of incidental or suspected GBCa determines the amenability and likely success of resection for curative intent. Cure depends on complete resection of malignant tissue (R0 resection), which can only be confirmed by histology. Adjuvant and neoadjuvant therapies have not improved the survival of patients with GBCa, as it remains poorly responsive to these modalities. Re-operations following failed R0 resections are associated with poor outcomes and have not been found to improve clinical outcomes.

Available therapies and their studied outcomes are discussed below. Simple cholecystectomy is curative for early GBCa. Patients with more advanced disease, however, fair very poorly, even after cholecystectomy. Hepatic involvement is an independent prognostic factor adversely affecting surgical resection with curative intent [30,63]. Jaundice, a contraindication for attempts at curative resection of other biliary tract tumours, is not a determinant of inoperability in GBCa [27,62,63,65]. For operable patients, extended resections with complex reconstructions in select cases (including jaundiced patients) are necessary to achieve R0 resection [66]. Surgical options are shown in Table 2-5 below.

Aggressive/extended surgical resection and, in selected cases, adjuvant chemotherapy and/or radiotherapy, have been recommended for T2 to T4 GBCa; however, outcomes and overall prognosis remain poor [67-68]. Oncologic resection options for tumours beyond T1b include extended cholecystectomy, en-bloc resection of liver lobes IVB and V, and lymphadenectomy of the porta-hepatis, gastro-hepatic ligaments, and retro-duodenal space [27,63,69,70]. It is recommended that these be performed via an open approach; if initially diagnosed during laparoscopic surgery, then conversion to open surgery with port-site tissue resection is obligatory to manage unintended tumour seeding and thereby prevent recurrence [71].

Table 2-5: Gallbladder cancer surgical management options

Simple cholecystectomy	Open procedure or conversion from laparoscopic to open approach
Extended cholecystectomy/ radical surgery for tumours > pT2	En bloc resection of the gallbladder in combination with wedge resection of 2 cm of adjacent liver tissue, usually segments IVB and V
	Formal anatomic resection of segments IVB and V
Extremely aggressive surgery	Bile duct resection
	Liver resection and extended right hepatectomy
	Hepato-pancreaticoduodenectomy
	Pancreatico-duodenectomy
	Portal pancreatico-duodenectomy and vein resection
(13) (65) (75) (76) (56) (63) (70) (84) (85)	

While a detailed description of available surgical techniques is beyond the scope of this dissertation, Jin *et al.* [63] noted that extensive surgery has not improved the prognosis of patients with advanced GBCa. Some authors have concluded that mortality rates following major GBCa resection remain prohibitively high [63]. Recent work by Chen and colleagues [72], however, indicated that prognosis was much improved if R0 resection was achieved, even if it required extensive locoregional resection.

The AJCC 2010 [60] report noted 5-year survival rates of 50% and 29% in patients with T1 and T2 tumours, respectively. Further, patients with stage III (lymph nodes positive) and locally advanced or metastatic disease (stage IV) survived for a short period of time [60]. Patients who underwent curative intent surgery had a 5-year survival rate of 63.2%, whereas 5-year survival for patients receiving palliative treatment was still an ominous 0% [30]. By contrast, patients with incidental GBCa who underwent operative resection had significantly longer survival than patients with GBCa

suspected pre-operatively who undergo similar surgery; median survival times were 25.8 vs. 4.4 months, respectively [30]. R0 resection is the only definitive therapeutic procedure for GBCa [63, 72].

2.1.3.7.2 Management of unsuspected or incidental GBCa

Unsuspected or incidental GBCa is classified according to whether it is diagnosed intra-operatively during cholecystectomy or postoperatively by histopathological examination of GBS [78]. In both instances, the cholecystectomy is performed for presumed benign gallbladder disease [78].

The rate of incidental GBCa was 4 (1.4%) of 282 in open cholecystectomy specimens in the 3-year study by Memon *et al.* [4]. In their study of incidental GBCa at a London (UK) tertiary hospital, Solaini *et al.* [77] noted rates of 2% (18 of 864) for dysplasia and 0.8% (7 of 864) for carcinoma. The increasing incidence of incidental or unsuspected GBCa is at least partially attributed to laparoscopic surgery, which has resulted in an increasing number of cholecystectomies [69]. Incidental GBCa has been reported in 0.09% to 2% of all GBS obtained during laparoscopic cholecystectomy [32].

According to Cavallaro *et al.* [78], GBCa is suspected preoperatively in only 30% of patients with confirmed malignancies; the other 70% are discovered in specimens submitted for presumed benign disease. Further, only 0.19% to 3% of all submitted cholecystectomy specimens are found to have incidental GBCa [78, 79].

According to the National Comprehensive Cancer Network (NCCN) guidelines [52], T1b and higher stage incidental GBCa require extended hepatic resection and lymphadenectomy, with or without bile duct excision. No objective evidence is available to justify extended resection for less invasive tumours, so the guidelines do not recommend it [52].

The management of unsuspected (as opposed to incidental) GBCa is contentious, with no clear guidelines regarding re-operations and further resection, such as lymphadenectomy, bile duct resection, and other surgical procedures [78]. Simple cholecystectomy may be curative for early

GBCa (TNM Tis and T1a); however, curative intent re-resections are recommended if R0 resection is considered possible [78]. From their retrospective analysis, Watson *et al.* [80] concluded that completion cholecystectomy (re-resection) following a diagnosis of incidental GBCa was primarily performed for staging and conferred no significant improvement in prognosis of any of their patients. These authors supported the use of intraoperative histological specimen examination to enable additional or radical resection during the index operation in uncertain cases rather than later re-operations, which are more surgically challenging and provide no significant added benefit [80].

Nevertheless, there are many obstacles for immediate conversion to radical cholecystectomy for incidentally discovered or previously unsuspected GBCa. According to Isambert *et al*. [81], more than 20% of specimens submitted for histopathology revealed macroscopic suspicious lesions when the pathologist opened the GBS, which the surgeons had failed to appreciate. These authors also noted other issues of concern regarding optimal management of incidental or unsuspected GBCa [81]:

- Frozen section histopathological assessment services may not be immediately or readily available.
- TNM stage may be indeterminate, especially in the setting of acute inflammation.
- Lymph node dissections are associated with significant risk of injury to adjacent structures and vasculature.
- Technical limitations may exist, including the surgeon's ability, availability of surgical assistants, anaesthetic concerns, and post-operative care issues.
- There may be issues regarding the patients' general condition or informed consent, which may not have been adequately addressed pre-operatively.

The above points, the assessed resectability of the GBCa, and the surgeon's training and ability to perform more extensive surgical resections formed part of the algorithm by Misra *et al.* [82] published in The Lancet Oncology, highlighting the slow pace of progress in the management of

incidentally discovered and unsuspected GBCa. These authors also elaborated on the key role of diagnostic laparoscopy for assessing metastatic disease in patients with intra-operatively diagnosed GBCa, which they note could prevent unnecessary laparotomy if advanced metastatic disease is detected [82].

2.1.3.7.3 Neoadjuvant and adjuvant therapies for gallbladder cancer

A recent phase III trial using adjuvant combination chemotherapy of mitomycin C and 5-fluorouracil (5-FU) in bile duct cancers and GBCa suggested improved outcomes for more advanced malignancies [83]. In this study, the benefits were more pronounced for GBCa than for bile duct cancers [83]. Subset analysis of 149 patients with GBCa revealed 5-year disease-free survival rates of 20.3% with the combination vs 11.6% for the controls (p=0.02). Likewise, 5-year overall survival rates were 26.0% with the combination vs 14.4% for the controls (p=0.04) [52,83].

Chemotherapy and radiotherapy response rates have been consistently below 30% as palliative adjuvant therapies, but combination therapy using gemcitabine and cisplatin showed, on average, 3.6 months improvement in overall survival compared to treatment with gemcitabine alone [53]. The NCCN [52,53] now recommends combination therapy using these two agents as standard of care for patients with advanced biliary tree cancers, including GBCa. The 2015 NCCN guidelines [52] note the limited amount of data regarding the effectiveness of adjuvant chemotherapy and chemo-radiation for the management of GBCa; however, they recommend 5-FU- or gemcitabine-based regimens for patients with non-curative resections. A multi-disciplinary, multinational phase III trial evaluating adjuvant chemotherapy (gemcitabine and cisplatin) in advanced GBCa has been initiated by Wege *et al*. [86]; the results were pending at the time of submission of this dissertation.

Radiotherapy is generally regarded as an 'all-comers' palliative treatment for patients unsuitable for surgery or those who have undergone inadequate surgical resection [53,56]. Pilgrim *et al*. [53] indicated

that a major factor limiting assessment of the clinical effectiveness of systemic therapies in GBCa is the inadequacy of locoregional disease control.

In the NCCN 2015 guidelines [52], when phase III trial data were still pending, a phase II trial of adjuvant combination chemotherapy followed by chemo-radiotherapy for GBCa showed a 2-year overall survival rate of 56%, disease-free survival rate of 47%, and local relapse rate of 13%. The NCCN 2017 guidelines [87], however, report data from a phase III trial supporting gemcitabine/cisplatin for patients with advanced or metastatic hepatopancreaticobiliary cancers. Nevertheless, participation in clinical trials is still recommended in 2017 [87].

Fluoropyrimidine chemo-radiation, followed by either fluoropyrimidine- or gemcitabine-based combination chemotherapeutic regimens, is recommended as adjuvant therapy [52]. There have been no studies comparing adequate R0 resection alone to adequate R0 resection followed by chemo-radiotherapy [53]. The efficacy of neoadjuvant therapies in down-staging tumours has neither improved survival nor shown any clinical benefit, and according to Pilgrim *et al*. [53], complete surgical resection is an independent prognostic factor for optimal GBCa management [53,88]. Failure to achieve this continues to be associated with dismal outcomes.

2.2 Paediatric Cholecystectomies

A review of the disease profile afflicting the paediatric age group is necessary because of the number of GBS resected and subsequently submitted for histological assessment. Clarity regarding the utility of submitting specimens for histology in this age group is, therefore, sought.

Cholelithiasis is uncommon in the paediatric group, with a reported prevalence of less than 0.5% in paediatric population studies [89]. While the aetiology of cholelithiasis in this age group is multi-factorial, some authors note certain factors associated with its increased prevalence [19,90]. A higher incidence of gallstone disease is associated with prematurity, childhood pregnancy, history of

necrotizing enterocolitis, cystic fibrosis, haemolytic diseases, and certain interventions, such as ileal resection and total parenteral nutrition [90-91].

Biliary dyskinesia is a diagnosis based on the presence of biliary colic symptoms with no choledocholithiasis visualised on ultrasonography, a cholecystokinin-stimulated gallbladder ejection fraction of less than 40% at 30 minutes, and a lack of any clear alternative cause. It is reported with increasing frequency in some studies [90,92]. Other indications for cholecystectomy in children include acute cholecystitis, cholangitis, choledocholithiasis, pancreatitis, choledochal cysts, biliary atresia, liver transplantation, and liver resection for various indications [89,91].

Stringer *et al.* [93] classified polypoid lesions of the gallbladder in children as either benign or malignant and offered a histological classification of each group. According to these authors, children with symptomatic lesions and those with asymptomatic lesions larger than 10 mm require cholecystectomy [93]. Other rare diseases of the gallbladder, such as leiomyosarcoma, have been described in children [22]. However, according to Hansel *et al* [94], suspicious lesions in the paediatric gallbladder are more likely to be rhabdomyosarcoma. Gallbladder malignancy in the paediatric group is rare and there is a paucity of literature on the subject.

The increasing awareness of complications associated with gallstones has resulted in more liberal use of cholecystectomy in children who present with biliary colic from stones and those with chronic vague upper abdominal pain [91]. Nevertheless, cholecystectomy is still an uncommon procedure in children, performed in approximately 2 to 3 per 100 000 children under the age of 15 years [89].

2.3 Costs

In their 5-year study, Darmas *et al*. [16] calculated the cost of detecting GBCa as £5437.50 per case. In this study, malignancy was diagnosed in 4 of the 1452 (0.27%) GBS examined, and the cost was £14.9 for each examination.

More recently, Elshaer *et al*. [95] calculated the total cost for histopathologic examination of GBS by multiplying the cost of preparing standard haematoxylin and eosin slides for each GBS by the total number of specimens examined. They estimated the cost of slide preparation as £10.4 to £15.0 per GBS [95]. In this study, spanning a period of 9 years, a total of 3330 cholecystectomies were performed; in 3041, the indication was documented gallstone disease. Incidental GBCa was diagnosed in 13 patients. Thus, the authors estimated that the cost of detecting each case of incidental GBCa was between £2664.0 and £3842.30 when GBS were routinely submitted for histology [95]. Of note, GBCa was only diagnosed in patients above the age of 51, and if a selective approach for GBS submission were based on age, cost savings would be significant [95]. If only specimens from patients over 51 were submitted, the estimated costs of diagnosing the 13 incidental GBCa cases would be £1492.80 to £2153.0 per case, resulting in estimated savings of £15225.60 to £21960.0 [95].

Romero-Gonzalez *et al.* [49], in their aforementioned paper, concluded that 46% of post-cholecystectomy GBS could be safely *not* submitted for histopathological examination without compromising the patient safety; this would reduce the GBS histology examination budget by 50%. These authors indicated that adequate assessment requires sampling from three areas in each GBS: the fundus, body, and neck [49].

The 2016 histology fee structure of the Department of Labour of the Republic of South Africa (RSA) [96] indicates the costs according to clinical pathology unit values (Table 2 6). Based on this cost structure and the required assessment of at least three sections of gallbladder, the total

pathologists' cost would be R933.12 (= R432.00 + R250.56 + R250.56) [96]. Additional costs may include immunofluorescence studies or frozen sections performed in the operating theatre, which are sometimes requested. In the research described in the next section, the minimum total cost for the analysis of GBS analysed is estimated from the value of R933.12, without considering the use of any other specimens or further special tests.

Table 2-6: Fee Structure for Histopathology in the Republic of South Africa, 2016

Item	Pathologists	Other Specialists or General Practitioners
Histology per sample	R432.00	R287.28
Histology per each additional block	R250.56	R166.32
Histology and frozen section in the laboratory	R490.32	R326.16
Histology and frozen section in the operating theatre	R1944.00	R1 296.00
Second and subsequent frozen sections, each	R432.00	R289.44
Attendance in the operating theatre; no frozen section performed	R568.08	R378.00
Histology consultation	R218.16	R144.72
Special stains	R144.72	R97.20
Immunofluorescence studies	R447.12	R298.08
Electron microscopy	R2030.40	R360.80

3 RESEARCH

3.1 Research Questions

i. What is the histopathologic disease profile of GBS from three academic hospitals affiliated with Wits over the study's 3-year period?

ii. What is the overall incidence of GBCa in the above group?

iii. What is the incidence of incidental GBCa in patients who undergo cholecystectomy for presumed benign disease?

3.2 Methodology

3.2.1 Study design

Descriptive retrospective study.

3.2.2 Study setting

i. Chris Hani Baragwanath Academic Hospital (CHBAH)

ii. Charlotte Maxeke Johannesburg Academic Hospital (CMJAH)

iii. Helen Joseph Hospital (HJH)

iv. South African National Health Laboratory Service (NHLS)

3.2.3 Study population and sampling method

The study population included all GBS from patients who underwent cholecystectomy and whose specimens were sent to NHLS for histopathology assessment from three Wits academic hospitals from January 1, 2010 to December 31, 2012. Patient information was collected from the NHLS, with relevant permission.

3.2.4 Data collection and data management

Data were acquired from the NHLS database using GBS coding. The data were entered into a data collection sheet and then transferred to Excel™ spreadsheets for analysis. The data included demographic and clinical information sent to NHLS with each specimen on the request forms, as well as the final histopathology reports of the submitted GBS. See Appendix 1.

3.2.4.1 Data sets, inclusion criteria, and exclusion criteria

The NHLS Disa™ and Trackcare™ databases were the data sources. Inclusion criteria were all specimens from patients who underwent cholecystectomy at the three hospitals during the defined study period. All specimens were registered in the NHLS database for histopathology analysis after cholecystectomy, which was performed for any indication. Specimens in which GBCa or any regional malignancy was suspected pre-operatively were included.

Exclusion criteria were specimens submitted but with incomplete details, rendering the specimens unsuitable for histology analysis and reporting. Specimens from outside the study parameters – study period and study setting – were excluded. The NHLS laboratory examines specimens from other regional hospitals in the greater Johannesburg and Gauteng regions, such as the Sebokeng and Natalspruit hospitals, but these centres were not part of the study setting and were thus excluded from the study.

3.2.4.2 Data analysis

Microsoft Excel 2010™ datasheets and IBM SPSS Statistics 22 Software™ were used for data capture and statistical analysis. Multivariate analysis of the data, also executed using the IBM SPSS™ statistics analysis tools, was used to elucidate the complex relationships between multiple data variables, as discussed in the Results.

3.2.4.3 Budget, resources, and time schedule

Costs related to this research project were borne by the researcher. Microsoft Office Project 2010™ was used to generate a Gantt chart for planning and delivering this research project.

3.2.4.4 Ethical and legal considerations

This study conformed to Wits' ethical, data protection, and privacy guidelines. Relevant authorisation obtained prior to commencement of this study included the following:

i. Wits Ethics Committee approval (see Appendix 2 Human Research Ethics Committee (Medical) Clearance Certificate in Chapter 8.2)
ii. Approval from Prof M. Smith, Academic Head of Surgery at Wits and Chief of Surgery at the CHBAH.
iii. Approval from Prof M. Hale, Academic Head of Pathology at Wits and Head of Pathology at the Gauteng NHLS.
iv. Approval from the Chief Executive Officers (or representatives) of the three hospitals included in this study.

3.2.4.5 Declarations

There are no conflicts of interests concerning this research project or concerning any other research or academic undertaking in relation to this or any other study conducted by the researcher and author of this dissertation.

4 RESULTS

The results of this study are categorised according to these headings:

i. Adult demographic results
ii. Adult histopathologic profile results
iii. Adult data analysis results
iv. Paediatric results

4.1 Adult Demographic Results

The three hospitals sent GBS from 1194 adult patients to the laboratory for histology over the 3-year study period. The male to female ratio was 1:3.4, with 925 (77.5%) and 269 (22.5%) specimens originating from female and male patients, respectively (Figure 4.1 and Table 4 1). The mean age of the patients was 46.62 years (standard deviation [SD], 17.81), with a range of 34 to 87 years (Figure 4.2 and Figure 4.3).

Table 4-1: Proportions of Gallbladder Specimens from Adult Males and Females

Sex	Number	Percent
Female	925	77.5%
Male	269	22.5%
Total	1194	100.0%

Figure 4.1: Number of specimens from adult male and female patients

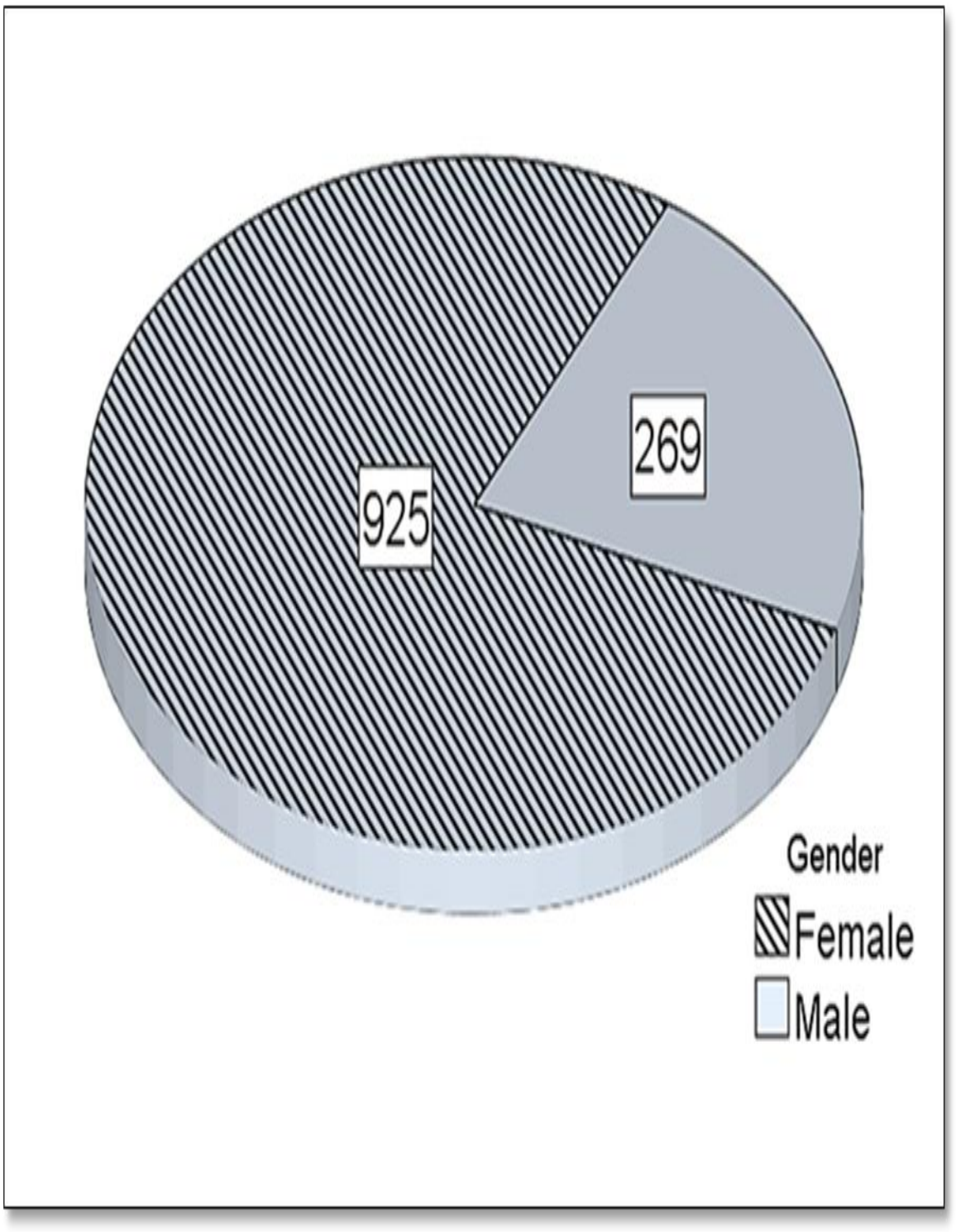


Figure 4.2: Histogram of age and sex of adult patients

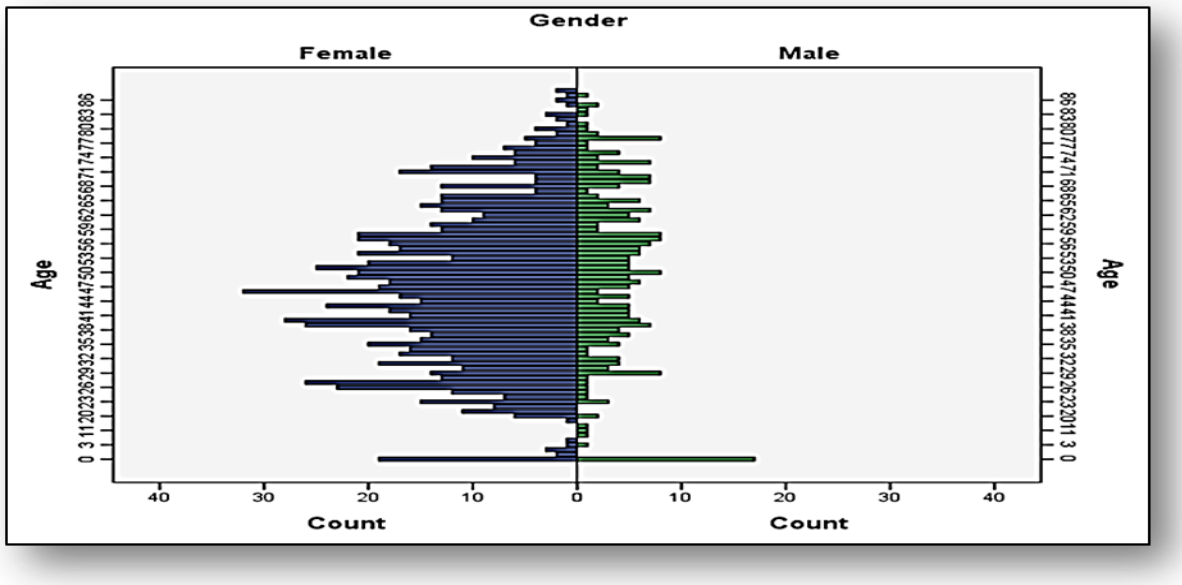
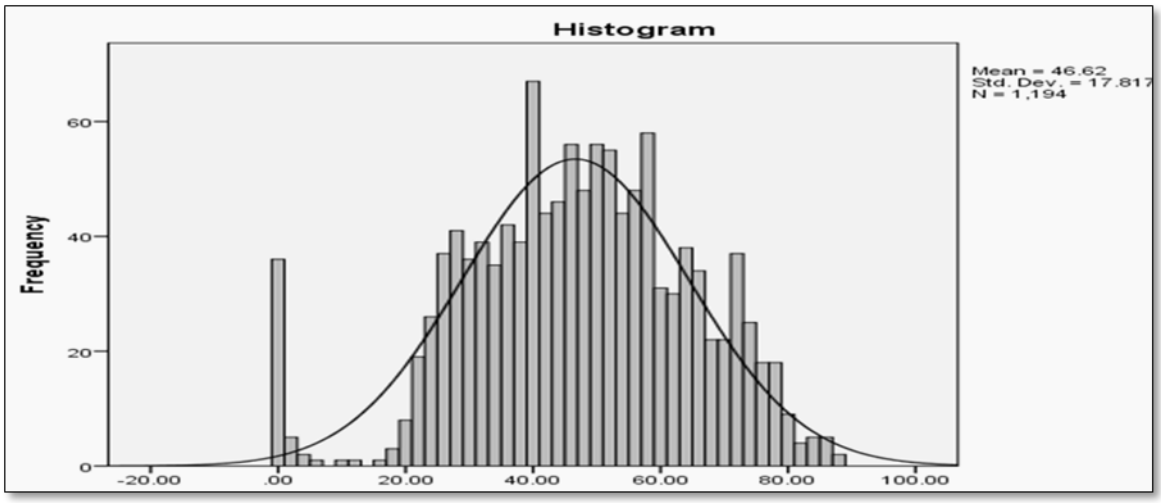


Figure 4.3: Age (years) distribution curve and histogram of adult patients



Abbreviations: N, number; SD, standard deviation

As shown in Table 4-2, CHBAH sent the most GBS for analysis: 507 (42.5%) specimens. This was followed by CMJAH, which sent 407 specimens (34.1%), and HJH, which sent 280 specimens (23.5%).

Table 4-2: Number of Adult Specimens Sent from Each Hospital

Hospital	Number	Percent
CHBAH	507	42.5%
CMJAH	407	34.1%
HJH	280	23.5%
Total	1194	100.0
Abbreviations: CHBAH, Chris Hani Baragwanath Academic Hospital; CMJAH, Charlotte Maxeke Johannesburg Academic Hospital; HJH, Helen Joseph Hospital		

4.2 Adult Histopathologic Profile Results

Of the 1194 specimens, 1159 (97.1%) were found to have benign disease, 20 (1.7%) had malignant disease, and 8 (0.7%) had premalignant disease. The histology results according to sex and histopathology group are shown in Table 4-3.

4.2.1 Gallbladder histopathologic disease profile

Acute cholecystitis was diagnosed in 705 GBS (59.04%) and chronic cholecystitis in 401 GBS (33.58%), 16 of which had chronic granulomatous cholecystitis. Complicated cases with perforations or fistulae were found in 26 specimens. Forty-five GBS (4.43%) had normal histology. Benign tumours were found in 33 GBS and choledochal cysts were noted in 4 specimens.

Table 4-3: Sex and Histopathology Group of Adult Gallbladder Specimens

Gallbladder specimens according to sex		Primary histopathology group			Total
		Benign	Premalignant	Malignant	
Male	Number of specimens sent for histology	257	4	8	269
	% diagnosed within male group	95.5%	1.5%	3.0%	100.0 %
	% of total number of specimens sent	21.5%	0.3%	0.7%	22.5%
Female	Number of specimens sent for histology	902	11	12	925
	% diagnosed within female group	97.5%	1.2%	1.3%	100.0 %
	% of total number of specimens sent	75.5%	0.9%	1.0%	77.5%
Total	Number of specimens sent for histology	1159	15	20	1194
	% diagnosed in both groups combined	97.1%	1.3%	1.7%	100.0 %

4.2.1.1 Results based on sex

4.2.1.1.1 Results for males

As shown in Table 4-4, acute cholecystitis was diagnosed in 156 males (56.5%), and complicated acute cholecystitis was diagnosed in five males (1.9%). Chronic cholecystitis occurred in 69 male patients (25.7%). GBCa was diagnosed in eight male patients and benign tumours in two.

Table 4-4: Histopathology Results in Adult Males

Primary histopathology result	Number	Percent
Acute cholecystitis	156	56.5
Chronic cholecystitis	69	25.7
Normal	13	4.5
Benign tumour	2	0.7
Carcinoma in situ, epithelial dysplasia, or cytological atypia	1	0.4
Gallbladder cancer	8	3.7
Trauma/stab wound	3	1.1
Congenital biliary abnormality	11	4.1
Complicated acute cholecystitis	5	1.9
Total	269	100.0

4.2.1.1.2 Results for females

The most frequently diagnosed gallbladder diseases in females were acute cholecystitis (549 specimens; 59.4%) and chronic cholecystitis (281 specimens; 30.4%) (Table 4-5). GBCa was diagnosed in 12 female patients and benign tumours in 12.

4.2.1.1.3 Combined male and female results

Combined results revealed gallbladder polyps in 17 specimens, cholesterolosis in nine specimens, and a porcelain gallbladder in two specimens. GBCa was associated with premalignant lesions in six specimens, only one of the 20 specimens diagnosed with GBCa was associated with a polyp. Atypia or dysplasia was noted in 8 specimens; four of these were associated with GBCa. The trauma units sent three specimens, none of which revealed any underlying pathology (the primary histopathology result was thus trauma/stab wound).

Table 4-5: Histopathology Results in Adult Females

Primary histopathology results	Number	Percent
Acute cholecystitis	549	59.4
Chronic cholecystitis	281	30.4
Normal	40	4.3
Gallbladder cancer	12	1.5
Benign tumour	12	1.3
Congenital biliary abnormality	14	1.3
Complicated acute cholecystitis	8	0.9
Carcinoma in situ, epithelial dysplasia, or cytological atypia	4	0.4
Gallbladder empyema	2	0.2
Acute haemorrhagic cholecystitis	1	0.1
Granulomatous inflammation	1	0.1
Other malignancy	1	0.1
Total	925	100.0

4.2.1.2 Histology results of lymph nodes

Lymph nodes were not routinely reported in GBS. Of the 42 specimens sent with lymph nodes, nine contained metastases from the primary GBCa. Eight lymph node specimens were acutely inflamed.

4.2.1.3 Gallbladder cancer histopathology data

In the 3-year study period, GBCa was detected in 20 specimens (1.67%). None of these contained 'premalignant' lesions. Twelve specimens were from females and eight were from males. The mean age of patients with GBCa was 58.8 years (SD, 16.179), with a range of 34 to 83 years. The mean age for females diagnosed with GBCa was 62.8 years (range, 34 to 83), whereas the mean age for males was 63.6 years (range, 48 to 78).

The female to male ratio for the GBCa subgroup was 60:40, with nine GBCa specimens obtained from CHBAH, nine from CMJAH, and two from HJH. In seven specimens, cancers from other

body areas involved the gallbladder secondarily: four from pancreatic cancer, two from liver cancer, and one from melanoma. Benign gallbladder disease was found in 12 GBS excised during surgical resection of another malignancy (pancreas, stomach, and colon); none of these revealed any secondary malignant involvement. Detailed histopathology results of the 20 specimens are summarised in Table 4 6.

For the group of 20 patients diagnosed with GBCa:

- A gallbladder mass was the indication for cholecystectomy in nine cases.
- Complicated cholecystitis was found in four specimens.
- Macroscopic features of the GBS were documented in only four reports.
- Twelve (12) reports conformed to AJCC 2010 reporting; reporting for the other eight specimens was inadequate.
- Concurrent polyps were found in one specimen.
- Adenocarcinoma was diagnosed in 15 specimens.
- Undifferentiated carcinoma (stage IV cancer) was found in 4 specimens.
- Squamous GBCa was detected in 1 specimen.
- Biliary intra-epithelial neoplasia was found in association with adenocarcinoma in 1 case.

There were no patients in whom a second set of specimens was submitted, suggesting there were no re-operations or further operative interventions performed in any patient diagnosed with GBCa.

Table 4-6: Histopathology and Other Details of the 20 Cancer Specimens

#	Age Sex	Indication	Pathology Forms Information	Specimens Submitted	*Primary Ca* (site of primary)	Histopathology Type and Details	TNM
1	78 F	Acute cholecystitis (severe)	Cholecystitis, GB adhered to colon	GB + Transverse Colon + Liver Bx	GBCa 40 * 25* 20 mm. Fundus of GB adhered to colon and omentum.	Moderately-differentiated intestinal GBCa, adenocarcinoma. R1 resection. GB involving liver bed and colon + N1/ 14 nodes +ve.	T4N1M1 Stage IVB
2	65 F	GB mass	Mass	GBS	GBCa, No Macro.	GBCa, adenocarcinoma indeterminate. No details about margin status.	No details.
3	48 M	GB polyp	Mass	GBS + Hepatic parenchyma	GBCa, GB = 65 * 14 * 29 mm. Hepatic parenchyma, GB polyp = 19 * 11 * 8 mm.	GBCa, adenocarcinoma, invasive intestinal type. No hepatic involvement.	T1bNxMx Stage 1
4	72 F	OJ and distended GB mass	Mass	GBS	GBCa. No Macro.	GBCa, adenocarcinoma. No details about margin status.	No details.
5	73 M	GB mass	Mass (CT), sub-hepatic collection	GBS	GBCa, 54 * 30 * 25 mm. Thickened irregular wall.	GBCa, undifferentiated carcinoma. Sub-hepatic collection = extensive tumour necrosis. No details about margin status.	No details. Stage IV
6	59 F	OJ and cholangitis + mass	Mass	GBS	GBCa, GB = 125 * 65 * 30 mm.	GBCa, adenocarcinoma. No detail about margin status.	No details.
7	83 F	Biliary colic, intra-operative suspicion	Biliary colic. Intra-op suspicious GB infiltrating liver. Sonar = stones.	GBS	GBCa, 72 * 26 * 24 mm. Ulcerated mucosa. Indurated wall. Chronic cholecystitis. Positive nodes (2/2).	Invasive well-differentiated GBCa, adenocarcinoma. +ve transmural invasion, +ve cystic nodes. No details about margin status.	T2N1M0 Stage IIIB
8	61 M	Biliary colic and cholecystitis	Biliary colic	GBS	GBCa, GB = 120 * 40* 15 mm. Partially ulcerated mucosa.	GBCa, adenocarcinoma + biliary intra-epithelial neoplasia (bili N 2-3) + moderate to severe dysplasia. No details about margin status.	T1bNxMx TcinN0M0
9	68 M	Chronic cholecystitis	Cholecystitis	GBS + porta hepatis LND Bx	GBCa 65 * 40 * 15 mm. No Macro.	GBCa, adenocarcinoma. Extensive perineural invasion. Positive resection margins. Porta hepatis lymph node deposits.	T4N1Mx Stage Iva
10	61 F	Symptomatic GBS	Biliary colic	GBS	GBCa 60 * 25 * 6 mm. No Macro.	GBCa, adenocarcinoma/cholesterolosis	T1NxMx
11	75 F	Acute cholecystitis + emphysematous GB	Complicated cholecystitis	GBS	GBCa 125 * 50 * 35 mm. Perforations + irregular ulcerated tumour at fundus (30 * 45 mm) with extension to liver bed.	Squamous GBCa/ulcerated tumour/chronic active cholecystitis/ cholesterolosis + liver parenchyma invasion with positive margins + additional biliary intra-epithelial neoplasia. Peritoneal fluid: malignant cells (squamous carcinoma).	T3N1Mx Stage IIIb
12	55 M	Acute cholecystitis	Cholecystitis	GBS	GBCa 50 * 35 * 10mm. No Macro.	GBCa, undifferentiated carcinoma + glandular atypia. Biliary intra-epithelial neoplasia Grade 2 + xanthogranulomatous inflammation with ulceration.	No details. Stage IV
13	34 F	GBCa on CT	Mass	GBS	GBCa adenocarcinoma 90 * 35 * 30 mm/fundal fungating tumour 42 * 26 * 25 mm/friable tumour.	GBCa, adenocarcinoma ulcerated poorly differentiated with uninvolved margins intraepithelial neoplasia grade iii + low-grade dysplasia/pancreatic metastatic spread.	T4N1M1 Stage IV
14	54 M	GBCa on CT	Mass	GBS	GBCa adenocarcinoma 90 * 35 * 30 mm.	GBCa, undifferentiated carcinoma	T3N1Mx Stage IV
15	49 F	OJ and hilar stricture	Mass	GBS + peritoneal Bx	GBCa adenocarcinoma 90 * 40 * 40 mm.	Metastatic moderately-differentiated GBCa, adenocarcinoma/peritoneal metastatic lesions	T4N1M1 Stage IV
16	58 M	Acute cholecystitis + empyema	Complicated cholecystitis	GBS	GBCa adenocarcinoma 112 * 52 * 31 mm, Fibropurulent exudate. Ulcerated mucosal surface. Wall thickness 9 mm.	Moderately-differentiated GBCa, adenocarcinoma. Cystic duct and node involved + chronic cholecystitis.	T2N1M0 Stage III
17	72 F	Gangrenous GB	Complicated cholecystitis	GBS + Omentum+ Liver Bx	GBCa adenocarcinoma (subtotal cholecystectomy) 30 * 25 * 20 mm. No Macro.	Metastatic well-differentiated GBCa, adenocarcinoma + cystic node. Omental metastasis.	T4N1M1 Stage IVB
18	74 F	Cholecystitis	Cholecystitis	GBS	GBCa 55 * 32 * 7 mm. No Macro.	GBCa, adenocarcinoma. No details about margin status.	No details.
19	69 M	GB mass	Mass	GBS + Liver Bx	GBCa 47 * 12 * 14 mm. No Macro.	GBCa, undifferentiated carcinoma. No details about margin status/liver.	No details. Stage IV
20	76 F	Cholecystitis	Cholecystitis	GBS	GBCa. No Macro.	GBCa, adenocarcinoma. No details about margin status.	No details.

Interpretation is from clinical information provided on request forms.
Undifferentiated carcinoma is inherently Stage IV disease, according to AJCC 2010.
Abbreviations: Bx, biopsy; CT, computed tomography; GB, gallbladder; GBCa, gallbladder cancer; GBS, gallbladder specimen; LND, lymph nodes; No macro, macroscopic details not provided or incomplete; OJ, Obstructive Jaundice; TNM, tumour, node, metastasis

4.3 Adult Data Analysis Results

Further analyses of the results were conducted to identify relationships between these factors:

i. Correlations between age and sex, and histopathologic diagnoses

ii. Correlations between histopathologic diagnoses and GBCa

iii. Multivariate relationships between the above. For example, are elderly males with complicated cholecystitis more likely to have GBCa than the total population of this study?

4.3.1 Correlation statistical analysis

With the IBM SSPS software, multiple result sets were analysed using the cross-tabulations statistical analysis tool. The cross-tabulation results according to sex are shown in Table 4-7.

Table 4-7: Cross-tabulation Results for Sex and Histopathology Group

Histology Group ↓	Sex					
	Male			Female		
	Number of specimens with disease	% of gallbladder diseases in sex	% of total number with disease	Number of specimens with disease	% of gallbladder diseases in sex	% of total number with disease
Benign	257	95.5%	21.5%	902	97.5%	75.5%
Premalignant	4	1.5%	0.3%	11	1.2%	0.9%
Malignant	8	3.0%	0.7%	12	1.3%	1.0%
Total	269	100%	22.5%	925	100.0%	77.5%

4.3.1.1 Cross-tabulation results for males

The number of GBS with malignancy in the male subgroup was eight, which represented 3% of gallbladder diseases in males but only 0.7% of the total number of specimens analysed in males. Using the IBM software cross-tabulation analysis tool to analyse all data collected for this study, the expected number of GBCa cases in males was 4.5, suggesting that a higher number of

malignancies were diagnosed than expected. However, these small numbers prevent firm conclusions.

4.3.1.2 Cross-tabulation results for females

The observed number of specimens with GBCa in the female subgroup was 12, representing 1.3% of female specimens but only 1% of the total specimens sent in females. From the cross-tabulation analysis, the expected number of specimens with GBCa was 15.5. Again, the small numbers prohibit definitive conclusions.

4.3.1.3 Cross-tabulation results for both sexes

Previous reports indicated that GBCa is more common among older women, with a 3:1 female to male prevalence ratio [32-34]. This contrasts with the results of the current study, in which the relative prevalence of GBCa was higher in males: malignancy was more common than expected in males and less common than expected in females. The reason(s) for this disparity from the literature are unclear, and the numbers are too small to generate firm conclusions; however, the discrepancy may reflect a different disease distribution in the South African population and the possible importance of other variables, such as genetics or HIV infection, which were not evaluated in this research project.

Age, as an independent variable, was significantly associated with the development of GBCa (see Table 4.8). This is consistent with clear association between GBCa and advanced age reported in the literature.

4.3.1.4 Multivariate analysis.

Multivariate nonparametric analyses, including Spearman's and Kendal's correlation analyses, showed statistically significant relationships between age and GBCa when sex was factored in as an added dependent variable, as shown in Table 4 8.

Table 4-8: Spearman's and Kendall's Correlation Analysis Results

Statistical Test			*Malignancy Diagnosis*
Kendall's Tau-B	Age	Correlation coefficient	0.059*
		Significance (2-tailed)	0.013
Spearman's Rho (combined age and sex)	Age	Correlation coefficient	0.072*
		Significance (2-tailed)	0.013
	Sex	Correlation coefficient	-0.049 (female likelihood)
		Significance (2-tailed)	0.089
		Number of variables analysed	1194
*** Correlation is significant at the 0.05 level (2-tailed).**			

Female sex seems to be a risk factor for GBCa, but only in combination with age as a covariable, as shown by a linear-by-linear association with a statistical significance of 0.058 for the risk of finding GBCa in females (Table 4-9). This observation is consistent with findings published in the literature [17,33-34]. There was no statistically significant correlation between age and sex with regard to the frequency of GBCa.

Table 4-9: Sex Chi-square Tests

Test	*Value*	*Df*	*Asymptotic Significance (2-tailed)*
Pearson chi-square	3.728	2	0.155
Likelihood ratio	3.277	2	0.194
Linear-by-linear association	3.584	1	0.058
Abbreviation: DF, degrees of freedom			

4.3.1.5 Multinomial regression analysis

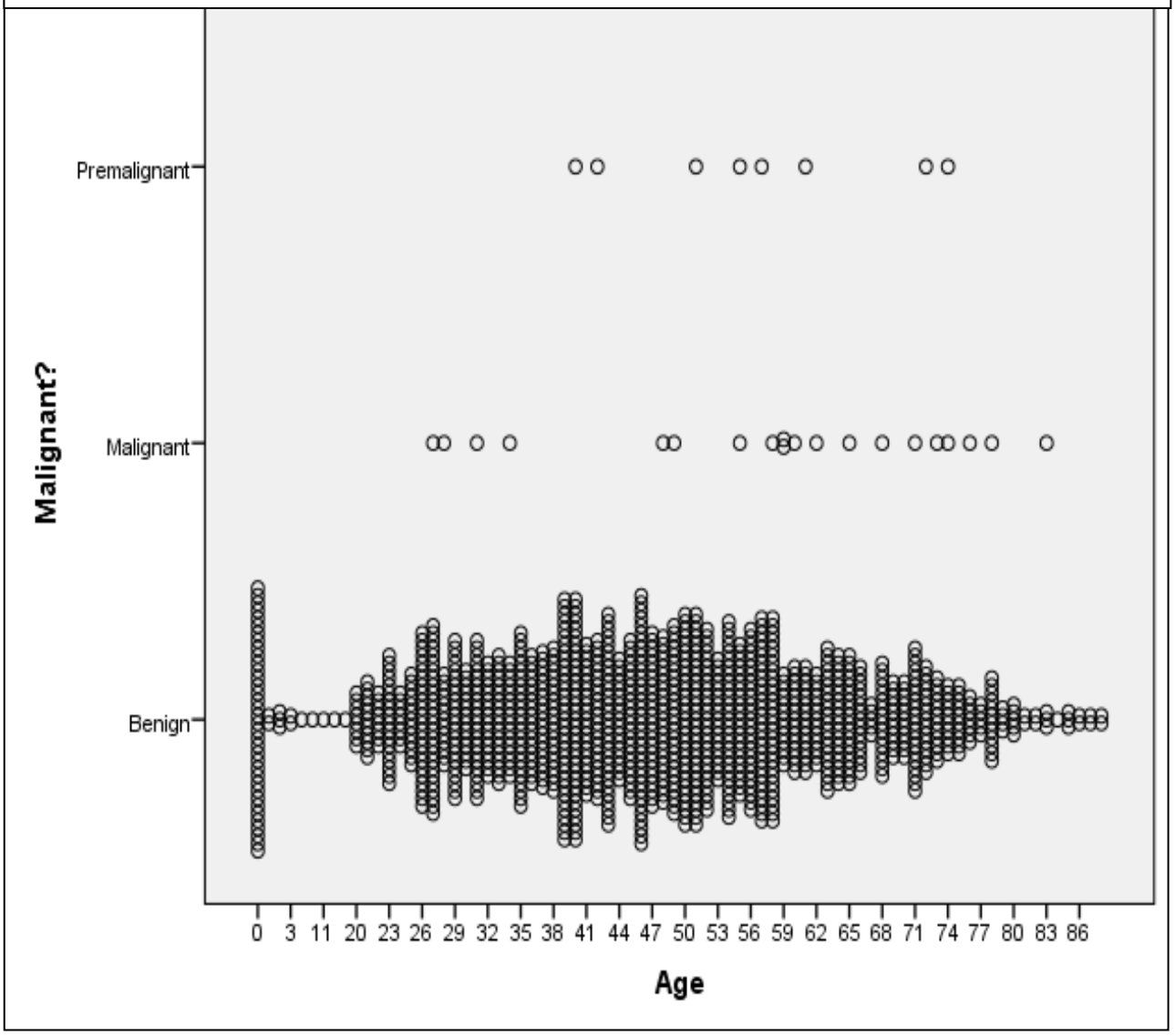
Multinomial regression analysis of the data confirmed advanced age and female sex as linked co-factors associated with GBCa. Using Kendall's Tau-B^{TM} tool, advanced age as a single variable was significantly associated with the diagnosis of GBCa, as shown in Table 4-8. Using the Spearman's

Rho^{TM} analysis tool, the significant association between advanced age alone and the diagnosis of GBCa was confirmed.

When age and sex were combined as co-factor variables using the Spearman's Rho^{TM} analysis tool, advanced age and female subgroup were found to be significant covariables (Table 4.-8). A specific age at which risk was increased could, however, not be determined because of the small sample size of patients diagnosed with GBCa.

There was no statistically significant relationship between GBCa and premalignant lesions in this study and notably, GBCa was diagnosed more commonly than ‘premalignant’ disease, as shown in Figure 4.4. This result is consistent with the reported poor association between GBCa and premalignant lesions. The lower prevalence of premalignant diseases also suggests that natural progression of GBCa from these disorders is unlikely, and the aetiology and pathogenesis of this malignancy remain unknown.

Figure 4.4: Scatter distribution of histopathology groups



The number of incidental cases of GBCa was low, and statistical analyses provided little information in terms of associations. Future studies in this regard, spanning a longer period and including larger data subsets, may increase the yield and significance of such analyses. Efforts to unmask associations were, therefore, abandoned for the purposes of this study.

4.4 Paediatric Results

Forty-eight (48) specimens were sent from paediatric patients (age <18 years) who underwent cholecystectomy; 27 specimens were from females and 21 were from males. The indications for cholecystectomy were not documented, but key observations in the paediatric subgroup include the following:

- 44 specimens were from children under 10 years of age, with 36 obtained from children under the age of 1 year.
- 39 specimens were received from CHBAH, 8 from CMJAH, and 1 from HJH.
- The most frequent histopathology finding was acute cholecystitis, noted in 25 specimens.
- Congenital abnormalities were diagnosed in 23 specimens.
- Choledochal cysts were found in two specimens.
- Granulomatous inflammation was found in two specimens.
- Cytological atypia was reported for one specimen.
- No malignancies were found.

5 DISCUSSION

The number of adult GBS sent for histopathology examination from the three hospitals over the 3-year study period was 1194. This is consistent with other reports regarding the histology of GBS, as listed in Table 1-1; however, there were no similar published studies from South African institutions.

The most common histopathologic finding in adults was acute cholecystitis. It was diagnosed in 705 specimens, representing 59.04% of the total number of specimens analysed. Chronic cholecystitis was found in 33.58% of the specimens. These results are consistent with those of previous similar research studies [7,9,16]. Other conditions detected in this study results were rare, not associated with any malignancy, and required no further treatment beyond simple cholecystectomy.

The incidence of GBCa in this study was 1.67%. This is consistent with the reported incidence range of 0.27% to 3.46%, as shown in Table1-1. Twelve of the 20 cancer specimens exhibited evidence of locally advanced disease, but none of the 20 patients with GBCa underwent extended resection after the first cholecystectomy. Twelve specimens (60% of the GBCa diagnosed in this study) were at least stage III or IV, according to the 2010 TNM AJCC classification. This stage assignment was based on the tumour subtype and extent of spread observed in the histopathology sections. No clinical records were analysed. Eight specimen reports did not have enough information for correct disease staging.

Thirteen of the 20 patients with GBCa had features suspicious for malignancy, thus the number of incidental GBCa in this study group was seven, representing a rate of 0.59%. The most common feature raising suspicion for GBCa was a pre-operative or intra-operative finding of a gallbladder mass. One of the 13 specimens had only suspicious lymph nodes, with no documented gallbladder mass.

Adenocarcinoma and undifferentiated carcinoma were diagnosed in 15 and four GBS, respectively. Further review of clinical notes and patient follow-up was not performed. However, current outcomes of GBCa are generally poor, regardless of the therapeutic modality.

Some authors have recommended the selective submission of GBS based on various factors [7] [9][16]:

- Patient characteristics and/or
- Specimen macroscopic appearance (immediately post-cholecystectomy),
- Patient age and sex (as previously discussed), and
- Abnormal features suspicious for GBCa, such as gallbladder mass, polyp, thickened wall, or evidence of calcification (using pre-operative imaging or gallbladder examination immediately post-cholecystectomy by the surgeon).

Eight cases diagnosed with early disease in the current study may have been effectively cured with cholecystectomy. A review of post-therapy clinical notes and radiological reports was not performed but may have provided information regarding this. It is worth noting that after searching the NHLS database, no subsequent GBS were submitted for patients found to have GBCa on the initial specimens, suggesting that no further surgery was performed. However, this would require review of clinical notes for confirmation.

The numbers observed in this study suggested a higher relative prevalence of GBCa in males than in females, with eight of the 20 diagnosed cases being male (males had a higher than expected rate of GBCa diagnosis). Multivariate analysis of all data, however, revealed that female sex and advanced age, when combined, were associated with a higher likelihood of GBCa. The specific age associated with an increased risk could not be inferred because of the small number of patients diagnosed with GBCa. As independent variables, sex was not significantly associated with GBCa. Correlation statistical analysis evaluating demographic data, and benign or pre-malignant

gallbladder diseases did not reveal any significant other associations with GBCa. The natural history of GBCa arising from 'premalignant' lesions was not supported by this study's data and, therefore, seems unlikely. A premalignant lesion was detected in only one specimen with GBCa. Of note, the reliability and generalisability of any findings relating to GBCa in this study are limited by the small sample size.

5.1 Cost Implications

The cost of histology examination of a GBS according to the RSA Department of Labour for 2016 [96] is R432.00 per specimen and R250.56 for each additional section. Some pathology units routinely process three sections per GBS, but quantifying the exact cost of processing the 1194 adult specimens sent in this study was not possible, as the number of sections processed per specimen was unknown. Based on three analysed sections, a prudent minimum total cost for the analysis of 1194 GBS is estimated at R1114 145.28 (1194 × R933.12), without considering the possibility of analysing more sections or performing additional special investigations. These numbers are, however, crude estimates. A health economics costing study of this process and escalating annual healthcare costs would be necessary to provide more exact values [96,98]. The cost associated with histological diagnosis of the seven cases of incidental GBCa is estimated to be approximately R159163.61.

Additional implications of the time taken to process GBS, as previously discussed by the Royal College of Pathologists in the UK [5], were not quantified but need to be weighed against the training of pathology registrars and specialists.

Other issues worthy of consideration in future studies would be cost-effectiveness analyses, determination of quality adjusted life years, game theories, and other health economic assessment tools to determine the utility of the expenditure for histopathologic analysis of GBS when the utility gains seem questionable [20,99]. The opportunity costs are certainly not minor when one considers

that this paper considered only three hospitals; there are also many other private and public institutions in which surgeons routinely submit GBS as standard practice.

5.2 Study Limitations

This study has several limitations, including the following:

i. The breadth of information regarding the patients' demographic and other health status data was limited. Variables such as race, ethnicity, and HIV status were either not recorded or unavailable. The effect of these important variables on gallbladder disease profile, especially in the South African context, remains unknown and would provide valuable healthcare insight.

ii. The level of details in the pathology request forms was not standardised. In particular, documentation of indications for surgery, pre-operative imaging results (modalities and standardised format findings), and intra-operative findings were not routinely documented on the histology request forms. Reporting standards in general require urgent attention across the medical fraternity, possibly commencing at the medical undergraduate level with intense incorporation of technology into clinical practice. This, it is firmly believed, would significantly improve research output, accuracy, and generalisability.

iii. The level of details pertinent to accurate staging was variable. Macroscopic features, lesion size, resection margin status, and histopathologic tumour subtype were not uniformly reported. Information regarding the surgeons' intra-operative evaluation of the GBS was not available or documented.

5.3 Recommendations

I. A detailed and thorough report of the clinical, radiologic, and surgical findings should accompany all GBS when submitted to the laboratory for examination.

II. A standardised histopathology reporting format for GBS should be adopted.

III. Cost-effectiveness studies regarding submission of GBS should be conducted to determine the utility and true cost implications of this practice, given the apparent low positive outcomes of these examinations, as it remains unclear whether further treatments based on the histopathology results would offer any tangible clinical benefit.

6 CONCLUSION

The research described in this report sought to answer three questions. The spectrum of gallbladder diseases diagnosed by histologic analysis of GBS was discussed and compared to the existing literature. Acute cholecystitis was the most commonly diagnosed gallbladder disease. Acute and chronic cholecystitis were found in 56.5% and 25.7% of all adult specimens, respectively. GBCa was found in 20 (1.67%) of the 1194 adult GBS submitted for histopathology analysis over the 3-year study period, providing answers to the first and second questions. To answer the third question, incidental GBCa was found in 7 (0.59%) of the 1194 adult specimens. The most common feature raising suspicion for GBCa was a gallbladder mass noted pre- or intra-operatively, although one of the 13 specimens had only suspicious lymph nodes.

Advanced age, in combination with female sex, was found to be associated with GBCa, although the number of cancers in this study was small, prohibiting generalisability of these data. The most common histological type in GBCa specimens was adenocarcinoma. Macroscopic features suggestive of GBCa in GBS could not be determined.

With the poor outcomes associated with GBCa, routine histopathologic examination of GBS to unmask the seven incidental GBCa cases confers little clinical benefit when one considers the poor prognosis and poor responsiveness of this cancer to all available therapies. The routine submission of all GBS, therefore, cannot be justified based on the literature review and the results of this study.

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8 APPENDICES

8.1 Appendix 1 Data Collection Sheet and Legend for Analysis Coding

Specimen ID #

Gender	M
	F
Operation centre	1 CMJAH
	2 HJH
	3 CHBAH
Operation date	
Histo-pathological diagnosis	1. Normal
	2. Acute cholecystitis
	3. Granulomatous inflammation
	4. Chronic cholecystitis
	5. Gallbladder empyema
	6. Acute haemorrhagic cholecystitis
	7. Xantomatous deposits
	9. Choledochal cysts
	10. Benign tumours (detail) polyps, cholesterolosis, adenoma, adenomyosis, heterotopia, + supporting (lipoma, leiomyoma, haemangioma, granular cell tumours)
	11. Cis, epithelial dysplasia and cytological atypical (premalignant)
	12. Kaposi's sarcoma
	13. Gb malignancy (detail)
	14. Trauma/ stab wounds
	15. Congenital biliary abnormality
	16. Complicated acute cholecystitis
	17. Other malignancy
	19. Lymph node - inflammation
	20. Secondary colitis
	21. Acute pancreatitis
	22. Porcelain gallbladder (calcified)
Basic tumour type	1. None
	2. Premalignant
	3. Malignant
TNM staging	1. T 1-4
	2. N 1-
	3. M 1
Margins involved	1. Yes
	2. No
Macroscopic features/ lesions	

8.2 Appendix 2 Human Research Ethics Committee (Medical) Clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Academic & Research)

MEMORANDUM

TO: **Dr Teboho T Mahlobo**
Division of General Surgery
EMAIL tt.mahlobo@gmail.com

FROM: Ms Anisa Keshav
Secretary: Human Research Ethics Committee (Medical)
Tel 717-1234 fax 0865532280
e-mail: anisa.keshav@wits.ac.za

DATE: 2 September 2013

REF: ***R14/49 (FOR OFFICE USE ONLY)***

The protocol below was considered at a meeting of the Human Research Ethics Committee (Medical) **on Friday 30 August 2013. Below please see the Committee's decision:**

M130841
Review of Histopathological Disease Profile of Gallbladder Specimens after Cholecystectomy

Approved subject to:

-Providing written permission to do the study from the head of anatomical pathology, and the CEOs of CHBAH and CMJAH.

-Removing identifiers from data collection sheet and keeping separate coding list with study numbers.

Please submit the above so I may issue your clearance certificate.

Please highlight any changes made and send two hard copies including a cover letter to this office.

Certificate

8.3 Appendix 3 Turnitin Report

