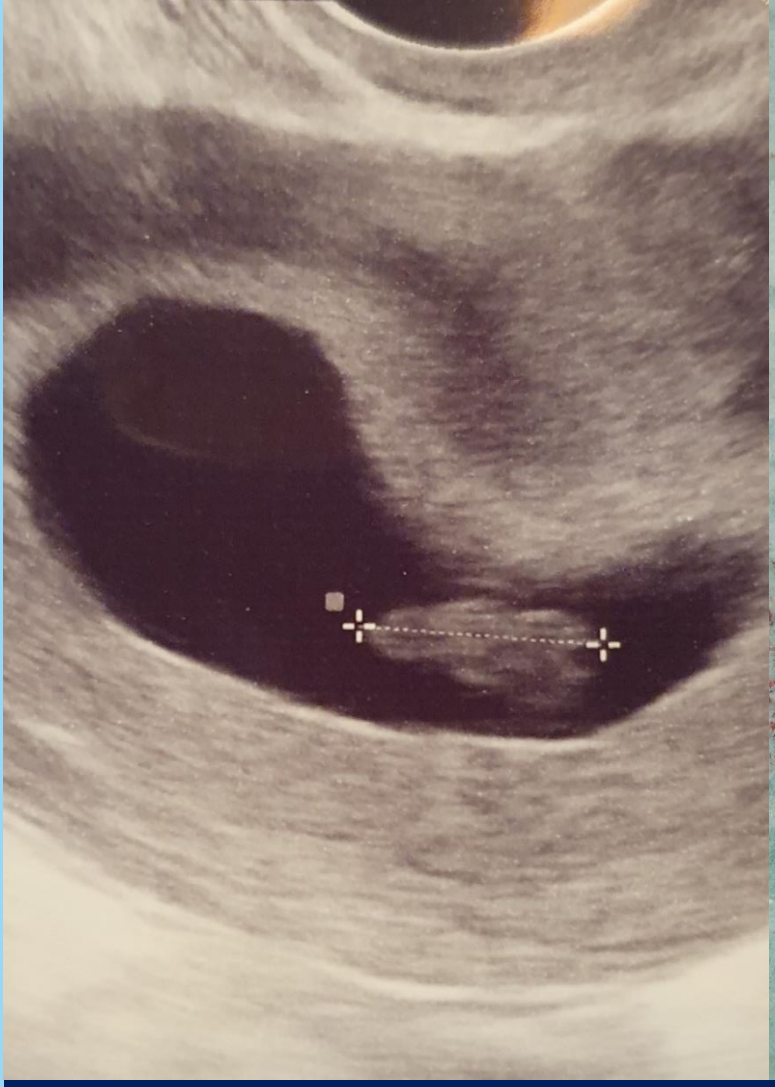

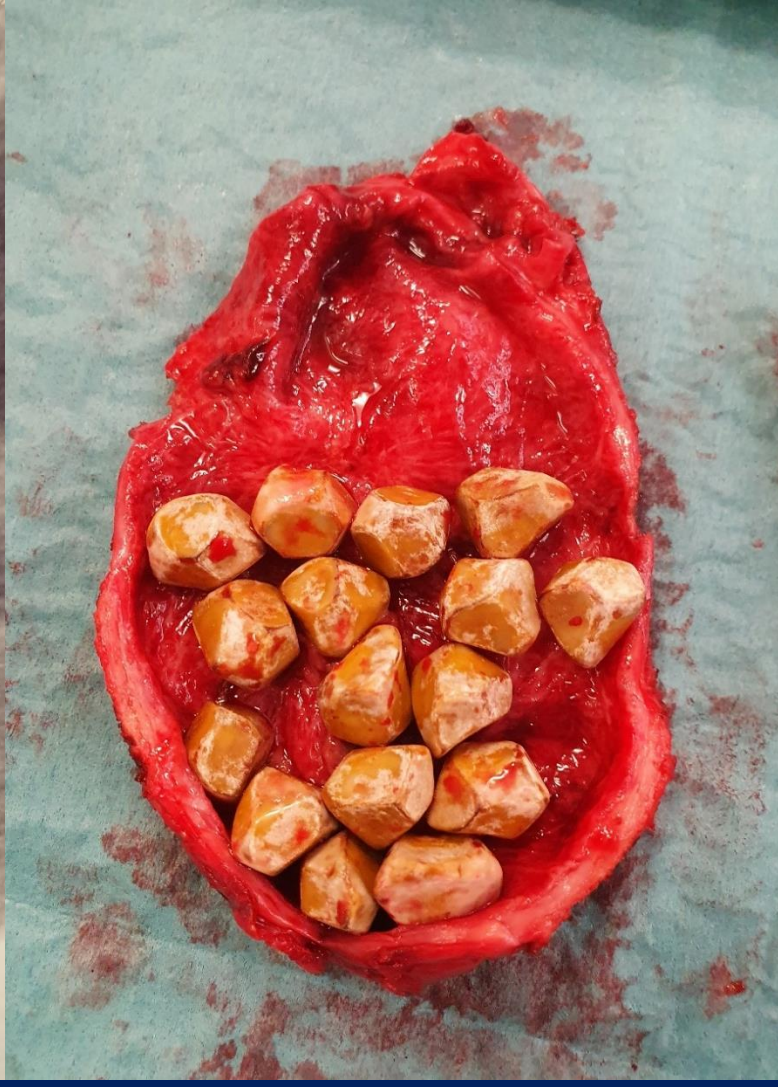

Gallstones:
From screen-detection or treatment to long-term morbidity

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V. Shabanzadeh DM, Skaaby T, Sorensen LT, Jorgensen T. Screen-detected gallstone disease and cardiovascular disease. Eur J Epidemiol. 2017;32:501-10.
VI. Shabanzadeh DM, Linneberg A, Skaaby T, Sorensen LT, Jorgensen LT. Screen-detected gallstone disease and autoimmune diseases - A cohort study. Dig Liver Dis. 2018;50:594-600.
VII. Shabanzadeh DM, Sorensen LT, Jorgensen T. Association between screen-detected gallstone disease and cancer in a cohort study. Gastroenterology. 2017;152:1965-74.
VIII. Shabanzadeh DM, Martinussen T, Sørensen LT. Development of upper gastrointestinal cancer in patients with symptomatic gallstones, cholecystectomy, and sphincterotomy: A nationwide cohort study. Scand J Surg. 2022;111:39-47.

Roman numerals are used for references to studies in the text.

Contents

Preface

The studies of this thesis were performed in the period 2014-2022 at the Research Unit of the Digestive Disease Center and at the Center for Clinical Research and Prevention at Bispebjerg and Frederiksberg Hospital. During my time as a Ph.d. fellow, a continuous stream of ideas for new projects and support from mentors provided the pillars for the extension of my research. The studies could not have been performed without some persons that require special mentioning.

My warmest and heartfelt gratitude to Torben Jørgensen and Lars Tue Sørensen. Your selfless mentoring during my junior years was priceless and continues to be a huge inspiration to me. Thanks for the companionship, commitment, friendship, endless discussions, and for always providing your honest opinions. Torben, thank you for teaching me rigorous scientific methodology and that scientific progress should be driven by idealism, sustainability, and humanity – I shall remember it! Also, for having the determination to perform the first gallstone screening cohort in Denmark over 40 years ago. Lars, thanks for guiding directions in both science and surgery for, once, a very young medical student and for, still, inviting me in on your ideas. I hope to continue our teamwork.

I send my appreciations to the people at the Center for Clinical Research and Prevention for your open doors and helpfulness. Especially to Allan Linneberg and Tea Skaaby for your rewarding collaboration. To the many people at or passing through the Research Unit of the Digestive Disease Center including research nurses and office buddies. Thanks for numerous talks over coffee – both clarifying and off-topic. Particularly, to Lars Nannestad Jørgensen and Bonna Leerhøy for all your help. To Torben Martinussen at the University of Copenhagen for helping me handling my largest dataset so far and for your highly specialized statistical insights.

Thanks to my great colleagues at the departments for surgery and gastroenterology in both Hillerød and Hvidovre Hospital for providing surgical training during years of research and for still supporting the ongoing collaborations in research and work.

My dearest gratitude to my dear mother Karin for your endless helpfulness and for proofreading my manuscripts. Finally, my deepest appreciation to my beloved partner Allan and our kids Ester and Adam. Thank you for your support despite periods of distractions and absences.

Daniel Mønsted Shabanzadeh
Copenhagen, November 2023

Introduction

The natural course of gallstones has been debated since the beginning of the 20th century and the idea of the innocent asymptomatic gallstone has ranged from acceptance to myth[1-3]. The course of gallstones has been studied in both clinical and general populations and reported with high incidence rates for development of symptomatic gallstones[4-7]. The natural course of gallstones in a population uninformed of gallstone status has not been reported before. Inadequate knowledge on the natural course of gallstones and a lack of determinants for symptomatic gallstones have limited the development of management strategies for gallstones including indications for surgical treatment. The overall aim of this thesis was to describe the natural course and consequences of gallstones in a population uninformed of gallstone status including the development from asymptomatic to symptomatic gallstones and the development of long-term morbidity and mortality for surgically treated and untreated gallstones (Figure 1).

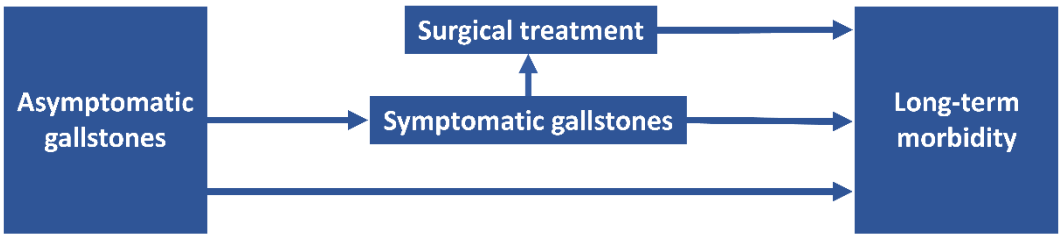


Figure 1: The natural course and consequences of gallstones

Gallstones are common in the general population of Denmark with a prevalence of 10-20% depending on age and sex[8]. The standard treatment for symptomatic gallstones is cholecystectomy – a surgical removal of the gallbladder[9]. In the presence of common bile duct stones (CBDS), the most performed and recommended treatment is endoscopic retrograde cholangiography (ERC) with the aim of performing therapeutic sphincterotomy of the sphincter of Oddi and stone extraction[9, 10]. Annual cholecystectomies have more than doubled in the period 1995-2015 in Denmark and over 9000 cholecystectomies were performed in 2015[11, 12]. Similar developments have been seen in other European countries[13, 14]. The increase has partly been ascribed to the introduction of laparoscopic cholecystectomy in the 1990'ies[15, 16]. The gain in popularity for laparoscopic surgery may partly be due to a significantly shorter hospital stay and a quicker convalescence when compared to the traditional open cholecystectomy[17]. ERC rates have also been rapidly increasing within the recent decade, especially in North America[18, 19].

The clinical course of gallstones can be asymptomatic or symptomatic. Symptomatic gallstones may have an uncomplicated or complicated course. Complications include acute cholecystitis, CBDS, cholangitis, pancreatitis, and rarely also bowel obstruction. Clinical uncomplicated gallstones include symptomatic gallstones in the absence of complications[20]. Symptoms ascribed to gallstones have traditionally been termed "biliary colic" and were described by Sir William Osler. This complex of symptoms consists of pain attacks localized in the upper right abdominal quadrant (right hypochondrium or epigastrium), with projection to the back, of longer duration, and of higher intensity[2]. Similar definitions of biliary pain in symptomatic gallstones have been accepted in later studies of screen-detected gallstones[7, 21]. It has generally been challenging to associate gallstone prevalence with abdominal symptoms in studies of general or clinical populations[22]. Mechanisms believed to cause symptoms are not fully understood, but may include gallstone passage to the duodenum or obstruction of the gallbladder or bile ducts causing biliary distention[1, 2] which is believed to activate visceral sensory neurons and sensation of pain[23].

Cholecystectomy is effective for treatment of biliary colic as identified in systematic reviews with meta-analysis of prospective clinical trials[24]. However, high proportions of 30-40% will experience remaining symptoms of upper abdominal pain or other abdominal symptoms following cholecystectomy[25, 26]. Functional bowel symptoms such as diarrhea, constipation[25], irritable bowel syndrome[27] will persist, dyspeptic symptoms may persist or debut[27-29], while others such as flatulence may debut following cholecystectomy[25]. Selection of patients for cholecystectomy with uncomplicated gallstones and presumed symptoms due to gallstones is currently limited by insufficient knowledge about which patients are likely to improve after cholecystectomy[24, 30]. Non-operative observation for symptomatic uncomplicated gallstones has been demonstrated feasible in a randomized controlled trial (RCT)[31, 32]. The indication for cholecystectomy is therefore relative in presence of uncomplicated disease[33]. On the other hand, recommendations for treatment of clinically complicated gallstones generally includes cholecystectomy[9, 33]. Intra- and postoperative complications for cholecystectomy as seen in larger clinical databases are about 8% overall. The risk of bile duct injuries requiring supplemental interventions is about 0.3%[34-36].

Gallstones are associated with long-term morbidities and the earliest reported associations were for gallbladder cancer and cardiovascular disease (CVD)[1, 37]. Since then, numerous studies based on clinically diagnosed gallstones have been published on the development of other gastrointestinal cancers and CVD[38-40]. These associations have been suggested due to common etiological factors, mimicry in clinical presentations explaining short-term associations, or other detection bias rather than being causal[37, 41-44]. The theory that cholecystectomy rather than gallstones promote mechanisms of cancer development was also suggested early[43, 45].

Common mechanisms for gallstones and long-term morbidities have not been identified, but more recent studies have linked gallstones to a variety of disease pathways. These include biomarkers for obesity such as insulin resistance and systemic inflammation[46-49]. Genetic studies including genome-wide exploration have also found associations for gallstones and genetic variations for cellular receptors such as the ATP-binding cassette transporters G5 and G8 *(ABCG5/ABCG8)* which facilitates biliary and intestinal cholesterol transport[50], the UDP glucuronosyltransferase *(UGT1A1)* which conjugates bilirubin[51], and the farnesoid X receptor (FXR) *(NR1H4)* which regulates intestinal reuptake and hepatic secretion of bile acids[52, 53]. Changes in cholesterol and bilirubin metabolism due to these variants have also been associated with gallstones[54, 55].

The objectives of this thesis were to

1. determine the cumulative incidence proportion (CIP) for development from asymptomatic to symptomatic gallstones
2. identify determinants of symptomatic gallstones
3. determine mortality and long-term development of CVD, autoimmune disease, and gastrointestinal cancer for persons with treated and untreated gallstones as compared to persons without gallstones
4. determine the impact of treatments for symptomatic gallstones on the development of upper gastrointestinal cancer (biliary, gallbladder, hepatic, pancreatic, esophageal, gastric, duodenal, and small bowel) when compared to the background population.

The thesis comprises epidemiological cohort studies. A general population sample screened for gallstones without being informed about gallstone status was used for objectives 1.-3. and a nationwide registry-based cohort was used for objective 4.

Methodological considerations

Terminology for gallstones in this thesis

Due to the existence of several sources for identification of gallstone presence in cohort studies, the following terms for gallstones are used:

1. *Clinically diagnosed gallstones* are derived from clinical registries or hospital records and identified during hospital admissions. Entries may be based on registry codes for diagnosis or treatments, positive radiology examinations, or surgical findings.

2. *Self-reported gallstones* are derived from study questionnaires and defined as either having a previous positive examination or cholecystectomy.
3. *Screen-detected gallstones* are derived from systematic screening in an entire population with ultrasound or other radiology examinations performed.

The term *gallstones* refer to gallbladder stones (cholecystolithiasis).

Symptomatic and asymptomatic gallstones in cohort studies

An early observation for symptomatic gallstones was that once symptoms have developed, they will prevail unless gallstones are surgically treated[56]. More recently, two RCTs from Norway explored observation versus cholecystectomy for symptomatic gallstones and demonstrated the feasibility of observation, but also that a high proportion will need cholecystectomy. Of those allocated to observation at baseline, 51% with uncomplicated gallstones[31, 32] and 33% with complicated acute cholecystitis needed cholecystectomy during a 14-year follow-up[57, 58]. A clinical observational study from Sweden which included predominantly patients with symptomatic uncomplicated gallstones not treated at baseline presentation showed that 41% needed surgery during six years of follow-up[59]. In a large study from the US of insurance claims from persons with acute cholecystitis initially treated non-operatively, 29% needed cholecystectomy during a two-year follow-up[60]. Thereby, the course of clinically diagnosed gallstones, both uncomplicated and complicated, seems to be eventful with high risk of readmission and treatment during follow-up. Further, from a clinical point of view one may speculate that clinically diagnosed gallstones are identified through a positive clinical examination which most likely was indicated by symptoms caused by gallstones.

Cohort studies exploring the impact of gallstones on long-term morbidity have traditionally included clinically diagnosed or self-reported gallstones such as the Framingham study[61]. However, clinically diagnosed gallstones represent only a smaller selected population with symptomatic gallstones whereas most persons with gallstones remain unidentified and thereby misclassified. Furthermore, only a fraction of persons with gallstones will develop symptoms and we assume that this subgroup differs from those that remain asymptomatic. Consequently, including only clinically diagnosed gallstones is with risk of differential misclassification bias. Such a bias may cause estimates to become both significant and non-significant[62].

Clinically diagnosed gallstones are therefore inappropriate for exploring the natural course of gallstones and symptom development, due to symptoms being present at baseline with a higher risk of future clinical events and due to misclassification of asymptomatic gallstones as no gallstones causing differential misclassification bias.

Screening of an entire population is the only method to identify all persons with gallstones in a population, both the asymptomatic and symptomatic. Based on the bias caused by clinically diagnosed gallstones, screen-detected gallstones are considered superior for studying long-term gallstone exposures in this thesis.

The definite verification for presence of gallstones is surgery. However, surgery is inappropriate and too invasive for the purpose of screening in a study. At ultrasound examination, gallbladder stones are echo-dense structures in the gallbladder lumen (see figures on front page), that cast acoustic shadows and move with gravity unless impeded by size or wedged into the infundibulum. Non-visualization of the gallbladder lumen may occur, but gallstones may still be found as echo-dense structures with acoustic shadows[8]. Ultrasound examination is the most accurate non-invasive diagnostic examination for gallbladder stones with the highest sensitivity and specificity of 97% and 95%, respectively[63]. Ultrasound therefore is the superior method for screening larger populations. Misclassification of gallstone status is minimized at ultrasound when compared to alternative radiology modalities.

At the outcome level, symptomatic gallstones have in previous cohort studies of screen-detected gallstones been defined as either a cholecystectomy or other surgical treatments during follow-up[64, 65]. Alternatively, by presence of specific gallstone-related symptoms in questionnaires[6, 7, 66] and vice versa for the asymptomatic gallstones. Answering symptom questionnaires when gallstone status is known following ultrasound examination at either baseline or follow-up examinations is with risk of recall bias causing overestimation of symptomatic gallstones. Studies included in this thesis defined symptomatic gallstones as hospital admissions with gallstone events – a method free or recall bias.

Study design

For the study of the natural course of gallstones and the long-term morbidity of untreated and treated gallstones, the epidemiological cohort study design was considered the most appropriate for the studies of this thesis. Although other observational study designs such as the case-control or cross-sectional frequently have been performed in the past[67-69], they cannot explore the long-term temporal associations of interest in this thesis. The RCT study design is generally considered superior for exploration of causality and could, in theory, explore the long-term morbidity of treated versus untreated gallstones. However, the large sample size and long follow-up period required for the outcomes of interest to occur is challenging, if not impossible, to perform in the more tightly scheduled RCT design.

To determine development of symptomatic gallstones, mortality, and long-term morbidity in Studies I-VII, general population samples from the Western part of suburban Copenhagen were explored. These were randomly drawn from the Danish Civil Registration System. The cohorts were part of the international MONICA (Multinational mONItoring of trends and determinants in CArdiovascular disease) collaboration which explored general populations. Participants were informed about the aim of the study but were not informed about findings of gallstone disease or other benign conditions in the gallbladder following ultrasound examination to avoid unnecessary treatment and worrying. The study conformed to ethical guidelines and was accepted by the local research ethic committee[8]. At baseline, detailed examinations for gallstones, cholecystectomy, and physiological variables were performed and data on lifestyle, abdominal complaints, medical history and social status were collected[70]. Examinations were performed on the Research Centre for Prevention and Health at Glostrup Hospital which now has become the Centre for Clinical Research and Prevention at Frederiksberg Hospital. Detailed descriptions of the baseline cohorts of MONICA I[8], re-examination 1[71], re-examination 2[16], 1914 Cohort[72], and MONICA III[16] have been published before. To explore the development of the subgroup of upper gastrointestinal cancers following treatments for symptomatic gallstones required a very large population due to these cancers being rare. To gain high enough sample size for this purpose in Study VIII, a nationwide cohort of all persons born in Denmark 1930-84 was drawn from the Danish Civil Registration System (Table 1). Other studies based on MONICA I, re-examinations, and the 1914 cohort exploring outcomes of gallstone prevalence and incidence have been published and used in other theses before[73, 74].

Long-term follow-up was performed through national registries. The Danish Civil Registration System contains daily updated information on vital status and migration of the entire nation[75]. The National Patient Registry contains data on all hospital admissions from the year 1977 and emergency room visits were included from 1995. The National Patient Registry includes codes for diagnoses according to the International Classification of Disease (ICD 8 or ICD 10) and codes for surgical interventions according to the Nordic Classification of Surgical Procedures. Codes are registered at the time of discharge from the hospital[76]. The Danish Register of Causes of Death includes one underlying cause of death with up to three contributory causes according to the ICD and has been doing so since 1970[77]. Every permanent resident of Denmark has a unique personal registration number which enables linkage between registries. Since clinical registries are dependent on correct coding performed by the clinician that treats the patient, there may be a potential risk for outcome misclassification when using registry follow-up. However, this risk may be outweighed by the unique possibility to perform complete long-term follow-up evaluation of larger population samples as performed in studies of this thesis.

	Sampling year	Baseline examination period	Age at baseline (years)	Invited (N)	Participants (N)	Gallstones at ultrasound (N)	Cholecystectomy at baseline (N)
MONICA I	1982	1982-84	30-60	4807	3785	216	106
MONICA I, re-examination 1	1982	1987-88		3608	2987	+68	-
MONICA I, re-examination 2	1982	1993-94		4130 including re-invitation of 1982 sample	2656	+194	-
1914 cohort	1984	1984-85	70	540	374	59	32
MONICA III	1991	1991-92	30-70	2500	1777	127	51
Nationwide cohort	2016	-	≥30	-	4 465 962	-	-

Table 1: Cohorts used in the studies of this thesis

Statistical methods

Descriptive analyses were reported as unadjusted CIPs of outcome events and with death of unrelated causes as the competing event. Persons were censored when they had an outcome event, died, emigrated, or completed follow-up. Gray's test[78] was performed for comparing CIPs where relevant and significance level was set at a p-value < 0.05. Statistical analyses were performed with Cox regression[79]. As the underlying time scale, time followed-up was used in Studies I-VII and age in Study VIII. Further, time-varying exposures for gallstones, cholecystectomy and sphincterotomy were included in analyses of Study VIII. Hazard ratios (HR) with 95% confidence intervals (CI) were reported and significant associations were defined as a 95% CI not including one. Unadjusted or age- and sex-adjusted models were reported. Multivariable adjusted models were reported to control for possible confounding including variables presumed to be associated with both gallstones and outcome. At least 10 outcome events per parameter were required in multivariable models. Plots of scaled Schoenfeld or Martingale residuals were inspected to test goodness of fit and the assumption of proportional hazards[80]. The "R-studio" software (RStudio Inc, Boston MA) with packages "survival", "cmprsk", and "prodlim" were used for analyses and figures. Statistical analyses were planned and performed by the author of this thesis under supervision by a statistician for Studies I-VII. Analyses were performed by a statistician for Study VIII.

Results and discussion

The studies of this thesis are presented in the following sections including a review of other relevant published studies. The sections are structured according to the following order 1) what was known already, 2) what were the novel findings of studies in this thesis, 3) a discussion of the novel findings compared to other studies, 4) what has been published since the studies of this thesis, and 5) a short section summary.

Development of symptomatic gallstones

The natural history of gallstones with focus on the progression from asymptomatic to symptomatic gallstones has been the subject of numerous studies for decades. A variety of incidence rates for development of symptoms have been reported due to differences in study populations and methodology.

Two early reports from the US published in the 1940ies described the course of patients with clinically diagnosed gallstones identified during surgery for unrelated causes. During the primary operation, 12% of women had surgery for gallstones and 24% returned for cholecystectomy at a later stage due to development of symptoms[81]. The other study reported that 46% developed symptoms that could be ascribed to gallstones and that 21% required cholecystectomy[4]. Several studies published in the 1960ies followed patients with clinically diagnosed gallstones that were left untreated and identified through x-ray examinations such as cholecystography in hospital records. A Danish study following patients for 5-20 years found that one-third to one-half developed symptoms and that 25% required operation[5]. A study from the US with 15-30 years follow-up found that 51% eventually required cholecystectomy due to symptoms and that an additional 6% had symptoms, but remained unoperated[82]. A Swedish study followed patients for 11 years and found that 51% developed symptoms and that 35% needed cholecystectomy[83]. These early studies all included clinically diagnosed gallstones from clinical populations and comprised a mixture of patients being asymptomatic, symptomatic, or with unknown presence of symptoms at baseline. The reported incidence rates for symptom development and surgery were exceptional high compared with later reports.

It was not until the 1980ies that larger cohort studies were published. These included persons with clinically diagnosed gallstones identified at cholecystography and derived from larger databases. Persons were assumed to be asymptomatic at baseline due to no gallstone treatments registered

or no symptoms reported in their records. A study from Gracie and Ransohoff from 1982 included predominantly male and white faculty members from the University of Michigan and followed them up to 24 years. The cumulative probability of symptom development was 18% at 15 years follow-up. Need of cholecystectomy due to symptoms was 11% during the entire period. Prophylactic cholecystectomy without presence of symptoms was performed in 28%[84]. A study of subscribers of the Health Insurance Plan of greater New York followed for a mean of five years reported symptom development in 10% and need of surgery in 7%[85]. A study including patients from the Kaiser Permanente Medical Care Program of Northern Carolina with up to 25 years follow-up found that 19% developed clinical gallstone events and that 16% required surgery. The cumulative probability for an event was 41% at 20 years[86]. Although these populations may have been outpatients when compared to previous studies, the recruitment was still from health maintenance organizations and all gallstones were clinically diagnosed. Reasons for performing cholecystography were not reported. Although efforts were made only to identify the asymptomatic gallstones, these retrospective studies have most likely also included symptomatic gallstones at baseline.

With the development of the ultrasound examination technique, gallstones could be identified without x-ray exposure or surgery. From the 1980ies the technique had become readily available. Specific ultrasound screening-studies of larger general population samples could now be performed. The novelty of these studies was that they did not include selected clinical populations as previous studies did, but more representative general populations. Further, populations from screening-studies could now be followed prospectively as cohorts as compared to previous studies which had depended on retrospective data from hospital records or insurance claims.

Long-term follow-up reports of screen-detected gallstones were published from the 1990ies. The Group for Epidemiology and Prevention for Cholelithiasis in Italy (GREPCO) included civil servants in Rome and performed a 10-year follow-up through visits and questionnaires. Of the persons assessed as asymptomatic at baseline, a cumulative proportion of 26% developed symptoms based on questionnaires. Cholecystectomy was performed in 24% of which 13% were prophylactic and without presence of symptoms[6]. The same research group performed a cohort study with a 10-year follow-up of females sampled from electoral rolls in a rural town of central Italy. Of the initially asymptomatic women, 15% developed symptoms and 23% had cholecystectomy performed[66]. The Multicenter Italian Study on Cholelithiasis (MICOL) included persons through systemic samplings from electoral rolls in eight Italian regions and followed them through re-examinations with questionnaires. Of the initially asymptomatic persons, 22% developed symptoms during a mean follow-up of 9 years. A cholecystectomy rate for this subgroup was not reported, but the authors reported that a total of 24% of the cohort including both symptomatic and asymptomatic persons

at baseline had cholecystectomy during follow-up. Further, 14% of persons who remained or became asymptomatic had prophylactic cholecystectomy[7]. A study from Sweden performed follow-up through hospital records of a random sample of persons from one municipality. Of the persons with screen-detected gallstones, polyps, or sludge at baseline, a total of 11% had hospital admissions with gallstone symptoms during median seven years of follow-up, and surgery was required in 10%. This corresponded to a cumulative proportion of 8% that developed symptoms during the first five years[64]. A study from Norway followed a random sample of persons drawn from the National Peoples Registry in Bergen and performed a 24-year follow-up through hospital or national records. Of the original cohort with screen-detected gallstones, only 47% were alive and available for follow-up of which 43% had interim or current pain symptoms and 7% had cholecystectomy performed[65]. The study was limited by most persons being lost to follow-up and by not reporting CIPs. A study from Japan followed persons with screen-detected gallstones from a mass screening with ultrasound. During a mean of five years follow-up, 8% required cholecystectomy[87]. For a summary of findings from the available cohort studies, see Table 2.

All these previous cohort studies of ultrasound screen-detected gallstones informed their populations about presence of gallstones at baseline. It was argued that information about gallstone status was due to ethical reasons and to enable follow-up procedures such as cholecystography and ultrasound[6]. Some studies even referred persons with screen-detected gallstones to their family physician or recommended medical follow-up within one year[6, 66, 87]. One study discussed that this may have caused a protopathic bias increasing consultations with physicians and risk of symptom development and cholecystectomy[6].

Study I was the first published cohort study on the natural course of gallstones including a population uninformed of gallstone status following ultrasound examination. It included all 664 persons with screen-detected gallstones from MONICA I, MONICA I re-examinations, 1914 cohort, and MONICA III. Persons were followed-up for gallstones events for median 17.4 years (range 0.1-29.1 years) with a completion of 99.7%. A total of 20% had gallstone events during follow-up which corresponded to a cumulative proportion of 18% at 20 years. Complicated events such as acute cholecystitis, CBDS, or pancreatitis were found in 8%. Uncomplicated events defined as a gallstone diagnosis without a diagnosis indicating a complicated event were found in 12% where 7% had cholecystectomies. A total of 10% (n=69/664) had awareness of their gallstones at baseline. Awareness was found statistically associated with development of gallstone events during follow-up in both CIPs (Figure 2) and multivariable analysis (I). Awareness may reflect the higher risk of symptoms in persons with previous symptomatic gallstones as discussed earlier. It may also be

suggested that risk of symptom development is higher in persons aware of the presence of gallstones.

	Population	N subjects followed	Gallstone diagnosis	Folow-up (years)	Symptom development (total / CIP)	Cholecystectomy (symptoms / prophylactic)
Truesdell 1944 USA[81]	St. Luke's Hospital New York, female patients	50	Surgery for other causes	≈20	24%	24%
Comfort 1948 USA[4]	Mayo clinic patients	112	Surgery for other causes	10-20	46%	21%
Lund 1960 Denmark[5]	Copenhagen County Hospital Gentofte patients	526	X-ray, cholecystography	5-20	33-50%	25%
Ralston 1965 USA[82]	Mayo clinic patients	116	X-ray, surgery	15-30	57%	51%
Wenckert 1966 Sweden[83]	Patients at Allmänna Sjukhuset, City of Malmö	781	Cholecystography	11	51%	35%
Gracie and Ransohoff 1982 USA[84]	University of Michigan Faculty members	123	Cholecystography	11-24	13% / 18% at 15 y	11% / 28%
McSherry 1985 USA[85]	Health Insurance Plan of Greater New York subscribers	135	X-ray, cholecystography, ultrasound	Mean 5	10%	7%
Friedman 1989 USA[86]	The Keiser Permanente Medical Care Program, Northern California	123	Cholecystography	≈25	19% / 41% at 20 y	16%
Attili 1995 Italy[6]	Group for Epidemiology and Prevention of Cholelithiasis (GREPCO), civil servants from Rome	118	Ultrasound	10	24% / 26% at 10 y	11% / 13%
Angelico 1997 Italy[66]	GREPCO, random sample of females from electoral rolls in one rural town of central Italy	26	Ultrasound	10	15%	23%
Halldestam 2004 Sweden[64]	Randomly sample aged 35-85 from one municipality	123	Ultrasound	Median 7	11% / 8% at 5 y	10% had surgery
Festi 2010 Italy[7]	Multicenter Italian Study on Cholelithiasis (MICOL), systematic sampling from electoral rolls in 10 Italian regions	580	Ultrasound	Mean 9	22%	24% of entire cohort (793 persons) / 14% for those remaining asymptomatic
Schmidt 2011 Norway[65]	Random sample from National Peoples Registry in Bergen	134/285 alive and followed	Ultrasound	≈24	43% of living persons	7% of living persons

Inui 2016 Japan[87]	Oriental Clinic mass screening program	720	Ultrasound	Mean 5	NA	8%
Shabanzadeh 2016 Denmark (I)	MONICA cohorts, random samples from suburban Copenhagen	664	Ultrasound	Median 17	20% / 18% at 20 y	7% due to symptomatic uncomplicated disease
CIP, cumulative incidence proportion; NA, not available; y, years						

Table 2: Cohort studies exploring the development from asymptomatic to symptomatic gallstones and the need for cholecystectomy

Although definitions of symptomatic gallstones may differ between previous cohort studies, the incidence rate for development of symptomatic gallstones in Study I is the lowest reported. This suggests gallstones to be an uneventful condition with an asymptomatic course for the majority of carriers. The high incidence rates for development of symptomatic gallstones and especially for the uncomplicated ones in previous studies were in populations aware of gallstones. Further, they represent a more aggressive treatment approach towards gallstones with even prophylactic cholecystectomy in asymptomatic persons. Previous cohort studies most likely have pathologized gallstones and introduced protopathic bias which have caused higher incidence rates for symptom development and cholecystectomy.

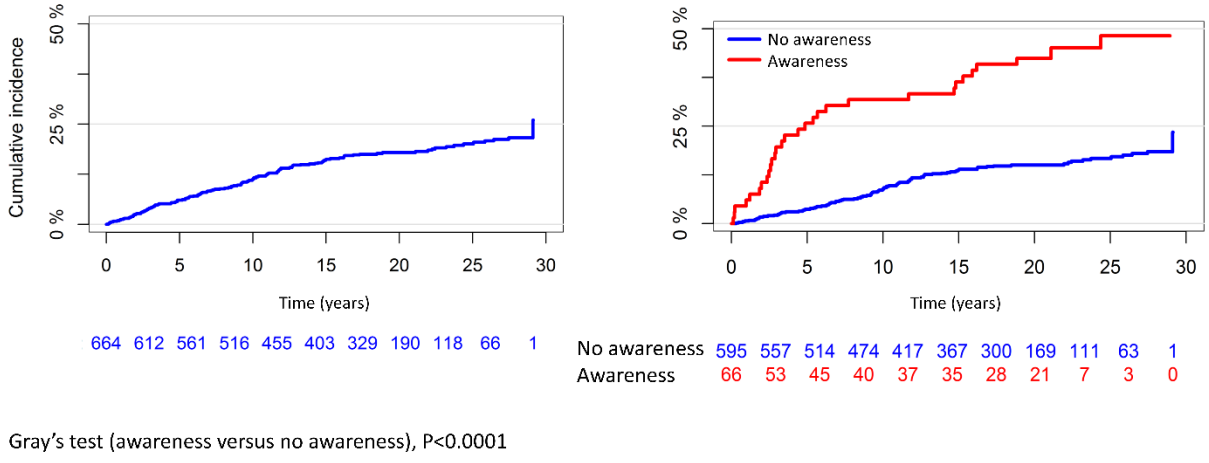


Figure 2: Cumulative incidence curves for development of symptomatic gallstone events and for awareness of gallstone status

Determinants of symptomatic gallstones

A stone size above 1.5 cm was the only determinant of symptomatic gallstones found in a previous cohort study of screen-detected gallstones[7]. Another study found a lower median age at baseline in treated compared to untreated patients[64], but the study used descriptive statistical analysis only without time-to-event analyses. Other cohort studies based on ultrasound screening have failed to identify determinants in multivariable analyses[6, 65, 66, 87]. The earlier cohort studies including clinically diagnosed gallstones from the 1980ies also did not identify significant determinants for development of symptomatic galstones[85, 86].

Study I confirmed stone size above 1 cm as a determinant for symptomatic gallstones. Novel determinants for symptomatic gallstones were identified and included multiple stones, female sex, younger age, and gallstone awareness. Further, complicated events were determined by multiple stones and awareness. Complication subgroups could only be explored in unadjusted analyses due to low number of events. A stone size above 1 cm and a gallstone age > 5 years were associated with acute cholecystitis. Multiple stones were associated with CBDS. Uncomplicated events were determined by gallstone size above 1 cm, younger age, female sex, and awareness (I).

To study the natural course of gallstones in a population unaware of gallstone status at baseline, persons with gallstone awareness were excluded in Studies II and III. This reduced the study population to 595 unaware persons with screen-detected gallstones. Follow-up procedures for symptomatic gallstones were the same as described for Study I. Symptomatic gallstones developed in 16.6% during a follow-up of median 17.5 years of which 7% were complicated and 9% uncomplicated clinical events.

Study II explored the impact of anthropometric, lifestyle, and social status variables at baseline. Body mass index (BMI) was identified as determinant for symptomatic gallstones. Further, a sedentary as compared to a light to vigorous activity level determined complicated events. These were also novel determinants compared to previous studies.

Study III explored the impact of abdominal symptoms at baseline. Symptom development was determined by pain localized in the epigastrium, of longer duration, of higher intensity, at night, and with need of analgesics. Uncomplicated events were determined by the same as for all events except for pain at night. Complicated events were determined by pain of higher intensity and at night only. Functional symptoms such as the irritable bowel syndrome or dyspepsia of the nausea or regurgitation type did not determine symptomatic gallstones. The identified baseline symptoms associated with development of symptomatic gallstones in persons unaware of gallstones in Study

III share similarities with the previously described symptom complex of biliary colic. However, biliary colic has previously only been described in historical expert opinions, found associated with gallstone prevalence, or part of consensus statements defining biliary pain (see Introduction). Prospective studies exploring incident gallstones at repeated ultrasound examinations have associated symptoms of upper abdominal pain, with projection, and of longer duration[88] or with pain in the right hypochondrium or epigastrium[89] – the study did not explore associations for clinical events. Study III was the first published cohort study to identify associations for baseline symptoms and clinical events in a population unaware of gallstones.

Two clinical studies published after the publication of Studies I-III have explored selection of patients with symptomatic uncomplicated gallstones for cholecystectomy based on presumed symptom determinants. A large RCT allocated patients to either usual care in which selection was left to the discretion of the surgeon or a restrictive strategy. In the latter, cholecystectomy was advised for those patients who fulfilled a symptom complex of five criteria which was presumed to define biliary pain including 1) severe pain attacks, 2) pain lasting 15–30 min or longer, 3) pain located in epigastrium or right upper quadrant, 4) pain radiating to the back, and 5) a positive pain response to simple analgesics. At one-year follow-up, no significant difference in proportions of patients that were pain free following cholecystectomy for the restrictive strategy or usual care (64% and 63%, respectively) were found. Less patients had cholecystectomy in the restrictive strategy arm. The study concluded suboptimal pain reduction in both strategies for selection of patients with gallstones and abdominal pain for cholecystectomy[90]. A prospective cohort study performed in extension of this RCT included patients undergoing cholecystectomy for symptomatic uncomplicated gallstones defined as presence of at least three out of the five symptoms mentioned above. The study developed a score to predict a clinically relevant pain reduction after cholecystectomy defined as a decline of four in the visual analogue scale (VAS) at six months follow-up. Overall, 57% of patients were pain free and 75% had a relevant pain reduction. Variables for the score were identified through a literature review. The final score included seven determinants which consisted of older patients, without a history of abdominal surgery, with a high VAS at baseline, with pain radiation to the back, a positive pain response to simple analgesics, nausea during pain attacks, and no heartburn. The score was externally validated in the subgroup of patients undergoing cholecystectomy in the above mentioned RCT and concluded a good prediction for clinically relevant pain reduction following surgery[91]. Although these studies may have identified a prediction score for clinically relevant pain reduction, the reported proportions of patients with remaining pain following surgery were high. Results from these studies indicate, that selection of patients for cholecystectomy based on patient-reported symptoms of pain only may be insufficient.

It was possible to identify novel determinants for symptomatic gallstones in uninformed or unaware persons with gallstones in Studies I-III. These determinants including gallstone characteristics (stone size above 1 cm and multiplicity), younger age, BMI, and symptoms of pain should be validated in future prospective clinical trials of cholecystectomy for uncomplicated gallstone patients presumed to be symptomatic.

Gallstones and mortality

The earliest report exploring gallstones and mortality was from the Framingham study which was initiated in 1949. Persons were followed for 10 years and no excess mortality was found in those with clinically diagnosed gallstones when compared to those without[61]. In a later cohort study of Pima Indians with clinically diagnosed gallstones and follow-up period of 20 years, a higher overall mortality was found in those with gallstones. An excess mortality caused by cancer was also identified in multivariable analysis. Further, mortality rates for those with gallstones did not differ from those who had cholecystectomy performed[92]. Two general population cohort studies have since explored mortality by comparing screen-detected gallstones at ultrasound or cholecystectomy at baseline to general population controls. A study from the US with mean follow-up 14 years found higher all-cause mortality in persons with gallstones or cholecystectomy in age-adjusted models. Mortality caused by cancer was associated with gallstones but not cholecystectomy, by CVD with both gallstones and cholecystectomy, and by diabetes with cholecystectomy but not gallstones[93]. A Norwegian study with follow-up of median 25 years found significantly higher mortality for persons with gallstones, but no significant association for persons with cholecystectomy in age- and sex-adjusted models[94].

Study IV included all 5928 persons with complete ultrasound examinations from MONICA I, 1914 cohort, and MONICA III. At baseline 7% had screen-detected gallstones, 3% cholecystectomy, and 90% no gallstones (controls). Persons were followed-up for median 25 years (range 0.08-33.1). Completion was 99% and lost to follow-up was mostly due to emigration. The same population and follow-up methods were also used for Studies V, VI, and VII.

CIPs for overall mortality at 30 years were significantly increased for screen-detected gallstones (72%) and cholecystectomy (77%) when compared to controls (42%). There were no apparent differences in mortality proportions for persons having gallstones or cholecystectomy (Figure 3). These associations were confirmed in multivariable models. Mortality caused by CVD was associated with gallstones and cholecystectomy (Table 3).

Study IV confirmed previous findings of an increased overall and CVD mortality and that no differences were found between persons with screen-detected gallstones or cholecystectomy.

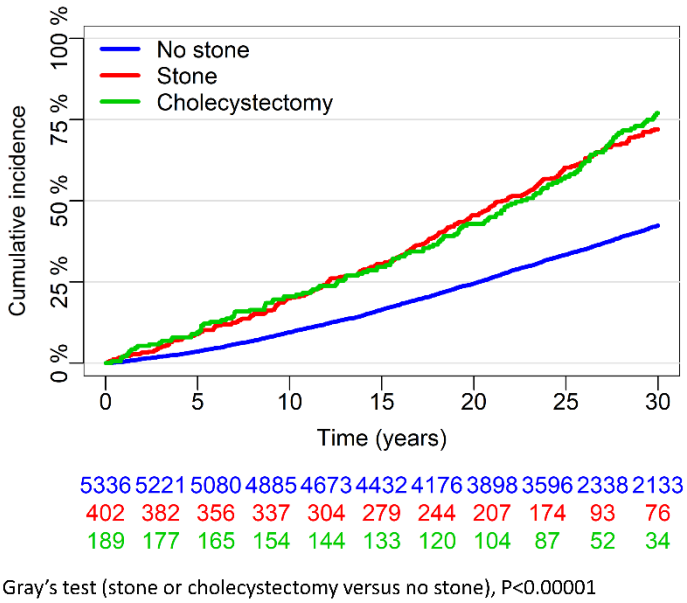


Figure 3: Cumulative incidence curves for gallstones and overall mortality

When exploring causes of death due to gallstones in the persons with screen-detected gallstones or cholecystectomy at baseline in the cohorts (n=853), a total of three persons (0.35%) had gallstone disease registered. Two had CBDS and gallbladder perforation registered as the underlying causes of death. The third had myocardial infarction as underlying cause of death with gallstone disease as a contributing cause (III). One person without screen-detected gallstones at baseline had died of gallbladder cancer (IV).

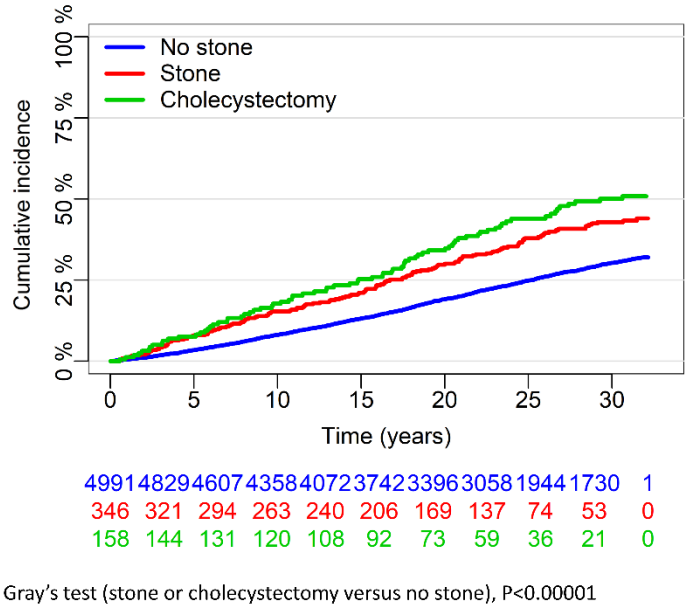
Screen-detected gallstones are associated with both higher overall and CVD-caused mortality. Mortality caused by symptomatic gallstones seems very low with scarcity of cases in the explored cohorts. Cholecystectomy does not seem to modify the increased overall mortality risk in presence of gallstones.

Gallstones and development of CVD

Several cohort studies with self-reported and clinically diagnosed gallstones have found associations for CVD[40] including coronary heart disease[95-97], stroke[98], and pooled CVD[99, 100] when

compared to general population controls. At the time when Study V was published, no cohort studies exploring screen-detected gallstones and CVD were identified.

In study V, the CIP for CVD at 30 years were significantly increased for persons with screen-detected gallstones (43%) and cholecystectomy (50%) when compared to controls (30%). There were no apparent differences in CVD proportions for gallstones or cholecystectomy (Figure 4). These associations were confirmed in multivariable models including age, sex, cohort, BMI, systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, concentrations of serum high-density and non-high-density lipoprotein cholesterol, smoking status, alcohol consumption, dietary habits, physical activity level, and social group for gallstones and cholecystectomy. When exploring subgroups of CVD, coronary artery disease was associated with cholecystectomy and non-significantly with gallstones (HR 1.22, 95% CI 0.96-1.56), cerebrovascular disease with cholecystectomy and non-significantly with gallstones (HR 1.11, 95% CI 0.84-1.46), and peripheral artery disease with cholecystectomy and non-significantly with gallstones (HR 1.43, 95% CI 0.99-2.07) (Table 3). The apolipoprotein E4 allele has previously been associated with both gallstones and CVD and could serve as a possible confounder in the identified association[101, 102]. Apolipoprotein E genotypes were measured in MONICA I and explored as genotypes apolipoprotein E4 versus other genotypes (E4 allele homozygote or heterozygote versus E2 or E3 alleles) as covariate in a multivariable model. Associations for CVD were confirmed for both gallstones and cholecystectomy (V).



Gray's test (stone or cholecystectomy versus no stone), P<0.00001

Figure 4: Cumulative incidence curves for gallstones and development of cardiovascular disease

Atherosclerosis has been suggested a common mechanism for CVD and gallstones since stenosis of carotid or coronary arteries found at angiography have been associated with gallstones in cross-sectional studies[103, 104]. Genetic variants for the cholesterol transporter ABCG5/8 have been associated with both atherosclerosis and gallstones[50, 105, 106]. However, a large and more recent study exploring genetic variation of *ABCG5/8* associated with decreased plasma levels of low-density lipoprotein cholesterol identified an association for clinically diagnosed gallstones and an inverse association for CVD[55]. Therefore, a common mechanism through the ABCG5/8 cholesterol transporter seems unlikely at this point. Biomarkers of systemic inflammation are elevated in persons with gallstones indicating a possible link to gallstone development[49]. For a full discussion on mechanisms of inflammation, see below (Gallstones and development of autoimmune disease). Although the role of the immune system in gallstone development is only an emerging field of research, inflammation has for long been associated with atherosclerosis, and targeted treatments are currently being developed[107]. The gut microbiota has been suspected as being part of gallstone development through the production of lithogenic fecal secondary bile acids[108]. More recently, a possible link to atherosclerosis was also suggested[109]. Mechanisms of systemic inflammation and the microbiota may thereby include candidate mechanisms for gallstones and CVD.

Study V was the first to report temporal associations for screen-detected gallstones, cholecystectomy, and CVD and to report associations for subgroups of CVD. The identified association cannot be explained through coexistence of common risk factors for gallstones and CVD due to multivariable adjusting including cardiometabolic risk factors and the apolipoprotein E4 allele. It also cannot be ascribed to short-term associations due to mimicry in clinical conditions or to detection bias (see Introduction), since the population was drafted from the general population and participants were uninformed about ultrasound findings.

Screen-detected gallstones and cholecystectomy are associated with development of CVD. Non-significant associations for screen-detected gallstones in Study V were most likely due to insufficient number of CVD events in subgroups rather than a causal difference in associations for gallstones or cholecystectomy and CVD. The CVD risk in persons with gallstones does not seem to be altered by cholecystectomy.

Gallstones and development of autoimmune disease

Associations for gallstones and immunological diseases have previously only been explored in a case-control study[110], in a cohort with self-reported or clinically diagnosed gallstones[111], or explored as the reverse temporal association with incident gallstones as the outcome[112, 113]. These studies

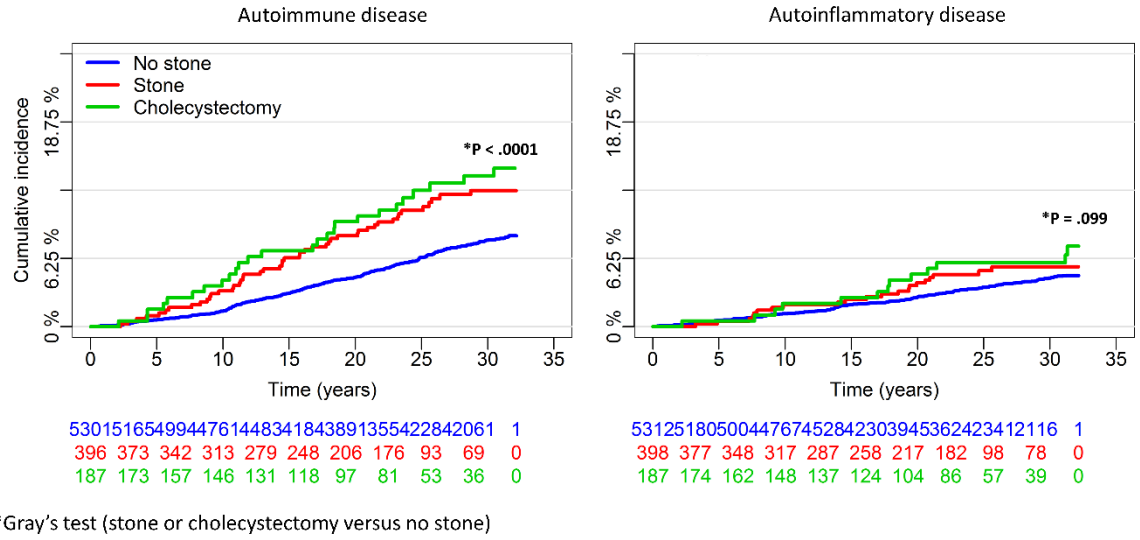
have explored only few immunological diseases, including the autoimmune disease rheumatoid arthritis[110] and the autoinflammatory diseases psoriasis[111, 113] and inflammatory bowel disease[112].

Study VI explored associations for screen-detected gallstones, cholecystectomy, autoimmune disease, and autoinflammatory disease. Autoimmune disease included systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, dermatopolymyositis, rheumatoid arthritis, diabetes mellitus type 1, autoimmune thyroid disease, primary adrenocortical insufficiency, celiac disease, autoimmune liver disease, myasthenia gravis, pemphigoid, pemphigus, antibody-associated vasculitis, and autoimmune anemia. Autoinflammatory disease included inflammatory bowel disease, psoriasis and psoriasis arthritis, ankylosing spondylitis, vitiligo, multiple sclerosis, and non-antibody-associated vasculitis. The CIPs for autoimmune disease at 30 years were significantly increased for screen-detected gallstones (13%) and cholecystectomy (14%) when compared to controls (8%). There were no apparent differences in autoimmune disease proportions for gallstones or cholecystectomy (Figure 5). In multivariable models including the same covariates as in Study V, associations were confirmed for gallstones and non-significantly for cholecystectomy (HR 1.50 95% CI 0.99-2.29). When exploring subgroups of autoimmune disease, diabetes mellitus type 1 was associated non-significantly with gallstones (HR 1.57 95% CI 0.98-2.52) but not with cholecystectomy, and autoimmune thyroid disease was associated with gallstones and cholecystectomy (Table 3). No associations were found for rheumatoid arthritis. The CIPs for autoinflammatory disease at 30 years were not significantly different for screen-detected gallstones (6%) and cholecystectomy (6%) when compared to controls (4%) (Figure 5). No significant associations were found in analyses of pooled autoinflammatory disease (Table 3) or subgroups thereof including inflammatory bowel disease, non-antibody-associated vasculitis, or psoriasis and psoriasis arthritis.

Although no identified common mechanisms can describe associations for gallstones and autoimmune disease at this point, some candidate mechanisms may be suggested including insulin resistance, functions of cholecystokinin – the hormone stimulating gallbladder contractions, and systemic inflammation. Insulin resistance and obesity have been associated with attenuated gallbladder contractions, gallstone development[114], and also hypothesized to cause latent autoimmune diabetes in adults[115]. Cholecystokinin knockout mice exert enhanced gallstone formation[116]. Celiac disease patients have higher levels of serum cholecystokinin when compared to controls, suggesting an attenuated response in gallbladder smooth muscle tissue in autoimmune disease[117]. Cholecystokinin has also been demonstrated to attenuate the adaptive immune response of B cells and antibody production[118]. Elevated levels of C-reactive protein, a biomarker of systemic inflammation, has been found in persons with gallstones compared to controls[49, 119].

Further, local inflammation with infiltration of inflammatory cells and gallbladder wall thickening has been demonstrated at the time of bile cholesterol crystallization during gallstone formation[120, 121]. These mechanisms are suggestive of early presence of inflammation in gallstone development. The role of the immune system in gallstone development has only recently caught attention with no specific mechanisms identified yet, and ongoing efforts are needed for a better understanding[122]. Autoimmune disease may be triggered by a number of factors in genetically susceptible individuals, and the process from an initiated autoimmunity to clinically overt disease takes many years[123]. Whether gallstones and autoimmune disease are linked through both being a product of systemic inflammation, or gallstones may act as a trigger for the development of autoimmune disease is yet to be explored.

Study VI was the first to report temporal associations for screen-detected gallstones, cholecystectomy, and autoimmune disease and associations for the subgroups diabetes mellitus type 1 and autoimmune thyroid disease. The associations cannot be explained through possible coexistence of common risk factors for gallstones and autoimmune disease or through detection bias due to the same reasons as given for study V.



*Gray's test (stone or cholecystectomy versus no stone)

Figure 5: Cumulative incidence curves for gallstones and development of autoimmune and autoinflammatory disease

Screen-detected gallstones and cholecystectomy are associated with development of autoimmune disease largely driven by diabetes mellitus type 1 and autoimmune thyroid disease. Non-significant associations for screen-detected gallstones were most likely due to insufficient number of events in

subgroups or cholecystectomy at baseline rather than a causal difference in associations for gallstones or cholecystectomy and autoimmune disease. The risk for autoimmune disease does not seem to be altered by cholecystectomy.

	Screen-detected gallstones	Cholecystectomy
Mortality (IV)	+	+
CVD mortality	+	+
CVD (V)	+	+
Coronary artery disease	NSA	+
Cerebrovascular disease	-	+
Peripheral artery disease	NSA	+
Autoimmune disease (VI)	+	NSA
Diabetes mellitus type I	NSA	-
Autoimmune thyroid disease	+	+
Autoinflammatory disease (VI)	-	-
Gastrointestinal cancer (VII)	NSA	+
Right-side colon cancer	+	-
NSA, non-significant association indicated by a p-value ≤ 0.1		

Table 3: Associations for screen-detected gallstones, cholecystectomy and long-term morbidity as identified in multivariable adjusted analyses in Studies IV-VII

Gallstones and development of gastrointestinal cancer

Only few cohort studies of clinically diagnosed gallstones and cancer have explored separate exposures for gallstones and cholecystectomy when compared to general population controls[42, 124-133]. These studies identified associations for gallstones and hepatic[124, 125, 131], biliary[126], gallbladder[125, 131], pancreatic[125, 133], gastric[125], small intestines[127], and colon[132] cancer and for cholecystectomy and hepatic[125], pancreatic[125, 127, 131], esophageal[128], gastric[125], duodenal[131], small intestines[125, 127, 129], and colon[129, 132] cancer. By the time Study VII was published, only two studies had explored associations for screen-detected gallstones, cholecystectomy at baseline and cancer in general population samples. These studies had found associations for mortality caused by cancer and pooled cancer incidence for screen-detected gallstones, but not for cholecystectomy[93, 94].

Study VII found higher CIPs for pooled gastrointestinal cancer at 30 years for screen-detected gallstones (10.7%) and cholecystectomy (12.4%) when compared to controls (6.6%). There were no apparent differences in proportions for gallstones or cholecystectomy (Figure 6). Associations were confirmed for pooled gastrointestinal cancers in age, sex, and cohort adjusted models. In multivariable models also including BMI, high-density and non-high-density lipoprotein cholesterol, smoking status, alcohol consumption, dietary habits, physical activity level, and social group, associations were significant for cholecystectomy and non-significant associations were found for

gallstones (HR 1.42, 95% CI 0.998-2.01). When exploring subgroups of cancer, right-side colon cancer (coecum, ascending colon to right flexure, and appendix) was associated with gallstones, but not with cholecystectomy (HR 1.63, 95% CI 0.58-4.72) in a multivariable model adjusted for age, sex, cohort, and BMI. Adding dietary habits to the model did not change estimates significantly (Table 3). Although the association for cholecystectomy and right-side colon cancer did not reach the level of significance, the CIPs were equally high for gallstones (2.7%) and cholecystectomy (2.3%) when compared to no gallstones (0.96%). Colorectal cancer or the remaining colon cancer sub-sites were not associated with gallstones or cholecystectomy. Other gastrointestinal cancers of higher incidence such as pancreatic, esophageal, and gastric cancers were not found associated with pooled screen-detected gallstones. However, these cancers occurred infrequently in the cohorts, and exposures for gallstones and cholecystectomy could therefore not be explored separately (VII). None of the persons with screen-detected gallstones developed gallbladder cancer (I).

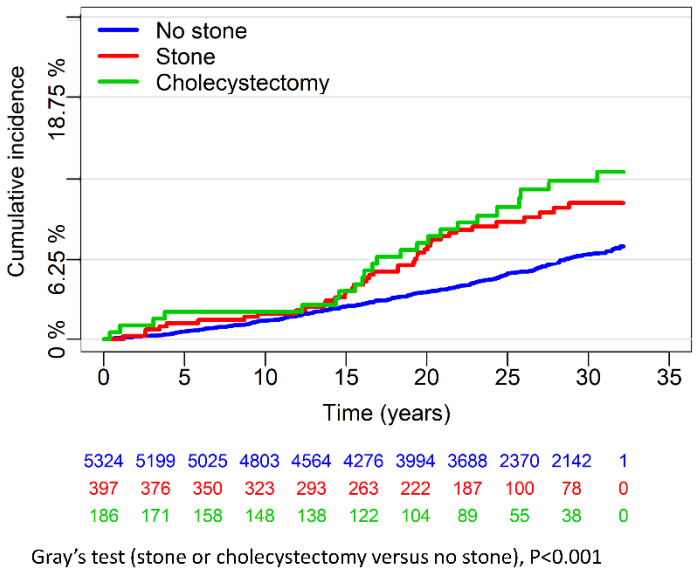


Figure 6: Cumulative incidence curves for gallstones and development of pooled gastrointestinal cancer

Only previous cohort studies with clinically diagnosed gallstones and cholecystectomy have identified an association for pooled colon cancer[132] and an association for cholecystectomy and right-side colon cancer before[129].

Mechanisms involved in right-side colon cancer development in persons with gallstones or previous cholecystectomy may involve the entero-hepatic metabolism of bile acids. Secondary bile acids are produced by the colonic bacteria through 7α-dehydroxylase activity and have been considered carcinogenic for long[134, 135]. Cellular mechanisms for the carcinogenesis of secondary bile acids and colon cancer are not fully understood, but may involve antagonism of the FXR, which besides its most prominent role of regulating bile acid circulation also acts as a tumor suppressor[136, 137]. The predilection of the right-side colon has been suggested through a greater proximal colonic absorption of and exposure to secondary bile acids[45]. Persons with gallstones have elevated amounts of secondary bile acids and elevated amount and activity of 7α-dehydroxylase colonic bacteria[108, 138-140]. Alternatively, mechanisms after cholecystectomy include a continuous bile flow into the bowel which also have been suggested[141, 142]. Most previous cohort studies have referred to the mechanisms of cholecystectomy with higher output of secondary bile acids to the proximal colon to explain associations for cholecystectomy and colon cancer[129, 132]. However, mechanisms of cholecystectomy do not explain the association for screen-detected gallstones and right-side colon cancer as found in Study VII. The identified association calls for further research on the impact of the microbiota and the FXR on the development of gallstones and colon cancer.

Study VII was the first to report a temporal association for screen-detected gallstones and right-side colon cancer. The association for cholecystectomy or gallstones and colon cancer has previously been suggested as noncausal and as an epiphenomenon to dietary habits[143, 144] with higher consumption of fats causing increased fecal secondary bile acids[145]. Short-term associations have previously also been interpreted as a detection bias since early symptoms of cancer may mimic symptomatic gallstones (see Introduction). The identified association for screen-detected gallstones cannot be explained as an epiphenomenon since dietary habits and other possible confounding variables were adjusted for. It can also not be ascribed to detection bias due to the same reasons as explained for Studies V and VI.

Screen-detected gallstones and cholecystectomy seem to be associated with development of gastrointestinal cancer and mostly driven by right-side colon cancers. Non-significant associations were more likely due to few cholecystectomies at baseline rather than due to altered cancer risks following cholecystectomy. Cholecystectomy does not seem to alter the increased risk for pooled gastrointestinal cancer and right-side colon cancer seen for gallstones.

Gallstone treatments and development of upper gastrointestinal cancer

Previous studies including clinically diagnosed gallstones have found associations for cholecystectomy and cancer when compared to general population controls[125, 127-129, 131, 132]. Few smaller studies including clinical populations with CBDS have only discussed and proposed mechanisms for sphincterotomy and biliary cancer without performing controlled analysis or explore temporal associations[146-149]. The exploration of whether gallstones, cholecystectomy, or sphincterotomy cause cancer development has generally been limited by unavailability of life-long follow-up data, not exploring the impact of sphincterotomy, and by not including the time-varying changing exposures for gallstones and treatments in time-to-event analyses during long-term follow-up.

Studies of screen-detected gallstones and long-term morbidity included in this thesis have found some associations for cholecystectomy that did not reach the level of significance (VI, VII). First, too low baseline sample size of cholecystectomy in MONICA and 1914 cohorts were suspected which could have caused a type II error. Second, exploration of the less frequent upper gastrointestinal cancer outcomes generally requires a very large sample size (see Methodological considerations, Study design). Consequently, to obtain a sufficient sample size for exploration of gallstone treatments, a nationwide cohort was sampled for Study VIII. Persons included counted 4 465 962, were born in Denmark, and were followed from age 30 years in the period 1977-2014. Separate exposures for gallstones or treatments thereof included a diagnose of clinically diagnosed gallstones, cholecystectomy, sphincterotomy or a combination of the latter two. The remaining general population comprised the controls (VIII).

Cumulative incidence curves for sphincterotomy were much higher for biliary, gallbladder, hepatic, and pancreatic cancer, and higher for both cholecystectomy and sphincterotomy for biliary and pancreatic cancers. Cholecystectomy caused lower incidence curves for pancreatic and gastric cancer when compared to controls (Figure 7). Multivariable analyses including sex, socio-economic status, civil status, level of education, and personal annual income were performed. Sphincterotomy and biliary cancer was the only association which persisted above five years of follow-up. Other associations at two to five years only were found for sphincterotomy with both gallbladder and pancreatic cancer, for cholecystectomy with both duodenal and small bowel cancer, and for both cholecystectomy and sphincterotomy with biliary and small bowel cancer. Inverse associations persisting above five years of follow-up were found for cholecystectomy and biliary, pancreatic, esophageal, and gastric cancer (VIII).

A cohort study from Sweden found a significantly increased standardized incidence ratio for biliary cancers in patients with gallstones but found no significant differences for patients with cholecystectomy when compared to general population controls. The authors concluded that biliary cancer risk decreased with time after cholecystectomy and returned to the level of the background population after 10 years[126]. Similar arguments of diminished cancer risks based on non-significant estimates following gallstone treatments have been suggested for pancreatic cancer after cholecystectomy or sphincterotomy[133]. Such results are not consistent with the findings of a significant inverse association for cholecystectomy and cancer as found in Study VIII. However, they do suggest a protective mechanism of cholecystectomy for biliary and pancreatic cancer in patients with gallstones. Population-based cohort studies from Sweden have found significantly higher standardized incidence ratio for small bowel cancer in patients with cholecystectomy[129] just as in study VIII. Another study from the same research group found a significantly higher incidence for esophageal cancer in patients with cholecystectomy[128], which was not found in Study VIII.

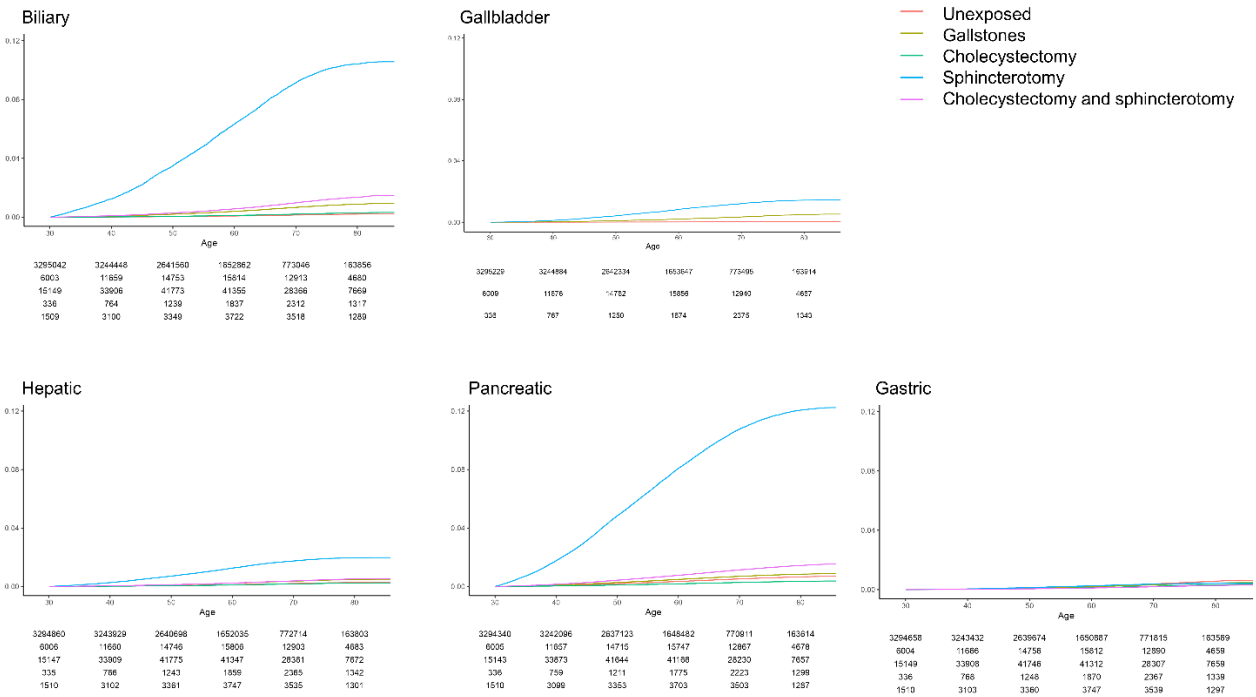


Figure 7: Cumulative incidence curves for symptomatic gallstones, cholecystectomy, and sphincterotomy and the development of upper gastrointestinal cancers

Previous smaller clinical series exploring development of biliary cancer following sphincterotomy in patients with CBDS have found no significant associations for development of biliary cancer. They report no or very few incident biliary cancers following sphincterotomy during long-term follow-up[150-152], no significant associations for sphincterotomy versus no sphincterotomy[153, 154], or no significant associations for sphincterotomy versus cholecystectomy[155]. Cohort studies with general population controls report no significant associations for sphincterotomy and biliary cancer[156-158].

Sphincterotomy causes several changes in bile and in the common bile duct as found in experimental studies including colonization with bowel bacteria[159], higher amounts of secondary bile acids and cytotoxic agents, histological chronic inflammation[160], and atypical cells indicating cell proliferation[161]. These observations support a plausible and coherent mechanism for biliary cancer transformation following sphincterotomy. On the level of discussion in smaller clinical series, mechanisms of duodeno-biliary reflux with chronic biliary inflammation for biliary cancer transformation following sphincterotomy have been suggested[146, 148]. Similarly, cohort studies have discussed carcinogenic chronic inflammation of bile ducts in presence of gallstones which may be altered by cholecystectomy[126, 133]. The latter does, however, still not explain the inverse associations for cholecystectomy and biliary, pancreatic, esophageal, and gastric cancer when compared to the general population as found in Study VIII.

A strong statistical association was found for sphincterotomy and biliary cancer. Possible associations were found for sphincterotomy with pancreatic and gallbladder cancer (VIII). These associations have not been reported in cohort studies of general populations before. Persons with primary sclerosing cholangitis have much higher incidence rates of biliary cancer when compared to the general population[162]. The association for sphincterotomy and biliary cancer was confirmed in a supplementary adjusted model including primary sclerosing cholangitis, thereby minimizing the most obvious potential confounding. Diagnostic mimicry and detection bias were avoided by not considering incident cancers during immediate follow-up period from zero to two years as causal. Therefore, only estimates from two years of follow-up and above were reported. Results for treated gallstones are based on the largest and most completely followed-up data reported (VIII). Previous studies may have been limited by smaller population samples insufficient for identification of significant associations.

Untreated clinically diagnosed gallstones had higher cumulative incidence curves for biliary and gallbladder cancer. Associations were found for biliary and small bowel cancer at two to five years follow-up, and for gallbladder cancer at above five years. An inverse association for gallstones and pancreatic cancer at above five years follow-up was found (VIII). Interpretation of such results based

on clinically diagnosed gallstones should be with caution (see Symptomatic and asymptomatic gallstones in cohort studies).

Study VIII concluded that sphincterotomy most likely is associated with development of biliary cancer. Consequently, caution with use of sphincterotomy is suggested in patients with CBDS. Alternative surgical treatments should be used until further studies are performed. A cautious treatment approach should at least be for the younger patients with longevity and thereby higher risk for developing consequences of a sphincterotomy. More studies are needed to confirm the possible protective mechanisms for cholecystectomy and development of biliary, pancreatic, esophageal, and gastric cancer.

Prophylactic cholecystectomy for asymptomatic gallstones

The earliest reports on gallstones assumed to be asymptomatic in the years 1920-65 recommended prophylactic cholecystectomy due to a high risk of symptomatic gallstones observed in clinical series from hospitals in both the US and Denmark[4, 5, 163, 164]. The cohort study from Gracie and Ransohoff in 1982 concluded a more uneventful natural course of gallstones compared to earlier reports (see Development of symptomatic gallstones) and argued against prophylactic cholecystectomy in the asymptomatic[84]. Currently, prophylactic cholecystectomy for asymptomatic gallstones is generally not recommended in consensus or guideline papers[9, 165-174] and decision analysis studies[175, 176].

Prophylactic cholecystectomy for asymptomatic gallstones is suggested for only few clinical sub-populations of morbid patients due to a higher risk of symptomatic complicated gallstones found in clinical observational studies. These include patients with sickle-cell disease[9, 169, 174, 177] or short-bowel syndrome[178]. Also patients with carcinoid tumors scheduled for treatment with somatostatin analogs[179] and those undergoing heart transplantation[169, 180, 181] are suggested to undergo prophylactic cholecystectomy. Regarding morbidly obese patients and prophylactic cholecystectomy for asymptomatic gallstones concomitant with bariatric surgery, available evidence is controversial with the most recently published larger observational study and RCT recommending it[182, 183], but current guidelines recommending against it[9, 184, 185].
Prophylactic cholecystectomy for asymptomatic gallstones based on gallstone characteristics in otherwise healthy persons has been suggested more recently. Smaller sized gallstones determined pancreatitis in a study including clinically diagnosed gallstones and a decision analysis concluded that life-years may be gained or lost by prophylactic cholecystectomy, depending on incidence and mortality of pancreatitis[186]. Based on this study, Italian surgical societies suggest that prophylactic

cholecystectomy may be advisable for microcalculi or biliary sludge in the presence of a functioning gallbladder[169]. Prophylactic cholecystectomy is currently also recommended for some ultrasound findings which have been associated with gallbladder cancer. These include a gallstone size above 3 cm[168, 171, 174, 187] due to an increased cancer risk observed in a clinical cholecystectomy series including American Indians and non-Indians[188]. Prophylactic cholecystectomy for gallbladder polyps of 1 cm is generally recommended in most international guidelines[189-193]. Recommendations for the calcified "porcelain" gallbladder are controversial[9, 165, 166, 170, 171, 174, 193] which is most likely due to the broad variety in cancer risk assessments in available clinical series[194].

A recent cohort study suggested that prophylactic cholecystectomy for asymptomatic gallstones may be performed based on a prediction score. The study included a general medical population with incidental clinically diagnosed gallstones at ultrasound or computed tomography examinations. The CIP for symptomatic gallstones was 22% at 10 years and determinants were younger age, female sex, larger and multiple stones, gallbladder polyps, and chronic hemolytic anemia. The study's prediction score was both developed and validated in the same cohort[195]. The study was published several years after Study I. When compared to Study I, CIP was about twice as high (18% at 20 years in Study I, see Development of symptomatic gallstones), the determinants younger age, female sex, larger and multiple stones were confirmed, and two novel determinants were identified including gallbladder polyps and chronic hemolytic anemia. The most obvious differences between the studies, however, is that the study cohort comprised clinically diagnosed gallstones and also did not report what recommendations or information about presence of gallstones were given to patients. The high CIP for symptomatic gallstones were comparable to similar cohort studies of clinically diagnosed gallstones previously reported[85, 86] and therefore expected. Further, most symptomatic gallstones requiring treatment were for chronic cholecystitis which included all symptomatic uncomplicated gallstone disease. As mentioned before, the indication for cholecystectomy in persons with symptomatic uncomplicated gallstones is both relative (see Introduction) and questionable (see Determinants of symptomatic gallstones). A prediction score for development of symptomatic gallstones was also presented in Study I which suggested that men of higher age with single gallstones of 1 cm or less had the lowest risk when compared to women of younger age and with multiple gallstones above 1 cm. It was suggested that the identified determinants and prediction score should be validated in future prospective clinical studies (I). Although the new study also recommended validation in the discussion, it concluded that the prediction score could easily be adapted for use in clinical care to aid in shared decision-making regarding prophylactic surgery for asymptomatic gallstones[195].

Prophylactic cholecystectomy to avoid long-term morbidity has also been suggested. Following the publication of Study VII, a journal correspondence suggested a generalized screening program for gallstones in the general population and that the indication to treat only symptomatic gallstones may change if asymptomatic gallstones carry the increased risk of developing colon cancer[196]. In the response, it was emphasized that Study VII suggested that all gallstones – whether asymptomatic, symptomatic or cholecystectomy – are associated with development of right-side colon cancer[197].

The Taiwan National Health Insurance Research Database has provided data to cohort studies exploring the impact of cholecystectomy on long-term morbidity in patients with clinically diagnosed gallstones. Cholecystectomy was associated with colorectal and gastric cancer within and persisting above five years of follow-up, respectively[198]. However, cholecystectomy was also found inversely associated with colorectal cancer which persisted after six months follow-up in another study[199]. Regarding non-cancer morbidity, inverse associations for cholecystectomy and acute myocardial infarction were found to persist for more than five years[200]. An inverse association was also found for cholecystectomy and stroke[201]. The study groups did not recommend prophylactic cholecystectomy in persons with gallstones due to the observational study designs, high numbers needed to treat, and they generally concluded that more studies were needed[199-201].

Regarding screening for asymptomatic gallstones in the general population, a recent cost-effect and decision analysis study found that ultrasound screening is worthwhile and recommended that the Chinese population should be routinely screened annually[202]. However, screening implies treatment of asymptomatic persons including prophylactic cholecystectomy. The discussion of screening is therefore related to and dependent on the discussion of prophylactic cholecystectomy.

Based on the studies in this thesis, prophylactic cholecystectomy for asymptomatic gallstones cannot be recommended for prevention of symptomatic gallstones due to a low CIP at 20 years follow-up (I). Second, in the presence of determinants for symptomatic gallstone disease (I-II), prophylactic cholecystectomy is still not advisable in the asymptomatic. Cholecystectomy did not seem to decrease the excess risk for mortality (IV) or long-term morbidities including CVD (V-VII) in persons with gallstones when compared to general population controls. Cholecystectomy may be inversely associated with biliary, pancreatic, esophageal, and gastric cancers indicating a protective mechanism for cancer development. But cholecystectomy may potentially also cause duodenal or small bowel cancer (VIII). Proportions of these cancers are low in the general population. In addition, prophylactic cholecystectomy for asymptomatic gallstones in the general population for prevention of long-term morbidity is also not justified.

Consequently, symptomatic gallstone disease remains as the only indication for cholecystectomy until we have validated determinants and prediction scores for development of symptomatic gallstones. Selection for cholecystectomy in presence of symptomatic uncomplicated disease is still a challenge, and the indication will, until further, be based on presence of symptoms and shared decision-making. Alternatively, one may quote Carl Langenbuch – the surgeon who performed the first cholecystectomy in 1882 – that cholecystectomy is at the moment suitable only for those cases in which the patient and physician have reached the end of their patience[203].

Study limitations

Studies I-III used hospital admissions with gallstone diagnosis or intervention codes to distinguish between uncomplicated and complicated gallstones. This has a potential risk of non-differential misclassification which, consequently, may cause non-significant estimates[62]. A sensitivity analysis exploring this possible misclassification was performed in Study I. The few complicated events that were only based on presence of interventions such as ERC or cholecystostomy, a drainage of the gallbladder mostly used for treatment of acute cholecystitis, but without a registered complicated diagnosis were re-classified as uncomplicated events. The sensitivity analysis showed similar estimates as the original analysis. However, misclassification of complicated and uncomplicated gallstone events can probably not be completely avoided. Emphasis was therefore on all gallstone events as the outcome in these studies, thereby minimizing potential misclassification.

Studies IV-VII included long-term follow-up of a population sample screened for gallstones at baseline. This may be with risk of underestimating gallstones, which could have developed during the follow-up period. Long-term morbidities were dependent on those in registries and therefore limited to those clinically detected or verified post-mortem by the few autopsies performed. Cholecystectomies may also have been performed during follow-up in the group with screen-detected gallstones. All these limitations carry a potential risk of non-differential misclassification bias[62]. However, based on findings from Study I, we know that less than one-fifth of gallstones in the general population will be discovered clinically as symptomatic gallstones during long-term follow-up. The risk is therefore most likely negligible. Baseline cholecystectomies were few and a risk of a type II error causing non-significant associations was therefore present and likely in studies VI and VII.

Study VIII used registry data to identify exposures of symptomatic gallstones, cholecystectomy, and sphincterotomy in the general population. As described above, the use of symptomatic gallstones is with risk of differential misclassification bias (see Symptomatic versus asymptomatic

gallstones in cohort studies). Estimates from symptomatic gallstones were therefore interpreted with caution in Study VIII. However, estimates based on the gallstone treatments cholecystectomy and sphincterotomy were without such risk of bias in Study VIII, and emphasis was therefore on these estimates in the conclusions. Further, data on anthropometry and lifestyle habits were not available in registries and therefore not adjusted for in multivariable analysis with the potential risk of residual confounding. This limitation was addressed through adjusting for socio-economic variables which served as a proxy.

Perspectives for future research

Questions yet to be answered prevail in the management of gallstones that will require future research efforts, including both clinical, epidemiological, and experimental studies. Studies from the many previous decades have estimated risk for development of symptomatic gallstones with inconsistency, which probably have caused changing views upon the risk of symptomatic gallstones and need for treatment. Much of this inconsistency in research may have been due to lack of knowledge about gallstone risk in persons uninformed about presence of gallstones. Also, likely due to definitions of symptomatic and asymptomatic gallstones and lack of knowledge about specific symptoms caused by gallstones. A suggestion for future studies is not to focus on patient-reported symptoms only in the selection for surgery.

Regarding the specific clinical challenge in selection of patients with symptomatic uncomplicated gallstones for laparoscopic cholecystectomy, the most recent clinical studies including a RCT with more restrictive selection still show unacceptably high proportions of about 40% with remaining pain symptoms and 25% without a clinically relevant pain reduction following cholecystectomy[90, 91]. To better identify the subgroup of patients with gallstones that may benefit from cholecystectomy, future clinical cohorts should validate the determinants identified in Studies I-III including gallstone characteristics (stone size above 1 cm and multiplicity), BMI, and symptoms of pain for symptomatic gallstones with outcomes of pain and symptom relief following surgery. Such studies are feasible now and may improve clinical practice within a near future. If determinants are validated, a further RCT could explore patients selected for cholecystectomy accordingly versus usual clinical care. A similar RCT has been performed before, however the selection in the restrictive strategy was based on patient-reported symptoms only[90]. A future RCT should include a primary outcome of relevant pain reduction but should also explore quality of life and functional symptoms as secondary outcomes. If a more relevant population for cholecystectomy will be identified, other future RCTs could include patients with modest or no symptoms and explore if prophylactic surgery is justified. Such studies may be feasible within a near future.

Based on the studies of this thesis, cholecystectomy did not seem to change risk for mortality or long-term morbidity including CVD, autoimmune disease, and pooled gastrointestinal or colon cancer. However, inverse associations for cholecystectomy and biliary, pancreatic, esophageal, and gastric cancer were suggested indicating protective mechanisms. Common mechanisms for gallstones and both CVD and colon cancer such as pathways of inflammation, the microbiota, and the FXR should be explored much further in experimental and epidemiological studies. Such studies

will aim in identifying the persons with gallstones who possibly may benefit from cholecystectomy for prevention of disease development or progression. Only such knowledge may justify possible future clinical studies of prophylactic cholecystectomy for prevention of non-symptomatic gallstone associated morbidity. Alternatively, such studies may aim in identifying more detailed therapeutic targets for prevention of disease beyond cholecystectomy.

Recently, patients with gallstones were found to have high amounts of the fecal bacteria of the order *Desulfovibrionales* compared to patients without gallstones at 16S rRNA sequencing. When transplanting feces from patients with gallstones to mice, a sequence of events was demonstrated including higher colonic 7α-dehydroxylase activity, higher amounts of secondary bile acids, regulation of FXR proteins, elevated expression of *ABCG5/8* genes, increased hepatic cholesterol synthesis and bile cholesterol, and gallstone formation[204]. Further, transplantation of *Lactobacillus* strains has shown to improve dysbiosis of the gut microbiome, change bile acid composition, activate the FXR signaling pathways, and prevent gallstone formation in mice[205]. Ongoing research efforts using emerging technologies connect the dots of gallstone formation. They may also, at some point, reveal links to mechanisms of other related morbidities.

Sphincterotomy was strongly associated with biliary cancer. More cohort studies may be requested, but these should similarly include very large general population samples instead of repeating the limitations of inadequate population samples in previous cohort studies. Due to the inability of exploring this association any further in RCTs, it may be more feasible to change clinical practice for treatment CBDS, at least for the younger patients for a start.

Finally, the novel findings identified in the studies of this thesis were derived from large population samples with a detailed registry follow-up. Most of the above-mentioned questions can only be answered through large data samples. The creation of larger data sources for the purpose of research including clinical databases for gallstone disease is requested and strongly encouraged.

Summary and conclusions

In this thesis, the natural course and long-term morbidity of gallstones was explored in a general population cohort uninformed of the presence of screen-detected gallstones which has not been reported previously. Less than one out of five persons with gallstones will develop symptomatic gallstones over 20 years of follow-up – this is the lowest cumulative incidence proportion reported to date. The conclusion is that the natural course of gallstones is more uneventful than previously anticipated.

Development of symptoms was determined by gallstone size above 1 cm, multiple stones, gallstone awareness, higher BMI, female sex, and younger age. The first determinant was confirmed based on a previous cohort study and the remaining were novel. Further, pain localized in the epigastrium, of longer duration, of higher intensity, at night, and with need of analgesics were identified determinants in a cohort unaware of gallstone status which also were novel findings.

Screen-detected gallstones were associated with higher mortality, CVD, autoimmune disease, gastrointestinal cancer, and especially right-side colon cancer when compared to general population controls. Associations for screen-detected gallstones found in this thesis were confirmed for mortality, CVD and cancer, and were novel for autoimmune disease. Cholecystectomy did not change associations for mortality or long-term morbidity.

In patients with gallstone treatments compared to population controls, sphincterotomy had a strong association with development of biliary cancer which persisted after five years of follow-up. Caution with the use of sphincterotomy in the younger patients is recommended. Possible protective mechanism of cholecystectomy against biliary, pancreatic, esophageal, and gastric cancer were suggested. These were all novel associations identified in this thesis.

Common mechanisms for development of gallstones, CVD, autoimmune disease, and cancer should be further explored in future experimental and epidemiological studies. Target mechanisms are suggested in the pathways of inflammation, the microbiota, and cellular receptors of cholesterol and bile metabolism.

Efforts should be made to improve selection of patients with symptomatic uncomplicated gallstones for cholecystectomy in future clinical studies. First, the impact of identified determinants from this thesis should be explored in clinical cohorts on symptom relief following cholecystectomy.

If determinants are confirmed, RCTs should explore selection of patients based on these determinants compared with usual clinical care.

Prophylactic cholecystectomy for asymptomatic gallstones in the general population is not recommended. Challenges for future studies include the identification of the subgroup of persons with gallstones that may benefit from cholecystectomy in the long-term. In the light of such future findings, cholecystectomy indications may either expand or be reappraised. For the time being, cholecystectomy is still indicated for symptomatic gallstones only.

Dansk resumé

Afhandlingen undersøger spontanforløb og morbiditet af galdesten ved langtidsopfølgning af en befolkningskohorte, som ikke er blevet informeret om tilstedeværelsen af galdesten efter screening med ultralydsundersøgelse. Lignende er ikke blevet rapporteret tidligere. Mindre end én ud af fem personer med galdesten vil udvikle galdestensudløste symptomer i løbet af 20 års opfølgning – dette er den laveste kumulative incidens rapporteret til dato. Spontanforløbet af galdesten konkluderes at være mindre aggressivt end tidligere antaget.

Udvikling af symptomatiske galdesten blev determineret af en galdestensstørrelse over 1 cm, multiple sten, viden om tilstedeværelsen af galdesten, højere BMI, kvindeligt køn og yngre alder, hvoraf kun stenstørrelse er identificeret i ét tidligere studie. Yderligere determinerede smerter lokaliseret i epigastriet, af længere varighed, af højere intensitet, om natten og med behov for analgetika udvikling af symptomatiske galdesten i en kohorte, der ikke var informeret om tilstedeværelsen af galdesten, hvilket ligeledes var nye fund.

Galdesten identificeret ved screening forårsagede højere dødelighed, kardiovaskulær sygdom, autoimmun sygdom, mave-tarmkræft og især højresidig tyktarmskræft, når der sammenlignes med befolkningskontroller. De fundene associationer for galdesten og dødelighed, kardiovaskulær sygdom og cancer blev bekræftet, mens associationen for autoimmun sygdom var et nyt fund. Kolecystektomi ændrede ikke associationer for dødelighed eller morbiditet.

Hos patienter, der havde fået foretaget behandling for galdesten, blev der fundet en stærk sammenhæng for sfinkterotomi og udvikling af galdegangskræft, når der sammenlignes med befolkningskontroller. Sammenhængen persisterede over fem år. Der anbefales forsigtighed ved brug af sfinkterotomi hos yngre patienter. En mulig beskyttende effekt af kolecystektomi blev fundet for kræft i galdegange, bugspytkirtel, spiserør og mavesæk. De identificerede associationer for galdestensbehandlinger og udvikling af kræft er nye, når der sammenlignes med tidligere studier.

Fælles mekanismer for udvikling af galdesten, kardiovaskulær sygdom, autoimmun sygdom og cancer bør undersøges yderligere i fremtidige eksperimentelle og epidemiologiske studier. Potentielle mekanismer foreslås at omfatte inflammation, tarmflora og cellulære receptorer involveret i omsætningen af kolesterol og galdesyrer.

Det foreslås at, udvælgelsen af patienter med symptomatiske ukomplicerede galdesten til kolecystektomi bør optimeres igennem fremtidige kliniske studier. De i denne afhandling identificerede determinanter bør undersøges i kliniske kohorter med henblik på symptomlindring efter kolecystektomi. Såfremt determinanterne kan definere en subgruppe, som har bedre symptomlindring, bør fremtidige RCT'er undersøge udvælgelse af patienter baseret på disse over for sædvanlig klinisk praksis.

Profylaktisk kolecystektomi ved asymptomatiske galdesten i almenbefolkningen anbefales ikke. Udfordringen for fremtidige studier bliver at identificere subgruppen af personer med galdesten, som har gavn af kolecystektomi på længere sigt. I lyset af sådanne fremtidige fund kan indikationen for kolecystektomi enten udvides eller revurderes. Indtil videre er kolecystektomi stadigvæk kun indiceret ved symptomatiske galdesten.

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