Accepted Manuscript

Similarities and differences between biliary sludge and microlithiasis: Their clinical and pathophysiological significances

Helen H. Wang, Piero Portincasa, Min Liu, Patrick Tso, David Q-H. Wang

Please cite this article as: Wang HH, Portincasa P, Liu M, Tso P, Wang DQH, Similarities and differences between biliary sludge and microlithiasis: Their clinical and pathophysiological significances, *Liver Research* (2018), doi: https://doi.org/10.1016/j.livres.2018.10.001.

Similarities and differences between biliary sludge and microlithiasis: their clinical and pathophysiological significances

Helen H. Wang,[1] Piero Portincasa,[2] Min Liu,[3] Patrick Tso,[3] David Q.-H. Wang[1,4]

[1]Department of Medicine, Division of Gastroenterology and Liver Diseases, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA;

[2]Department of Biomedical Sciences and Human Oncology, Clinica Medica "A. Murri", University of Bari "Aldo Moro" Medical School, Bari, Italy; and

[3]Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, OH 45237, USA

Running title: biliary sludge and microlithiasis

[4]Address correspondence to David Q.-H. Wang, M.D., Ph.D., Department of Medicine, Division of Gastroenterology and Liver Diseases, Marion Bessin Liver Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA. Phone: (718) 430-8865, Fax: (718) 430-8568, Email: david.wang@einstein.yu.edu

Abbreviations: ABC, ATP-binding cassette (transporter); ERCP, endoscopic retrograde cholangiopancreatography; MRI, magnetic resonance imaging; TPN, total parenteral nutrition; UDCA, ursodeoxycholic acid.

Key words: acute cholecystitis; acute pancreatitis; biliary colic; cholesterol monohydrate crystals; lithogenic bile

There is no conflict of interest to disclose for all authors.

Author's contribution: HHW and DQ-HW: literature search, drafting of the manuscript, drawing of Figures 4 and 7, and revision of the manuscript. PP, ML, and PT: critical revision of the manuscript. DQ-HW: collection of Figures 2 and 3. PP: collection of Figures 1, 5, 6, and 8.

Similarities and differences between biliary sludge and microlithiasis: their clinical and pathophysiological significances

1

Abbreviations: ABC, ATP-binding cassette (transporter); ERCP, endoscopic retrograde cholangiopancreatography; MRI, magnetic resonance imaging; TPN, total parenteral nutrition; UDCA, ursodeoxycholic acid.

Key words: acute cholecystitis; acute pancreatitis; biliary colic; cholesterol monohydrate crystals; lithogenic bile

There is no conflict of interest to disclose for all authors.

Abstract

The terms biliary sludge and microlithiasis were originated from different diagnostic techniques and may represent different stages of cholesterol gallstone disease. Although the pathogenesis of biliary sludge and microlithiasis may be similar, microlithiasis could be preceded by biliary sludge, followed by persistent precipitation and aggregation of solid cholesterol crystals, and eventually, gallstone formation. Many clinical conditions are clearly associated with the formation of biliary sludge and microlithiasis, including total parenteral nutrition, rapid weight loss, pregnancy, organ transplantation, administration of certain medications, and a variety of acute and chronic illnesses. Numerous studies have demonstrated complete resolution of biliary sludge in approximately 40% of patients, a cyclic pattern of disappearing and reappearing in about 40%, and progression to gallstones in nearly 20%. Although only a minority of patients with ultrasonographic demonstration of biliary sludge develop gallstones, it is still a matter of controversy whether microlithiasis could eventually evolves to cholesterol gallstones. Biliary sludge and microlithiasis are asymptomatic in the vast majority of patients; however, they can cause biliary colic, acute cholecystitis, and acute pancreatitis. Biliary sludge and microlithiasis are most often diagnosed ultrasonographically and bile microscopy is considered the gold standard for their diagnosis. Specific measures to prevent the development of biliary sludge are not practical or cost-effective in the general population. Laparoscopic cholecystectomy offers the most definitive therapy on biliary sludge. Endoscopic sphincterotomy or surgical intervention is effective for microlithiasis-induced pancreatitis. Ursodeoxycholic

acid (UDCA) can effectively prevent the recurrence of solid cholesterol crystals and significantly reduce the risk of recurrent pancreatitis.

1. Introduction

Many clinical studies have found that biliary sludge and cholesterol microlithiasis (hereafter referred to as microlithiasis) could cause either an uncomplicated symptomatic gallstone-like disease (biliary colic), or a complicated gallstone-like disease (acute cholecystitis and acute pancreatitis).[1-12] Furthermore, both biliary sludge and microlithiasis could represent the early stage of cholesterol gallstone disease because approximately 20% of patients with biliary sludge or microlithiasis can eventually develop cholesterol gallstones.[3] However, in the vast majority (~60%) of subjects, biliary sludge and microlithiasis can spontaneously disappear after pathogenic conditions are cured, and under these circumstances, they are completely dissolved.[13] If the gallbladder displays a well-regulated motility function, biliary sludge and microlithiasis can be totally eliminated into the intestine. Moreover, they can be completely dissolved in almost all of the individuals who are treated with ursodeoxycholic acid (UDCA) and/or cholecystokinin. This indicates that biliary sludge and microlithiasis are a reversible morbid condition during the development of cholesterol gallstone disease.[2] Although the terms biliary sludge and microlithiasis are often used interchangeably in the literature, the former is frequently described in pregnant women and in patients on total parenteral nutrition (TPN), while the latter is usually considered as a pathogenic factor in patients with idiopathic acute pancreatitis.[14-21] In clinical practice, physicians and surgeons have used many different definitions of biliary sludge and microlithiasis, which have made it difficult to compare

various studies, including therapeutic strategies and effects.[22] The diagnosis of biliary sludge is almost always based on ultrasonographic findings, while the exact diagnosis of microlithiasis requires the direct microscopic examination of bile. On transabdominal ultrasonography, biliary sludge has been defined as an amorphous mixture of particulate matter and bile, which occurs when various solutes in bile precipitate.[23] Biliary sludge is echogenic and does not cast an acoustic shadow (**Figure 1**). Although sludge is dependent on gravity, it slowly moves to the dependent portion of the gallbladder. Bile is usually collected for microscopy in patients without gallstones on ultrasonography, and is often aspirated from the duodenum, common bile duct, or gallbladder via an endoscope channel or a plastic cannula during the procedure of endoscopic retrograde cholangiopancreatography (ERCP).[24-27] Bile collected in this context frequently contains classical birefringent solid cholesterol monohydrate crystals (**Figure 2**). Bile microscopy has assumed a role in clinical diagnosis of hepato-pancreato-biliary diseases.

During clinical practice, the criteria for differentiating between biliary sludge and microlithiasis are not entirely clear because they are very tiny and are not detected easily by current imaging techniques. It has been suggested that a gallstone be defined as a particle with a diameter greater than 3 mm that cannot be crushed by digital compression. [28-30] Moreover, microlithiasis is a hard, ball-like object, and light yellow in color with smooth curved surface (**Figure 3**). Because of scattered and absorbed light, microlithiasis is opaque, and black in color under the microscope. In contrast, biliary sludge is soft, non-birefringent amorphous strands under phase contrast and polarizing light microscopy.[31-33] Furthermore, it is unclear whether there are differences in the

5

natural history, fate, the resulting complications, the responses to medical treatment, and prognosis between biliary sludge and microlithiasis. It has been questioned whether there is a difference in chemical composition of biliary lipids as well as pro-nucleating and anti-nucleating proteins between biliary sludge and microlithiasis. It is still unclear whether biliary sludge or microlithiasis is more predisposed to evolve into macroscopic stones and to cause complications. Nevertheless, biliary sludge and microlithiasis could have the same meaning and could result in the same complications. Thus, they could display the same fate and natural history. In this review article, we discuss similarities and differences between biliary sludge and microlithiasis and summarize their pathophysiological and clinical significances.

2. Etymology of biliary sludge and microlithiasis

Although a white crystalline substance, i.e., solid cholesterol monohydrate crystals, has been found to be the major component of cholesterol gallstones during the second half of the 18th century, it was until the early 20th century that biliary cholesterol monohydrate crystals were identified by light microscopy to be colorless, transparent, thin plate-like solid crystals with parallel edges, as well as with 79.2° and 100.8° angles, and often a notched corner (**Figure 2**).[34-40] This microscopic finding provided an imaging basis for clinical diagnosis of gallbladder cholesterol gallstone disease before modern gallbladder imaging techniques such as ultrasonography, x-ray film, and computed tomography were available.[28, 29, 41-48] Classical plate-like birefringent rhomboids were often detected in duodenal aspirates of patients with cholesterol gallstone disease although investigators also found some other forms of solid

6

cholesterol crystals, i.e., in the form of needle-like or arc-like crystals, thin plates with irregular margins, or agglomerates.[49-52]

With the advent of abdominal ultrasonography in the early 1970s, the term biliary sludge was first used for diagnosis of gallbladder disease.[23, 53, 54] Ultrasonographic examination found that biliary sludge appears as low-level echoes that layer in the dependent portion of the gallbladder without acoustic shadowing and it generally shifts slowly with positioning (**Figure 1**). Because abdominal ultrasonography is a noninvasive and inexpensive technique, it has been extensively used for clinical diagnosis of biliary diseases. In contrast, microlithiasis-induced acute pancreatitis is difficultly diagnosed by abdominal ultrasonography because the pancreas is located in the abdominal cavity behind the stomach. After alcohol is excluded as a causative factor for acute pancreatitis, ERCP should be performed and bile is aspirated from the duodenum. After being centrifuged, bile samples are examined by phase contrast and polarizing light microscopy.[38, 55-57] If solid cholesterol crystals are found, the diagnosis of microlithiasis-induced acute pancreatitis is established.[58-62] Bile is now collected for microscopy in patients without gallstones on ultrasonography, and is usually aspirated from the duodenum or common bile duct via an endoscope channel or a 5-Franch plastic catheter during ERCP. Because the diameter of tubing is too small, large size (>5 mm) of gallstones, if existed, cannot be aspirated during the ERCP procedure for microscopic examination. Although it is possible that a tiny gallstone could be found in the duodenal lumen and/or the common bile duct during the examination directly by ERCP, the probability of finding such a small stone is very low.

7

Obviously, the terms biliary sludge and microlithiasis were originated from the clinical applications of different diagnostic techniques.

3. Definition and chemical composition of biliary sludge and microlithiasis

Biliary sludge is most commonly composed of classical solid plate-like cholesterol monohydrate crystals, as well as varying sizes of aggregated liquid crystals, calcium bilirubinate granules, and other calcium salts, most of which are embedded in strands of mucin gel produced by the gallbladder.[31-33] Of note is that the chemical composition of biliary sludge varies with the clinical condition. For example, biliary sludge consists primarily of cholesterol monohydrate crystals in pregnant women,[63-67] whereas calcium bilirubinate predominate in patients receiving TPN, a predisposing condition to gallbladder stasis and precipitation of solid cholesterol crystals from bile. Moreover, biliary sludge is composed mainly of calcium-ceftriaxone complexes (i.e., so-called pseudocholelithiasis) in patients receiving high-dose ceftriaxone therapy.[68]

Biliary sludge also contains a large proportion of undefined residues, protein-lipid complexes, soluble mucin proteins, and some minerals.[69] In addition, xenobiotics such as ceftriaxone and biliary proteins are found to be important components. Calcium bilirubinate granules are almost invariably present, and bilirubin is usually found in its unconjugated, least soluble form. The source of the unconjugated bilirubin is controversial. Bilirubin is excreted by the liver mainly in its diglucuronide form, but small amounts of the monoglucuronide and unconjugated forms are also found. β-glucuronidase may be important for increasing unconjugated bilirubin because this enzyme can deconjugate bilirubin. Some pathogenic conditions, such as chronic low-

8

grade biliary infection, are associated with pigment gallstones, in which the activity of this enzyme is increased. β-glucuronidase has also been identified in uninfected bile, probably having originated in the biliary epithelium. Non-enzymatic hydrolysis of bilirubin may occur.

On microscopy, microlithiasis is often defined as a tiny stone with a diameter <3 mm, which cannot be crushed by digital compression. Under polarizing light microscopy, these tiny stones always display rounded contours and black centers from light scattering/absorption. To date, such petty stones are often missing or undetected by conventional ultrasonography or current imaging techniques.

4. Pathogenesis

Human bile is a golden yellow, dark green, or brownish complex solution that is composed mainly of water, organic solutes (i.e., lipids), inorganic salts, and some proteins. In bile, cholesterol, phospholipids, and bile acids are three major lipid components, which account for approximate 99% of total lipids by weight. Bilirubin is a minor solute and represents less than 1% of biliary lipids in bile. Approximately 95% of the cholesterol molecule in bile is in the unesterified form and <5% of the sterols are cholesterol precursors and dietary sterols.[70-72] In contrast, the concentrations of cholesteryl esters are negligible in human bile. Because bile is an aqueous solution and cholesterol is basically insoluble in water, the physical-chemical mechanisms for cholesterol solubilization in bile are complex. Bile acids, which are synthesized in the liver through the conversion of cholesterol, can form simple and mixed micelles in bile. These micelles can aid in solubilizing cholesterol in bile.[73-78] In addition, the vesicles

9

that are composed primarily of phospholipids also greatly enhance the solubilization of cholesterol in bile.[39, 79-84] The precipitation of solid cholesterol monohydrate crystals represents a failure of biliary cholesterol homeostasis in which the physical-chemical balance of cholesterol solubility in bile is disturbed, thereby leading to the formation of cholesterol monohydrate crystals and gallstones. Over the past five decades, a lot of accumulated evidence from human and animal studies has greatly increased our understanding of the molecular, cellular and genetic mechanisms underlying the pathogenesis of cholesterol gallstone disease.

Based on clinical and epidemiological studies and animal experiments, we have proposed a novel concept that interactions of five primary defects play a critical role in the pathogenesis of cholesterol gallstone disease.[85] These defects include (i) genetic factors and *Lith* genes; (ii) hepatic hypersecretion of biliary cholesterol, leading to supersaturated bile; (iii) rapid phase transitions of cholesterol in bile; (iv) impaired gallbladder motility accompanied with hypersecretion of mucins and accumulation of mucin gel in the gallbladder lumen, as well as immune-mediated gallbladder inflammation; and (v) increased amounts of cholesterol of intestinal origin due to high efficiency of cholesterol absorption and/or slow intestinal motility, which aids "hydrophobe" absorption and augments synthesis of secondary bile acids by the anaerobic intestinal microflora.[70, 85, 86] On the basis of numerous human and animal studies, hepatic cholesterol hypersecretion has been recognized to be the primary pathophysiologic defect, leading to the formation of cholesterol-supersaturated bile and solid cholesterol crystals, as well as the aggregation and growth of these solid crystals into microscopic stones first and then macroscopic gallstones. These abnormalities are

caused by multiple *Lith* genes, with insulin resistance as part of the metabolic syndrome working with cholelithogenic environmental factors to induce the phenotype.[70, 87, 88] The growth of solid cholesterol crystals starts as soon as cholesterol nucleation and crystallization occurs and this process is greatly accelerated by mucin gel, a potent pro-nucleating agent. Rapid growth and agglomeration of solid plate-like cholesterol monohydrate crystals into microlithiasis and eventually gallstones is a consequence of persistent hepatic hypersecretion of biliary cholesterol together with both gallbladder mucin hypersecretion and incomplete evacuation by the gallbladder due to its impaired motility dependent on defective smooth muscle in response to neuro-hormonal stimuli.[89] Over the past decades, new progress has been made in the genetic analysis of *Lith* genes and the pathophysiology of gallstone disease. Many excellent review articles on these topics have been extensively published and interested readers can further read these papers.[71, 85, 89-96]

The extent to which patients with no identifiable risk factors develop biliary sludge and microlithiasis remains controversial. However, there are many clinical conditions that are clearly associated with the formation of biliary sludge and microlithiasis.[71] These include parenteral supplementation, conditions associated with weight loss, pregnancy, a variety of acute and chronic illnesses, organ transplantation, and administration of certain medications. Numerous studies have found that physical and chemical interactions of gallbladder contents together with altered gallbladder motility and mucosal function serve as a nidus for the formation of biliary sludge and microlithiasis.[2] It is likely that microlithiasis is preceded by the formation of biliary sludge, followed by microprecipitate aggregation and eventual stone formation.[97-100]

This hypothesis is supported by the typical finding of biliary sludge before stone recurrence in chemical dissolution studies. Others repudiate this hypothesis because of the infrequent development of gallstones in persons with biliary sludge and the spontaneous resolution of biliary sludge in most individuals. Of note, a genetic defect in the *ABCB4* gene is the cause of a subtype of progressive familial intrahepatic cholestasis called PFIC3, in which the phenotypic spectrum of PFIC3 ranges from neonatal cholestasis to cirrhosis in young adults.[101-103] In addition, the *ABCB4* defect is involved in two other human liver diseases such as intrahepatic cholestasis of pregnancy[104-106] and cholesterol gallstone disease, i.e., low phospholipid-associated cholelithiasis (LPAC).[107-110] It is still unclear why these three human liver diseases are induced by a single gene (*ABCB4*) deficiency, and its pathogenic mechanism is under an extensive investigation.[111-113]

The formation of biliary sludge greatly depends on the physical-chemical interactions of the three major lipid constituents, abnormalities of gallbladder mucosal function, and gallbladder dysmotility.[85, 89, 90, 93, 114] It is most likely that cholesterol gallstones are formed initially from biliary sludge by persistent precipitation and aggregation of solid cholesterol crystals, and it is generally thought that biliary sludge is a necessary precursor to gallstones. For example, after chemical dissolution of gallstones, biliary sludge in the gallbladder lumen is usually seen on ultrasonography before gallstone recurrence. Thus, the pathogenesis of biliary sludge could be similar to that of cholesterol gallstones. However, controversial results have also been found from clinical studies that biliary sludge can spontaneously resolve in most persons and that gallstones form in only a small minority of persons with biliary sludge. This suggests that

12

biliary sludge could not be an essential precursor to gallstones and that the pathogenesis of the two entities could differ. Nevertheless, it is impossible that gallstones could form from clear bile without the intermediate formation of microprecipitates, including classical cholesterol monohydrate crystals, anhydrous cholesterol crystals and sandy stones. It is still imperative to investigate how these microprecipitates aggregate and grow to form well-ordered, structured gallstones.

It is likely that microlithiasis may form through a different mechanism compared to the formation of cholesterol gallstones although both could start from gallbladder bile supersaturated with cholesterol. The natural history of microlithiasis is not completely defined yet. Although clinical studies have found that only a minority of patients with ultrasonographic demonstration of biliary sludge develop gallstones, it is still a matter of controversy whether microlithiasis could evolves to cholesterol gallstone disease.

Although the pathogenesis of microlithiasis could be the same to that of cholesterol gallstones, there are distinct differences. Rapid precipitation of solid cholesterol crystals from cholesterol-supersaturated bile is the key factor in the formation of microlithiasis.[115] In bile aspirated from the duodenum after intravenous infusion of the cholecystokinin analogue ceruletide, patients with gallbladder microlithiasis have significantly lower concentrations of phospholipids compared to that in patients with macroscopic gallbladder stones.[29] Relative phospholipid deficiency is caused mostly by missense mutations in the ATP-binding cassette (ABC) subfamily B member 4 (*ABCB4*) gene, also known as the multidrug resistance protein 3 (*MDR3*) gene. The *ABCB4* gene encodes for an energy-dependent phospholipid efflux translocator at the canalicular membrane of the hepatocytes, which facilitates the

transport of phospholipids from the inner to the outer canalicular membrane of hepatocytes for hepatic secretion into canalicular bile.[116-119] Interestingly, some cases of acute pancreatitis caused by microlithiasis are associated with point mutations in the *ABCB4* gene.[120] Obviously, relative phospholipid deficiency in bile is related to rapid cholesterol crystallization and microlithiasis, as well as a high risk of developing acute pancreatitis. These findings can be explained by the physical-chemical properties of biliary cholesterol, phospholipids and bile acids in bile. Because cholesterol is poorly soluble in an aqueous environment, it is solubilized in bile in mixed micelles by bile acids and phospholipids. In case of cholesterol supersaturation, excess cholesterol is also solubilized in vesicles that are composited mainly of phospholipids. If excess cholesterol cannot be dissolved by micelles or vesicles, it could precipitate as solid cholesterol crystals.[121] The importance of the relative amounts of bile acids vs phospholipids in the model bile system has found for crystallization behavior.[37] In contrast to usual cholesterol gallstone patients, biliary lipid composition of patients with microlithiasis plots in the left two-phase zone, in which besides classical plate-like cholesterol monohydrate crystals, various intermediate cholesterol crystals such as arc-like and needle-like crystals and tubular crystals can be detected, probably related to a faster crystallization process.[37, 51, 122] In addition, these patients with microlithiasis have an increased cholesterol saturation index similarly to those with cholesterol gallstones. However, bile supersaturation of patients with microlithiasis is due to a decrease in biliary phospholipid concentrations and not to an increase in biliary cholesterol concentrations.

Hypersecretion of gallbladder mucin, including release of excess mucin gel, is also strongly associated with microlithiasis.[98] Mucin gel can promote rapid cholesterol crystallization possibly by triggering cholesterol nucleation and offering low affinity binding sites for phospholipids and cholesterol.[123] On the other hand, mucin gel may increase bile viscosity, thus leading to the formation of a gel matrix which can entrap solid cholesterol crystals and enhance aggregation and growth of solid cholesterol crystals into microlithiasis in the gallbladder.[40, 124-128]

As discussed above, it is highly likely that biliary sludge and microlithiasis could represent two different steps during the early stage of cholesterol gallstone formation. Further studies on both the natural history and the pathogenesis of biliary sludge and microlithiasis are strongly needed to address these questions.

5. Epidemiology, natural history and clinical course

The prevalence of biliary sludge varies widely as reported in the literature. Most studies have examined patients in a specific clinical situation such as pregnancy or critical illness.[3] Moreover, clinical investigations on the natural history of biliary sludge are usually based on abdominal ultrasonographic diagnosis, without microscopic examination or chemical composition analysis. Many of these studies are also limited by inadequate follow-up. In addition, it is not known whether the natural history of cholesterol monohydrate crystal-predominant sludge differs from that of calcium bilirubinate-predominant sludge.

Only one large study has been done with a well-defined follow-up protocol. In that study, three clinical outcomes are found: (i) complete resolution, (ii) a waxing and

waning course, and (iii) gallstone formation.[65] More importantly, biliary sludge found in patients with abdominal pain can spontaneously disappear in about 50% of cases and persist asymptomatically in about 20% of cases over a 3-year period. Over the same period, symptoms develop in 10% to 15% of patients and gallstones are formed in 5% to 15% of patients. If a specific precipitating cause for the formation of biliary sludge exists, biliary sludge usually resolves upon the removal of that cause. If the precipitating event recurs or persists, gallstones can form eventually. For instance, biliary sludge and gallstones often coexist after multiple pregnancies or prolonged TPN administration.

Similar to gallstones, biliary sludge is often asymptomatic in most patients. However, in addition to predisposing to gallstone formation, biliary sludge can lead to complications such as biliary colic.[16] Approximately 31% of patients with nonalcoholic pancreatitis have biliary sludge, and up to 74% of patients with "idiopathic" pancreatitis have been found to be associated with biliary sludge, in which excess alcohol use, gallstones, metabolic abnormalities and drug-related causes have been excluded. Other complications of biliary sludge include acute cholangitis and acute acalculous cholecystitis.

If biliary sludge causes symptoms, a more provocative hypothesis is that the biliary pain and inflammation found in gallstone disease are mediated by the presence of biliary sludge. For example, symptomatic patients with gallstones who receive UDCA treatment have a striking alleviation of the symptoms in three months, although the number and size of their gallstones do not change. It is likely that solid cholesterol crystals in bile are dissolved before the gallstones do. Asymptomatic patients with gallstones who are receiving shock-wave lithotripsy can develop biliary colic, acute

cholecystitis, or acute pancreatitis. In these patients, biliary sludge may have been created iatrogenically.

Our understanding of the clinical significance of biliary sludge has been limited by short-term studies involving patients with known risk factors, many of whom have coexisting gallstones and gallbladder pathologic condition. However, many clinical studies have demonstrated complete resolution of biliary sludge in approximately 40% of patients, a cyclic pattern of disappearing and reappearing in about 40%, and progression to gallstones in approximately 20%.

Potential outcomes of microlithiasis include remaining asymptomatic, developing biliary colic, or experiencing biliary and/or pancreatic complications such as acute cholecystitis, acute cholangitis, papillary stenosis, and acute pancreatitis. Although the course cannot be reliably predicted, removal of the precipitating event correlates with resolution of microlithiasis. Persistence of an underlying risk factor often leads to gallstone formation and complications such as acute pancreatitis.

Microlithiasis may lead to acute pancreatitis through several mechanisms. Small gallstones may transiently impact at the papilla, leading to pancreatic duct obstruction and eventual pancreatitis. Repeated exposure to microlithiasis may result in papillary stenosis and sphincter of Oddi dysfunction, both of which are often associated with acute pancreatitis. The smaller size of stones comprising microlithiasis (vs. macrolithiasis) does not imply a lower likelihood of acute pancreatitis. In contrast, there are some data suggesting that smaller stones may correlate with a greater tendency for the development of acute pancreatitis. However, there is also some uncertainty regarding the causal relationship between microlithiasis and acute pancreatitis. Some

investigations found that microlithiasis does not cause acute pancreatitis, but instead indicates the prior presence of larger gallstones that leads to the pancreatitis.[129] Pancreatitis itself may induce the formation of microlithiasis as a result of edema of the head of the pancreas that leads to biliary compression and gallbladder hypomotility. Gallbladder stasis also results from fasting, parenteral nutrition, and weight loss, with all these conditions being common in patients with pancreatitis. Also, gallbladder content is modified and may become more lithogenic as a result of pancreatitis.

6. Clinical manifestation and diagnosis

When patients have abdominal pain, true biliary colic should be distinguished from nonspecific abdominal discomfort.[130] A cholecystectomy done for true biliary colic is usually curative, but symptoms often persist if the procedure is done in patients with nonspecific abdominal pain such as dyspepsia, gastric ulcer, duodenal ulcer, irritable bowel syndrome, causalgia, dysmenorrhea, psychosomatic pain, constipation, viral gastroenteritis, mesenteric adenitis, or choledocholelithiasis.[131-134] Nevertheless, despite the availability of many imaging techniques that can be used to uncover biliary sludge and gallstones, the diagnosis of biliary colic is ultimately based on clinical judgment.

When biliary sludge is suspected, the choice of diagnostic method must be based on the clinical setting and the sensitivity, specificity, and cost of the diagnostic tests available (**Figure 4**). In general, given its relatively low cost and noninvasiveness, transabdominal ultrasonography should be the initial test. However, because the sensitivity of this test is only approximately 60%,[3, 135] further imaging exams should be considered if the ultrasonographic result is negative and clinical suspicion remains high.

For example, in a patient with recurring attacks of idiopathic pancreatitis.[17] If the diagnosis is to be pursued, either endoscopic ultrasonography or bile microscopy should be chosen.

Clinically, biliary sludge is most often diagnosed ultrasonographically, according to the criteria defined above. The ultrasonographic pattern can be produced by calcium bilirubinate granules or aggregated cholesterol monohydrate crystals as small as 0.5 to 1 mm in diameter mixed with bile and mucin gel, independent of bile viscosity.[1] Other entities, including blood, necrotic debris, multiple small gallstones, or pus, can also have an appearance similar to that of biliary sludge. However, the sensitivity of transabdominal ultrasonography for biliary sludge is only about 60%, and this procedure cannot define the chemical composition of biliary sludge.[136] The appearance of biliary sludge on endoscopic ultrasonography is similar to that on transabdominal ultrasonography, and the sensitivity of endoscopic ultrasonography is approximately 96%.[19, 137-140] On computed tomography, biliary sludge has greater attenuation than normal bile and layers within the gallbladder. Its appearance on magnetic resonance imaging (MRI) has not been well characterized.[141-144]

The evaluation usually begins with transabdominal ultrasonography because it is noninvasive and relatively inexpensive.[145-147] The combination of mucin gel and solid cholesterol crystals greater than 50 μm in size produce low-amplitude echoes without a postacoustic shadow that layer in the dependent portion of the gallbladder and shift with positional changes. Larger gallstones produce high-amplitude echoes and a postacoustic shadow (**Figure 5**). The use of transabdominal ultrasonography is limited by its low sensitivity (~50% to 60%) and inability to determine the chemical composition

of bile or its microprecipitates. The low sensitivity may be due to the operator-dependent nature of this procedure, the tendency for classical plate-like cholesterol monohydrate crystals, but not calcium bilirubinate crystals, to be imaged, and the intermittent presence of biliary sludge or microlithiasis. Repeat examination could improve the diagnostic rate. Notably, compared to fundamental ultrasonography, harmonic ultrasonography shows better conspicuity of gallbladder microlithiasis.[148]

Generally, bile microscopy is considered the gold standard for diagnosis. Bile may be collected from the duodenum (during endoscopy), bile duct (during ERCP), or the gallbladder itself (during ERCP or by percutaneous aspiration). The sensitivity of this test for biliary sludge or gallstones varies from 67% to 86%, and the specificity ranges from 88% to 100%.[52, 149] The sensitivity is 83% when bile is obtained directly from the common bile duct during ERCP.[61] The sensitivity of bile microscopy would be even higher if bile is obtained through duodenal intubation than through endoscopy. If gallbladder bile is collected, the sensitivity of bile microscopy is probably the highest among all sampling methods. The diagnostic rate of bile microscopy is the highest when bile is collected from the gallbladder, followed by from the bile duct and then from the duodenum. Although the sensitivity of bile microscopy varies according to the site of bile aspiration, there is likely minimal difference between sampling techniques when gallbladder bile is collected. To enhance gallbladder contractility, improve the collection of gallbladder bile, and increase the diagnostic rate, cholecystokinin is often administered before bile aspiration.

Direct microscopic examination of the content of the gallbladder is more sensitive than ultrasonography in the detection of biliary sludge. Thus, even though it is less

clinically applicable than ultrasonography, the microscopic examination of bile is considered the diagnostic gold standard. In addition, bile microscopy allows the chemical composition of biliary sludge to be defined by precipitate structure.

Although many different protocols for bile microscopy have been described, some measures can maximize the sensitivity of this test. It should be recognized that gallbladder bile is preferred for bile microscopy. Although hepatic bile is supersaturated with cholesterol, rapid transit through the biliary ductal system rarely allows enough time for hepatic bile to form a solid cholesterol crystal large enough to be detectable on polarizing light microscopy. Hepatic bile is invariably yellow and free of precipitate, even when the gallbladder contains biliary sludge, microlithiasis or gallstones. Thus, using ductal bile to look for biliary sludge in patients with intact gallbladders is inappropriate.

As discussed above, cholesterol monohydrate crystals demonstrate birefringence under polarizing light microscopy and are classically rhomboidal-shaped with a notched corner (**Figure 2**). Calcium bilirubinate granules are non-birefringent and appear as brown or reddish-brown minute participates (**Figure 2**). The number of solid cholesterol crystals required to establish a positive study varies widely among clinical investigations. Bile microscopy is a semiquantitative study with the number of solid cholesterol crystals being influenced by the method of sampling. Grading systems have been developed to quantify the number of solid cholesterol crystals, but this is mostly for research purposes and of little clinical utility. It has never been shown that the quantity of solid cholesterol crystals correlates with clinical outcome. Most investigators regard the presence of even a small number of solid cholesterol crystals as a positive result.

Endoscopic ultrasonography has been less widely studied than bile microscopy.[150] The sensitivity of endoscopic ultrasonography is 96% compared with 67% for duodenal drainage. However, the specificities of these two methods are similar from 86% to 91%. The sensitivity of endoscopic ultrasonography and bile microscopy combined is approximately 92%. More importantly, endoscopic ultrasonography operates at a higher frequency than transabdominal ultrasonography and minimizes the influence of bowel gas or subcutaneous tissue on image quality.[151-153] Endoscopic ultrasonography produces higher image resolution and greater diagnostic sensitivity (~95%). Stimulated biliary drainage can be performed at the time of endoscopic ultrasonography, which has been shown to further enhance diagnostic capability.

If patients with suspected biliary sludge have a negative result of transabdominal ultrasonography, further testing largely depends on the clinical situation. We recommend bile sampling for patients in whom the clinical suspicion of biliary sludge is high and in whom further treatment, such as cholecystectomy, would be considered. The choice of a method of bile sampling depends on the clinical situation.[154] For example, if upper gastrointestinal tract disorders remain in the differential diagnosis, an upper gastrointestinal endoscopy should be performed and bile should be aspirated for bile microscopy during the procedure. If patients have no indication for upper gastrointestinal endoscopy, a bile sample could be obtained through duodenal intubation. Patients with recurrent episodes of idiopathic acute pancreatitis generally undergo ERCP, and bile can be sampled from the duodenum or the common bile duct during the procedure.[155-157] If patients have an indication for endoscopic ultrasonography, such as evaluation of abnormalities found from previous imaging

exams, they should undergo this procedure first. If biliary sludge is not identified on imaging exams, bile can be collected for microscopy. If patients have no indication for endoscopic ultrasonography, the test is not recommended because it has relatively low availability and high cost. Notably, ERCP is very helpful in finding gallstones in the common bile duct, as illustrated in **Figure 6**.

As shown in **Figure 7**, the optimal methods for detecting microlithiasis and the extent to which a diagnosis is sought largely depend on the clinical setting and prior evaluation.[158-161] The two most widely used techniques are transabdominal ultrasonography and bile microscopy. Although computed tomography and MRI may detect microlithiasis, their cost and unproven utility should preclude their use solely for this purpose unless otherwise indicated.[17, 162-164] As shown in **Figure 8**, a gallstone is found by computed tomography scans in the papilla of Vater in a patient with acute pancreatitis.

Bile microscopy is the gold standard for the diagnosis of microlithiasis with an overall sensitivity of about 65% to 90%. Bile sampling should be performed only if less invasive studies are negative, the clinical suspicion of microlithiasis is high, and the results could be used to guide management. Techniques vary with respect to the site of bile aspiration, use of cholecystokinin, processing of samples, and criteria for a positive test.

7. Treatment

Specific measures to prevent the development of biliary sludge are not practical or cost-effective in the general population. Although many studies have been performed

concerning two potential preventive strategies, including use of the hydrophilic tertiary bile acid UDCA and an intestinal hormone cholecystokinin, none of these methods can be recommended for routine clinical use, even in high-risk populations.[165]

UDCA is an orally administered bile acid that has been extensively studied for the dissolution of cholesterol gallstones and the treatment of primary biliary cirrhosis.[166-169] It reduces hepatic cholesterol secretion into bile and prolongs the time of cholesterol nucleation and crystallization. Some studies have investigated the effect of UDCA on the treatment of biliary sludge. In patients who are rapidly losing weight, UDCA reduces the incidence of gallstones by 50% to 100%.[170, 171] In patients with biliary sludge and idiopathic acute pancreatitis, after the initial treatment with UDCA to dissolve solid cholesterol crystals, sustaining maintenance therapy successfully prevents the recurrence of biliary sludge and pancreatitis. Notably, UDCA effectively prevents the recurrence of solid cholesterol crystals and significantly reduces the risk of recurrent pancreatitis.[30] The UDCA therapy may be a reasonable alternative in poor operative candidates or the very elderly.

Cholecystokinin or the cholecystokinin analogues could be used for prophylaxis against the development of biliary sludge in patients in whom gallbladder stasis is an underlying cause of biliary sludge, particularly in patients receiving prolonged total parenteral nutrition.[172, 173] Clinical studies have found that after patients initially free of biliary sludge or gallstones are randomly assigned to be treated with a daily intravenous infusion of cholecystokinin or placebo, no patients treated with cholecystokinin develops biliary sludge or gallstones.[172] In contrast, 62% of the patients receiving placebo suffer from either biliary sludge or gallstones. Moreover, few side effects of cholecystokinin or

cholecystokinin analogues are found in these patients. However, the safety and efficacy of cholecystokinin and the cholecystokinin analogues in other clinical settings have not been investigated yet.

In general, patients should be evaluated specifically for biliary sludge or gallstones only after they develop symptoms. Regularly monitoring asymptomatic patients for the development of biliary sludge or gallstones has not been generally done, even in high-risk patients. However, biliary sludge is often found incidentally on imaging exams performed for other reasons, and its management in these circumstances has been disputed. If a specific precipitating cause of biliary sludge is present, attempts should be made to eliminate it. For management purposes, biliary sludge and gallstones should be considered similar in almost all respects. Thus, asymptomatic patients with biliary sludge should be observed and managed expectantly (**Figure 4**).

When symptoms or complications of biliary sludge occur, therapy should be considered immediately. Patients with uncomplicated biliary colic are at moderate risk for future pain and more serious complications, but up to 30% of patients have no further symptoms. Thus, clinical judgment must be used to decide whether to proceed with therapy in these patients.[115] If more serious complications such as acute pancreatitis have occurred, therapy should be considered more strongly. The definitive therapy for biliary sludge is cholecystectomy, using either the laparoscopic or the open route.[115] However, if patients are under poor surgical conditions, nonsurgical interventions, such as oral UDCA dissolution or percutaneous cholecystostomy for drainage, should be considered (**Figure 4**).[115] The long-term efficacy of these treatments has not been proven. Therefore, these treatments should be used only in

patients who require therapy but are not under good surgical conditions. The recurrence rate of biliary sludge after oral UDCA dissolution is not known, but gallstones recur in approximately 50% of patients.[167] The efficacy of percutaneous cholecystostomy and drainage in the treatment of biliary sludge has not been well established. Other therapeutic options include surgical intervention, endoscopic sphincterotomy, and chemical dissolution. Notably, the benefits of these therapies have been demonstrated by a significant decrease in recurrent episodes of pancreatitis after therapy (<10%) versus a recurrence rate of approximately 66% to 75% in untreated patients.[62, 170, 174-176]

Laparoscopic cholecystectomy offers the most definitive therapy and is generally considered the treatment of choice (**Figure 4**).[122, 177-179] Laparoscopic cholecystectomy is indicated in good operative candidates of almost any age because the risks of future gallbladder-related symptoms and complications outweigh the operative risks.[90] Endoscopic sphincterotomy is an effective alternative and may obviate the need for cholecystectomy in the very elderly or those with significant comorbid illness.[180-184] The benefits of sphincterotomy include enhanced gallbladder motility and reduced gallbladder stasis on the evidence given by a significant reduction in gallbladder fasting volume and residual volume and by an increase in gallbladder ejection fraction. This improvement in gallbladder motility after endoscopic sphincterotomy may persist for at least 5 years. Endoscopic sphincterotomy may reduce bile lithogenicity by modifying biliary lipid composition, leading to a significant prolongation in time of cholesterol nucleation and crystallization.

There is no demonstrable difference in the clinical benefit of cholecystectomy or endoscopic sphincterotomy for microlithiasis-induced pancreatitis.[185] However, the use

of endoscopic therapy is favored only in patients with poor operative conditions or the very elderly, unless their clinical course justifies more aggressive intervention.[186] The continued risk of gallbladder-related symptoms or complications such as biliary colic, cholecystitis, and cholangitis after endoscopic sphincterotomy could need more definitive therapy in patients with good surgical conditions and with a reasonable life expectancy. The safety of laparoscopic cholecystectomy and the risk of immediate and late complications of endoscopic sphincterotomy further argue for the roles of these surgeries.

For the treatment of microlithiasis, endoscopic sphincterotomy is performed in 25 patients and cholecystectomy is carried out in two patients. Notably, this is partially explained by the coexistence of sphincter of Oddi dysfunction in many of these patients, which served as an indication for endoscopic sphincterotomy. However, in another studies on 12 patients with microlithiasis alone, only 2 are offered cholecystectomy. Interestingly, although endoscopic sphincterotomy is performed in all 5 patients with common bile duct stones, 4 of the 5 patients also undergo elective cholecystectomy. Biliary sphincter manometry has found that in 60 patients with biliary-type pain after cholecystectomy, 25 of whom ultimately prove to have elevated sphincter pressures. In addition, solid cholesterol monohydrated crystals are found in 3 patients, 2 without and 1 with elevated sphincter pressure. Of special note, in this selected group of patients, the vast majority of them have symptoms caused by sphincter of Oddi dysfunction.[175, 187] Taken together, the treatments of these patients may help us understand the choice of surgical intervention for microlithiasis.

In patients with acute recurrent pancreatitis, should the search for microlithiasis be abandoned or should such efforts be intensified? It is highly likely that such a decision is largely made based on the conditions including the clinical manifestation, approach to diagnosis, and indications for therapeutic intervention (**Figure 7**).[188-190] The presence of biliary sludge or microlithiasis in patients with acute recurrent pancreatitis usually leads to therapeutic intervention because of the tendency for pancreatitis to recur and the associated morbidity and mortality. Because of the prevalence of microlithiasis in this group of patients and the limited sensitivity of diagnostic studies, it is reasonable to proceed with laparoscopic cholecystectomy.[191-195] Endoscopic sphincterotomy and UDCA administration are options for patients with poor operative conditions or the very elderly. If this approach is followed, there is no need to search for microlithiasis, because a negative evaluation would not alter your management plan.

However, for the patients who refrain from recommending therapeutic intervention without clear evidence of microlithiasis, transabdominal ultrasonography or laboratory evidence of microlithiasis may suffice. If stronger evidence is desired, bile microscopy during ERCP should be then considered. If the imaging exams continue to show no signs of microlithiasis, surveillance should be carried out by endoscopic ultrasonography with bile microscopy or repeating transabdominal ultrasonography in several months. If microlithiasis cannot be found despite all efforts and patients still experience recurrent pancreatitis, elective cholecystectomy should be reconsidered.[191-195]

8. Conclusions and future research directions

Biliary sludge is often found in diverse clinical situations, but has not yet been well understood. Its clinical course varies in which evolution to gallstones, a waxing and waning course, and complete resolution are all possible outcomes. In addition, biliary sludge could lead to biliary and/or pancreatic complications, including biliary colic, acute pancreatitis, and acute cholecystitis. It is most often diagnosed on transabdominal ultrasonography, although bile microscopy is considered the gold standard for diagnosis. If patients with biliary sludge are asymptomatic, they can be managed expectantly. If patients develop symptoms or complications, cholecystectomy should be considered as the definitive therapy.

Much is still to be learned about biliary sludge and microlithiasis. Continuing controversies involve the natural history of biliary sludge vs. microlithiasis, risk factors for their formation, and their true importance in the spectrum of biliary tract disease. Future prospective clinical studies may help clarify these issues and further increase our understanding of the pathogenesis of these biliary tract abnormalities.

Long-term and larger prospective studies are strongly needed to provide insights into the natural history, pathogenesis, clinical significance, ideal means of diagnosis, and optimum form of therapy on patients with biliary sludge or microlithiasis. Only then can our efforts be focused on the management of these patients.

References

1. Wang DQ, Portincasa P. Gallstones: Recent advance in epidemiology, pathogenesis, diagnosis and management. New York: Nova Biomedical, 2017.
2. Wang DQ, Afdhal NH. Gallstone Disease. In: Feldman M, Friedman LS, Brandt L, eds. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 10 ed. Philadelphia: Elsevier Saunders, 2014:1100-1133.
3. Ko CW, Sekijima JH, Lee SP. Biliary sludge. Ann Intern Med 1999;130:301-11.
4. Lee SP, Nicholls JF, Park HZ. Biliary sludge as a cause of acute pancreatitis. N Engl J Med 1992;326:589-93.
5. Venneman NG, van Brummelen SE, van Berge-Henegouwen GP, et al. Microlithiasis: an important cause of "idiopathic" acute pancreatitis? Ann Hepatol 2003;2:30-5.
6. Gerke H, Baillie J. Biliary microlithiasis: a neglected cause of recurrent pancreatitis and biliary colic? J Gastroenterol Hepatol 2005;20:499-501.
7. Shaffer EA. Gallbladder sludge: what is its clinical significance? Curr Gastroenterol Rep 2001;3:166-73.
8. Peery AF, Crockett SD, Barritt AS, et al. Burden of Gastrointestinal, Liver, and Pancreatic Diseases in the United States. Gastroenterology 2015;149:1731-1741 e3.
9. Mitchell RM, Byrne MF. Biliary emergencies: pancreatitis, cholangitis, and more. Semin Gastrointest Dis 2003;14:77-86.
10. Mitchell RM, Byrne MF, Baillie J. Pancreatitis. Lancet 2003;361:1447-55.
11. Vidarsdottir H, Moller PH, Vidarsdottir H, et al. Acute pancreatitis: a prospective study on incidence, etiology, and outcome. Eur J Gastroenterol Hepatol 2013;25:1068-75.
12. McNabb-Baltar J, Ravi P, Isabwe GA, et al. A population-based assessment of the burden of acute pancreatitis in the United States. Pancreas 2014;43:687-91.
13. Jungst C, Kullak-Ublick GA, Jungst D. Gallstone disease: Microlithiasis and sludge. Best Pract Res Clin Gastroenterol 2006;20:1053-62.
14. Lee SP, Hayashi A, Kim YS. Biliary sludge: curiosity or culprit? Hepatology 1994;20:523-5.
15. Angelico M, Della Guardia P. Review article: hepatobiliary complications associated with total parenteral nutrition. Aliment Pharmacol Ther 2000;14 Suppl 2:54-7.
16. Janowitz P, Kratzer W, Zemmler T, et al. Gallbladder sludge: spontaneous course and incidence of complications in patients without stones. Hepatology 1994;20:291-4.
17. Levy MJ, Geenen JE. Idiopathic acute recurrent pancreatitis. Am J Gastroenterol 2001;96:2540-55.
18. Messing B, Bories C, Kunstlinger F, et al. Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? Gastroenterology 1983;84:1012-9.
19. Bolondi L, Gaiani S, Testa S, et al. Gall bladder sludge formation during prolonged fasting after gastrointestinal tract surgery. Gut 1985;26:734-8.

20. Houssin D, Castaing D, Lemoine J, et al. Microlithiasis of the gallbladder. Surg Gynecol Obstet 1983;157:20-4.
21. Goodman AJ, Neoptolemos JP, Carr-Locke DL, et al. Detection of gall stones after acute pancreatitis. Gut 1985;26:125-32.
22. Cucher D, Kulvatunyou N, Green DJ, et al. Gallstone pancreatitis: a review. Surg Clin North Am 2014;94:257-80.
23. Conrad MR, Janes JO, Dietchy J. Significance of low level echoes within the gallbladder. AJR Am J Roentgenol 1979;132:967-72.
24. Kaw M, Brodmerkel GJ, Jr. ERCP, biliary crystal analysis, and sphincter of Oddi manometry in idiopathic recurrent pancreatitis. Gastrointest Endosc 2002;55:157-62.
25. Cooperman M, Ferrara JJ, Carey LC, et al. Idiopathic acute pancreatitis: the value of endoscopic retrograde cholangiopancreatography. Surgery 1981;90:666-70.
26. Feller ER. Endoscopic retrograde cholangiopancreatography in the diagnosis of unexplained pancreatitis. Arch Intern Med 1984;144:1797-9.
27. Geenen JE, Nash JA. The role of sphincter of Oddi manometry and biliary microscopy in evaluating idiopathic recurrent pancreatitis. Endoscopy 1998;30:A237-41.
28. Sharma BC, Agarwal DK, Dhiman RK, et al. Bile lithogenicity and gallbladder emptying in patients with microlithiasis: effect of bile acid therapy. Gastroenterology 1998;115:124-8.
29. Fracchia M, Pellegrino S, Secreto P, et al. Biliary lipid composition in cholesterol microlithiasis. Gut 2001;48:702-6.
30. Ros E, Navarro S, Bru C, et al. Occult microlithiasis in 'idiopathic' acute pancreatitis: prevention of relapses by cholecystectomy or ursodeoxycholic acid therapy. Gastroenterology 1991;101:1701-9.
31. Lee SP, Nicholls JF. Nature and composition of biliary sludge. Gastroenterology 1986;90:677-86.
32. Lee SP, Maher K, Nicholls JF. Origin and fate of biliary sludge. Gastroenterology 1988;94:170-6.
33. Angelico M, De Santis A, Capocaccia L. Biliary sludge: a critical update. J Clin Gastroenterol 1990;12:656-62.
34. Chevreul ME. Examen des graisses d'homme, de mouton, de boeuf, de jaguar et d'oie. Annales de Chimie et de Physique 1816;2:339-372.
35. Berthelot M. Sur plusiers alcools nouveaux. Combinaisons des acides avec la cholesterine, l'ethal, le camphre de Borneo et la meconine. Annales de Chimie et de Physique 1859;56:51-98.
36. Reinitzer F. Beitrage zur Kenntniss des Cholesterins. Sitzber Akad Wiss Wien 1888;97:167-187.
37. Wang DQ, Carey MC. Complete mapping of crystallization pathways during cholesterol precipitation from model bile: influence of physical-chemical variables of pathophysiologic relevance and identification of a stable liquid crystalline state in cold, dilute and hydrophilic bile salt-containing systems. J Lipid Res 1996;37:606-30.

38. Wang DQ, Carey MC. Characterization of crystallization pathways during cholesterol precipitation from human gallbladder biles: identical pathways to corresponding model biles with three predominating sequences. J Lipid Res 1996;37:2539-49.

39. Gantz DL, Wang DQ, Carey MC, et al. Cryoelectron microscopy of a nucleating model bile in vitreous ice: formation of primordial vesicles. Biophys J 1999;76:1436-51.

40. Wang HH, Portincasa P, Liu M, et al. Effect of gallbladder hypomotility on cholesterol crystallization and growth in CCK-deficient mice. Biochim Biophys Acta 2010;1801:138-46.

41. Juniper K, Jr., Burson EN, Jr. Biliary tract studies. II. The significance of biliary crystals. Gastroenterology 1957;32:175-208; discussion, 208-11.

42. Ramond MJ, Dumont M, Belghiti J, et al. Sensitivity and specificity of microscopic examination of gallbladder bile for gallstone recognition and identification. Gastroenterology 1988;95:1339-43.

43. Burnstein MJ, Vassal KP, Strasberg SM. Results of combined biliary drainage and cholecystokinin cholecystography in 81 patients with normal oral cholecystograms. Ann Surg 1982;196:627-32.

44. Porterfield G, Cheung LY, Berenson M. Detection of occult gallbladder disease by duodenal drainage. Am J Surg 1977;134:702-4.

45. Foss DC, Laing RR. Detection of gallbladder disease in patients with normal oral cholecystograms. Results using a simplified biliary drainage technique. Am J Dig Dis 1977;22:685-9.

46. Sigman HH, Goldberg N, Niloff PH, et al. Use of biliary drainage in diagnosis of biliary tract disease. Am J Gastroenterol 1977;67:439-43.

47. Reisberg IR, Mabee GW. Endoscopic biliary drainage for detection of gallbladder disease. Gastrointest Endosc 1979;25:6-9.

48. Susann PW, Sheppard F, Baloga AJ. Detection of occult gallbladder disease by duodenal drainage collected endoscopically. A clinical and pathologic correlation. Am Surg 1985;51:162-5.

49. Abeysuriya V, Deen KI, Navarathne NM. Biliary microlithiasis, sludge, crystals, microcrystallization, and usefulness of assessment of nucleation time. Hepatobiliary Pancreat Dis Int 2010;9:248-53.

50. Landi K, Sinard J, Crawford JM, et al. Cholesterol crystal morphology in acalculous gallbladder disease. J Clin Gastroenterol 2003;36:364-6.

51. Neoptolemos JP, Davidson BR, Winder AF, et al. Role of duodenal bile crystal analysis in the investigation of 'idiopathic' pancreatitis. Br J Surg 1988;75:450-3.

52. Buscail L, Escourrou J, Delvaux M, et al. Microscopic examination of bile directly collected during endoscopic cannulation of the papilla. Utility in patients with suspected microlithiasis. Dig Dis Sci 1992;37:116-20.

53. Filly RA, Allen B, Minton MJ, et al. In vitro investigation of the origin of echoes with biliary sludge. J Clin Ultrasound 1980;8:193-200.

54. Lee SP. Pathogenesis of biliary sludge. Hepatology 1990;12:200S-203S; discussion 203S-205S.

55. Sedaghat A, Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. N Engl J Med 1980;302:1274-7.

32

56. Mouiel J, Chauvin P, Borelli JP, et al. [Role of biliary microlithiasis in acute pancreatitis. Apropos of a series of 31 cases]. Chirurgie 1975;101:258-65.
57. Marks JW, Bonorris G. Intermittency of cholesterol crystals in duodenal bile from gallstone patients. Gastroenterology 1984;87:622-7.
58. Block MA, Priest RJ. Acute pancreatitis related to grossly minute stones in a radiographically normal gallbladder. Am J Dig Dis 1967;12:934-8.
59. Negro P, Flati G, Flati D, et al. Occult gallbladder microlithiasis causing acute recurrent pancreatitis. A report of three cases. Acta Chir Scand 1984;150:503-6.
60. Goldstein F, Kucer FT, Thornton JJ, 3rd, et al. Acute and relapsing pancreatitis caused by bile pigment aggregates and diagnosed by biliary drainage. Am J Gastroenterol 1980;74:225-30.
61. Delchier JC, Benfredj P, Preaux AM, et al. The usefulness of microscopic bile examination in patients with suspected microlithiasis: a prospective evaluation. Hepatology 1986;6:118-22.
62. Saraswat VA, Sharma BC, Agarwal DK, et al. Biliary microlithiasis in patients with idiopathic acute pancreatitis and unexplained biliary pain: response to therapy. J Gastroenterol Hepatol 2004;19:1206-11.
63. Maringhini A, Marceno MP, Lanzarone F, et al. Sludge and stones in gallbladder after pregnancy. Prevalence and risk factors. J Hepatol 1987;5:218-23.
64. Maringhini A, Ciambra M, Baccelliere P, et al. Biliary sludge and gallstones in pregnancy: incidence, risk factors, and natural history. Ann Intern Med 1993;119:116-20.
65. Ko CW, Beresford SA, Schulte SJ, et al. Incidence, natural history, and risk factors for biliary sludge and stones during pregnancy. Hepatology 2005;41:359-65.
66. Everson GT. Pregnancy and gallstones. Hepatology 1993;17:159-61.
67. Valdivieso V, Covarrubias C, Siegel F, et al. Pregnancy and cholelithiasis: pathogenesis and natural course of gallstones diagnosed in early puerperium. Hepatology 1993;17:1-4.
68. Park HZ, Lee SP, Schy AL. Ceftriaxone-associated gallbladder sludge. Identification of calcium-ceftriaxone salt as a major component of gallbladder precipitate. Gastroenterology 1991;100:1665-70.
69. Ko CW, Schulte SJ, Lee SP. Biliary sludge is formed by modification of hepatic bile by the gallbladder mucosa. Clin Gastroenterol Hepatol 2005;3:672-8.
70. Wang DQ, Cohen DE, Carey MC. Biliary lipids and cholesterol gallstone disease. J Lipid Res 2009;50 Suppl:S406-11.
71. Wang HH, Portincasa P, Wang DQ. Molecular pathophysiology and physical chemistry of cholesterol gallstones. Front Biosci 2008;13:401-23.
72. Carey MC, Lamont JT. Cholesterol gallstone formation. 1. Physical-chemistry of bile and biliary lipid secretion. Prog Liver Dis 1992;10:139-63.
73. Carey MC, Cohen DE. Update on physical state of bile. Ital J Gastroenterol 1995;27:92-100.
74. Freeman JB, Meyer PD, Printen KJ, et al. Analysis of gallbladder bile in morbid obesity. Am J Surg 1975;129:163-6.

75. Shaffer EA, Braasch JW, Small DM. Bile composition at and after surgery in normal persons and patients with gallstones. Influence of cholecystectomy. N Engl J Med 1972;287:1317-22.

76. Shaffer EA, Small DM. Biliary lipid secretion in cholesterol gallstone disease. The effect of cholecystectomy and obesity. J Clin Invest 1977;59:828-40.

77. Shaffer EA, Small DM. Gallstone disease: pathogenesis and management. Curr Probl Surg 1976;13:3-72.

78. Donovan JM, Carey MC. Separation and quantitation of cholesterol "carriers" in bile. Hepatology 1990;12:94S-104S; discussion 104S-105S.

79. Small DM. Cholesterol nucleation and growth in gallstone formation. N Engl J Med 1980;302:1305-7.

80. Bourges M, Small DM, Dervichian DG. Biophysics of lipid associations. 3. The quaternary systems lecithin-bile salt-cholesterol-water. Biochim Biophys Acta 1967;144:189-201.

81. Bourges M, Small DM, Dervichian DG. Biophysics of lipidic associations. II. The ternary systems: cholesterol-lecithin-water. Biochim Biophys Acta 1967;137:157-67.

82. Brecher P, Chobanian J, Small DM, et al. The use of phospholipid vesicles for in vitro studies on cholesteryl ester hydrolysis. J Lipid Res 1976;17:239-47.

83. Carey MC. Aqueous bile salt-lecithin-cholesterol systems: equilibrium aspects. Hepatology 1984;4:151S-154S.

84. Hay DW, Cahalane MJ, Timofeyeva N, et al. Molecular species of lecithins in human gallbladder bile. J Lipid Res 1993;34:759-68.

85. Wang HH, Portincasa P, Afdhal NH, et al. Lith genes and genetic analysis of cholesterol gallstone formation. Gastroenterol Clin North Am 2010;39:185-207, vii-viii.

86. Wang DQ, Afdhal NH. Gallstone Disease. In: Feldman M, Friedman LS, Brandt L, eds. Sleisenger and Fordtran's Gastrointestinal and Liver Disease. 9 ed. Philadelphia: Elsevier Saunders, 2010:1089-1120.

87. Mendez-Sanchez N, Chavez-Tapia NC, Motola-Kuba D, et al. Metabolic syndrome as a risk factor for gallstone disease. World J Gastroenterol 2005;11:1653-7.

88. Nervi F, Miquel JF, Alvarez M, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. J Hepatol 2006;45:299-305.

89. Portincasa P, Di Ciaula A, Wang HH, et al. Coordinate regulation of gallbladder motor function in the gut-liver axis. Hepatology 2008;47:2112-26.

90. Lammert F, Gurusamy K, Ko CW, et al. Gallstones. Nat Rev Dis Primers 2016;2:16024.

91. Krawczyk M, Wang DQ, Portincasa P, et al. Dissecting the genetic heterogeneity of gallbladder stone formation. Semin Liver Dis 2011;31:157-72.

92. de Bari O, Wang TY, Liu M, et al. Cholesterol cholelithiasis in pregnant women: pathogenesis, prevention and treatment. Ann Hepatol 2014;13:728-45.

93. Portincasa P, Moschetta A, Di Ciaula A, et al. Pathophysiology and Cholesterol Gallstone Disease. In: Borzellino G, Cordiano C, eds. Biliary Lithiasis: Basic Science, Current Diagnosis and Management. 1 ed. Milano: Springer Italia S.r.l., 2008:19-49.

94.   Wang DQ, Afdhal NH. Genetic analysis of cholesterol gallstone formation: searching for Lith (gallstone) genes. Curr Gastroenterol Rep 2004;6:140-50.
95.   Lammert F, Miquel JF. Gallstone disease: from genes to evidence-based therapy. J Hepatol 2008;48 Suppl 1:S124-35.
96.   Lammert F, Matern S. The genetic background of cholesterol gallstone formation: an inventory of human lithogenic genes. Curr Drug Targets Immune Endocr Metabol Disord 2005;5:163-70.
97.   Wang DQ, Paigen B, Carey MC. Phenotypic characterization of Lith genes that determine susceptibility to cholesterol cholelithiasis in inbred mice: physical-chemistry of gallbladder bile. J Lipid Res 1997;38:1395-411.
98.   Lee SP, LaMont JT, Carey MC. Role of gallbladder mucus hypersecretion in the evolution of cholesterol gallstones. J Clin Invest 1981;67:1712-23.
99.   Marks JW, Bonorris GG, Albers G, et al. The sequence of biliary events preceding the formation of gallstones in humans. Gastroenterology 1992;103:566-70.
100.   Shiffman ML, Sugerman HJ, Kellum JM, et al. Gallstone formation after rapid weight loss: a prospective study in patients undergoing gastric bypass surgery for treatment of morbid obesity. Am J Gastroenterol 1991;86:1000-5.
101.   de Vree JM, Jacquemin E, Sturm E, et al. Mutations in the MDR3 gene cause progressive familial intrahepatic cholestasis. Proc Natl Acad Sci USA 1998;95:282-7.
102.   Deleuze JF, Jacquemin E, Dubuisson C, et al. Defect of multidrug-resistance 3 gene expression in a subtype of progressive familial intrahepatic cholestasis. Hepatology 1996;23:904-8.
103.   Jacquemin E, Hadchouel M. Genetic basis of progressive familial intrahepatic cholestasis. J Hepatol 1999;31:377-81.
104.   Jacquemin E, Cresteil D, Manouvrier S, et al. Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. Lancet 1999;353:210-1.
105.   Lammert F, Marschall HU, Glantz A, et al. Intrahepatic cholestasis of pregnancy: molecular pathogenesis, diagnosis and management. J Hepatol 2000;33:1012-21.
106.   Dixon PH, Sambrotta M, Chambers J, et al. An expanded role for heterozygous mutations of ABCB4, ABCB11, ATP8B1, ABCC2 and TJP2 in intrahepatic cholestasis of pregnancy. Sci Rep 2017;7:11823.
107.   Rosmorduc O, Hermelin B, Poupon R. MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. Gastroenterology 2001;120:1459-67.
108.   Rosmorduc O, Hermelin B, Boelle PY, et al. ABCB4 gene mutation-associated cholelithiasis in adults. Gastroenterology 2003;125:452-9.
109.   Rosmorduc O, Poupon R. Low phospholipid associated cholelithiasis: association with mutation in the MDR3/ABCB4 gene. Orphanet J Rare Dis 2007;2:29.
110.   Poupon R, Rosmorduc O, Boelle PY, et al. Genotype-phenotype relationships in the low-phospholipid-associated cholelithiasis syndrome: a study of 156 consecutive patients. Hepatology 2013;58:1105-10.

111. Jacquemin E. Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: one gene for three diseases. Semin Liver Dis 2001;21:551-62.
112. Sundaram SS, Sokol RJ. The Multiple Facets of ABCB4 (MDR3) Deficiency. Curr Treat Options Gastroenterol 2007;10:495-503.
113. Lucena JF, Herrero JI, Quiroga J, et al. A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. Gastroenterology 2003;124:1037-42.
114. Di Ciaula A, Wang DQ, Portincasa P. An update on the pathogenesis of cholesterol gallstone disease. Curr Opin Gastroenterol 2018;34:71-80.
115. Portincasa P, Wang DQ. Gallstones. In: Podolsky DK, Camilleri M, Fitz JG, Kalloo AN, Shanahan F, Wang TC, eds. Yamada's Textbook of Gastroenterology. Volume 2. 6 ed. Hoboken, New Jersey: Wiley-Blackwell, 2015:1808-1834.
116. Smit JJ, Schinkel AH, Oude Elferink RP, et al. Homozygous disruption of the murine mdr2 P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. Cell 1993;75:451-62.
117. Oude Elferink RP, Ottenhoff R, van Wijland M, et al. Regulation of biliary lipid secretion by mdr2 P-glycoprotein in the mouse. J Clin Invest 1995;95:31-8.
118. Groen AK, Van Wijland MJ, Frederiks WM, et al. Regulation of protein secretion into bile: studies in mice with a disrupted mdr2 p-glycoprotein gene. Gastroenterology 1995;109:1997-2006.
119. Oude Elferink RP, Ottenhoff R, van Wijland M, et al. Uncoupling of biliary phospholipid and cholesterol secretion in mice with reduced expression of mdr2 P-glycoprotein. J Lipid Res 1996;37:1065-75.
120. Fein F, Hermelin B, Becker MC, et al. Acute recurrent biliary pancreatitis associated with the ABCB4 gene mutation. Gastroenterol Clin Biol 2007;31:106-9.
121. Lammert F, Wang DQ, Hillebrandt S, et al. Spontaneous cholecysto- and hepatolithiasis in Mdr2-/- mice: a model for low phospholipid-associated cholelithiasis. Hepatology 2004;39:117-28.
122. Alexakis N, Lombard M, Raraty M, et al. When is pancreatitis considered to be of biliary origin and what are the implications for management? Pancreatology 2007;7:131-41.
123. Wang DQ, Cohen DE, Lammert F, et al. No pathophysiologic relationship of soluble biliary proteins to cholesterol crystallization in human bile. J Lipid Res 1999;40:415-25.
124. van Erpecum KJ, Wang DQ, Moschetta A, et al. Gallbladder histopathology during murine gallstone formation: relation to motility and concentrating function. J Lipid Res 2006;47:32-41.
125. Wang HH, Liu M, Portincasa P, et al. Lack of endogenous cholecystokinin promotes cholelithogenesis in mice. Neurogastroenterol Motil 2016;28:364-375.
126. Wang HH, Portincasa P, Wang DQ. The cholecystokinin-1 receptor antagonist devazepide increases cholesterol cholelithogenesis in mice. Eur J Clin Invest 2016;46:158-69.
127. Wang TY, Portincasa P, Liu M, et al. Mouse models of gallstone disease. Curr Opin Gastroenterol 2018;34:59-70.

128. Pazzi P, Gamberini S, Buldrini P, et al. Biliary sludge: the sluggish gallbladder. Dig Liver Dis 2003;35 Suppl 3:S39-45.
129. Diehl AK. Gallstone size and the risk of gallbladder cancer. JAMA 1983;250:2323-6.
130. Shaffer E. Acalculous biliary pain: new concepts for an old entity. Dig Liver Dis 2003;35 Suppl 3:S20-5.
131. Heaton KW. Diagnosis of acute non-specific abdominal pain. Lancet 2000;355:1644.
132. Thompson WG, Longstreth GF, Drossman DA, et al. Functional bowel disorders and functional abdominal pain. Gut 1999;45 Suppl 2:II43-7.
133. Hearing SD, Thomas LA, Heaton KW, et al. Effect of cholecystectomy on bowel function: a prospective, controlled study. Gut 1999;45:889-94.
134. Poulin EC, Schlachta CM, Mamazza J. Early laparoscopy to help diagnose acute non-specific abdominal pain. Lancet 2000;355:861-3.
135. Venu RP, Geenen JE, Toouli J, et al. Endoscopic retrograde cholangiopancreatography. Diagnosis of cholelithiasis in patients with normal gallbladder x-ray and ultrasound studies. JAMA 1983;249:758-61.
136. Portincasa P, Wang DQ. Gallstones. In: Podolsky DK, Camilleri M, Fitz JG, Kalloo AN, Shanahan F, Wang TC, eds. Yamada's Atlas of Gastroenterology. 5 ed. Hoboken, New Jersey: Wiley-Blackwell, 2016:335-353.
137. Dahan P, Andant C, Levy P, et al. Prospective evaluation of endoscopic ultrasonography and microscopic examination of duodenal bile in the diagnosis of cholecystolithiasis in 45 patients with normal conventional ultrasonography. Gut 1996;38:277-81.
138. Cooperberg PL, Gibney RG. Imaging of the gallbladder, 1987. Radiology 1987;163:605-13.
139. Cano N, Cicero F, Ranieri F, et al. Ultrasonographic study of gallbladder motility during total parenteral nutrition. Gastroenterology 1986;91:313-7.
140. Gafa M, Sarli L, Miselli A, et al. Sludge and microlithiasis of the biliary tract after total gastrectomy and postoperative total parenteral nutrition. Surg Gynecol Obstet 1987;165:413-8.
141. Calvo MM, Bujanda L, Heras I, et al. Magnetic resonance cholangiography versus ultrasound in the evaluation of the gallbladder. J Clin Gastroenterol 2002;34:233-6.
142. Calvo MM, Bujanda L, Calderon A, et al. Comparison between magnetic resonance cholangiopancreatography and ERCP for evaluation of the pancreatic duct. Am J Gastroenterol 2002;97:347-53.
143. Calvo MM, Bujanda L, Calderon A, et al. Role of magnetic resonance cholangiopancreatography in patients with suspected choledocholithiasis. Mayo Clin Proc 2002;77:422-8.
144. Cavdar F, Yildar M, Tellioglu G, et al. Controversial issues in biliary pancreatitis: when should we perform MRCP and ERCP? Pancreatology 2014;14:411-4.
145. Surlin V, Saftoiu A, Dumitrescu D. Imaging tests for accurate diagnosis of acute biliary pancreatitis. World J Gastroenterol 2014;20:16544-9.

146. Fusaroli P, Kypraios D, Caletti G, et al. Pancreatico-biliary endoscopic ultrasound: a systematic review of the levels of evidence, performance and outcomes. World J Gastroenterol 2012;18:4243-56.

147. De Lisi S, Leandro G, Buscarini E. Endoscopic ultrasonography versus endoscopic retrograde cholangiopancreatography in acute biliary pancreatitis: a systematic review. Eur J Gastroenterol Hepatol 2011;23:367-74.

148. Choi CS, Ku YJ, Yoon DY, et al. Harmonic ultrasonography for the detection of microlithiasis in the gallbladder. Ultrasonography 2014;33:275-82.

149. Moskovitz M, Min TC, Gavaler JS. The microscopic examination of bile in patients with biliary pain and negative imaging tests. Am J Gastroenterol 1986;81:329-33.

150. Wilcox CM, Seay T, Kim H, et al. Prospective Endoscopic Ultrasound-Based Approach to the Evaluation of Idiopathic Pancreatitis: Causes, Response to Therapy, and Long-term Outcome. Am J Gastroenterol 2016;111:1339-48.

151. Anderloni A, Galeazzi M, Ballare M, et al. Early endoscopic ultrasonography in acute biliary pancreatitis: A prospective pilot study. World J Gastroenterol 2015;21:10427-34.

152. Anderloni A, Repici A. Role and timing of endoscopy in acute biliary pancreatitis. World J Gastroenterol 2015;21:11205-8.

153. Anderloni A, Ballare M, Pagliarulo M, et al. Prospective evaluation of early endoscopic ultrasonography for triage in suspected choledocholithiasis: results from a large single centre series. Dig Liver Dis 2014;46:335-9.

154. Levy MJ. The hunt for microlithiasis in idiopathic acute recurrent pancreatitis: should we abandon the search or intensify our efforts? Gastrointest Endosc 2002;55:286-93.

155. Dill JE. Endosonography/bile drainage combination for difficult-to-diagnose gallbladder disease. J Laparoendosc Adv Surg Tech A 1998;8:361-5.

156. Dill JE, Dill BP. EUS for idiopathic pancreatitis. Gastrointest Endosc 2006;64:845; author reply 845-7.

157. Dill JE, Hill S, Callis J, et al. Combined endoscopic ultrasound and stimulated biliary drainage in the diagnosis of cholecystitis and microlithiasis. Endoscopy 1995;27:218.

158. Andersson R, Andersson B, Haraldsen P, et al. Incidence, management and recurrence rate of acute pancreatitis. Scand J Gastroenterol 2004;39:891-4.

159. Ardengh JC, Malheiros CA, Rahal F, et al. Microlithiasis of the gallbladder: role of endoscopic ultrasonography in patients with idiopathic acute pancreatitis. Rev Assoc Med Bras (1992) 2010;56:27-31.

160. Dill JE, Dill BP. Microlithiasis and pancreatitis. Gastrointest Endosc 2002;56:784; author reply 784-5.

161. Al-Haddad M, Wallace MB. Diagnostic approach to patients with acute idiopathic and recurrent pancreatitis, what should be done? World J Gastroenterol 2008;14:1007-10.

162. Thevenot A, Bournet B, Otal P, et al. Endoscopic ultrasound and magnetic resonance cholangiopancreatography in patients with idiopathic acute pancreatitis. Dig Dis Sci 2013;58:2361-8.

163. Venu RP, Geenen JE, Hogan W, et al. Idiopathic recurrent pancreatitis. An approach to diagnosis and treatment. Dig Dis Sci 1989;34:56-60.

164. Sarva RP, Farivar S, Fromm H, et al. Study of the sensitivity and specificity of computerized tomography in the detection of calcified gallstones which appears radiolucent by conventional roentgenography. Gastrointest Radiol 1981;6:165-7.

165. Working Group IAPAPAAPG. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology 2013;13:e1-15.

166. Di Ciaula A, Wang DQ, Garruti G, et al. Therapeutic reflections in cholesterol homeostasis and gallstone disease: a review. Curr Med Chem 2014;21:1435-47.

167. Di Ciaula A, Wang DQ, Wang HH, et al. Targets for current pharmacologic therapy in cholesterol gallstone disease. Gastroenterol Clin North Am 2010;39:245-64, viii-ix.

168. Portincasa P, Ciaula AD, Bonfrate L, et al. Therapy of gallstone disease: What it was, what it is, what it will be. World J Gastrointest Pharmacol Ther 2012;3:7-20.

169. Portincasa P, Di Ciaula A, de Bari O, et al. Management of gallstones and its related complications. Expert Rev Gastroenterol Hepatol 2016;10:93-112.

170. Shiffman ML, Kaplan GD, Brinkman-Kaplan V, et al. Prophylaxis against gallstone formation with ursodeoxycholic acid in patients participating in a very-low-calorie diet program. Ann Intern Med 1995;122:899-905.

171. Broomfield PH, Chopra R, Sheinbaum RC, et al. Effects of ursodeoxycholic acid and aspirin on the formation of lithogenic bile and gallstones during loss of weight. N Engl J Med 1988;319:1567-72.

172. Sitzmann JV, Pitt HA, Steinborn PA, et al. Cholecystokinin prevents parenteral nutrition induced biliary sludge in humans. Surg Gynecol Obstet 1990;170:25-31.

173. Wu ZS, Yu L, Lin YJ, et al. Rapid intravenous administration of amino acids prevents biliary sludge induced by total parenteral nutrition in humans. J Hepatobiliary Pancreat Surg 2000;7:504-9.

174. Portincasa P, Moschetta A, Puglisi F, et al. Medical Treatment of Gallstone Disease. In: Borzellino G, Cordiano C, eds. Biliary Lithiasis: Basic Science, Current Diagnosis and Management. 1 ed. Milano: Springer Italia S.r.l., 2008:149-157.

175. Testoni PA, Caporuscio S, Bagnolo F, et al. Idiopathic recurrent pancreatitis: long-term results after ERCP, endoscopic sphincterotomy, or ursodeoxycholic acid treatment. Am J Gastroenterol 2000;95:1702-7.

176. Barton P, Steininger R, Maier A, et al. Biliary sludge after liver transplantation: 2. Treatment with interventional techniques versus surgery and/or oral chemolysis. AJR Am J Roentgenol 1995;164:865-9.

177. Stevens CL, Abbas SM, Watters DA. How Does Cholecystectomy Influence Recurrence of Idiopathic Acute Pancreatitis? J Gastrointest Surg 2016;20:1997-2001.

178. Garg PK, Tandon RK, Madan K. Is biliary microlithiasis a significant cause of idiopathic recurrent acute pancreatitis? A long-term follow-up study. Clin Gastroenterol Hepatol 2007;5:75-9.

179. Raty S, Pulkkinen J, Nordback I, et al. Can Laparoscopic Cholecystectomy Prevent Recurrent Idiopathic Acute Pancreatitis?: A Prospective Randomized Multicenter Trial. Ann Surg 2015;262:736-41.

180. Carr-Locke DL. Role of endoscopy in gallstone pancreatitis. Am J Surg 1993;165:519-21.
181. Carr-Locke DL. Acute gallstone pancreatitis and endoscopic therapy. Endoscopy 1990;22:180-3.
182. Neoptolemos JP, Carr-Locke DL, London N, et al. ERCP findings and the role of endoscopic sphincterotomy in acute gallstone pancreatitis. Br J Surg 1988;75:954-60.
183. Neoptolemos JP, London N, Slater ND, et al. A prospective study of ERCP and endoscopic sphincterotomy in the diagnosis and treatment of gallstone acute pancreatitis. A rational and safe approach to management. Arch Surg 1986;121:697-702.
184. Soetikno RM, Carr-Locke DL. Endoscopic management of acute gallstone pancreatitis. Gastrointest Endosc Clin N Am 1998;8:1-12.
185. Yaghoobi M. ERCP for gallstone pancreatitis. N Engl J Med 2014;370:1955-6.
186. Ricci F, Castaldini G, de Manzoni G, et al. Minimally invasive treatment of acute biliary pancreatitis. Surg Endosc 1997;11:1179-82.
187. Elton E, Howell DA, Parsons WG, et al. Endoscopic pancreatic sphincterotomy: indications, outcome, and a safe stentless technique. Gastrointest Endosc 1998;47:240-9.
188. Jain R. Biliary Sludge: When Should It Not be Ignored? Curr Treat Options Gastroenterol 2004;7:105-109.
189. Das R, Yadav D, Papachristou GI. Endoscopic Treatment of Recurrent Acute Pancreatitis and Smoldering Acute Pancreatitis. Gastrointest Endosc Clin N Am 2015;25:737-48.
190. Monkhouse SJ, Court EL, Dash I, et al. Two-week target for laparoscopic cholecystectomy following gallstone pancreatitis is achievable and cost neutral. Br J Surg 2009;96:751-5.
191. Kamal A, Akhuemonkhan E, Akshintala VS, et al. Effectiveness of Guideline-Recommended Cholecystectomy to Prevent Recurrent Pancreatitis. Am J Gastroenterol 2017;112:503-510.
192. Hernandez V, Pascual I, Almela P, et al. Recurrence of acute gallstone pancreatitis and relationship with cholecystectomy or endoscopic sphincterotomy. Am J Gastroenterol 2004;99:2417-23.
193. Uhl W, Warshaw A, Imrie C, et al. IAP Guidelines for the Surgical Management of Acute Pancreatitis. Pancreatology 2002;2:565-73.
194. Toouli J, Brooke-Smith M, Bassi C, et al. Guidelines for the management of acute pancreatitis. J Gastroenterol Hepatol 2002;17 Suppl:S15-39.
195. Tenner S, Baillie J, DeWitt J, et al. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol 2013;108:1400-15; 1416.

Figure Legends

Figure 1. Longitudinal (top) and transversal (bottom) ultrasonographic scans of the gallbladder from a 56-year-old woman with drinking habits and a recent history of right hypochondrium pain. The finding is suggestive of chronic cholecystitis, as indicated by a thickened gallbladder wall (up to 5 mm). Biliary sludge in the gallbladder is visible as an amorphous echogenic image that is distinctly different from the anechoic bile, and it can slowly move to the most dependent part of the gallbladder, as indicated by a red asterisk. A clear acoustic shadow is missing at the level of the distal gallbladder wall. Intra- and extra-hepatic bile ducts are not visible and therefore not dilated. Abbreviation: L, liver. We sincerely thank Drs. Francesco Minerva and Marica Noviello for providing us with this picture.

Figure 2. Fresh human gallbladder bile is examined by phase contrast and polarizing light microscopy, showing biliary sludge that is composed mainly of classical solid plate-like cholesterol monohydrate crystals, as well as varying sizes of calcium bilirubinate granules in golden color, and mucin gel. Magnifications are 800x by polarizing light microscopy.

Figure 3. On microscopy, microlithiasis is often defined as a tiny stone with a diameter <3 mm, which cannot be crushed by digital compression. Under phase contrast and polarizing light microscopy, microlithiasis always display rounded contours and black

centers from light scattering/absorption. Magnifications are 100x by polarizing light microscopy.

Figure 4. Suggested algorithm for the management of patients with biliary sludge. Abbreviation: CT, computed tomography; ERC, endoscopic cholangiography; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; MRCP, magnetic resonance cholangiography; UDCA, ursodeoxycholic acid; US, ultrasonography. See text for further details.
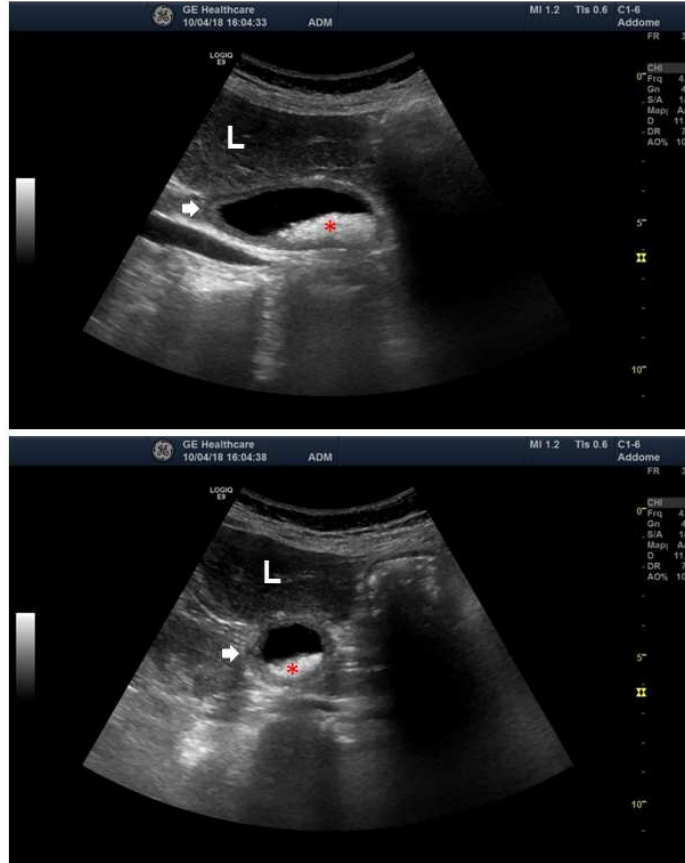
Figure 5. Transversal (left) and longitudinal (right) ultrasonographic scans of the gallbladder from a 64-year-old man with occasional discovery of a solitary stone. The thickness of gallbladder wall is normal (<3 mm) and the gallstone is visible as a round-shaped hyperechoic image (8 mm in diameter, as pointed out by a red asterisk), which is dependent with gravity and is producing a distal echoic shadow, as indicated by a red arrow. Abbreviation: L, liver.

Figure 6. Endoscopic retrograde cholangiopancreatography (ERCP) shows a filling defect image of a gallstone (as pointed out by a red arrow) of ~15 mm in diameter within the proximal common bile duct (CBD). We sincerely thank Dr. Giuseppe Scaccianoce for providing us with this picture.
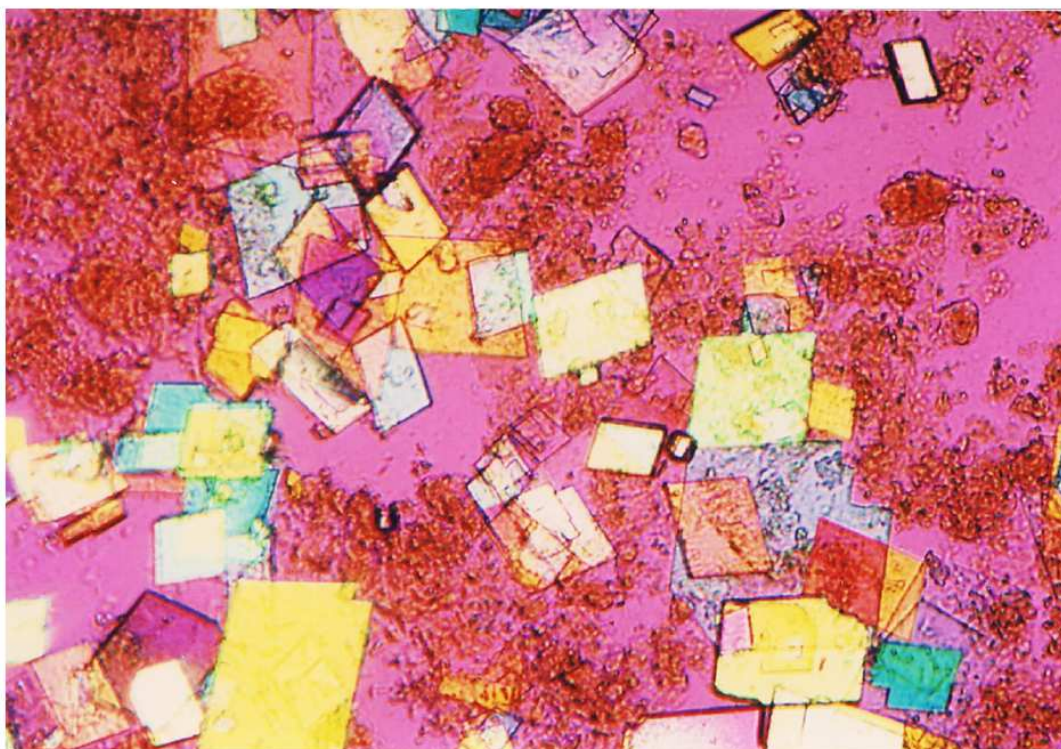
Figure 7. Suggested algorithm for treatment in patients with microlithiasis-induced pancreatitis. Abbreviation: CT, computed tomography; ERC, endoscopic

cholangiography; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography; MRCP, magnetic resonance cholangiography; MRI, magnetic resonance imaging; US, ultrasonography. See text for details.

Figure 8. Computed tomography (CT) scans were performed in a patient with acute abdominal pain and elevated pancreatic enzymes. Because this patient was allergic, the CT scans were performed in emergency without contrast medium injection. The final diagnosis was biliary pancreatitis. **A** and **B**: A radiopaque gallstone of ~10 mm in diameter (arrow) is visible in the distal part of the common bile duct (i.e., the papilla of Vater). **A** and **C**: The pancreas (PA) is enlarged due to edema. **B**: The common bile duct (CBD) is dilated above the stone to a diameter of 12.2 mm. **D**: The gallbladder (GB) wall shows a normal thickness. We sincerely thank Dr. Cristina Jablonska for providing us with this picture.

Patient with suspected biliary sludge

Choice of diagnostic methods is largely based on (i) clinical symptoms; (ii) sensitivity; (iii) specificity; and (iv) costs.

Abdominal US, EUS, ERCP, ERC, CT, MRCP and/or bile microscopy

Diagnosis of biliary sludge

Symptoms and/or complications

No

Yes

Reversible risk factor present

Surgical candidate

No

Yes

No

Yes

"Watch-and-see" policy and expectant management

Cholecystectomy if biliary sludge persists and symptoms or complications develop

Nonsurgical therapy (such as UDCA)

Cholecystectomy

```
                    ┌─────────────────────────────────┐
                    │ Patient with suspected pancreatitis │
                    └─────────────────────────────────┘
                                    │
                    ┌─────────────────────────────────┐
                    │ Abdominal plain film, US, CT,    │
                    │ ERCP, MRI, and lab tests         │
                    └─────────────────────────────────┘
                                    │
                    ┌─────────────────────────────────┐
                    │ Diagnosis of pancreatitis        │
                    └─────────────────────────────────┘
                                    │
                          ◇ Causes identified ◇
                    No │                      │ Yes
```

- Obstruction (gallstones, tumors, parasites, duodenal diverticula)
- Alcohol and other toxins
- Medications
- Metabolic abnormalities
- Trauma
- Infection
- Vascular disorders
- Postoperative state
- Post-ERCP

ERCP, EUS, ERC, bile microscopy, and sphincter of Oddi manometry

Microlithiasis-induced pancreatitis

No → Idiopathic pancreatitis → Treat appropriately

Yes → Cholecystectomy or biliary sphincterotomy

Treat appropriately