Best Practice & Research Clinical Gastroenterology
Vol. 20, No. 6, pp. 1053–1062, 2006
doi:10.1016/j.bpg.2006.03.007
available online at http://www.sciencedirect.com

5

Microlithiasis and sludge

Christoph Jüngst MD
Research Fellow
Department of Medicine I, Universitätsklinikum Bonn, Sigmund-Freud Str. 25, 53105 Bonn, Germany

Gerd Achim Kullak-Ublick MD
Professor of Medicine
Universitäts Spital Zürich, Division of Clinical Pharmacology and Toxicology, Rämistr. 100, 8091 Zürich, Switzerland

Dieter Jüngst* MD
Professor of Medicine
Department of Medicine II, Klinikum Grosshadern, Marchioninistr. 15, 81377 Munich, Germany

'Sludge' is the solid material which results from the slow settling of particles dispersed in a liquid medium. Biliary sludge in the gallbladder can be detected by transabdominal ultrasonography, and the typical echoes derive mainly from pigment precipitates mixed with cholesterol crystals. A portion of biliary sludge contains comparatively large particles (1–3 mm) called microliths, the formation of which is an obligatory intermediate step in the development of all types of gallstone. Microlithiasis and sludge in bile may cause colicky pain, cholecystitis, cholangitis, and acute pancreatitis, and are thus of clinical relevance. In these patients treatment follows the guidelines of symptomatic gallstone disease, and strategies include long-term application of ursodeoxycholic acid, endoscopic papillotomy, or preferably laparoscopic cholecystectomy.

Key words: bile; sludge; gallstones; microlithiasis; cholecystitis; pancreatitis; treatment.

CHEMICAL COMPOSITION

Biliary sludge comprises a suspension of precipitated 'particulate matter' in bile dispersed in a viscous, mucin-rich liquid phase. The most common precipitates in gallbladder bile are cholesterol monohydrate crystals, calcium bilirubinate

* Corresponding author. Tel.: +49 89 7095 2376; Fax: +49 89 7095 5374.
E-mail address: dieter.juengst@med.uni-muenchen.de (D. Jüngst).

1521-6918/$ - see front matter

granules, calcium phosphate and calcium carbonate crystals, and calcium salts of fatty acids.

Ultracentrifugation of bile allows quantification of the important components of biliary sludge by determining the differences in the concentrations of cholesterol, protein, mucin and bilirubin between native and ultracentrifuged gallbladder bile samples.[1] The chemical composition of the precipitates correlates well with the composition of the associated stones.[2]

DIAGNOSIS

Transabdominal ultrasound examination of the gallbladder permits the visualisation of particles in bile, usually those of >2–3 mm in diameter (Figure 1). These represent the larger components of biliary sludge and consist of aggregated crystals or microliths embedded in the mucin-rich liquid phase. As a consequence of the sedimentation of sludge, the gallbladder may contain layered bile, with the lowest layer demonstrating low-amplitude echogenicity on ultrasound. The precise nature of the origin of echoes within biliary sludge has been reported in different in-vitro studies which showed that filtration of bile converts echogenic bile into echo-free bile.[3–5] Examination of the filtered residue by light microscopy revealed that the source of echoes in biliary sludge was predominantly pigment precipitates mixed with cholesterol crystals. The pigment precipitates consisted mainly of calcium bilirubinate which may be detected by high-resolution computer tomography.

For microscopical analysis, duodenal bile may be collected during duodenoscopy after stimulation of the gallbladder with cholecystokinin.[6] The particulate matter visible under the microscope comprises mainly cholesterol monohydrate crystals and calcium bilirubinate granules and, less often, microspherolites consisting of calcium carbonate.[7] The procedure is cumbersome and of limited clinical value, and has thus not reached a broad acceptance. Microlithiasis in bile may be overlooked by conventional

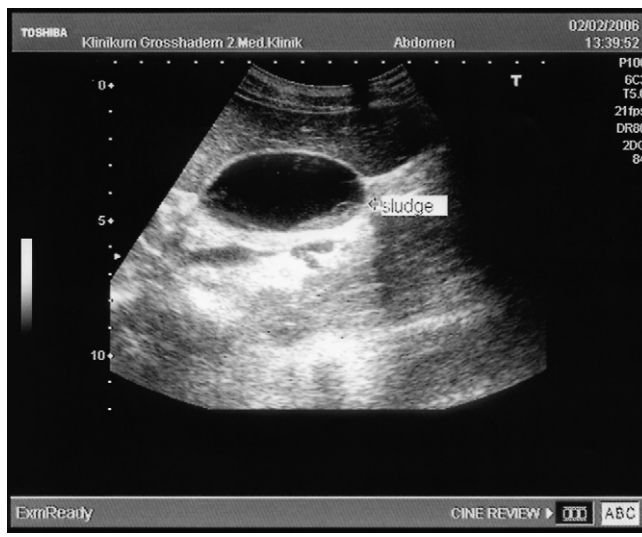

Figure 1. Transabdominal ultrasonography of a gallbladder containing sludge in a young patient presenting with acute cholangitis.

abdominal ultrasound, but can be detected with higher sensitivity using endoscopic ultrasonography.[8,9]

EPIDEMIOLOGY AND RISK FACTORS

In the largest series of patients with different internal diseases, including cholecystolithiasis, gallbladder sludge was found by transabdominal ultrasound in only 1.7%.[10] Higher prevalence of sonographically detectable biliary sludge in the gallbladder has been reported during pregnancy (30%), parenteral nutrition (50%), weight loss (25%), biliary stasis, or after intravenous administration of ceftriaxone (40%).[11–19]

Because biliary sludge may dissolve spontaneously, the cumulative prevalence of sludge does not necessarily increase with age.

Female gender is a prominent risk factor for cholesterol gallstone formation, but of 286 patients in whom biliary sludge was detected by ultrasound, 157 were women and 129 were men.[10] Thus female gender seems not to be a specific risk factor, at least for the formation of ultrasonographically detectable biliary sludge.

Obesity is a well-known risk factor for cholesterol gallstones.[20] Because of the low prevalence of sonographically detectable biliary sludge, the relationship between obesity and biliary sludge formation is unknown. Rapid weight loss has more recently been recognised as a risk factor for cholesterol gallstone formation. Approximately 25% of obese patients develop gallstones during dietary weight loss. After gastric bypass surgery, as many as 50% of patients form biliary sludge or gallstones.[16,21] The pathogenesis of biliary sludge and gallstone formation after rapid weight loss and gastric bypass surgery appears to be multifactorial. It has been shown that hepatic cholesterol secretion increases during caloric restriction.[22,23] Additional factors may include increased mucin production (a potent stimulator of cholesterol crystal nucleation) and decreased gallbladder motility.[23,24] Impaired gallbladder motility also appears to be of major importance for the development of biliary sludge during total parenteral nutrition (TPN). As many as 23% of patients under TPN for a minimum of 3 months were found to develop biliary sludge.[12,15,25] Moreover, in a prospective study a 50% incidence of biliary sludge was detected by ultrasound within 4–6 weeks of TPN. Ten days of fasting following gastrointestinal surgery caused sludge in 32% of patients.[21,24]

The high percentage of sludge formation during fasting is believed to be primarily caused by gallbladder hypomotility and bile stasis.

Pregnancy is another well-known risk factor for the development of biliary sludge and subsequent gallstones.[26] During pregnancy, bile becomes more lithogenic as a result of increased serum oestrogen levels which cause increased cholesterol secretion and supersaturation of bile.[27] In addition, because of gallbladder hypomotility, the residual volume of the gallbladder increases and stasis develops, and this also promotes sludge and stone formation.[26] The altered gallbladder motility during pregnancy is predominantly due to the inhibitory effect of progesterone on smooth muscle and perhaps to diminished contraction in response to cholecystokinin.[28] Sludge formation begins in the first trimester and increases up to 4–6 weeks post-partum.[29]

In addition to impaired motility of the gallbladder, bile stasis is an important cause of sludge formation. Sludge was analysed in six patients with common duct obstruction caused by chronic pancreatitis, pancreatic pseudocyst or carcinoma.[17] Microscopy of sludge showed calcium bilirubinate granules but no cholesterol crystals. During stasis of gallbladder bile, bilirubin glucuronide is hydrolysed to bilirubin, which then forms the pigment precipitate. When unconjugated bilirubin precipitates from

bile it forms complexes with inorganic ions, mostly calcium. In an animal model it has been shown that ligation of gallbladders resulted in the formation of pigment sludge and stone.[30]

Octreotide, the somatostatin analogue, has been shown to cause a 28% incidence of gallstones in patients receiving this drug for the treatment of acromegaly. Decreased gallbladder motility is the most probable cause for this octreotide effect.[31,32] Ceftriaxone, a third-generation cephalosporin with a long duration of action, is excreted into urine. However, as much as 40% of the drug is secreted in unmetabolised form into bile, reaching biliary concentrations of 100–200-fold higher than the concentration in serum.[33] Once the biliary saturation level is exceeded, ceftriaxone complexes with calcium and forms an insoluble salt.[19,34]

The development of biliary sludge is a well-known complication after liver transplantation. In the largest series, filling defects of the bile duct were detected in cholangiographies in 94 (5.7%) of 1650 patients.[35] On the basis of the cholangiographic appearance, the bile-duct filling defects were categorised as sludge or cast in 53 (56%), stones in 32 (34%), and necrotic debris in nine (10%) patients. The formation of biliary sludge is considered to be a serious, life-threatening complication. The major reasons for sludge formation are biliary strictures and prestenotic dilatations of the bile ducts.

PATHOGENESIS

The formation of cholesterol monohydrate crystals is believed to be crucial in the pathogenesis of cholesterol gallstones.[36,37] Although virtually insoluble in water, cholesterol is made soluble in bile through carriers which include bile salts and phospholipids. In unsaturated bile, cholesterol is primarily transported in simple and mixed micelles. As cholesterol saturation increases in bile, more cholesterol is carried in larger phospholipid cholesterol vesicles.[38] Unilamellar vesicles can coalesce into multilamellar vesicles, which tend to be less stable and allow the growth of cholesterol crystals from the surface.[39,40] These vesicles may interact with soluble mucin which acts as an annealing agent, favouring the further nucleation and agglomeration of cholesterol monohydrate crystals.[41–43] These crystals are entrapped in the soluble or gel form of mucin. Thus, one form of biliary sludge represents an early event in cholesterol gallstone formation with cholesterol monohydrate crystals embedded in biliary mucin.[2]

Calcium bilirubinate granules represent another major component of biliary sludge. These pigment granules are typically found in gallbladder bile of patients with pigment stones.[7] In liver cirrhosis, the risk of developing biliary sludge consisting of calcium bilirubinate granules is increased.[44] Hypersecretion of bilirubin conjugates (especially monoglucuronides) into the bile is the most important factor for the formation of biliary sludge consisting of calcium bilirubinate granules. Unconjugated monohydrogenated bilirubin is formed by the action of endogenous β-glucuronidase, which can co-precipitate with calcium as a result of supersaturation.[45]

NATURAL HISTORY

In a prospective study, 96 patients with biliary sludge detected sonographically were followed for a mean of 3 years by serial ultrasound scans.[46] In 17 patients (18%) biliary sludge disappeared but recurred during the observation period, while eight patients (8.3%) developed asymptomatic gallstones. Only six patients (6.3%) developed

symptomatic gallstones which required treatment by cholecystectomy. Another six patients (6.3%) suffered from biliary pain attacks, in part associated with recurrent pancreatitis, and also had to be treated by elective cholecystectomy.

In a further study, 56 patients with gallbladder sludge were followed up using ultrasound.[10] Within a mean of 2 months 40 patients (71.4%) were free of sludge and showed normal sonographic gallbladder findings. Gallbladder stones without sludge developed in five patients (8.9%) within a mean of 2.5 months, and gallstones with persistent sludge were observed in two further patients (3.6%) after 6.1 and 30.7 months, respectively. None of these patients became symptomatic in the course of the follow-up period. Acute cholecystitis developed in a further four patients (7.1%). No cases of acute pancreatitis were observed in this study.[10] The incidence of sludge or stone formation during pregnancy is 5.1% in the second trimester, 7.9% in the third trimester, and 10.2% 4–6 weeks post-partum.[29] Both sludge and stones are usually 'silent', but when biliary pain develops it is generally associated with the presence of stones and not with sludge. After delivery, gallbladder motility returns to normal and sludge disappears in 60–70% of the women.[47] The incidence of gallbladder sludge amounts to 50% in patients with total parenteral nutrition of more than 6 weeks' duration.[21] Normal nutrition causes disappearance of sludge, in most cases within a few weeks; however, because of the development of acalculous cholecystitis or symptomatic gallstones, cholecystectomy is required in 15% of cases.[21]

In conclusion, biliary sludge may be a reversible condition. Upon withdrawal of the noxious agent or condition, sludge dissolves or is discharged in the majority of patients. However, a minority of patients develop gallstones which may become symptomatic.

CLINICAL MANIFESTATIONS OF BILIARY SLUDGE

The clinical manifestations of biliary sludge and microlithiasis are only poorly understood. Both can cause symptoms similar to a typical biliary colic, mainly via obstruction of either the cystic or the common bile duct.

Biliary sludge or microlithiasis may cause so-called acalculous biliary pain and cholecystitis. The clinical features of acute so-called acalculous cholecystitis differ from those of cholecystitis resulting from stone disease. Although right upper quadrant pain, fever, localized tenderness over the gallbladder, and leukocytosis may be evident in classic presentations, some or all of these features are frequently lacking in elderly postoperative patients. Compared to ordinary calculous cholecystitis, the clinical course of acute acalculous cholecystitis is more fulminant. By the time the diagnosis is made, at least half of the patients have already experienced complications such as gangrene or localized perforation of the gallbladder.[48]

Cholesterolosis of the gallbladder is an acquired histological abnormality of the gallbladder mucosa, with excessive accumulation of cholesterol ester within epithelial macrophages. Although the cause of the accumulation of cholesterol esters and cholesterol in the gallbladder mucosa is unclear, it has been unequivocally shown that the gallbladder epithelium is capable of absorbing cholesterol from bile.[49–51] However, it is unknown why in some patients resorbed biliary cholesterol is esterified and then stored in foamy macrophages, resulting in cholesterolosis.[49]

However, the most relevant clinical manifestation of microlithiasis or biliary sludge is the obstruction of the common biliary duct with acute cholangitis or pancreatitis.

Gallstone pancreatitis is usually related to small stones, which may not be detected by transabdominal ultrasound.[52–57]

Therefore, occult microlithiasis should be strongly suspected in cases of acute pancreatitis of unknown origin, especially when frequent relapses occur.[58]

TREATMENT OF BILIARY SLUDGE OR MICROLITHIASIS

Similar to overt gallstone disease, treatment is only necessary in patients in whom biliary sludge or microlithiasis cause symptoms or complications. In accidentally detected biliary sludge with no biliary symptoms, expectant management is warranted. In accordance with the natural history of the majority of such patients, biliary sludge will dissolve or be discharged spontaneously. However, in patients in whom biliary sludge is responsible for biliary pain or for complications such as cholecystitis, cholangitis or recurrent pancreatitis, immediate treatment — preferably by laparoscopic cholecystectomy — is necessary.

For patients with biliary sludge and recurrent biliary outflow obstruction associated with either recurrent cholangitis or pancreatitis, an alternative approach is endoscopic papillotomy,[59–61] which may be combined with maintenance treatment with ursodeoxycholic acid or laparoscopic cholecystectomy.[62–64]

Biliary sludge after liver transplantation is a serious complication and should be treated primarily by oral chemolysis with ursodeoxycholic acid. Unfortunately, medical treatment often fails, and more aggressive approaches such as percutaneous transhepatic biliary drainage followed by irrigation with heparinised saline solutions, intraluminal chemolysis with glyceryl mono-octanoate and bile salt EDTA, or basket extraction become necessary. Endoscopic intervention and surgery are alternative approaches.[35,65]

In patients with symptomatic sludge after weight loss or total parenteral nutrition, treatment strategies such as bile acid dissolution therapy or laparoscopic cholecystectomy may be applied. However, since the development of sludge in patients undergoing weight loss and total parenteral nutrition is highly predictable, and in view of the potentially serious complications, prophylactic therapy is indicated.[12,13] In patients on a low-calorie diet, 500 mg ursodeoxycholic acid daily will minimize the risk of sludge development.[66] In patients with total parenteral nutrition a daily stimulation of gallbladder contraction with intravenous cholecystokinin is highly effective in the prevention of biliary sludge.[67]

SUMMARY

Biliary sludge was first described with the advent of ultrasonography and comprises a mixture of particulate matter formed by the precipitation of solutes in bile. Cholesterol monohydrate crystals, calcium bilirubinate, and other calcium salts are the most common components. The natural course of biliary sludge varies between complete resolution and progression to gallstones. Biliary sludge — and particularly microlithiasis — may cause complications, including biliary colic, acute pancreatitis, and acute cholecystitis. Clinical conditions and events associated with the formation of biliary sludge include rapid weight loss, pregnancy, ceftriaxone therapy, octreotide therapy, and liver transplantation.

Microlithiasis or sludge may be diagnosed on transabdominal ultrasonography or, with higher sensitivity, by endoscopic ultrasound. There are proven methods for the

prevention of sludge formation, even in high-risk patients. Asymptomatic patients with sludge can be managed expectantly. If patients with sludge or microlithiasis develop symptoms or complications, long-term treatment with ursodeoxycholic acid, endoscopic papillotomy or cholecystectomy are treatment options. Further studies of the pathogenesis, natural history, and clinical associations of biliary sludge will be essential to our understanding of gallstones and other biliary tract abnormalities.

Practice points

- endoscopic ultrasound is superior to transabdominal ultrasound in the diagnosis of microlithiasis and sludge in bile
- microlithiasis and sludge in bile are the dominant causes of 'acalculous' biliary pain and cholecystitis and so-called 'idiopathic' pancreatitis
- treatment of microlithiasis and sludge in bile follows the guidelines of treatment in symptomatic gallstone disease, and includes long-term application of ursodeoxycholic acid, endoscopic papillotomy, and preferably laparoscopic cholecystectomy

Research agenda

- improvements in the non-invasive diagnosis of biliary sludge or microlithiasis are urgently needed to increase our understanding of the pathophysiological role of this entity for the development of gallstones or other biliary tract abnormalities

REFERENCES

1. Jüngst D, del Pozo R, Christoph S et al. Sedimentation of biliary 'sludge'. Effect of composition of gallbladder bile from patients with cholesterol, mixed or pigment stones. *Scand J Gastroenterol* 1996; **31:** 273–278.
2. de la Porte PL, Lafont H, Domingo N et al. Composition and immunofluorescence studies of biliary 'sludge' in patients with cholesterol or mixed gallstones. *J Hepatol* 2000; **33:** 352–360.
3. Conrad MR, Jones RO & Dietchy J. Significance of low level echoes within the gallbladder. *AJR Am J Roentgenol* 1979; **132:** 967–972.
4. Filly RA, Allen B, Minton MJ et al. In vitro investigation of the origin of echoes within biliary sludge. *J Clin Ultrasound* 1980; **8:** 193–200.
5. Jennings WC, Drabek GA & Miller KA. Significance of sludge and thickened wall in ultrasound evaluation of the gallbladder. *Surg Gynecol Obstet* 1992; **174:** 394–398.
6. 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:** 162–165.

*7. Ros E, Navarro S, Fernádez I et al. Utility of biliary microscopy for the prediction of the chemical composition of gallstones and the outcome of dissolution therapy with ursodeoxycholic acid. *Gastroenterology* 1986; **91:** 703–712.

*8. Thorboll J, Vilmann P, Jacobsen B et al. Endoscopic ultrasonography in detection of cholelithiasis in patients with biliary pain and negative transabdominal ultrasonography. *Scand J Gastroenterol* 2004; **39:** 267–269.

*9. Mirbagheri SA, Mohamadnejad M, Nasiri J et al. Prospective evaluation of endoscopic ultrasonography in the diagnosis of biliary microlithiasis in patients with normal transabdominal ultrasonography. *J Gastrointest Surg* 2005; **9:** 961–964.
*10. Janowitz P, Kratzer W, Zemmler T et al. Gallbladder sludge: spontaneous course and incidence of complications in patients with stones. *Hepatology* 1994; **20:** 291–294.
11. Maringhini A, Ciambra M, Bacceliere P et al. Sludge, stones and pregnancy. *Gastroenterology* 1988; **95:** 1160–1161.
12. Messing B, Bories C, Kunstlinger F et al. Does total parenteral nutrition induce gallbladder sludge formation and lithiasis? *Gastroenterology* 1983; **84:** 1012–1019.
13. Bolondi L, Gaiani S, Testa S et al. Gallbladder sludge formation during prolonged fasting after gastrointestinal tract surgery. *Gut* 1985; **26:** 734–738.
14. Cano N, Cicero F, Ranieri F et al. Ultrasonographic study of gallbladder motility during total parenteral nutrition. *Gastroenterology* 1986; **91:** 313–317.
15. Gafá 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:** 413–418.
16. Liddle RA, Goldstein RB & Saxton J. Gallstone formation during weight-reduction dieting. *Arch Intern Med* 1989; **149:** 1750–1753.
17. Allen B, Bernhoft R, Blanckaert N et al. Sludge is calcium bilirubinate associated with bile stasis. *Am J Surg* 1981; **141:** 51–56.
18. Pitt HA, Doty JE, den Besten LW et al. Stasis before gallstone formation: altered gallbladder compliance or cystic duct resistance. *Am J Surg* 1982; **143:** 144–149.
19. 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:** 1665–1670.
20. Stampfer MJ, Maclure KM, Colditz GA et al. Risk of symptomatic gallstones in women with severe obesity. *Am J Clin Nutr* 1992; **55:** 652–658.
21. 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:** 1000–1005.
22. Marks JW, Bonorris GG, Albers G et al. The sequence of biliary events preceding the formation of gallstones in humans. *Gastroenterology* 1992; **103:** 566–570.
23. Shiffman ML, Shambruck RD, Schwartz CC et al. Gallbladder mucin, arachidonic acid and bile lipids in patients who develop gallstones during weight reduction. *Gastroenterology* 1993; **105:** 1200–1208.
24. Inoue K, Fuchigami A, Higashide S et al. Gallbladder sludge and stone formation in relation to contractile function after gastrectomy. *Ann Surg* 1992; **215:** 19–26.
25. Pitt HA, King 3rd W, Mann LL et al. Increased risk of cholelithiasis with prolonged total parenteral nutrition. *Am J Surg* 1983; **145:** 106–112.
26. Tsimoyiannis EC, Antoniou NC, Tsaboulas C et al. Cholelithiasis during pregnancy and lactation. Prospective study. *Eur J Surg* 1994; **160:** 627–631.
27. Lynn J, Williams L, O'Brien J et al. Effects of estrogen upon bile: implications with respect to gallstone formation. *Ann Surg* 1973; **178:** 514–524.
28. Hould FS, Fried GM, Fazekas AG et al. Progesterone receptors regulate gallbladder motility. *J Surg Res* 1988; **45:** 505–512.
29. Ko CW, Beresford SAA, Schulte SJ et al. Incidence, natural history, and risk factors for biliary sludge and stones during pregnancy. *Hepatology* 2005; **41:** 359–365.
30. Bernhoft RA, Pellegrini CA, Broderick WC et al. Pigment sludge and stone formation in the acutely ligated dog gallbladder. *Gastroenterology* 1983; **85:** 1166–1171.
31. Van Liessum PA, Hopman WPM, Pieters GFFM et al. Postprandial gallbladder motility during long term treatment with the long-acting somatostatin analog SMS 201-995 in acromegaly. *J Clin Endocrinol Metab* 1989; **69:** 557–562.
32. Montini M, Gianola D, Pagani MD et al. Cholelithiasis and acromegaly: therapeutic strategies. *Clin Endocrinol* 1994; **40:** 401–406.
33. Arvidsson A, Alvan G, Angelin B et al. Renal and biliary excretion and effect on the colon microflora. *J Antimicrob Chemother* 1982; **10:** 207–215.
34. Schaad UB, Wedgwood-Krucko J & Tschaeppeler H. Reversible ceftriaxone-associated biliary pseudolithiasis in children. *Lancet* 1988; **2:** 1411–1413.

35. Sheng R, Ramirez CB, Zajko AB et al. Biliary stones and sludge in liver transplant patients: a 13-year experience. *Radiology* 1996; **198:** 243–247.
36. Sedaghat A & Grundy SM. Cholesterol crystals and the formation of cholesterol gallstones. *N Engl J Med* 1980; **302:** 1274–1277.
37. Holan KR, Holzbach RT, Hermann RE et al. Nucleation time: a key factor in the pathogenesis of cholesterol gallstone disease. *Gastroenterology* 1979; **77:** 611–617.
38. Jüngst D, Gussmann E, Zündt B et al. Solubility of cholesterol in the crystal-free gallbladder bile of gallstone patients. *J Lab Clin Med* 2004; **144:** 134–140.
39. Sömjen GJ & Gilat T. Changing concepts of cholesterol solubility in bile. *Gastroenterology* 1986; **91:** 772–775.
40. Schriever CE & Jüngst D. Association between cholesterol–phospholipid vesicles and cholesterol crystals in human gallbladder bile. *Hepatology* 1989; **9:** 541–546.
41. Smith BF. Human gallbladder mucin binds biliary lipids and promotes cholesterol crystal nucleation in model bile. *J Lipid Res* 1987; **28:** 1088–1097.
42. Afdahl NH, Niu N, Gantz D et al. Bovine gallbladder mucin accelerates cholesterol monohydrate crystal growth in model bile. *Gastroenterology* 1993; **104:** 1515–1523.
43. Wilhelmi M, Jüngst C, Mock M et al. Effect of gallbladder mucin on the crystallization of cholesterol in bile. *Eur J Gastroenterol Hepatol* 2004; **16:** 1301–1307.
44. Acalovschi M, Badea R & Pascu M. Incidence of gallstones in liver cirrhosis. *Am J Gastroenterol* 1991; **86:** 1179–1181.
45. Cahalane MJ, Neubrand MW & Carey MC. Physical–chemical pathogenesis of pigment gallstones. *Semin Liver Dis* 1988; **8:** 317–328.
*46. Lee SP, Maher K & Nicholls JF. Origin and fate of biliary sludge. *Gastroenterology* 1988; **94:** 170–176.
47. 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:** 116–120.
48. Johnson LB. The importance of early diagnosis of acute acalculous cholecystitis. *Surg Gynecol Obstet* 1987; **164:** 197–203.
49. Sahlin S, Stahlberg D & Einarsson K. Cholesterol metabolism in liver and gallbladder mucosa of patients with cholesterolosis. *Hepatology* 1995; **21:** 1269–1275.
50. Jacyna MR, Ross PE, Bakar MA et al. Characteristics of cholesterol absorption by human gall bladder: relevance to cholesterolosis. *J Clin Pathol* 1987; **40:** 524–529.
51. Tilvis RS, Aro J, Strandberg TE et al. Lipid composition of bile and gallbladder mucosa in patients with acalculous cholesterolosis. *Gastroenterology* 1982; **82:** 607–615.
52. Negro P, Flati G, Flati D et al. Occult gallbladder microlithiasis causing acute recurrent pancreatitis. *Acta Chir Scand* 1984; **150:** 503–506.
*53. Lee SP, Nicholls JF & Park HZ. Biliary sludge as a cause of acute pancreatitis. *N Engl J Med* 1992; **326:** 589–593.
54. Diehl AK, Holleman DR, Chapman JB et al. Gallstone size and risk of pancreatitis. *Arch Intern Med* 1997; **157:** 1674–1678.
55. Kohut M, Nowak A, Nowakowska-Dulawa E et al. The frequency of bile duct crystals in patients with presumed biliary pancreatitis. *Gastrointest Endosc* 2001; **54:** 37–41.
*56. Venneman NG, Renooij W, Rehfeld JF et al. Small gallstones, preserved gallbladder motility, and fast crystallization are associated with pancreatitis. *Hepatology* 2005; **41:** 738–746.
57. Venneman NG, Buskens E, Besselink MGH et al. Small gallstones are associated with increased risk of acute pancreatitis: potential benefits of prophylactic cholecystectomy. *Am J Gastroenterol* 2005; **100:** 2540–2550.
58. Levy MJ & Geenen JE. Idiopathic acute recurrent pancreatitis. *Am J Gastroenterol* 2001; **96:** 2540–2555.
59. Carr-Locke DL. Role of endoscopy in gallstone pancreatitis. *Am J Surg* 1992; **165:** 519–521.
60. Welbourn CRB, Beckly DE & Eyre-Brook IA. Endoscopic sphincterotomy without cholecystectomy for gallstone pancreatitis. *Gut* 1995; **37:** 119–120.
61. Ricci F, Castaldini G, de Manzoni G et al. Minimally invasive treatment of acute biliary pancreatitis. *Surg Endosc* 1997; **11:** 1179–1182.
*62. 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:** 1701–1709.

*63. 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:** 1206–1211.
64. Testoni PA, Caporuscio S, Bagnolo F et al. Idiopatic recurrent pancreatitis: long-term results after ERCP, endoscopic sphincterotomy, or ursodeoxycholic acid treatment. *Am J Gastroenterol* 2000; **95:** 1615–1618.
65. 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:** 865–869.
66. 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.
*67. Ko CW, Sekijima JH & Lee SP. Biliary sludge. *Ann Intern Med* 1999; **130:** 301–311.