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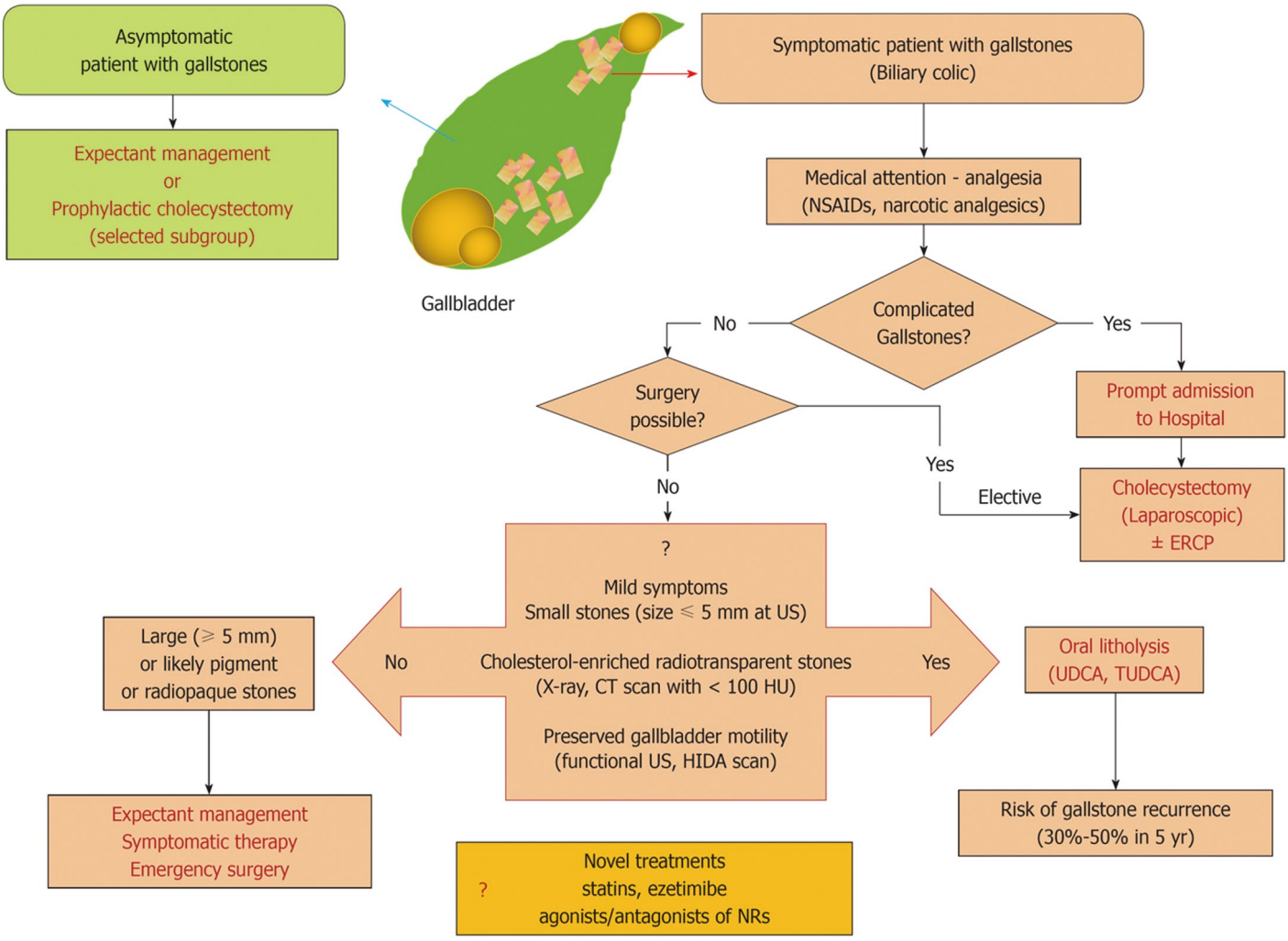

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TOPIC HIGHLIGHT

Angelo Zullo, MD, *Series Editor*

Therapy of gallstone disease: What it was, what it is, what it will be

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Abstract

Cholesterol gallstone disease is a common clinical condition influenced by genetic factors, increasing age, female gender, and metabolic factors. Although laparoscopic cholecystectomy is currently considered the gold standard in treating patients with symptomatic gallstones, new perspectives regarding medical therapy of cholelithiasis are currently under discussion, also taking into account the pathogenesis of gallstones, the natural history of the disease and the analysis of the overall costs of therapy. A careful selection of patients may lead to successful non-surgical therapy in symptomatic subjects with a functioning gallbladder harboring small radiolucent stones. The classical oral litholysis by ursodeoxycholic acid has been recently paralleled by new experimental observations, suggesting that cholesterol-lowering agents which inhibit cholesterol synthesis (statins) or intestinal cholesterol absorption (ezetimibe), or drugs acting on specific nuclear receptors involved in cholesterol and bile acid homeostasis, might be proposed as additional approaches for treating cholesterol gallstones. In this review we discuss old, recent and future perspectives on medical treatment of cholesterol cholelithiasis.

Key words: Gallstones; Dissolution therapy; Cholecystectomy; Bile acids; Ezetimibe; Statins; Gallbladder; Bile; Nuclear receptors

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INTRODUCTION

The prevalence of gallstones increases with age, and is associated with a number of major risk factors (Table 1)[1-3].

In westernized countries, well known risk factors are: obesity, type 2 diabetes, dyslipidaemia, and hyperinsulinaemia, which are often components of the metabolic syndrome[4-8]. Although the majority of stones in the gallbladder remain "silent" and do not require medical or surgical treatment, gallstone disease is still one of the most common digestive diseases requiring hospital admission and financial resources, since its prevalence ranges from 10% to 15% in adults and medical expenses for gallstone treatment exceeded $6 billion in the year 2000 in the United States[1,9-11].

To know exactly the composition of gallstones is an essential step to select patients responsive to oral litholysis with bile acids (see below). In principle, the only gallstones amenable to litholysis are cholesterol-enriched, calcium-free stones. Cholesterol gallstones represent about 75% of the gallstones in westernized countries[12-14] and can be dissolved when no calcium has deposited in the stones[1,15]. Historically, the Renaissance physician, botanist, alchemist and astrologer Paracelsus (Philippus Aureolus Theophrastus Bombastus von Hohenheim) was the first one to hypothesize that gallbladder concrements were originating from the precipitation of solid material made of tartaric acid[16,17]. To date, we know that specific pathogenetic factors contributing to the formation of cholesterol gallstones must include: hepatic hypersecretion of cholesterol into bile leading to a supersaturated bile, accelerated nucleation/crystallization of cholesterol, defective gallbladder motility (a form of leiomyopathy) leading to gallbladder stasis, increased absorption of intestinal cholesterol, and influence of *LITH* genes[1,18-24]. The remaining gallstones are pigment stones that contain less than 30% cholesterol, i.e., black pigment stones which are about 20% of all gallstones found in the gallbladder and/or bile duct (containing mainly insoluble bilirubin pigment polymer mixed with calcium phosphate and carbonate, and cholesterol) and brown pigment stones which are about 5% of all gallstones, found in bile ducts (containing calcium bilirubinate, calcium palmitate, stearate and cholesterol)[25].

Patients presenting with a typical colicky pain ("symptomatic") do need treatment because of the high rates of complications (e.g., acute cholecystitis, acute biliary pancreatitis or cholangitis), and early recurrence of symptoms. The high costs of both surgical and medical therapeutic interventions and the natural history of the disease indicate restricting the treatment to a subgroup of symptomatic patients with specific symptoms[1,23,26].

The first cholecystectomy was performed in 1882 by Carl Langenbuch in Berlin[27,28], which was the first milestone in the treatment of gallstones. Initial experiments on the dissolution of gallstones were already happening at the end of the 19th century[29,30] and in the first half of the 20th century[31]. However, it was Danzinger *et al*[32] in 1972 who reported that the primary bile acid chenodeoxycholic acid (CDCA) could dissolve cholesterol gallstones in humans when given orally for 6 mo. These days, oral litholysis by ursodeoxycholic acid (UDCA) plays a limited role in cholesterol gallstone treatment. However, some novel and interesting therapeutic options have been suggested by data from pathogenetic and pharmacological studies[1], in particular in subjects permanently or temporarily at risk for gallstone disease (Table 1). Experimental data on the capacity of the Niemann-Pick C1-like 1 (NPC1L1) protein inhibitor ezetimibe to reduce intestinal absorption of cholesterol[33], the effects of statins to inhibit cholesterol synthesis[34], or drugs acting on specific nuclear receptors (NRs) involved in cholesterol and bile acid homeostasis[35] may offer an integrate, potent and innovative strategy for the medical treatment of cholesterol gallstones[36]. Major updated therapeutic aspects in patients with gallstones will be reviewed in this paper.

MANAGING GALLSTONE DISEASE

The therapeutic option of gallstone disease is based on few crucial steps, i.e., presence/absence of typical symptoms (i.e., colicky pain), presence of complications, and gallbladder function, as well as composition and size of gallstones (Figure 1).

Bearing in mind data on epidemiology and overall costs of both medical and surgical therapies, it is not routinely recommended to treat asymptomatic gallstone patients[37-39]. Thus, an expectant management (medical attention) is currently considered the most appropriate choice in patients with gallstones of any type without specific symptoms (i.e., biliary colic). Indeed, approximately 60%-80% of patients with gallstones are completely asymptomatic[40-42] and stones are frequently found during routine abdominal ultrasonography[40-42]. In general, the risk of developing typical biliary pain is low (2.0%-2.6% per year[43-46]) although microlithiasis or biliary sludge in the gallbladder lumen puts patients at risk for colicky pain or acute pancreatitis[47,48]. Nevertheless, the overall risk rate for complications (yearly incidence 0.3%) and gallbladder cancer (0.02%) are very low[49,50]. If biliary pain and/or complications are present, cholecystectomy represents the gold standard (see below), as oral litholysis with hydrophilic bile acids have a limited role, and are reserved to symptomatic patients with small radiolucent gallstones in a well functioning gallbladder with a patent cystic duct[1,23]. Before cholecystectomy, however, careful medical attention and analgesia are often required. Major features of the uncomplicated biliary colic are depicted in Table 2, concerning pathogenesis, onset, intensity, localization, duration, radiation, associated features, relief of pain, and therapeutic aspects. The chemical formula of drugs currently used to induce analgesia in patients with colicky pain is depicted in Figure 2.

Cholecystectomy

Cholecystectomy can be performed by laparoscopy, by a small-incision (< 8 cm in length), or by open operation, and several meta-analyses indicate surgical procedures as the gold standard for the treatment of symptomatic gallstones[51-53]. Laparoscopic cholecystectomy, or alternatively, small incision cholecystectomy[53], are both safe with

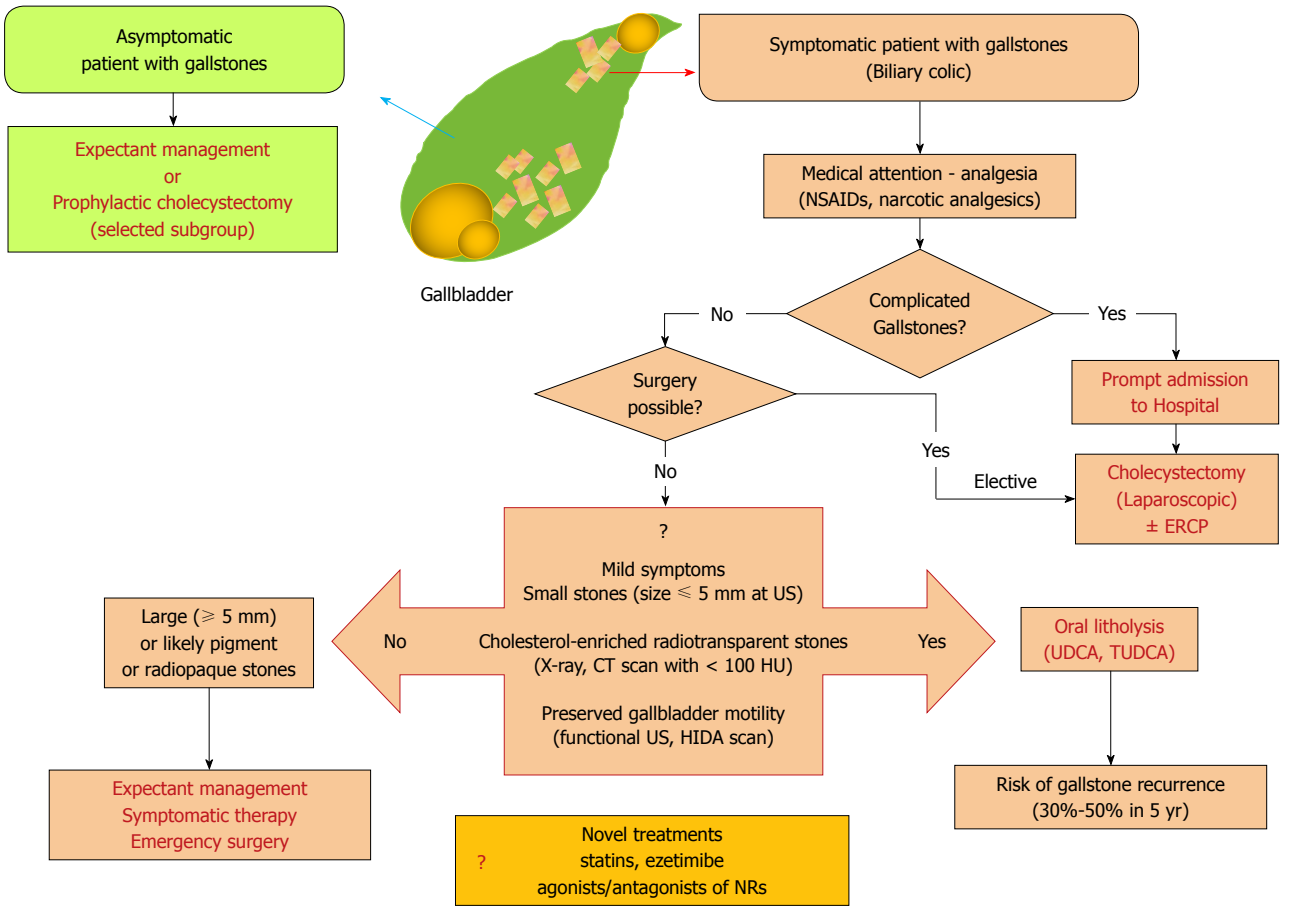

Figure 1 Flow-chart depicting the standard therapies of gallstone disease (adapted from Portincasa *et al*[1,15,23,148]). As a starting point, at the top the gallbladder containing "supersaturated" biliary cholesterol is depicted. Typical solid plate-like monohydrate cholesterol crystals form first and aggregate after, to grow as cholesterol stones. Left: flow-chart reserved to asymptomatic patients with gallstones (i.e., when stones/crystal aggregates are not impacted within the cystic duct). Best choice is expectant management, while few indications for prophylactic cholecystectomy exist and are reported in Table 2; Right: the complex flow-chart reserved to symptomatic gallstone patients is shown. This is the case when stones/crystal aggregates are impacted within the cystic duct. A key step is to identify the "symptomatic" patients with or without complications. In this respect, documenting the presence of biliary colic is of key importance. Meta-analyses indicate that surgery (cholecystectomy) is the gold standard for treating symptomatic gallstones[51-53]. For treatment of uncomplicated and complicated biliary colic, see also Tables 3 and 4. CT: Computed tomography; ERCP: Endoscopic retrograde cholangiopancreatography; HIDA: 99mTc-N-(2,6-dimethylacetanilide)-iminodiacetic acid; HU: Hounsfield Unit; NSAIDs: Non-steroidal anti-inflammatory drugs; NRs: Nuclear receptors; TUDCA: Tauroursodeoxycholic acid; UDCA: Ursodeoxycholic acid; US: Abdominal ultrasonography. The HU is an arbitrary unit of X-ray attenuation used for CT scans. Each voxel is assigned a value on a scale in which air has a value of -1000; water, 0; and compact bone, +1000.

Table 1 Non-genetic risk factors for gallbladder stones

Age
Female gender
High-calorie, low-fiber diet
High-carbohydrate diet, dietary glycemic load
Obesity
Physical inactivity
Rapid weight loss/surgery for obesity
Total gastrectomy with lymph node dissection
Spinal cord injury
Infections: enterohepatic *Helicobacter* species, malaria
Biliary strictures
Drugs: estrogens, calcineurin inhibitors, fibrates, octreotide, ceftriaxone
Total parenteral nutrition
Duodenal diverticulum
Extended ileal resection (black pigment stones)
Vitamin B_{12}/folic acid deficient diet (black pigment stones)
Pancreatic insufficiency
Cholangitis (brown pigment bile duct stones)

Adapted from Portincasa *et al*[1] and Grünhage *et al*[160] with permission.

a similar mortality rate ranging from 0.1% to 0.7%[52,54]. Both these procedures are cost-effective compared with open cholecystectomy[52]. Hospital stay and convalescence are shorter, as is the total cost lower for laparoscopic cholecystectomy compared with open cholecystectomy[54]. The overall incidence of bile duct injuries requiring corrective surgery varies between 0.1% and 0.3%[55-57] and both laparoscopic and open cholecystectomies yield similar complication rates[52,54].

Principally due to the low rate of complications, it is currently under discussion if cholecystectomy may be suggested also for patients with asymptomatic gallstones, but it is generally conceived that surgical procedures are not recommended routinely in symptom-free patients (Figure 1). Few indications for prophylactic cholecystectomy in asymptomatic patients with gallstones are reported in Table 3. For example, cholecystectomy should be considered in children with asymptomatic gallstones[58] (in particular with sickle cell disease[59,60], spherocytosis,

Table 2 Major features of the uncomplicated biliary colic

Pathogenesis	Visceral pain caused by the impaction of the stone in the cystic duct or the ampulla of Vater, followed by distension of the gallbladder and/or biliary tract with activation of visceral sensory neurons[161]
Onset	Not exclusively postprandial, typically intermittent
Intensity	Mean visual analogue scale of 9 cm on a 0-10 cm scale
Localization	Most frequently right upper quadrant of the abdomen and/or the epigastrium (representative dermatomes T8/9)
Duration	Generally longer than 15-30 min. Can last several hours and be associated non-specific symptoms of indigestion
Radiation	Angle of the right scapula and/or shoulder (about 60% of cases), retrosternal area (less than 10% of cases)
Associated features	Urgency to walk[162] (two-third of patients), nausea or vomit[42,161,162]
Relief	If the stone returns into the gallbladder lumen, passes through the ampulla of Vater into the duodenum or migrates back to the common bile duct[26]
First-line therapy	Fast-acting narcotic analgesics (meperidine[163]) or non-steroidal anti-inflammatory drugs (NSAIDs) (im or iv ketorolac or ibuprofen po) which could also reduce the risk of evolution towards acute cholecystitis[164-167]
Second-line therapy	Antispasmodic (anticholinergic) agents like hyoscine (scopolamine). Less effective than NSAIDs[164]
Recommendations	Fasting, to avoid release of endogenous cholecystokinin and further gallbladder contraction

Adapted from[25,63,148] with permission.

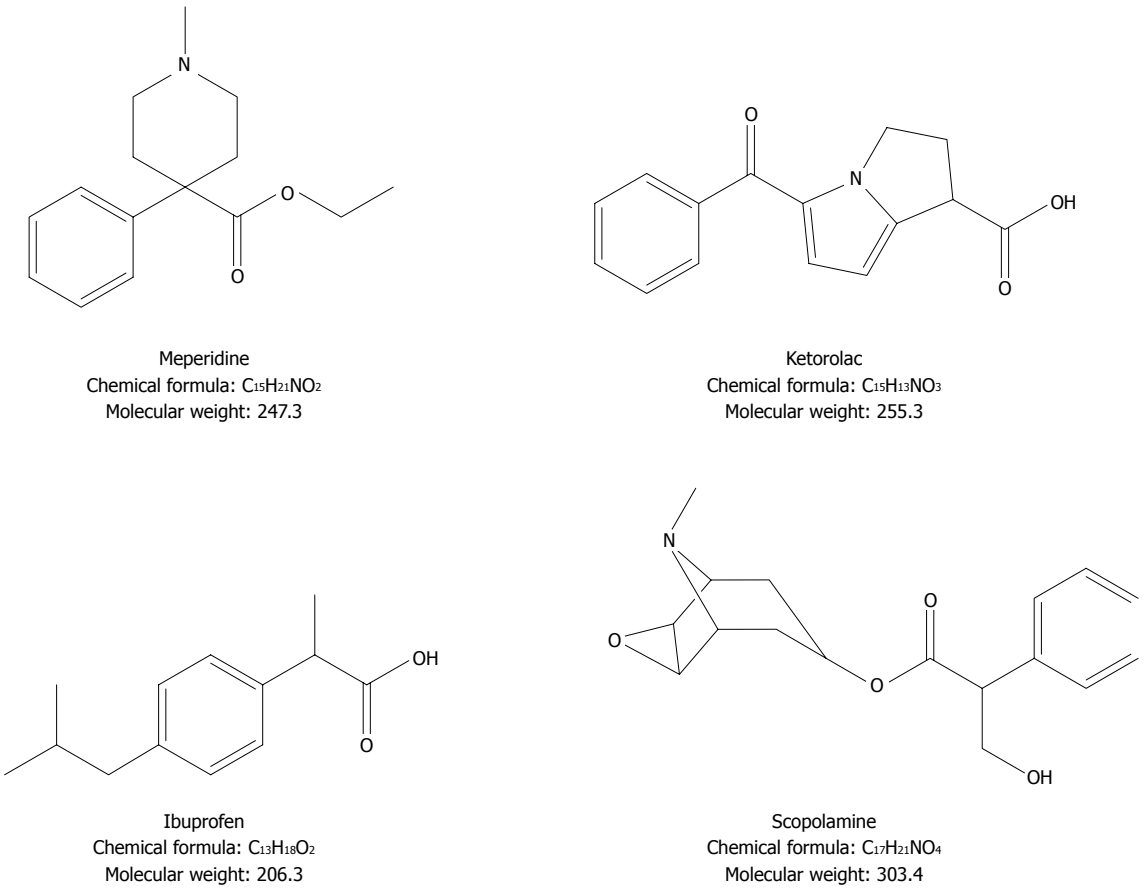

Figure 2 Chemical formula of drugs currently used to induce analgesia in patients with colicky pain. The three categories are: narcotic analgesics, non-steroidal anti-inflammatory drugs, and antispasmodics.

and elliptocytosis[60]) who are exposed to the risk of pain and complications. In this group the natural history of gallstones is not well known[61], although a recent study suggests that clinically silent gallstones in children and infants are associated with low rates of complications and can be therefore managed conservatively[60], as in adults. Other groups in which prophylactic cholecystectomy must be considered are the morbidly obese undergoing bariatric surgery, patients at high risk for gallbladder cancer, patients with sickle cell anemia, and coexistence of small gallstones and gallbladder dysmotility[47,62-68]. A totally different approach is necessary in the case of complicated biliary colic, as also shown in Table 4.

OLD AND NEW NON-SURGICAL OPTIONS

There is no established medical therapy for dissolution of pigment stones or calcified stones of any type. For

Table 3 Indications for "prophylactic" cholecystectomy (i.e., asymptomatic gallstone patients bearing a high risk of becoming symptomatic)

Children (because they are exposed to the long-term physical presence of stones[58])
Morbid obese patients undergoing bariatric surgery (high risk to become symptomatic during rapid weight loss[62])
Increased risk for gallbladder cancer[63]
Patients with large gallstones (greater than 3 cm)[64,65]
A "porcelain" gallbladder[66] or gallbladder polyps rapidly growing or larger than 1 cm
Native Americans with gallstones (risk of gallbladder cancer 3 to 5 percent)[67]
Gallstone patients with sickle cell anemia (formation of calcium bilirubinate gallstones due to chronic hemolysis. Patients may become symptomatic with recurrent episodes of abdominal pain[68])
Coexistence of small gallstones and gallbladder dysmotility (increased risk of pancreatitis[47])

Adapted from[25,63,148] with permission.

Table 4 Major features of the complicated biliary colic

Additional findings compared to uncomplicated biliary pain	Leukocytosis, nausea, jaundice, vomiting, fever
Underlying potential complications	Acute pancreatitis, acute cholecystitis, biliary obstruction and cholangitis, gallbladder perforation, abscess formation, mucocele of the gallbladder
Decision	Quick admission to the hospital
Therapies	Antibiotics or invasive procedures with or without surgical procedures (Figure 1)
	Early laparoscopic cholecystectomy recommended between 2 and 4[168] in mild and moderate acute cholecystitis

Adapted from[25,63,148] with permission.

cholesterol gallstones, current medical treatment includes oral litholysis with bile acids (see below). Medical therapies alternative to oral bile acids have been proposed in the past, including direct stone dissolution with methyl tert-butyl ether (MTBE), a potent organic cholesterol solvent[69], extracorporeal shock-wave lithotripsy (ESWL)[70], or in combination[71], followed by oral litholysis with bile acids. The interest in such options, however, has vanished due to their invasiveness, potential toxicity (MTBE) or traumatic (ESWL) side effects and, for both, the high post-dissolution recurrence rate[1,72,73]. Novel treatments to be discussed include statins, ezetimibe, and agonists/antagonists of NRs.

Oral dissolution therapy

The first successful dissolution of cholesterol gallstones was achieved in 1972 by oral administration of the natural primary tri-hydroxy bile acid CDCA[32] (Figure 3). The use of CDCA was abandoned because side effects were noticed, including a dose-dependent increase in serum liver enzymes, an increase in serum low-density lipoprotein (LDL) cholesterol, and diarrhea.

A further step was to use the more hydrophilic tri-hydroxy bile acid UDCA[74]. UDCA is more hydrophilic and less toxic than CDCA, and is currently employed for oral litholysis of small cholesterol gallstones in patients with a functioning gallbladder (Figure 3). This bile acid, in a dose of 10-14 mg/kg per day, increases its proportion in the bile acid pool (it originally accounts for less than 8%-10% of the biliary bile acid pool in healthy subjects), inducing a decreased hepatic secretion of biliary cholesterol and the formation of unsaturated gallbladder bile (cholesterol saturation index of less than 1)[75-77], the key factor which promotes the dissolution of cholesterol crystals and gallstones.

The fine mechanisms involved in UDCA-induced dissolution of cholesterol stones are rather complex. The so-called ternary phase diagram is used to explain the molecular effects of UDCA on bile composition and cholesterol solubility[78]. A group of the equilibrium phase diagram of cholesterol-lecithin-taurine-conjugated bile acid systems (37 ℃, 0.15 M NaCl, pH 7.0, total lipid concentration 7.5 g/dL) are drawn to display varied positions and configuration of crystallization regions due to decreasing bile acid hydrophobicity, with the lipid components being expressed in moles percent. At the bottom, the one-phase micellar zone exists (i.e., high bile acid-lecithin moles percent), while above this zone two-phase zones exist on both sides from a central three-phase zone. The study of solid and liquid crystallization sequences present in bile shows that different regions exist within each zone, namely A, B in the left two-phase, C, D in the central three-phase regions, and E in the right 2-phase zone. The number of phases given represents the equilibrium state and develop as cholesterol monohydrate crystals and saturated micelles for crystallization regions A and B; cholesterol monohydrate crystals, saturated micelles and liquid crystals for regions C and D; and liquid crystals of variable compositions and saturated micelles for region E[78]. As the bile acid hydrophobicity decreases, the maximum micellar cholesterol solubility is reduced and crystallization pathways A-E move to the left. This change results in an enlarged region E that extends to the left and overlaps pathophysiological compositions as ex-

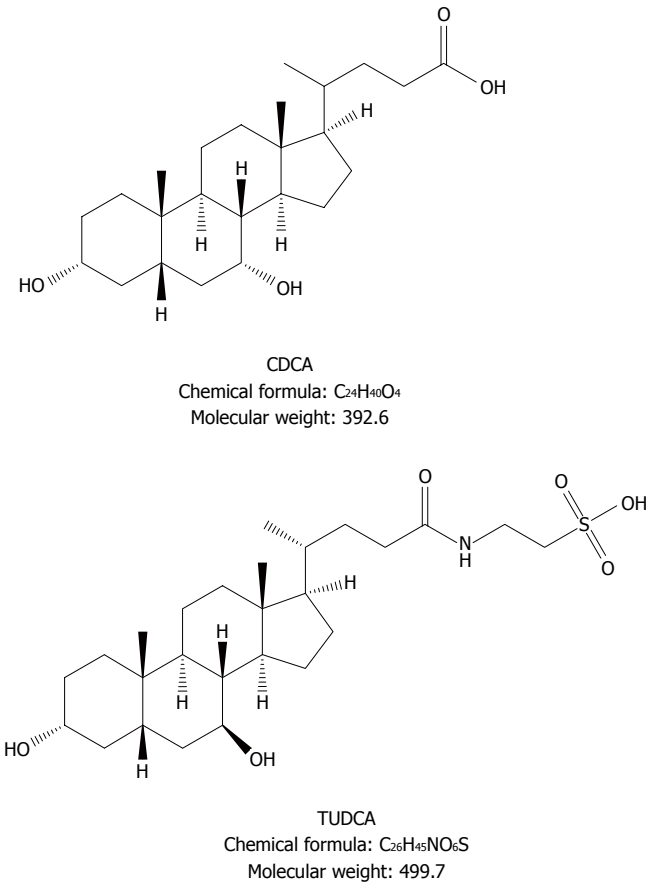

Figure 3 Chemical formula of bile acids used for oral litholysis of small, radiotransparent, cholesterol-enriched gallstones in a functioning gallbladder with a patent cystic duct of patients with symptomatic gallstones. CDCA: Chenodeoxycholic acid; UDCA: Ursodeoxycholic acid; TUDCA: Tauroursodeoxycholic acid.

emplified in the tauroursodeoxycholate (TUDC)-lecithin-cholesterol system. This event induces a greatly reduced chance for the formation of solid plate-like cholesterol monohydrate crystals in bile.

A bedtime administration of UDCA or TUDCA, is recommended since it maintains hepatic bile acid secretion rate overnight, thus reducing secretion of supersaturated bile and increasing the dissolution rate[79,80]. The hydrophilic bile acid UDCA is also able to act as a litholytic agent through the reduction of intestinal cholesterol absorption[81-83] and as a possible "prokinetic" agent capable of ameliorating postprandial gallbladder emptying as suggested by observations *in vitro* on isolated gallbladder smooth muscle strips from both animals and gallstone patients[84,85]. The improvement of gallbladder smooth muscle contractility probably also results from the prevention of the impairment of smooth muscle contractility induced by the more hydrophobic and toxic deoxycholate[86,87].

However, although the majority of gallstones (about two-thirds) in westernized countries are mainly composed of cholesterol, only a minority of patients (less than 10% of total) with cholesterol-enriched gallstones is amenable to oral dissolution therapy with UDCA or with its taurine-conjugates TUDCA[1,26]. In fact, dissolution therapy with oral bile acids can be only suggested to symptomatic gallstone patients who are unfit for surgery and have small (equal to or less than 5 mm in size), uncalcified (radiolucent), and cholesterol-enriched (i.e., more than 80%) stones in a functioning gallbladder with a patent cystic duct[88]. A number of diagnostic techniques provide essential information for appropriate selection of patients.

Gallbladder ultrasonography allows the accurate visualization of gallstone number, size, burden, biliary sludge[39,89,90] and explores the morphology and contractile property of the gallbladder, the features of the gallbladder wall with respect to the (acute-chronic) inflammatory status, and the patency of the cystic duct[91-97]. An abdominal plain radiography or a computed tomography (CT) scan[98,99] are needed to exclude the presence of calcified stones[25]. By CT scan, in particular, values of < 100 Hounsfield Units predict radiolucent cholesterol rich, dissolvable stones[100] (see also Figure 1 for explanation).

An accurate selection of gallstone patients with the characteristics described above offers a higher chance of successful oral litholysis alone or after ESWL inducing stone fragmentation[93-96,101], with an expected dissolution rate of about 1 mm decrement in stone diameter per month[102].

The complete disappearance of stones with a diam-

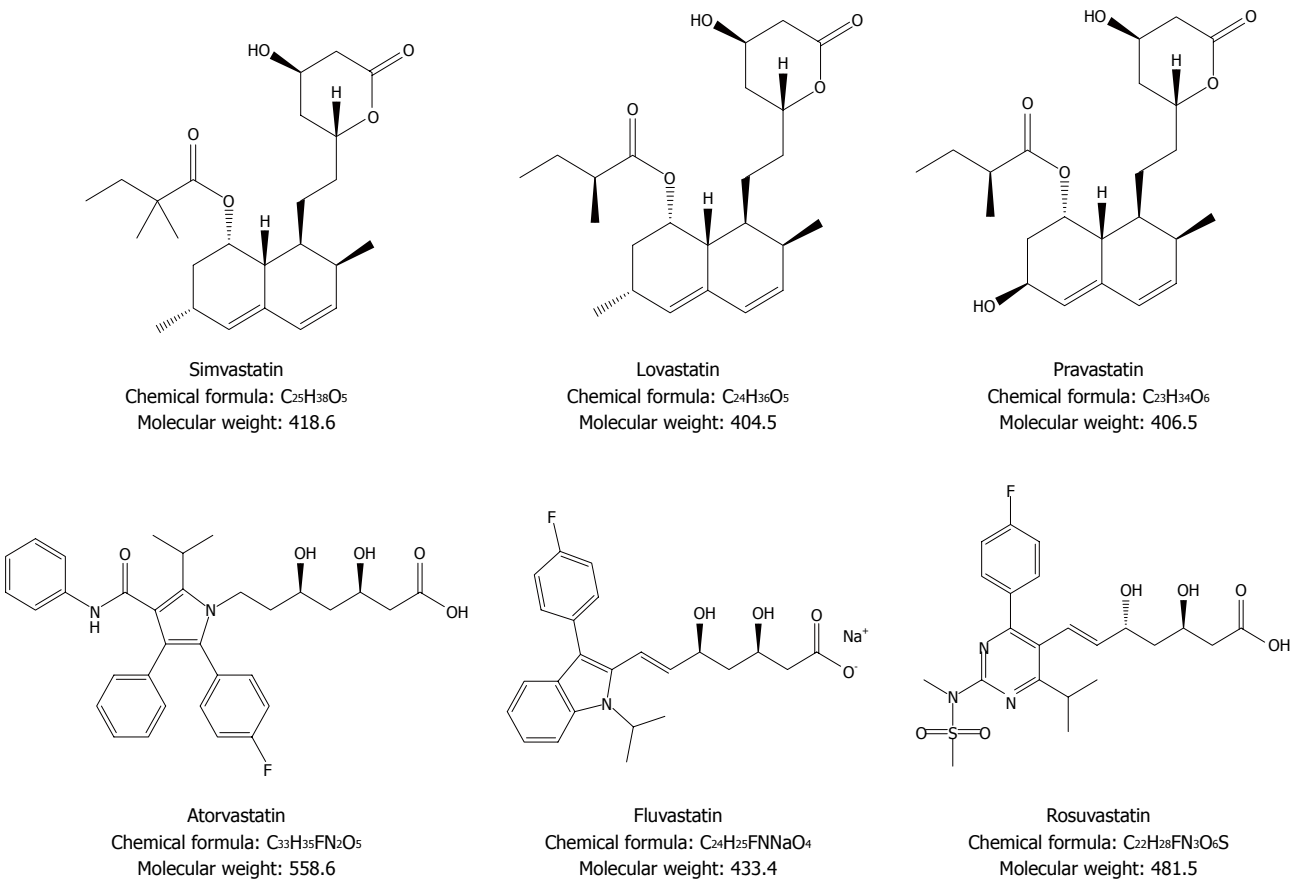

Figure 4 Chemical formula of different statins used to inhibit hepatic cholesterol synthesis[119-122].

eter of less than 5 mm has been described after 6 mo of UDCA administration in about 90% of cases[103]. The chance of dissolution is significantly lower (less than 40%-50% after 1 year of the treatment) in patients with larger or multiple stones[49,104].

Main limits of the dissolution therapy by oral bile acids are the possibility of gallstone recurrence (about 10% per year up to 5 years[105,106]) and the risk of appearance of a surface calcification on cholesterol gallstones during bile acid therapy in about 10% of cases[107]. A recurrence rate of 30%-50% at 5 years is seen after bile acid therapy or lithotripsy[94,108-110], particularly in patients with multiple gallstones[109]. After gallstone disappearance, the persistence of the same pathogenetic factors inducing gallstone formation is principally responsible for their recurrence[1]. It has to be underlined, however, that recurrent gallstones respond well to a re-treatment[99,111].

Although limited to a relatively small subgroup of patients, the dissolution therapy with UDCA or TUDCA still remains at present an interesting tool in patients who form gallstones as a consequence of transient and non-genetic risk factors (i.e., pregnancy, convalescence from abdominal surgery, obese patients during rapid weight loss[1,112-114], Table 1) and, thus, have a minimum risk of recurrence. Early non-randomized or placebo-controlled studies[115-117] suggested that UDCA might also reduce the risk of biliary colic. A large randomized, double-blind, placebo-controlled trial on the effects of UDCA in highly symptomatic gallstone patients scheduled for cholecystectomy, however, found that UDCA was ineffective on biliary colic. In fact, the likelihood of remaining colic-free is comparable in patients with strong or weak baseline gallbladder contraction as determined by ultrasonography after a standard mixed meal[118].

CHOLESTEROL LOWERING AGENTS

Bile supersaturation with cholesterol is a key factor for cholesterol gallstone formation, and it is principally related to a sustained hepatic hypersecretion of cholesterol depending on the source; from hepatic cholesterol biosynthesis, intestinal cholesterol absorption and HDL-derived cholesterol[18]. As a consequence, all drugs targeting these steps are potentially able to influence both cholesterol gallstone formation and dissolution. Statins and ezetimibe have interesting effects.

Statins are competitive inhibitors of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, a rate-limiting enzyme for cholesterol biosynthesis, and they are able to reduce biliary cholesterol independently of their ability to suppress hepatic cholesterol synthesis[119-122]. Several statins are being used (Figure 4) and their pharmacological properties modulate cholesterol homeostasis both in bile and in the liver, potentially leading to a reduction of

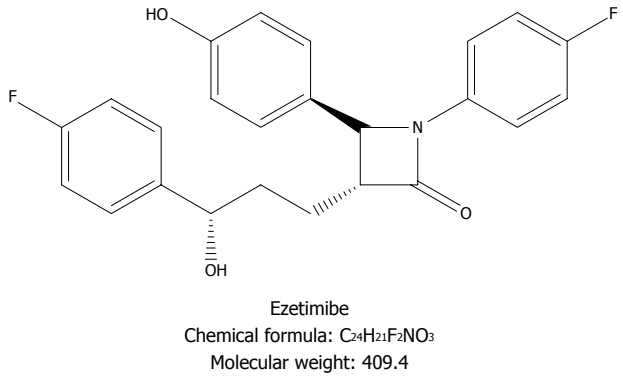

Figure 5 Chemical formula of ezetimibe, the specific inhibitor of the Niemann-Pick C1-like 1 protein.

cholesterol gallstone formation[123-125], as clearly demonstrated by animal studies[126,127]. In humans, by contrast, the potential beneficial effects of statins on cholesterol gallstones are not so clear.

The risk of cholecystectomy decreased slightly in a cohort of US women self-reporting long-term use of statins[128]. Similar results were suggested by a case-control analysis using the UK-based General Practice Research Database and evaluating incident patients between 1994 and 2004. In this study the long-term use of statins (1 to 1.5 years) was associated with a decreased risk of gallstones followed by cholecystectomy, compared with patients without statin use[129]. Furthermore, a recent population-based control study using medical databases from northern Denmark showed a decreased odds ratio for gallstone disease in current statin users (1-2 years of statin use), as compared with nonusers[130]. However, experimental studies show controversial results, since a decreased biliary cholesterol concentration, a reduced gallstone formation, or gallstone dissolution has been found by some[131-134] but not all studies[124,135-138].

Recent advances underscore the role of intestinal factors as a key factor for cholesterol absorption, biliary secretion and cholesterol gallstones[1,139]. In fact, it has been found experimentally that if dietary cholesterol is absent, all biliary cholesterol derives mainly from a limited *de novo* synthesis (less than 15%). Thus, the small intestine must be seen as a unique organ providing dietary and reabsorbed biliary cholesterol to the body[139]. This step plays a crucial role in cholesterol gallstone pathogenesis, since animal studies demonstrate that there is a significant positive correlation between the efficiency of intestinal cholesterol absorption and the prevalence of cholesterol gallstone formation[21].

Ezetimibe, in this respect, is an interesting drug since it has novel hypocholesterolemic effect[140] (Figure 5). Ezetimibe has a strong inhibitory effect on intestinal cholesterol absorption; cholesterol is indeed the most effective substrate of the NPC1L1 protein, the protein that governs intestinal absorption of cholesterol by recycling between the endocytic recycling compartment and plasma membrane[141]. NPC1L1 is highly expressed in the small intestine and localized along the brush border in both humans and mice[142,143], but also present in the human liver[143,144]. In mice, ezetimibe largely reduces cholesterol, and to some extent phospholipid content, but not the bile acid content in gallbladder bile. However, all crystallization pathways and phase boundaries on the bile phase diagram are essentially similar, regardless of whether animals are treated with or without ezetimibe[36]. By inhibiting both the cholesterol absorption in the intestine and the hepatic uptake of chylomicron remnants, ezetimibe might lower biliary cholesterol secretion and saturation[145]. Furthermore, it has been also demonstrated that increasing doses of ezetimibe lead the relative lipid composition of gallbladder bile to a progressive shift down and to the left of the phase diagram, which goes into the one-phase (protective) micellar zone, with an abundance of unsaturated micelles but never solid cholesterol crystals or liquid crystals. As a consequence, in gallbladder bile the micellar cholesterol solubility is increased, with more cholesterol molecules transferred from the cholesterol monohydrate surface into unsaturated micelles. In this environment, gallstones are reduced in size and can be completely dissolved[36,146]. Ezetimibe might therefore act as a new tool in treating/preventing cholesterol gallstones[147] but also induce amelioration of gallbladder motility, as a consequence of bile desaturation[36]. Ezetimibe is also effective in humans, since it has been demonstrated in a Mexican population that this drug in a dosage of 20 mg po/d for 1 mo, is able to significantly reduce cholesterol saturation and cholesterol saturation index and to retard cholesterol crystallization in gallstone patients[36].

In the near future, well designed experimental studies might confirm the efficacy of statins and ezetimibe, alone and/or in association with hydrophilic bile acids, in symptomatic patients without genetic risk of gallstone formation but in the presence of several predisposing conditions (Table 1). Obesity, in particular, is associated with an increased cholesterol biosynthesis in the liver, mostly due to higher levels of HMG-CoA reductase activity. Thus, in obese patients, the administration of statin might be potentially useful to prevent gallstone formation[148]. It may be also useful in patients with rapid weight loss, a condition characterized by an increased hepatic secretion of biliary cholesterol, an increase in mucin production by the gallbladder epithelium, and a significant impairment of gallbladder motility[149].

AGONISTS AND ANTAGONISTS OF NRS

Multiple physiological, developmental, and toxicological processes in the body are regulated by sets of genes, which are coordinated and activated by ligand-activated transcription factors, the NRs[150]. Lipid sensing NRs drive lipid homeostasis in the hepatobiliary and gastrointestinal systems. A key function is exerted by the oxysterol receptor liver X receptor (LXR) and by the bile acid receptor farnesoid X receptor (FXR); both are involved in the

molecular regulation of hepatic and biliary lipid metabolism, and modulate bile flow and cholesterol gallstone formation. LXR acts as the intracellular "sensor" of cholesterol[151], while FXR is the intracellular sensor of bile acids[152,153]. To maintain lipid homeostasis, cells synthesize oxysterols under conditions of cholesterol overload, and oxysterols, in turn, bind and activate LXR, which acts to reduce the systemic cholesterol burden[154]. In the enterohepatic system, FXR highly determines expression levels of genes involved in the maintenance of cholesterol, bile acid and triglyceride homeostasis[155].

FXR also up-regulates hepatic expression of bile acid and lipid transporters on the canalicular membrane of hepatocytes and increases activity of regulatory enzymes responsible for bile acid detoxification. These biochemical properties characterize FXR as a potential suitable target for drugs to be employed in the treatment of both cholestasis and cholelithiasis[156]. Animal studies confirmed a direct role of LXR and FXR in the processes leading to cholesterol precipitation in bile. FXR-null mice are prone to cholesterol gallstone formation, while the activation of FXR *via* specific synthetic ligands such as GW4064 restores a normal homeostasis between cholesterol, bile acids and phospholipids in bile[157]. This mechanism depends on FXR-induced activity of the energy-dependent ATP-Binding Cassette (ABC) transporters ABCB11 for bile acids and ABCB4 for phospholipid[158] and it is linked to a better cholesterol solubilization in bile, thus preventing the formation of cholesterol crystals and gallstones. The activation of FXR promotes an increase in cholesterol secretion by a direct up-regulation of the main hepatocyte canalicular transporters (ABCG5 and ABCG8) leading to increased biliary cholesterol saturation and precipitation of cholesterol crystals, gallstone formation and growth[159]. Such innovative and intriguing results from animal studies have not been confirmed in humans, so far. Future studies are required to assess the usefulness and safety of synthetic, liver-specific FXR agonists and LXR antagonists in humans, not only targeting gallstone disease but also type Ⅱ diabetes, dyslipidaemia and several cancers[35].

CONCLUSION

The gold standard for treating symptomatic gallstones remains laparoscopic cholecystectomy. Oral litholysis (basically restricted to few oral hydrophilic bile acids) has a limited role in a scant subgroup of selected patients with symptomatic cholesterol gallstones, but is complicated by the high rate of gallstone recurrence after dissolution treatment and a negative cost-benefit balance. As a consequence of novel and recent animal and human studies, the research agenda in the field of non-surgical therapy of cholesterol cholelithiasis is filled with several possibilities. Drugs affecting cholesterol synthesis and intestinal absorption (i.e., statins, ezetimibe) and agonists/antagonists of the NRs FXR/LXR involved in biliary lipid secretion may offer, in the near future, promising agents to treat cholesterol gallstones or to prevent their formation in populations at risk.

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TOPIC HIGHLIGHT

Angelo Zullo, MD, *Series Editor*

'Les liaisons dangereuses': Hepatitis C, Rituximab and B-cell non-Hodgkin's lymphomas

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Abstract

Rituximab has provided a revolutionary contribution to the treatment of B-cell non-Hodgkin's lymphomas (NHL). A high prevalence of hepatitis C virus (HCV) infection has been described in B-cell NHL patients. Cases of liver dysfunction in HCV-positive patients have been reported with Rituximab-containing regimens. In this paper we review the recent data regarding the effects of Rituximab in NHL patients with HCV infection. We also added a section devoted to improving communication between oncohaematologists and hepatologists. Furthermore, we propose a common methodological ground to study hepatic toxicity emerging during chemotherapy.

Key words: Rituximab; B-cell non-Hodgkin's lymphoma; Hepatitis C virus; Immunochemotherapy; Methodology

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INTRODUCTION

Rituximab was the first monoclonal antibody approved by the Food and Drugs Administration (November 1997) for the treatment of a human neoplasia: CD20-positive B-cell non-Hodgkin's lymphoma (NHL)[1]. NHL is the most common haematological cancer in adults, and approximately 85% of NHL in adults are of B cell origin.

Hepatitis C virus (HCV) infection is highly prevalent among B-cell NHL patients as compared to controls (15% *vs* 1.5%)[2,3]. Epidemiological studies and meta-analyses indicate that HCV-positive patients have a 2.5-fold increased risk to develop NHL than HCV-negative controls[4]. This figure is highly suggestive of a causative role for HCV infection in the outbreak of lymphomas. The number of B cell NHL attributable to HCV infection varies greatly by country, but can be as high as 10% in highly endemic areas[4,5]. The relative risk of lymphoma development in HCV-positive individuals is similarly increased for all major NHL subtypes and sites of presentation[6]. It has also been shown that HCV-infected patients on inter-

feron therapy who reach a sustained viral response have an hazard ratio of lymphomagenesis significantly lower than untreated patients[7]. Several biological mechanisms linking HCV infection with lymphoma development have been proposed and are still under debate[7,8].

Rituximab is highly selective against CD20+ NHL cells with limited toxic side effects. Nevertheless, untoward reactions have been reported and recently reviewed[1,9]. The reactivation of viral infections is an important adverse event associated with Rituximab administered alone or in combination with chemotherapy (R-CHT). The occurrence of acute hepatitis and even death[10] due to hepatitis B virus (HBV) reactivations in NHL patients treated with R-CHT has been reported since the introduction of Rituximab. Currently these patients are prophylaxed/treated with nucleot(s)ide analogues[10,11]. Data describing the possible role of Rituximab or R-CHT in inducing hepatic toxicity (HT) in HCV-positive B-cell NHL patients have became available only recently.

In this paper we will review the available data regarding this issue and the proposed mechanisms of liver impairment. We also added a section committed to improving communication between oncohaematologists and hepatologists. Furthermore, we propose the basis for a common methodological ground to approach the study of HT emerging during chemotherapy.

SEARCH STRATEGY, STUDY SELECTION CRITERIA AND DATA EXTRACTION

A computerized literature search of MEDLINE was performed using the following search terms: (HCV) AND (Rituximab) AND (Lymphoma), considering English-written literature only. To identify additional studies, the bibliographies of the identified papers were searched for further relevant articles. From the review process were excluded all those studies that (1) described patients not affected with B-cell NHL; (2) from which it was impossible to extract the exact number of NHL patients and/or of HCV-positive patients from heterogeneous series; (3) from which it was not possible to confidently attribute the grade of HT to a specific patient or to a disease group; (4) did not clearly presented the data on HT; and (5) were reported only in abstract form.

STUDIES

Pre-Rituximab era

In the pre-Rituximab era, a few full papers addressed the issue of HCV-positive status as a potential risk factor for the development of liver-related side effects in NHL patients receiving chemotherapy treatments.

In two studies from the far-east, incidence of moderate-severe HT occurred in 18% of HCV-positive B-cell NHL patients treated with standard chemotherapy[12,13]. Conversely, from the available data in the HCV-negative group the incidence of moderate-severe HT ranged from 0% to 14%[12-14]. According to the Takai paper, this difference was not statistically significant[13].

Contrasting data were obtained in a French study conducted on a large population of patients with diffuse large B-cell NHL (DLBCL). Chemotherapy induced the emergence of moderate-severe HT events in 12/23 HCV-positive patients[15]. This percentage (52%) was larger than that observed previously[16], and in addition HCV-positive status was shown to have a negative effect on overall survival (OS, $P = 0.02$), but not on event-free survival. Development of HT determined treatment modification in 47% of HCV positive patients. However, in this study more aggressive chemotherapy regimens were admittedly adopted. No association between initial severity of hepatic disease and subsequent development of HT was described. In the papers previously examined, data on HCV-RNA trends were not systematically collected or reported, thus hampering the identification of an unambiguous relationship between HCV replicative activity and HT development.

Thus, in the pre-Rituximab era no clear indications had emerged to define the HCV-positive B-cell NHL a group at higher risk of developing HT as the result of standard chemotherapy. The occurrence of severe HT was considered to be so rare as to deserve the publication of case reports[17].

Into the Rituximab era

In the early B-cell NHL Rituximab trials, HCV-positive status was not an exclusion criteria for treatment, and no HT[18], or only mild elevation of liver enzymes had been described[15]. In addition, the trials did not report the HCV-status of the patients. At any rate, no data had emerged suggesting a possible role for HCV-positive status as a risk factor for HT during Rituximab treatment.

Initial experiences with R-CHT in HCV-positive B-cell NHL were published at the beginning of the new millennium. Emergence of HT was rarely reported in earlier case reports or case series, and HCV-RNA levels, not consistently determined, did not show uniform trends as the result of Rituximab treatment[19-26]. A large retrospective study performed in Italy on a group of consecutive HCV-positive patients with DLBCL, showed that only 15% (20/132) of patients developed moderate-severe HT, reconfirming the low incidence reported in previous studies[25]. Occurrence of HT determined a modification of the scheduled therapy: dose reduction/prolongation of intervals between chemotherapy cycles in case of grade 2-3 HT (11%), treatment interruption in the most severe cases (4%, grade 3-4 HT). In this study Rituximab was combined with standard chemotherapy protocols in 26.5% of patients (35/132). None of the Rituximab-treated patients developed moderate-severe HT, and only five developed a mild liver enzymes increase not requiring treatment discontinuation. No data on HCV-RNA trends were provided. Progression free survival (PFS) and OS at 5 years were respectively 51% and 72%. Unfortunately, lack of a control group did not allow for comparison of

clinical outcome with HCV-negative patients. However, results suggested that Rituximab might be combined safely with chemotherapy in HCV-positive NHL patients, but careful monitoring of liver function and viremia was recommended[27].

In 2008, Ennishi published an interesting paper dealing with HCV-positive B-cell NHL patients from Japan treated with Rituximab; HCV genotype was characterized, and HCV-RNA was tested monthly during and after chemotherapy[28]. Serum transaminases, albumin and total bilirubin were also monitored. Five of the six anti-HCV antibody-positive patients were HCV-RNA positive at baseline. An increase of HCV-RNA (ranging 0.73-1.06 $\log_{10}$) during treatment was observed in all these 5 patients. However, only one patient, genotype 2a, developed severe HT. In this patient HCV-RNA decreased below the limit of detection at the time of maximal serum transaminases peak, to increase again after R-CHT was stopped. The authors were, however, unable to explain this phenomenon and why in the remaining 4 patients, despite an increase of HCV-RNA during Rituximab treatment, there was no evidence of HT[28]. Recently, a letter from Italy also suggested that genotype 2 might represent a specific risk factor for the development of HT in NHL patients treated with Rituximab-containing regimens[29]. However, data were limited and it was not clear if pre-treatment HCV-RNA were available for all patients, and not all patients developing an increase in HCV-RNA developed HT.

In 2010, another retrospective study from Italy reported the data on 160 HCV-positive patients with NHL[30]. In this paper HCV-positive patients were carefully studied for liver and haematological disease status, and chemotherapy treatment used. The common terminology criteria for adverse events (CTCAE) were used to define HT, with a non-standardized adaptation for patients with elevated transaminases at baseline[31]. HCV genotype was available in 60 of the 146 HCV-RNA positive patients (41%). Significant HT occurred in 24 patients (15%), and 8 (5%) did not complete the planned treatment because of it. Five (18%) of the 28 patients treated with R-CHT developed HT: 3 stopped therapy, while the other 2 had to postpone it. Nine of 132 (7%) patients treated with Rituximab-free regimens developed HT. Thus, even if barely missed, HT incidence was however not significantly different between the two groups ($P = 0.07$), even if limitations due to sample size cannot be excluded. HCV-RNA quantification did not correlate with ALT levels, and was thus defined not useful to predict the occurrence of HT. Severe HT developed more frequently in genotype 1 patients (26%), than among genotype 2 (3%), with 85% of moderate-severe HT events developing in the former group ($P = 0.02$). Maximum increase of HCV-RNA over the baseline levels was more frequently reported among genotype 1 patients than genotype 2 ($P = 0.05$). Five years OS was significantly lower in patients who had developed significant HT (62% *vs* 84%, $P = 0.006$). Also median PFS was shorter for patients developing HT (2 years *vs* 3.7 years, $P = 0.03$). However, lymphoma relapse and progression were related to a previous episode of significant HT only in aggressive NHL subtypes ($P = 0.01$). The authors concluded that Rituximab use is related to a slightly higher occurrence of toxicity that does not circumvent its use in HCV-positive NHL. They however underlined that occurrence of HT in HCV-positive NHL patients caused a significant limitation in the delivery of an effective immunochemotherapy[31].

In 2010, we published a retrospective study on a group of Italian patients with CD20-positive, B-cell NHL treated with Rituximab-CHT[32]. Nine patients (8.6%) were HCV positive and viremic at baseline. Two were also HBsAg-positive but HBV-DNA-negative at baseline and received appropriate prophylaxis[10], remaining HBV-DNA negative thereafter. Three (33%) of the 9 HCV-positive patients and none of the 95 negative developed HT ($P < 0.001$). All had normal ALT before treatment. In two, ALT peak developed approximately 5 mo after the end of treatment. One of them, also in this case a genotype 2a, developed icteric hepatitis (total bilirubin 7.8 mg/dL) without relevant prothrombin time alteration during the acute phase. The remaining patient developed HT while on treatment, but chemotherapy was not stopped, and he completed the full course of treatment. In the patients developing HT, HCV-RNA did not follow the ALT trend, with two patients showing increases and one a decrease over baseline. No significant correlation was detected between ALT and HCV-RNA levels before, during, and 12 mo after HT development. Only one patient had advanced liver fibrosis. At the 12-mo follow-up, no liver-related death or complication had developed in HCV-positive patients developing HT, ALT had decreased but not regressed to normal, and patients were alive and in remission for their haematological disease. We concluded proposing HCV-positive status as a risk factor for the development of HT in B-cell NHL patients receiving Rituximab-containing regimens[32].

By the end of 2010, Ennishi published the results of the currently largest, multicenter retrospective study on HT and the prognosis of patients with DLBCL treated with Rituximab-containing regimens[33]. They analyzed 553 patients: 131 HCV-positive and 422 HCV-negative. HCV-positive patients were significantly older, had more advanced disease (higher international prognostic index, > 1 extranodal site and spleen involvement) than those HCV-negative. Thirty-six (27%) HCV-positive and 13 (3%) HCV-negative patients developed severe HT ($P < 0.0001$). HT determined dose modification in 12% and chemotherapy withdrawal in 4.6% of HCV-positive patients. Six HCV-positive patients died of hepatic failure, caused by hepatocellular carcinoma in four. Similar to our results, HCV infection was confirmed as a significant risk factor for the development of severe HT at multivariate analysis (hazard ratio: 14.72, 95% CI: 6.37-34.03, $P < 0.001$). Increased pre-treatment transaminases levels were predictive for the development of severe HT. However advanced haematological disease stage, and not HCV-

positive status or development of HT, affected negatively PFS and OS. Dose delays and liver failure associated with severe HT development were the likely explanations for the non-significant trend toward the reduced late survival (> 2 years) observed after therapy ($P = 0.07$)[34], and it could be speculated that if a larger sample size would have been available, this difference in late survival among HCV-positive patients might have been significant. HCV-RNA levels (collected before, during treatment, 1 mo after treatment and 2-6 mo after having stopped treatment) increased significantly during immunochemotherapy ($P = 0.006$). However, complete data at all the 3 time points were available only for 26% of patients, and median HCV-RNA increase on treatment was of 0.5 $\log_{10}$ only. It was also not clear if the HCV-RNA increase did at all correlate with ALT levels and HT events. No data on genotypes were available[35,36]. Ennishi's data are indeed stimulating, but to clarify the possible role of viremia in determining liver injury, further studies are needed to prospectively evaluate viral load variations and parameters of liver damage in Rituximab-treated patients developing and not developing HF before, during and after therapy.

POSSIBLE MECHANISMS OF HT DEVELOPMENT IN B-CELL NHL PATIENTS TREATED WITH RITUXIMAB-CONTAINING REGIMENS

Some insights on HT development in B-cell NHL patients treated with Rituximab can be derived from the literature. The spontaneous occurrence of hepatitis flares in HCV patients have been described. Rumi *et al*[37] observed that in a group of HCV patients, genotype 2c and 1b, followed for 71 mo, the incidence of hepatitis flare was more frequent among the former group as compared to the latter (31% *vs* 7.5%), and that these episodes correlated with fibrosis progression[37]. Such observations may suggest that a similar phenomenon could be occurring in HCV-positive NHL patients, a speculation further supported by papers suggestive of a specific role for genotype 2 in determining the development of HT in these patients[24,29,32]. However, spontaneous fluctuations of HCV-RNA in chronic infection, usually of limited magnitude (maximum 1 $\log_{10}$), have been described[38]. Interestingly, when we transformed to $\log_{10}$ the available HCV-RNA data from the above listed studies, the delta over basal differences observed were mostly comprised within this range or slightly over it (data not shown). However, the data by Rumi were derived from immunocompetent HCV-infected patients, and thus differences in study design and population do not allow either for direct comparison or to conclude that HT occurring in Rituximab-treated patients are part of the natural history of HCV infection. As far as genotype is concerned, more data are needed to establish a major role for genotype 2 in determining HT in Rituximab-treated HCV-positive NHL patients.

It is a widely accepted concept that immunosuppressive treatment might determine an increase in hepatitis virus replication, leading to an expansion of infected hepatocytes. Following treatment interruption and immunoreconstitution infected hepatocytes are lysed. This mechanism holds true for hepatitis B infection[39,40], but has not yet been demonstrated for HCV infection. Since these viruses markedly differ in their virological characteristics and in their immune escape and survival strategies[41], these hypotheses need to be tested and verified. For instance, after years in which it was widely believed that glucocorticosteroids enhanced HCV-RNA replication, it has instead been recently demonstrated *in vitro* that these drugs actually reduce it, increasing instead HCV entry into hepatocytes[42]. Similar data on Rituximab are however not available.

According to another widely cited letter, Rituximab treatment could affect viral replication inducing a decrease of immunoglobulin of the M class[43]. In chronic infection, HCV circulates usually bound to IgM, so its decrease, secondary to Rituximab treatment, might determine a loss of immune control over the virus allowing for an increase of HCV-RNA. Again, HCV-RNA increase has not been clearly associated with the development of HT. In addition, this report regarded a patient with HCV-related cryoglobulinemia, and modifications of viral load were determined during concomitant treatment with pegylated interferon, further hampering data interpretation.

Direct drug-related liver toxicity[44], or development of Rituximab-immunomediated phenomena[9,45,46] should also be considered as possible alternative causative mechanisms. Rituximab might induce HT per se, with HCV infection representing a risk factor for its development, as reported for antiretroviral drugs[47].

Even if intriguing and reasonable, the currently available data do not provide evidence to support any of the above speculations.

EVALUATION OF HT

In order to supply a common background between oncohaematologists and hepatologists, to better communicate and manage our patients, we felt it opportune to develop a section commited to the evaluation of hepatotoxicity.

BASELINE EVALUATION

When performing baseline evaluation of oncohematological patients scheduled to undergo chemotherapy, a search for liver disease and viral hepatitis infection is recommended. Patient evaluation and history-taking should address relevant issues such as previous history/diagnosis of liver disease, drug or alcohol abuse, previous blood transfusion, use of prescription and non-prescription drugs, herbal remedies[48]. Signs and symptoms of liver disease should also be searched for.

Table 1 Clinical significance of commonly performed biochemical liver tests

Liver test	Clinical significance
AST, ALT	Cytolysis
Total bilirubin, γGT, ALP	Cholestasis
Prothrombin time/INR, Albumin, pCHE	Synthesis
Decreased platelet number	Portal hypertension

AST: Aspartate-aminotransferase; ALT: Alanine aminotransferase; γGT: γ-glutamyl transpeptidase; ALP: Alkaline phosphatase; INR: International normalized ratio; pCHE: Pseudo-cholinesterase.

Successively, evaluation of serum enzymes, even with its well known limitations[49,50], is commonly employed. Using these tests we tentatively explore and categorize the possible presence of signs of liver dysfunction. A list of the most commonly performed biochemical liver function tests and the function they explore/express is provided in Table 1.

Presence of chronic infection with hepatitis viruses should be investigated by testing for viral serum markers: antibodies to HCV, hepatitis B surface antigen, and hepatitis B core antigen, and hepatitis B surface antigen. HBV-DNA testing is performed to differentiate active from inactive carriers. In the case of hepatitis B, management guidelines for oncohaematological, and immunosuppressed patients have been proposed[10], while no defined strategies have been provided for HCV-positive oncohaematological patients. Patients positive for HCV-antibodies undergo qualitative HCV-RNA testing to verify the presence of active infection. Order of magnitude of viral replication and genotyping are not clinically relevant, unless an antiviral treatment is scheduled[38]. Routinely testing HCV-RNA levels during oncohematological treatment remains a debated issue, and considering its cost, it should preferably be done in a controlled clinical study setting until relevant data can support its use in clinical practice.

Imaging studies are part of the baseline staging of haematological disease. These can also provide important information to detect signs of underlying cirrhotic liver disease. A list of the information provided by liver ultrasound, the most commonly performed imaging test, is summarized in Table 2. Similar information can also be provided by computed tomography and magnetic resonance.

Hepatic biopsy, with its intrinsic limitations and risks[51], is still regarded as the gold standard to define the extent of liver damage. It can provide additional information, such as hepatic involvement secondary to the haematological disease, and is used to confirm the presence of cirrhosis, but it is rarely performed in this setting given the priorities and reduced times imposed by the need of treatment typical of oncohematological diseases.

Transient eleastography (Fibroscan®) is useful to determinate non-invasively and with sufficient accuracy the presence/absence of liver cirrhosis in HCV patients[52]. However its use in oncohematological patients has never been studied in detail.

Table 2 Liver ultrasound: information for the management of liver diseases

Parenchymal signs	
Dimensions	Hepatomegaly
	Caudate lobe hypertrophy
	Quadrate lobe hypotrophy
Echo pattern	Coarse: typical of liver fibrosis
	Coarse nodular: micronodular cirrhosis
	Attenuation sign/bright liver: typical of hepatic steatosis
Nodules	Benign *vs* malignant lesions
	Skip areas (fatty liver)
Surface	Nodular *vs* smooth
Extraparenchymal signs	
Indirect signs of portal hypertension	Splenomegaly
	Ascites
	Collateral vessels
	Lack of splenic and/or superior mesenteric vein diameter variations during breathing
	Increased portal vein diameter
Doppler ultrasound	Inversion/reduction of portal vein flow
	Portal vein thrombosis/cavernomatosis

If cirrhosis has been diagnosed, its severity should be defined. Several scoring systems have been validated, and the most used are the Child-Pugh-Turcotte (CPT), and the model of end stage liver disease[53-55]. The former is based on the evaluation of 3 biochemical, i.e., albumin, bilirubin and prothrombin time/international normalized ratio (PT and INR respectively), and two clinical variables (ascites and portal-systemic encephalopathy), while the latter also requires creatinine levels in addition to bilirubin and INR. Even if limitations for chemotherapy administration are suggested only for CPT stage "C" cirrhotic patients[50], stratification by grade of impaired liver function may provide an additional tool to estimate disease burden at baseline and during follow up.

ON-TREATMENT MONITORING

Patients undergoing chemotherapy are followed up to estimate the effects of treatment on disease course. Physical examination, laboratory tests, and imaging studies concur to the early detection of side effects. However, not only chemotherapy can cause HT. In case of alterations to liver biochemistry, use of drugs other than chemotherapy, especially antibiotics, novel hepatitis virus infection or reactivation, and possible liver involvement by haematological disease progression should be considered and ruled out.

In case HT is caused by chemotherapy, events should be described and categorized by standardized grading systems. Oncohaematologists usually adopt the US National Cancer Institute CTCAE[49,50]. Toxic effects grades are scored 1 to 5. The CTCAE also provides descriptors for definite hepatic events (i.e., liver dysfunction, viral hepatitis), but these definitions are composite, more complex and not easy to adopt. By definition, when serum transaminases [aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT), respectively], alkaline phospatase, or γ-glutamyl transpeptidase increase > 5-20

Table 3 Patterns of drug induced liver disease according to laboratory tests

Pattern	ALT	ALP	ALT/ALP
Hepatitis pattern	⩾ 3 ULN	--	⩾ 5 ULN
Cholestatic pattern	--	⩾ 2 ULN	⩽ 2 ULN
Mixed pattern	> 3 ULN	> 2 ULN	> 2 ULN to < 5 ULN

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ULN: Upper limit of normal. Adapted from Verma *et al*[56].

Table 4 Mild, moderate and marked elevations of serum liver enzymes

Enzyme	Mild	Moderate	Marked
sAT	> 2-3	2, 3-20	> 20
ALP	< 1.5.2	1.5-5	> 5
γGT	> 2-3	2-3-10	> 10

Numbers are times (X) upper limit of normal value. sAT: Serum aminotransferases; ALP: Alkaline phosphatase; γGT: γ-glutamiltranspeptidase. Adapted from Ahmed *et al*[57].

× upper normal level (ULN), the adverse event is defined as grade 3 and categorized as "severe", while increases > 20 × ULN are grade 4 and are defined as "life threatening or disabling". It is thus a descriptive terminology trying to express a clinical interpretation with a numeric grade. However, sometimes patients with these toxicities can be asymptomatic.

In a more hepatological perspective, severity of HT is commonly derived from the biochemical pattern determined by drug exposure (Table 3)[56]. Patients with the hepatocellular pattern can be asymptomatic or report fatigue and right upper quadrant pain. Serum transaminases increment can be variable, but all patients with clinical or laboratory evidence of moderate/severe acute hepatitis (Table 4)[57] should have immediate measurement of PT/INR, serum bilirubin and careful evaluation for subtle alterations in mentation to exclude the presence of acute liver failure (ALF)[58]. While the degree to which transaminases are elevated does not adequately mirror liver impairment, jaundice instead represents a good predictor of mortality in drug-related liver injury. Bilirubin persistently > 3 × ULN (biliary obstruction and Gilbert's syndrome having been ruled out), is burdened with a 10% mortality (range, 5%-50%)[56]. When INR ⩾ 1.5), and there is evidence of an altered sensorium, the diagnosis of ALF is established. Hospital admission in this setting is mandatory[58]. Extrapolating these data to the oncology setting is difficult, but as a general rule any drug associated with increase of serum AST/ALT > 3 × ULN should be stopped if jaundice has developed[59].

The cholestatic pattern can mimic biliary obstruction or the course can be more indolent with jaundice and pruritus. Mortality appears to be less than in the hepatocellular pattern (1%-7.8%), and death is usually not liver-related[56]. The mixed pattern has probably the lowest mortality (around 2%)[60,61].

CONCLUSION

HCV-positive status seems to represent a risk factor for the development of HT in patients with B-cell NHL treated with Rituximab. The degree of possible HT is variable ranging from moderate to severe. However, larger prospective studies, designed with a strong methodological basis and using standard descriptive terminologies for HT are warranted. These studies should properly define hepatological events and are needed to clarify the causative relationship, and to uncover toxicity mechanisms. Until then, HCV-positive patients receiving Rituximab should be carefully followed up to rapidly detect and properly manage the possible development of liver-related side-effects.

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Events Calendar 2012

January 13-15, 2012
Asian Pacific Helicobacter pylori Meeting 2012
Kuala Lumpur, Malaysia

January 19-21, 2012
American Society of Clinical Oncology 2012 Gastrointestinal Cancers Symposium
San Francisco, CA, United States

January 20-21, 2012
American Gastroenterological Association Clinical Congress of Gastroenterology and Hepatology
Miami Beach, FL, United States

January 26-27, 2012
2nd Annual Pediatric Pharmacology Conference
Philadelphia, PA, United States

February 12-15, 2012
4th International Conference on Drug Discovery and Therapy
Dubai, United Arab Emirates

February 23, 2012
Management of Barretts Oesophagus: Everything you need to know
Cambridge, United Kingdom

March 8-9, 2012
British Pharmacological Society Focused Meeting - Challenges in Neurotherapeutics: From Animal Models to Clinical Needs
Dublin, Ireland

March 14-16, 2012
85th Annual Meeting of the Japanese Pharmacological Society
Kyoto, Japan

March 14-17, 2012
Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics
Washington DC, United States

March 19-21, 2012
The Biomedical Basis of Elite Performance: A joint meeting of the British Pharmacological Society and the British Physiological Society
London, United Kingdom

March 20-22, 2012
78th Annual Meeting of the German Society for Experimental and Clinical Pharmacology and Toxicology Association
Dresden, Germany

March 26-27, 2012
26th Annual New Treatments in Chronic Liver Disease
San Diego, CA, United States

March 31-April 1, 2012
27th Annual New Treatments in Chronic Liver Disease
San Diego, CA, United States

April 4-6, 2012
7th P2T Congress Organized by the French Society of Pharmacology and Therapeutics
Dijon, France

April 19, 2012
Spring Meeting of the Swiss Society of Pharmacology and Toxicology
Bern, Switzerland

April 21-25, 2012
Experimental Biology '12, Sponsored by the American Society for Pharmacology and Experimental Therapeutics
San Diego, CA, United States

April 23-24, 2012
4th British Pharmacological Society Meeting on Cell Signalling
Leicester, United Kingdom

May 18-19, 2012
Pancreas Club Meeting
San Diego, CA, United States

June 6-9, 2012
3rd International Congress on Pharmacology of Natural Products (Fapronatura 2012) Sponsored by the Cuban Society of Pharmacology
Topes de Collantes, Cuba

June 18-21, 2012
Pancreatic Cancer: Progress and Challenges
Lake Tahoe, NV, United States

August 22-25, 2012
27th Annual Meeting of the Federation of Societies of Experimental Biology Cosponsored by the Brazilian Society of Pharmacology and Experimental Therapeutics
Águas de lindóia, Brazil

September 10-14, 2012
8th Congress of Toxicology in Developing Countries (CTDC8) by the International Union of Toxicology
Bangkok, Thailand

September 15-16, 2012
Current Problems of Gastroenterology and Abdominal Surgery
Kiev, Ukraine

October 30 - November 2, 2012
12th ISoP Annual Meeting - New Landscapes in Pharmacovigilance
Cancun, Mexico

November 6-9, 2012
44th Brazilian Congress of Pharmacology and Experimental Therapeutics
Foz do Iguaçu, Brazil

November 15-17, 2012
IV Hospital Week of Clinical Pharmacology Organized by the Serbian Medical Association Section for Clinical Pharmacology
Belgrade, Serbia

December 1-4, 2012
Advances in Inflammatory Bowel Diseases
Hollywood, FL, United States

December 18-20, 2012
British Pharmacological Society Winter Meeting
London, United Kingdom

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GENERAL INFORMATION

World Journal of Gastrointestinal Pharmacology and Therapeutics (*World J Gastrointest Pharmacol Ther*, *WJGPT*, online ISSN 2150-5349, DOI: 10.4292), is a bimonthly, open-access (OA), peer-reviewed journal supported by an editorial board of 188 experts in gastrointestinal pharmacology and therapeutics from 36 countries

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

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The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJGPT* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article *via* online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJGPT* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJGPT* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

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The major task of *WJGPT* is to rapidly report the most recent results in basic and clinical research on gastrointestinal pharmacology & therapeutics, including the effects of drugs on the gastrointestinal, pancreatic and hepatobiliary systems, particularly with relevance to clinical practice. *WJGPT* accepts papers on the following aspects related to gastroenterology or hepatology: (1) Clinical pharmacological research articles on specific drugs, concerning with pharmacodynamics, pharmacokinetics, toxicology, clinical trial, drug reactions, drug metabolism and adverse reaction monitoring, *etc*; (2) Research progress of clinical pharmacology; (3) Introduction and evaluation of new drugs; (4) Experiences and problems in applied therapeutics; (5) Research and introductions of methodology in clinical pharmacology; and (6) Guidelines of clinical trial. Specifically, this journal welcome research and review articles associated with both Western medicine and Chinese herbs as well as their combinations in basic and clinical application.

Columns

The columns in the issues of *WJGPT* will include: The columns in the issues of *WJGPT* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Articles: To report innovative and original findings in gastrointestinal pharmacology & therapeutics; (9) Brief Articles: To briefly report the novel and innovative findings in gastrointestinal pharmacology & therapeutics; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJGPT*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastrointestinal pharmacology & therapeutics; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on the research in gastrointestinal pharmacology & therapeutics.

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Statistical review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

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In the interests of transparency and to help reviewers assess any potential bias, *WJGPT* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

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Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

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Title: Title should be less than 12 words.

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There are unstructured abstracts (no less than 256 words) and structured abstracts (no less than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no less than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…"; MATERIALS AND METHODS (no less than 140 words); RESULTS (no less than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/2150-5349/g_info_list.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

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Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as

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Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

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Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

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Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as

χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, ρ (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/2150-5349/g_info_20100315090437.htm.

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Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

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