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Iatrogenic Bile Duct Injuries Following Laparoscopic Cholecystectomy: Myth or Reality? A Recent Literature Review from 2006 to 2011

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1. Introduction

At the end of 1980’s, the introduction of miniminvasive surgery in clinical practice represented a significant achievement of science and technology research, and laparoscopic cholecystectomy (LC) is nowadays considered a gold standard in the treatment of symptomatic cholelithiasis, according to well known and acknowledged advantages. Although experience is essential to avoiding rates of morbidity in any surgical procedure, in LC the effect of the learning curve does not seem to be the most important factor in minimizing the possibility of iatrogenic bile duct injuries (IBDI) because most of them are related to anatomic misdiagnoses and lapses from basic principles of biliary surgery. IBDI are still a severe complication of biliary surgery, characterized by high morbidity and in some cases significant mortality, often due to the onset of septic complications. They cause a costs rise, related to diagnostic and therapeutic procedures, and they are often associated with distressing litigations, frustrating for surgeons. Factors that may be related to IBDI include certain pitfalls believed to be inherent in the laparoscopic approach: the two-dimensional view and the absence of tactile sensation. However, an analysis of literature until 2005 shows that the “mini-invasive” approach is related to a higher incidence of iatrogenic bile duct injuries (IBDI), both of the main and accessory bile ducts. In 2002 Nuzzo (Nuzzo, 2002), by the means of an Italian survey, proved a three times higher incidence of IBDI than in open cholecystectomy (OC), showing about 300 bile duct injuries out of every 100000 cholecystectomies per year in Italy, pointing out in that country a considerable mortality, in most of cases related to sepsis, with a significant rise of morbidity and healthcare costs ensuing from hospital stay, instrumental investigations, and medium and long-term clinical follow-up. Moreover, IBDI are reported to have late severe aftermath, causing the surgeon frustration and expensive litigation. After accomplishing a learning curve for mini-invasive approach, led by an experienced surgeon, the most significant etiopathogenetic factors of IBDI are the misidentification of the main biliary tree (BT) and wrong manoeuvres to manage bleedings. Treatment of IBDI requires a multidisciplinary approach – namely endoscopy, interventional radiology and surgery – in referral centres,
because in most cases the proper repair represents often the ultimate intervention to the patient. Whenever a surgical treatment is required – for “major” injuries – hepaticojejunostomy represents the best choice, but options are described as T-tube drainage, liver resections and even liver transplantation, confirming their potential extreme seriousness. Patients undergoing reparative interventions, following IBDI, require frequently a cholestasis evaluation by long-term clinical, biochemical and imaging follow-up (> 10 years), because of the possible onset of long term complications (anastomotic strictures, secondary biliary cirrhosis), that produce a significant growth of expenditures. Authors performed a meta-analysis of most recent reviews articles of the last five years, with the aim of assessing the real incidence of this issue during these late years.

2. Methods

A Pubmed database search was performed to identify the most recent articles from 2006 to 2011, about IBDI following cholecystectomy, using the keywords “laparoscopic cholecystectomy”, “small-incision cholecystectomy”, “open cholecystectomy”, “iatrogenic bile duct injuries”, “biliary fistulas”, “iatrogenic biliary strictures”, and “cholestasis”. Additional papers, among the most important and cited in literature, reporting incidence of BDI following LCs, were individually searched for, excluding case report articles. These data are purely descriptive and no statistical analysis was performed. We excluded “Single-Port cholecystectomy” from our research. By the means of this research, authors examined the incidence, the main risk factors, the mechanisms of bile duct injuries, the diagnostic work-up and the management, in order to provide the most recent results about this issue.

3. Classification of IBDI

At present several classifications of IBDI have been proposed with the aim of standardizing the assessment, planning treatment and evaluating the outcome. Nevertheless, none of these have been universally accepted as a standard. Some of these classifications are based on the anatomical level of the lesion (Strasberg et al., 1995), (Bismuth, 1982), (Neuhaus et al., 2000), (Csendes et al., 2001), (McMahon et al., 1995), (Siewert et al., 1994), (Frattaroli et al., 1996) and (Bergman et al., 1996) and on the kind of repair required. On the other hand, other classifications - (Bektas et al., 2007), (Lau & Lei, 2007), (Kapoor, 2008),(Stewart-Way, 2004) - also assess hilar vascular lesions associated in IBDI, which could jeopardize their management and overall morbidity. None of them include factors such as sepsis, haemodynamic status or comorbidities, which affect remarkably long-term outcome. The most commonly used is Strasberg classification (1995), which incorporates the previous classification of Bismuth (1982). The development of these two classifications kept up with the operative technique, because thanks to LC, IBDI became more complex and more proximal than in OC, so requiring an assessment both of site and type of lesion, from the transection through wrong closures, up to strictures. The authors prefer Strasberg classification not only for its feasibility to figure out the proper treatment, but also because most vascular injuries occurring in LC don’t modify the repair technique of the biliary tree. Type A injuries include cystic duct leakage or leakage from canalicula in the liver bed, and these can be treated by endoscopy (papillotomy and prosthesis). Type B consists of partial occlusion of the biliary tree, mainly caused by the closure of an aberrant right hepatic duct. Type C includes the transection without ligation of the aberrant right hepatic duct. Type D describes partial damages to a major hepatic duct. Types E
Injuries are further subdivided into E1 through E5 according to the previous Bismuth classification: E1, transection of bile duct > 2 cm from the hilum; E2, transection of bile duct < 2 cm from the hilum; E3 and E4 represent stricture at the same level or above the confluence of hepatic ducts; E5 injuries describe involvement of an aberrant right sectoral duct injury concomitant with a common hepatic duct stricture.

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Table 1. Incidence of IBDI reported in the literature from 2006 to 2011. Median incidence: 0.2% - Total amount of patients: 412585.
4. Analysis of IBDI incidence

Since its introduction over 20 years ago, population-based studies have reported a significant increase of IBDI incidence following LC, compared to open technique, ranging from 0.1% to 1.7%. About this issue, in Italy Nuzzo (Nuzzo et al., 2002) reported a three times higher incidence (0.3% vs 0.1%) compared to the conventional approach. Consequently, IBDI during LCs became an important issue in the field of surgical pathology. Although there was no evidence of superiority over open technique, soon after its introduction LC became the standard treatment of cholecystectomy (NIH Consensus Conference 1993). The rising popularity was based on assumed lower morbidity and complication proportions, and a quicker postoperative recovery compared to open technique (Sheha, 1996). However, the reviewed studies were non-randomised trials, with no fair assessment of the effects of the interventions. Authors carried out a survey of studies published from 2006 to 2011 (Table 1) (Figure 1), showing in the several surgical experiences an higher incidence during the early stage of learning, a following period of stabilisation, and an eventual decrease when the amount of LCs rose (Figure 2). In disagreement with this point of view, Giger (Giger, 2011) in a retrospective ten years survey (from 1995 to 2005) did not report a decrease of IBDI rates (0.3%), in spite of the assistance of the intraoperative cholangiography (IOC), considered useful for preventing iatrogenic injuries. However,

Fig. 1. IBDI incidence: analysis of literature from 2006 to 2011 C.I.: Confidence Interval 95%

Fig. 2. IBDI incidence: relationship with surgical experience
several studies (with no statistical evidence) hypothesize that the regular practice of critical view of safety (CVS)- a codified dissective technique showing the anatomic elements of Calot’s triangle- could cause a decrease of IBDI rates (Yamashita 2010, Yegiyants 2008, Avgerinos 2009, Sanjay 2010). Instead, Strasberg himself mentioned the lack of evidence of CVS for preventing IBDI, although surely a great step toward a safer LC, but it is unclear whether the CVS alone is sufficient as a technique to minimize the risk of IBDI. Also, by way of example, major IBDI continue to occur in the Netherlands despite increasing adoption of the CVS technique (de Reuver, 2007). Gurusamy (Gurusamy, 2010), in a meta-analysis of randomized clinical trials of LCs for acute cholecystitis, reports lower IBDI rates in early treated patients (0,5%) compared to delayed interventions (1,4%). Minor IBDI rates are also decreased according to Yüksel (Yüksel, 2006) (3% vs 4,6%). Boddy (Boddy, 2007) in a ten year series of 4139 LCs, reported lower rates of IBDI for hepatopancreaticobiliary surgeons (0,1%) in comparison to general surgery consultants (0,9%). Another controversial factor is the use of IOC: some authors recommend its routinary employment (Nickholgh, 2006 - BDI 0,09%), others (Sarli, 2006) report no disadvantages with a selective use (IBDI 0,16%). About the influence of workload on surgeons efficiency, Yaghoubian (Yaghoubian, 2008) reports a decrease from 1% to 0,4% of IBDI, after implementation of the 80-hour workweek in his hospital. Two studies examine surgeon’s experience as factor affecting IBDI incidence: Hobbs (Hobbs, 2006) reports a decrease of IBDI from 1994 to 1998 (from 0,35% to 0,13%), ascribing 1/3 of IBDI of his centre to surgeons with < 200 operations in the previous 5 years; Harrison (Harrison, 2011), after analysing a 234280 LCs series, does not report any difference (IBDI 0,24% vs 0,26%) between rates of experienced surgeons compared to residents. Concerning the surgical approach, Keus’ (Keus, 2010) Cochrane Review compares open, small-incision, and laparoscopic cholecystectomies, evaluating only randomized trials. In this study there is no statistical evidence of IBDI rates among the three approaches, with laparoscopic IBDI rates that range from 0,2% to 1,4%. However, the trials analysed in the review, statistically compelling, do not include acute cholecystitis as indication, but embrace minor injuries in IBDI rates, as self-limiting bile leakage, whereupon rates are as high as 1,4%. Intraoperative ultrasonography (IOUS) seems to be an effective tool in reducing IBDI rates (Machi, 2009 - BDI: 0%, 1381 LCs), but unfortunately there are few studies about this technique, which requires a long learning curve (according to the author, > 100 procedures) discouraging its widespread. As one can infer from Table 1, showing incidences from the total amount of 412585 LCs performed in several international centres, the median estimated incidence is 0,2%, certainly lower than those reported before 2006. Nevertheless, it’s difficult to establish if currently this value of incidence is equal to that reported in OCs, sometimes reported as low as 0,1%. In conclusion, according to authors’ analysis, it seems that during the last years IBDI incidence following LCs is lower than that reported in the previous fifteen years since the approach was introduced in 1987. This occurrence could be related to the wider experience of the several centres, to the spread of better technologies, and likely a greater concern and a careful sensitivity of surgeons’ community toward this issue.

5. Risk factors

Several risk factors are related to IBDI, which can be classified as patient factors, local factors due to illness, and extrinsic factors, related to the surgeon and his operative technique. Patient factors are male gender, advanced age and obesity (Waage, 2006). Local factors include: congenital malformations, such as partial liver agenesis (Fields, 2008); anatomical
anomalies of the biliary tract (BT) (Colovic, 2009), concerning both the proximality and the confluence between the cystic duct and the main BT, which may be angular (75%), parallel (20%) or spiral (5%), so jeopardizing its dissection (Strasberg, 2008); inflammatory conditions, such as acute cholecystitis, that increases up to three times the risk of IBDI with a 5% rate, are the most important predisposing factor for IBDI in LC (Kitano, 2002). The reason of this rate is what Strasberg name “hidden cystic duct syndrome”, or otherwise “second cystic duct syndrome”. When the surgeon dissects the infundibulum to identify the cystic duct as first step (infundibular technique), without first isolating Calot structures, the hepatobiliary duct may be confused with a false cystic duct, and sectioned. This misidentification is more likely with acute and chronic inflammation, large stones impacted in the infundibulum, adhesions between gallbladder and choledochus or intrahepatic gallbladder (Chapman, 2003). These last conditions are related to extrinsic factors, too. In fact, when anatomy is not clear, disective technique must be meticulous. So surgeon must not only isolate infundibulum, but also accurately prepare Calot triangle before cutting any structure, in order to avoid IBDI. In LC they are more proximal, and sometimes associated with vascular injuries, often depending on the right hepatic artery. The incidence of vascular injuries during LC is 61% in the series by Koffron (Koffron, 2001) and 47% in the series by Belghiti (Alves, 2003). It is still controversial the possible influence of the associated vascular lesion on the outcome of the surgical biliary diversion. In LC the learning curve seems to be the most important factor for minimising IBDI rates (Archer, 2001). Even surgeon’s tiredness has been implicated as a risk factor for IBDI (Yaghoubian, 2008), as testified by decreased incidence with work hour restrictions.

6. How to prevent IBDI

Principles for a correct technique in laparoscopic approach, for preventing IBDI, were largely described since the beginning of laparoscopic era. Use of a 30° camera, avoiding thermocoagulation near the main BT, a meticulous dissection, an accurate haemostasis and eventually conversion to open surgery, whenever is not possible to figure out clearly the anatomy, all of these represent well-known and precious dogmas (Troidl, 1999). During LC correct identification of the cystic duct can made easier in complex cases by several methods. Routinely intraoperative Cholangiography (Traverso, 2006) can show the whole biliary tract, including any anomaly; it can verify the existence of IBDI, leading quickly to treatment. Many authors state that unfortunately many lesions have already been made at time of radiological investigation, but nonetheless this represent a meaningful step because an early diagnosis is helpful in the outcome of repair. The critical view technique, described by Strasberg in 1995, consists of the identification of the cystic duct and cystic artery through dissection of the upper border of the Calot triangle along the underside of the gallbladder, thereby exposing the base of the liver. Once this view has been achieved, these two structures will be the only entering the gallbladder. Exposing the inner layer of the subserosa could be useful, further optimising the critical view, either for aberrant ducts or swollen gallbladder (Mirrizzi, 1932). The infundibular technique is based on the identification of the cystic duct where it joins the infundibulum. Some authors still recommend this technique, if routinely assisted by the intraoperative cholangiography. It is currently the most widely used and maybe quickest technique, but it has the drawback of not preventing IBDI because of lacking of the contemporary assessment of cystic duct and
main BT (an hot point of the hidden cystic duct syndrome). The Fisher method consists of dissecting the gallbladder from the gallbladder bed starting from the bottom to the infundibulum, as in open surgery. Since cystic artery is ligated at the end of this step, this procedure is more bleeding, thereby more dangerous. The use of intraoperative laparoscopic ultrasound represents another method to prevent IBDI. It is a really interesting diagnostic tool, even if it requires an equipped operating room and highly trained staff. A multicentre study by Machi (Machi, 2009) highlighted his usefulness. However, its advantages are unclear compared to the intraoperative cholangiography, neither they submit a standardized technique. So its effectiveness has to be demonstrated, because it is more expensive than cholangiography, highly operator-dependent and not available in every hospital.

7. Diagnosis

Early diagnosis of IBDI is of primary importance, and decisive as well for the long-term results after treatment. IBDI are noticed intraoperatively only in few cases, often by means of bile leaking, allowing an early repair. Unfortunately, in most cases, at times after days or weeks after intervention, diagnosis is accomplished lately, due to the onset of abdominal pain, fever, jaundice, and septic syndrome. An unusual postoperative course may be a warning sign of IBDI. Postoperative diagnosis must be assessed by clinical exam, lab values (cholestasis) and imaging techniques. Liver function tests after bile duct injury may show cholestasis by the rise of Gamma-Glutamyl Transpeptidase (GGT) and Alkaline Phosphatase (ALP). However, these tests may often be in the normal range. In patients with biliary stricture or complete occlusion, bilirubin is elevated, while in bile leakage, bilirubin may be normal or only mildly elevated, after absorption of bile from the peritoneal cavity (Lau, 2010). Intraoperative recognition of bile duct injuries and its immediate repair is highly advantageous in preventing serious complications (sepsis) and increase repair success rates (Lohan, 2005). Unfortunately, diagnosis is intraoperative only in 10% to 30% of cases (Lee, 2000), while most of them are recognized postoperatively, with patients sometimes complaining of vague abdominal symptoms, 48h after the intervention (biliary abdominal collections, jaundice, anorexia, elevation of cholestasis and liver enzyme values) (Lillemoe, 2006). Finally, we can have delayed diagnosis for cases that often become more complex and with poor prognosis, recognized after a week to months since intervention, with recurrent cholangitis, obstructive jaundice up to secondary biliary cirrhosis (Sicklick, 2005). Ultrasonography is the first investigation which can show fluid collections and dilatation of the biliary tract, but reveals neither the site and seriousness of the lesion nor a coexisting vascular injury, for what Computed tomography with contrast agent is recommended. Scanning with iminodiacetic acid (HIDA scan) can only diagnose bile leaks. MR-cholangiography is at present a fundamental investigation that allows to identify leaks from small biliary radicals or cystic duct stump, together with the presence of arterial injuries and choledocholithiasis. MR-cholangiography with manganese is an efficient method to reveal IBDI, but series in literature (Khalid, 2001) are few. Cholangiography, both percutaneous transhepatic (PTC) and endoscopic (ERCP), is the gold standard for evaluating bile duct injuries, often essential to plan therapeutic procedure. Sometimes they represent the ultimate treatment. In cases of lesion proximal to the hilum, with either transection or aberrant ducts leaking, ERCP cannot show the biliary tract, so that an anterograde Cholangiography by PTCA is indicated (Pawa, 2009).
8. Management

Management of bile duct injuries is complex and a multidisciplinary approach in tertiary centres is recommended. In any case, early recognition and treatment of septic complications is of paramount importance. Management can be categorized into non-surgical and surgical, and has to be performed in referral centres because often repair intervention represents the only chance of care for the patient. The therapeutic approach and its timing depend on several factors: the extent of lesion, the experience of the surgeon, the inflammatory and haemodynamic status, all of them jeopardize outcome. It is well known that, an intraoperative recognition of the lesion and its immediate repair offers the best long-term results with a low morbidity, reduced hospital stay and costs. The experience of referral hepato-biliary surgery centres plays an important role, as demonstrated by the better success rates of repair (de Reuver, 2007), especially in case of proximal IBDI associated with vascular injury (Bilge, 2003). Non-surgical management is based on endoscopic procedures (ERCP) and on interventional radiology, often mandatory for repair and less expensive. They require bilioenteric continuity. They are less invasive and more appropriate in patients who are not candidates for surgery (Misra, 2004). Recently the development of different types of biliary prostheses contributed hugely to simplify the management of biliary benign strictures (Ramos-De la Medina, 2008). Endoscopic treatment (papillotomy with stenting) is indicated for type A lesions. Its effectiveness decreases if leaks are more proximal, because of differences in the basal or intraductal pressure, of CD length and BT diameter (Marks, 1998). About surgical management, in case of the complete transection of the common hepatic duct or of choledochus, end-to-side Roux-en-Y hepaticojejunostomy represents the best bilio-enteric anastomosis. The defunctionalised loop avoid intestinal reflux into the BT and prevents ascending cholangitis. The hepatoduodenal anastomosis has an increased anastomotic tension and reflux, and a higher rate of developing high debt biliary fistula (Mercado, 2008). Still controversial is the effectiveness and the duration of a transanastomotic tutor, and it depends on surgeon’s experience and choice. In case of complete section, an end-to-end anastomosis on T-tube is not indicated because in almost 50% of cases is followed by biliary stricture (Jarnagin, 2009). Biliary diversion should be preferred when vascular damage is expected, even if section of the bile duct is just partial. A T-tube has therefore only a decompressive function and does not act as a tutor for a bilio-biliary hazardous anastomosis; it can therefore be used only in selected cases such as partial section or lesion with a proper vascularisation. Finally, unfortunately, there are some complications that may require liver transplantation: IBDI associated with recurrent episodes of cholangitis, chronic cholestasis and secondary biliary cirrhosis, and lesions of the hepatic hilar vessels, especially the hepatic artery, which according to some authors can lead to an acute hepatic failure (Fernández, 2004), although this occurs rarely in a liver otherwise healthy because of its double blood supply (Stewart, 2004).

9. Hepatico-jejunal anastomosis

A regular evaluation of cholestasis is indicated in patients undergone hepaticojejunostomy, by over ten years lasting follow-up due to the potential onset of long-term complications. The goal of surgical repair of the injured biliary tract is the restoration of a durable bile flow, and the prevention of short- and long-term complications such as biliary fistula, intra-
abdominal abscess, and subsequently biliary stricture, recurrent cholangitis, and secondary biliary cirrhosis. A tension-free Roux-en-Y hepaticojejunostomy is the preferred procedure for the majority of bile duct injuries. Tension-free end-to-end anastomoses are rarely possible, even if the duodenum has been largely mobilized. Moreover, a high rate of restricture has been reported for end-to-end repair of laparoscopic bile duct injuries (Stewart, 1995), sometimes up to 100% of cases. For diathermy injury to the bile duct, the anastomosis should be made proximally near to the confluence of the bile ducts to avoid stricture formation, as a consequence to coagulation injury to the collateral network of blood vessels supplying the CBD/CHD (Lau, 2009). Outcome after HJ is evaluated by cholestasis and liver function tests - the clinical presentation of an anastomotic stricture is cholangitis in almost half of the case, and jaundice or abnormal liver function tests in the other half (Goykhman, 2008), with the potential onset of long-term complications (> 10 years) requiring a further intervention, often transhepatic percutaneous stenting. Factors as ischemia, inflammation, and fibrosis may play a fundamental role in the development of strictures. About long-term outcome, a poor prognostic factor is serum ALP levels higher than 400 UI six months after intervention (Huang, 2003). Independent poor prognostic factors of the HJ outcome are: surgical repair in the presence of active inflammation (peritonitis), injuries at or above the biliary bifurcation, and bile duct injury with concomitant vascular injury. These factors are associated to a significant higher risk of developing severe biliary complications, such as strictures, hepatic abscess, and secondary biliary cirrhosis (Schmidt, 2004). Two thirds of recurrences occur within the first two years from the intervention, but stricture recurrence even after 10 years has also been reported (Sutherland, 1999). The long interval from reconstruction to symptomatic late stricture and liver failure underlines the need for long-term follow-up.

10. Conclusions

Analysis of literature related to IBDI following LCs is still difficult and inaccurate, because bile duct injuries are not always standardized, according to the several existing classifications, and as consequence authors do not always specify the type of injury reported. Several papers report laparoscopic techniques with the aim of reducing IBDI, but none of them is absolutely perfect or even better than others, because as a matter of fact IBDI may occur even to the most skilled laparoscopic surgeons. Accordingly, we might suppose that these complications are not exclusively due to unskilfulnes below standards, but they are probably related to the limits of the minimvasive approaches themselves – lack of three-dimensional view and loss of tactile feedback – which could increase operative risks. In other words, compared to a recent pastime, from 1987 to 2005, during which IBDI following LCs had a huge rise, estimated in Italy as high as between 0.3% vs 0.1% (an amount of 300 IBDI per year out of every 90000 LCs), according to the most recent literature a decreasing trend is reported. Everyone must trust in a leading concept: during the first dissective phases, in case of unclarified anatomy, the surgeon must not hesitate about converting, with the aim of carrying out a safer intervention, because of the potential hazard of IBDI. LC is still an ideal approach for symptomatic cholelithiasis, but a cholecystectomy through a “wide subcostal laparotomy” is indicated as well, if preserving from a bile duct injury, which is at present a dreadful complication in terms of morbidity, mortality and social costs. Diffusion and sharing of such principles among surgeons and patients likely represent the
most efficient strategy to prevent IBDI. IBDI following LC represent a delicate and interesting chapter in hepato-biliary surgery. They are characterized by high morbidity and sometimes mortality, especially caused by septic complications. The pastime higher incidence seems decreasing and close to that described in traditional surgery, likely due to a better knowledge of the mini-invasive approach and an improved sensitivity toward this issue by the surgical community. Nonetheless they are still causing an increase in healthcare costs because of the investigation, the management and the hospital stay, and moreover for legal issues frustrating to surgeons. Among diagnostic tools, MR Cholangiography is thought to be very important for a suitable biliary-vascular study, but is often followed by second level invasive procedures, that sometimes represent an effective therapeutic option. An early diagnosis is directly related to outcome of the repair, that must be multidisciplinary - endoscopy, interventional radiology, and surgery - in referral centres, because frequently the patient has an unique therapeutic chance. For “major" lesions, Hepaticojejunostomy is the intervention of choice, whereas a T-tube diversion is indicated for selected patients, because of long-term biliary strictures. A regular evaluation of cholestasis is indicated in these cases by means of over ten years follow-up. In case of IBDI, irrespective of biliary repair, the early recognition and treatment of the septic complications, that often rise up mortality, is the most important aim on planning diagnostic and therapeutic work-up.

11. References


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Iatrogenic Bile Duct Injuries Following Laparoscopic Cholecystectomy: Myth or Reality? A Recent Literature Review from 2006 to 2011


This book covers different aspects on the understanding of mechanisms, effects, and management of cholestasis. This unique compendium contains important citations, an invaluable amount of research work, and many applications, which are outstanding resources for clinicians, pharmacists, biochemists, upper-level undergraduate, graduate, and continuing-education students who are dedicated to discovering new knowledge on cholestasis.

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