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^[1]Passed away on October 20, 2007

^[2]Passed away on June 14, 2008



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Overview of immunosuppression in liver transplantation

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Abstract

Continued advances in surgical techniques and immunosuppressive therapy have allowed liver transplantation to become an extremely successful treatment option for patients with end-stage liver disease. Beginning with the revolutionary discovery of cyclosporine in the 1970s, immunosuppressive regimens have evolved greatly and current statistics confirm one-year graft survival rates in excess of 80%. Immunosuppressive regimens include calcineurin inhibitors, anti-metabolites, mTOR inhibitors, steroids and antibody-based therapies. These agents target different sites in the T cell activation cascade, usually by inhibiting T cell activation or *via* T cell depletion. They are used as induction therapy in the immediate peri- and post-operative period, as long-term maintenance medications to preserve graft function and as salvage therapy for acute rejection in liver transplant recipients. This review will focus on existing immunosuppressive agents for liver transplantation and consider newer medications on the horizon.

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Key words: Immunosuppression; Liver transplantation; Induction therapy; Rejection

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INTRODUCTION

Due to advances in immunosuppression and improvements in surgical techniques, liver transplantation has become an extremely successful treatment option for patients with end-stage liver disease, with one-year graft survival rates exceeding 80%^[1]. Currently, there are eight patients worldwide who have survived more than three decades after liver transplantation^[2].

Organ transplantation initially came to light with the first successful kidney transplantation in 1954 on monozygotic twins; however, immunosuppression was limited to total body irradiation which was largely fatal^[3,4]. With the invention of 6-mercaptopurine (6-MP) and azathioprine (AZA) in the 1950s along with the introduction of corticosteroids as combination therapy by Starzl in the 1960s, there was noticeable improvement in kidney allograft survival, although one-year survival still did not exceed 50%^[4]. Multiple interventions including splenectomy, thymectomy and thoracic duct drainage were employed with minimal success.

The first successful human liver transplant was performed by Thomas Starzl in Denver in 1967 on an 18-month-old child with unresectable hepatoblastoma^[2]. The immunosuppressive regimen included anti-lymphocyte globulin (ALG), AZA and prednisolone and the child survived for more than a year.

However, the next significant breakthrough in immunosuppression did not occur until the discovery of cyclosporine (CYA) in 1972 from the soil fungus *Tolypocladium inflatum*. Borel *et al*^[5] first described its remarkable immunosuppressive properties in 1976 and by the 1980s there was international affirmation of its effectiveness. CYA quickly became the standard of care for maintenance immunosuppression in solid organ transplant recipients. This paved the way for the current era of liver transplantation, which has since continued to evolve with the discovery of multiple novel immunosuppressive agents.

IMMUNOSUPPRESSION

Effective immunosuppression in transplantation relies on preventing the immune system from rejecting the allograft while preserving immunologic control of infection and

Table 1 Drugs that increase CNI and sirolimus levels

Drugs that increase CNI levels

Macrolides: clarithromycin, erythromycin, azithromycin
 Antifungals: fluconazole, itraconazole, ketoconazole, voriconazole, clotrimazole
 Calcium channel blockers: verapamil, diltiazem, nifedipine
 Others: cisapride, metaclopramide, amiodarone, cimetidine, protease inhibitors

Drugs that decrease CNI and sirolimus levels

Antibiotics: rifabutin, rifampin
 Anticonvulsants: carbamazepine, phenobarbital, phenytoin, fosphenytoin
 Others: St. John's Wort

CNI: Calcineurin inhibitor.

neoplasia. Although the mechanism is not completely understood, transplanted livers rarely reject compared to other organs, do not require an HLA-matched donor, and may offer a protective effect for other simultaneously transplanted organs^[6,7]. Both micro- and macrochimerism models have been used to explain this phenomenon, as well as that of hepatic dissolution of donor specific antibodies. Ideally, the long-term objective is to achieve immune tolerance or the ability to alter the recipient's immune system in order to promote long-term graft function without immunosuppressive therapy, while maintaining immunity to infectious agents^[8]. Unfortunately, except for a small minority of patients (approximately 20%) who have been successfully weaned off immunosuppressive medications, most experience immunologic rejection with the discontinuation of these drugs and have to be maintained on at least low doses of these medications^[9-13].

Immunosuppressive regimens include calcineurin inhibitors, anti-metabolites, mTOR inhibitors, steroids and antibody-based therapies. These agents target different sites in the T cell activation cascade, usually by inhibiting T cell activation or proliferation or *via* T cell depletion. The selection of agents is based on an individual's medical history as well as on institution experience and preference. Most immunosuppressive regimens combine drugs with different sites of action of T cell response, allowing for dosage adjustments to minimize side effects and toxicities. Currently, the mainstay of maintenance immunosuppressive regimens are calcineurin inhibitors (CNIs), used in greater than 95% of transplant centers upon discharge, although there is a known increased risk of renal impairment^[14,15], metabolic derangements, neurotoxicity and *de novo* malignancies^[16] with the long-term use of these medications.

CALCINEURIN INHIBITORS

CYA and tacrolimus are the two CNIs approved for use in organ transplantation and are the principal immunosuppressives used for maintenance therapy. The routine use of these medications in liver transplant recipients has dramatically decreased the incidence of rejection and graft loss. The primary mode of action is inhibition of T cell activation. CYA binds to

Table 2 Common side effects of immunosuppressive agents

Drug	Adverse effects
Tacrolimus	Nephrotoxicity, neurotoxicity ¹ , diabetes ¹ , hyperkalemia, metabolic acidosis, hypertension, hyperlipidemia
Cyclosporine	Nephrotoxicity, neurotoxicity, diabetes, hyperlipidemia ¹ , hypertension ¹ , hyperkalemia, metabolic acidosis, gingival hyperplasia, hypertrichosis
MMF	Myelosuppression, gastrointestinal side effects, viral infections (CMV, HSV), spontaneous abortions in pregnant women
Sirolimus	Hyperlipidemia, myelosuppression, proteinuria, poor wound healing, pneumonitis, skin rash
Corticosteroids	Diabetes, hypertension, obesity, osteoporosis, avascular necrosis, growth retardation, Cushingoid features, psychosis, poor wound healing, adrenal suppression, cataracts

¹More common in respective agent. MMF: Mycophenolate mofetil; CMV: Cytomegalovirus; HSV: Herpes simplex virus.

cyclophilin which results in inhibition of the calcium/calmodulin-dependent phosphatase, calcineurin. The binding to cyclophilin interferes with calcineurin's de-phosphorylation of nuclear factor of activated T cells (NFAT), preventing translocation of NFAT into the nucleus and up-regulation of pro-inflammatory cytokines. The end result is the inhibition of IL-2 gene transcription and T cell activation and proliferation^[4,8]. Tacrolimus also inhibits calcineurin but binds specifically to FK506-binding protein (FKBP-12).

The immunosuppressive effects of the CNIs are related to total drug exposure which can be estimated by measuring blood 12-h troughs. The potency of tacrolimus is estimated to be 100 times greater on a molar level^[8] when compared to CYA. Although several earlier studies showed tacrolimus to be superior to CYA in the prevention of cellular rejection^[17-19], another more recent multi-center trial showed no significant differences between the two medications with regard to acute rejection episodes, death or graft loss^[20]. Both CNIs are metabolized principally by the cytochrome P450 system and therefore have significant interactions with multiple medications requiring careful monitoring of drug levels (Table 1).

CNIs have a wide range of toxicities, many of which are dose-dependent (Table 2). Nephrotoxicity is a well-recognized side effect and it has been documented that nearly 20% of liver transplant recipients experience chronic renal failure within 5 years^[15]. This can be best managed by either discontinuation or reduction of the medication. Neurotoxicity is another common problem; one which is more predominant with tacrolimus. The clinical presentation varies from headaches and tremors to agitation, confusion, hallucinations or overt psychosis. Hypertension, hyperlipidemia, hyperkalemia, metabolic acidosis and diabetes are also frequent side effects. Diabetes is more common with tacrolimus use, whereas hypertension and hyperlipidemia tend to be more prominent with CYA use. Gingival hyperplasia and hypertrichosis are specific side effects of CYA only.

Another important feature of CNIs is their interaction with transforming growth factor- β (TGF- β), a cytokine that augments fibrosis development and promotes tumor cell invasiveness^[21]. TGF- β transcription is increased with CNI use, which is of concern given the possibility of hepatocellular carcinoma (HCC) recurrence or the emergence of post-transplant lymphoproliferative disorder (PTLD).

ANTIMETABOLITES

Both mycophenolate mofetil (MMF) and mycophenolate sodium (MPS) undergo immediate first-pass metabolism in the liver into the active compound mycophenolic acid (MPA), which was first discovered in 1893^[22]. However, the immunosuppressive properties of MPA were not recognized until the 1990s. MPA inhibits inosine-5'-monophosphate dehydrogenase (IMPDH)^[23], the rate-limiting enzyme in the *de novo* synthesis of guanosine nucleotides. Inhibition of the IMPDH pathway results in selective blockade of lymphocyte proliferation^[24].

The major advantage in using the MPAs is their lack of renal toxicity. In patients with pre-existing renal disease, they have been used in conjunction with low-dose CNIs as part of a renal-sparing protocol with promising results^[25,26]. Ideally, these medications should be initiated when renal dysfunction is first noted, although emerging data suggests the benefits of MPAs in reversing long-standing renal disease due to its association with decreased TGF- β levels^[27-29]. MPAs are rarely used as monotherapy in transplant recipients given their higher rates of rejection compared to the CNIs^[30,31], although more recent data demonstrate the safety of this approach when carried out carefully^[32,33]. However, in patients previously on CNIs or mTOR inhibitors with evidence of acute rejection, MPAs are often added as supplemental immunosuppressive therapy.

The predominant side effects of MPAs are related to gastrointestinal disorders and bone marrow suppression (Table 2). Diarrhea is the most common dose-limiting adverse effect, although abdominal pain, nausea and vomiting can frequently occur^[34]. Studies have also shown increased incidences of cytomegalovirus (CMV)^[35-37], herpes simplex virus (HSV)^[38,39], *Candida* infections, and, rarely, progressive multifocal leukoencephalopathy (PML)^[40] with the use of MPAs. In pregnant patients, increased risks of spontaneous abortions during the first trimester and serious congenital malformations have also been reported (www.fda.gov). Routine monitoring of MPA levels is not generally employed in clinical practice.

Azathioprine is another antimetabolite which was predominantly used for the prevention of rejection in the 1960s but has since been largely replaced by the MPAs. It is selectively used in a few centers in combination with other immunosuppressive medications, primarily CNIs and steroids.

mTOR INHIBITORS

The two mTOR inhibitors approved for organ trans-

plantation are sirolimus (SRL) and everolimus (EVL), although neither has been approved for use in liver transplantation to date. They bind intracellularly to FK506 binding protein (FKBP12) but unlike tacrolimus, they do not inhibit calcineurin activity. Rather, the complex is a highly specific inhibitor of mammalian target of rapamycin complex 1 (mTORC1)^[41] which has a direct effect on the cell signaling pathway required for cell cycle progression. This subsequently inhibits IL-2 signaling to T cells, thus preventing T cell proliferation. Similar to the CNIs, sirolimus is metabolized by the cytochrome P450 system and requires therapeutic drug monitoring (Table 1).

The first reported study illustrating the effectiveness of sirolimus monotherapy for maintenance of immunosuppression in liver transplantation was in 1999 by Watson *et al*^[42]. However, two subsequent large studies examining sirolimus *de novo* therapy with tacrolimus and corticosteroids were terminated early due to excess hepatic artery thrombosis (HAT). As a result, sirolimus carries a black box label warning which cautions against the possible development of early post-transplant HAT. Subsequent studies have since disputed this finding^[43-45], however, mTOR inhibitors are rarely used as *de novo* therapy.

Importantly, in patients with CNI-induced nephrotoxicity, conversion to sirolimus therapy has proved to be effective with ensuing improvements in renal function^[46-48]. Again, sirolimus conversion should be initiated early since late conversion rarely improves chronic renal dysfunction^[49]. In fact, several studies have shown that in patients with pre-existing renal disease, sirolimus can worsen nephrotoxicity and promote proteinuria^[50-52].

Recent studies have also shown potential anti-tumor properties of sirolimus^[53-56] which might be of importance in patients undergoing liver transplantation for HCC. Zimmerman *et al*^[57] examined the role of sirolimus-based maintenance therapy in post-transplant recipients with a history of HCC and found that overall survival was increased in the sirolimus arm compared to the CNI arm. Clinical trials examining the anti-cancer effects of mTOR inhibitors in liver transplant recipients with HCC have been encouraging^[44,58] and new trials are ongoing.

Metabolic side effects of mTOR inhibitors include proteinuria and increases in serum cholesterol and triglycerides (Table 2). Bone marrow suppression, interstitial pneumonitis, peripheral edema, dermatological effects (acne, mouth ulcers) and delayed wound healing are all well-documented. Inhibition of fibroblast growth factor by sirolimus impairs tissue repair and plays a role in delayed wound healing^[59]. Interstitial pneumonitis is rarely life-threatening, is dose-dependent and resolves on withdrawal of the drug^[60].

CORTICOSTEROIDS

Corticosteroids are well-known for their anti-inflammatory properties such as suppression of prostaglandin synthesis,

stabilization of lysosomal membranes and reduction of histamine and bradykinin release^[30,31]. They also exhibit various immunomodulatory effects including effects on antigen presentation by dendritic cells and induction of a decrease in the number of circulating CD4+ T cells, IL-1 transcription and IL-1-dependent lymphocyte activation^[4,8].

High-dose corticosteroids were used judiciously in the 1960s in post-transplant recipients, with resulting increased morbidity due to their well-known deleterious side effects. This led to several studies in the 1980s on renal transplant recipients which confirmed that graft and patient survivals, as well as rejection episodes, were similar in the high- and low-dose steroid groups as long as AZA was also used^[61-63]. Currently, intravenous corticosteroids are predominantly used as first-line therapy for the treatment of acute cellular rejection. Regarding maintenance therapy, they are often successfully withdrawn within 3-6 mo post-transplantation in patients without evidence of rejection or liver disease attributed to autoimmune disorders^[64]. The primary concern with corticosteroid use is exacerbation of hepatitis C virus (HCV) recurrence and liver fibrosis with high-dose pulsed therapy^[65]; however, this has not been evident with low, gradually tapered doses^[66,67].

Well-documented side effects of corticosteroids include diabetes, hypertension, central obesity, Cushingoid features, osteoporosis, avascular necrosis, psychosis, poor wound healing, adrenal suppression and cataracts (Table 2).

ANTIBODY-BASED THERAPIES

Polyclonal antibodies

Polyclonal antibodies, including anti-thymocyte (ATG) and anti-lymphocyte globulins (ALG), have been used since the early days of liver transplantation and are prepared by inoculating rabbits or horses with human lymphocytes or thymocytes^[4]. Their mechanism of action is rapid lymphocyte depletion due to complement-mediated cell lysis and uptake by the reticulo-endothelial system (RES) of opsonized T cells^[68]. In addition, they may also cause partial T cell activation and blockade of T cell proliferation^[69]. Polyclonal antibodies were routinely used as induction therapy in liver transplantation along with corticosteroids and AZA before the discovery of CYA.

Lymphocyte depletion is believed to play a role in preparing the recipient's immune system to adapt and recognize the transplanted organ as self and prevent destruction of the allograft. Accordingly, studies have shown that ATG administration results in regulatory T cell (Treg) expansion *in vitro* and *in vivo*^[70-72]. Tregs or suppressor T cells are responsible for preventing activation of the immune system and maintaining tolerance to self-antigens.

Currently, approximately 20% of transplant centers use these agents for induction purposes^[73] and recent data support the administration of thymoglobulin induction to delay CNi use and avoid renal toxicity without increasing the risk of rejection or HCV recurrence^[74-76]. A few

studies have also successfully shown the benefit of using these medications as induction therapy to avoid post-transplant corticosteroid use^[77,78] without an increased incidence of acute rejection. This is especially important in HCV recipients where high-dose pulsed corticosteroid therapy can significantly accelerate liver fibrosis. At present, anti-lymphocyte antibodies are used extensively to treat steroid-resistant acute rejection and are successful in 70%-96% of patients^[79-81].

The main side effect of these medications, affecting 80% of patients, is a "first-dose reaction" and febrile episode which can often be ameliorated by pre-medication with antipyretics, antihistamines and intravenous steroids. This effect is likely due to pyrogen release from the massive destruction of lymphocytes^[69,82]. Other adverse effects include thrombocytopenia, anemia, CMV infection, PTLT, pruritic skin rashes, serum sickness and anaphylaxis^[83-85].

Monoclonal antibodies

Monoclonal antibodies include the anti-IL-2 receptor (CD25) antibodies, anti-CD52 antibody and muromonab-CD3 (OKT3). The two anti-IL-2 receptor antibodies approved for clinical use are basiliximab (Simulect), a chimeric protein, and daclizumab (Zenapax), a humanized protein. Both antibodies are specific for the α chain of the IL-2 receptor, CD25, which is only expressed on activated T cells^[8]. These antibodies remain in the circulatory system for weeks after initiation of therapy and have been used successfully with low-dose CNIs in preventing acute rejection in the early post-transplant period^[86-88]. They also have fewer side effects compared to the anti-lymphocyte globulins, rarely cause the typical first-dose infusion reactions and are associated with less risk of opportunistic infections and PTLT.

Muromonab-CD3 (OKT3) targets the CD3 molecule on T cells and causes depletion of lymphocytes by massive T cell lysis^[89] and cytokine release^[90]. This profound cytokine release can lead to pulmonary edema and acute respiratory distress and rarely, intra-graft thrombosis and aseptic meningitis^[91,92]. As a result, antihistamines and intravenous steroids are routinely used as pre-medication to reduce this "cytokine release syndrome". Several days after OKT3 administration, T lymphocytes no longer express CD3 and are considered to be immunologically incompetent^[93]. OKT3 is primarily used in liver transplantation for steroid-resistant acute rejection^[94,95] and has a success rate of complete recovery in 50% of patients. OKT3 use should be limited in the HCV population as several studies have confirmed exacerbation of disease recurrence with this agent^[96,97].

The humanized anti-CD52 antibody, alemtuzumab (Campath-1) targets lymphocytes, monocytes, macrophages, natural killer cells and thymocytes but spares plasma cells and memory lymphocytes^[8,98]. It is unique in that it depletes lymphocytes from the circulation as well as peripheral lymph nodes. Several studies in renal transplant patients have shown its efficacy in preventing rejection when used in

combination with low-dose CNIs or sirolimus^[99-101]. Tzakis *et al.*^[102] compared the use of alemtuzumab induction therapy combined with low-dose tacrolimus in liver transplant recipients receiving standard doses of tacrolimus and corticosteroids. Although patients who received alemtuzumab had less renal dysfunction and acute rejection in the first two months post-transplant, the overall incidence of rejection was not significantly different between the two groups. Similarly, Marcos *et al.*^[103] proposed that alemtuzumab, in conjunction with minimal CNI use, is a successful treatment strategy in liver transplant recipients, improving overall graft and patient survival, especially in HCV-infected subjects.

FUTURE DIRECTION OF IMMUNOSUPPRESSION

Costimulation blockade (Belatacept)

Belatacept is a soluble cytotoxic T-lymphocyte antigen-4 (CTLA-4) agent which binds CD80 and CD86 and inhibits T cell activation^[4,8]. Belatacept competes with the CD28 receptor on T cells which normally binds CD80 and CD86 on the antigen presenting cell (APC) as a co-stimulatory signal required for T cell activation. Belatacept is administered intravenously once a month and does not carry the renal toxicity of CNIs. Clinical trials in liver transplant patients are currently ongoing with this agent.

Efalizumab

Efalizumab is a humanized leukocyte function-associated antigen-1 (LFA-1; CD11a) specific monoclonal antibody that inhibits T cell-APC stabilization and blocks lymphocyte adhesion to endothelial cells^[104,105]. This agent was approved for the treatment of psoriasis in 2003 and has not yet been used in liver transplantation, although a few clinical trials have been carried out in renal transplant patients with mixed results^[106]. Although the results regarding immunosuppression were promising, an increased risk for PTLT was shown when efalizumab was used in combination with high-dose CYA.

Other newer agents on the horizon undergoing phase II/III trials include Janus Kinase (JAK) 3 inhibitors, AEB071 (a protein kinase C (PKC) isoforms inhibitor), and Alefacept (a LFA3-IgG1 fusion receptor protein). JAKs are intermediaries between cytokine receptors and signal transducers and activators of transcription (STATs) which lead to immune cell activation^[107,108]. JAK-3, a cytoplasmic tyrosine kinase, is primarily found on hematopoietic cells and its stimulation is specific for the IL-2 family of cytokines which makes it a very attractive target for immunosuppression. Clinical trials are underway in renal transplant patients using these agents. AEB071 (PKC inhibitor) is an oral agent that blocks early T cell activation and IL-2 production^[109]. Three phase II renal transplant trials using AEB were started, two of which had to be stopped due to increased episodes of acute rejection; the third trial is ongoing

in Europe^[110]. Alefacept, a LFA3-IgG1 fusion receptor protein initially approved for the treatment of psoriasis, interferes with T-cell activation and produces a dose-dependent reduction in T-effector memory cells^[111]. A multi-center clinical trial in renal transplant recipients is currently underway.

CONCLUSION

The current era of immunosuppressive therapy continues to evolve with the discovery of novel agents, targeting different sites of the immune cascade. Important objectives when using these medications include decreasing the incidence of renal toxicity from CNIs while preserving graft function as well as optimizing immunosuppression without creating an environment for increased infections, aggressive recurrence of hepatitis C or triggering PTLT and other malignancies.

At our institution, high-dose intravenous corticosteroids are used in the immediate peri- and post-operative period and then tapered accordingly. In patients without renal dysfunction post-transplantation, CNIs are the mainstay of therapy with the long-term goal of low levels of immunosuppression and minimization of medication. In patients with renal insufficiency, we have had success with a combination of low-dose CNI therapy and MPAs or a switch to mTOR inhibitors to preserve graft function and prevent further renal deterioration. We typically avoid the switch to mTOR inhibitors within the first 3-6 mo post-transplantation given the risk of hepatic artery thrombosis, rejection, and wound healing. Patients are weaned off corticosteroids within 6 mo to 1 year, providing they do not have evidence of autoimmune disease or recurrent episodes of rejection.

As evidenced by prior studies, the recommended approach to the patient with HCV infection is gradual, cautious weaning of corticosteroids within the first 3-6 mo while continuing low levels of maintenance immunosuppression, typically with CNIs. While HCV recurrence is universal after liver transplantation, avoiding excessive and erratic changes in the immunosuppressive regimen should prevent clinically aggressive disease.

The ultimate goal remains the ability to induce tolerance in transplant recipients. While this is not a current available practice, data from selected patients demonstrate that it may become a viable option with advances in future research and improved understanding of the genetic make-up and predisposition of this population. Until then, finding the balance between preserving graft function and optimizing immunosuppression while minimizing toxicities remains a challenge.

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EDITORIAL

Prevalence, predictors, and clinical consequences of medical adherence in IBD: How to improve it?

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Abstract

Inflammatory bowel diseases (IBD) are chronic diseases with a relapsing-remitting disease course necessitating lifelong treatment. However, non-adherence has been reported in over 40% of patients, especially those in remission taking maintenance therapies for IBD. The economical impact of non-adherence to medical therapy including absenteeism, hospitalization risk, and the health care costs in chronic conditions, is enormous. The causes of medication non-adherence are complex, where the patient-doctor relationship, treatment regimen, and other disease-related factors play key roles. Moreover, subjective assessment might underestimate adherence. Poor adherence may result in more frequent relapses, a disabling disease course, in ulcerative colitis, and an increased risk for colorectal cancer. Improving medication adherence in patients is an important challenge for physicians. Understanding the different patient types, the reasons given by patients for non-adherence, simpler and more convenient dosage regimens, dynamic communication within the health care team, a self-management package incorporating enhanced patient education and physician-patient interaction, and identifying the predictors of non-adherence will help devise suitable plans to optimize patient adherence. This editorial summarizes the available literature on frequency, predictors, clinical consequences, and strategies for improving medical adherence in patients with IBD.

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Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Therapy; Adherence; Compliance; 5-aminosalicylate; Mesalazine; Azathioprine

INTRODUCTION

Inflammatory bowel disease (IBD) is a multifactorial entity with both genetic and environmental factors contributing to disease pathogenesis^[1]. Worldwide, the incidence rates for IBD vary from 0.5 to 24.5 per 100 000 person-years^[2], with the majority of patients being disabled during various parts of their lives. This characteristic may also suggest poor adherence (i.e. a percentage of the prescribed doses is not taken) outside the clinical trial settings^[3-5].

Treatment of IBD can involve several medications with varying regimens, dietary modifications, and potentially, surgery, depending on symptoms, severity of illness, and response to treatment. Adherence to the pharmacological treatment is a complex process, where the doctor-patient relationship, treatment regimen and other disease-related factors play key roles. The undesirable side effects of some medications (e.g. weight gain, cushingoid appearance, and immune suppression) and the complex treatment regimens for IBD patients (e.g. varying dosing schedules and pill quantities for each medication) are likely to disrupt adherence and the effective management of this condition. These data are consistent with the hypothesis that many patients engage in an implicit cost-benefit analysis in which beliefs about the necessity of their medication are weighed against concerns about the potential adverse effects of taking it, and that these beliefs are related to medication adherence, as in other chronic conditions^[6]. This scenario has been also proven in patients with IBD in a very recent paper^[7]. In contrast, the impact of medication non-adherence on the hospitalization risk and health care costs in chronic conditions (e.g. diabetes, hypertension, and congestive heart failure) is enormous^[8]. It has been estimated to cost as much as \$100 billion in the US annually, and accounts for 10% of all hospital admissions.

However, there are no studies that directly assess the costs associated with non-adherence in patients with IBD. Recently, it has been estimated in the UK that relapse was associated with a two- to threefold increase in the costs for those who did not require hospital care and a 20-fold increase for those who were hospitalized^[9].

Research on adherence in IBD is limited. Studies in adults have revealed medication non-adherence rates ranging from 35% to 45%^[3,5,10]. Unfortunately, most of these studies used different, unimodal indirect assessment methods including non-standardized self-report questionnaires, non-standardized patient/parent interviews, pill counts, pharmacy records, and measurements of health outcomes. A drawback of this method is that it can overestimate adherence, and its accuracy depends on the patient's cognitive abilities, the honesty of replies, as well as the interviewer's correct interpretation of responses. The patient may forget doses taken or missed. Prescription refills are also considered questionable for assessing dosing compliance because they provide no information on timing or quantity of pills ingested. In addition, pill counts are often erroneous because patients do not always return bottles with leftover pills. Thus, until now, the methods for assessing compliance have varied in terms of prospective *versus* retrospective or objective *versus* subjective measurement, target behaviour assessed (e.g. consumption of medication and refill of prescription), medication assessed, and method of assessment. This variability resulted in different estimates of the prevalence of non-adherence and diminished generalizability and the validity of data. Furthermore, these studies were limited by potential response bias in the self-report measures or behavioural manipulation, such as discarding pills to influence pill count data^[11].

In general, direct methods for measuring medication adherence include drug concentration monitoring through blood and urine assays. This strategy is expensive and inconvenient for patients, and, moreover, only a limited number of drugs can be monitored in this manner. The bioavailability and completeness of absorption of various drugs, as well as the rate of metabolism and excretion, are factors that make it difficult to correlate drug concentrations in blood or urine with adherence. The ability of direct methods to identify non-adherence also depends on the accuracy of the test and the degree to which the patient was non-adherent before the urine or blood sample was taken. Drug concentration monitoring can also be misleading because most drugs are rapidly absorbed following administration. Thus, even if numerous doses were omitted, yet a few doses were taken immediately prior to the blood test, the results would show the presence of a moderate amount of drug, or *vice versa*.

In IBD, bioassays measuring 6-thioguanine nucleotide (6-TGN) and 6-methylmercaptopurine nucleotide (6-MMPN) levels have been suggested as potentially useful objective adherence markers for 6-mercaptopurine (6-MP)/azathioprine (AZA)^[11,12]. However, they have not been validated against traditional measures of adherence. Moreover, like other bioassays, they are subject to pharmacokinetic variation in absorption, metabolism,

and excretion. Despite their limitations, bioassays provide key adherence data in that they can confirm ingestion. Non-therapeutic metabolite levels can suggest either non-adherence or pharmacokinetic influence, or both; cases where both 6-TGN and 6-MMPN levels are subtherapeutic/unquantifiable are likely to indicate non-adherence. Thus, although there is no gold standard in adherence assessment, and limitations exist with any measure of adherence, both behavioural and biological measures offer unique data that could be used to better understand non-adherence. Moreover, determining the most advantageous approach to assessing adherence is critical to the clinical care of these patients. This editorial summarizes the available literature on frequency, predictors, clinical consequences, and strategies for improving medical adherence in patients with IBD.

PREVALENCE OF NON-ADHERENCE IN IBD

In normal clinical practice, adult studies have revealed medication non-adherence prevalence rates ranging from 35% to 72%^[3,5,10,13,14]. For example a cross-sectional study of US outpatients with quiescent ulcerative colitis (UC) found that only 40% were adherent to maintenance mesalazine (mesalamine) therapy^[3]. In the UK, approximately 15% of patients fail to even redeem prescriptions at the pharmacy^[15]. Moreover, treatment non-adherence rates might vary considerably between countries. In Europe, a survey of 203 IBD patients revealed self-reported non-adherence rates ranging from 13% in France, to 26% in Italy, 33% in the UK and 46% in Germany. The overall non-adherence rate was 29% across Europe^[4], where non-adherence was defined as taking < 80% of prescribed medication. Similarly high rates of non-adherence were reported from Eastern Europe. Overall intentional non-adherence was reported by 38.9% of patients, and 18.6% of the patients at least once discontinued the treatment^[5]. In a Canadian study, UC diagnosis was associated with higher risk of non-adherence (OR: 4.42)^[16].

Significant differences may exist in children and adolescents, given the complex developmental challenges unique to childhood and adolescence, including the maturation of cognitive and behavioral patterns (e.g. health beliefs) that affect self-management. However, only a few studies have examined adherence rates in pediatric IBD, with the results indicating the prevalence of non-adherence ranging from 50% to 66%^[17,18]. Moreover, special attention should be paid to the method of assessment, because significant differences may be present in objective methods *versus* subjective self-report methods. In a recent paper, Hommel *et al*^[11] reported an objective non-adherence frequency of 38% for 6-MP/AZA and 49% for 5-ASA medications, while the subjective non-adherence frequency was reported to be as low as 6% for 6-MP/AZA and 3% for 5-ASA. In contrast, in a prospective, single-center study from Germany^[19] both objective (9.2%) and self-reported (7.1%) non-adherence rates were low in 65 adult Crohn's disease (CD) patients.

PREDICTIVE FACTORS FOR NON ADHERENCE AND CLINICAL CONSEQUENCES

Gender

Conflicting data are available on the role of gender in predicting non-adherence to medical therapy. Kane *et al*^[3] and Mantzaris *et al*^[20] related poor adherence with the male gender. In the study by Kane *et al*^[3] non-adherent patients were statistically more likely to be males (67% *vs* 52% in adherent patients). Gender interactions also proved relevant in a recent population-based study, in which young females proved to be less adherent than males^[17], while other studies could not find a significant difference^[5]. In addition there may be different factors affecting medication adherence in men and women. In the study by Ediger *et al*^[16] a diagnosis of ulcerative colitis (*vs* Crohn's disease) having high scores on the Obstacles to Medication Use Scale and a low level of the personality trait of agreeableness, were important predictors of low adherence in males. For women, important predictors of low adherence included an age younger than 30 years, having high scores on the Obstacles to Medication Use Scale, and having a low level of the personality trait of agreeableness. Immunosuppressant use was associated with high adherence in women.

Similarly, data are conflicting with regards to marital status, type of education, employment status, or type of disease. A higher education level and full time employment was also associated with a non-adherent patient behavior in some^[5,16,21], but not all, studies^[14].

Age and disease duration

Age seems to be an important factor, as younger patients tend to be less adherent than older patients^[10,18]. In a recent Italian study^[22] non-adherence was 43% in patients < 40 years old compared to 34% in those older than 40 years ($P = 0.041$, OR: 1.5, CI: 1.01-2.13). Recently, diagnosis and disease duration shorter than 5 years was also associated with significantly worse adherence (24% of the patients) than a longer-standing disease (15% of the patients; $P = 0.001$, OR: 2.1, CI: 1.30-3.39) in the same study. Moreover, non-adherence increased to 75% when both age (< 40 years) and disease duration (< 5 years) were considered. This may have to do with the fact that IBD primarily affects young individuals with greater personal and social goals, being busy at work, and having some degree of rebelliousness, but it may also be that a younger age is associated with a more recent diagnosis, with less experience with the burden of relapse or surgery. This was, however, not a universal finding^[5].

Phenotype, disease activity and surgery

In UC, Kane *et al*^[3] reported by means of univariate analysis, that male gender, being without a relationship partner, left-sided disease, and a history of more than four concomitant medications, were negatively associated with adherence. Conversely, being married, having a recent colonoscopy, and a greater extent of disease supported

adherence. A UK-based cross-sectional study, using data extracted from general practitioner (GP) clinical records, examined the usage of long-term aminosalicylate therapy in patients with UC^[13]. It was found that 38% of the patients with extensive colitis, 37% of the patients with left-sided colitis and 46% of those with proctitis did not take medications for maintenance therapy. This was not, however, confirmed in all studies^[5].

An association between medical adherence and complicated disease course in CD was reported by Spanish authors^[14]. Better adherence was significantly associated with a more complicated disease course (steroid dependency, steroid refractoriness, need for infliximab treatment, hospitalization, or surgery) in patients with short disease duration. Similarly, in a recent Hungarian study^[23], a higher number of previous surgeries was associated with improved self-reported adherence in patients with CD.

Active disease was associated with higher adherence, even if steroids were included in the treatment regimen in both CD and UC^[10]. In contrast, other studies reported low adherence rates after long-term remission^[3,22]. Very high non-adherence rates (74.3%) were reported for azathioprine in CD patients who were in long-term (> 48 mo) clinical remission^[20].

Moreover, a direct association between adherence and risk of relapse was reported in UC. Kane *et al*^[24] prospectively studied the risk factors associated with relapse among 99 patients who were in remission for more than six months and prescribed 5-ASA maintenance therapy. The clinical recurrence of UC was defined as four or more bowel movements per day. At a 12-mo follow-up, 19 of 86 patients had recurrent disease, 13 (68%) of whom were non-adherent. Patients who were non-adherent with medication had a greater risk of recurrence than adherent patients (OR: 5.5, 95% CI: 2.3-13). A Kaplan-Meier curve constructed to compare outcomes stratified by adherence status for 24 mo also showed that UC patients adherent to their 5-ASA therapy had a significantly greater chance of remaining in remission than those who were non-adherent (89% *vs* 39%; $P = 0.001$).

Drug type and dosing regimes

Non-adherence to therapy might also be due to the drug formulation causing discomfort (difficulty in swallowing tablets or using enemas) or side effects (pain, abdominal distension, or difficulty in retaining enemas). Most studies are consistent in finding that topical therapy with enemas, suppositories or foams is more likely to be associated with non-adherence than oral therapy. In an Italian study^[22], topical therapy with enemas was associated with significantly more non-adherence (68% of users) than oral therapy (40% of users; $P = 0.001$, OR: 0.25, CI: 0.11-0.60). Similarly, analyzing a national prescription-based database also showed that overall adherence to mesalazine was unexpectedly low and the rectal formulation was among the factors influencing non-adherence^[25]. Enemas were judged difficult to use, painful or to cause bloating, and were difficult to manage during working hours.

The association between the type of oral medications and non-adherence is more controversial. The undesirable

side effects of some medications (e.g. weight gain, cushingoid appearance, or immune suppression) and the complex treatment regimens for IBD patients (e.g. varying dosing schedules and pill quantities for each medication) are likely to disrupt adherence and effective management of this condition. Interestingly, some studies did not report a direct association. For example, in the study by Cervený *et al*^[5], the non-adherence rate at any time point was 40% on aminosalicylates, 29% in patients on systemic steroids, and 31% in patients on immunosuppressants in CD. Similar data were reported in UC, supporting the notion that adherence is influenced by multiple parallel factors, including gender, age, disease activity, and so on. Interestingly, the same study, using a factor analysis, reported a strong influence of adverse drug effects on adherence. Intentional non-adherent behavior due to adverse drug effects was the second most common cause reported during a patient interview. In addition, adverse drug effects were independently proven by factor analysis to affect a patient's confidence in treatment.

Reasons for non-adherence to oral therapy include multiple daily doses and a high number of concomitant medications. In the study by Kane *et al*^[3], besides being males, single, and having left-sided disease, non-adherent patients were statistically more likely to be taking four or more concomitant medications (60% *vs* 40%). Similarly, in the study of Shale and Riley^[21], in addition to being young, having education beyond the age of 16 years and being in full-time employment, being prescribed a 3-times-a-day regimen was identified as predictor for non-adherence. The need to take medicine during working hours ($P = 0.001$, OR: 3.5, 95% CI: 2.27-5.26), and multiple daily doses ($P = 0.045$, OR: 2.8, 95% CI: 0.99-7.70) were significantly associated with non-adherence in adults^[22], which was also confirmed by other studies^[20,21]. Similarly, adolescents whose regimen involved more than one daily medication administration had more adherence barriers^[26]. In addition, lack of time and medication side effects were also commonly reported barriers. Other adolescent-reported barriers included missing medication due to feeling well or discontinuing medication based on the belief that the medication was not working. In contrast, a recent retrospective cohort study suggests that adherence in UC patients is independent of drug formulation^[27]. Magowan *et al*^[27] used records from multiple US health plans to compare the refill prescription profiles of 1680 UC patients who had initiated 5-ASA therapy with one of four formulations: delayed-release mesalamine (Asacol), controlled-release mesalamine (Pentasa), sulfasalazine (Azulfidine), or balsalazide (Colazal). Upon initiation of treatment, the median daily dose and respective tablet/capsule load were 2.4 g (6 tablets) for delayed-release mesalamine, 4.0 g (16 capsules) for controlled-release mesalamine, 2.0 g (4 tablets) for sulfasalazine, and 6.75 g (9 capsules) for balsalazide. Comparison of the refill profiles over 12 mo, however, indicated that adherence in these patients was not affected by formulation type and/or dose regimen.

The use of once-daily treatment for improving medical compliance is further supported by a recent randomized, multicentre, investigator-blinded study of 362

patients who were randomised to receive mesalazine granules (Pentasa®) 2 g once daily or 1 g twice daily. It showed an 11.9% greater remission rate at one year (73.8% *vs* 63.6%, respectively) in the single daily dose group^[28]. Patient questionnaires showed significantly greater self-reported compliance ($P < 0.05$) and acceptability ($P < 0.001$) in the once-daily group. High compliance rates were reported for the once-daily MMX mesalazine and Salofalk® granules^[29,30]; therefore the effect is likely to be generic rather than compound-specific. Thus, new mesalazine formulations offer a simplified dose regime, resulting in presumably improved long-term compliance that can be considered an important advantage in the management of UC patients.

Patient-doctor relationship

The partnership between patient and the treating physician is of utmost importance in determining medical adherence, where effective patient-physician dialog is central to promoting patient adherence^[22]. Studies have also shown that the interaction between the patient and the physician has a huge impact on health outcomes and costs. Both the quality and quantity of the visits are important. Sewitch *et al*^[10] found an increased risk of intentional non-adherence to be associated with being treated by the same physician for more than one year, not scheduling another appointment, and greater total discordance between the patient and the physician. Similarly, a higher degree of intentional non-adherence in the study by López San Román *et al*^[31] was associated with greater patient depression and patient-physician discord. Patients trusted their physician less, and considered themselves to be less informed about their treatment.

A direct association between the total number of health care visits and medical adherence was proven in children with CD^[18]. In addition, patients under specialist care were significantly more likely to be taking an aminosalicylate than those definitely discharged to general practitioner's care in adults with UC^[13]. In contrast, however, a European cohort showed no correlation between the number of times an IBD patient had seen the physician and self-reported medication adherence rates^[4].

STRATEGIES TO IMPROVE ADHERENCE

A large body of evidence supports the key role of the physician-patient relationship in achieving higher patient medication-adherence rates. Psychology literature points out to using COPE principles as a way for physicians to improve their relationship with patients and optimize patient adherence to their medication. The COPE principles encompass the following: communicate with patients; obtain patient's commitment to therapeutic objectives; promote emotional/psychological/physical support as necessary; educate the patient and their family. In addition, trust in the physician and continuity of care by the same doctor are also important to patients.

In the everyday practice, the physician's willingness to allow patients to contribute input and become involved in their illness during the medical visits was suggested to

facilitate treatment decisions that are meaningful to both parties^[32]. The consultation style adopted by the physician is also an important factor in building the physician-patient relationship. Indeed, when physicians adopted a mutual, co-operative relationship, and exhibited less control dominance, a reported increase in patient adherence and satisfaction was observed.

During consultations, all factors affecting adherence need to be explored, including the patient's level of knowledge, belief systems and support environment, for example their network of family and friends.

Written and oral education (on the disease, management algorithm and medications) has been shown to increase adherence by approximately 6%-25%^[33]. Written information is more effective when verbally reinforced. In addition, a study of 69 patients with IBD demonstrated improved knowledge, patient satisfaction, and a positive trend towards greater adherence in patients who had undertaken the IBD education program (consisting of pamphlets and ad hoc physician education), which is the standard of care in many referral centers, compared with patients who received standard care^[34].

Guided self-management involving the provision of a shared set of guidelines containing action plans for the prevention of disease activity and/or symptom relief, have been used in the management of many chronic illnesses. A randomized, controlled study evaluating guided self-management programs in patients with IBD has demonstrated, a reduction in hospital visits without an increase in morbidity and greater confidence in the patient's ability to cope with IBD^[35]. Further studies are needed in order to assess whether such interventions will improve adherence to medication and clinical outcomes in patients with IBD. Furthermore, a special form of patient education could be successfully implemented using an internet-based patient education platform, as suggested by Elkjaer *et al*^[36].

Another approach that could be used to optimize patient adherence involves individualized therapy, where physicians review the patient's disease and therapeutic history, and identify which treatment(s) were effective/ineffective in the past to avoid prescribing the same unsuccessful medication. Simplification of treatment (e.g. reduced dosing frequency and the use of long-acting agents) and avoidance of unnecessary multiple concomitant medications is preferable, where feasible, and are associated with better adherence and improved clinical outcome^[3,21,24]. Furthermore, this patient review process could also provide predictive information on medication non-adherence behavior, and thus help identify those patients at high risk who might require longer consultation slots than those at low risk. Patients could also be prompted to take their medications via simple pill-taking cues, such as placing pills close to something they use daily, for example the toothpaste, breakfast table, glasses/contact lenses case, and so on. In addition, telephone support, postal reminders, and setting alarms on watches/mobile phones have been suggested. Nevertheless, combining education and behavioral interventions has been suggested to be the most effective approach to improving adherence.

CONCLUSION

Non-adherence is common in IBD and has been reported in 40%-60% of patients, especially those in remission and taking maintenance therapies for IBD. The economical impact of medication non-adherence, including absenteeism, hospitalization risk, and health care costs in chronic conditions, is enormous. The causes of medication non-adherence are multi-factorial, including forgetfulness, gender, new diagnosis, disease phenotype, patient-physician relationship, complicated dosing regimens, side-effect profile of the drugs, and treatment delivery methods. The associated factors may vary in each country because of the difference in the healthcare systems and the population. Moreover, a gold standard method to estimate the prevalence of non-adherence does not exist. Subjective assessment may underestimate adherence, while recent episodes of non-adherence may result in high non-adherence rates if measured by direct methods (e.g. drug concentration monitoring using blood and urine assays). Moreover, this latter strategy is expensive and inconvenient for patients, and only a limited number of drugs can be monitored in this way. Poor adherence may result in more frequent relapses, disabling disease course, and in ulcerative colitis, in increased risk for colorectal cancer. Improving medication adherence in patients is an important challenge for physicians. Understanding the different patient types, the reasons given by patients for non-adherence, simpler and more convenient dose regimens, dynamic communication within the healthcare team, self-management package incorporating enhanced patient education and physician-patient interaction and identifying the predictors of non-adherence, will help devise suitable plans to optimize patient adherence.

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REVIEW

Advances in diagnosis, treatment and palliation of cholangiocarcinoma: 1990-2009

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Abstract

Several advances in diagnosis, treatment and palliation of cholangiocarcinoma (CC) have occurred in the last decades. A multidisciplinary approach to this disease is therefore recommended. CC is a relatively rare tumor and the main risk factors are: chronic inflammation, genetic predisposition and congenital abnormalities of the biliary tree. While the incidence of intra-hepatic CC is increasing, the incidence of extra-hepatic CC is trending down. The only curative treatment for CC is surgical resection with negative margins. Liver transplantation has been proposed only for selected patients with hilar CC that cannot be resected who have no metastatic disease after a period of neoadjuvant chemo-radiation therapy. Magnetic resonance imaging/magnetic resonance cholangiopancreatography, positron emission tomography scan, endoscopic ultrasound and computed tomography scans are the most frequently used modalities for diagnosis and tumor staging. Adjuvant therapy, palliative chemotherapy and radiotherapy have been relatively ineffective for inoperable CC. For most of these patients biliary stenting provides effective palliation. Photodynamic therapy is an emerging palliative treatment that seems to provide pain relief, improve biliary patency and increase survival. The clinical utility of other emerging therapies such as transarterial chemoembolization, hepatic arterial chemoinfusion and high intensity intraductal ultrasound needs further study.

INTRODUCTION

Cholangiocarcinomas (CC) are malignant tumors originating from epithelial cells lining the biliary tree and gallbladder^[1]. Intrahepatic CCs (ICC) arise within the liver and extra-hepatic CCs (ECC) originate in the bile duct along the hepato-duodenal ligament. ICCs usually present as masses in the liver while jaundice is the most common presentation of ECCs. CCs are relatively rare tumors although their incidence is rising worldwide^[2,3]. Several advances in the diagnosis, therapy and palliation of patients affected by CC have occurred during the last decades. The aim of this article is to review the most recent high quality literature on this topic.

EVIDENCE ACQUISITION

We sought studies that reported at least one of the following aspects of CC: epidemiology, diagnosis, therapy (e.g. surgery, radiotherapy, chemotherapy, phototherapy), and palliation. Preference was given to randomized controlled trials (RCT) and prospective observational studies. For each of these topics we searched MEDLINE, Ovid MEDLINE In-Process, Cochrane Database of Systematic Reviews, Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, EMBASE, PubMed, National Library of Medicine Gateway by established systematic review methods (Jadad Scale for RCT controlled studies, Downs and Black checklist for observational studies^[4-6]). We further reviewed reference lists and articles from the authors' libraries. We limited our search to English-language articles published from January 1990 to June 2009. We then developed a comprehensive

Table 1 Summary of the terms used singly or in combination for evidence acquisition

Primary MeSH terms	Secondary MeSH terms (Epidemiology, diagnosis)	Secondary MeSH terms (treatment, palliation)
Cholangiocarcinoma(s)	Epidemiology	Hepatectomy
Adenocarcinoma(s)	Classification	Resection
Carcinoma(s)	Diagnosis	Therapeutic(s)
Bile duct neoplasm(s)	Differential diagnosis	Treatment outcome(s)
Biliary tract neoplasm(s)	Early diagnosis	Surgery
Common bile duct neoplasm(s)	Risk factor(s)	Transplantation
Liver neoplasm(s)	Diagnostic imaging	Biliary tract
Bile duct(s)	Magnetic resonance imaging	Surgical procedures
Common bile duct	Endosonography	Liver transplantation
Intrahepatic bile duct(s)	Ultrasonography	Organ transplantation
Extrahepatic bile duct(s)	Emission computed tomography	Clinical trial
Biliary tract disease(s)	Radionuclide imaging	Controlled clinical trial(s)
Bile duct disease(s)	Positron emission tomography	Randomized controlled trial(s)
	X-ray	Clinical trial (phase I)
	Computed tomography	Clinical trial (phase II)
	Biopsy (needle)	Clinical trial (phase III)
	Biopsy (fine needle)	Clinical trial (phase IV)
	Cytology	Drug therapy
	Cytodiagnosis	Chemotherapy
	Tumor markers (biological) antigen(s)	Adjuvant
	Carcinoembryonic antigen	Antineoplastic agent(s)
	Ca 19-9 antigen	Combined modality therapy
	Ca 125 antigen	Antineoplastic
	Endoscopic retrograde cholangiopancreatography	Combined chemotherapy protocols
	Cholangiography	Neoadjuvant therapy
	<i>In situ</i> hybridization	Radiotherapy
	Fluorescence <i>in situ</i> hybridization	Adjuvant embolization
	Nucleic acid hybridization	Portal vein embolization
	Computed assisted image processing	Drainage
		Cholestasis
		Obstructive jaundice

and current database to catalog the medical literature on CC. The evidence database for the catalog was assembled only for CC arising in the intra- and extra-hepatic bile ducts. Our review did not include the management of gallbladder cancer, as several other comprehensive articles had already covered this topic^[7-10]. To identify all potential papers, we searched medical subject headings reported in Table 1. Two authors (Aljiffry M and Molinari M) independently performed the selection of the articles based on the content of titles and abstracts. When in doubt, each article was reviewed entirely. The decision to include articles in this review was reached by consensus. For conciseness, a full list of search strategies, search results, and quality assessment for each included study are available on request from the corresponding author.

EPIDEMIOLOGY

The incidence of CC is rising in most countries and it is the second most common primary malignancy of the liver after hepatocellular carcinoma^[1]. In the USA, approximately 5000 new cases are diagnosed every year^[11] accounting for almost 3% of all tumors of the gastrointestinal tract^[12]. While the incidence of ICC is rising, the occurrence of ECC is trending down^[13,14] suggesting that different risk factors may be involved^[15]. Caution should be used when interpreting these results as misclassification bias may have influenced these observations^[2,16]. In fact, analysis of the Surveillance Epidemiology and End Results database from 1975 until

1999 has shown that most hilar tumors (more than 90%) were classified as ICC^[2,16] while ECC were often combined with gallbladder cancers^[2,13]. Nevertheless, evidence that ICC and ECC may be dissimilar tumors is supported by the recent discovery that, *in vitro*, they express diverse cellular proteins and have different cellular shape, doubling time, chromosome karyotype and chemosensitivity^[17]. Similarly, researchers from France showed that hilar CC (HCC) express higher levels of MUC5AC (60% *vs* 22%), Akt2 (64% *vs* 36%), CK8 (98% *vs* 82%), annexin (56% *vs* 44%) and less vascular epithelial growth factor (22% *vs* 78%) in comparison to ICC^[18]. These findings support the hypothesis that the heterogeneous protein and receptor expression of ECC and ICC may be due to different carcinogenic pathways^[17,18].

ICC

The estimated age-adjusted incidence rates of ICC in the USA has increased by 165% over the last thirty years (from 0.32 per 100 000 in 1975-1979 to 0.85 per 100 000 in 1995-1999^[2,19] accounting for 10% to 15% of all primary hepatic cancers^[20]. The average age at presentation is the seventh decade of life^[2] with a male to female ratio of 1.5^[20]. The mortality rate and incidence of ICC have parallel trends^[13] as age-adjusted mortality rate increased from 0.07 per 100 000 in 1973 to 0.69 per 100 000 in 1997^[21].

ECC

In the USA, the age-adjusted incidence of ECC has

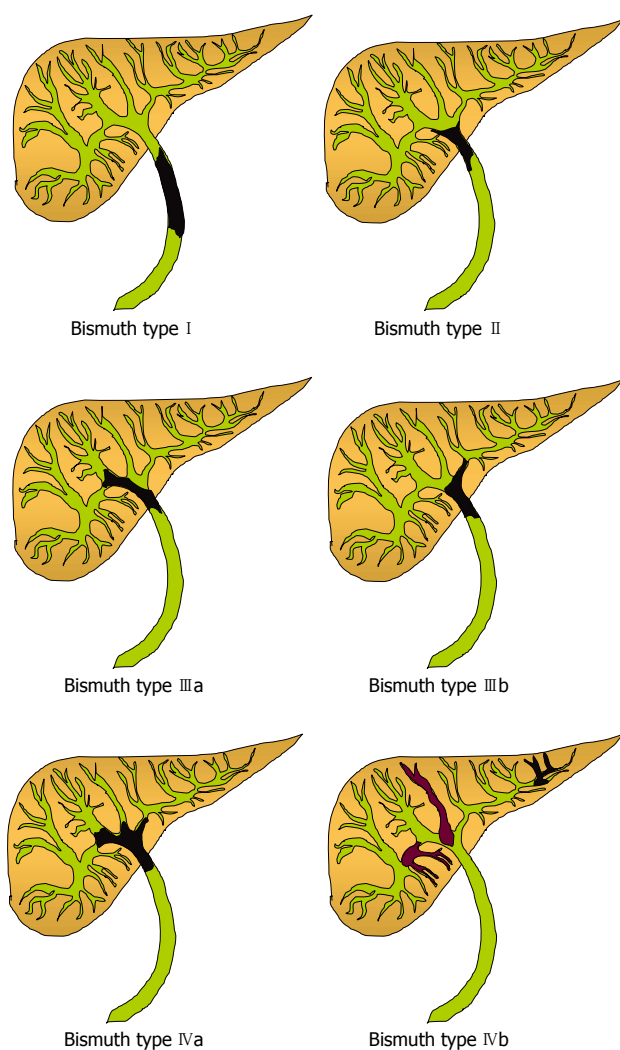


Figure 1 Bismuth's classification of cholangiocarcinomas. Type I: Cholangiocarcinoma is confined to the common hepatic duct; Type II: Cholangiocarcinoma involves the common hepatic duct bifurcation; Type IIIa: Cholangiocarcinoma affects the hepatic duct bifurcation and the right hepatic duct; Type IIIb: Cholangiocarcinoma affects the hepatic duct bifurcation and the left hepatic duct; Type IV: Cholangiocarcinoma is either located at the biliary confluence with both the right and left hepatic ducts involvement or has multifocal distribution.

decreased by 14% compared to data from two decades earlier. Currently it is 1.2 per 100 000 in men and 0.8 per 100 000 in women^[2,22]. Similarly to ICC, 65% of ECC present in the seventh decade of life^[22]. The mortality rate of ECC parallels its incidence and in the USA, the age-adjusted mortality rates for ECC declined from 0.6 per 100 000 in 1979 to 0.3 per 100 000 in 1998^[14,21].

CLASSIFICATION

Anatomical classification

ICCs arise within the liver parenchyma while ECCs involve the biliary tree within the hepatoduodenal ligament and gallbladder. ECCs are further divided into hilar or distal tumors. HCC, also called Klatskin tumors, are located within 2 cm from the bifurcation of the common duct and were described for the first time by Klatskin in 1965^[22]. Ten years later, Bismuth and Corlette proposed

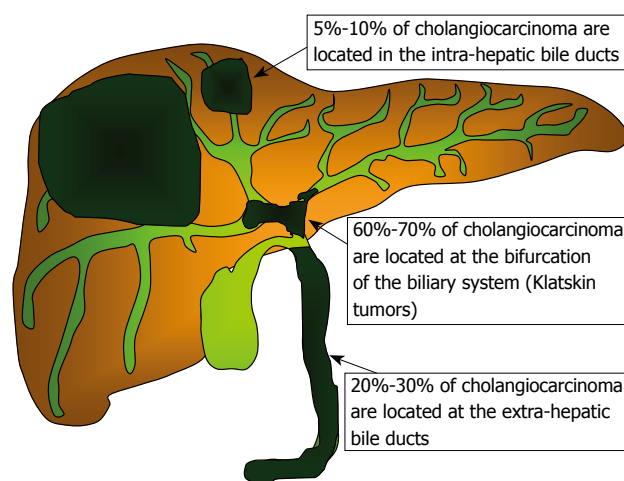


Figure 2 Anatomical presentation of cholangiocarcinomas. The majority of cholangiocarcinomas (60%-70%) present in the area of the biliary duct bifurcation and are called Klatskin tumors. The extra-hepatic bile duct is involved in 20%-30% of cases while intrahepatic cholangiocarcinomas represent 5%-10% of the tumors originating from the biliary system.

a clinical classification that stratifies these tumors by anatomical location (Figure 1)^[23]. Approximately 60% to 70% of CC are located in the hylum, 20% to 30% are ECC, and 5% to 10% are ICC (Figure 2)^[24,25].

Pathological classification

More than 90% of CC are well- to moderately-differentiated adenocarcinomas^[26,27] with tendency to develop desmoplastic reaction and early perineural invasion. Macroscopically, ICC may develop in solid masses, infiltrate periductal tissues, grow intraductally or have mixed characteristics. On the other hand, ECC develop nodular lesions, sclerosing strictures, or papillary growth patterns. Sclerosing CC are the most common^[28] while papillary adenocarcinomas are rare and associated with more favorable prognosis^[22].

RISK FACTORS FOR CHOLANGIOCARCINOMA

Only a minority of patients presenting with CC have known risk factors such as chronic biliary inflammation, cholestasis or congenital abnormalities (Table 2)^[29].

Primary sclerosing cholangitis (PSC)

In Western countries, PSC is the most important predisposing factor for CC^[30,31]. The cumulative annual risk of CC in patients with PSC is 1.5% per year after the development of jaundice^[32] and the prevalence of CC in patients with PSC ranges between 8% and 40%^[30,33,34]. A recent epidemiological study from the Netherlands has shown that the risk of CC for patients with PSC is 9% after 10 years from the time of the diagnosis^[35]. In patients with concomitant inflammatory bowel disease, the 10-year and 20-year risks for CC are 14% and 31% respectively, which are significantly higher than patients without inflammatory bowel disease (2% and 2%

Table 2 Known risk factors for cholangiocarcinomas

General risk factors
Old age (older than 65 years)
Smoking
Obesity
Diabetes
Post surgical
Biliary-enteric anastomosis
Chronic inflammatory diseases
Primary sclerosing cholangitis (PSC)
Hepatolithiasis (Oriental Cholangiohepatitis)
Hepatitis C
Hepatitis B
Human Immunodeficiency Virus (HIV)
Liver cirrhosis
Parasitic infections
<i>Opisthorchis viverrini</i>
<i>Clonorchis sinensis</i>
Congenital
Choledochal cysts
Caroli's disease
Congenital hepatic fibrosis
Chemical agents
Thorotrast
Dioxin
Nitrosamines
Asbestos
Medications
Oral Contraceptive Pills
Isoniazid

respectively; $P = 0.008$)^[35]. Individuals with PSC frequently develop CC at younger age (30-50 years) compared to the general population (60-70 years)^[50,32]. The diagnosis of CC in this group is challenging because clinical presentation and radiological findings of CC and PSC are similar. As a result, most cases of CC complicating PSC are detected at advanced stages and have poor prognosis^[36]. Predictive factors of CC in PSC patients are: sudden progressive jaundice, unintentional weight loss, marked dilation of bile ducts proximal to biliary strictures, serum level of Ca 19-9 tumor marker above 100 U/mL, and presence of cellular dysplasia on cytological specimens obtained by brushing of the biliary ducts^[37].

Parasitic infections

Infestation with liver flukes (*Opisthorchis viverrini* and *Clonorchis sinensis*) has been strongly associated with an increased risk of CC in South-East Asia^[3,38,39]. In areas where *Opisthorchis viverrini* is endemic, the adjusted prevalence for CC by age and gender is as high as 14%^[40]. The pathophysiology causing CC in these patients is not completely understood, however it is hypothesized that parasites colonize the biliary system causing chronic inflammation and predisposing to malignant transformation.

Intrahepatic biliary stones (Hepatolithiasis)

Oriental cholangiohepatitis (also known as recurrent pyogenic cholangiohepatitis) has a prevalence of 20% in South-East Asia^[41] and almost 10% of affected patients develop ICC^[42-45]. Recurrent episodes of cholangitis aggravate the chronic inflammatory process that persists

between flare ups. Risk factors associated with CC in these patients are: age over 40 years, long history of hepatolithiasis, unintentional weight loss, increasing serum alkaline phosphatase, decreasing serum albumin, and serum CEA tumor marker above 4.2 ng/mL^[46].

Congenital biliary cystic diseases

Patients with choledochal cysts have low risk for CC if the cyst is excised early in their life^[46]. On the other hand, the incidence of malignant degeneration is between 10% and 20% if the cyst is not excised by the age of 20 years^[28,47]. The mechanism of malignant transformation is not totally understood although biliary stasis and reflux of pancreatic secretions are suspected of causing neoplasia through chronic inflammation^[48]. CC can occur years after resection of the cyst suggesting that there might be a genetic defect predisposing to tumors of the biliary system^[49].

Liver cirrhosis and viral infections

The risk of developing CC in cirrhotic patients is ten-fold higher than the general population (0.7% *vs* 10.7%)^[2,50]. Among patients with CC in the USA, the prevalence of hepatitis C viral infection (HCV) was found to be four times higher than the general population (0.8% *vs* 0.2%)^[20]. These results have been confirmed in Italy^[51], in Taiwan^[52] and in Japan where HCV and viral hepatitis B (HBV) infection were detected in 23% and 11.5% of CC compared to 6% and 5.5% of controls, respectively, with cumulative rates 1000-times greater than the general population^[53]. Similar results were recently confirmed in a case-control study performed in China, where researchers found that at multivariate analysis, significant risk factors for the development of ICC were hepatolithiasis (adjusted OR: 5.7; 95% CI: 1.9-16.8) and HBV infection (adjusted OR: 8.8; 95% CI: 5.9-13.1)^[15]. A large epidemiological study from the United States validated that HCV infection is a risk factor for ICC (hazard ratio 2.55; 95% CI: 1.3-4.9) but not for ECC (hazard ratio 1.5; 95% CI: 0.6-1.85)^[54]. Although human immunodeficiency virus (HIV) does not cause cirrhosis *per se*, 0.5% of patients infected with HIV have been found to have CC in comparison to 0.1% among controls, confirming previous observations which suggested that chronic viral infections might predispose to neoplastic transformation of some cell lines^[20].

Chemical agents

Several compounds have been suspected of causing CC. Thorotrast (thorium dioxide) needs a special mention because it was used as a radiological contrast in the period between 1920-1950 and was found to increase the risk of CC up to 300-times in comparison to the general population^[16,55]. Because of its long biological half-life (400 years), the latency period of thorotrast-induced CC ranges between 16 to 45 years^[56], with the highest incidence between 20 to 30 years after exposure^[57]. A few studies have shown an association between CC and other chemical agents such as asbestos^[58], vinyl chloride^[59] and nitrosamines^[60]. Medications such as isoniazide^[61] and first generation of oral contraceptives^[62] are also suspected of increasing the risk of CC.

Other risk factors

Tobacco smoking is weakly associated with CC in the general population^[20] while it appears to be a strong risk factor for PSC patients^[63]. Other predisposing factors for CC are diabetes, obesity, presence of bile duct adenomas and biliary papillomatosis^[64,65]. Although there is no evidence that endoscopic sphincterotomy increases the risk of CC, biliary-enteric bypasses may do so^[66].

DIAGNOSIS

Clinical presentation

CCs are usually clinically silent or associated with nonspecific symptoms in early stage (Table 3)^[67,68]. ICCs are often diagnosed by imaging tests, and rarely during physical exams, as asymptomatic hepatic masses^[26]. On the other hand, ECC usually present with painless jaundice^[69] and symptoms related to biliary obstruction such as itching, clay-colored stool and hyperpigmented urine^[69]. Only 10% of cases present with ascending cholangitis^[70]. Jaundice is usually persistent and progressive while intermittent biliary obstruction may be observed in patients with papillary lesions that cause a ball-valve effect^[71]. Physical examination of patients with CC may reveal hepatomegaly, palpable gallbladder (Courvoisier sign), or signs of portal hypertension due to portal vein thrombosis secondary to tumor invasion or compression^[33,69].

Laboratory investigations

Serum biochemical tests usually support the clinical suspicion of CC but they are rarely diagnostic. Jaundice occurs only if there is obstruction of the two main intra-hepatic biliary ducts or common bile duct. In these circumstances, elevation of the serum levels of bilirubin and markers of biliary epithelial injury, such as alkaline phosphatase (ALP) and gamma glutamyltransferase (GTT)^[33], are common^[72,73]. On the other hand, in the presence of unilateral intrahepatic biliary obstruction, elevation of ALP or GTT may be present without increase in the serum bilirubin level^[33]. Other abnormal laboratory findings include hypoalbuminemia and prolonged prothrombin time, which reflect the combination of diminished hepatic synthetic function, cachexia and malabsorption of vitamin K^[33].

Serum tumor markers

Several tumor markers may support the diagnosis of CC, although none of them is sensitive enough to be used for screening purposes. The most commonly used markers are carbohydrate antigen (Ca 19-9) and carcinoembryonic antigen (CEA)^[73]. These tumor markers are not very specific as they can be elevated in the presence of other malignancies (e.g. pancreas and stomach) and with benign conditions such as cholangitis and hepatolithiasis^[73-75]. In patients without PSC, serum Ca 19-9 values above 100 U/mL have a sensitivity of 53% and specificity of 75%-90% for the diagnosis of CC^[74]. In patients with PSC, serum Ca 19-9 levels above 100 U/mL have sensitivity of 75%-89% and specificity of 80%-86% for the diagnosis

Table 3 Presenting symptoms of patients affected by cholangiocarcinomas

Symptoms	Percentage (%)
Jaundice	84
Weight loss	35
Abdominal pain	30
Nausea and vomiting	20
Fever	10

of CC^[75-77]. In a recent study from the Mayo Clinic, the optimal cutoff value for serum Ca 19-9 in patients with PSC was 20 U/mL which provided a sensitivity of 78%, specificity of 67%, positive predictive values of 23% and negative predictive value of 96%^[78]. Serum Ca 19-9 combined with either ultrasonography, computed tomography, or magnetic resonance imaging provided a sensitivity of 91%, 100% and 96% respectively for CC diagnosis^[78]. The levels of Ca 19-9 seem to correlate with the stage of the disease. Patel *et al*^[72] reported that the sensitivity of Ca 19-9 above 100 U/mL for the diagnosis of CC in patients with resectable tumors was 33% compared to 72% in patients with unresectable tumors. Using more than one tumor marker for patients with PSC may improve the detection rate of CC. In one study, using Ca 19-9 levels above 180 U/mL in combination with CEA levels above 5.2 ng/mL had a sensitivity of 100% and a specificity of 78.4%^[79]. Several new markers are currently being investigated. The human mucin 5, subtypes A and C (MUC5AC) are the most promising for future clinical use with sensitivity and specificity of 71% and 90%, respectively^[80].

Imaging modalities

Imaging modalities are essential for the diagnosis and treatment planning of patients with CC^[73].

Abdominal ultrasound (US)

US is usually the initial imaging test performed to evaluate patients with biliary obstruction^[81]. The sensitivity and accuracy of US for ECC diagnosis are 89%^[82] and 80%-95%, respectively^[83,84]. On the other hand, ICC are difficult to distinguish from other solid intra-hepatic masses as they lack specific US features^[83,84]. The use of duplex US with color Doppler technology is helpful in assessing portal venous invasion and hepatic parenchymal involvement. In a small series of patients with HCC, duplex US detected portal vein invasion correctly in 86% of patients^[85]. In a larger study, duplex US was 93% sensitive and 99% specific for detecting portal vein involvement^[86]. As the sensitivity and specificity of US are operator-dependent, most patients with suspected CC undergo further imaging modalities to confirm and stage suspected tumors^[87]. The sensitivity of US improves significantly in the presence of elevated serum tumor marker Ca 19-9^[80]. Serum level of Ca 19-9 above 20 U/mL in patients with PSC has been shown to increase the diagnostic sensitivity of abdominal US up to 91%, with specificity of 62%, positive predictive value of 23%, and negative predictive value of 98%^[80].

Computed tomography (CT)

Triple-phase CT scan is widely used to diagnose and stage CC^[88] as it provides valuable information regarding local spread, vascular invasion, lymph node involvement and presence of distant metastases^[89,90]. On CT scans, ICC usually present as hypodense lesions with irregular margins on initial images and a variable degree of delayed venous phase enhancement^[86]. These characteristics have been shown to correlate with prognosis as hyperattenuating CC have a more aggressive behavior^[91]. Other CT findings of ICC include dilatation and thickening of the peripheral intra-hepatic bile ducts and liver capsular retraction^[92]. ECC may be seen as a focal thickening of the ductal wall with various enhancement patterns^[93]. However, in many cases of ECC, visualization of the neoplasms is not definitive because they are too small to be detected. More recent studies^[92,94] have shown that modern contrast-enhanced multidetector row computed tomography was 78.6%-92.3% accurate for the diagnosis of ECC, although there was a strong tendency to underestimate the longitudinal extension of the tumor (77.8%) in comparison with the pathological results of the excised specimens^[95,96]. Four-channel multidetector-row CT has been shown to correctly diagnose hepatic artery invasion with 100% sensitivity and 90% specificity and portal vein invasion with 92.3% sensitivity and 90.2% specificity^[96]. Regular enhanced CT can be extremely useful by showing indirect signs of ECC such as biliary ductal dilatation and hepatic lobar atrophy. Atrophy of one hepatic lobe could be associated with hypertrophy of the opposite lobe, a condition known as the atrophy-hypertrophy complex. This phenomenon is seen when CC obstruct the biliary outflow of a single lobe and invade the ipsilateral portal vein causing compensatory hypertrophy of the opposite hepatic lobe^[97]. The sensitivity of triple-phase helical CT in the detection of HCC is in the range of 90% to 100%^[92,98] and it is even more sensitive in detecting ICC greater than 1 cm in size^[90]. These results show a marked improvement in the diagnostic yield of CT compared to previous reports in which the tumor detection rate was only 60%^[99]. CT is also useful for assessing the vascular and lymph node status of patients affected by CC. In a series of 55 patients with HCC, CT accurately predicted portal vein invasion, arterial invasion, and lymph node involvement in 86%, 93%, and 84% of patients, respectively^[100]. The overall accuracy of CT for determining resectability of CC is in the range of 60% to 85%^[90,100,101]. Recently, CT cholangiography (CTC) has been shown to be a promising modality for delineating the biliary tree. In a large study, CTC was superior to conventional CT or US and equal to endoscopic retrograde cholangiopancreatography (ERCP) for the diagnosis of HCC^[102]. In another smaller study, the sensitivity and specificity of CTC for malignant biliary obstruction were both 94%^[103]. One of the limitations of CTC is that optimal imaging quality depends on the secretory function of the liver^[104]. For patients affected by PSC, the combination of tumor serology (serum level of Ca 19-9 above 20 U/mL) and

contrast-enhanced abdominal CT scan has been shown to improve the diagnostic sensitivity (100%), specificity (38%), positive predictive value (22%) and negative predictive value (100%) of the test^[80].

Magnetic resonance imaging (MRI) and Magnetic resonance cholangiopancreatography (MRCP)

MRI with concurrent MRCP can provide three-dimensional reconstruction of the biliary tree by using magnetic resonance technology^[105]. Multiple studies have demonstrated the utility of MRCP in evaluating patients with CC^[106,107]. MRCP has diagnostic accuracy comparable to invasive cholangiographic techniques such as ERCP or percutaneous transhepatic cholangiography (PTC)^[108-111]. A further advantage of MRCP over invasive cholangiographies is that it does not require biliary instrumentation^[112]. Therefore, MRI along with MRCP is considered the radiological modality of choice for evaluating patients with suspected CC^[113]. MRCP/MRI allows definition of the anatomy and extent of CC within the hepatobiliary system^[108,110,114] vascular invasion, local lymphadenopathy and distant metastases^[108,113,115]. Ideally, MRCP should be performed before decompressing the biliary tree^[86]. ICC appear as a hypointense lesion on T1- and hyperintense on T2-weighted images with pooling of contrast within the tumor on delayed pictures as seen with CT^[116,117]. On MRCP, ECC may appear as extrahepatic lesions with similar signal intensity of ICC on both T1- and T2-weighted images, in addition to proximal biliary dilatation^[106,116]. A meta-analysis of 67 studies (4711 patients) evaluating MRCP performance in patients with suspected biliary diseases showed an overall sensitivity of 88% and specificity of 95%^[107]. In a series of 99 patients with HCC, MRCP accurately determined the longitudinal extension of the tumor in 88% of patients^[118]. In another smaller study, MRCP predicted the extent of biliary ductal involvement in 96% of cases with malignant hilar obstructions^[115]. Regarding surrounding structures, MRI has been shown to have 66% accuracy for detection of lymph node metastases^[119], 78% sensitivity and 91% specificity for portal vein invasion, 58%-73% sensitivity^[120] and 93% specificity for arterial invasion^[121]. In a comparative study the relationship of ICC to the vessels and surrounding organs was more easily evaluated on CT compared to MRI^[89]. For patients affected by PSC and CC, the diagnostic capacity of MRI is enhanced by the presence of serum tumor marker Ca 19-9 above 20 U/mL; as a recent study has shown that the sensitivity of the test in this case was 96%, specificity 37%, positive predictive value 24%, and negative predictive value 98%^[80].

Cholangiography

ERCP and PTC provide dynamic images but require invasive access to the biliary system. Both techniques can detect biliary abnormalities and determine the location and extent of ECC within the biliary tree. The choice between PTC and ERCP is generally dictated

by the availability of local expertise and the anatomical characteristics of the tumor^[69]. In patients with complete biliary obstruction, ERCP often cannot assess the proximal biliary tree while PTC cannot assess the distal extent of the tumor^[33,122].

The sensitivity and specificity of cholangiography range between 75%-85%, and 70%-75%, respectively^[110,116] with accuracy of 95%^[123]. Recent data have shown that in the presence of PSC, the association of an elevated level of serum Ca 19-9 increases the diagnostic utility of ERCP as its sensitivity was 91%, specificity 69%, positive predictive value 42%, and negative predictive value was 96%^[80]. A drawback of these invasive procedures is the risk of complications such as post-ERCP pancreatitis (4%-10%)^[123], bacteriobilia (30%-100%)^[73], bleeding, sepsis, vascular injury and death^[124]. On the other hand, ERCP and PTC have the advantage of providing brush cytology and bioptic specimens that can confirm the diagnosis of CC. The sensitivity of biopsy and brush cytology for diagnosing CC has been low due to the desmoplastic reaction associated with the tumor which is characterized by the presence of few malignant cholangiocytes within an extensive fibrous stroma^[11]. In a large prospective study, the sensitivity of routine cytology varied from 9%-24% and the specificity varied from 61%-100% with a high rate of inter-pathologist variation; the best diagnostic yield was obtained when the pathologists were aware of the patient's clinical condition^[125]. This was recently confirmed by a study from the Mayo Clinic which showed that in patients affected by PSC, the simultaneous presence of an elevated serum tumor marker level (Ca 19-9 above 20 U/mL) increased the sensitivity (50%), specificity (97%), positive predictive value (86%) and negative predictive value (88%) of cytological specimens^[80]. In another study, repeated brushing appeared to be a valuable strategy to improve the sensitivity of cytological analysis up to 44%^[126]. Endoscopic transpapillary forceps biopsies had a diagnostic sensitivity of 52% and a specificity of 100%^[127]. Advanced cytologic techniques, including digitized image analysis (DIA) and fluorescence *in situ* hybridization (FISH), have been recently used to increase the sensitivity of cytology^[128] especially in patients with PSC^[80]. The DIA technique quantitates nuclear DNA *via* special stains to assess the presence of aneuploidy, whereas FISH analysis detects chromosomal polysomy by using fluorescent probes. In a prospective study, DIA increased the sensitivity from 18% to 39% but decreased the specificity from 98% to 77%^[129]. In another comparative study, FISH increased the sensitivity from 15% to 34% compared to routine cytology, with similar specificities (91% for FISH and 98% for routine cytology)^[130]. For patients with PSC, the presence of elevated serum level of Ca 19-9 (above 20 U/mL) increases the diagnostic capacity of DIA and FISH; as a recent study measuring these parameters has shown that their sensitivity was 57% and 86%, specificity 94% and 83%, positive predictive value 89% and 80%, and negative predictive value was 74% and 88%, respectively^[80]. The use of peroral cholangioscopy

(POCS) or choledochoscopy has been shown to improve the diagnostic capacity of ERCP by directing tissue sampling. Fukuda *et al*^[131] reported a sensitivity of 100% and a specificity of 87% for diagnosing the etiology of bile duct strictures by adding POCS to ERCP. At this moment, the availability of POCS is limited to a few centers due to lack of expertise and the high costs of instrumentation. The introduction of new technologies such as SpyGlass[®], a single operator peroral cholangiopancreatography, has eliminated the need for two ERCP operators and has the potential of becoming an important tool to improve the diagnostic capacities of endoscopic techniques, and it is currently under investigation^[132-134].

Endoscopic ultrasound (EUS)

EUS is performed by using high frequency ultrasound probes placed on the endoscope. EUS has the advantage of interrogating tissues and organs in direct proximity to the stomach and duodenum, increasing the ability to detect abnormalities that would not be easily identified by percutaneous approach. In a prospective study of patients with suspected CC, EUS had a diagnostic sensitivity of 79% and specificity of 62%^[111]. This was confirmed in a recent meta-analysis where EUS had sensitivity and specificity of 78% and 84%, respectively^[135]. Two of the most attractive features of EUS are the ability to perform direct-guided fine needle aspirations (FNA) of the tumors in patients with negative cytology or the ability to sample enlarged lymph nodes for preoperative staging^[136-138]. However, caution should be applied in patients with potentially curative CC as this approach has some risk of peritoneal seeding^[65,130]. A recent prospective study evaluated the diagnostic yield of EUS-guided FNA of suspected HCC in potentially operable patients with negative brush cytology. The study showed sensitivity and specificity of 89% and 100%, respectively, and changed the preplanned surgical approach in 61% of patients^[136]. In another prospective study, EUS-guided FNA of suspected CC reported a diagnostic sensitivity of 86%, with a specificity of 100%. In the same study, EUS-guided FNA had a positive impact on the treatment management of 84% of patients^[139].

Intraductal ultrasound (IDUS)

IDUS is performed by using high frequency US probes placed into the common bile duct under ERCP guidance^[140]. Malignant biliary strictures often appear on IDUS as a hypoechoic infiltration of the ductal wall with irregular margins^[141,142]. In a prospective study of 62 patients with biliary strictures, IDUS had a diagnostic sensitivity of 90% and specificity of 93%^[143]. In another study by Stavropoulos *et al*^[144], IDUS increased the diagnostic accuracy of ERCP from 58% to 90% in a series of patients with biliary strictures and no mass detected on CT.

Positron emission tomography (PET)

PET is a non-invasive imaging modality that provides

functional images by detecting radiotracer 18F-fluorodeoxyglucose (FDG) uptake in neoplastic cells^[145]. Currently it is considered a standard modality for the staging of many malignancies^[146]. In the last decade, integrated PET and CT imaging systems (PET/CT) have combined the ability to obtain anatomical and functional images^[146,147]. PET and PET/CT are proven to be useful in the diagnosis and staging of CC. In a recent study, PET showed sensitivity and specificity of 90% and 78% respectively^[148]. In another study by Anderson *et al.*^[149], PET had sensitivity of 85% for CC measuring at least 1 cm in size although its sensitivity was only 18% for infiltrating CC. These values were confirmed by Kluge *et al.*^[150] who reported sensitivity of 92% and specificity of 93% for the detection of any type of CC by PET scan. A more recent study has shown that the sensitivity of PET/CT is correlated with the stage of CC as the sensitivity of the study was 25% for T2 tumors, 70% for T3 tumors and 66.7% for T4 tumors^[151]. The rate of detecting distant metastases by PET and PET/CT in patients with CC is in the range of 70% to 100%, while the detection of regional lymph node metastases is only about 12%^[152]. The sensitivity and specificity of PET/CT for detecting lymph node metastasis and distant metastasis were 41.7% and 80%, and 55.6% and 87.5%, respectively^[146]. Another study from the Memorial Sloan Kettering Cancer Center has shown that PET/CT had an overall sensitivity for identifying the primary tumor of 80% (78% for CC and 86% for gallbladder cancer) and changed management in nearly a quarter of all patients^[153]. PET has been shown to be useful in monitoring tumor response to treatment. In a small series by Chikamoto *et al.*^[154], PET had a sensitivity of 80% for detecting local recurrence after resection in patients with HCC. One of the limitations of PET is that patients with biliary inflammatory conditions such as PSC or cholangitis may have false positive results^[152,155] while patients with mucinous CC may have falsely negative scans due to poor uptake of FDG^[155].

Optical coherence tomography (OCT)

OCT is a new technique that produces cross-sectional images using infrared light. Preliminary studies have demonstrated the ability of OCT to generate high resolution images of the biliary tree that correlate with histological findings^[156,157]. OCT has the potential to identify early CC^[104] but it is not widely available except in a few centers. Therefore the role of OCT in the diagnostic workup of CC is not yet established.

Non diagnostic work-up

Non-diagnostic cytology or biopsy results should not rule out CC in the presence of appropriate clinical and radiological findings^[73]. In the absence of other explainable causes of biliary strictures, patients should be considered to have CC and treated as such, accepting that 10% to 15% will have benign lesions on final pathology^[158,159]. For high risk patients, no surveillance or screening programs have been validated. Some authors

advocate annual follow up with non-invasive modalities (tumor markers and radiological tests), reserving invasive methods only when cytology and bioptic specimens or stenting are indicated^[160].

TUMOR SPREAD

Understanding the patterns of spread of CC is essential for staging and treatment planning. CC can spread along biliary ducts, invade perineural and vascular tissues, spread directly into adjacent structures, invade lymph nodes or develop distant metastasis. Longitudinal extension of CC consists of mucosal (superficial) or submucosal (invasive) infiltration depending on the tumor growth pattern. Mucosal extension is predominantly seen with papillary (intraductal) and nodular (mass-forming) tumors, while submucosal extension is mainly seen with sclerosing (infiltrating) tumors^[161]. The length of longitudinal extension is determined by the type of invasion, with a mean length of 6-10 mm for the submucosal spread and 10-20 mm for the mucosal spread^[162]. Therefore, a gross surgical margin of more than 1 cm in the infiltrating type and more than 2 cm in the papillary and nodular types is recommended to achieve negative microscopic resection margins. One of the special characteristics of CC is the presence of perineural invasion that is seen in about 75% of cases^[163,164]. Perineural invasion is a prognostic factor for poor survival^[164,165]. In a retrospective review by He *et al.*^[164], the 5-year survival rate was 47% for patients without perineural invasion compared to 13% for those with perineural invasion. HCC can spread directly into the hepatic parenchyma and the hepatoduodenal ligament where the proper hepatic artery and the portal vein are in close proximity to the bile duct, while distal ECC may directly infiltrate into the pancreas or the duodenum^[166]. Up to 80% of HCC have extension into the liver parenchyma^[166,167] by direct infiltration or by longitudinal extension along the biliary ducts^[168]. The latter mechanism explains the caudate lobe involvement by HCC and tumors involving the left hepatic duct^[169]. Hence, the practice of partial hepatectomy with caudate lobectomy for the surgical treatment of patients with hilar tumors is associated with improved survival^[168]. Tumors at the biliary confluence involve the portal vein in 30% of cases and often result in hepatic lobar atrophy^[166,167]. The significance of portal vein involvement in patients' survival is controversial. Some studies have shown that tumor invasion of the portal vein has a negative impact^[170,171] while other investigators reported opposite findings^[166]. This is most likely due to the fact that patients with portal vein invasion may tolerate more extensive surgical resections as the contralateral lobe becomes hypertrophied, therefore decreasing the risk of perioperative mortality and enhancing the chances of negative resection margins. Lymph node metastases usually involve the regional hilar nodes and to a lesser extent the para-aortic lymphatic nodes^[172]. The prevalence of lymph node involvement is approximately 45% for all CC with distal ECC having the highest incidence of nodal metastases^[68,172]. Several studies have confirmed

Table 4 AJCC staging of ICC

Stage	Tumor	Node	Metastasis
I	T1	N0	M0
II	T2	N0	M0
III A	T3	N0	M0
III B	T4	N0	M0
III C	Any T	N1	M0
IV	Any T	Any N	M1

T1: Solitary tumor without vascular invasion; T2: Solitary tumor with vascular invasion or multiple tumors none > 5 cm; T3: Multiple tumors > 5 cm or tumor involving a major branch of the portal or hepatic vein(s); T4: Tumor(s) with direct invasion of adjacent organs other than the gallbladder or with perforation of visceral peritoneum; N0: No regional lymph node metastasis; N1: Regional lymph node metastasis; M0: No distant metastasis; M1: Distant metastasis. AJCC: American Joint Committee on Cancer; ICC: Intrahepatic cholangiocarcinoma.

that lymphatic tumor involvement is an important prognostic factor. Survival rates for patients undergoing surgical resection with positive lymphatic invasion at 5 years are 10% to 15% in comparison to 30% to 40% for patients without lymph node metastasis^[164,172]. Presence of distant metastases (e.g. lung, bone, peritoneal, distant lymph nodes) is observed in 30% of patients at the time of diagnosis and is associated with survival of only a few months^[166].

STAGING

ICC and ECC are staged differently.

ICC

ICC are classified as primary liver malignancies in the new American Joint Committee on Cancer (AJCC) staging system, also known as the TNM staging (Table 4)^[173]. The AJCC staging system for primary liver tumors was based on data provided by patients affected by hepatocellular carcinomas and therefore is not sufficiently accurate for ICC^[174]. A new staging system for ICC was proposed by Nathan *et al*^[174] based on the number of tumors, vascular invasion, lymph node status and presence of metastatic disease. The presence of multiple tumors may be indicative of satellite neoplastic deposits or intrahepatic metastatic disease from hematogenous or lymphatic spread; similarly to vascular and lymph node invasion, it is associated with poor survival.

ECC

Giving the proximity of ECCs to the portal vein and hepatic artery, the goal of staging is to determine the local extent of the disease as it predicts resectability and the extent of the resection. The AJCC staging system for ECC (Table 5)^[173] is based on pathological data useful in identifying the patients' prognosis but with little applicability for assessing the feasibility of surgical treatment^[174]. Bismuth-Corlette classification for HCC is useful for describing the tumor location and its spread within the biliary tree but it is not predictive of resectability. The Memorial Sloan-Kettering Cancer

Table 5 AJCC staging of ECC

Stage	Tumor	Node	Metastasis
0	Tis	N0	M0
I A	T1	N0	M0
I B	T2	N0	M0
II A	T3	N0	M0
II B	T1-T3	N1	M0
III	T4	Any N	M0
IV	Any T	Any N	M1

Tis: Carcinoma *in situ*; T1: Tumor confined to the bile duct histologically; T2: Tumor invades beyond the wall of the bile duct; T3: Tumor invades the liver, gallbladder, pancreas, and/or unilateral branches of the portal vein (right or left) or hepatic artery (right or left); T4: Tumor invades any of the following: main portal vein or its branches bilaterally, common hepatic artery, or other adjacent structures, such as the colon, stomach, duodenum, or abdominal wall; N0: No regional lymph node metastasis; N1: Regional lymph node metastasis; M0: No distant metastasis; M1: Distant metastasis.

Table 6 Proposed T-Stage criteria for hilar cholangiocarcinomas (MSKCC)

Stage	Criteria
T1	Tumor involving biliary confluence with or without unilateral extension to second-order biliary radicles
T2	Tumor involving biliary confluence with or without unilateral extension to second-order biliary radicles and ipsilateral portal vein involvement with or without ipsilateral hepatic lobar atrophy
T3	Tumor involving biliary confluence with bilateral extension to second-order biliary radicles; or unilateral extension to second-order biliary radicles with contralateral portal vein involvement; or unilateral extension to second-order biliary radicles with contralateral hepatic lobar atrophy; or main or bilateral portal vein involvement

Center (MSKCC) has proposed a staging system known as T-stage criteria^[174]. The MSKCC staging system is based on the location and extent of ductal involvement, presence or absence of portal vein invasion, and presence or absence of hepatic lobar atrophy irrespective of metastases or lymph node status (Table 6). The MSKCC staging system for HCC correlates with resectability and survival, as 59% of T1 lesions are resectable with median survival of 20 mo compared to 0% resectability for T3 lesions with a median survival of only 8 mo^[174].

THERAPY

Surgical resection

Tumor resection is the only potential cure for CC and the median survival of patients with unresectable disease is 6 to 12 mo^[26]. All patients with resectable ICC and HCC, and the majority of patients with ECC, require partial hepatectomy to increase the chances of negative resection margins. Preoperative patients' evaluation includes an extensive assessment of their fitness for major surgery, the absence of any metastatic disease and the possibility of resection margins free from cancer^[175]. If any of these conditions are not satisfied, surgical therapy is not indicated and palliative modalities should be recommended.

Table 7 Criteria for unresectability of HCC

Local tumor invasion
Bilateral hepatic duct involvement up to secondary biliary radicles
Encasement or occlusion of the main portal vein
Unilateral tumor extension to secondary biliary radicles with contralateral portal vein or hepatic artery encasement or occlusion
Hepatic lobar atrophy with contralateral portal vein or hepatic artery encasement or occlusion
Hepatic lobar atrophy with contralateral tumor extension to secondary biliary radicles
Metastatic disease
Lymph node metastases beyond the hepatoduodenal ligament (N2 lymph nodes) ¹
Distant metastasis (e.g. lung, liver, peritoneal)

¹Peripancreatic, periduodenal, periportal, celiac, or superior mesenteric lymph nodes.

Preoperative patient preparation

Many patients are not considered surgical candidates because of the presence of comorbidities or advanced age. A patient's performance status, nutritional conditions, and comorbidities need to be carefully evaluated before considering surgery^[176]. A retrospective review of patients with resected HCC showed that the presence of preoperative serum albumin less than 3 g/dL was a significant predictive factor for high postoperative mortality^[177]. In the same study, a preoperative serum total bilirubin above 10 mg/dL was associated with lower survival rates^[177]. The role of preoperative biliary drainage (PBD) in jaundiced patients remains controversial. A recent meta-analysis failed to demonstrate any benefit^[178]. Furthermore, PBD seems to increase the risk of perioperative infections and a longer postoperative hospital stay^[124,178].

Nevertheless, prolonged preoperative biliary obstruction is associated with increased postoperative morbidity and mortality after hepatic resection due to the presence of severe cholestatic liver dysfunction^[177,179]. Currently, preoperative PBD is not routinely recommended, but it has been shown to be beneficial in the presence of cholangitis, severe malnutrition, coagulation abnormalities^[180,181] and when patients require major hepatic resection^[175,182]. When preoperative drainage is performed, definitive surgery should be deferred for a few weeks to allow sufficient restoration of hepatic function^[168]. The use of liver volumetric and/or hepatic functional studies is warranted when anticipating an extended hepatic resection, to estimate the future liver remnant and minimize the risk of liver failure caused by insufficient function or small residual liver parenchyma.

Assessment of resectability

Meticulous interpretation of all the available clinical and radiological data is recommended to determine resectability and avoid unnecessary interventions. Despite the improvement of diagnostic modalities, about 16%-25% of patients are found to have more extensive disease preventing resection at the time of laparotomy^[68,183]. The major determinants of resectability

Table 8 Survival rates after resection of ICC

Author (yr)	Resections (n)	Overall 5-year survival (%)	R0 5-year survival (%)
DeOliveira <i>et al</i> ^[67] , 2007	34	40	63
Miwa <i>et al</i> ^[195] , 2006	41	29	36
Jan <i>et al</i> ^[196] , 2005	81	15	NR
Ohtsuka <i>et al</i> ^[197] , 2003	50	23	NR
Uenishi <i>et al</i> ^[198] , 2001	28	27	67
Inoue <i>et al</i> ^[199] , 2000	52	36	55
Yamamoto <i>et al</i> ^[200] , 1999	83	23	53
Madariaga <i>et al</i> ^[201] , 1998	34	35	41

NR: Not reported.

are the extent of tumor within the biliary tree, the amount of hepatic parenchyma involved, vascular invasion, hepatic lobar atrophy, and metastatic disease^[166,179]. A review of 294 cases of CC demonstrated that resectability rates are higher for more distal tumors^[69]. The determination of resectability is most challenging in patients with HCC. It is reported that about half of patients with HCC deemed to be resectable preoperatively have unresectable disease when explored^[174]. The radiological criteria defining unresectability in patients with HCC are listed in Table 7^[174,175]. With regard to distal ECC and ICC, AJCC stages III and IV are generally considered unresectable (Table 8).

Generally, invasion of the main portal vein or invasion of the vasculature supplying the planned hepatic remnant preclude resection. Nevertheless, recent reports have shown that en-bloc resection with vascular reconstruction may achieve negative margins and potential cure with only 10% perioperative mortality in very selected patients^[184,185]. The application of staging laparoscopy has been recently advocated as it can reduce the number of unnecessary laparotomies by identifying metastatic lesions in the liver and in the peritoneal cavity^[186]. The yield of laparoscopy for detecting unresectability in patients with potentially resectable CC on preoperative imaging modalities is about 25% with an overall accuracy of 50%^[187,188]. Moreover, laparoscopy offers the addition of intraoperative hepatic US, which can increase the diagnostic yield up to 42%^[189]. One of the limitations of laparoscopy is the inability to detect vascular or nodal involvement^[188,189]. Peritoneal washings to obtain cytology specimens have not been shown to predict occult metastasis in patients with CC^[190]. Ultimately, true resectability is determined after a complete abdominal exploration.

Operative procedures and survival

The goal of surgery is to obtain complete excision of the tumor with negative histological margins (R0 resection), as this is associated with marked survival advantages compared to margin positive resections (R1 or R2 resection)^[26,68,176,191]. To confirm histologically-negative margins, many authors advocate the use of intraoperative frozen section examinations of the bile ducts^[174]. A very important study from the MSKCC has recently evaluated the clinical significance of intraoperative frozen section for patients affected by HCC^[192]. The primary aim of

Table 9 Survival rates after resection of HCC

Author (yr)	Resections (n)	Liver resection (%)	R0 resection (%)	Overall 5-year survival (%)	R0 5-year survival (%)
Hasegawa <i>et al</i> ^[213] , 2007	49	92	78	40	50
DeOliveira <i>et al</i> ^[67] , 2007	173	20	19	10	30
Dinant <i>et al</i> ^[214] , 2006	99	38	31	27	33
Hemming <i>et al</i> ^[205] , 2005	53	98	80	35	45
Rea <i>et al</i> ^[208] , 2004	46	100	80	26	30
Kawasaki <i>et al</i> ^[182] , 2003 ¹	79	96	68	NR	40
Kawarada ^[215] , 2002	87	75	64	26	NR
Jarnagin <i>et al</i> ^[166] , 2001	80	78	78	37	NR
Tabata <i>et al</i> ^[216] , 2000	75	71	60	23	40
Kosuge <i>et al</i> ^[217] , 1999	65	88	52	35	52
Miyazaki <i>et al</i> ^[218] , 1998	76	86	71	26	40

¹Five-year survival for patients with R1 resection is 6%; NR: Not reported.

this study was to assess the importance of obtaining frozen sections of the bile duct margins for the planning of the extent of the surgical dissection. Frozen sections were obtained in 101 patients: among them 20 (19.8%) had positive and 81 (80.1%) had negative results. Among the patients who had negative frozen sections, 8 (9.8%) individuals were found to have positive margins at subsequent histopathology. In this study, intraoperative frozen section was shown to be 71.4% sensitive, 100% specific, and with a positive predictive value of 100% and negative predictive value of 80.2%^[192].

ICC

Surgical therapy for ICC is based on the same principles used for hepatic resections performed for hepatocellular carcinomas or secondary tumors. The operative approach should be aimed at ensuring R0 resection margins whenever it is possible. Lymph node dissection during resection of ICC is not recommended as it does not improve patients' survival^[193,194]. Current outcomes after surgical resection have improved in comparison to historical data with 5-year survival rates ranging from 20% to 40% (Table 9)^[195-201]. Predictors of poor outcomes include: positive resection margins, lymphatic and vascular invasion and periductal infiltrating disease^[202,203]. The most common site of recurrence after surgical resection is within the liver^[196].

HCC

Curative surgery of HCC usually requires the excision of the extrahepatic bile duct, regional lymphadenectomy, cholecystectomy and in most cases some sort of partial hepatectomy including the caudate lobe, especially for tumors mainly extending in the left hepatic duct^[174,207]. The rationale behind performing partial hepatectomies in HCC is to ensure histologically negative margins. Several studies have shown that this strategy increases R0 resections in up to 80% of patients^[174,182,205]. Extended lymphadenectomy is not recommended as there is no evidence showing survival advantage^[168,172].

Radical resection of HCC has 5%-10% perioperative mortality rate, especially when extended hepatectomy (5 or more segments) is required^[174,206-208]. This partly

Table 10 Survival rates after resection of distal ECC

Author (yr)	Resections (n)	Overall 5-year survival (%)	R0 5-year survival (%)
DeOliveira <i>et al</i> ^[67] , 2007	229	23	27
Cheng <i>et al</i> ^[219] , 2007	112	25	26
Murakami <i>et al</i> ^[224] , 2007	36	50	62
Yoshida <i>et al</i> ^[225] , 2002	26	37	44
Fong <i>et al</i> ^[222] , 1996	45	27	54 ¹

¹Patients had node negative tumors as well.

relates to the increased rate of postoperative liver failure with major hepatic resections. Portal vein embolization (PVE) is a valuable preoperative measure when anticipating extensive liver resections with subsequent small hepatic residual volume^[209]. A compensatory hypertrophy of the remnant hepatic parenchyma is induced by selectively occluding the main portal vein branch to the lobe that will be resected. This can increase the volume of the anticipated liver remnant by 12%-20%, thereby reducing the rate of postoperative liver dysfunction^[210,211]. PVE is useful when the anticipated liver remnant volume is less than 20%-25% of the total liver volume in patients with normal liver function, and when the anticipated liver remnant volume is 40% or less in patients with compromised liver function^[212].

The average 5-year survival rates post-resection for HCC are 25%-40% (Table 10)^[68,174,182,209,213-218]. Factors associated with favorable outcome include; R0 resection, no lymph node metastasis, absence of perineural invasion, and well-differentiated histological grade^[174,185].

ECC

The same principle of achieving a negative resection margin applies to ECC. The resectability rate has been reported as being up to 90% with distal extrahepatic tumors^[68,219]. Complete removal of distal ECC usually requires a pancreaticoduodenectomy (Whipple procedure)^[73,220,221]. Even in these circumstances, extended lymphadenectomy is not justified as it does not provide survival advantages and it is associated with increased perioperative morbidity^[222]. Segmental bile duct excision is rarely an option, except for CC located in the middle of the common duct in the absence of periductal invasion or spread to the surrounding structures. Only 10% of patients undergoing bile duct excision alone obtain curative resection margins on final pathology^[222,223]. Most commonly, when approaching patients with CC arising midway along the extrahepatic duct, surgeons should assess whether a pancreaticoduodenectomy or a partial hepatectomy is more appropriate with regard to the tumor extension. In these patients, curative resections are associated with a 25%-50% 5-year survival rate (Table 10)^[68,220,223-225]. The main determinants of poor outcomes are positive surgical margins and lymph node involvement^[220,223]. Other factors associated with unfavorable prognosis include pancreatic invasion, duodenal invasion, perineural invasion, and a poorly-differentiated histology^[68,220].

Liver transplantation

Transplantation is an emerging therapy for unresectable CC without evidence of metastatic disease. Candidates are individuals who would require a total hepatectomy to achieve clear margins and those with underlying liver failure precluding hepatic resection. The early experience of transplantation for CC reported early recurrence rates of more than 50% and a 5-year survival of 10%-20%^[226-228]. More recently, in highly selected patients undergoing neoadjuvant protocols, promising results have been reported. In 2002, Sudan *et al*^[229] reported a series of 11 patients transplanted for CC after neoadjuvant chemoradiation with 45% tumor free survival and median follow up of 7.5 years. Similar reports have been reported by Becker *et al*^[230] who observed a 45% 5-year survival for patients who were diagnosed as being affected by CC prior to undergoing transplantation, and a 33% 5-year survival was observed by Sotiropoulos *et al*^[231] in Germany. At the Mayo Clinic, Rosen *et al*^[232,233] have developed a liver transplantation protocol for HCC that provides a disease-free 5-year survival of 82%. This protocol is aimed at treating unresectable HCC or CC in PSC patients. To be eligible for this protocol, the diagnosis of CC is confirmed histologically, considered unresectable and with no evidence of metastatic disease. Eligible patients receive neoadjuvant chemoradiation therapy followed by staging laparotomy to rule out metastatic disease followed by living-related or cadaveric liver transplantation. Currently, the use of liver transplantation for the treatment of CC is reserved only for highly selected patients in specialized centers.

ADJUVANT THERAPY

The use of postoperative chemotherapy, radiotherapy or chemoradiation therapy have been evaluated as means of improving disease-free survival in patients with resected tumors since CC have high rates of local and distant recurrence.

Adjuvant chemotherapy

Postoperative chemotherapy has failed to show significant survival benefits^[234,235]. A recent multicenter RCT evaluated the effect of postoperative chemotherapy with mitomycin C and 5-fluorouracil (5FU) versus surgery alone for individuals affected by cancers of the pancreas and biliary system^[236]. Among 508 patients post-R0 resection, 139 individuals were affected by CC and for these individuals no survival benefit was seen after chemotherapy treatment^[237].

Adjuvant radiotherapy

The use of postoperative external beam radiation with or without intraoperative radiotherapy and intraluminal radiotherapy (brachytherapy) has been explored in the adjuvant setting without significant benefits after R0 resections^[125,237,238]. On the other hand, several studies showed that adjuvant radiotherapy may benefit patients with positive resection margins^[239-241]. Todoroki *et al*^[241] showed that the 5-year survival in patients with

R1 resections was 34% when adjuvant radiotherapy (intraoperative and external beam) was used compared to 14% with surgery alone.

Adjuvant chemoradiation therapy

The radiosensitizing effect of chemotherapeutic agents has been evaluated in the adjuvant setting with positive results only for distal ECC. In a retrospective cohort study of 94 individuals who underwent resection for CC, 34% received postoperative chemoradiation^[242]. Longer survival was seen in patients who received adjuvant therapy (median survival 41 mo *vs* 24 mo)^[243]. Other retrospective studies demonstrated similar results and showed that patients with distal ECC had a superior survival advantage in comparison to more proximal CC following adjuvant therapy^[243,244]. Recently, Hughes *et al*^[245] have confirmed a slight 5-year survival advantage with postoperative chemoradiation therapy in patients with distal ECC compared with surgical resection alone (35% *vs* 27%). In line with these findings, Figueras *et al*^[246] did not demonstrate a significant survival benefit with adjuvant chemoradiation therapy for HCC. These results need to be confirmed further with larger prospective trials. For ICC, evidence to support the use of adjuvant chemoradiation therapy is very limited. In a recent retrospective study of 3839 patients, Shinohara *et al*^[247] have shown that the overall survival rate was significantly different between groups receiving surgery alone and surgery plus adjuvant radiation therapy ($P = 0.014$) and between radiation therapy alone and no treatment ($P < 0.0001$). The combination of surgery and adjuvant radiation therapy conferred the greatest benefit on overall survival (HR: 0.40; 95% CI: 0.3-0.47), followed by surgery alone (HR: 0.49; 95% CI: 0.44-0.54) and radiation therapy alone (HR: 0.68; 95% CI: 0.59-0.77) compared with no treatment.

There is a lack of RCT evaluating the utility of adjuvant therapy following R0 resections of CC. Moreover, most of the current studies are small and retrospective in nature and incorporated CC with cancers of the gallbladder and pancreas. Therefore, no standard adjuvant modalities are universally embraced for the treatment of CC.

Neoadjuvant therapy

The role of preoperative chemoradiation therapy has been evaluated in a small series of patients with ECC^[248]. Among nine patients who underwent neoadjuvant therapy, McMasters *et al*^[248] observed pathological complete response in 3 individuals and negative resection margins were obtained in all subjects. More recently, neoadjuvant therapy has been used in the setting of liver transplantation for CC with promising results. Further trials are required to better assess its efficacy.

PALLIATION

Nearly half of the patients with CC are considered candidates only for palliative treatments due to the advanced stage of their disease at the time of diagnosis or the

presence of significant comorbidities that prevent surgical therapy^[68,174]. Therefore, palliation plays an important role in the management of these individuals. The primary aim of palliative interventions is to improve quality of life by relieving symptoms and prolonging survival by preventing cholestatic liver failure. In the presence of incurable CC, tissue diagnosis should be obtained whenever possible to direct palliative therapy planning.

Biliary drainage

Biliary obstruction is the major cause of morbidity and mortality in patients with CC. The goals of biliary decompression are to relieve jaundice, pain, pruritus, and to prevent cholangitis and cholestatic liver failure^[249]. Different modalities are currently available to drain the biliary system and these include: endoscopic, percutaneous and surgical bypass. The ideal palliative biliary decompression should be effective, provide durable results, and have low risks of morbidity and mortality.

Biliary endoprosthesis (stenting)

Biliary stenting can be achieved endoscopically or percutaneously. Endoscopic biliary stenting is the most widely used method and the percutaneous approach is usually performed when endoscopic drainage fails or cannot be performed. Percutaneous stents can be either internal, external or both. External stents have the disadvantage of draining bile without the ability of enteric recycling and are associated with more discomfort and reduced quality of life. Little is known as to whether percutaneous or endoscopic biliary drainage have different overall efficacy in palliating patients with unresectable disease. Generally, only patients with advanced tumors that are totally obstructed are candidates for percutaneous external biliary drainage. A recent multicenter retrospective study from South Korea has shown that the placement of percutaneous self-expanding metallic stents across HCCs is associated with a higher success rate and lower risk of procedure-induced cholangitis^[250].

Endoscopic stents can be either self-expanding metallic or plastic (polyethylene). Metal stents are more expensive than plastic stents but have larger diameters and provide better patency rates^[251]. Metal stents can be either uncovered or covered by sealing the metallic mesh with a membrane which prevents tumor growth through the stent, increasing patency rates. Plastic stents often need to be changed at 2 to 3-mo intervals, while metal stents can remain patent up to 9 mo^[252]. Several RCT have compared metal to plastic stents for the treatment of patients with inoperable malignant biliary obstruction^[253,254]. These studies concluded that metal stents are more cost-effective for patients who are expected to survive more than 5 mo as they need less interventions and shorter hospitalizations^[254,255]. Patency rates are generally higher for ECC^[255] and metal stents provide superior palliation for HCC as compared to plastic stents^[256,257]. Draining about 25% of the hepatic parenchyma is usually sufficient for adequate palliation in the absence of infection^[250]. A RCT comparing unilateral versus bilateral drainage in patients with malignant hilar obstruction found that

drainage of one functional hepatic lobe is sufficient to relieve obstruction with no difference in complication and survival rates^[258]. It is important to note that stents placed for hilar lesions will require re-intervention in about 30% of patients due to stent occlusion^[259,260]. A RCT comparing covered to uncovered stents in patients with unresectable distal biliary malignancies showed that the patency of covered stents was significantly higher than that of uncovered stents^[261]. However, multiple studies report an increased risk of cholecystitis (5%) with the covered stents due to cystic duct occlusion^[262].

Surgical biliary drainage

Biliary-enteric anastomosis can be performed by open or laparoscopic approach. Studies comparing surgical to non-surgical biliary drainage showed similar overall palliative effects but with higher perioperative morbidity and mortality^[263,264]. Surgical drainage has the advantage of superior patency rates and prevents the need for stent exchanges required when using endoscopic or percutaneous stents due to clogging^[265]. Currently, the main candidates for surgical drainage are patients found to have unresectable CC at the time of exploration, individuals who are not able to undergo repeat endoscopic or percutaneous stent exchanges, and those who have long expected survival and who are fit for surgery^[183,266].

Palliative radiotherapy

Palliative radiotherapy may benefit patients with locally advanced unresectable CC or those who have undergone palliative bypass in the absence of distant metastases. The use of palliative radiotherapy has beneficial effects on pain relief, biliary patency and overall patient survival^[267,268]. The two most commonly used radiotherapy modalities are external beam radiation with 30 to 50 Gy, intraluminal brachytherapy with 10 to 20 Gy or the combination of both. Intraluminal brachytherapy is delivered by using iridium-192 seeds mounted on a catheter that is deployed across the tumor by endoscopic or percutaneous approach^[269]. It appears that brachytherapy is able to deliver more effective doses of radiation without damaging the surrounding organs. Generally, the majority of studies that demonstrated benefit of radiotherapy used combinations of both modalities with median patient survival ranging between 9 and 14 mo^[270-273]. Palliative radiotherapy is associated with increased incidence of complications such as cholangitis, gastroduodenitis and longer hospital stay in comparison to best supportive care and therefore it is not routinely used in many centers^[274]. Moreover, higher doses of radiation (more than 55 Gy) may be required to obtain an improved survival, with increased toxicity rates^[275]. Controlled studies are required to better evaluate the effectiveness and safety of these palliative treatments. For ICC, brachytherapy can be delivered by radioembolization with yttrium-90 microspheres^[276]. This approach has been shown to provide partial response in 27% of patients and stable disease in 68% with limited side effects; therefore it is not surprising that it has become the leading modality for palliation of CC in centers where this technique is available^[277].

Palliative chemotherapy

There is no standard chemotherapy option for patients with CC. Patients with widespread disease considered for palliative chemotherapy undergo treatment in an attempt to control the disease and improve their overall survival. Owing to the lack of RCT and the retrospective nature of observational studies with heterogeneous patient populations currently available, the interpretation of the survival benefit of palliative chemotherapy is difficult. Various chemotherapeutic agents with different dosing regimens have been tested with overall poor survival improvement. Historically, 5FU was the first chemotherapeutic agent used for palliation of CC patients with only 10% response rates when used alone. Several subsequent studies have evaluated 5FU in combination with other agents such as leucovorin, interferon-alpha, cisplatin, and oxaliplatin, with an overall response rate of 25% to 55% and a median survival ranging between 6 and 12 mo^[278-281]. Multiple phase II trials have evaluated the use of oral 5FU prodrugs (uracil-tegafur and capecitabine) in patients with advanced CC^[282,283]. For example, the combination of capecitabine and cisplatin had mild toxicity and produced a response in 41% of patients with a median survival of 12 mo^[283]. Gemcitabine proved to have good efficacy as a single agent in biliary malignancies with response rates of 30%^[284]. Several gemcitabine-based combinations, including cisplatin, capecitabine, and oxaliplatin have reported response rates up to 36% and median survival of 10 to 15 mo^[285-287]. Finally, a recent analysis of all the published chemotherapy trials in individuals affected by advanced CC from 1985 to 2006 concluded that gemcitabine combined with platinum compounds (cisplatin or oxaliplatin) had the best patient response rates^[288,289]. Recently, the roles of transcatheter arterial chemoembolization (TACE) and transcatheter arterial chemoinfusion (TACI) have been assessed for patients affected by unresectable ICC^[290,291]. Although TACE and TACI with gemcitabine, cisplatin and doxorubicin in different combinations^[292] are well tolerated, survival benefits have not been proven in large studies and will require further evidence before becoming widely accepted in the scientific community.

Photodynamic therapy (PDT)

PDT is an emerging palliative strategy based on the intravenous administration of photosensitizing agents that preferentially accumulate in malignant cells. After the delivery of these photosensitizing agents, specific wavelengths of light are administered causing activation of the photosensitizer and thus tumor cell necrosis^[293]. The depth of tumor necrosis obtained by this technique is between 4 mm and 6 mm^[294]. This modality is currently used as a palliative measure in conjunction with biliary stenting for nonresectable CCs. Improvements in quality of life, biliary drainage, and survival in patients with advanced CCs post-PDT have been reported in several case series^[294]. Furthermore, a RCT compared PDT with endoscopic stenting to stenting alone in patients with unresectable CC^[295]. The

study was terminated prematurely because PDT proved to be markedly superior to simple stenting. The PDT group in that trial had higher median survival (493 d *vs* 98 d), improved biliary drainage and better quality of life than the stenting alone group^[296]. Recently, PDT was investigated as a neoadjuvant modality before surgical resection of advanced HCC in 7 patients^[296]. Tumor-free resection margins were achieved in all patients with a 1-year recurrence-free survival rate of 83%^[297]. A recent study confirmed that the use of PDT as a neoadjuvant therapy is safe and can downstage tumors from unresectable to resectable^[297]. The main side effects of PDT include photosensitivity caused by the administration of photosensitizer agents and cholangitis related to biliary instrumentation^[294,298].

Other palliative measures: Several other palliative modalities have shown some benefits in selected groups of patients. Radiofrequency ablation has been used for patients unfit for surgery who have small intrahepatic CC^[299]. In a single-centre cohort of patients with unresectable intrahepatic CC, TACE has shown some survival advantage in comparison to best supportive care (median survival: 23 mo)^[300]. Hepatic arterial chemoinfusion offers tumor-directed chemotherapy and it has been proven to be safe^[301] as has localized ablation of tumor cells by high intensity intraductal ultrasound^[302]. Another promising area is the use of molecular targeting agents for chemoprevention and adjuvant therapy of CC such as cyclooxygenase-2 and nitric oxide inhibitors^[303]. The clinical utility of these emerging therapies needs further investigation before gaining wide acceptance.

CONCLUSION

Over the last decades several advances have occurred in the fields of epidemiology, diagnostic modalities, medical and surgical treatment of CC as well as in palliation. The diagnosis, staging and further management of patients affected by this disease may be a complex issue and requires expertise in many fields. To optimize the outcome of patients with suspected or proven CC, a multidisciplinary approach is recommended.

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Emerging treatments for complex perianal fistula in Crohn's disease

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INTRODUCTION

Crohn's disease is a chronic inflammatory disease of the intestine of unknown etiology. It is characterized by focal or segmental transmural inflammation which can occur in any part of the digestive tract with occasional granuloma formation. This transmural inflammation disrupts intestinal mucosal integrity, favoring the development of abscesses and fistulas. When fistulas form, they can track between intestinal segments or between an intestinal segment and other organs (bladder, vagina), adjacent tissue or the skin. Fistulas are classified as internal when they communicate with adjacent organs (e.g. entero-enteric and rectovaginal fistulas) and external when they communicate with the dermal surface (e.g. enterocutaneous, peristomal and perianal fistulas).

The cumulative incidence of perianal fistulas in Crohn's disease varies between 20% and 25% in population studies^[1-3]. Perianal disease is associated with high morbidity and, typically, with local pain and discharge; it therefore has a very negative impact on the quality of life of the affected patients. In Crohn's patients, perianal disease is more common when the colon is affected and particularly when the rectum is involved^[2].

The ideal therapeutic goal in perianal fistulizing Crohn's disease is complete and sustained closure of the fistulas without the development of abscesses, thereby avoiding the need for surgical interventions, and improving the patients' quality of life. In an appreciable number of patients, complete closure cannot be achieved despite intensive medical treatment (including infliximab) and surgery in accordance with normal practice. Currently the goal of therapy for these patients has shifted from complete fistula closure to reduction in drainage from the fistula tract in order to improve their

Abstract

Complex perianal fistulas have a negative impact on the quality of life of sufferers and should be treated. Correct diagnosis, characterization and classification of the fistulas are essential to optimize treatment. Nevertheless, in the case of patients whose fistulas are associated with Crohn's disease, complete closure is particularly difficult to achieve. Systemic medical treatments (antibiotics, thiopurines and other immunomodulatory agents, and, more recently, anti-tumor necrosis factor- α agents such as infliximab) have been tried with varying degrees of success. Combined medical (including infliximab) and less aggressive surgical therapy (drainage and seton placement) offer the best outcomes in complex Crohn's fistulas while more aggressive surgical procedures such as fistulotomy or fistulectomy may increase the risk of incontinence. This review will focus on emerging novel treatments for perianal disease in Crohn's patients. These include locally applied infliximab or tacrolimus, fistula plugs, instillation of fibrin glue and the use of adult expanded adipose-derived stem cell injection. More well-designed controlled studies are required to confirm the effectiveness of these emerging treatments.

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quality of life. In these patients with complex perianal fistulizing disease that persists despite intensive medical and surgical treatment, a therapeutic gap exists for new treatments which aim for complete and sustained closure of the perianal fistulas.

In this review, after a brief discussion of the diagnosis, characterization, classification, and current systemic treatments of perianal fistulas in patients with Crohn's disease, we shall discuss some of the newer local therapies and their potential applications.

DIAGNOSIS AND CHARACTERIZATION

The starting point for management of perianal fistulas is a complete and accurate diagnosis of the lesions, which requires careful exploration of the anal and perianal region. An inadequate examination which fails to detect occult lesions (abscesses or fistula branches) may result in perianal disease becoming persistent or recurrent. An endoscopic examination is needed to determine the presence of macroscopic inflammation in the rectum and/or rectal stenosis, as such findings are important for the prognosis and treatment of the disease. There is consensus among the American Gastroenterological Association (AGA)^[4,5] and European Crohn's & Colitis Organization (ECCO)^[6] working groups concerning the need to complement the study of perianal disease with other diagnostic tools such as examination under anesthesia (EUA), magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS).

In the hands of expert surgeons, EUA is considered the gold standard against which other techniques are compared. EUA has an accuracy of 90% for diagnosis and classification of fistulas and abscesses^[7]. With this technique, it is possible to perform concomitant surgery of the lesions: incision and drainage of abscesses with seton placement, and other procedures to treat fistulas. MRI has an accuracy of between 76% and 100% for diagnosis and classification of perianal fistulas^[7,8]. With MRI, the surgeon who performs EUA can obtain important additional information in 15%-21% of patients^[7,9]. In view of its harmless nature and the additional information it provides, MRI is the initial diagnostic technique of choice according to the ECCO consensus statement^[6]. EUS offers a diagnostic accuracy of between 56% and 100%, and the findings change the surgical approach in 10%-15% of cases^[7,10], helping to guide medical-surgical treatment of perianal fistulas in Crohn's disease, resulting in a high response rate^[11]. Sometimes, the pain caused by the lesions or stenosis makes EUS difficult.

The combination of either of these imaging techniques (MRI or EUS) with EUA yields a diagnostic accuracy of 100% for perianal disease^[7]. Imaging techniques are essential to provide surgeons with a virtual view which allows them to treat all lesions during the surgical procedure.

CLASSIFICATION OF PERIANAL FISTULAS

A number of classification systems have been proposed

in the past^[12,13], but perhaps the most anatomically accurate is the Parks classification^[14], which takes the external anal sphincter as the central reference point and describes 5 types of perianal fistula: superficial, inter-sphincteric, trans-sphincteric, supra-sphincteric and extra-sphincteric. The Parks classification is however limited in that it does not take into account the presence of abscesses and/or connections with other organs such as the vagina or bladder, even though such information is important for determining the medical and surgical management of the disease.

The AGA technical review proposed a more clinically useful classification system with just 2 categories: simple and complex fistulas^[4,5]. Simple fistulas are low (superficial, low inter-sphincteric or low intra-sphincteric), have a single external opening and are not associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis. In contrast, complex fistulas are high (high inter-sphincteric, high intra-sphincteric, supra-sphincteric or extra-sphincteric) and/or can have several external openings and may be associated with perianal abscess, connection to the vagina or bladder, rectal stenosis or macroscopic proctitis. This classification has greater clinical relevance: simple fistulas respond better to treatment whereas complex ones have lower cure rates with medical treatment and, with this type of fistula, an aggressive surgical procedure will often lead to incontinence.

MEASURES OF HEALING

The Crohn's Disease Activity Index (CDAI) is widely used in Crohn's disease as an outcome measure but it is not designed or able to assess perianal fistulous disease activity, thus the Perianal Disease Activity Index (PDAI) is often used as an equivalent of this index for measurement of morbidity associated with perianal disease^[15]. This index assesses 5 categories related to fistulas: discharge, pain, restriction of sexual activity, type of perianal disease and degree of induration. The advantage of the PDAI is that it assesses aspects of the quality of life that are most affected in patients with perianal disease and that it has been validated in recent clinical studies^[16].

The most widely used instrument for assessing treatment outcomes in clinical trials is the Fistula Drainage Assessment. This measure classifies fistulas as open (i.e. purulent material is expelled with gentle pressure) or closed^[17]. A fistula has to remain closed for 2 consecutive visits (at least 4 wk apart) to be considered in remission. The Fistula Drainage Assessment does not consider changes in anal pain, which is also an important marker of treatment response.

Consideration of complete re-epithelization of the external openings supported by MRI studies could represent a major improvement in the assessment of fistula closure. Long term maintenance of the healing is of great therapeutic relevance. Indeed, the drafted guideline of the European Medicines Agency (EMA)

on “The clinical development of new medicinal products for the treatment of Crohn's Disease (Doc. Ref. CPMP/EWP/2284/99 Rev. 1)” states that “the therapeutic goals of management of fistulizing Crohn's disease are to close fistulas and maintain their closure, to reduce the incidence of infections in persisting fistulas, and to limit the need for surgical interventions. Clinical studies in fistulizing Crohn's disease should reflect this. The primary endpoint of the clinical trials should then be complete closure of fistulas and maintenance of a closed fistula without development of new fistulas.”

An MRI-based activity score was developed to assess the anatomical evolution of perianal fistulas in Crohn's disease^[18]. MRI imaging demonstrates that despite closure of draining external orifices after infliximab treatment, inflammatory changes in the fistula track persist for a long time. It has been suggested that this residual activity may cause recurrent fistulas and pelvic abscesses but a *post hoc* analysis of the ACCENT II study showed that maintenance infliximab therapy does not result in increased abscess development in patients with fistulizing Crohn's disease^[19].

TREATMENT

Crohn's disease cannot be cured by medical or surgical treatment. The aim of therapy is to alleviate symptoms and treat complications of the disease in order to improve the patients' quality of life. The strong negative impact of symptomatic perianal disease on quality of life justifies aggressive treatment to facilitate healing. The spontaneous cure rate for perianal fistulas is very low, ranging from 6% to 13% in the placebo arm of 3 controlled studies^[17,20,21].

Medical treatments

Antibiotics: Bacteria may in theory play a role in the appearance and persistence of perianal fistulous disease. Thus antibiotics are sometimes used as first-line therapy for fistula healing. In other cases antibiotics, in view of their prophylactic effects against infections and abscesses, are used as adjuvant (or bridging) therapy. Most of the studies of perianal fistulizing disease treated with antibiotics are uncontrolled and the sample sizes are small. In these studies, both metronidazole^[22-24], and ciprofloxacin^[25], as well as a combination of the 2 drugs^[26], showed an initial beneficial effect on the perianal fistula. Response typically occurs after 6 to 8 wk of treatment and is usually manifest in the form of decreased drainage. Fistula closure is uncommon and symptoms tend to recur after suspending treatment^[24].

Recently, a small randomized, double-blinded, placebo-controlled study evaluated ciprofloxacin or metronidazole for the treatment of perianal fistulas in patients with Crohn's disease^[27]. Twenty-five patients were randomized to ciprofloxacin 500 mg (10 patients), metronidazole 500 mg (7 patients) or placebo (8 patients) twice daily for 10 wk. Response ($\geq 50\%$ reduction in the number of draining fistulas) at week 10 was seen in 4 patients (40%) treated with ciprofloxacin,

1 patient (14.3%) treated with metronidazole, and 1 patient (12.5%) treated with placebo ($P = 0.43$). One patient from both the ciprofloxacin and placebo arm and 5 (71.4%) treated with metronidazole dropped out of the study ($P < 0.02$). This study was probably too small to detect differences between treatment arms.

In two studies, antibiotics were used as an adjuvant or a bridge to other drugs. The use of metronidazole and/or ciprofloxacin induced a response ($\geq 50\%$ reduction in the number of draining fistulas) at week 8 with fistula closure occurring in 25% of cases^[28]. At week 20, those patients who received additional azathioprine therapy had a better medium-term response (48% *vs* 15%). It should be pointed out that most of the patients in that study had simple fistulas and that only 9 of the 52 cases were classed as complex fistulas. In a placebo-controlled study, all patients received infliximab (3 induction doses at weeks 6, 8 and 12) and were randomized to receive either 500 mg ciprofloxacin twice daily or a placebo for 12 wk^[16]. The response rate (defined as $\geq 50\%$ reduction from baseline in the number of draining fistulas) at week 18 showed a tendency in favor of ciprofloxacin in combination with infliximab compared to infliximab alone (OR: 2.37; 95% CI: 0.94-5.98)^[16].

Thiopurines: Azathioprine and 6-mercaptopurine have shown efficacy in the treatment of Crohn's perianal fistulas. In a meta-analysis of 5 controlled studies, a response (defined as complete closure or decreased drainage) was found in 54% of the patients treated with azathioprine or 6-mercaptopurine compared to 21% in the placebo group (OR: 4.44; 95% CI: 1.50-13.2)^[29]. This meta-analysis is limited in that fistula response was a secondary endpoint and not the primary one in all of the studies included. There have been no controlled trials in which the primary endpoint was assessment of the effect of thiopurines on the closure of fistulas in patients with Crohn's disease.

Anti-tumor necrosis factor (TNF)- α agents:

The efficacy of the anti-TNF- α antibody infliximab in fistulizing perianal disease refractory to 3 mo of conventional treatment has been shown in a controlled clinical study^[19]. The most favorable outcomes were obtained at doses of 5 mg/kg body weight and 3 induction infusions at 0, 2 and 6 wk. This regimen achieved complete fistula closure (no drainage in 2 visits 4 wk apart) in 55% of the patients compared to only 13% in the placebo group. The mean time to response was 2 wk and the mean duration of response was 12 wk after the last infusion. The ACCENT II study later confirmed the response rate to induction (69% at week 14) in the open-label extension^[30]. Responders were randomized to infliximab 5 mg/kg body weight or placebo every 8 wk. At week 54, 36% of the patients on infliximab were able to maintain complete closure compared to 19% of those on placebo ($P = 0.009$). Similar results have also been reported in clinical practice in a large uncontrolled series^[31].

Infliximab maintenance treatment has been shown to decrease the use of hospital resources (fewer hospitalizations and less need for surgery) in patients with fistulizing Crohn's disease^[32]. Nevertheless, it has been reported that in perianal disease, early relapse was common after stopping infliximab treatment, with only 34% of patient maintaining remission at 1 year^[33].

Adalimumab, another anti-TNF- α antibody, may also prove effective in perianal Crohn's disease. In the CHARM study, 33% of the patients randomized to adalimumab achieved long term complete fistula closure versus 13% in the placebo group (secondary endpoint; placebo *vs* adalimumab group combined; $P = 0.043$)^[34]. Complete fistula healing was sustained for up to 2 years by most of the patients in the open extension trial ADHERE. In a prospective open-label study in patients with active perianal fistulous disease who stopped responding or developed intolerance to infliximab, adalimumab induction therapy (160 mg at week 0 and 80 mg at week 2) induced complete fistula closure at week 4 in 23% of the cases^[35].

Other immunomodulators: Randomized studies designed specifically to assess the efficacy of cyclosporine in the closure of fistulas in patients with fistulizing Crohn's disease have not been published. However, there are several uncontrolled case series which used continuous cyclosporine infusion in patients who had failed conventional therapy^[36]. Many patients showed an initial response and were switched to oral cyclosporine; however the response was rapidly lost on drug withdrawal.

Uncontrolled case series suggested that tacrolimus may be useful in the treatment of perianal disease^[37,38]. In a small controlled clinical trial, patients treated with tacrolimus (0.2 mg/kg per day) had a higher response rate (defined as closure of at least 50% of fistulas) at week 4 compared to placebo (43% *vs* 8%), but no differences were observed in terms of complete fistula closure (10% *vs* 8%)^[21].

In a retrospective study methotrexate was used in patients with fistulizing Crohn's disease; after 6 mo 44% of the patients had partial or complete fistula closure^[39]. An early case series suggested that the antimetabolite agent mycophenolate mofetil could be effective in Crohn's perianal disease^[40]. In a more recent uncontrolled study from the same group mycophenolate mofetil induced a partial response in 7 out of 8 patients with perianal fistulas, but the response was subsequently lost in 5 of these 7 patients for several reasons including side effects^[41].

In refractory Crohn's disease, small uncontrolled series showed that thalidomide may be effective in treating complex perianal fistulas^[42,43]. Severe side effects, including neuropathy, were common and limited the long term use of the drug. Lenalidomide, an analogue of thalidomide, with lower toxicity and powerful anti-TNF properties was not effective in active luminal Crohn's disease^[44], and has not yet been tested for perianal Crohn's disease.

Miscellaneous therapies: A pilot open-label study provided data suggesting granulocyte colony-stimulating factor (GM-CSF) is a safe and potentially effective agent for the treatment of active perianal Crohn's disease^[45]. GM-CSF has been used in a placebo-controlled study in patients with luminal Crohn's disease, some of whom had draining fistulas at study entry. At 6 mo, 4 out of 8 patients in the GM-CSF group and 2 out of 5 in the placebo group had complete fistula closure^[46].

Octreotide, a somatostatin analogue, may have a role in treating Crohn's enterocutaneous fistulas, but has not been used in perianal disease^[47]. The effect of elemental diet on perianal Crohn's disease has been studied in a small retrospective series. Fistulas improved in some patients but early relapse occurred in almost all the cases^[48]. In a review of 22 patients with active and refractory perianal Crohn's disease treated with hyperbaric oxygen, 73% achieved a response^[49]. In a randomized, placebo-controlled trial oral, spherical adsorptive carbon was effective for the control of perianal fistulas in patients with Crohn's disease (remission rates were 29.6% *vs* 6.7% for placebo)^[50]. There is not sufficient evidence for any of these agents to support their use in patients with Crohn's perianal fistulas outside of clinical trials.

Summary of medical treatment in current guidelines: Despite methodological limitations in the supporting studies, antibiotics and azathioprine or 6-mercaptopurine are considered first-line therapy in complex perianal disease in the ECCO consensus statement^[6], and infliximab is reserved as a second-line treatment in case of failure. In the AGA technical review^[4,5] infliximab is recommended for treatment of complex perianal disease along with azathioprine or 6-mercaptopurine and antibiotics for the induction phase. Maintenance is recommended with azathioprine or 6-mercaptopurine, in association with infliximab in some cases.

Surgical treatment

Surgical treatment of complex perianal fistulizing disease aims to control sepsis through abscess drainage and intervention in the fistula tracts, including placement of non-cutting setons^[51]. Fistulectomy or fistulotomy are rarely indicated in complex fistulas in view of the high rate of proctectomy because of nonhealing or incontinence associated with the procedure^[6,51,52]. In severe cases with high fistulas, endorectal flaps are useful^[51,53]. In patients with severe refractory disease, diversion with ostomy (loop ileostomy or end sigmoid colostomy) or even proctectomy might be necessary.

In an uncontrolled study carbon dioxide laser ablation has been used as an alternative treatment in patients with perianal Crohn's disease^[54].

Combined medical and surgical treatment

The ideal treatment goal for complex perianal fistulas associated with Crohn's disease is the closure of all the fistulas and the prevention of recurrence. The

best outcomes have been achieved in studies using a combination of medical and surgical therapy^[55,56].

Surgery may offer some advantages when combined with medical treatment, for example, infliximab. There is concern, however, that use of infliximab may cause abscesses by inducing rapid closure of the fistulas. This problem might be reduced by performing MRI or EUS-guided EUA to detect all fistula tracks and to insert draining setons. Regueiro *et al.*^[55] observed that patients who underwent an EUA before infliximab administration were significantly less likely to have fistula recurrence compared to those treated with infliximab alone (44% *vs* 79%).

Hyder *et al.*^[57] investigated such a strategy in patients with perianal Crohn's disease. After EUA, 12 out of 22 patients required abscess drainage and 17 out of 22 had at least one drainage seton inserted. The short-term efficacy of that strategy was as high as 85% as measured by the PDAI; although the authors noted that long term healing rates were low. Talbot *et al.*^[58] reported a similar strategy in patients with complex fistulas-setons were inserted and removed after the second infliximab infusion. Complete healing of the perianal fistula was obtained in 47% of the patients and all showed at least a partial response.

The presence of active proctitis has a negative impact on the outcome of the surgical treatment of Crohn's fistulas. A pilot study suggested that infliximab treatment has a beneficial additive effect in the multistep treatment to first improve the proctitis before performing surgery in complex perianal Crohn's disease with active proctitis^[59].

In one recent retrospective study, 21 patients with Crohn's perianal fistulas and symptomatic perianal disease were treated according to a treatment protocol of serial EUS examinations^[60]. Surgical and medical therapy was tailored to the results of the EUS findings with seton placement and incision and drainage procedures performed when appropriate. Follow-up EUS examination guided when to remove setons or when to stop infliximab or antibiotics. Median follow-up was 68 wk (35-101 wk). No abscesses developed in any patient. Eighteen out of 21 patients (86%) had complete drainage cessation initially, and 16 out of 21 (76%) had long term cessation of drainage. Eleven (52%) had no persistent fistula activity on EUS. In 7 of these, the fistula remained closed after stopping infliximab or antibiotics while the remaining 4 continued infliximab for mucosal disease. This study showed that EUS-guided combination surgical and medical treatment with infliximab had high short and long term fistula response rates^[60].

In a recent randomized prospective study, 10 patients with active Crohn's perianal fistulas were randomized to either EUS guidance or control^[61]. All patients underwent an initial EUS. Patients in the EUS cohort were evaluated by a colorectal surgeon who had access to the EUS findings. The surgeon was blinded to the results of the initial EUS for those in the control group. EUA with seton placement or incision and drainage

was done at the surgeon's discretion, and all patients received optimal medical therapy including antibiotics, azathioprine or 6-mercaptopurine and infliximab. Patients in the control group received further therapy at the surgeon's discretion without EUS guidance. Those in the EUS cohort had additional EUS evaluations at weeks 22 and 38 with further therapy based on EUS results. One of 5 (20%) in the control group and 4 of 5 (80%) in the EUS group had complete cessation of drainage. In this small study, EUS guidance for combination medical and surgical therapy in perianal Crohn's disease appeared to improve outcomes.

Local treatments

Fistula healing is not possible in a significant percentage of patients with complex fistulizing Crohn's disease managed according to the currently accepted treatment algorithms^[4-6]. In addition, systemic medical treatments may be subject to intolerance or loss of response and surgical treatments such as fistulotomy should be used with caution given the risk of incontinence. Thus there is a therapeutic gap in the management of perianal Crohn's disease, and a number of local therapies which aim to achieve complete closure are under development. Table 1 summarizes studies of these new local treatments. The following sections will discuss these new local treatments in more detail.

Topical tacrolimus: Topical tacrolimus has been used successfully in the treatment of skin diseases with an immune component such as atopic dermatitis^[62]. Casson *et al.*^[63] therefore decided to investigate whether an in-house-prepared topical formulation could be beneficial in a series of pediatric patients with different manifestations of Crohn's disease including one case of perianal fistula; the patient responded to treatment although details of fistula healing were not presented.

The efficacy of topical tacrolimus in perianal Crohn's disease was recently investigated in a randomized placebo-controlled study^[64]. In that study, 19 patients, 12 of whom had fistulizing perianal Crohn's disease, were randomized to topical tacrolimus (1 mg in 1 g ointment applied twice daily) or placebo for 12 wk. In the case of patients with fistulas, the primary outcome measure was improvement defined as $\geq 50\%$ decrease in actively draining fistulas on 2 consecutive visits. Treatment showed a beneficial effect on anal and perianal ulcerating disease but lacked efficacy in the treatment of fistulizing Crohn's disease.

Fibrin glue: Instilling fibrin glue into fistulas is a simple and safe procedure which does not preclude the use of other techniques or repeat procedures in the case of failure^[65]. Several studies have been published of series of patients treated with fibrin glue and success rates vary from 0% to 80% (Table 1). This variability can be attributed, among other things, to the different types of fistulas treated (simple or complex; cryptoglandular, Crohn's, or traumatic etiology), and the differences in the definition of healing.

Table 1 Summary of studies of local treatments

Intervention reference	Study design and patients	Main findings
Topical tacrolimus		
Casson <i>et al</i> ^[63] , 2000	Case study of series of pediatric Crohn's patients including 1 patient with perianal fistula	Response reported in patient with perianal fistula
Hart <i>et al</i> ^[64] , 2007	Randomized, placebo-controlled study in 19 patients (12 Crohn's)	Treatment found not to be beneficial in perianal fistulas
Fibrin glue		
Abel <i>et al</i> ^[79] , 1993	Uncontrolled study of use of fibrin glue in 10 patients (2 Crohn's)	0/2 patients with Crohn's disease achieved healing
Cintron <i>et al</i> ^[80] , 2000	79 patients (6 Crohn's) assigned to 3 types of fibrin glue treatment	2/6 Crohn's patients (33%) achieved healing (no drainage)
Lindsey <i>et al</i> ^[66] , 2002	Randomized trial comparing fibrin glue with conventional surgery (fistulotomy or loose seton placement) in 42 patients (6 Crohn's and complex perianal fistula)	Healing (no drainage) in 2/6 Crohn's patients (33%) who received fibrin glue. No Crohn's patients received conventional surgery
Sentovich ^[81] , 2003	Uncontrolled study: 48 patients (5 Crohn's) underwent seton placement followed by instillation of fibrin glue	Healing in 4/5 (80%) Crohn's patients
Zmora <i>et al</i> ^[82] , 2003	Retrospective review of 37 patients with perineal fistula (7 Crohn's) treated with fibrin glue	Healing in 3/7 Crohn's patients (43%) (2 patients also treated with endorectal advancement flap)
Loungnath <i>et al</i> ^[83] , 2004	Retrospective review of 42 patients with perianal fistula (13 Crohn's) treated with fibrin glue	Lasting fistula healing in 4/13 (31%)
Singer <i>et al</i> ^[84] , 2005	Randomized trial comparing fibrin glue + antibiotics, fibrin glue + surgery, and fibrin glue + antibiotic and surgery in 75 patients (3 Crohn's)	Treatment failed in all 3 Crohn's patients (fibrin glue + antibiotic in 1 patient and fibrin + antibiotic and surgery in 2 patients)
Intralesional infliximab		
Poggioli <i>et al</i> ^[68] , 2005	Uncontrolled study of 15 Crohn's patients with complex perianal fistulas	Healing in 10/15 patients after 3-12 infusions
Asteria <i>et al</i> ^[69] , 2006	Uncontrolled study of 11 Crohn's patients with complex perianal fistulas naïve to infliximab	8/11 patients responded ($\geq 50\%$ reduction in fistula drainage) to treatment
Adipose-derived stem cell (ASC) therapy with fibrin glue		
García-Olmo <i>et al</i> ^[72] , 2005	Uncontrolled proof-of-concept study in patients with fistulizing Crohn's disease, including 1 perineal fistula	Perineal fistula healed after 8 wk
García-Olmo <i>et al</i> ^[73] , 2009	Randomized controlled phase II study comparing fibrin glue + ASCs with fibrin glue in 49 patients with complex perianal fistula (14 Crohn's)	Healing in 5/7 Crohn's patients (71%) in fibrin glue + ASCs group compared to 1/7 (14%) in the control group
Fistula plugs		
O'Connor <i>et al</i> ^[75] , 2006	Uncontrolled study of fistula plug in 20 Crohn's patients with fistula tracts not amenable to fistulotomy	Success rate of 80%, lower in the case of complex fistulas
Schwandner <i>et al</i> ^[76] , 2008	Uncontrolled study of 19 patients (7 Crohn's) with trans-sphincteric anorectal fistulas	Treatment success in 6/7 patients with Crohn's disease (86%)
Ky <i>et al</i> ^[77] , 2008	Prospective analysis of 45 patients (20 with complex fistulas and 14 with Crohn's disease) receiving anal fistula plug	Healing in 4/14 Crohn's patients (29%) after a median follow-up of 6.5 mo

Only one controlled study with patients with Crohn's disease has compared fibrin glue with surgical treatment not involving fibrin glue. In that study, Lindsey *et al*^[66] randomized patients with simple and complex fistulas to treatment with fibrin glue or conventional treatment (fistulotomy or loose seton placement with or without subsequent flap advancement). For the purposes of the study, complex fistulas were defined as high fistulas, fistulas associated with Crohn's disease, and low fistulas with compromised sphincters. Both Crohn's patients with complex fistulas reported healing, in one case after a second procedure. Healing among Crohn's patients in the other arm was not reported.

Intralesional infliximab: Although systemic infliximab administration is considered one of the more efficacious therapeutic options available for complex perianal fistulas associated with Crohn's disease, several authors have investigated the efficacy of local application of this drug. The main rationale for this approach is to try and avoid the potential systemic toxicity associated with infliximab. The first study to employ this approach was

published by Lichtiger *et al*^[67] in 2001. Nine patients with perianal Crohn's disease refractory to antibiotics or 6-mercaptopurine were treated with a circumferential and intrafistulous injection of infliximab at 0, 4, and 7 wk. Remission or partial response was achieved in 83% of the patients.

Since then, a number of uncontrolled studies have been conducted to assess the feasibility of local infliximab therapy. Poggioli *et al*^[68] included 15 patients with complex perianal fistulas associated with Crohn's disease. In 9 of these, intravenous infusion of infliximab was felt to be contraindicated because of fibrostenotic disease. The patients were injected with 15-21 mg of infliximab at the internal and external openings and along the fistula tract. The injections were well tolerated and 10 of the 15 patients achieved healing after 3 to 12 injections. A similar study was reported by Asteria *et al*^[69], although patients were excluded if they had received prior treatment with infliximab. Up to 3 injections of 20 mg of infliximab were made along the fistula tract and at both openings. The efficacy endpoint was reduction in fistula drainage of 50% or more (response)

or complete cessation of fistula drainage for at least 4 wk (remission). Overall, 8 patients achieved a response (73%) and, of these, 3 achieved remission (27%). After a longer follow-up (mean 10.5 mo, range 7-18 mo), 6 patients were responders and 4 were in remission.

Adipose-derived stem cell therapy: Adult stem cell therapy has promising applications in a number of areas of medicine and has no ethical concerns. Given that liposuction is a relatively safe procedure, an appealing source of adult stem cells is lipoaspirate^[70]. The stromal cells obtained are subsequently cultured and expanded to produce autologous adipose-derived adult stem cells (ASCs). Trials of ASCs in the treatment of fistulizing Crohn's disease have delivered the expanded ASCs by injecting them around the fistula opening and directly into the fistula tract.

The first procedure of this type published in the literature was a case report of a 33-year-old woman with Crohn's disease and a rectovaginal fistula refractory to treatment^[71]. ASCs were injected into the rectal mucosa, close to the sutured internal opening. After resection of the posterior vaginal wall and construction of an advancement vaginal flap, the accessory perineal hole was sealed with 2 mL of fibrin glue. One week after the intervention, the wound had completely healed. In 3 mo of follow-up, no recurrence of the rectovaginal fistula was reported.

A subsequent phase I study assessed 9 ASC injection procedures in 4 patients with fistulizing Crohn's disease^[72]. The series included 3 rectovaginal fistulas and 1 perineal fistula. As before, cells were injected into the rectal mucosa, close to the sutured opening and fistula tracks were then filled with fibrin glue. Two of the 3 rectovaginal fistulas and the perineal fistula had healed after 8 wk.

One randomized clinical trial using ASCs has also been conducted. In a recently completed phase II study, 49 patients with perianal fistula-14 of whom had Crohn's disease-were randomized to receive ASC therapy and fibrin glue or to receive fibrin glue alone (control group)^[73]. The primary outcome measure was the proportion of patients with complete fistula closure. An investigator blinded to the treatment confirmed healing by examination of a digital photograph. Five of the 7 patients with Crohn's disease assigned to ASC therapy achieved healing compared to one of 7 patients in the control group. The difference was not statistically significant but the study was not powered to detect differences in small subgroups such as those with Crohn's disease. Healing in this trial was defined as the absence of drainage, as well as complete epithelization of external openings. No severe adverse events related to ASCs have been reported when utilized for fistulizing Crohn's disease.

Fistula plugs: Recently, the use of bioprosthetic plugs made from porcine intestinal submucosa has been tried in patients with perianal fistula. In a prospective study which excluded patients with Crohn's disease, Johnson

et al^[74] randomized patients with high transsphincteric or deeper fistulas to either fistula plug or fibrin glue therapy. Of the 10 patients who underwent fibrin glue treatment, 6 (60%) had persistence of one or more fistulas at 3 m compared to 2 out of 15 (13%) of those who underwent the procedure with the fistula plug ($P < 0.05$). In a subsequent prospective but uncontrolled study, 20 Crohn's patients with a total of 36 fistula tracts not amenable to fistulotomy were treated with fistula plugs^[75]. After irrigation with hydrogen peroxide, each primary opening was occluded with a fistula anal plug. The authors found an overall success rate of 80%, although they noted that patients with complex fistulas with multiple primary openings were less likely to achieve success. Success appeared to be independent of the presence of setons or the use of anti-TNF- α therapy.

Schwandner *et al*^[76] have reported their experience in a series of 19 patients with transsphincteric anorectal fistulas. Seven of these patients had Crohn's disease. The surgical procedure comprised irrigation of the fistula tract and placement and internal fixation of the anal fistula plug without flap advancement or excision of the fistula tract. Success was defined as closure of both the internal and external openings with no further interventions and absence of abscess formation. Six of the 7 patients with Crohn's disease achieved success (85.7%).

In another retrospective study, anal fistula plugs were more successful in the treatment of simple anorectal fistulas but were associated with a high failure rate in complex perianal fistulas and particularly in patients with Crohn's disease (closure rate of 26.6% of fistulas in this group)^[77].

In a recent retrospective review reported at the 2008 Digestive Diseases Week conference^[78], the use of anal fistula plugs was associated with a lower success rate than previously reported: only 2 of the 22 (15%) Crohn's disease-associated fistulas healed. In 87% of the procedures the reason for failure was sepsis. These controversial results in an uncontrolled series await confirmation by randomized trials.

CONCLUSION

Symptomatic perianal fistulas in patients with Crohn's disease can have a large negative impact on quality of life. Treatment of complex perianal fistulas remains a difficult problem. Use of anti-TNF- α antibody therapy is widespread and supported by randomized clinical trials. Nevertheless, some patients fail anti-TNF- α treatment and, given the risk of incontinence associated with aggressive surgical procedures, there remains an unmet therapeutic need. Some of the emerging local therapies have obtained promising results in patients with fistulizing Crohn's disease in uncontrolled studies and case series but, for the most part, this promise has still to be confirmed in randomized trials. Adipose-derived stem cell therapy has compared favorably with fibrin glue alone in a randomized phase II trial, and high

healing rates were observed. Once the efficacy of these new local therapies has been confirmed, further effort will be required to optimize their use in the management of fistulizing Crohn's disease, which is necessarily complex and multidisciplinary.

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Meta-analysis and systematic review of colorectal endoscopic mucosal resection

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Abstract

AIM: To evaluate the proportion of successful complete cure *en-bloc* resections of large colorectal polyps achieved by endoscopic mucosal resection (EMR).

METHODS: Studies using the EMR technique to resect large colorectal polyps were selected. Successful complete cure *en-bloc* resection was defined as one piece margin-free polyp resection. Articles were searched for in Medline, Pubmed, and the Cochrane Control Trial Registry, among other sources.

RESULTS: An initial search identified 2620 reference articles, from which 429 relevant articles were selected and reviewed. Data was extracted from 25 studies ($n = 5221$) which met the inclusion criteria. All the studies used snares to perform EMR. Pooled proportion of *en-bloc* resections using a random effect model was 62.85% (95% CI: 51.50-73.52). The pooled proportion for complete cure *en-bloc* resections using a random effect model was 58.66% (95% CI: 47.14-69.71). With higher patient load (> 200 patients), this complete cure *en-bloc* resection rate improves from 44.19% (95% CI: 24.31-65.09) to 69.17% (95% CI: 51.11-84.61).

CONCLUSION: EMR is an effective technique for the resection of large colorectal polyps and offers an alternative to surgery.

INTRODUCTION

The use of endoscopic mucosal resection (EMR), pioneered in Japan for the treatment of early gastric cancer, has expanded to include therapy of other early gastrointestinal malignancies and pre-cancerous lesions such as adenomas. At the same time, this technique has gained acceptance in Europe and in the US, especially for the treatment of Barrett's esophagus with high grade dysplasia^[1-3]. Several variations of the EMR technique have been devised such as inject-lift-cut, strip biopsy, suction cup (EMRC), and EMR with a ligating device.

Throughout the world, adenomas of the colorectum represent the single most important premalignant lesion of the GI tract. Large (> 2 cm) colorectal polyps have been found in 0.8%-5.2% of patients undergoing colonoscopies for different indications^[4].

Large sessile and flat polyps represent a major technical challenge to conventional snare resection. Additional procedures and therapies such as Argon plasma coagulation are frequently needed to destroy remnant tissue after resection^[5]. When these techniques are not used or possible, patients are frequently referred for surgical resection^[6].

EMR has been shown to be useful in the removal of large colorectal sessile and flat lesions^[7]. However, there are limits to the size of lesions which can be removed *en-bloc* with the various EMR techniques, with 1.5-2 cm generally being the upper limit^[8].

En-bloc removal of large polyps is desirable as it facilitates thorough histological evaluation related to the

completeness of resection, and is associated with a lower recurrence rate as compared to piecemeal removal^[19-14].

MATERIALS AND METHODS

Study selection criteria

Studies using EMR technique to resect large (> 2 cm) colorectal polyps were selected. Successful cure *en-bloc* resection was defined as one piece removal with tumor-free vertical and lateral margins.

Data collection and extraction

Articles were searched for in Medline, Pubmed, Ovid journals, Japanese language literature, Cumulative Index for Nursing & Allied Health Literature, ACP journal club, DARE, International Pharmaceutical Abstracts, old Medline, Medline non-indexed citations, OVID Healthstar, and the Cochrane Controlled Trials Registry. The search terms used were EMR, endoscopic mucosal resection, colon polyps, lateral spreading tumors, large polyps, nonpolypoid colon lesions, flat colon polyps, and flat adenomas. Two authors (SP and YK) independently searched and extracted the data for revising into an abstracted form. Any differences were resolved by mutual agreement.

Quality of studies

Clinical trials with a control arm can be assessed for the quality of the study. A number of criteria have been used to assess the quality of a study (e.g. randomization, selection bias of the arms in the study, concealment of allocation, and blinding of outcome)^[15,16]. There is no consensus regarding how to assess studies without a control arm. Hence, these criteria do not apply to studies without a control arm^[16]. Therefore, for this meta-analysis and systematic review, studies were selected based on completeness of data and inclusion criteria.

Statistical methods

This meta-analysis was performed by calculating pooled proportions, i.e. pooled proportion of *en-bloc* resections and complete cure *en-bloc* resections. Firstly, the individual study proportions of successful resections were transformed into a quantity using Freeman-Tukey variant of the arcsine square root transformed proportion. The pooled proportion was calculated as the back-transform of the weighted mean of the transformed proportions, using inverse arcsine variance weights for the fixed effects model and DerSimonian-Laird weights for the random effects model^[17,18]. Forrest plots were drawn to show the point estimates in each study in relation to the summary pooled estimate. The width of the point estimates in the Forrest plots indicated the assigned weight to that study. The heterogeneity among studies was tested using Cochran's Q test based upon inverse variance weights^[19]. If P value was > 0.10 , the null hypothesis was rejected that the studies were heterogeneous. The effects of publication and selection bias on the summary estimates were tested by Begg-Mazumdar bias indicator^[20]. Also, funnel plots were constructed to evaluate potential publication bias using the standard error and diagnostic odds ratio^[21,22].

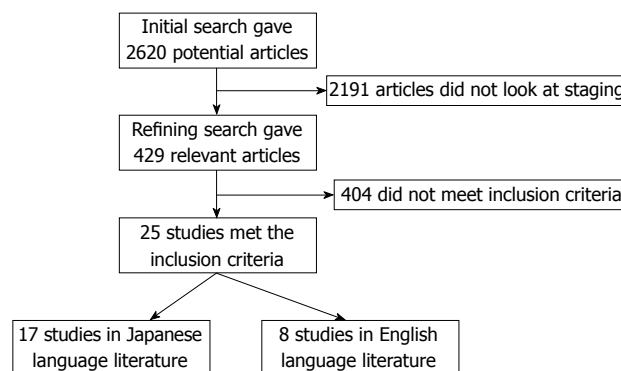


Figure 1 Search results.

RESULTS

An initial search identified 2620 reference articles from which 429 relevant articles were selected and reviewed. Data was extracted from 25 studies ($n = 5221$) which met the inclusion criteria^[23-46]. The search results are shown in Figure 1. All the studies used snare to perform EMR. Two studies used a strip biopsy technique^[42,43]. The mean size of the polyps was 22.48 ± 4.52 mm. There were 3755 successful *en-bloc* resections. The study characteristics are shown in Table 1.

The pooled proportion of *en-bloc* resections using a random effect model was 62.85% (95% CI: 51.50-73.52). Forest plot in Figure 2A depicts the individual study proportion of successful *en-bloc* resections in relation to the pooled estimate. The pooled proportion for complete cure *en-bloc* resections using a random effect model was 58.66% (95% CI: 47.14-69.71). Figure 2B shows Forrest plot depicting the individual study successful cure *en-bloc* resections in relation to the pooled estimate. The fixed effect model was not used because of the heterogeneity of studies.

Subgroup analysis was carried out by grouping studies according to the study population. This was done because the expertise needed to perform procedures might have affected the outcome. Studies were categorized into three groups: < 100 patients, 100-200 patients and > 200 patients. The proportions for successful *en-bloc* and successful cure *en-bloc* resections are shown in Table 2.

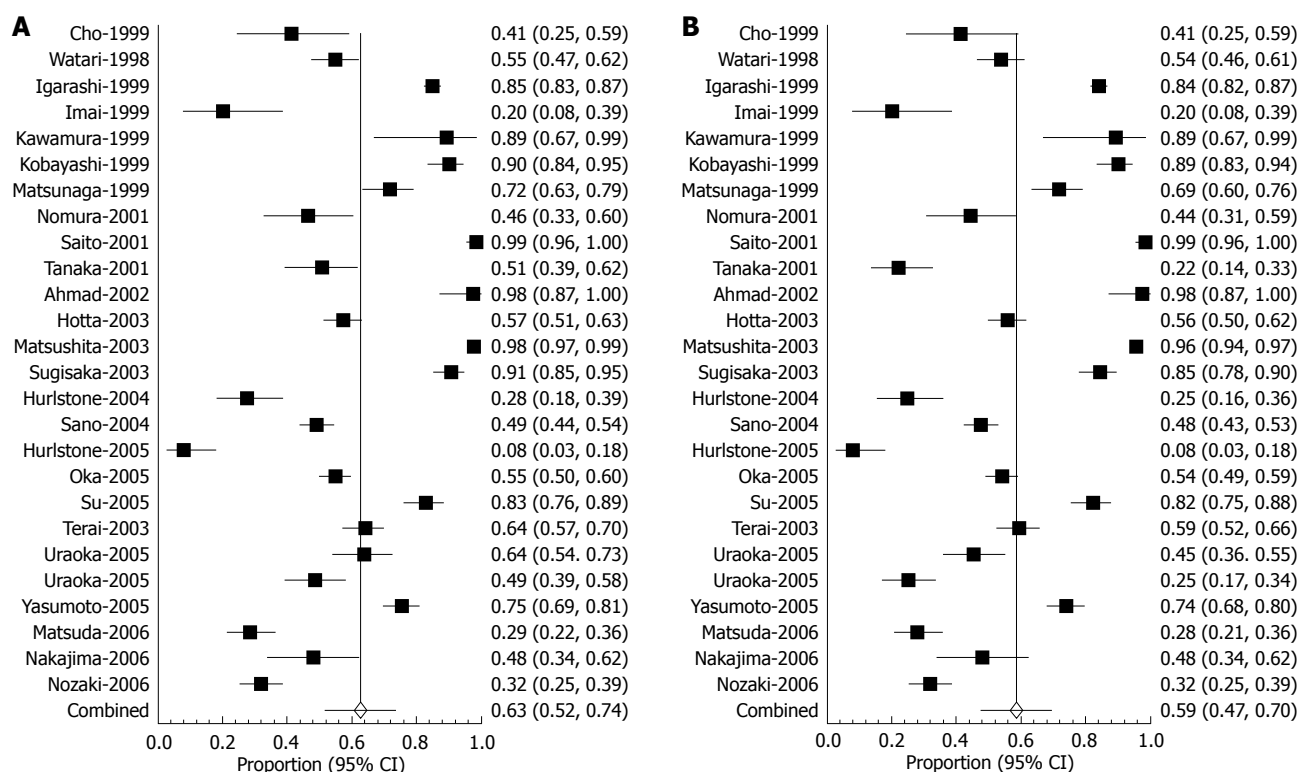
The publication bias calculated by Begg-Mazumdar bias indicator for successful cure *en-bloc* resections concluded that the Kendall's tau b value was -0.19 ($P = 0.17$). The funnel plot in Figure 3 shows that there was no publication bias for successful cure *en-bloc* resections.

DISCUSSION

Some colorectal cancers develop from adenomas. The risk of high grade dysplasia and cancer increases with the size of the lesion. Endoscopic removal of large (> 2 cm) sessile and flat polyps represents a difficult challenge for conventional snare resection and they are frequently managed by piecemeal resection or surgically^[6,47]. EMR was the definitive procedure in all the collated studies. The data for complications was not available for the majority of the studies, so this data was not collected. EMR is a technique that can be applied to sessile and flat

Table 1 Study characteristics

	Author, yr	Instrument used	n	Type of polyp	Technique
1	Matsushita <i>et al</i> ^[23] , 2003	Snare	935	No information	EMR
2	Imai <i>et al</i> ^[24] , 1999	Snare	30	No information	EMR
3	Igarashi <i>et al</i> ^[25] , 1999	Snare	884	No information	EMR
4	Oka <i>et al</i> ^[26] , 2005	Snare	410	Lateral spreading tumor	EMR
5	Sano <i>et al</i> ^[27] , 2004	Snare	392	Lateral spreading tumor	EMR
6	Hotta <i>et al</i> ^[28] , 2003	Snare	284	Protrusion 68, flat 213, depressed 3	EMR
7	Matsuda <i>et al</i> ^[29] , 2006	Snare	154	Is, Isp 33, LST-G 96, NG 25	EMR
8	Yasumoto <i>et al</i> ^[30] , 2005	Snare	240	LST-G 180, NG 60	EMR
9	Terai <i>et al</i> ^[31] , 2003	Snare	223	Lateral Spreading tumor	EMR
10	Nozaki <i>et al</i> ^[32] , 2006	Snare	198	Ip 3, Isp 34, Is 7, LST-G 85, NG 28	EMR
11	Watari <i>et al</i> ^[33] , 1998	Snare	186	Lateral spreading tumor	EMR
12	Sugisaka <i>et al</i> ^[34] , 2003	Snare	162	No information	EMR
13	Matsunaga <i>et al</i> ^[35] , 1999	Snare	134	No information	EMR
14	Nomura <i>et al</i> ^[36] , 2001	Snare	54	No information	EMR
15	Kobayashi <i>et al</i> ^[37] , 1999	Snare	131	No information	EMR
16	Nakajima <i>et al</i> ^[38] , 2006	Snare	52	No information	EMR
17	Cho <i>et al</i> ^[39] , 1999	Snare	34	No information	EMR
18	Saito <i>et al</i> ^[40] , 2001	Snare	170	Lateral spreading tumor	EMR
19	Tanaka <i>et al</i> ^[41] , 2001	Snare with needle spike	81	Lateral spreading tumor	EMR
20	Ahmad <i>et al</i> ^[42] , 2002	Snare with suction	41	Colon and rectum	EMR
21	Hurlstone <i>et al</i> ^[43] , 2004	Strip technique of Karita	80	Rectal villous adenoma	EMR
22	Hurlstone <i>et al</i> ^[43] , 2005	Strip technique of Karita	62	Rectal villous adenoma	EMR
23	Su <i>et al</i> ^[44] , 2005	Snare with needle spike	152	Colonic nonpolypoid lesions	EMR
24	Uraoka <i>et al</i> ^[45] , 2005	Snare	113	Lateral spreading tumor	EMR
25	Kawamura <i>et al</i> ^[46] , 1999	Snare	19	Submucosal invasive colorectal cancers	EMR

Figure 2 Forrest plot showing successful *en-bloc* (A) and cure *en-bloc* (B) resection.

lesions. Though initially used for the treatment of early gastric cancer in Japan, the technique has been expanded to the therapy of large colorectal neoplasms^[7].

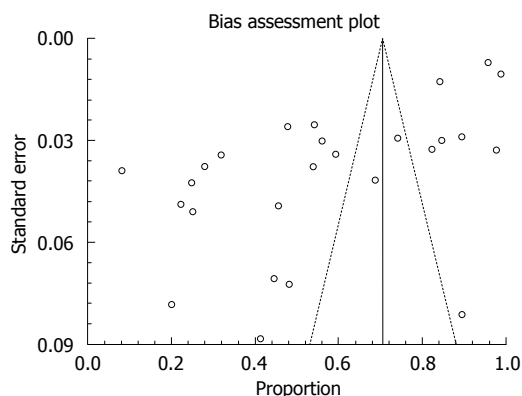
This meta-analysis revealed that *en-bloc* resection was achieved in 62.85% of lesions and tumor-free vertical and lateral margins were achieved in 58.6%. These results compare well to *en-bloc* resection rates achieved by conventional polypectomy snare, which have been reported

to be between 7% and 34% for large sessile polyps^[6,48].

Furthermore, our meta-analysis revealed that experience performing EMR plays an important role in achieving a better *en-bloc* resection and cure *en-bloc* tumor-free rate. Studies reporting more than 200 lesions removed reported a 71.39% *en-bloc* resection of lesions and tumor-free vertical and lateral margins in 69.17% of cases, while studies reporting less than a 100 lesions reported a

Table 2 Results based on study size

Study size	No. of studies	Successful <i>en-bloc</i> resection (95% CI)	Successful cure <i>en-bloc</i> resection (95% CI)
< 100 patients	9	48.07% (28.36-68.09)	44.19% (24.31-65.09)
100-200 patients	9	68.93% (50.39-84.76)	63.32% (43.50-81.04)
> 200 patients	7	71.39% (52.24-87.20)	69.17% (51.11-84.61)

Figure 3 Funnel plot showing publication bias for successful cure *en-bloc* resection.

48.07% *en-bloc* removal and tumor-free vertical and lateral margins in 44.19% of cases. This indicates that experience in the technique of EMR increase the cure *en-bloc* rate.

In the present meta-analysis we searched the world literature which included articles published in Japanese language literature. We believe that our results are a reasonable reflection of the status of EMR in the therapy of large colorectal polyps.

EMR is an effective technique for resection of large colorectal polyps. The technique offers an alternative to surgery. This meta-analysis shows that the success rate for *en-bloc* margin-free resection is not high but improves with experience. Improvements in techniques and equipment are needed to increase complete cure *en-bloc* resection rates.

COMMENTS

Background

Endoscopic mucosal resection (EMR) has emerged as an alternative to surgery for the resection of large colorectal polyps. Complete cure with tumor-free lateral and vertical margins would prevent further therapy. Published data regarding successful *en-bloc* resection with tumor-free margins by EMR has been varied.

Innovations and breakthroughs

EMR has been shown to be useful in the removal of large colorectal sessile and flat lesions. However, there are limits to the size of lesions which can be removed *en-bloc* with the various EMR techniques, with 1.5-2 cm generally being the upper limit. *En-bloc* removal of large polyps is desirable as it facilitates thorough histological evaluation related to the completeness of resection, and is associated with a lower recurrence rate as compared to piecemeal removal.

Applications

EMR is an effective technique for resection of large colorectal polyps and offers an alternative to surgery. This meta-analysis shows that the success rate for *en-bloc* margin-free resection is not high but improves with experience. Improvements in techniques and equipment are needed to increase complete cure *en-bloc* resection.

Peer review

The authors evaluated the proportion of successful complete cure *en-bloc*

resections of large colorectal polyps achieved by EMR. They found that EMR is an effective technique for resection of large colorectal polyps. This article is well written and easy to read.

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BRIEF ARTICLES

Sirolimus, bevacizumab, 5-Fluorouracil and irinotecan for advanced colorectal cancer: A pilot study

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irinotecan, bevacizumab and sirolimus in advanced colorectal carcinoma after failure of classical treatment is feasible and promising. Further evaluation of this combination is required.

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Abstract

AIM: To evaluate the efficacy and the safety of combined 5-Fluorouracil, irinotecan, bevacizumab and sirolimus in refractory advanced colorectal carcinoma.

METHODS: We initiated a regimen with at day 1 an injection (*iv*) of bevacizumab at 5 mg/kg, followed by 180 mg/m² irinotecan, followed by Leucovorin 400 mg/m², followed by a 5-Fluorouracil bolus 400 mg/m² and a 46-h infusion 2400 mg/m². Sirolimus was given orally as continuous administration of 2 mg twice a day every days. This treatment was repeated every 14 d.

RESULTS: A total of 12 patients were enrolled. All patients presented with metastatic disease that had failed at least three lines of chemotherapy that contained oxaliplatin, irinotecan and bevacizumab. Cetuximab failure was also observed in all K-Ras wild-type patients. The median number of cycles was 8.5 (range 2-20) and clinical benefit was observed in eight patients. The median time to progression was 5 mo and the median survival was 8 mo. Grade 3 neutropenia developed in four patients, and grade 3 diarrhea and stomatitis in two.

CONCLUSION: The combination regimen of 5-Fluorouracil,

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide^[1]. Approximately 25% of patients with colorectal cancer present with overt metastatic disease, and metastatic disease develops in 40%-50% of newly diagnosed patients. Standard first-line treatments include fluorouracil (5-FU) with leucovorin and irinotecan^[2,3] or oxaliplatin^[4], alone or combined with bevacizumab^[5]. Cetuximab, a chimeric IgG1 monoclonal antibody against epidermal growth factor receptor (EGFR), has efficacy as monotherapy and in combination with irinotecan in irinotecan-resistant patients, if the tumor expresses wild-type K-RAS and B-RAF^[6,7]. However if these standard treatments fail, there are no accepted treatment options, and for such patients historical estimation of progression-free survival (PFS) and overall survival (OS) are about 2 and 4 mo, respectively^[8].

Recent results have suggested that inhibitors of mTor (mammalian target of rapamycin) signal are able to improve survival and induce tumor response in patients with poor-prognosis renal cell carcinoma^[9], and combination of mTor inhibitors and classical

chemotherapy is currently under clinical investigation. In colorectal carcinoma cell lines *in vitro*, rapamycin enhances the antitumor effect of irinotecan through Hypoxia-inducible factor-1 α inhibition (a key transcription factor that regulates angiogenesis) and by disruption of tumor vasculature^[10]. Therefore, we hypothesize that, in multi-treated colorectal patients, multimodal angiogenesis targeting using irinotecan, bevacizumab and the mTor inhibitor sirolimus can have a therapeutic effect.

The goal of this pilot study was to evaluate the safety and efficacy of sirolimus, bevacizumab, leucovorin, 5-FU, and irinotecan (FOLFIRI) in patients with advanced colorectal cancer that had progressed after treatment with 5-FU, irinotecan, oxaliplatin, bevacizumab and anti-EGFR therapy.

MATERIALS AND METHODS

Eligibility criteria

The eligibility and exclusion criteria, and pretreatment characteristics of the patients are presented in Table 1. Written informed consent was required before chemotherapy.

Treatment protocols and dose modification

On day 1, irinotecan (180 mg/m²) was administered as a 2-h *iv* infusion. Then leucovorin 400 mg/m² followed by a 5-Fluorouracil bolus 400 mg/m² was administered as a bolus *iv* injection. Then 5-Fluorouracil 2400 mg/m² was given as a 46-h *iv* infusion. Bevacizumab was given at 5 mg/kg as an *iv* infusion every two weeks over 60 min before the beginning of the chemotherapy. Sirolimus was given orally as continuous administration of 2 mg twice a day every days. The doses used for fluorouracil, irinotecan and bevacizumab were the classical recommended doses^[2-5]. For sirolimus, a dose of 4 mg/d was chosen because a phase I study has demonstrated that this is tolerable and sufficient to achieve mTor inhibition^[11]. Dose modifications of irinotecan or 5-FU were made for hematological or non-hematological toxicity, on the basis of the most severe grade of adverse effect that occurred during the previous cycle. Treatment was delayed until the absolute number of neutrophils was > 1000/ μ L, platelets were > 100 000/ μ L, and mucositis, diarrhea, or skin toxicity had recovered to grade 1 or less. The 5-FU dose was reduced after the occurrence of National Cancer Institute Common Toxicity Criteria (NCI-CTC) grade 3 diarrhea, stomitis or dermatitis. For toxicity of grade 3 or higher, a 20% dose reduction for irinotecan was prescribed by the protocol. Bevacizumab was retained for uncontrolled hypertension or proteinuria of > 3 g in 24 h. Bevacizumab was discontinued for grade 3 or 4 hemorrhage, thromboembolic events that required full dose anticoagulation, or any grade 4 toxicity. Sirolimus was discontinued for grade 3 or 4 diarrhea or stomatitis. Treatment was administered until the disease progressed, unacceptable toxic effects developed, or the patient refused further treatment.

Table 1 Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Histologically confirmed colorectal cancer (adenocarcinoma)	Thromboembolism that required therapeutic anticoagulation
Measurable disease	Central nervous system metastasis
No secondary malignancy	Major surgery within 6 wk
Age > 18 years	Nonhealing wounds
ECOG performance status 0-2	Uncontrolled hypertension
Adequate hematological, hepatic and renal function (including urinary excretion of no more than 500 mg protein/d)	Pregnant or lactating women
Failure of bevacizumab, oxaliplatin and irinotecan-based treatment	Bleeding diathesis, active or recent
For wild-type K-Ras: failure with cetuximab and irinotecan combination was required	Cardiovascular disease
Failure caused by significant intolerance to either drug was allowed	Cerebrovascular accident

Pretreatment and follow-up evaluation

Pretreatment evaluation included physical examination, complete blood cell counts, blood chemistry, tumor marker level (carcinoembryonic antigen, CEA), and computed tomography (CT) within 15 d of starting chemotherapy. Tumor responses were determined by RECIST and Choi criteria^[12,13]. Complete blood cell counts, serum chemistry, including liver and renal function, were performed at least every two weeks, and tumor assessment by CT was performed every three cycles.

Statistical analysis

Efficacy analysis was performed according to the intention-to-treat principle. Patients were considered assessable for response if they were eligible, had measurable disease, and had received at least one dose of study therapy. In the analysis of survival and subsequent treatment, all patients were followed until death, loss to follow-up, or termination of the study. PFS and OS were calculated using the Kaplan-Meier method. PFS was calculated from the date therapy started to the date of disease progression, and OS was calculated from the date therapy started to the date of death.

RESULTS

Patient characteristics

Between January 2008 and December 2008, a total of 12 patients were included in this pilot study at the Department of Medical Oncology, Georges-Francois Leclerc Cancer Center, Dijon, France. Demographic details of the patients included in the study are shown in Table 2. There were seven male and five female patients, median age 61 years (range 51-75). All patients had progressed after prior 5-FU, irinotecan and bevacizumab therapy, and 5-FU, oxaliplatin and bevacizumab therapy. Importantly, all patients had been treated previously with FOLFIRI bevacizumab chemotherapy and experienced progression according to the RECIST criteria following this treatment. Nine patients harbored wild-type K-Ras tumor and progressed on irinotecan plus cetuximab

Table 2 Patient characteristics

Characteristics	n
Median age (Range) (yr)	62 (51-75)
Sex	
Male	7
Female	5
ECOG performance status	
0-1	8
2	4
CEA level (range) (ng/mL)	882 (21-6327)
Primary site	
Colon	10
Rectum	2
Sites of metastasis	
Liver	9
Lung	7
Lymph nodes	6
Others	5
K-Ras status	
Wild-type	9
Mutated	3

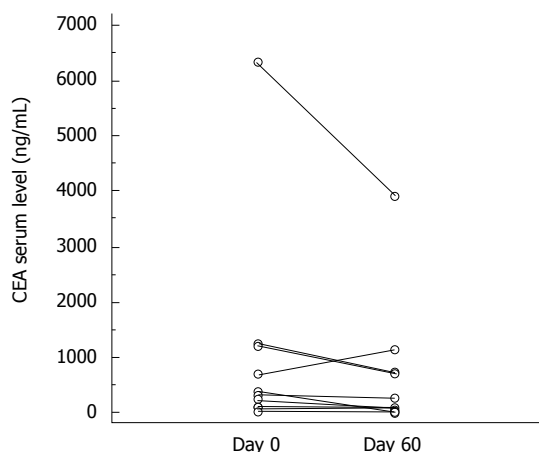


Figure 1 Carcinoembryonic antigen (CEA) serum level before and 2 mo after the beginning of the treatment.

therapy. All 12 patients were assessable for response, for toxicity and survival.

Objective tumor responses and survival

There were a median 8.5 cycles (range 2-20) of chemotherapy. Chemotherapy was stopped because of disease progression in 10 patients, and two discontinued because of toxicity (grade 3 diarrhea and stomatitis). Median follow-up duration was 9 mo. At 2 mo, CEA level decreased in all except one patient (mean level 882 ± 510 vs 579 ± 320 ; $P = 0.025$, Wilcoxon test; Figure 1). According to RECIST criteria, one patient had a partial response, seven had stable disease for > 3 mo, and four had progressive disease. According to the Choi criteria, three patients had a partial response, five had stable disease, and four had progressive disease (Figure 2). PFS was 5 mo (95% CI: 2.5-6), and median OS was 7 mo (95% CI: 4-NR). Figure 3 shows PFS and OS curves.

Toxicity

A total of 116 cycles of chemotherapy were administered.

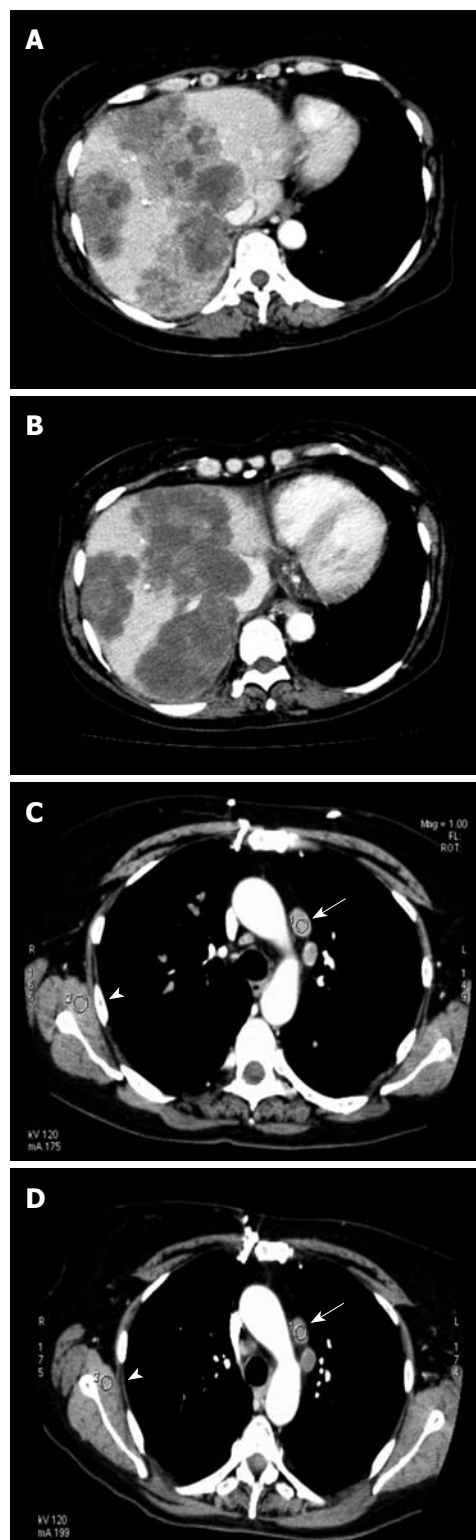


Figure 2 Representative CT scan of patients responding according to Choi criteria. A: (Patient 5) Contrast-enhanced CT-scan (arterial phase) showed three large liver metastases before inclusion; B: Two months later, a significant decrease in the enhancement of the metastases was observed by CT performed under the same conditions (arterial phase). Although lesions were classified as stable disease using RECIST criteria, they could be considered as a partial response using Choi criteria; C: (Patient 8) Contrast-enhanced CT-scan (arterial phase) showed two latero aortic lymph node metastases. Density was 82.8 UH (arrow). To assure comparability, density was also measured in muscle (72.8 UH) (arrow head); D: After 2 mo, although size of lymph node metastases was stable, there was a significant decrease in density (59 UH) (arrow), which led to a partial response using Choi criteria. In comparison, muscle exhibited no decrease in density (73 UH) (arrow head).

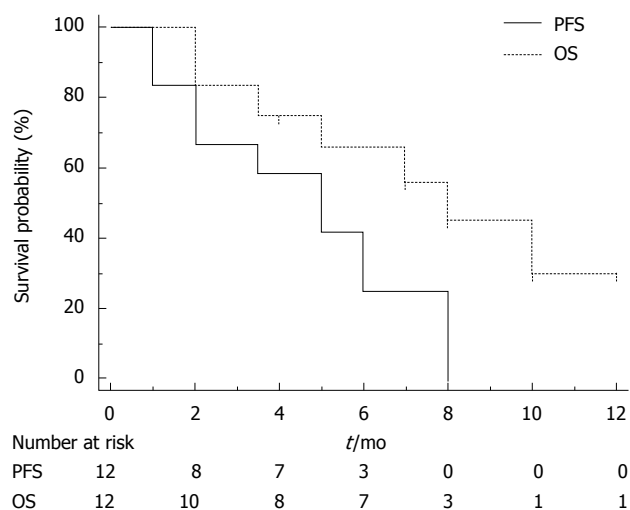


Figure 3 Kaplan-Meier curves of progression-free survival (PFS) and overall survival (OS).

All patients received at least 1 mo of the chemotherapy and sirolimus regimen. Dose modifications or interruptions were required in three patients. The incidence of hematological and non-hematological toxicity is summarized in Table 3. The major grade 3/4 hematological toxicity included neutropenia in three patients (25%) and thrombocytopenia in two (16%). No neutropenic fever was observed. Grade 1/2 nausea, vomiting and diarrhea developed in five patients; however, this toxicity was mild and manageable. In two patients, sirolimus-related grade 3 stomatitis led to treatment discontinuation. Only grade 2 nose bleeding were observed and did not require therapeutic modification. Hypertension occurred in three patients and was managed without drug modification. Proteinuria > 2 g/24 h occurred in one patient and was treated by angiotensin-converting enzyme inhibitors. All patients with stable disease had minor grade 1/2 manageable adverse effects. There was no treatment-related death. Eight deaths were caused by disease progression.

DISCUSSION

Recent trials with advanced colorectal carcinoma have shown that patients may survive for 24–30 mo^[14]. However, after failure of irinotecan, oxaliplatin, bevacizumab and anti-EGFR therapy, there are no accepted treatment options. Estimated PFS is about 2 mo and OS is 4 mo^[8].

Few nonrandomized trial have demonstrated that association of fluorouracil or capecitabine with mitomycin may give some response in heavily pretreated patients^[15–18]. Such treatments give an objective response rate of about 10% and PFS of 2–3 mo. Although, in these trials, patients did not benefit from new target therapies.

Recently, target therapy such as sorafenib and sunitinib have given some promising results in patients with end-stage colorectal cancer. Sunitinib was tested in a phase II trial in patients with previously treated colorectal metastases at a dose of 50 mg/d for 4 wk, followed by 2 wk off treatment^[19]. Median time to progression was 2.5 mo and median OS was 7 mo in bevacizumab-

Table 3 Observed toxicity according NCI-CTC grading (*n* = 12)

	NCI-CTC grade			
	1	2	3	4
Hematological				
Anemia	1	3		
Leucopenia		2	4	
Neutropenia		2	4	
Thrombocytopenia		1	2	
Non hematological				
Nausea/vomiting	2	5		
Mucositis	3	4	2	
Diarrhea		5	2	
Proteinuria		1		
Asthenia	3	5		
High blood pressure		3		

pretreated patients and 10 mo in those not pretreated with bevacizumab. Sorafenib has been tested in association with oxaliplatin in a phase I trial in patients with oxaliplatin-refractory colorectal cancer. The recommended dosage was 130 mg/m² oxaliplatin and continuous sorafenib 400 mg twice daily. This treatment showed promising efficacy^[20].

The phosphoinositide 3-kinase/mTOR axis is a pivotal pathway in cell growth and cell-cycle progression, in response to different stimuli, such as nutrients and growth receptors^[21]. The mTor pathway is activated aberrantly in around half of human tumors and plays a crucial role in angiogenesis. mTor inhibition is now considered as an important target for new anticancer drugs^[22]. In a phase I trial, mTor inhibitors showed minor efficacy in colorectal carcinoma^[23]. *In vitro* and preclinical studies have demonstrated the synergistic efficacy of combined rapamycin and irinotecan^[10]. In fact, irinotecan and rapamycin act on tumor growth by inhibiting angiogenesis by acting on Hypoxia inducible factor-1 α ^[24,25]. Recently, combination of everolimus and bevacizumab has demonstrated some activity in patients with refractory metastatic colorectal cancer who had progressed on a bevacizumab-based regimen, which suggests that bevacizumab and mTor inhibitors overcome resistance to bevacizumab^[26]. Therefore, we hypothesized that combination of sirolimus, bevacizumab and irinotecan may provide some synergistic antiangiogenic effects that can reverse resistance to bevacizumab- and irinotecan-based chemotherapy.

In our pilot study, combination of sirolimus, bevacizumab, 5-FU and irinotecan demonstrated that, for patients with advanced colorectal cancer, some clinical stabilization and prolonged stable disease can be obtained. CT monitoring suggested an antiangiogenic effect of the therapy, with tumor necrosis obtained in three patients. Median PFS of 5 mo and median OS of 8 mo suggest a potential clinical benefit of a such treatment. Despite its potent efficacy, this target therapy can have adverse effects, and we have to balance the efficacy, toxicity and cost of such therapy. Further studies will be needed to confirm our results.

COMMENTS

Background

Novel combinations of new chemotherapeutic agents and target therapies have demonstrated an improvement in tumor response and overall survival in patients with advanced colorectal cancer. Although, after failure of irinotecan, oxaliplatin, bevacizumab and cetuximab, no treatment demonstrates efficacy.

Research frontiers

The phosphoinositide 3-kinase/mTOR (mammalian target of rapamycin) axis is activated aberrantly in around half of human tumors and plays a crucial role in angiogenesis. In a phase I trial, mTor inhibitors showed minor efficacy in colorectal carcinoma. *In vitro* and preclinical studies have demonstrated the synergistic efficacy of combined rapamycin and irinotecan. Recently, combination of everolimus (an mTor inhibitor) and bevacizumab have demonstrated some activity in patients with refractory metastatic colorectal cancer who had progressed on a bevacizumab-based regimen, which suggests that bevacizumab and mTor inhibitors overcome resistance to bevacizumab. Therefore, the authors hypothesized that combination of sirolimus, bevacizumab and irinotecan provides some synergistic antiangiogenic effects that can reverse resistance to bevacizumab- and irinotecan-based chemotherapy.

Innovations and breakthroughs

This is believed to be the first study to investigate the capacity of an mTor inhibitor to reverse resistance to conventional therapies. The data suggest that both treatment regimens demonstrate efficacy and tolerable toxicity in this setting.

Applications

These data are in accordance with the preliminary findings from ASCO 2009, which demonstrate that sirolimus may reverse resistance of colorectal cancer to bevacizumab therapy. The data suggest that the therapy may have some clinical efficacy but substantial adverse effects.

Terminology

Sirolimus, also called rapamycin, is an mTor inhibitor. This drug is used as an immunosuppressant but demonstrates antitumor efficacy by inhibiting the mTor/AKT pathway. Irinotecan kills cells by inhibiting the enzyme topoisomerase I, which is also involved in DNA synthesis in proliferating cells. Bevacizumab is a monoclonal antibody that inhibits VEGF, which is expressed in some cases of colorectal cancer and aids the blood supply to tumors; bevacizumab inhibits this process and tumor cell growth.

Peer review

This is an interesting small pilot study that investigated fourth-line chemotherapy in patients with advanced metastatic colorectal cancer that had failed at least three previous chemotherapeutic regimens. A major finding was that clinical benefit was observed in 8/12 patients with severe adverse effects and relatively short overall survival.

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BRIEF ARTICLES

Effect of early propranolol administration on portal hypertensive gastropathy in cirrhotic rats

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Abstract

AIM: To investigate any protective effect of early propranolol administration in the development of portal hypertensive gastropathy in cirrhotic rats.

METHODS: For the development of liver cirrhosis and portal hypertensive gastropathy, 60 rats underwent ligation of the left adrenal vein and complete devascularization of the left renal vein, followed by phenobarbital and carbon tetrachloride (CCl₄) administration. After two weeks of CCl₄ administration, the rats were randomly separated into two groups. In group A, propranolol was continuously administered intragastrically throughout the study, whereas in group B normal saline (placebo) was administered instead. Hemodynamic studies and vascular morphometric analysis of gastric sections were performed after complete induction of cirrhosis.

RESULTS: Vascular morphometric studies showed higher numbers of vessels in all mucosal layers in

the control group. Statistical analysis revealed a significantly higher total vascular surface in the control group compared to the propranolol group, but with no statistically significant difference between the mean vascular surfaces between the groups. Our study clearly shows that the increased mucosal blood flow is manifested by a marked increase of vessel count.

CONCLUSION: Early propranolol's administration in portal hypertensive cirrhotic rats seems to prevent intense gastric vascular congestion that characterizes portal hypertensive gastropathy.

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Key words: Portal hypertension; Portal hypertensive gastropathy; Hepatic cirrhosis; Carbon tetrachloride; Gastric mucosal lesion

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INTRODUCTION

Portal hypertension is a clinical syndrome characterized by elevation of portal pressure and accompanies most cases of hepatic cirrhosis. Most liver cirrhosis complications are attributable to concomitant portal hypertension and the consequent development of portosystemic collaterals and hyperdynamic circulation^[1-4]. Portal hypertensive

gastropathy (PHG) represents a clinical entity in portal hypertension and is endoscopically characterized by a mosaic-like or snake skin pattern of the gastric mucosa, mainly in the body and fundus of the stomach and more rarely in the gastric antrum^[5,6]. These gastric mucosal lesions represent another frequent cause of upper gastrointestinal bleeding, even though esophagogastric varices remain the major source of bleeding in patients with portal hypertension^[5,7].

Non-selective β -blockers have largely been used for primary prophylaxis of bleeding from gastroesophageal varices^[8-12]. Their effect though, as well as of many other agents, on the development of varices has yet to be clarified because there are conflicting results from several studies, both clinical and experimental^[13-17]. On the other hand, development of PHG seems to follow a different pathophysiological pathway and there are relatively few studies investigating drugs' effect on PHG. Propranolol, for example, has been shown to reduce bleeding related to PHG in small studies^[11,12] and these observations were confirmed in a randomized controlled trial of 56 patients with PHG^[18]. We therefore decided to investigate propranolol's effect on PHG and to clarify more precisely if early propranolol administration has any preventive effect on the development of PHG in rats with carbon-tetrachloride (CCl₄)-induced cirrhosis.

MATERIALS AND METHODS

Animals

Sixty four-month-old-male Wistar rats, weighting 280-350 g, were used. They were housed one per cage, kept on an artificial 12-h light-dark cycle and at stable room temperature of 20-22°C. They had free access to tap water and standard laboratory pulverized rat chow throughout the study.

For all animal experiments the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) were followed. The study was approved by the Ethical Committee of the Aristotles University of Thessaloniki.

Experimental model

Liver cirrhosis, portal hypertension and esophagogastric varices were induced using a model, originally developed in our department, which has been proved to be very effective for the induction of cirrhotic portal hypertension as well as of esophageal and gastric varices^[19].

Briefly, all animals underwent ligation of the left adrenal vein and complete devascularization of the left renal vein. Two weeks later, induction of liver cirrhosis started according to the model of weekly intragastric administration of CCl₄ in the phenobarbitone-induced rat^[20,21].

Animal groups and drug administration

Two weeks after the beginning of carbon tetrachloride administration, the rats were randomly separated into two groups. In Group A, comprising 30 rats, propranolol was continuously administered throughout the study, whereas in Group B (30 rats), normal saline (placebo)

was continuously administered instead of propranolol. This early commencement of drug administration, before the full development of liver cirrhosis, aimed to simulate clinical practice, where any kind of preventive treatment should begin soon after initiation of the effect of a hepatotoxic agent. Propranolol (Inderal®, Wyeth Pharmaceuticals Inc., USA), dissolved in normal saline, was administered intragastrically, at a dose of 30 mg/kg per day.

Experimental period - animal sacrifice

CCl₄ was administered weekly until stable ascites developed (8-10 wk) as previously described^[16,17,19,22]. Ascites development was easily recognized by an abrupt increase in body weight and confirmed by the abdominal distention observed in the anesthetized rat in the prone position. Once stable ascites developed, CCl₄ administration was discontinued; one week later, rats were re-operated, portal pressure was measured and then the animals were sacrificed with an *iv* bolus administration of 0.5 mL of potassium chloride. The liver, stomach, and esophagus were carefully dissected and removed.

Portal pressure measurement

Portal pressure measurements were performed before animal sacrifice under light ether anesthesia; the rats were kept fasting for 12 h, with free access only to water. The peritoneal cavity of the animal was carefully accessed through the old midline incision, the presence of ascites was confirmed and ascitic fluid was carefully collected and measured. Portal pressure measurement was conducted by catheterization of a mesenteric vein with a PE-50 catheter, which was advanced until its tip reached the origin of the portal vein, while its other end was connected to a Space Labs, Inc. (Model 90308-11-14) pressure recorder. The external zero reference point was placed at the mid portion of the rat.

Histopathological study

The liver, stomach, and esophagus were fixed in 10% buffered formalin solution and embedded in paraffin soon after their removal.

Two sections of the stomach, the first at the cardioesophageal junction and the second at the body of the stomach, were stained with hematoxylin-eosin and initially examined on a light microscope (magnification $\times 4$ and $\times 10$). A liver section was also examined to confirm development of liver cirrhosis.

Morphometric analysis

Following light microscopy, all sections to be studied were scanned by a high resolution frame capture camera (JVC TK-F7300U), processed with computer software (Tema v1.00) and reproduced on a high-contrast, high-resolution PC monitor.

By use of the above mentioned software, delineating the outlines of vessels led to an easy calculation of the following parameters per optical field: (1) Total number of veins counted in gastric submucosa; (2) Total submucosal area occupied by vessels; (3) Mean

cross sectional vessel area (this variable was calculated by dividing total submucosal vessel area by the number of submucosal vessels); (4) Total number of superficial vessels in the gastric mucosa; (5) Total area of superficial vessels in the gastric mucosa; (6) Mean cross sectional vessel area of superficial gastric mucosal vessels; (7) Total number of deep gastric mucosal vessels; (8) Total area of deep gastric mucosal vessels; and (9) Mean cross sectional area of deep gastric mucosal vessels

All calculations were performed blindly by an experienced pathologist who was not informed as to the origin of the preparations.

Statistical analysis

Statistical version 6.0 (Stat Soft Inc.) was used for statistical analysis. First, the distribution of each parameter was determined according to its histograms and normal plots and was confirmed by application of the Shapiro-Wilk W test. Results were expressed as mean \pm SD for variables with normal distribution and as median - interquartile range for skewed distribution. Comparison between groups was performed using Student's t -test for unpaired data to evaluate differences in portal pressure and total submucosal area occupied by vessels; values of these variables followed a normal distribution. For all other variables, the non-parametric Mann Whitney U -test was applied. $P < 0.05$ were considered statistically significant.

RESULTS

Mortality

Forty-eight rats survived the study. There were no significant differences in body weight of rats among the two groups. There were seven deaths in group A and five in group B. As shown in Table 1, one of the propranolol group rats and two of the control group died from variceal bleeding before the end of the study (large amounts of blood were found in the stomach and upper jejunum). Two rats of group A and three rats of group B died from improper manipulation (administration of carbon tetrachloride into the tracheal-bronchial tree), while the deaths of four group A rats were attributed to CCl_4 toxicity.

Ascites

No significant difference in the amount of ascitic fluid was observed between the two groups ($P > 0.05$).

Portal pressure

Portal pressure values followed a normal distribution in both groups. Mean portal pressure was lower in the propranolol group (11.6 ± 1.36) compared to mean portal pressure of control group (14.61 ± 1.84) (Table 2). Comparison between groups revealed a portal pressure decrease of 21.5% in the propranolol group, which was proved to be statistically significant ($P < 0.05$).

Liver cirrhosis

All rats developed micronodular cirrhosis within 8-10 wk.

Table 1 Number of rats and causes of death

	Total number of deaths <i>n</i> (%)	Cause of death		
		Variceal bleeding	CCl_4 toxicity	Improper manipulation
Group A (<i>n</i> = 30)	7 (23.3)	1	4	2
Group B (<i>n</i> = 30)	5 (16.67)	2	-	3

Table 2 Portal pressure in group A and B

	Portal pressure (mmHg)			
	Mean	Minimum	Maximum	SD
Group A (<i>n</i> = 23)	11.60	9.20	14.3	1.36
Group B (<i>n</i> = 25)	14.61	11.3	18.2	1.84

Propranolol causes significant decrease ($P < 0.05$) in portal pressure.

Regenerating nodules surrounded by thickened septa of connective tissue with obvious architectural distortion were present on all hepatic sections. There was no obvious difference in the degree of hepatic fibrosis between the groups.

Gastric sections on light microscopy

Microscopic examination of the stomach revealed excessive mucosal and submucosal vascular congestion. Besides congestion, animals of group B (placebo treated groups) were found with more mucosal and submucosal vessels, while in some of them the development of smooth muscle cells in the mucosa was noticed.

Morphometric analysis of gastric mucosa and submucosa

Measurements and calculations were performed by image analysis in both groups. From the variables studied, only total and mean cross sectional area of superficial gastric mucosal vessels followed a normal distribution in both groups, while all other variables studied presented skewed distributions in either group or were non-continuous scale variables. Comparison between groups was performed using Student's t -test for unpaired data for the variables "total area of gastric superficial mucosal vessels" and "mean cross sectional area of gastric superficial mucosal vessels", and the non-parametric Mann Whitney U -test for all other variables. The summarized analysis and comparison of data are shown in Table 3. Statistically significant differences ($P < 0.05$) between groups were revealed for the variables "total area occupied by vessels" and "total number of counted veins" in the submucosa, the deep and superficial layers of gastric mucosa. On the other hand, the variable "mean cross sectional area" of gastric submucosal vessels as well as of deep and superficial gastric mucosal vessels did not differ significantly between the groups.

DISCUSSION

Esophageal varices have long been considered the major cause of upper gastrointestinal hemorrhage in patients

Table 3 Results of morphometric analysis and comparison between groups

	Group A	Group B	P
Total area of submucosal vessels (μm^2)	47 441.37 \pm 24 299.48	47 539.12 (33 295.84, 55 931.07)	0.0037
Mean cross sectional area of submucosal vessels (μm^2)	4682.70 (3571.92, 6350.68)	5911.17 \pm 1963.34	0.09
Number of submucosal vessels	5 (4, 7)	8 (7, 9)	0.01
Total area of superficial gastric mucosal vessels (μm^2)	7004.03 \pm 2438.37	10 994.49 \pm 3746.56	0.0001
Mean cross sectional area of superficial mucosal vessels (μm^2)	642.78 \pm 432.59	573.70 (475.08, 623.39)	0.25
Number of superficial gastric mucosal vessels	12 (9, 14)	20 (17, 24)	0.00001
Total area of deep gastric mucosal vessels (μm^2)	6916.76 (3694.98, 8016.40)	19 367.84 \pm 7034.08	0.0008
Mean cross sectional area of deep gastric mucosal vessels (μm^2)	834.88 (554.05, 953.04)	982.90 (697.11, 1249.35)	0.1
Number of deep gastric mucosal vessels	9 (7, 11)	20 (16, 22)	0.00001

Values are mean \pm SD or median (lower, upper quartiles).

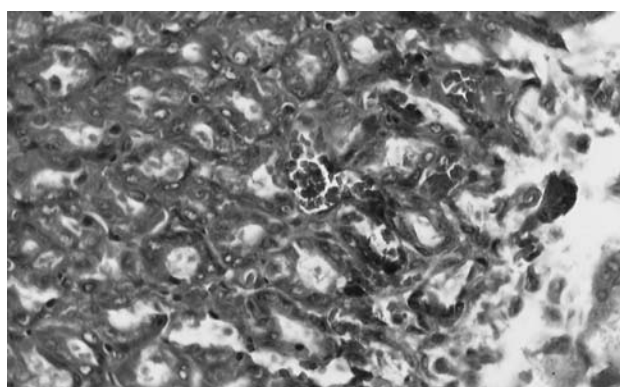


Figure 1 Vascular ectasia and congestion of gastric submucosa in group B (HE, \times 400).

with portal hypertension. However, gastric mucosal lesions have lately been considered as another frequent cause of upper gastrointestinal bleeding in these patients, accounting for 20% to 40% of all cases^[23,24]. Dilated precapillaries, capillaries and submucosal veins, extensive submucosal edema, thickening of the submucosal arteriolar walls and submucosal veins showing features of arterIALIZATION, are all observed in patients with portal hypertension^[5,25-28], while morphometric analyses have shown an increase of mean mucosal capillary cross-sectional area^[29-32]. Clinically significant bleeding is seen in association with severe portal hypertensive gastropathy (PHG) and non-selective beta-blockers, such as propranolol and nadolol, have been shown to reduce portal pressure and gastric mucosal blood flow. Previous experimental studies using propranolol^[13,14,33,34] and clonidine^[35] early in the process of portal hypertension induction have been proven effective in prevention of complications.

However, these studies, mainly based on hemodynamic measurements, are very sensitive and easily affected by a number of imponderable and in many cases unknown factors^[36-38]. This might explain why similar studies from various research centers often resulted in completely different conclusions^[39-43].

To avoid these problems, we decided to directly investigate the effects of early propranolol administration on gastric mucosal and submucosal pathology. The gastric mucosal and submucosal vein plexus (Figure 1) was meticulously studied and measurements of vessels'

and submucosa's areas were carefully performed with the aid of an image analysis system. Portal pressure was the only hemodynamic parameter studied. Measurements revealed a 21.5% decrease of portal pressure in propranolol treated rats; these results are fully compatible with literature data^[13,14,33,34]. On the other hand, careful analysis of morphometric data revealed that early propranolol administration significantly affects the total area of gastric submucosal and mucosal vessels as well as the number of gastric submucosal and mucosal vessels of cirrhotic rats, while the mean cross sectional area does not seem to be significantly affected.

In clinical practice, cirrhosis represents the major cause of portal hypertension. Induction of cirrhotic portal hypertension by carbon tetrachloride administration was therefore considered to be an appropriate experimental model for our study. In this model, portal hypertensive syndrome is fully developed after a reasonable time, permitting the study of chronic and early propranolol administration. All similar studies presented in the literature^[13,14,33-35], were carried out either in prehepatic portal hypertension or in cases of schistosomiasis, probably due to lack of reliable models capable of developing esophagogastric varices in cirrhotic rats. This is mainly due to the development of extended collaterals from the portal vein to the left renal vein via the left adrenal vein. These collaterals, which are non-functional in normal rats, prevent portosystemic shunt through the gastric and lower esophageal veins in case of portal hypertension. We overcame this problem by using a modification of the well-known model of carbon tetrachloride induced cirrhosis. This included the induction of cirrhosis in rats that had previously undergone ligation of the left adrenal vein and complete devascularization of the left renal vein. The effectiveness of this model has already been demonstrated in previous studies^[15-17,19].

Gastric submucosal vessels, as well as superficial and deep gastric mucosal vessels, were meticulously studied, and various measurements were carefully performed using an image analysis system, which permitted objective determination of numerous parameters. All gastric submucosal, as well as deep and superficial gastric mucosal vessels per optical field, were counted, and their borders were carefully delineated, to calculate the total and mean cross sectional area of submucosal gastric

veins, and deep and superficial gastric mucosal vessels. An accurate method of measuring vessel cross-sectional areas and comparing them would be to perfuse fix the vessels with a controlled perfusion pressure; however, the significant in vivo differences in portal pressure between the groups, and their effect on gastric vessels, would be masked. We also thought to perfuse fix vessels with pressures comparative to portal pressures; however, this was technically difficult. Thus, the final choice was to use no perfusion fixation and compare simple sections, by measuring several parameters, including vessel numbers. Several factors, besides portal pressure, affect vein development and gastric mucosal and submucosal congestion in cirrhotic animals. It is a general belief that portal pressure increase is the main causative factor for the development of portosystemic collaterals^[1-3], which are considered to be the result of widening, distension, and hypertrophy of pre-existing vessels. Additionally, active angiogenesis can also participate in their formation. Propranolol, by reducing the hepatic venous pressure gradient and azygos blood flow, seems to contribute to the reducing opening of pre-existing blood vessels. On the other hand, neoangiogenesis seems to be prevented by abolishing the norepinephrine inducing effect on vascular endothelial growth factor (VEGF) expression^[44-46]. Morphometric analysis in our study revealed a statistically significant difference ($P < 0.05$) between groups in the number of mucosal and submucosal vessels, as well as in the total area occupied by vessels, which was significantly greater ($P < 0.05$) in the placebo group compared to the propranolol treated group. On the other hand, there was no difference in mean cross sectional area of submucosal and mucosal vessels between the groups.

We can therefore claim, based on the results of this experimental study, that early propranolol administration in portal hypertensive cirrhotic rats could be useful in prevention of portal hypertensive gastropathy and its complications.

COMMENTS

Background

Patients with portal hypertension are at substantial risk of bleeding from small gastric mucosal lesions that have been largely described as portal hypertensive gastropathy.

Research frontiers

Propranolol is a well-known and extensively used beta-blocker that has been shown to reduce bleeding related to portal hypertensive gastropathy in small studies. In the area of prevention of portal hypertensive gastropathy, previous experimental studies have shown that early continuous administration of non-selective β -blockers, such as propranolol, could ameliorate portosystemic hemodynamics and therefore reduce complications. The sensitive and easily affected hemodynamic measurements used in previous studies have resulted in different conclusions and led to an effort to directly investigate the effects of early propranolol administration on gastric mucosal and submucosal pathology.

Innovations and breakthroughs

The concept that early continuous administration of drugs is capable of reducing portal pressure and could prevent the development of extended portosystemic collaterals and consequently esophagogastric varices or portal hypertensive gastropathy, has led to a series of experimental and clinical studies. Many agents with a known lowering effect on portal pressure (including propranolol, nadolol, clonidine, octreotide, isosorbide mononitrate and, more recently, endothelin

receptor antagonists) have already been tested for their effect on portosystemic shunting and development of esophageal varices, when administered early, that is before the full development of portal hypertension syndrome. Several studies have been published, both clinical and experimental, with controversial results on the protective role of non-selective beta-blockers. To avoid complicated and easily affected hemodynamic measurements the authors decided to directly investigate the effects of early propranolol administration on gastric mucosal and submucosal pathology. The gastric mucosal and submucosal vein plexus was meticulously studied and measurements of vessels' and submucosa's area were carefully performed with the aid of an image analysis system. Portal pressure was the only hemodynamic parameter studied in order to confirm the already known propranolol's effect on it.

Applications

The study results suggest that early propranolol administration in portal hypertensive cirrhotic rats could prevent intense gastric vascular congestion, which characterizes portal hypertensive gastropathy and could therefore be useful in preventing its complications.

Peer review

This is a well designed study to look at the effects of a non selective β blocker in the prevention of portal hypertensive gastropathy in a rat model for cirrhosis and portal hypertension.

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BRIEF ARTICLES

Does *Helicobacter pylori* eradication therapy for peptic ulcer prevent gastric cancer?

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Abstract

AIM: To investigate the effects of *Helicobacter pylori* (*H. pylori*) eradication therapy for treatment of peptic ulcer on the incidence of gastric cancer.

METHODS: A multicenter prospective cohort study was conducted between November 2000 and December 2007 in Yamagata Prefecture, Japan. The study included patients with *H. pylori*-positive peptic ulcer who decided themselves whether to receive *H. pylori* eradication (eradication group) or conventional antacid therapy (non-eradication group). Incidence of gastric cancer in the two groups was determined based on the results of annual endoscopy and questionnaire surveys, as well as Yamagata Prefectural Cancer Registry data, and was compared between the two groups and by results of *H. pylori* therapy.

RESULTS: A total of 4133 patients aged between 13 and 91 years (mean 52.9 years) were registered, and 56 cases of gastric cancer were identified over a mean follow-up of 5.6 years. The sex- and age-adjusted incidence ratio of gastric cancer in the eradication group, as compared with the non-eradication group, was 0.58 (95% CI: 0.28-1.19) and ratios by follow-up period (< 1 year, 1-3 years, > 3 years) were 1.16 (0.27-5.00), 0.50 (0.17-1.49), and 0.34 (0.09-1.28), respectively. Longer follow-up tended to be associated with better prevention of gastric cancer, although not to a significant extent. No significant difference in incidence of gastric cancer was observed between patients with successful eradication therapy (32/2451 patients, 1.31%) and those with treatment failure (11/639 patients, 1.72%). Among patients with duodenal ulcer, which is known to be more prevalent in younger individuals, the incidence of gastric cancer was significantly less in those with successful eradication therapy (2/845 patients, 0.24%) than in those with treatment failure (3/216 patients, 1.39%).

CONCLUSION: *H. pylori* eradication therapy for peptic ulcer patients with a mean age of 52.9 years at registration did not significantly decrease the incidence of gastric cancer.

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Key words: *Helicobacter pylori*; Peptic ulcer; Gastric cancer; Eradication therapy; Cancer prevention

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Mabe K, Takahashi M, Oizumi H, Tsukuma H, Shibata A, Fukase K, Matsuda T, Takeda H, Kawata S. Does *Helicobacter pylori* eradication therapy for peptic ulcer prevent gastric cancer? *World J Gastroenterol* 2009; 15(34): 4290-4297 Available from: URL: <http://www.wjgnet.com/1007-9327/15/4290.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.4290>

INTRODUCTION

Yamagata Prefecture is an area in which gastric cancer

is particularly common. In Cancer Incidence in Five Continents, published by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO), it is reported that the incidence of gastric cancer for men in Yamagata Prefecture, was the second highest in the world, at 91.6/100 000 (ASR world) in 1993-1997^[1]. Yamagata Prefecture therefore has attempted aggressively to achieve secondary prevention of gastric cancer. In 1994, IARC/WHO concluded that *Helicobacter pylori* (*H pylori*) is a definite carcinogen in humans^[2], and the results of a prospective cohort study^[3] and animal studies^[4-6] have demonstrated that *H pylori* causes gastric cancer. The possible role of *H pylori* eradication therapy in primary prevention of gastric cancer has thus attracted substantial interest in Yamagata Prefecture. In November 2000, when coverage of *H pylori* eradication for patients with *H pylori*-positive peptic ulcer by the National Health Insurance system in Japan began, the Yamagata *H pylori* Clinical Study Group was established to design a multicenter prospective cohort study to investigate whether *H pylori* eradication therapy for patients with peptic ulcer can decrease the incidence of gastric cancer.

Although animal studies have revealed that primary prevention of gastric cancer by *H pylori* eradication is more effective as the duration between *H pylori* infection and eradication is decreased^[7], the effects in humans of this type of prevention have not been determined sufficiently. Non-randomized prospective studies^[8,9] and retrospective studies^[10,11] in Japan have suggested that *H pylori* eradication therapy prevents the development of gastric cancer, while a large-scale randomized controlled study in China did not support this conclusion. Although, a sub-population analysis of patients who did not have precancerous change at the time of eradication therapy has suggested gastric-cancer-preventive effects of *H pylori* eradication therapy^[12]. No significant reduction in the incidence of gastric cancer by *H pylori* eradication therapy was observed in a meta-analysis^[13]. In a multicenter, randomized controlled study in patients who underwent endoscopic resection of early gastric cancer and were thus at high risk of secondary gastric cancer, occurrence of secondary gastric cancer was prevented significantly by *H pylori* eradication therapy^[14]. Evidence of prevention of gastric cancer by *H pylori* eradication therapy thus needs to be obtained.

This report describes the results of a multicenter, prospective cohort study that investigated whether *H pylori* eradication therapy in patients with peptic ulcer, living in an area where the incidence of gastric cancer is especially high, was effective in primary prevention of gastric cancer. The results of the present study, including endoscopy findings, were reconciled with those of the Yamagata Prefecture Cancer Registry to ensure accurate detection of gastric cancer.

MATERIALS AND METHODS

Study design

The present study was designed at Yamagata Prefectural Central Hospital, where the office of the Yamagata

H pylori Clinical Study Group was located in May 2000. It included 82 participating institutions, 26 hospitals and 56 clinics, in Yamagata Prefecture. For ethical reasons, we selected performance of a non-randomized, multicenter, prospective cohort study in which patients decided themselves whether to receive *H pylori* eradication therapy (eradication group) or conventional antacid therapy (non-eradication group) for the treatment of *H pylori*-positive peptic ulcer. The sample size was calculated to detect a significant difference in incidence of gastric cancer in patients who received *H pylori* eradication therapy or conventional antacid treatment over a 7-year period (a 2-year registration period and a 5-year follow-up period), with a power of 90% and an alpha error of 5% on the basis of the following assumptions: the incidence of gastric cancer in patients who received conventional antacid therapy was 0.5%; *H pylori* eradication therapy decreased the incidence of gastric cancer by 50%-90%; the percentage of withdrawals was 20%; and patients were allocated to the non-eradication and eradication groups at a ratio of 1:5. We estimated that 560-2467 patients and 2797-12 333 patients were required for the non-eradication and eradication therapy groups, respectively. All tests and treatments performed were covered by the National Health Insurance (NHI) as determined by the Ministry of Health and Welfare of Japan (currently the Ministry of Health, Labor, and Welfare of Japan), and the study protocol was approved by the Ethics Committee of Yamagata Prefectural Central Hospital. All patients received a full explanation of the study using a standardized document, and provided written informed consent before registration in the study.

Patients with *H pylori*-positive peptic ulcer were considered eligible. Patients with a history of gastric cancer and those in whom endoscopy or biopsy at the time of registration revealed gastric cancer were excluded from the study. Patients were registered between November 2000 and December 2003, and followed up until the end of December 2007.

Diagnosis of *H pylori*-positive peptic ulcer and treatment

Prior to registration, all patients underwent upper gastrointestinal endoscopy and biopsy, if necessary, to diagnose peptic ulcer and exclude gastric cancer. During endoscopy, biopsy samples were collected from the greater curvature of the upper body and antrum of the stomach. The presence/absence of *H pylori* infection was evaluated by rapid urease test.

H pylori eradication therapy consisted of 30 mg lansoprazole or 20 mg omeprazole, plus 750 mg amoxicillin and 200 or 400 mg clarithromycin, all twice daily for 7 d. At least 1 mo after the completion of eradication therapy, patients underwent upper gastrointestinal endoscopy with the rapid urease test and urea breath test (with a cut-off value of 2.5‰; Ubit, Otsuka Pharmaceuticals, Tokyo, Japan). Successful *H pylori* eradication was defined as negative results on the rapid urease and urea breath tests. When the results of the two tests were inconsistent, retesting was performed.

Conventional antacid therapy consisted of antacids such as proton-pump inhibitors and histamine- H_2 blockers.

Detection of gastric cancer

During the follow-up period up to December 2007, endoscopy was performed annually, in principle, to determine the presence/absence of gastric cancer. When follow-up endoscopy was performed, the investigators reported its results to the study office using a follow-up report form to provide information on the date of endoscopy, stage of ulcer, results of *H pylori* testing, and presence/absence of newly developed gastric cancer or other gastrointestinal diseases (such as reflux esophagitis, erosive gastritis/duodenitis, and esophageal adenocarcinoma).

To avoid overlooking gastric cancer due to the absence of annual endoscopy, a questionnaire survey was conducted and the data obtained in the present study were reconciled with those of the Yamagata Prefecture Cancer Registry. In October 2006, a questionnaire was mailed to all registered patients to determine the presence/absence of gastric cancer diagnosed after registration. The results of the questionnaire were compared with the data at registration to identify patients who might have developed gastric cancer after registration. Such cases were referred to the participating medical institutions to confirm the diagnosis of gastric cancer. In March 2008, record linkage between the cohort and Yamagata Prefectural Cancer Registry was conducted for identification of previous and new gastric cancer cases during the follow-up period up to the end of December 2007.

Statistical analysis

Person-years were calculated from the date of recruitment to the date of incidence of gastric cancer; end of follow-up in December 2007; date of change of residence to outside Yamagata Prefecture; death from causes other than gastric cancer; or the initiation of *H pylori* eradication therapy for patients in the antacid therapy group, whichever came first. For comparison between groups at baseline, Fisher's exact test was used for sex and type of peptic ulcer, and Student's *t* test or χ^2 test for histological type, location and stage of cancer, and treatment, with a level of significance of $P < 0.05$ (two-tailed).

Poisson regression was used to estimate the relative risk of gastric cancer in relation to eradication therapy for *H pylori*. Analyses were adjusted routinely for sex and age (< 60 , $60-70$, > 70 years), and stratified for duration of follow-up (< 1 , $1-3$, > 3 years) and location of ulcer (gastric, gastroduodenal or duodenal). We also examined the effects of adjustment for other risk factors including location of ulcer, intake of salt, and smoking. These statistical analyses were performed using Intercooled Stata 8.0 for Windows software (StataCorp LP, College Station, TX, USA).

The accumulated incidence of gastric cancer in patients with successful and unsuccessful *H pylori*

eradication was determined using data for patients for whom the results of *H pylori* eradication therapy were confirmed using the Kaplan-Meier method, and were tested for significance of difference between patients with and without successful eradication using the log-rank method. These analyses were performed using Dr. SPSS II for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Baseline characteristics of study subjects

A total of 4203 patients were registered, and 70 patients who had a history of gastric cancer or had gastric cancer at the time of registration were excluded. Intention-to-treat (ITT) analysis was performed for the data from the remaining 4133 patients (2964 male and 1169 female), who were aged between 13 and 91 years (mean 52.9 years).

H pylori eradication therapy was administered to 3781 patients (91.5%; eradication group) and conventional antacid therapy to 352 patients (8.5%; non-eradication group). Table 1 summarizes the characteristics of the 4133 patients included in the present study. There were no significant differences between the eradication and non-eradication groups in baseline characteristics such as distribution of sex or location of ulcers, while mean age was lower in the eradication than in the non-eradication group (52.4 years *vs* 58.1 years). No results of *H pylori* eradication therapy were reported for 691 (18.3%) of the 3781 patients who received *H pylori* eradication therapy. The eradication rate evaluated in the ITT analysis and the per-protocol (PP) analysis were 64.8% (2451/3781) and 79.3% (2451/3090), respectively. There were no significant differences in any factors, including age, between patients with and without successful *H pylori* eradication (mean ages of patients with and without successful *H pylori* eradication were 52.5 ± 12.3 years and 52.1 ± 13.5 , respectively; $P = 0.51$).

Development of gastric cancer

During a total of 22 900 person-years of follow-up (mean follow-up period: 5.6 years), gastric cancer was found in 56 patients, including 47/3781 patients (1.24%, 0.21%/year) who received *H pylori* eradication therapy and 9/352 patients (2.56%, 0.50%/year) who received conventional antacid therapy. There were no differences in sex distribution; location of ulcer lesions; histological type, location or stage of cancer; or type of treatment for gastric cancer between patients with and without *H pylori* eradication therapy (Table 2). Poisson regression analysis of factors that affected the incidence of gastric cancer revealed that sex, age group, and location of ulcers were independent factors that affected the differences in incidence rate ratio (IRR) of gastric cancer between patients receiving and not receiving *H pylori* eradication therapy (Table 3). The IRR of gastric cancer adjusted for sex and age group was 0.58 (95% CI: 0.28-1.19). *H pylori* eradication therapy decreased the incidence of gastric cancer by about 40%, although this change was not statistically significant. IRR was

Table 1 Baseline characteristics of 4133 patients

	Eradication group (n = 3781)	Non-eradication group (n = 352)	
Male/Female	2715/1066	249/103	$P = 0.67^1$
Male (%)	71.80	70.70	
Mean age	52.4 ± 12.7	58.1 ± 12.6	$P < 0.001^2$
Min-max	13.2-85.9	22.2-91.9	
Age (yr)			$P < 0.001^1$
< 60	2732 (72.3)	186 (52.8)	
60-70	701 (18.5)	102 (29.0)	
> 70	348 (9.2)	64 (18.2)	
Location of ulcer			$P = 0.41^1$
GU	2048 (54.2)	195 (55.4)	
GDU	418 (11.1)	45 (12.8)	
DU	1265 (33.5)	110 (31.3)	
Unknown	50 (1.3)	2 (0.6)	
Salt consumption			$P < 0.001^1$
Restricted	1377 (36.4)	173 (49.1)	
No interest in salt consumption	729 (19.3)	29 (8.2)	
Not restricted	1185 (31.3)	105 (29.8)	
Unknown	490 (13.0)	45 (12.8)	
Smoking history			$P < 0.001^1$
Non-smokers	1174 (31.0)	120 (34.1)	
Past smokers	565 (14.9)	48 (13.6)	
Current smokers	1931 (51.1)	159 (45.2)	
Unknown	111 (2.9)	25 (7.1)	
Mean duration of follow-up (yr)	5.6 ± 1.1	5.2 ± 1.8	$P < 0.001^2$
Min-max	0.09-7.96	0.11-8.41	

¹ χ^2 test; ²*t* test. Eradication group: patients who received *Helicobacter pylori* eradication therapy with or without successful eradication. Non-eradication group: Patients who received conventional antacid therapy. GU: Gastric ulcer; GDU: Gastroduodenal ulcer; DU: Duodenal ulcer.

by duration of follow-up 1.16 (0.27-5.00) for patients followed up for < 1 year, 0.50 (0.17-1.49) for 1-3 years, and 0.34 (0.09-1.28) for > 3 years. There were no significant differences in the incidence of gastric cancer between any subgroups of the eradication and non-eradication groups, although the difference in incidence between the groups tended to increase as the duration of follow-up was prolonged (Table 4). Gastric cancer was found in 6/1375 patients with duodenal ulcer (0.44%). All six patients were > 50 years of age at the time of registration. No cases of Barrett's adenocarcinoma, for which the possibility of increase in occurrence after *H. pylori* eradication therapy was a concern, were found in either of the two groups.

In a separate analysis, 3090 patients who received *H. pylori* eradication therapy, with known results, were compared for incidence of gastric cancer according to presence/absence of successful eradication. Gastric cancer was detected in 43 of the 3090 patients, including 32/2451 patients (1.31%) and 11/639 patients (1.72%) with and without successful eradication, respectively. No significant difference in incidence of gastric cancer was observed between patients with and without successful eradication. Analysis by type of peptic ulcer at the time of registration revealed that the incidence of gastric cancer did not differ between patients with and without successful eradication in subgroups of patients

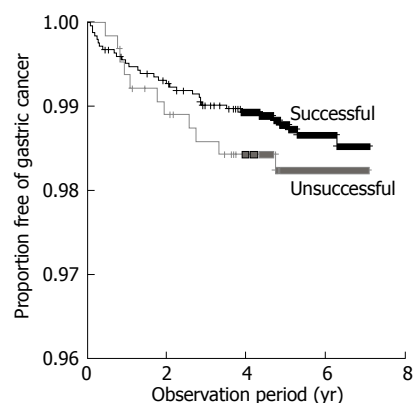


Figure 1 Proportion free of gastric cancer in the eradication group was compared according to results of eradication therapy, using Kaplan-Meier analysis. The incidence was 32/2451 (1.31%) in patients with successful eradication and 11/639 (1.72%) in patients with failure of eradication (log-rank test, $P = 0.43$).

with gastric or gastroduodenal ulcer, while successful eradication decreased the incidence of gastric cancer significantly in patients with duodenal ulcer (Figures 1 and 2).

DISCUSSION

This was a prospective, multicenter, cohort study in patients with *H. pylori*-positive peptic ulcer, which was designed to evaluate whether *H. pylori* eradication therapy decreased the incidence of gastric cancer in Yamagata Prefecture, a region in which gastric cancer is especially common. Since continuous follow-up is often difficult in clinical observation studies, the data from the present study were reconciled with those of the Yamagata Prefecture Cancer Registry to avoid overlooking gastric cancer. This was a unique feature of the present study.

During the follow-up of 4133 patients with peptic ulcer (mean age: 52.9 years) for a mean of 5.6 years, the incidence of gastric cancer in patients who received *H. pylori* eradication therapy was decreased by about 40% compared with that in patients who did not receive eradication therapy, although the difference between the groups was not statistically significant. Longer follow-up period tended to be associated with better prevention of gastric cancer, albeit not to a significant extent. Although there was no significant difference in incidence of gastric cancer according to the result of eradication therapy (success/failure) in those patients who received this treatment, successful eradication therapy did decrease significantly the incidence of gastric cancer in patients with duodenal ulcer.

There are four limitations to the interpretation of the results of the present study: (1) it was not a randomized controlled trial, and the number of patients not receiving eradication therapy was small; (2) the eradication rate was only 80%; (3) the follow-up period was not sufficiently long; and (4) the mean age of participants was high, at 53 years.

Factors (1) and (2) are limitations of the present study, in which randomization of patients was impossible

Table 2 Distribution of cases of gastric cancer

Sex	Male	Female			Total		
Eradication group	38	9			47	$P = 0.328^1$	
Non-eradication group	9	0			9		
Type of peptic ulcer	GU/GDU	DU					
Eradication group	43	4			47	$P = 0.244^1$	
Non-eradication group	7	2			9		
Histological type of cancer	Intestinal	Diffuse	Unknown				
Eradication group	35	10	2			47	$P = 0.304^2$
Non-eradication group	5	4	0			9	
Location of cancer	L	M	U	Unknown			
Eradication group	21	18	6	2	47	$P = 0.759^2$	
Non-eradication group	3	5	1	0	9		
Stage	Early	Advanced	Unknown				
Eradication group	34	11	2			47	$P = 0.198^2$
Non-eradication group	9	0	0			9	
Treatment	Endoscopy (EMR/ESD)	Surgery	Chemotherapy				
Eradication group	12	31	2			47	$P = 0.763^2$
Non-eradication group	2	7	0			9	

¹Fisher's direct test; ²Student's *t* test or χ^2 test. ESD: Endoscopic submucosal dissection; L: Lower third of the stomach; M: Middle third of the stomach; U: Upper third of the stomach.

Table 3 Results of poisson regression analysis

	IRR					
	Univariate	95% CI	<i>P</i>	Multivariate	95% CI	<i>P</i>
Eradication group	0.45	0.22-0.92	0.03	0.61	0.29-1.27	0.18
Non-eradication group	1.00			1.00		
Sex						
Male	1.00		0.05	1.00		0.03
Female	0.49	0.24-1.00		0.39	0.17-0.88	
Age (yr)	1.09	1.05-1.12	< 0.01			
< 60	1.00			1.00		
60-70	3.22	1.74-5.95	< 0.01	2.59	1.37-4.91	< 0.01
> 70	5.18	2.69-10.0	< 0.01	4.23	2.09-8.53	< 0.01
Location of ulcer	0.69	0.40-1.19	0.18			
Stomach/Stomach + duodenum	1.00			1.00		
Duodenum	0.28	0.13-0.63	< 0.01	0.37	0.16-0.83	0.02
Unknown	1.16	0.16-8.43	0.88	1.69	0.23-12.48	0.61
Salt consumption	0.94	0.84-1.06	0.27			
Restricted	1.00			1.00		
No interest in salt consumption	0.43	0.16-1.11	0.08	0.65	0.25-1.73	0.39
Not restricted	0.78	0.43-1.41	0.41	1.03	0.56-1.90	0.93
Unknown	0.58	0.24-1.39	0.22	0.58	0.22-1.60	0.29
Smoking history	1.04	0.89-1.22	0.63			
Non-smokers	1.00			1.00		
Past smokers	1.56	0.74-3.29	0.25	0.97	0.43-2.20	0.95
Current smokers	0.94	0.50-1.76	0.85	0.78	0.38-1.61	0.50
Unknown	1.71	0.50-5.88	0.39	1.65	0.41-6.65	0.48

IRR: Incidence rate ratio.

Table 4 IRRs of gastric cancer by follow-up period and location of ulcer

	Eradication group			Non-eradication group			IRR		IRR (sex- and age-adjusted)	
	per 1000 person-years	<i>n</i>	Incidence	per 1000 person-years	<i>n</i>	Incidence	95% CI		95% CI	
Overall	21.2	47	2.22	1.82	9	4.93	0.45	0.22-0.92	0.58	0.28-1.19
Follow-up period (yr)										
< 1	3.77	17	4.51	0.34	2	5.81	0.78	0.18-3.33	1.16	0.27-5.00
1-3	7.48	20	2.67	0.64	4	6.27	0.43	0.15-1.25	0.50	0.17-1.49
> 3	9.92	10	1.01	0.84	3	3.56	0.28	0.08-1.03	0.34	0.09-1.28
Gastric/Gastric + duodenal ulcers	13.8	41	2.97	1.21	7	5.76	0.52	0.23-1.15	0.62	0.27-1.39
Duodenal ulcers	7.12	5	0.70	0.60	2	3.35	0.21	0.04-1.08	0.31	0.06-1.61

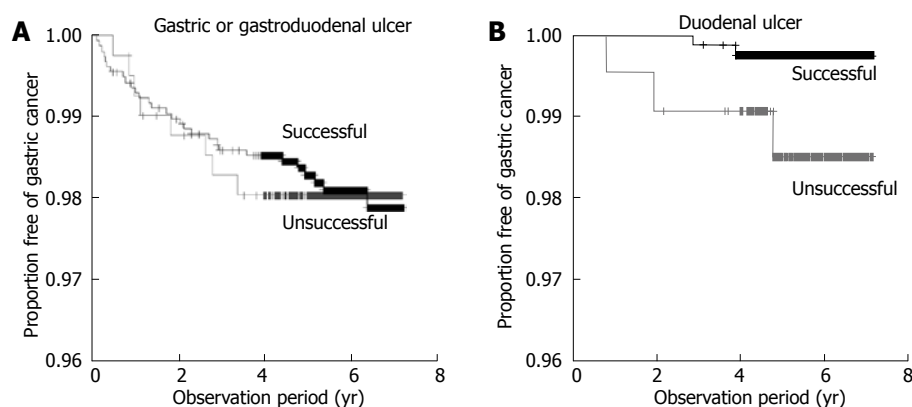


Figure 2 Proportion free of gastric cancer in patients with gastric or gastroduodenal ulcer (A) and duodenal ulcer (B) in the eradication group was compared according to results of eradication therapy, using Kaplan-Meier analysis. A: The incidence of gastric cancer was 29/1572 (1.85%) in patients with successful eradication and 8/413 (1.94%) in patients with failure of eradication (log-rank test, $P = 0.92$); B: The incidence of gastric cancer was 2/845 (0.24%) in patients with successful eradication and 3/216 (1.39%) in patients with failure of eradication (log-rank test, $P = 0.03$).

for ethical reasons, and secondary *H. pylori* eradication therapy was not covered by the NHI system. In addition to the four factors noted above, since the decrease in incidence of gastric cancer by *H. pylori* eradication was smaller than expected, and the numbers of patients did not reach those targeted, especially in the non-eradication group, the study did not have the statistical power required to detect a significant difference in the incidence of gastric cancer between patients who received eradication therapy and conventional antacid therapy.

The finding that the efficacy of *H. pylori* eradication in preventing gastric cancer tended to be better among patients with a longer follow-up period suggests that the length of follow-up in this study may have been insufficient. In a study of patients who underwent resection of gastric cancer, cancer in other locations was not detected upon preoperative evaluation^[15]. Although all patients evaluated in the present study underwent endoscopy and biopsy prior to registration, if required, the possibility cannot be ruled out that some patients had undetectable gastric cancer before registration. Gastric cancer lesions detected during the early phase after eradication therapy may in many cases have been present before therapy. More accurate determination of the efficacy of eradication therapy in preventing gastric cancer will require that patients be followed up for a long period of time. Since follow-up endoscopy cannot be continued for many years, it is important that our data be reconciled with those of the Yamagata Prefecture Cancer Registry to continue follow-up of the participants.

As pointed out by Wong *et al.*^[12], the precancerous state may represent the point of no return at which development of gastric cancer can no longer be prevented by *H. pylori* eradication. The participants in the present study were patients with peptic ulcer with a mean age of 53 years, and many patients with gastric ulcer also have atrophic gastritis or intestinal metaplasia. Among the registered patients, duodenal ulcer was more common in those < 50 years of age, while gastric ulcer

was common in patients > 50 years of age. The risk of gastric cancer was higher in patients with gastric or gastroduodenal ulcer than in those with duodenal ulcer, and a significant decrease in the incidence of gastric cancer according to successful *H. pylori* eradication was observed only in patients who underwent *H. pylori* eradication for the treatment of duodenal ulcer. Since *H. pylori* infection is usually established during childhood, it appears likely that antral gastritis and duodenal ulcer are common among young patients with a relatively short history of *H. pylori* infection, and that eradication of *H. pylori* may decrease the occurrence of gastric cancer. However, patients with a longer history of *H. pylori* infection often have corpus gastritis, and eradication therapy does not prevent gastric cancer to a significant extent. The results of an experiment in Mongolian gerbils has shown that eradication of *H. pylori* is more effective in preventing gastric cancer in animals with a shorter duration of *H. pylori* infection^[7]. These findings suggest the importance of the timing and target of *H. pylori* eradication therapy in preventing gastric cancer.

In a randomized clinical study on the effects of eradication of *H. pylori* after endoscopic mucosal resection of early gastric cancer, Fukase *et al.*^[14] have reported a significant decrease in the incidence of secondary gastric carcinoma during a 3-year follow-up period. The significant prevention of secondary gastric cancer by *H. pylori* eradication was considered to be caused by the following: (1) the risk of development of gastric cancer in patients following endoscopic mucosal resection for early gastric cancer is about 10-fold higher than in patients with *H. pylori*-positive peptic ulcer; (2) patients underwent accurate endoscopy several times before and after treatment for cancer; and (3) the number of cases of gastric cancer that were overlooked at the time of registration was therefore considered smaller than in other surveys.

Prior to initiation of the present study, there was concern regarding the possibility of a decrease in visits for endoscopy after symptomatic improvement, which could have resulted in an increase in advanced gastric

cancer. Although the investigators fully explained to subjects the risk of development of gastric cancer after *H pylori* eradication therapy and the importance of follow-up endoscopy, > 15% of them did not undergo examination to confirm the results of *H pylori* eradication therapy. The percentage of patients who received follow-up endoscopy as specified was significantly lower among those who received *H pylori* eradication therapy compared with conventional antacid therapy. Although this may have biased the rate of detection of gastric cancer, we attempted to decrease bias by reconciling our data with those of the Yamagata Prefecture Cancer Registry. Of the 56 cases of gastric cancer detected in the present study, 11 were detected as advanced gastric cancer. Cases of advanced gastric cancer mainly consisted of diffuse-type cancer and those with a mixture of intestinal and diffuse cancer cells. Careful observation is thus needed for the development of gastric carcinoma, especially diffuse-type gastric cancer, which may progress rapidly and is often difficult to detect.

Although it has been reported that gastric cancer does not develop in patients with duodenal ulcer^[3,8,9], gastric cancer developed in six patients with duodenal ulcer in the present study, including five aged ≥ 60 years and one 51-year-old patient. Since continuous infection with *H pylori* may result in the development of corpus gastritis in patients with antral gastritis associated with duodenal ulcer, middle-aged and elderly patients with duodenal ulcer often have gastritis in the corpus, and should thus be considered at high risk for development of gastric cancer, even after successful *H pylori* eradication therapy, especially in regions with a high incidence of gastric cancer.

In conclusion, *H pylori* eradication therapy for patients with peptic ulcer with a mean age of 52.9 years did not significantly decrease the incidence of gastric cancer during a mean follow-up period of 5.6 years. Although our results did not rule out a role for *H pylori* eradication therapy in preventing gastric cancer, the number of patients evaluated, duration of observation, and rate of eradication of *H pylori* were insufficient to obtain a significant difference in the incidence of gastric cancer between the groups with and without eradication therapy, and the mean age of patients was high. Further studies to clarify the efficacy of *H pylori* eradication in preventing gastric cancer will require eradication of *H pylori* as early as possible and careful and prolonged follow-up of patients.

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COMMENTS

Background

Yamagata Prefecture in Japan has the second highest incidence of gastric

cancer in the world. *Helicobacter pylori* (*H pylori*) plays an important role in the development of gastric cancer. It is thus of crucial importance to determine whether *H pylori* eradication therapy in this geographical area is effective in primary prevention of gastric cancer.

Research frontiers

Although it has been demonstrated that *H pylori* is a definite carcinogen in humans, previous studies that have examined the efficacy of eradication therapy in preventing gastric cancer have yielded inconsistent findings. In the present study, the authors found that eradication therapy in patients with peptic ulcer with a high mean age of 53 years did not significantly decrease the incidence of gastric cancer, at least over the mean 5.6-year follow-up period.

Innovations and breakthroughs

Single-center prospective studies and retrospective studies have reported significant prevention of gastric cancer by eradication therapy, while one randomized clinical trial has revealed no overall effects, but significant prevention of gastric cancer in patients without precancerous lesions. The present multicenter, prospective cohort study, conducted in an area where the incidence of gastric cancer is especially high, revealed no overall efficacy of *H pylori* eradication for preventing gastric cancer in patients with peptic ulcer. In contrast, it demonstrated that eradication was associated with a significant decrease of gastric cancer in patients with duodenal ulcer, which is known to be more prevalent in younger individuals.

Applications

Given the overall lack of efficacy of eradication therapy for peptic ulcer in preventing gastric cancer, the findings highlight the importance of longer and careful follow-up after eradication therapy. Furthermore, the significant efficacy of treatment observed in younger patients suggests the need to eradicate *H pylori* as early as possible.

Terminology

Conventional antacid therapy: a conventional method of treatment, covered by the National Health Insurance (NHI) as determined by the Ministry of Health, Labor, and Welfare of Japan, which consists of treatment with antacids including proton-pump inhibitors and histamine- H_2 blockers given over 6-8 wk.

Peer review

The innovative content, as well as readability, reflects the advanced level of clinical research in gastroenterology both at home and abroad.

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BRIEF ARTICLES

Small sphincterotomy combined with endoscopic papillary large balloon dilation *versus* sphincterotomy

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CONCLUSION: SES + ELBD did not show significant benefits compared to conventional EST, especially for the removal of large (≥ 15 mm) bile duct stones.

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Key words: Sphincterotomy; Endoscopic; Balloon dilatation; Cholelithiasis; Lithotripsy

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Abstract

AIM: To compare small sphincterotomy combined with endoscopic papillary large balloon dilation (SES + ELBD) and endoscopic sphincterotomy (EST) for large bile duct stones.

METHODS: We compared prospectively SES + ELBD (group A, $n = 27$) with conventional EST (group B, $n = 28$) for the treatment of large bile duct stones (≥ 15 mm). When the stone could not be removed with a normal basket, mechanical lithotripsy was performed. We compared the rates of complete stone removal with one session and application of mechanical lithotripsy.

RESULTS: No significant differences were observed in the mean largest stone size (A: 20.8 mm, B: 21.3 mm), bile duct diameter (A: 21.4 mm, B: 20.5 mm), number of stones (A: 2.2, B: 2.3), or procedure time (A: 18 min, B: 19 min) between the two groups. The rates of complete stone removal with one session was 85% in group A and 86% in group B ($P = 0.473$). Mechanical lithotripsy was required for stone removal in nine of 27 patients (33%) in group A and nine of 28 patients (32%, $P = 0.527$) in group B.

INTRODUCTION

The basic principle of common bile duct stone removal involves destruction or dilation of the bile duct orifice, which allows easy removal of the stone. Endoscopic sphincterotomy (EST) is accepted as the standard management for stone removal from the bile duct, but it is associated with serious complications such as hemorrhage, pancreatitis, perforation, and recurrent infection of the bile duct, which cause permanent functional loss of the sphincter of Oddi^[1-4]. Endoscopic papillary balloon dilation (EBD) was introduced by Staritz *et al*^[5] and has been accepted widely as an alternative to EST^[6-10]. It has similar outcomes for common bile duct stone removal compared to EST, and has the advantages over EST of preserving papillary sphincter function and causing minimal complications such as hemorrhage and perforation^[11-19]. Despite these advantages, EBD is associated with more severe and frequent occurrence of pancreatitis^[20-22]. In addition, EBD has some technical difficulties for removing large stones because the biliary opening is not enlarged to the same degree as with EST^[23].

To overcome these limitations, Ersoz *et al*^[24]

introduced EBD with conventional EST for the removal of large (≥ 15 mm) bile duct stones that are difficult to remove by EBD alone. They have reported that EBD with conventional EST is more effective for the retrieval of large stones and shortens the procedure time. Recently, this technique has been modified slightly to endoscopic papillary large balloon dilatation (ELBD) with small incision EST, and many studies have reported on the outcome of stone removal and complication rate^[25-27]. However, these studies on the efficacy of ELBD with SES have concentrated on small stones, of which the majority are ≤ 1 cm^[25,26]. Therefore, the effectiveness of SES with ELBD for large stone removal (≥ 15 mm) has not been established.

We conducted a prospective randomized study to compare the efficacy and safety of SES + ELBD with conventional EST for the treatment of large (≥ 15 mm) common bile duct stones.

MATERIALS AND METHODS

Patients

From June 2006 to December 2008, 55 patients were enrolled, and all patients were diagnosed as having common bile duct stones by endoscopic retrograde cholangiography (ERCP) or magnetic resonance imaging (MRI). In all patients, the stone was at least 15 mm in maximum diameter. The exclusion criteria for this study were the following: (1) bleeding tendency with INR > 1.5 ; (2) platelet count $< 50\,000/\text{mL}$; (3) anticoagulation therapy within 72 h of the procedure; (4) bilio-colic fistula; (5) stone size > 50 mm; (6) acute cholecystitis; (7) acute pancreatitis; (8) cholangitis; (9) intrahepatic duct stones; (10) pancreatobiliary malignancy; and (11) surgical history involving the biliary tree (not including the gall bladder) or gastrointestinal tract, such as the stomach or small bowel, which can alter the papillary location. Patients chosen for our study protocol were divided randomly into two groups according to the order of the procedure. Twenty-seven patients underwent SES + ELBD (group A) and 28 patients underwent conventional EST (group B). This study was approved by the institutional review board of our hospital, and all patients provide written informed consent before entering the study.

Methods

Management such as pharyngeal anesthesia and premedication before the procedure was carried out in the same manner as for general endoscopy, and ERCP was performed with a side-viewing endoscope (TJF240; Olympus, Tokyo, Japan). After the bile duct stones were visualized following cholangiography, the stone was removed according to each protocol. In group A, we made an incision to the mid-portion of the papilla with a pull-type sphincterotome (Figure 1A) and then inserted a CRE balloon (15, 16.5, or 18 mm; Boston Scientific, Natick, MA, USA) over a guidewire. Balloon dilation was performed using wire-guided hydrostatic balloon catheters placed across the papilla. The balloon was inflated with dilute contrast media until the waistline was

obliterated under fluoroscopic monitoring (Figure 1B). Initially, we performed dilation with a 15-mm-diameter balloon, and if the balloon was not large enough to remove the stones, we repeated it with a larger balloon in the order 15 mm \rightarrow 16.5 mm \rightarrow 18 mm. When the papillary orifice was dilated after balloon dilation (Figure 1C), the stones were retrieved using a Dormia basket (WebTM extraction basket; Wilson-Cook Medical, Winston-Salem, NC, USA) (Figure 1D) or retrieval balloon catheter (double lumen retrieval balloon catheter; Boston Scientific). When the stones were not extracted from the biliary tract with initial basket trapping, mechanical lithotripsy (BML-4Q; Olympus) was performed to fragment the stones. In group B, EST was performed with a pull-type sphincterotome (KD-6Q; Olympus) as the standard method, which was accomplished by extending the incision up to the major horizontal fold of the papillary orifice. After EST, the stones were removed in the same way as in group A. If the stones could not be removed completely in one session, we performed another stone removal session in each group. Complete stone removal was documented with a final cholangiogram. The procedure time was measured as the time between selective cannulation and complete stone removal in the cases of successful stone removal in the first session. The maximum procedure time for the first session was limited to 40 min if the stone was difficult to remove in one session.

Measurements

Stone size and number and bile duct size were documented on the cholangiogram during ERCP. Stone size was assessed by comparing the largest diameter of the stone with the diameter of the TJF240 endoscope, as measured on the cholangiogram. The primary endpoint was the success rate for complete removal of stones within the initial ERCP session. The secondary outcomes included the time for the procedure of these initial-success cases, frequency of mechanical lithotripsy, and associated complications such as bleeding, pancreatitis, cholangitis, and perforation. To observe the complications, blood samples involving a complete blood count, liver function test, amylase, and lipase concentrations were taken before the procedure and 1 and 2 d after ERCP. Post-ERCP pancreatitis was defined as persistent abdominal pain of more than 24 h duration, associated with serum amylase more than three times the upper limit of normal. Bleeding complication was deemed a decrease in hemoglobin concentration of > 2 mg/dL or clinical signs of bleeding after the procedure, such as melena or hematemesis. Cholangitis was defined as a fever accompanied by leukocytosis and right upper quadrant pain after the procedure. All complications were classified and graded according to the consensus guidelines with some modification^[28]. After the stones were removed, ductal clearance was confirmed with a cholangiogram during the procedure.

Statistical analysis

Statistical analysis was performed using the statistical

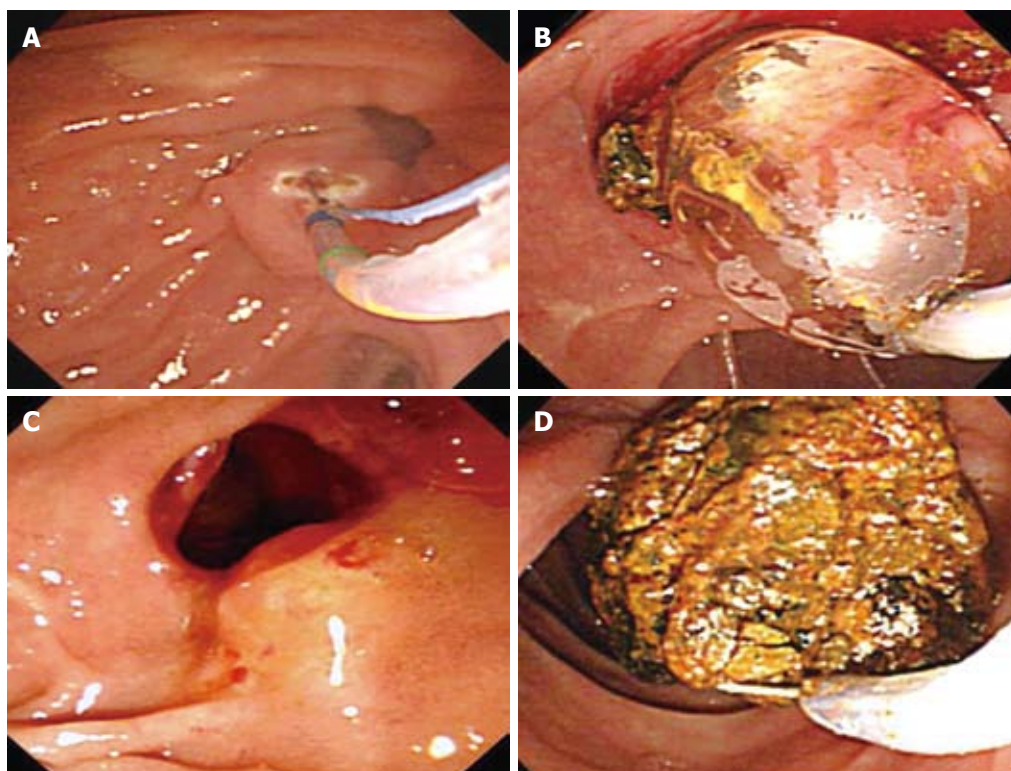


Figure 1 Endoscopic view. A: A small sphincterotomy using a pull-type sphincterotome; B: Endoscopic papillary balloon dilation with a large balloon after small endoscopic sphincterotomy; C: Dilated orifice after small EST + ELBD; D: Stone removal through the dilated orifice of the major papilla.

Table 1 Baseline characteristics of the patients

	Group A (<i>n</i> = 27)	Group B (<i>n</i> = 28)
Gender (M/F)	10/15	11/14
Mean age (yr) ¹	70.3 ± 8.7	69.8 ± 9.2
Mean diameter of stone (mm) ¹	20.8 ± 4.1	21.3 ± 5.2
Mean No. of stones ¹	2.2 ± 1.3	2.3 ± 1.2
Mean diameter of bile duct (mm) ¹	21.4 ± 6.3	20.5 ± 5.7
Periampullary diverticulum (%)	9 (33.3)	10 (35.7)
Previous cholecystectomy (%)	9 (33.3)	7 (25)
Distal CBD tapering (%)	11 (41)	10 (36)

¹mean ± SD; CBD: Common bile duct.

SPSS for Windows version 12.0 (Chicago, IL, USA). Data are presented as the mean ± SD or median with range. Categorical parameters were compared using the χ^2 or Fisher's exact test, and continuous variables were compared with Student's *t* test. *P* < 0.05 was considered statistically significant.

RESULTS

The gender ratio was similar in the two groups. The mean age was 70.3 years in group A and 69.8 in group B. The mean size of the stones was 20.8 mm (range 15-38.3 mm) in group A and 21.3 mm (range 15-48 mm) in group B. The mean number of stones was 2.2 in group A and 2.3 in group B. The maximal bile duct diameter did not differ significantly between the two groups. A peri-ampullary diverticulum was observed in nine patients in group A and 10 in group B. Sixteen

(29%) patients had a history of cholecystectomy. A tapered common bile duct was observed in 11 (41%) patients in group A and 10 (36%) in group B (Table 1).

Overall, complete removal of bile duct stones in the first session was achieved in 46 (84%) patients, while nine required additional sessions. The causes of failure in the first session were incomplete stone capture with the mechanical lithotripsy basket as a result of a large stone (two cases each in groups A and B), stone impaction (one case in group A), procedure-induced bleeding (one case in group B), and incomplete retrieval because of multiple stones (one case each in groups A and B). The stone clearance rate in the first session between the two groups did not differ significantly, and was 84% in both groups (*P* = 0.473). Mechanical lithotripsy was used for nine (33%) patients in group A and nine (32%) in group B (*P* = 0.527). The mean procedure time was compared in the cases involving successful removal of the stone in the initial session and did not differ statistically between the two groups. All stones were removed completely in all patients within three sessions (group A: 1.27 ± 0.53 sessions, group B: 1.31 ± 0.71 sessions, *P* = 0.714). The number of sessions of mechanical lithotripsy and mean procedure times did not differ significantly between the two groups (Table 2).

We also divided each group into subgroups according to the stone size (2 cm) and compared the stone removal rate and application of mechanical lithotripsy. The complete stone removal rate for each subgroup in the first session was similar in both groups: 85.7% (group A) and 86.6% (group B) in the subgroups with stones <

Table 2 Results of endoscopic stone removal after small EST + ELBD *vs* EST (stone size ≥ 15 mm)

	Group A (<i>n</i> = 27)	Group B (<i>n</i> = 28)	<i>P</i> value
Stone removal in the first session (%)	23 (85)	23 (86)	0.473
Mechanical lithotripsy (%)	9 (33)	9 (32)	0.527
Mean procedure time (min) ^{1,2}	18 \pm 12	19 \pm 13	0.917
Mean therapeutic session ¹	1.27 \pm 0.53	1.31 \pm 0.71	0.714

¹mean \pm SD; ²Calculated from initial success cases (*n* = 23 in both groups). Overall success rate of the first session: 84%.

Table 4 Comparison of stone removal in the first session and application of mechanical lithotripsy

	Group A (<i>n</i> = 11)	Group B (<i>n</i> = 10)	<i>P</i> value
Stone removal in the first session (%)	9 (81.8)	7 (70)	0.525
Mechanical lithotripsy (%)	6 (54.5)	6 (60)	0.801

2 cm in maximum diameter and 84.6% (group A) and 76.9% (group B) in the subgroups with stones ≥ 2 cm in maximum diameter. The rate of mechanical lithotripsy increased significantly with stone size irrespective of the group (*P* < 0.05 in each group, Table 3). Finally, we compared the stone removal rate in the first session and the need for mechanical lithotripsy in the cases with a tapered distal bile duct between the two groups. A tapered bile duct was deemed as one in which a portion of the distal common bile duct was narrowed with a steady curve on the cholangiogram. The stone removal rate was higher in group A (81.8%) than in group B (70%), but not significantly. In addition, the mechanical lithotripsy rate was similar between the two groups (Table 4).

Complications according to the consensus guidelines were not observed in either group, and we could not compare the complication rate between the two groups. Although mild amylase elevation less than three times the upper limit of normal was observed in four patients in group A (15%) and three in group B (11%), no instances of post-ERCP pancreatitis and cholangitis according to the consensus guidelines occurred in either group. We did not perform prophylactic pancreatic duct stenting during the procedure in any case. A small amount of bleeding was seen in four patients in group A (15%) and two in group B (7%). No procedure-related perforation was observed. Nine cases in which complete ductal clearance was not achieved in the first session underwent a second session on the next day or within a few days, and any additional protective procedure, such as biliary plastic stenting, was not performed until the next session.

DISCUSSION

EST is the most frequently used endoscopic technique for the clearance of stones from the bile duct. Its success rate exceeds 90%, and it has been accepted as the best nonsurgical treatment for common bile duct stones^[29-33]. However, EST is still associated with an 8%-12% rate

Table 3 Comparison of overall application of mechanical lithotripsy according to the size of the stone in each group

	Group A (<i>n</i> = 27)		Group B (<i>n</i> = 28)	
	< 2 cm (<i>n</i> = 14)	≥ 2 cm (<i>n</i> = 13)	< 2 cm (<i>n</i> = 15)	≥ 2 cm (<i>n</i> = 13)
Stone removal in the first session (%)	12 (85.7) ^a	11 (84.6) ^b	13 (86.6) ^c	10 (76.9) ^d
Mechanical lithotripsy (%)	2 (14.3) ^e	7 (53.8) ^f	2 (13.3) ^g	7 (53.8) ^h

Overall application of mechanical lithotripsy: 17/50 (34%). *P* value: a *vs* b, not significant; c *vs* d, not significant; e *vs* f, 0.018; g *vs* h, 0.008.

of acute complications, such as bleeding, perforation, cholangitis, and post-procedure pancreatitis^[11,25,34-37]. In addition, it permanently destroys the biliary sphincter, which can lead to chronic complications, such as duodenal biliary reflux, bacterial contamination, and chronic inflammation of the biliary system^[11].

EBD was introduced by Staritz *et al*^[5] in 1983 as an alternative method for the removal of bile duct stones. The main advantage of this technique is that it does not involve cutting the biliary sphincter, therefore preserving its function. However, major limitations of EBD exist, including difficulty in removing large stones and a high incidence of pancreatitis. Since balloon dilation does not enlarge the sphincter of Oddi to the same extent as EST, large stone removal with EBD is difficult, and mechanical lithotripsy is required more often than with EST^[11,21]. As a result, there is a need to modify the EBD technique to remove large bile duct stones and reduce the risk of pancreatitis. Similarly, EST is not a good method if the stones are too large to remove. Stone fragmentation procedures such as mechanical lithotripsy are required in this situation, regardless of the approach method. Ersoz *et al*^[24] first reported the use of EST followed by papillary balloon dilation. They reported an 83% success rate in the first session with a 7% rate of mechanical lithotripsy in 58 patients in whom endoscopic removal of bile duct stones using standard EST and balloon/basket extraction had failed. Recently, multiple published series have shown that the overall first session success rates of stone removal with EBD following EST ranged from 80% to 100%^[24,25,27,38], and these success rates were similar to those of EST. Although some recent studies have reported that the stone clearance rate for the initial session of EBD following EST is high, the outcome for large stone removal by ELBD following EST remains controversial. Since previous data from ELBD studies have included various sizes of stones, especially small stones < 1 cm, and comparison studies between SES + ELBD and conventional EST for large stone (≥ 15 mm) removal are not sufficient^[25,26,39]. Therefore, we could not clarify the effectiveness of ELBD following EST for large stone removal.

In our present study, we compared SES + ELBD to conventional EST in terms of usefulness and safety for the treatment of large stones. We also evaluated the number of applications of mechanical lithotripsy and compared this with previous studies, which reported

that EST + ELBD reduced the use of mechanical lithotripsy^[24,25,39]. The number of patients enrolled in our study was limited by the stone size and exclusion criteria. However, these criteria enabled us to compare the outcomes between the two groups more objectively.

Our findings showed that the initial success rate for the clearance of common bile duct stones was same in both groups and it was not significantly different. A previous series of EST + ELBD gave first session success rates of 70%-99% and mechanical lithotripsy rates of 1%-11%^[24-27,39]. In contrast, we had a 33% mechanical lithotripsy rate in group A and a 32% rate in group B. Compared to previous reports, the frequency of mechanical lithotripsy was markedly higher^[24-27,39], which might be attributable to the large stones (≥ 15 mm).

Previous studies likely reported lower rates of mechanical lithotripsy, because of smaller stones^[25,26,39], or a wider sphincterotomy^[24,40]. Of course, the frequency of mechanical lithotripsy might be related to various factors, such as the extent of EST, size of the stone and balloon, and shape of the stone and common bile duct. Removing large stones (≥ 15 mm) in patients with a tapered common bile duct without stone fragmentation might be difficult, despite orifice dilation using large balloon dilation. A retrospective pilot study of 50 patients revealed that patients that required mechanical lithotripsy were more often characterized by large stones combined with a tapered distal common bile duct rather than either of these features alone^[41].

Other recent studies have revealed that SES + ELBD reduced the frequency of mechanical lithotripsy and gave better results for the removal of stones^[25,27,39].

However, in our study, mechanical lithotripsy was not reduced with SES + ELBD, and no difference in the frequency of mechanical lithotripsy was observed between the two groups. We needed a stone fragmentation method such as mechanical lithotripsy, although we used a large balloon (maximum, 18 mm) to dilate the orifice; the larger stone size was associated with more frequent mechanical lithotripsy. The CRE balloon had a length of 8 cm, of which approximately half was positioned in the distal bile duct. Considering this point, we speculate that part of the terminal and distal bile duct could be dilated simultaneously using balloon dilation, and if the stone was small enough to pass through the dilated bile duct, it could be removed more easily. To remove large stones, however, some EST and large balloon dilation may help to dilate the sphincter of Oddi orifice, to allow the passage of large stones. Large balloon dilation alone cannot stretch the wall of the distal bile duct to the degree necessary for the effective removal of large stones. Hence, the configuration and wall lumen tension of the terminal bile duct may be more important factors for the removal of large stones than the size of the balloon and dilation of the bile duct. Therefore, if a stone is too large to remove *via* the dilated terminal bile duct and sphincter of Oddi, stone fragmentation using mechanical lithotripsy, for example, might be inevitable.

Complications according to the consensus guidelines did not occur in our study, which may be related to the

small number of patients enrolled. No procedure-related pancreatitis occurred. Only amylase elevation less than three times the upper limit of normal was observed in seven patients (four in group A and three in group B). Perforation did not occur in any patient.

A small amount of bleeding was observed in six patients in our study. Of these, stone removal was postponed to the next session for one patient in group B, but this case did not meet the criteria for bleeding complications according to the consensus guidelines^[28]. Other bleeding complications were easily controlled using argon-plasma coagulation, epinephrine spray, or compression by the balloon. Ersoz *et al*^[24] have reported a 9% bleeding rate in their EST + ELBD group, especially in patients with a tapered distal bile duct. With the larger balloon, the higher rate of bleeding could have been attributable to the moderate degree of EST. In addition, they performed major EST in their study. In the SES + ELBD group, the reported rate of bleeding ranged from 0% to 4.5%, and all of the cases were relatively mild^[25,39,40].

In conclusion, SES + ELBD did not show significant benefits compared to conventional EST and reducing the rate of mechanical lithotripsy, especially for the removal of large (≥ 15 mm) bile duct stones. Regarding the occurrence of complications, SES + ELBD showed a similar level of safety compared to conventional EST. Hence, SES + ELBD is a good alternative to conventional EST for the removal of large stones, especially for the unskilled endoscopist. However, a large-scale study of patients is required to clarify the difference in the efficacy of the two procedures.

COMMENTS

Background

Many recent studies on small sphincterotomy combined with endoscopic papillary large balloon dilation (SES + ELBD) have reported on the outcome of stone removal and the complication rate. As previous studies concentrated on the efficacy of small bile duct stone removal, the effectiveness of ELBD with SES for large stone removal (≥ 15 mm) has not been established.

Innovations and breakthroughs

Other recent studies have revealed that SES + ELBD reduced the frequency of mechanical lithotripsy and gave better results for the removal of stones. However, the present study found that SES + ELBD did not reduce the need for mechanical lithotripsy in removing large (≥ 15 mm) bile duct stones. Large balloon dilation alone cannot stretch the wall of the distal bile duct to the degree necessary for the effective removal of large stones. To remove large stones, the configuration and wall lumen tension of the terminal bile duct may be more important factors than the size of the balloon and dilation of the bile duct. Therefore, if a stone is too large to remove *via* the dilated terminal bile duct, stone fragmentation might be inevitable.

Applications

For the removal of large common bile duct stones, SES + ELBD is a good alternative to conventional endoscopic sphincterotomy (EST), especially for the unskilled endoscopist. However, a large-scale study is required to clarify differences in the efficacy of the two procedures.

Terminology

Endoscopic papillary balloon dilation (EPBD), an alternative method with similar outcomes compared to EST, is associated with frequent, severe pancreatitis. SES + ELBD is a modified EPBD technique.

Peer review

In this study, the numbers of the patients are too small to compare infrequent complications like bleeding or pancreatitis. However, it may be difficult to enroll many more patients with large bile duct stones.

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Prognostic analysis of patients with pancreatic head adenocarcinoma less than 2 cm undergoing resection

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between the two groups. During a follow-up period ranging from 1.0 to 122.7 mo (median, 10.9 mo), S-PAC and L-PAC patients had a similar prognosis after resection ($P = 0.4805$). Among the S-PAC patients group, patients with higher albumin level (> 3.5 g/dL) had more favorable survival than those with lower albumin levels, which was the only favorable predictive prognostic factor. Meanwhile, early-staged (stage I, II) S-PAC patients tended to have a more favorable outcome than late-stage (stage III, IV) S-PAC patients, but this was not statistically significant.

CONCLUSION: S-PAC patients should not be regarded as early PAC. Only higher albumin level (> 3.5 g/dL) and early stage disease (stage I, II) were the favorable prognosis factors for S-PAC patients.

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Abstract

AIM: To investigate the differences in clinicopathological features between patients with pancreatic cancer greater or less than 2 cm situated over the pancreatic head and the prognostic factors for survival of patients with pancreatic cancer < 2 cm over the pancreatic head.

METHODS: From 1983 to 2006, 159 patients with histologically proven pancreatic adenocarcinoma (PAC) at the pancreatic head undergoing curative resection at the Department of Surgery, Chang Gung Memorial Hospital, Taipei, Taiwan were reviewed, comprising 123 cases of large (L)-PAC (tumor > 2 cm) and 36 cases of small (S)-PAC (tumor ≤ 2 cm). We compared the clinicopathological characteristics and prognosis of L-PAC and S-PAC patients. The clinicopathological characteristics of S-PAC were investigated to clarify the prognosis predictive factors of S-PAC.

RESULTS: One hundred and fifty-nine PAC patients, aged 16-93 years (median, 59.0 years) with a tumor at the pancreatic head undergoing intentional curative resection were investigated. The S-PAC and L-PAC patients had similar demographic data, clinical features, and tumor markers (a similar positive rate of carcinoembryonic antigen and carbohydrate antigen 19-9). There were also similar rates of lymph node metastasis, portal vein invasion, stage distribution, tumor differentiation, positive resection margin, surgical morbidity and mortality observed

Key words: Prognostic factor; Pancreas; Pancreatic head area; Pancreatic cancer

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INTRODUCTION

Pancreatic adenocarcinoma (PAC), one of the most lethal malignant cancers, ranks fifth in mortality related to cancer worldwide with the 5-year survival after resection ranging from 10% to 29%^[1-3]. Thirty-two thousand new PAC cases have been found in America each year^[4].

Surgery remains the only curative treatment of PAC and detection of PAC with small size (≤ 2 cm) (S-PAC) seems to be essential to improve the outcome, which is demonstrated in some reports^[5-7]. The 5-year survival rate varied from 19% to 41% for PAC patients undergoing pancreatectomy with the highest survival in patients with small tumors confined to the pancreas.

But the controversial question is “Dose PAC with tumor diameters of 2 cm or less necessarily indicate early-stage disease or good prognosis?” Recent data have shown the different opinions about this issue^[8,9], which warrants further studies addressing this topic.

To collect an adequate number of resected cases of S-PAC was difficult due to limitations of diagnostic equipment, explaining why most of the previous studies were multi-institutional^[7,10,11]. Recent advances in diagnosis have made it possible to detect PAC earlier and to increase the number of resected cases; consequently evaluation of S-PAC cases in one center becomes feasible, which could provide more accurate information.

We retrospectively reviewed 159 PAC cases with pancreatic head cancer undergoing pancreaticoduodenectomy from 1983 to 2006. Among them, 36 cases were diagnosed with S-PAC, and the remainder were PAC with tumor diameter > 2 cm, classified as large PAC (L-PAC). We compared the clinicopathological features and surgical outcomes between S-PAC and L-PAC to find the favorable prognostic factors.

MATERIALS AND METHODS

From 1983 to 2006, 159 patients with histopathologically proven PAC located at the pancreatic head undergoing intentional curative resection at the Department of Surgery, Chang-Gung Memorial Hospital, Taipei, Taiwan were reviewed. Curative resection was defined as a negative resection margin observed during histopathological examination. The 159 PAC patients comprised 96 men and 63 women with a median age of 64.0 years (range, 16-93 years). Among them, 36 patients (22.6%) had a tumor size \leq 2 cm classified as S-PAC and 123 PAC patients had tumor size > 2 cm (L-PAC). Tumor size was defined by histopathological examination. Surgical mortality was defined as death occurring within 1 mo after surgery. Laboratory tests were conducted on the day before surgery. Serum carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) were measured by radioimmunoassay. The tumors were preoperatively evaluated by abdominal ultrasonography, endoscopic retrograde cholangiopancreatography, percutaneous transhepatic cholangiography, computed tomography, magnetic resonance image with cholangiopancreatography, and angiography, as appropriate. Tumor stage was defined according to the pathological tumor node metastasis (pTNM system) classification proposed by the UICC. Stages I and II were classified as early-stage, and stages III and IV as advanced stage PAC. Adjuvant chemotherapy was systemic administration of either 5-fluorouracil-based or gemcitabine-based regimen due to either the positive section margin or lymph node metastasis. Adjuvant radiotherapy was conducted by intra-operative radiotherapy, external beam radiotherapy and/or brachytherapy due to either a positive section margin or local recurrence.

Statistical analysis

All data are presented as percentage of patients or mean

with standard deviation. Numerical data were compared by independent two-sample *t*-tests. Nominal data were compared by Pearson chi-square test, or multiple forward stepwise logistic regression when appropriate. Survival was calculated and plots constructed according to the Kaplan-Meier method. Sixteen clinicopathological variables were selected for survival analysis, including demographic data, clinical features, laboratory data, operative findings, and pathological features. The log-rank test was performed for a univariate analysis for prognosis by using log-rank test and multivariate analysis was conducted with Cox's proportional hazard model. All statistical analyses were performed using the SPSS computer software package (Version 10.0, Chicago, IL, USA). A value of $P < 0.05$ was considered significant.

RESULTS

Clinicopathological features of L-PAC and S-PAC patients

The S-PAC group contained 22 men and 14 women, with a mean age of 61.9 ± 8.2 years. In the L-PAC group, there were 74 men and 49 women, with a mean age of 62.8 ± 10.5 years. Both groups had a similar age distribution, gender ratio, and laboratory data. In terms of symptoms and physical examination, both groups possessed a higher positive rate (> 80%) for non-specific symptoms and signs irrespective of the tumor size. Regarding tumor markers (CEA and CA 19-9), L-PAC and S-PAC groups had similar positive rates (35.9% and 75%, and 29.6% and 69%, respectively). In the light of lymph node metastasis and portal vein invasion, the two groups had similar positive rates. Even in the S-PAC groups, lymph node metastasis and portal vein invasion rates reached 63.9% and 11.1%, respectively. Both groups had almost the same curative resection rate. Portal vein invasion and retroperitoneal extension of the tumor explained the reasons for the positive margin. Both groups also had similar distributions of tumor differentiation and stage.

Morbidity and mortality rates between L-PAC and S-PAC groups

Surgical morbidity and mortality rates for the L-PAC group were 26.8% and 4.1%, respectively, similar to those of the S-PAC group which were 30.6% and 2.8%, respectively (Table 1). Almost the same percentage of patients received post-operative chemotherapy and radiation in the two groups, mainly for lymph node metastasis and positive margin.

Survival analysis between L-PAC and S-PAC

All of the 159 PAC patients undergoing resection were followed regularly until death with the duration of follow-up ranging from 1.1 to 213.5 mo (median, 16.4 mo). The 1-, 3- and 5-year survival rates of the 159 cases were 51.3%, 23.1% and 12.5%, respectively (Figure 1). The 3- and 5-year survival rates of the S-PAC and L-PAC patients were 26.4% and 6.6%, and 22.1% and 14.7%, respectively

Table 1 Clinicopathological features of 159 pancreatic head adenocarcinoma patients with tumor size smaller and larger than 2 cm *n* (%)

	S-PAC (<i>n</i> = 36)	L-PAC (<i>n</i> = 123)	<i>P</i>
Age (yr)	61.9 ± 8.2	62.8 ± 10.5	0.601
Sex (M/F)	22/14	74/49	0.918
Symptom (+/-)	35/1	123/0	0.226
Physical findings (+/-)	32/4	99/24	0.244
Albumin (g/dL)	3.74 ± 0.58	3.71 ± 0.61	0.827
AST (IU/L)	178.8 ± 258.5	138.1 ± 127.7	0.204
CEA (ng/mL) ≥ 5	8/27 (29.6)	33/92 (35.9)	0.549
CA 19-9 (IU/L) ≥ 37	20/29 (69.0)	72/96 (75.0)	0.518
Size (median)	1.5	3.5	0.0001
Operation time (min)	494.5 ± 141.5	469.2 ± 118.1	0.292
LN metastasis	23 (63.9)	72 (58.5)	0.565
PV invasion	4 (11.1)	12 (9.8)	0.760
Positive margin	10 (27.8)	35 (28.5)	0.244
Staging			0.459
I	12 (33.3)	39 (31.7)	
II	1 (2.8)	12 (9.8)	
III	22 (61.1)	71 (57.7)	
IV	1 (2.8)	1 (0.8)	
Tumor differentiation			0.723
W-D	11 (30.6)	38 (30.9)	
M-D	19 (52.8)	59 (48.0)	
P-D	6 (16.7)	26 (21.2)	
Morbidity	11 (30.6)	33 (26.8)	0.660
Postoperative CT	23 (63.9)	58 (47.2)	0.077
Postoperative RT	1 (2.8)	11 (8.9)	0.300
Mortality	1 (2.8)	5 (4.1)	0.760

AST: Aspartate aminotransferase; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9; LN: Lymph node; PV: Portal vein; W-D: Well-differentiated; M-D: Moderate-differentiation; P-D: Poor-differentiation; CT: Chemotherapy; RT: Radiotherapy.

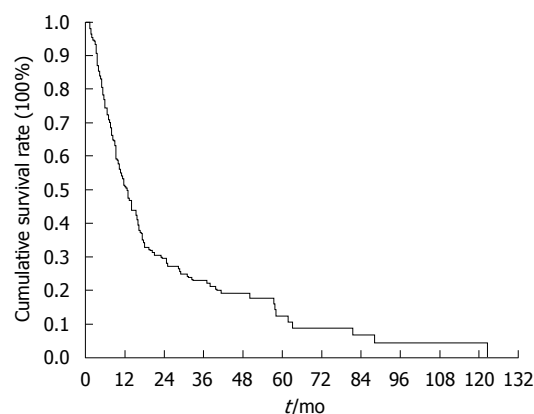
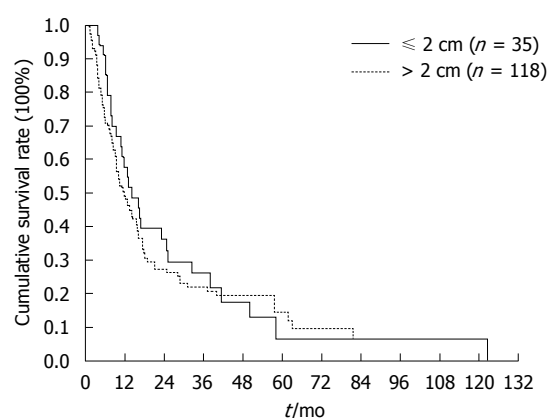
(Figure 2). The S-PAC patients had a similar overall survival rate to the L-PAC patients ($P = 0.48$) (Figure 2).

Prognosis predictive factors of S-PAC patients

S-PAC and L-PAC groups had the same prognosis in this study, which meant tumor size did not influence the outcome of this disease. So, we were interested in which factors could affect the prognosis of S-PAC patients. We chose 16 clinicopathological characteristics including demographic data, clinical biochemical laboratory values, tumor markers, operative procedure, tumor invasion, tumor differentiation, tumor stage, post-operative chemotherapy and radiation to determine which one could be the prognostic predictive factor (Table 2). Only the albumin level had a significant impact on survival of S-PAC patients ($P = 0.002$). The early stage S-PAC patients (Stage I, II) tended to have a favorable outcome compared with late stage S-PAC patients (Stage III, IV), but this difference did not reach statistical significance ($P = 0.0931$). Age, gender, operative procedures, biochemical data, tumor invasion, resection margin, and tumor differentiation did not associate with favorable prognosis.

DISCUSSION

PAC is one of most lethal human malignancies

**Figure 1** The overall survival rates of 159 pancreatic head adenocarcinoma patients.**Figure 2** The overall survival rates of S-PAC and L-PAC patients.

producing the fourth highest cancer-related mortality in the western world and the fifth highest cancer-related mortality rate worldwide^[4]. The overall 5-year survival rate of PAC has been reported to be around 1%-4%, which was attributed to its aggressive growth behavior, early local spread, early metastasis, and resistance to radiation and most systemic chemotherapies^[4]. Surgery remains the cornerstone of treatment. After resection, the 5-year survival rate can reach 10%-29%^[1-3].

For most malignancies, a smaller tumor size is usually deemed as a good indicator of early stage, and should be diagnosed and treated as soon as possible to improve the outcome. Some reports have demonstrated that tumor size was one of most important determinants of resectability and prognosis for pancreas cancer^[12-14]. Previously, Satake *et al*^[15] stated that PAC with tumor size < 40 mm represented a better prognosis. However, according to our report, even PAC with tumor size < 20 mm did not necessarily mean early stage disease and should not be treated by surgery alone^[7,9,16,17]. The reported 5-year survival for S-PAC patients ranges from 8% to 59% and is only 6.6% in our series^[3,7,9,16,18,19].

Several reasons could be found to explain this dismal outcome. S-PAC patients (tumor size < 2 cm) had similar clinicopathological features including positive clinical symptoms, tumor differentiation pattern and positive

Table 2 Univariate analysis of factors influencing the overall survival of the 35 small pancreatic head cancer patients undergoing resection

Factors	Survival time (mo)				P
	Median (mo)	Mean (mo)	3-yr (%)	5-yr (%)	
Gender					0.8417
Male (n = 21)	13.0	24.0	28.1	0	
Female (n = 14)	14.0	27.9	23.4	11.7	
Age (yr)					0.2931
≤ 60 (n = 13)	24.6	26.4	35.9	12.0	
> 60 (n = 22)	9.4	8.06-10.75	17.02	12.61	
Physical examination					0.9566
Positive (n = 31)	13.0	28.1	26.7	8.0	
Negative (n = 4)	16.1	22.5	25.0	0	
Bilirubin (mg/dL)					0.5049
≤ 2 (n = 8)	14.0	15.6	14.3	0	
> 2 (n = 27)	16.1	29.4	39.6	7.7	
Albumin (g/dL)					0.0002
≤ 3.5 (n = 13)	5.9	7.6	0	0	
> 3.5 (n = 22)	16.6	32.7	33.6	11.2	
Serum CEA (ng/mL)					0.6741
≤ 5 (n = 24)	16.6	22.4	23.6	7.9	
> 5 (n = 11)	9.1	19.8	25.0	15.42	
Serum CA 19-9 (IU/L)					0.7592
≤ 37 (n = 11)	16.6	19.4	11.1	11.1	
> 37 (n = 24)	14.0	23.5	33.6	0	
Operative procedure					0.6500
Whipple (n = 30)	16.1	27.9	28.8	7.2	
PPPD (n = 5)	14.0	15.5	0	0	
Portal vein invasion					0.1467
Positive (n = 2)	4.0	9.0	0	0	
Negative (n = 33)	16.1	28.0	28.1	7.0	
Resection margin					0.6704
Positive (n = 10)	10.9	19.5	30.0	0	
Negative (n = 25)	16.1	28.8	26.4	7.9	
TNM staging					0.0931
I + II (n = 13)	16.6	40.2	50.0	15.0	
III + IV (n = 22)	13.0	17.6	14.4	0	
Tumor differentiation					0.3202
W-D (10)	23.1	22.9	50.0	0	
M-D (19)	10.8	21.5	17.9	6.0	
P-D (6)	16.1	32.2	50.0	25.0	
Post-operative CT					0.7114
With (n = 23)	16.1	23.9	25.3	0	
Without (n = 12)	10.8	28.8	27.8	13.9	
Post-operative RT					0.9385
With (n = 1)	24.6	24.6	0	0	
Without (n = 24)	14.0	24.6	27.4	6.9	

IU: International unit; PPPD: Pylorus-preserving pancreaticoduodenectomy.

lymph node invasion rate to L-PAC patients (Table 1). Contrary to previous reports^[9,20], a well-differentiated type of adenocarcinoma is more frequently seen in PAC tumors with tumor size < 2 cm, but in our study, tumor size had no impact on tumor differentiation. S-PAC and L-PAC possessed the same distribution in terms of tumor differentiation. Jung *et al*^[20] also indicated that pancreas tumor size < 2 cm would tend to be symptomless, however, in our study, we could not find this difference. Both groups had a very low negative rate of symptoms. Contrary to Shimada's report^[8], we showed the incidence of lymph node metastasis was similar between S-PAC and L-PAC (as high as 63.9%), demonstrating that S-PAC with tumor size < 20 mm did not necessarily mean early stage disease. Such findings showed that PAC was attributed to its aggressive growth

behavior, early local spread and early metastasis no matter what its size.

CEA and CA 19-9 are widely used to screen malignancies in the general population. An 80% CA19-9 positive rate in pancreas cancer and high levels of CA19-9 associated with more advanced disease were reported^[21-23]. Jung *et al*^[20] reported that CA19-9 and CEA in patients with PAC tumor size > 6 cm would be higher than in smaller cancers. Steinberg revealed that CEA and CA 19-9 would not increase in patients with pancreas cancer < 2 cm^[24]. But in our study, we found the sensitivity of CEA and CA 19-9 for S-PAC and L-PAC patients were 29.6% and 69%, and 35.9% and 75%, respectively. Tumor marker values did increase in the S-PAC group and both groups had similar positive rates of CEA and CA 19-9.

Regarding prognostic analysis for S-PAC patients, only albumin level could be a favorable prognostic factor for S-PAC statistically ($P = 0.0002$). Early stage S-PAC (Stage I, II) tended to have a better prognosis than late stage S-PAC (Stage III, IV), but this was not statistically significant ($P = 0.0931$). In our study, most non-curative resection cases were due to portal vein invasion and retroperitoneum tumor extension. However, curative resection or not did not influence the final survival of S-PAC and tumor differentiation of S-PAC did not have any influence on survival either. Lymph node metastasis, poorly differentiated tumors, and positive margins were usually regarded as poor prognostic factors for pancreas cancer^[13,25,26]. In this study, we did not find these as unfavorable factors for prognosis. Limited case numbers should be the main cause. In terms of these issues, we need more time to collect more cases to answer these questions. Detection of PAC with small size (≤ 2 cm) (S-PAC) still warrants more efforts to improve the outcome.

In conclusion, S-PAC and L-PAC had similar clinicopathological characteristics. They expressed similar tumor biology, such as lymph node metastasis, portal vein invasion and tumor differentiation. So both groups had the same survival rate, explaining why S-PAC should not be deemed as an early stage disease and treated by surgery alone. For S-PAC groups, only albumin level could be a prognostic predictor. Early stage S-PAC tended to have a more favorable prognosis than late stage S-PAC, although this did not reach statistical significance.

COMMENTS

Background

Pancreatic cancer is a devastating cancer. Previously, cancer size has been deemed as an important prognostic factor for pancreatic cancer. However, growing evidence recently demonstrated the controversial conclusion against the previous viewpoint. Herein, the authors collected data from pancreatic cancer patients with cancer on the pancreatic head and they compared the clinicopathologic characteristics and survival between small pancreatic adenocarcinoma (S-PAC) and large (L)-PAC groups. In addition, they also tried to find out the prognostic factor for S-PAC patients.

Research frontiers

The tumor biology, including differentiation, lymph node metastasis, portal vein invasion and so on is similar between S-PAC and L-PAC patients, which would give rise to further studies of whether there are any different gene mutations in terms of the size of pancreatic cancer.

Innovations and breakthroughs

In this report, albumin level is the only prognostic factor for survival of S-PAC patients. Traditionally, lymph node metastasis and portal vein invasion are deemed as important prognostic factors for pancreatic cancer. Although such findings were not shown in their report, they considered these could be blamed partly on the limited number of cases.

Applications

Through the findings of their report, S-PAC should not be regarded as a early pancreatic cancer. Aggressive management such as post-operative chemotherapy and radiation are justified.

Peer review

This is an interesting clinical report. However, due to the poor prognosis of S-PAC patients and thus the small number of cases who survived for 5 years, we have to be careful in making extended conclusions from the data.

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Baseline predictors of virological response for chronic hepatitis B patients

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CONCLUSION: Baseline serum ALT, TSH, and TT4 levels, especially in combination, have high predictive values of virological response to Peginterferon α -2b in HBeAg-positive CHB patients.

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Key words: Chronic hepatitis B; Hepatitis B virus; Predictors; Virological response; Peginterferon

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Abstract

AIM: To determine which baseline factors of chronic hepatitis B patients are predictive of virological response to Peginterferon α -2b therapy.

METHODS: A total of 21 HBeAg-positive chronic hepatitis B (CHB) patients treated with Peginterferon α -2b were recruited. They were treated with Peginterferon α -2b (0.5-1.0 μ g/kg per week) for 24 wk and followed up for 24 wk. Clinical and laboratory data of the patients were determined at pretreatment and at week 12, at 24 during treatment, and at week 48 during follow up.

RESULTS: Ten patients achieved a virological response at the end of treatment. Their baseline serum alanine aminotransferase (ALT), thyroid-stimulating hormone (TSH), and total thyroxin (TT4) levels were significantly different from those who failed treatment. The positive predictive values (PPV) and negative predictive values (NPV) of ALT, TSH, and TT4 were 75% and 89 %, 75% and 89 %, and 75% and 75%, respectively. Moreover, combinations of the baseline ALT and TT4, ALT and TSH, TT4 and TSH levels had much higher PPV and NPV (86% and 88%, 89% and 100%, 83% and 100%, respectively).

INTRODUCTION

Chronic hepatitis B virus (HBV) infection is a worldwide health problem. More than 400 million people are chronically infected with HBV and are at risk of developing liver cirrhosis and hepatocellular carcinoma. Each year more than one million people die from HBV-related liver diseases^[1-4].

At present, the two main categories of antiviral drugs for chronic hepatitis B are interferon (including Peginterferon) and nucleoside/nucleotide analogs. Many studies have shown that an elevated serum ALT level is associated with virological response and HBeAg seroconversion in CHB patients^[5-8]. Besides higher serum ALT level, some studies also showed that higher aspartate aminotransferase (AST) level, increased histological activity in biopsy specimens, female sex, and lower serum HBV DNA levels are associated with a higher probability of HBeAg seroconversion in CHB patients treated with interferon-based therapies^[9-13]. It is also reported that the HBV genotype is an important predictor of response to interferon-based therapies^[14-17].

Recently, serum HBeAg levels have been used as

outcome predictors of sustained virological response to Peginterferon α -2a in HBeAg-positive CHB patients and showed high negative predictive values (NPVs) at week 24 of therapy^[18]. Another study showed that early serum HBsAg drops also had high predictive values of sustained virological response to Peginterferon α -2a in HBeAg-negative chronic hepatitis B patients both at week 12 and 24^[19].

However, the predictive values of other factors, especially the baseline factors for virological response to Peginterferon α -2b therapy are not clear. Therefore, in this study, we aimed to determine how well the baseline factors predicted the virological response to Peginterferon α -2b therapy in HBeAg-positive CHB patients.

MATERIALS AND METHODS

Ethics

The study was approved by the Investigation and Ethics Committee for Human Research at the Peking University First Hospital (Beijing, China). All patients provided informed written consent.

Patients and study design

Twenty-one consecutive HBeAg-positive chronic hepatitis B patients were evaluated. Patients were treated with Peginterferon α -2b at a dose of 0.5-1.0 μ g/kg per week for 24 wk. Clinical and laboratory data of the patients were determined before treatment, at week 12, and 24 during treatment. Thereafter they were scheduled for follow-up visits every 12 wk. End of treatment (EOT) response was defined as more than 2 log₁₀ IU/mL reduction in HBV DNA levels at the EOT. Non-response was defined as less than 2 log₁₀ IU/mL reduction in HBV DNA levels at the EOT.

Measurement of serologic markers of HBV

HBsAg, antibody to HBsAg, HBeAg, antibody to HBeAg and anti-HBc were measured using a microparticle enzyme immunoassay (Abbott Laboratories, North Chicago, IL). The HBV genotype was determined using the INNO LiPA HBV genotyping assay. Serum HBV DNA was measured using the TaqMan polymerase chain reaction assay [COBAS TaqMan, Roche Molecular System (lower limit of detection, 20 IU/mL)].

Measurement of biochemical markers

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured using a Hitachi Model 7600 Series Automatic Analyzer (Hitachi). Thyroid-stimulating hormone (TSH), total triiodothyronine (TT3), and total thyroxine (TT4) levels were measured using a Centaur Automated Chemiluminescence System (Bayer).

Statistical analysis

Quantitative variables were expressed as the median with interquartile ranges (IQR), and categorical variables

as frequencies. Comparisons between groups of quantitative and qualitative variables were performed using the Mann-Whitney *U* test and the Fisher's exact test, respectively. The accuracy of serum factors to predict virological response was assessed using the receiver operating characteristic curve. The cutoff value was chosen according to the receiver operating characteristic curve when the sensitivity and specificity were both relatively high for the selective baseline factor. All tests were two-sided and used a significance level of 0.05. Data handling and analysis were performed with SPSS software for windows, version 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Baseline characteristics of patients

The baseline characteristics of the 21 HBeAg-positive CHB patients are shown in Table 1. The median age was 25 years (range, 20-39), and 81% of them were male (17/21). The median value of serum HBV DNA levels was 8.2 log₁₀ IU/mL (IQR, 7.5-8.7 log₁₀ IU/mL). The distribution of HBV genotype was: B, 24%; C, 76%. The median values of serum ALT, AST, TSH, TT3, and TT4 level were 147 IU/L (IQR, 123-201 IU/L), 65 IU/L (IQR, 51-97 IU/L), 2.06 mIU/L (IQR, 1.41-3.10 mIU/L), 2.22 nmol/L (IQR, 2.04-3.03 nmol/L), and 111.4 nmol/L (IQR, 96.8-140.6 nmol/L) respectively. The baseline TT3 and TT4 values of one patient were not assayed at pretreatment. Serological tests were negative for hepatitis C virus, hepatitis D virus, and human immunodeficiency virus in all patients.

Virological response

Of the 21 patients, ten (48%) showed an EOT response, and eleven (52%) were non-responders. Four patients (19%) obtained HBeAg seroconversion at the end of treatment (week 24). However, two of the four HBeAg seroconversion patients lost anti-Hbe, while another six patients achieved HBeAg seroconversion at week 48. The median value of serum HBV DNA levels were 2.7 log₁₀ IU/mL (IQR, 1.9-4.0 log₁₀ IU/mL) and 3.1 log₁₀ IU/mL (IQR, 1.8-6.6 log₁₀ IU/mL) in responders at week 24 and 48 respectively. In non-responders, The median value of serum HBV DNA levels were 7.4 log₁₀ IU/mL (IQR, 6.8-7.9 log₁₀ IU/mL) and 7.6 log₁₀ IU/mL (IQR, 7.1-8.7 log₁₀ IU/mL) at week 24 and 48 respectively. The baseline ALT and TT4 level were significantly higher in responders than in non-responders (both *P* < 0.05, Table 1). However, the baseline TSH level was significantly lower in responders than in non-responders (*P* < 0.05, Table 1). The baseline age was similar between responders and non-responders.

Predictability

To determine how well the baseline ALT, TSH and TT4 levels predicted virological response to Peginterferon α -2b therapy, we performed receiver operating characteristic curves for each parameter. The areas under the curves of

Table 1 Baseline characteristics of patients

Characteristic	All patients (n = 21)	Responders (n = 10)	Non-responders (n = 11)	P value
Median age, range (yr)	25 (20-39)	25 (20-38)	25 (20-39)	0.749
Gender, male (%)	81	70	91	0.311
HBV genotype (%B, C)	24, 76	10, 90	36, 64	0.311
Median HBV DNA levels, range [log (IU/mL)]	8.2 (7.5-8.7)	7.7 (7.2-8.4)	8.4 (8.1-8.8)	0.090
Median ALT level, range (IU/L)	147 (123-201)	184 (146-247)	124 (112-148)	0.011 ^a
Median AST level, range (IU/L)	65 (51-97)	90 (57-132)	64 (45-73)	0.072
Median TSH levels, range (mIU/L)	2.06 (1.41-3.10)	1.82 (1.14-2.08)	2.55 (1.68-4.11)	0.035 ^c
Median TT3 levels, range (nmol/L)	2.22 (2.04-3.03)	2.85 (2.02-3.85)	2.20 (2.02-2.54)	0.305
Median TT4 levels, range (nmol/L)	111.4 (96.8-140.6)	132.7 (109.0-168.5)	107.8 (88.4-117.3)	0.037 ^c

Data are expressed as the median (IQR) and as percentages. HBV: Hepatitis B virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TSH: Thyroid-stimulating hormone. ^a $P < 0.05$ differences of baseline serum ALT level between responders and non-responders; ^c $P < 0.05$ differences of baseline serum TSH level between responders and non-responders; ^c $P < 0.05$ differences of baseline serum TT4 level between responders and non-responders.

Table 2 Predictive value of single and combined baseline factors

Parameters	Responders	Non-responders	Predictive value (%)
ALT \geq 140	9	3	PPV = 75
ALT < 140	1	8	NPV = 89
TSH < 2.4	9	3	PPV = 75
TSH \geq 2.4	1	8	NPV = 89
TT4 \geq 120	6	2	PPV = 75
TT4 < 120	3	9	NPV = 75
ALT \geq 140, TT4 \geq 120	6	1	PPV = 86
ALT < 140, TT4 < 120	1	7	NPV = 88
ALT \geq 140, TSH < 2.4	8	1	PPV = 89
ALT < 140, TSH \geq 2.4	0	6	NPV = 100
TT4 \geq 120, TSH < 2.4	5	1	PPV = 83
TT4 < 120, TSH \geq 2.4	0	7	NPV = 100

Data are expressed as numbers of instances. PPV: Positive predictive values; NPV: Negative predictive values.

ALT, TSH, and TT4 were 0.827 ($P = 0.011$), 0.773 ($P = 0.035$), and 0.778 ($P = 0.037$), respectively. Accordingly, we chose cutoff values of 140 IU/L, 2.4 mIU/L, and 120 nmol/L for ALT, TSH, and TT4, respectively. Correspondingly, their positive predictive values (PPV) and negative predictive values (NPV) were 75% and 89 %, 75% and 89 %, and 75% and 75% (Table 2). We further performed the combination of the baseline ALT and TT4, ALT and TSH, and TT4 and TSH to predict the virological response. We found that their PPV and NPV were 86% and 88%, 89% and 100%, and 83% and 100%, respectively (Table 2).

DISCUSSION

Nowadays, more and more doctors are taking the initiative in individualized treatment for chronic hepatitis B patients. With the purpose of taking individualized treatment, it is important to evaluate the baseline status of each patient at the start of treatment and to then decide which antiviral drug is the best choice. For those patients who are not likely to benefit from Peginterferon α -2b therapy, an early switch to nucleoside/nucleotide analogs is essential.

Recently, a study showed that HBeAg levels had high negative predictive values (NPVs) at week 24 of

sustained virological response to Peginterferon α -2a in HBeAg-positive CHB patients^[18]. While in HBeAg-negative CHB patients, early serum HBsAg drops also had high predictive values of sustained virological response to Peginterferon α -2a at week 12 and 24^[19].

In our study, 21 HBeAg-positive CHB patients were treated with Peginterferon α -2b for 24 wk and followed up for 24 wk. We found that baseline serum ALT, TSH, and TT4 levels, and especially the combination of these factors, had high predictive values of virological response to Peginterferon α -2b therapy.

To identify the baseline predictors of virological response, we performed univariate analysis and receiver operating characteristic curves for baseline serum ALT, TSH, and TT4 levels, and found that the cutoff value of 140 IU/L of baseline serum ALT level had a relatively high predictive value of virological response. The cutoff values for TSH and TT4 were 2.4 (mIU/L) and 120 (nmol/L), respectively. Moreover, we found that combinations of these factors could further improve the PPV and NPV scores.

Some studies have shown that the rates of HBeAg loss and seroconversion were correlated with the baseline level of ALT. In patients with a higher baseline level of ALT, the rates of HBeAg loss and seroconversion during lamivudine therapy were also significantly higher at the end of year three^[7]. A previous study showed that CHB patients with normal ALT levels respond very poorly to interferon α -2a therapy. However, the response was significantly better in patients with elevated ALT levels^[13]. In HBeAg-negative CHB patients treated with Peginterferon α -2a, with or without lamivudine, a high baseline ALT level was identified as a significant predictor of virological response at weeks 24 post-treatment^[8].

Besides high baseline serum ALT level, we also found that higher TT4 level and lower baseline serum TSH level were associated with better outcome of Peginterferon α -2b therapy in HBeAg-positive CHB patients.

Although no study exploring the predictive value of virological response for baseline serum TT4 in chronic hepatitis B patients has been reported, several studies have demonstrated a reciprocal relationship between the endocrine and immune systems. Recently a study showed that triiodothyronine and thyroxine concentrations were

positively associated with markers of inflammation, natural killer-like T cells, activated monocytes derived interleukin-6 (IL-6), higher expression of IL-2 receptor on CD3+ T-lymphocytes, and percentage expression of memory T-lymphocytes, memory T-helper lymphocytes and memory T-cytotoxic lymphocytes within normal physiological ranges^[20]. This is supported by previous findings that thyroid hormone was involved in primary and secondary lymphopoiesis, and blastogenic responses to T and B cell mitogens were also enhanced following thyroxine administration^[21,22]. Other studies showed that thyroxine did not induce resting T lymphocyte proliferation but increased mitogen ConA-induced stimulation after three days of culture, in a dose-dependent manner. Thyroxine substitutive treatment restored the euthyroid status and reversed the impairment of T-cell activation induced by chronic stress in mice^[23,24]. Interestingly, the age-dependent immunological deterioration in old mice could be recovered by thyroxine treatment^[25]. These results indicated that thyroxine could enhance the immune response. Thus, this may be the reason why the responders who had higher baseline TT4 level achieved virological response more easily during Peginterferon α -2b therapy in our study.

Another major finding was the lower baseline TSH level of responders was also associated with higher virological response rate. This could be caused by the negative feedback mechanism due to their higher baseline serum TT4 level.

In conclusion, the identification and application of baseline factors to predict virological response of chronic hepatitis B patients before antiviral therapy is important. Using this method, we can identify patients who will most likely benefit from Peginterferon α -2b therapy before treatment. However, because of the small cohort of patients enrolled in our study, large-scale studies are needed to further confirm our results and to identify simpler and more appropriate factors that have high predictive values of virological response in chronic hepatitis B patients.

COMMENTS

Background

Early prediction of virological response for chronic hepatitis B patients treated with antiviral drugs is important. Some factors such as HBsAg and HBeAg reduction have been found to have high predictive values of sustained virological response in chronic hepatitis B patients treated with Peginterferon α -2a. However, the predictive values of other factors, especially the baseline factors for virological response to Peginterferon α -2b therapy, are not clear.

Research frontiers

Many studies have shown that an elevated serum alanine aminotransferase (ALT) level was associated with virological response and HBeAg seroconversion in CHB patients. Recent studies showed a reciprocal relationship between the endocrine and immune system. In this study, the authors showed that baseline serum ALT, thyroid-stimulating hormone (TSH), and total thyroxine (TT4) levels, and especially combinations of these factors, have high predictive values of virological response to Peginterferon α -2b in HBeAg-positive CHB patients.

Innovations and breakthroughs

The present study demonstrated that baseline serum ALT, TSH, and TT4 levels, and especially combinations of these factors, have high predictive values of virological response to Peginterferon α -2b in HBeAg-positive chronic hepatitis B (CHB) patients before treatment.

Applications

This study might represent a future strategy for identifying chronic hepatitis B patients who will most likely benefit from Peginterferon α -2b therapy before treatment.

Terminology

ALT is an enzyme that is normally present in liver and heart cells. ALT is released into blood when the liver or heart is damaged. TSH is a peptide hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland which regulates the endocrine function of the thyroid gland. Thyroxine (T4) is a form of thyroid hormone which is the major hormone secreted by the follicular cells of the thyroid gland.

Peer review

This study is of interest as it describes the relationship of virological response to Peginterferon α -2b therapy and serum parameters at pretreatment, although this was obtained in a very small cohort of patients.

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BRIEF ARTICLES

Expression of thymidylate synthase and glutathione-s-transferase π in patients with esophageal squamous cell carcinoma

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Abstract

AIM: To investigate the expression of thymidylate synthase (TS) and glutathione-s-transferase π (GST- π) in esophageal squamous cell carcinoma and their association with the clinicopathologic characteristics.

METHODS: Immunohistochemical methods were used to detect the expression of TS and GST- π in surgically resected formalin-fixed, paraffin-embedded esophageal squamous cell carcinoma (ESCC) tissue sections from 102 patients (median age, 58 years) and in 28 normal esophageal mucosa (NEM) samples. The relationship between TS and GST- π expression and clinicopathologic factors was examined.

RESULTS: The expression of TS and GST- π was not statistically significantly associated with age of the patients, tumor size, lymph node metastasis, depth of invasion or tumor stage. TS staining was positive in 17.86% of normal esophageal mucosa and in 42.16% of ESCC samples ($P < 0.05$). The expression level of TS

was not only significantly lower in well-differentiated (21.88%) than in poorly-differentiated carcinomas (51.43%, $P < 0.05$), but was also significantly higher in samples from male patients (46.51%) than from female patients (18.75%, $P < 0.05$). GST- π was positively stained in 78.57% of normal esophageal mucosa and in 53.92% of ESCC samples ($P < 0.05$). The expression level of GST- π was also significantly higher in well-differentiated carcinomas (65.63%) than in poorly-differentiated carcinomas (35.00%, $P < 0.05$).

CONCLUSION: The expression of TS and of GST- π may be used as molecular markers for the characterization of ESCC. Poorly-differentiated cells showed increased expression of TS and reduced expression of GST- π .

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Key words: Esophageal squamous cell carcinoma; Glutathione-s-transferase π ; Immunohistochemistry; Thymidylate synthase; Tumor markers

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INTRODUCTION

Nearly 50% of patients with the diagnosis of esophageal cancer present with overt metastatic disease, and chemotherapy is the mainstay of palliation in this setting. With the increasing use of chemotherapy as an adjunct to surgical management, systemic chemotherapy will ultimately be used to treat the majority of patients with esophageal cancer. The combination of 5-fluorouracil

(5-FU) and cisplatin is widely used in the treatment of esophageal cancer. Alternatively, taxane or irinotecan is applied in combination with either 5-FU or cisplatin^[1]. The response to cisplatin and 5-FU has been low, ranging from 35% to 40%^[2]. Therefore, there is great interest in identifying additional biochemical markers that might be predictive of chemotherapy response and resistance. As Ilson *et al.*^[1] stated: "future strategies in the treatment of esophageal carcinoma will undoubtedly be based on advances in the understanding of the molecular biology of the disease".

Thymidylate synthase (TS) is a key enzyme for DNA and RNA synthesis. The anticancer activity of 5-FU is based on this molecular target. Glutathione-S-transferase π (GST- π) actively binds to platinum and allows it to be removed from the cytosol^[3]. Several studies have suggested that the expression of TS and GST- π could be associated with chemotherapy resistance and prognosis in esophageal cancer and gastric cancer patients^[3-6]. To our knowledge, the significance of TS and GST- π expression in esophageal squamous cell carcinoma (SCC) has not been reported to date in a Chinese population.

The current study was conducted (1) to investigate the expression characteristics of TS and GST- π in esophageal SCC (ESCC) in a Chinese population, and (2) to study the association between TS, GST- π and the clinical characteristics of the patients.

Immunohistochemical analysis has been used to assess the expression of molecular markers in malignant tumors. TS or GST- π has been shown to predict chemotherapy response and resistance in several cancers^[7-12].

MATERIALS AND METHODS

Patients and specimens

In this study, we determined the expression of TS and GST- π in surgically resected formalin-fixed, paraffin-embedded ESCC tissue sections from 102 patients and in 28 normal esophageal mucosa samples using immunohistochemical methods. The patients (86 males and 16 females) with ESCC underwent surgical resection at the Department of Thoracic Surgery, People's Hospital of Taizhou (Taizhou Medical School, Yangzhou & Nantong University), between August 2005 and September 2007. All patients had undergone a subtotal or total esophagectomy and radical lymph node dissection.

Histopathological specimens were fixed in 10% buffered formalin, routinely processed, and embedded in paraffin. All specimens were obtained from patients who had not received chemo- or radiotherapy prior to surgical resection. All hematoxylin and eosin stained sections were reviewed and reexamined by pathologists. The grade of tumor differentiation was determined according to the classification of the World Health Organization^[13], and staged according to the TNM classification^[14].

The patients were 35-76 years of age with a median age of 58.0 years. The location of the tumors was as

follows: upper intra-thoracic esophagus in 11 cases (10.7%); middle intra-thoracic esophagus in 55 cases (53.9%); lower intra-thoracic esophagus in 36 cases (35.2%). Histological degree of differentiation was well-differentiated in 32 cases (31.4%), moderately-differentiated in 50 cases (49.1%) and poorly-differentiated in 20 cases (19.6%). Five cases were Stage I, 47 cases were Stage II, 33 cases were Stage III and 17 cases were Stage IV. NEM samples were taken from 28 patients from an area more than 5 cm from the cancerous tissue, as control non-tumor samples.

Antibodies

The following antibodies were used in this study: mouse monoclonal antibody, anti-human TS and GST- π , the PV-9000 test kit (Zhongshan Goldenbridge Biotechnology Co., LTD, Beijing, China) was also used.

Immunohistochemical staining

The specimens with adjacent non-cancerous esophageal mucosa were cut into 4-5- μ m thick sections and mounted onto slides, deparaffinized with xylene, and rehydrated with graded concentrations of ethanol. Endogenous peroxidase activity was blocked by incubating with 3% hydrogen peroxide (H_2O_2) in deionized water for 10 min. The slides were washed three times with TBS buffer (10 mmol/L Tris-HCl, 100 mmol/L NaCl, pH 7.5) for 2 min. Before application of the TS primary antibody, an antigen retrieval technique was used (10 mmol/L sodium citrate solution, pH 6.0 in a rice cooker, at 640 W for 30 min). After three washes with TBS, an aliquot of 100 μ L of primary antibody was then applied to each section and incubated at 4°C overnight. It is not necessary to perform an antigen retrieval technique for GST- π . After washing 3 times with TBS and following the directions in the kit manual, agent one and then agent two (including the kit) were applied for 20 min at RT. Finally, the sections were washed 3 times with TBS, and the immunoreactions were visualized with 0.0067% diaminobenzidine as the substrate with 0.03% H_2O_2 in 100 mmol/L Tris-HCl buffer for 3 min. The sections were lightly counterstained in Harris hematoxylin solution for microscopic examination. Simultaneously, each section was incubated with TBS instead of the primary antibody as an internal negative control.

The immunostained specimens were analysed by two independent pathologists. Cytoplasm and/or nuclear staining (brown reaction product) was regarded as a positive result. Five fields in each tumor and non-tumor section were evaluated at medium power ($\times 200$) to determine the proportion of tumor cells and the staining intensity of the cytoplasm and/or nuclei in each section. The percentage of positive tumor cells was assigned to one of the following categories: 0 (0%-4%), 1 (5%-24%), 2 (25%-49%), 3 (50%-74%), or 4 (75%-100%). The intensity of immunostaining was determined as 0 (negative), 1+ (weak), and 2+ (strong). Additionally, an immunoreactive score was calculated by multiplying the percentage of positive cells and the staining intensity. The

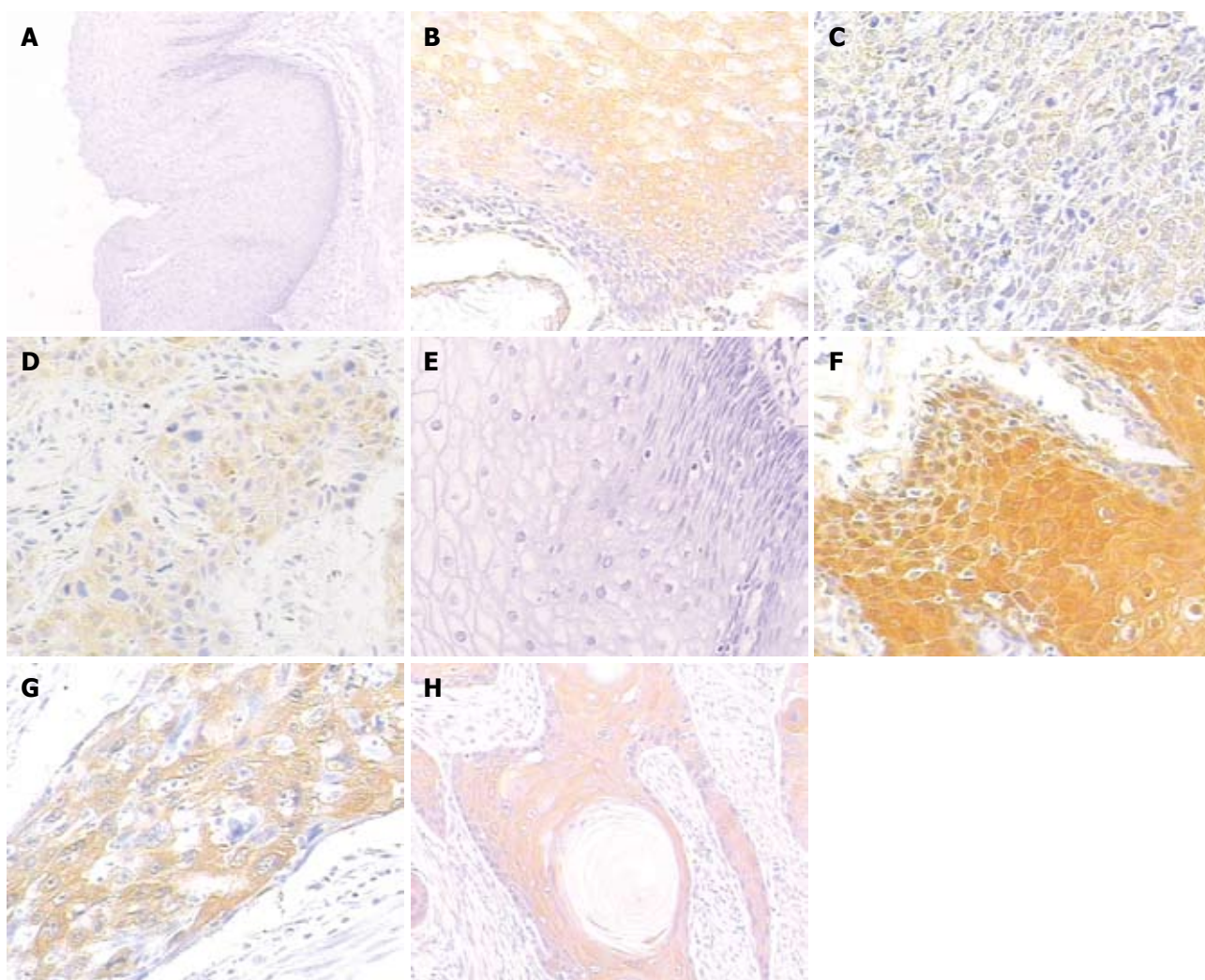


Figure 1 Expression of thymidylate synthase (TS) and glutathione-s-transferase π (GST- π) in normal esophageal mucosa and esophageal squamous cell carcinoma. A and E: Negative control for TS and GST- π in normal esophageal mucosa, positive staining can not be detected (using TBS as primary antibody) ($\times 100$); B and F: Positive staining was located in the cytoplasm in normal mucosa ($\times 200$); C and G: Poorly-differentiated SCC, note the diffuse strong TS immunostaining in esophageal SCC ($\times 200$), for GST- π , positive staining was shown in the cytoplasm ($\times 200$); D and H: Moderately-differentiated SCC, TS positive staining is in the cytoplasm ($\times 200$); Well-differentiated SCC, diffuse strong GST- π immunostaining can be seen in esophageal SCC ($\times 200$).

average score in each tumor and non-tumor section was calculated, and the expression was considered positive when the score was $> 2^{[15]}$. To confirm the reproducibility of the results, all sections were scored twice, the highest score between the 2 observers are thus reported.

Statistical analysis

The correlations between the expression of TS, GST- π and clinicopathological factors were determined using the χ^2 or Fisher test (SPSS 15.0 software package) at the 5% level.

RESULTS

Expression pattern of TS in normal esophageal mucosa and in ESCC

Without specific primary antibody to TS, no staining was observed in esophageal specimens (Figure 1A). The staining of TS was mainly concentrated in the cytoplasm of

cells, occasionally, the nuclei were also stained (Figure 1B). The expression rates of TS in normal esophageal mucosa and in ESCC were 17.86% (5/28) and 42.16% (43/102), respectively. This suggested that the expression level of TS in ESCC was significantly higher than that in normal esophageal mucosa ($\chi^2 = 5.50$, $P = 0.018$).

TS staining and clinicopathological factors

The correlations between the expression of TS and the clinicopathologic features of ESCC are summarized in Table 1. The expression of TS was not significantly associated with age of the patients, tumor size, lymph node metastasis, depth of invasion or tumor stage. The expression of TS was significantly higher in poorly- and moderately-differentiated ESCC (Figure 1C) than in well-differentiated ESCC (Figure 1D) ($\chi^2 = 7.866$, $P < 0.01$). Female patients had tumors with low TS expression (18.75%) more frequently than male patients (46.51%) ($\chi^2 = 4.264$, $P < 0.05$).

Table 1 Relationship between TS and clinicopathological characteristics of ESCC

Factor	n	(+)	(-)	Positive (%)	P value
Sex					
M	86	40	46	46.51	0.039
F	16	3	13	18.75	
Age (yr)					
< 60	58	27	31	46.55	0.302
≥ 60	44	16	28	36.36	
Histological grade					
Well	32	7	25	21.88	0.005
Moderate & poor	70	36	34	51.43	
Lymph node metastasis					
(-)	55	24	31	43.64	0.743
(+)	47	19	28	40.43	
Location ¹					
Upper	11	4	7	36.36	0.822
Middle	55	22	33	40.00	
Lower	36	17	19	47.22	
T Stage					
T ₀₋₁	7	4	3	57.14	0.405
T ₂₋₄	95	39	56	41.04	

¹Location: Upper, upper intra-thoracic esophagus; Middle, middle intra-thoracic esophagus; Lower, lower intra-thoracic esophagus. TS: Thymidylate synthase; ESCC: Esophageal squamous cell carcinoma.

Expression pattern of GST- π in normal esophageal mucosa and in ESCC

Without specific primary antibody to GST- π , no staining was observed in esophageal specimens (Figure 1E). While the staining of GST- π was mainly concentrated in the cytoplasm, occasionally, the nuclei of cells were also stained (Figure 1F). The positive expression rates of GST- π in normal esophageal mucosa and in ESCC were 78.57% (22/28) and 53.92% (55/102), respectively. This showed that the expression level of GST- π in normal esophageal mucosa was significantly higher than that in ESCC ($\chi^2 = 5.528$, $P < 0.05$).

GST- π staining and clinicopathological factors

The correlations between the positive expression rates of GST- π and the clinicopathologic features of ESCC are summarized in Table 2. The positive expression rates of GST- π were not significantly associated with the sex or age of the patients, tumor size, lymph node metastasis, depth of invasion or tumor stage. However, positive expression was significantly higher in well-differentiated ESCC (Figure 1H) than in poorly-differentiated ESCC (Figure 1G) ($\chi^2 = 4.645$, $P < 0.05$).

DISCUSSION

The present study was designed to evaluate the expression characteristics of TS and GST- π in ESCC, and to assess the relationship between TS, GST- π and clinical characteristics. Chemotherapy with cisplatin/5-FU is accepted as a standard treatment in squamous cell and adenocarcinoma of the esophagus. TS is the enzyme targeted by 5-FU, and this may be a potential marker of chemotherapy response, whereas an increase in expression of TS may indicate resistance to 5-FU^[16].

Table 2 Relationship between GST- π and clinicopathological characteristics of ESCC

Factor	n	(+)	(-)	Positive (%)	P value
Sex					
M	86	46	40	53.49	0.839
F	16	9	7	56.25	
Age (yr)					
< 60	58	33	25	56.90	0.489
≥ 60	44	22	22	50.00	
Histological grade					
Well	32	21	11	65.63	0.031
Moderate	50	27	23	54.00	
Poor	20	7	13	35.00	
Lymph node metastasis					
(-)	55	31	24	56.36	0.592
(+)	47	24	23	51.06	
Location ¹					
Upper	11	5	6	45.45	0.660
Middle	55	29	26	52.73	
Lower	36	21	15	58.33	
T Stage					
T ₀₋₁	7	3	4	2.86	0.543
T ₂₋₄	95	52	43	54.74	

¹Location: Upper, upper intra-thoracic esophagus; Middle, middle intra-thoracic esophagus; Lower, lower intra-thoracic esophagus. GST- π : Glutathione-s-transferase π .

Assessment of the probability of chemotherapy resistance using immunohistochemistry methods detecting TS and GST- π expression may allow for the selection of a more effective chemotherapeutic regimen in several cancer patients^[8-12].

TS plays an important role in folate metabolism. Using the methyltetrahydrofolic acid as a substrate, TS catalyses the methylation of deoxyuridylic acid, transferring it into deoxythymidylic acid, which is an important nucleotide in the synthesis and reparation of DNA^[17]. This study showed that the expression of TS in ESCC was higher than that in normal tissue, and that the expression in moderately- and poorly-differentiated ESCC was higher than that in well-differentiated ESCC. This study also revealed that there is a potential to select patients according to whether TS expression is correlated with chemosensitivity to 5-FU. Some studies have indicated that esophageal cancer in patients with low expression of TS is more sensitive to chemotherapy than those with high expression^[3,4].

In the current study, the expression level of TS was observed to be associated with gender. The expression level of TS was significantly higher in males than in females, contrary to the findings reported by Dong^[4]. More studies are needed to investigate the significance of this difference, if any, to the outcome of patients.

In our study, the proportion of male to female patients was 5.4 to 1, this was similar to that of Joshi (6.07 to 1)^[3]. If we can verify that the expression of TS is higher in male than female patients, then this result may help us to understand why esophageal squamous cell carcinoma is related to gender.

GST is a group of isozymes with the function of detoxification and combining proteins. In humans, GST

contains α , μ , π , σ and θ , 5 family constellations and 13 different enzymes. The constellations are encoded by GSTA, GSTM, GSTP, GSTS, GSTT, respectively, and the relationship between GST- π and tumors has attracted much attention^[18]. GST- π not only affects cisplatin by shifting it away from the cells, but it can also release oxygen free radicals, a mechanism which reduces radiation damage^[2]. The expression of GST- π was higher in normal esophageal mucosa than in ESCC, and was higher in well-differentiated tumors compared to poorly-differentiated tumors. These results suggest that the loss of GST- π expression in esophageal epithelium may be an early pre-cancerous sign. The expression of GST- π had no significant association with gender, age, location of the tumor, lymph node metastasis or T stage, consistent with a previous report^[19]. Recently, a study in gastric cancer indicated that the expression of GST- π may be associated with the efficacy of cisplatin^[6].

In conclusion, the present results indicate that expression of TS and GST- π in ESCC in a Chinese population may add to understanding tumor characteristics and to predict response to chemotherapy. It is possible to predict chemotherapy response and resistance by detecting these biological markers. Thus, we are planning to investigate the relationship between expression of TS, GST- π , the curative effect of chemotherapy, and survival rate in patients with ESCC in a future study.

COMMENTS

Background

Esophageal cancer is a serious threat to human health, and nearly 50% of patients with a diagnosis of esophageal cancer present with overt metastatic disease. Chemotherapy has played a crucial role in the treatment of esophageal cancer. The combination of 5-fluorouracil and cisplatin is widely used in these patients. The response to these two drugs has been low. Therefore, it is important to know which drugs may induce a response in these patients.

Research frontiers

Thymidylate synthase (TS) is a key enzyme for DNA and RNA synthesis. The anticancer activity of 5-FU is based on this molecular target. Glutathione-S-transferase π (GST- π) actively binds to platinum and allows it to be removed from the cytosol. In this study, we demonstrated that the expression of TS and GST- π was related to clinicopathological factors of esophageal cancer.

Innovations and breakthroughs

Recently, a number of studies have suggested that the expression of TS and GST- π is associated with chemotherapy resistance and prognosis in esophageal cancer and gastric cancer patients. However, the study of TS and GST- π in esophageal squamous cell carcinoma (ESCC) has not been reported in a Chinese population. Their study showed that the expression features of TS and GST- π were obviously different in normal esophageal mucosa and ESCC. Furthermore, the expression level of TS and GST- π is related to clinicopathological factors of ESCC in a Chinese population.

Applications

By understanding the anti-cancer molecular target of 5-FU and platinum and their relationships with TS and GST- π , this study indicated that it may be possible to predict response and resistance to chemotherapy by detecting TS and GST- π in patients with ESCC.

Terminology

The expression level of TS in ESCC was higher than in normal esophageal mucosa. In contrast, GST- π in normal esophageal mucosa was higher than that in ESCC. These enzymes can be used as diagnostic molecular markers for ESCC. The results demonstrated that tumor tissues were poorly differentiated when the expression of TS was increased and the expression of GST- π was reduced.

Peer review

This is well written article, describing the correlation between expression of TS and GST- π with clinicopathological features in patients with ESCC.

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BRIEF ARTICLES

Application of endoscopic hemoclips for nonvariceal bleeding in the upper gastrointestinal tract

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Author contributions: Guo SB and Gong AX designed and performed the research; Guo SB, Leng J, Ma J, and Ge LM collected and analyzed the data; Guo SB wrote the paper.

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an effective and safe method for acute nonvariceal bleeding in the upper GI tract with satisfactory outcomes.

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Abstract

AIM: To investigate acute nonvariceal bleeding in the upper gastrointestinal (GI) tract and evaluate the effects of endoscopic hemoclippping.

METHODS: Sixty-eight cases of acute nonvariceal bleeding in the upper GI tract were given endoscopic treatment with hemoclip application. Clinical data, endoscopic findings, and the effects of the therapy were evaluated.

RESULTS: The 68 cases (male:female = 42:26, age from 9 to 70 years, average 54.4) presented with hematemesis in 26 cases (38.2%), melena in nine cases (13.3%), and both in 33 cases (48.5%). The causes of the bleeding included gastric ulcer (29 cases), duodenal ulcer (11 cases), Dieulafoy's lesion (11 cases), Mallory-Weiss syndrome (six cases), post-operative (three cases), post-polypectomy bleeding (five cases), and post-sphincterotomy bleeding (three cases); 42 cases had active bleeding. The mean number of hemoclips applied was four. Permanent hemostasis was obtained by hemoclip application in 59 cases; 6 cases required emergent surgery (three cases had peptic ulcers, one had Dieulafoy's lesion, and two were caused by sphincterotomy); three patients died (two had Dieulafoy's lesion and one was caused by sphincterotomy); and one had recurrent bleeding with Dieulafoy's lesion 10 mo later, but in a different location.

CONCLUSION: Endoscopic hemoclip application was

INTRODUCTION

Bleeding in the upper gastrointestinal (GI) tract is very common. The majority of patients benefit from conservative treatments; however, for those who have active bleeding, or have a high risk of recurrence of bleeding, it is still a serious problem for both endoscopists and surgeons^[1]. At present, endoscopic therapy has been recommended as the first choice for the treatment of acute upper GI bleeding^[2]. Effective methods for the control of bleeding in the upper GI tract include local injection (epinephrine or ethanol), thermal coagulation (laser; heater probe), and mechanical methods (hemoclips; elastic bands)^[3,4]. Among these methods, hemoclips can achieve immediate hemostasis^[5] by obstructing the vessel and have the special advantage of lack of additional tissue damage^[6]. During January 2000 to January 2007, 68 patients were given endoscopic hemoclippping treatment for nonvariceal bleeding in the upper GI tract. In this retrospective study, clinical data and endoscopic findings are described, and the outcomes of the therapy are also evaluated.

MATERIALS AND METHODS

During January 2000 to January 2007, a total of 632 patients had emergent endoscopy for bleeding in the upper GI tract in our hospital, and 155 patients were given endoscopic therapy. Among them, 68 cases

with nonvariceal bleeding were given endoscopic hemoclip application. Written informed consent was obtained from all the patients or their relatives before the treatment. The 68 cases had ages ranging from 9 to 70 years (average 54.4, male:female = 42:26). The presenting manifestations were hematemesis in 26 cases (38.2%), melena in nine cases (13.3%), and both in 33 cases (48.5%). Some of the patients had basal disease, including cardiovascular disease (myocardial infarction, congestive heart failure, or significant cardiac arrhythmia) in eight cases (11.8%), liver cirrhosis in two cases (2.94%) and respiratory disease (chronic obstructive pulmonary disease) in six cases (8.82%). Twenty-eight cases were in a state of shock, and 44 cases were given blood transfusions of more than 400 mL; the systolic blood pressures of 12 cases were still less than 90 mmHg when they were given the endoscopic treatment. The electrocardiogram, blood pressure, and oxygen saturation were monitored for those who were in a severe condition.

The type of hemoclip applied was MD 850 (Olympus Corp.) with a rotatable clip application device (HX-5L, Olympus Corp.). After finding the bleeding point, we exposed the clip from the sheath, rotated it to a desired axis, and opened the clip to the maximum width. The clip was then pressed against the lesion and deployed. If needed, the procedure was repeated. The mean number of hemoclips applied was four. All of the patients were given physical care after endoscopic therapy, such as monitoring vital signs, fasting, intravenous fluid, intravenous administration of Histamine-2 receptor antagonists or proton pump inhibitors, hemostatic agents, and some were given blood transfusions.

RESULTS

The causes of the nonvariceal bleeding in the upper GI tract can be listed as followings: gastric ulcer in 29 cases, duodenal ulcer in 11 cases, Dieulafoy's lesion in 11 cases, Mallory-Weiss syndrome in six cases, post-operative in three cases, post-polypectomy bleeding in five cases, and post-sphincterotomy bleeding in three cases.

Hemostasis was defined as endoscopic cessation of bleeding for at least one minute after hemoclip application. Clinically, hemostasis was defined as no decrease in hemoglobin concentration, and correction of shock by blood transfusion and intravenous fluid. Hemostasis was obtained by hemoclip placement in 59 cases. Six patients underwent emergent surgery, in which three cases had peptic ulcers (two located in the posterior wall of the gastric body and one duodenal ulcer located in the posterior wall near the lesser curvature), one case had Dieulafoy's lesion, and two cases were caused by sphincterotomy. Three patients died due to cardiovascular failure and liver cirrhosis (two had Dieulafoy's lesion and one was caused by sphincterotomy). To evaluate the long-term outcomes of the treatment, the patients were followed-up for 30 d. All 59 cases achieved permanent hemostasis, and one of them had recurrent bleeding because of

Dieulafoy's lesion 10 mo later, but in a different location (initially in the proximal one third of the stomach and later in the duodena). The patient underwent endoscopic hemoclip application again, and also achieved a satisfactory result.

DISCUSSION

Despite the development of pharmacology and endoscopic therapy, nonvariceal bleeding in the upper GI tract remains a serious problem, especially for those who have active bleeding. It is associated with an approximately 20% rebleeding rate and its mortality ranges from 10% to 36%^[7-9]. The etiology of acute nonvariceal bleeding in the upper GI tract has changed little in the past 20 years, peptic ulcers (including gastric ulcer and duodenal ulcer) are still the most common causes of acute hemorrhage in the upper GI tract^[10]. In our group, it accounted for 58.8% of bleeding episodes. After endoscopic therapy, acid suppression is essential for those who have bleeding caused by peptic ulcer disease. In a low pH environment, platelets can lose their function, and blood clots might be dissolved by pepsin, resulting in further bleeding. Among the 40 bleeding peptic ulcers, 92.5% achieved permanent hemostasis, only three cases underwent emergent surgery.

Tears at the gastroesophageal junction (Mallory-Weiss syndrome) account for 5% to 15% of all cases of nonvariceal bleeding in the upper GI tract^[11]. These lesions are usually associated with repeated nausea and vomiting. For nonbleeding cases, conservative treatments are usually sufficient. In our group, six cases with active bleeding were given hemoclip application and all had excellent outcomes. Compared with other endoscopic treatments, such as sclerotherapy, epinephrine injection, and heater probe, the hemoclip is a safer choice, without adverse effects^[12].

Dieulafoy's lesion, an important cause of potentially life-threatening GI bleeding, was first described in 1896 and is a submucosal artery protruding from a minute defective mucosa surrounded by normal tissue^[13,14]. Its histopathologic description is "a caliber-persistent artery" in the submucosal tissue^[14]. It was regarded as a rare disease in the past because the caliber-persistent artery often retracts after bleeding^[9], but with the development of technology and familiarity with this disease, it is now estimated to represent about 5% the etiology of acute upper-GI bleeding^[9]. Endoscopic therapy is now considered the first-line method of achieving hemostasis, and hemoclip application has achieved satisfactory results with no reported ulcerative complications^[10]. It can cause occlusion of the bleeding vessel, which results of immediate local hemostasis and prevent delayed recanalization and recurrent bleeding^[5]. In most cases, the hemoclip can replace surgery as the first choice therapy for patients with Dieulafoy's lesion. However, because the lesions are often located in the proximal stomach, usually along the lesser curvature, it might be technically difficulty to apply a hemoclip. In our group, one case underwent emergent surgery and

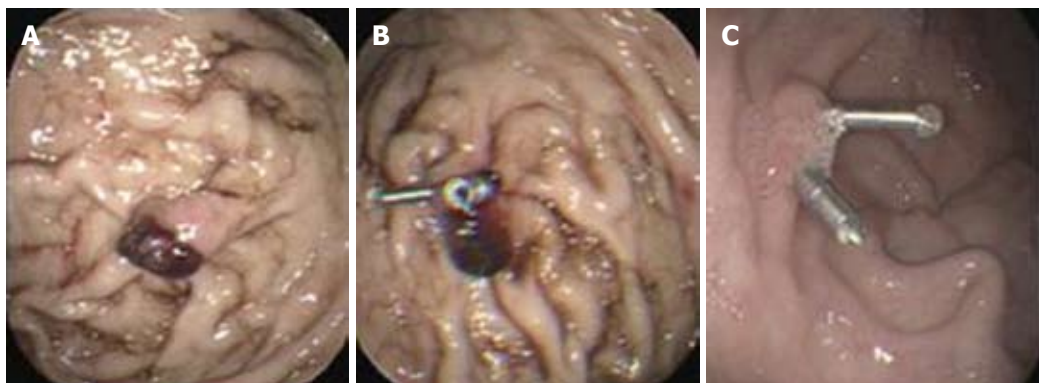


Figure 1 Endoscopic view of a Dieulafoy's lesion before and after endoscopic hemoclippping. A: Endoscopic view of a Dieulafoy's lesion with a protruding vessel in the gastric fundus; B: Endoscopic view showing complete closure of the mucosal defect with a protruding vessel by hemoclips; C: Endoscopic view of the same patient three months later.

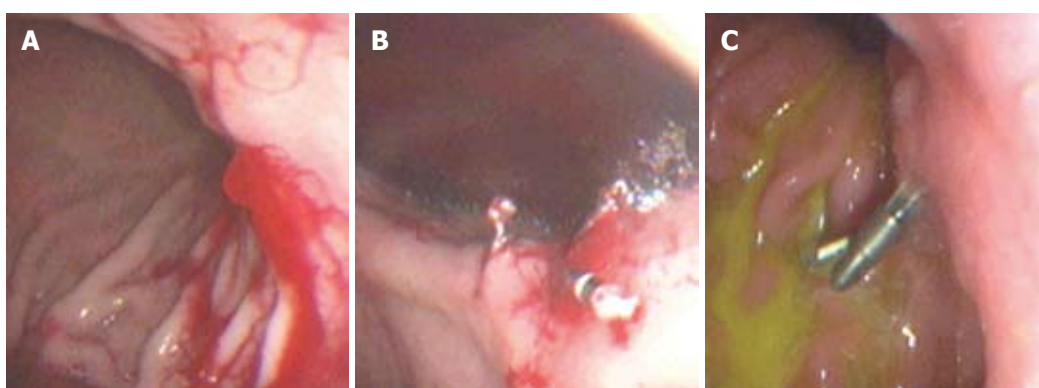


Figure 2 Endoscopic view of a Dieulafoy's lesion before and after endoscopic hemoclippping. A: Endoscopic view of a Dieulafoy's lesion with active bleeding at the posterior wall of the proximal one third of the stomach. B: View after hemoclips application to bleeding site; bleeding has stopped. C: Endoscopic view of the same patient three months later.

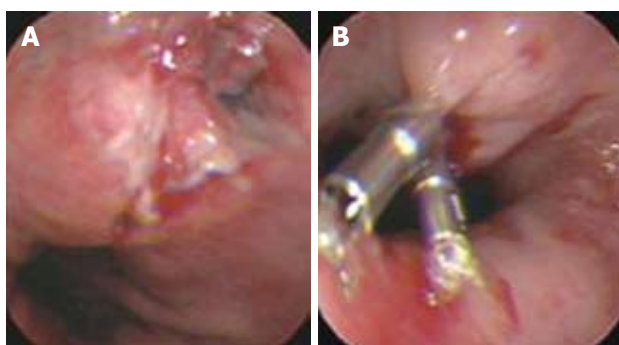


Figure 3 Endoscopic view of a Mallory-Weiss tear at the esophagogastric junction before and after endoscopic hemoclippping. A: Endoscopic view of a Mallory-Weiss tear at the esophagogastric junction with active bleeding; B: View after hemoclips application to bleeding vessel; bleeding has stopped.

two cases were dead due to cardiovascular failure.

Upper GI bleeding caused by endoscopic treatment, such as resection of polyps, mucosal resection, and sphincterotomy, is becoming more and more frequent in clinics due to the increased endoscopic treatments. In our group, five cases were caused by resection of polyps by endoscopy and three cases were caused by sphincterotomy by endoscopy. Among them, two cases underwent emergent surgery and one case died from GI bleeding. These three cases were all caused

by sphincterotomy. In most cases, hemoclippping can achieve satisfactory results; however, it is difficult to accomplish hemostasis through endoscopy in upper GI bleeding caused by sphincterotomy, even for experienced endoscopists, so if necessary, emergent surgery might be a better choice.

Patients who have active bleeding or have a high risk of recurrence of bleeding require effective hemostasis^[15]. At present, endoscopic therapy has been recommended as the first choice for the treatment of acute nonvariceal upper GI bleeding^[2]. Some endoscopic therapies, such as heat probe coagulation, injection of epinephrine, or sclerotic agency, have been proved to be effective for achieving hemostasis, but they might cause tissue injury at the same time, causing necrosis or even perforation^[16,17].

As a mechanical method of hemostasis, the hemoclip application was first introduced in 1975^[18,19]. Due to its simplicity, low cost, easy availability, repetition, minimal damage to the localized field, and reduced risk of adverse effects, hemoclips have been widely used for the treatment of nonvariceal bleeding in the upper GI tract, such as bleeding peptic ulcer^[20,21], Dieulafoy's lesion bleeding^[22,23] (Figures 1 and 2), Mallory-Weiss syndrome^[24,25] (Figure 3), post-polypectomy bleeding^[26], and post-sphincterotomy bleeding^[27]. It is suggested that

hemoclips are particularly helpful when active bleeding is encountered and/or there is a specific point of bleeding^[28]. In these cases, hemoclip application is fast and very effective in controlling bleeding. Theoretically^[6], clips can provide immediate hemostasis comparable with surgery by ligation of the bleeding vessel, with minimal injury to the adjacent tissue. DiMaio *et al*^[10] reported that Hemoclip application has excellent results for initial (97.6%) and permanent hemostasis (95.1%). An experimental study^[29] showed that only this mechanical method was effective for control of bleeding from vessels greater than 2 mm in diameter. Cipolletta *et al*^[30] also thought that clipping was superior to a standard therapy such as injection epinephrine, with significantly less further bleeding, fewer units of blood transfused, a shorter hospital stay, and limited damage to surrounding tissue. However, Gevers *et al*^[31] and Lin *et al*^[32] produced different results.

These inconsistent results in randomized controlled trials suggest that some other factors, such as age, the reasons and the locations of the lesions, shock, presence of multiple comorbidities, could all be associated with the failure of endoscopic hemoclips for bleeding^[33]. For example, some lesions are located in difficult-to-reach sites, which make it hard to apply the clips to the bleeding spot with a perpendicular angle^[34]. The tissue of an ulcer is very brittle, the clip can easily fall off if located on it, so the clip must be located on the normal tissue across the ulcer. If the ulcer is very large and beyond the width of the clip, we can not achieve hemostasis using a hemoclip. However, the experience of endoscopists appears to play a major role in successful clip application. In some cases, it is difficult to deploy hemoclips to the lesion with active bleeding, and only experienced endoscopists can accomplish this task. Thus, hemoclippping is more operator-dependent than other therapies, with some endoscopists achieving excellent results and others having less success. This could explain why the results have great variation in the hemoclip group. The devices themselves might also affect the final results. At present, a number of design improvements have been made to achieve better results. For example, the clip device can be rotated to a desired axis, which makes it easier to adjust the clip position before deployment. The installation of the clips is easier than before, which can save time, because in many cases, more than one clip is needed to achieve hemostasis. Of course, the results will be better if the device could deploy multiple clips at the same time and larger, stronger clips are designed to control the bleeding from large vessels. In summary, endoscopic hemoclip application is an effective and safe method for control nonvariceal bleeding in the upper GI tract with satisfactory outcomes, but the clip and the application device require further improvements.

COMMENTS

Background

Endoscopic hemoclippping has been proved to be effective for achieving hemostasis for nonvariceal gastrointestinal (GI) hemorrhage. However, the

efficacy for different causes of acute nonvariceal upper GI hemorrhage has been rarely reported. The aim of this study was to assess retrospectively the efficacy of endoscopic hemoclippping for different causes of acute nonvariceal upper GI hemorrhage, such as bleeding peptic ulcers, Dieulafoy's lesion bleeding, Mallory-Weiss syndrome, post-polypectomy bleeding, and post-sphincterotomy bleeding. The authors also wanted to determine the factors associated with failure of endoscopic hemoclippping to achieve hemostasis.

Research frontiers

The hemoclip has been widely used for the treatment of nonvariceal bleeding in the upper GI tract. The research hotspots is how to improve the success of hemostasis by endoscopic hemoclip application.

Innovations and breakthroughs

Many studies on the use of a hemoclip for the treatment of nonvariceal bleeding in the upper GI tract have been reported recently, but the results are inconsistent. In this article, The authors analyzed the common causes of acute nonvariceal bleeding in the upper GI tract, evaluated the efficiency of endoscopic hemoclippping for different causes of hemorrhage, and analyzed the factors associated with the failure of endoscopic hemoclippping to achieve hemostasis.

Applications

The study results suggest that endoscopic hemoclip application is an effective and safe method for controlling nonvariceal bleeding in the upper GI tract, with satisfactory outcomes and no adverse effects. Many factors, such as age, the causes and the locations of the lesions, shock, presence of multiple comorbidities, the devices and the experience of endoscopist might all be associated with the failure of endoscopic hemoclip to achieve hemostasis; and the clip and application device need to be further improved.

Peer review

It's a good idea to publish this clinical experience. This study is short (68 cases) but it's interesting to read it especially by endoscopists and it encourages other physicians to publish their experiences.

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Cytomegalovirus enteritis mimicking Crohn's disease in a lupus nephritis patient: A case report

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Abstract

Cytomegalovirus (CMV) infection of the gastrointestinal (GI) tract has been reported in both immunocompetent and, more frequently, in immunocompromised patients. We describe a case of a 19-year-old male who developed CMV infection of the terminal ileum while receiving immunosuppression for lupus nephritis. This was a distinctly unusual site of infection which clinically mimicked Crohn's ileitis. We note that reports of terminal ileal CMV infection have been infrequent. Despite a complicated hospital course, ganciclovir therapy was effective in resolving his symptoms and normalizing his ileal mucosa. This report highlights the importance of accurate histological diagnosis and clinical follow-up of lupus patients with GI symptoms undergoing intense immunosuppression.

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Key words: Cytomegalovirus; Enteritis; Lupus nephritis; Terminal ileitis

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INTRODUCTION

Cytomegalovirus (CMV) infection of the gastrointestinal (GI) tract has been reported in both immunocompetent and, more frequently, in immunocompromised patients^[1,2]. Sometimes the presentation of this infection can mimic other illnesses, making accurate diagnosis difficult. As the therapy for this infection can be very toxic, and discontinuation of therapeutic immunosuppression can cause worsening of the underlying pathology, accurate diagnosis is essential. The case presented here illustrates the difficulty in making an accurate diagnosis, while highlighting the fascinating mimicry which CMV can display.

CASE REPORT

A 19-year-old white male presented with a 3-wk history of increasing malaise, weakness, fever, and arthralgia. He had no significant past medical history or family medical history and was on no medications. Outpatient workup revealed elevated acute serum Lyme and Ehrlichia titers for which he received oral doxycycline. Two weeks later, he presented with fever, arthralgia and a malar rash, along with mild diffuse abdominal pain and diarrhea. Physical examination demonstrated hypertension (blood pressure 150/90 mmHg), lower extremity edema, and mild diffuse abdominal tenderness. A complete blood count and comprehensive chemistry profile revealed pancytopenia (white blood cells 3000/mm³, hematocrit 25% and platelets 100 000/mm³), elevated transaminases (aspartate transaminase 250 IU/L and alanine transaminase 271 IU/L), albumin 2 g/dL, nephrotic range proteinuria (5 g/d) and a urinalysis with red blood cells and red cell casts, consistent with glomerular hematuria. His creatinine was 0.8 mg/dL with an estimated glomerular filtration rate of 130 mL/min. Serologic examination was significant for depressed complement components C3 and C4, a high titer antinuclear antibody 1:320, a positive anti-double stranded DNA antibody, erythrocyte sedimentation rate of 65 with negativity for c-ANCA (antineutrophil cytoplasmic antibody) and p-ANCA. A bone marrow biopsy was non diagnostic. Renal biopsy revealed features of World Health Organization class IV and class V lupus nephritis. He was treated with pulsed methylprednisolone 1 g daily for 3 d along with 1.2 g (0.7 g/m²) cyclophosphamide by intravenous infusion.



Figure 1 Abdominal gastrointestinal series X-ray revealing the typical string sign.

He continued on prednisone 80 mg/d orally. His immediate course was complicated by a transient psychotic reaction which required rapid tapering of the steroids. Subsequently his blood counts, liver function and clinical symptoms improved, and he was discharged home with close outpatient follow-up.

Approximately one month after receiving cyclophosphamide, he developed fever, severe debilitating watery diarrhea with abdominal pain, and presented to the emergency room in acute renal failure (creatinine 2.0 mg/dL). The renal dysfunction resolved completely after the administration of intravenous saline, but the GI symptoms persisted. Urinalysis demonstrated persistent hematuria and proteinuria. Routine stool studies, including cultures, occult blood and *Clostridium difficile* toxin were negative. The diarrhea was intractable with a large stool osmolar gap, hypoalbuminemia of 1.4 g/dL, and depressed cyanocobalamin levels, consistent with malabsorption and protein-losing enteropathy. Abdominal computed tomography (CT) demonstrated edema of the small intestinal wall, particularly in the ileum. A small bowel barium study clearly demonstrated a “string sign” with long narrowed segments of distal jejunum and ileum consistent with Crohn’s jejuno-ileitis (Figure 1). At the time, the differential diagnosis also included lupus vasculitis of the GI tract, ileal tuberculosis, actinomycosis, lymphoma, amoebiasis, or viral infection. Stool acid-fast bacilli and amoebic serologies were negative. An initial colonoscopy showed an inflammatory stricture with friable, erythematous and edematous mucosa at the terminal ileum, 2 cm proximal to the ileocecal junction through which the scope could not be passed.

Multiple biopsies were obtained but were not diagnostic. A CT angiogram, performed to rule out lupus vasculitis demonstrated a normal mesenteric vasculature. A subsequent colonoscopy one week later demonstrated persistently inflamed and denuded mucosa with a persistent terminal ileal stricture. Terminal ileal biopsies clearly revealed intranuclear basophilic inclusions consistent with CMV infection which was confirmed by immunocytochemical staining (Figure 2). No evidence of granulomas or vasculitis was seen on histology. Based on this finding alone, further immunosuppression was

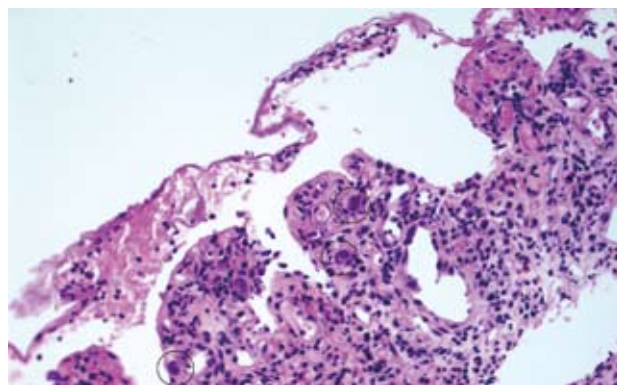


Figure 2 Hematoxylin and eosin staining of ileal tissue revealing cytomegalovirus inclusions, cytomegaly and intranuclear inclusions.

deferred, and the patient was started on intravenous ganciclovir 5 mg/kg twice daily. His subsequent hospital course was complicated by continued diarrhea, protein-losing enteropathy, severe malnutrition requiring total parenteral nutrition, fungal sepsis, and subclavian deep venous thrombosis. Ganciclovir was continued for 4 wk. Ultimately he recovered fully. His abdominal symptoms resolved, and he was able to tolerate an oral diet before discharge.

A repeat colonoscopy 4 mo later found resolution of the stricture, with no inclusion bodies seen on repeat biopsy. After this confirmation, cyclophosphamide and glucocorticoid therapies for lupus nephritis were resumed, with oral ganciclovir prophylaxis. There were no further infectious sequelae. He has had no further complications on follow-up and was switched to mycophenolate mofetil maintenance therapy. Currently he has normal renal function, no proteinuria or hematuria and continued quiescent lupus serology.

DISCUSSION

CMV is a double-stranded DNA virus and a member of the herpesviridae family.

During primary infection, T-cells are vital in controlling the viral replication, but do not eliminate the virus completely. This leads to a latent infection. Acute CMV infection in immunocompetent hosts can manifest with transient nonspecific symptoms, or as a systemic disease with significant organ involvement^[3]. It is estimated that 50%-80% of the adult population is seropositive for the virus^[4]. In immunocompromised hosts, re-activation or re-infection can lead to overt disease e.g., pneumonitis, hepatitis, pancreatitis, colitis, encephalitis, retinitis, or pericarditis leading to substantial morbidity and mortality. Transplant recipients and HIV infected patients with CMV enteritis had a mortality rate as high as 44% in one study^[5].

Immunosuppressive therapies for lupus have well documented infectious risks. Cyclophosphamide is a potent alkylating agent that impairs T-cell immunity at even low to moderate doses. In contrast to cancer chemotherapeutic doses, the doses given for lupus nephritis do not usually result in profound effects.

However, leukopenia and opportunistic infections can sometimes supervene. Data on the incidence of CMV disease with the cyclophosphamide induction protocol for lupus are scarce. The Euro-Lupus Nephritis Trial documented 3 cases out of 45 lupus nephritis patients on intravenous cyclophosphamide^[6].

The protean manifestations of lupus, together with the numerous possible complications of therapy, present unique problems for the treating physician. Lupus vasculitis has been reported to mimic Crohn's ileitis^[7]. The perplexing question in this case was whether the patient's symptoms represented this rare manifestation of GI lupus vasculitis, versus the coexistence of systemic lupus erythematosus (SLE) and Crohn's disease, an infectious complication of immunosuppression, or a fourth, less likely, possibility that the patient's CMV infection was the etiology of his abdominal pain at the time of his initial presentation with SLE, and thus predated his immunosuppression. The transient leukopenia and elevated transaminases on initial presentation, though not unusual for active systemic lupus, could have been the result of primary CMV infection in this spontaneously immunocompromised host.

The acute onset of CMV disease has been described in up to 46% of patients with connective tissue disease undergoing immunosuppressive therapy^[8]. It has also been noted that patients with connective tissue diseases treated with immunosuppression are at high risk for reactivation of latent CMV disease^[9]. Any part of the GI tract may be affected by CMV. Colitis is the most common manifestation of gastrointestinal CMV and can occur either alone or with other systemic involvement. CMV infection of the small bowel, though reported, is distinctly rare, especially in apparently immunocompetent hosts, and only involves 4.3% of CMV infections of the GI tract^[3,10]. CMV enteritis presents most commonly with fever, abdominal pain, diarrhea, or hemorrhage. The virus directly infects the bowel causing mucosal erosions or ulcerations. In severe cases, tissue necrosis and bowel wall perforation can occur^[1]. Histology of the affected mucosa shows a nonspecific inflammatory reaction and giant cells with ovoid nuclei containing basophilic "Cowdry" inclusion bodies. Mesenchymal cells are infected most frequently (97%) followed by endothelial cells (35%), smooth muscle cells (6%) and epithelial cells (3%). Mucosal ulcers are seen in more than half the cases^[10,11]. CMV ileitis in lupus patients is rare, but has been reported to cause ileal perforation^[12]. Though one case found CT evidence of bowel wall thickening^[7], we found no cases described in association with the radiographic "string sign". As mentioned, it is also possible that the initial presentation of abdominal pain and diarrhea in this case, prior to the diagnosis of lupus nephritis, may have actually been manifestations of CMV enteritis. Such infections have been reported to coincide with the immune dysregulation associated with SLE^[13,14].

Interestingly, there have even been reports suggesting that CMV disease itself may induce autoimmune abnormalities^[15]. There are a few case reports of acute CMV infection with elevated CMV antibody titers at

the time of diagnosis of SLE leading to speculation about a possible role in precipitating lupus activity^[16]. There have also been isolated case reports of SLE and Crohn's disease manifesting simultaneously in the same patient^[17], an association which could be attributed to the immunological basis of both diseases. CMV ileitis masquerading as Crohn's disease has also been reported, with documented mucosal and CT findings consistent with that diagnosis^[3]. However, the appearance of a radiographic "string sign" has never been described.

In this case, the persistently low complements and the ileal "string sign", in light of reports of lupus vasculitis of the GI tract mimicking Crohn's disease^[18], led to a therapeutic quandary. Specifically, it was uncertain whether the patient required intensification of his immunosuppression regimen for presumed vasculitis, or whether discontinuation of immunosuppression and concurrent antibiotic therapy was indicated. Given the well-documented infectious risks of therapies in both lupus and Crohn's disease, it was decided, despite decreasing complements and continued intractable diarrhea, to defer further immunosuppression pending definitive diagnosis of the underlying ileal pathology. This decision may have saved the patient from potentially catastrophic enhancement of his immunosuppression.

It is instructive to clinicians to be made aware of a rare complication of this common infection in an increasing number of potentially at-risk patients. A negative routine workup in a lupus nephritis patient with acute abdominal pain and diarrhea should provoke a high index of suspicion for occult CMV infection of the GI tract. Such symptoms may also result from mesenteric lupus vasculitis or from a manifestation of inflammatory bowel disease. Since morbidity increases with delay in initiation of effective therapy in all cases, early diagnosis and definitive treatment is vital for a favorable outcome. This case also raises the question in lupus patients as to whether CMV antigenemia and/or polymerase chain reaction for CMV DNA should be done routinely before initiating immunosuppression. The question whether empiric antiviral prophylaxis should be given to CMV seropositive patients remains unanswered.

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Rejection of Permacol® mesh used in abdominal wall repair: A case report

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Abstract

Permacol® mesh has shown promise when used in abdominal wall repair, especially in the presence of a contaminated surgical field. This biomaterial, derived from porcine dermis collagen, has proposed advantages over synthetic materials due to increased biocompatibility and reduced foreign body reaction within human tissues. However, we present a case report describing a patient who displayed rejection to a Permacol® mesh when used in the repair of abdominal wound dehiscence following an emergency laparotomy. Review of the English language literature using PubMed and Medline, showed only two previously published cases of explanation of Permacol® due to sepsis or wound breakdown. The authors believe this is the first case of severe foreign body reaction leading to rejection of Permacol®. Both animal and human studies show conflicting evidence of biocompatibility. There are several reports of successful use of Permacol® to repair complex incisional herniae or abdominal walls in the presence of significant contamination. It appears from the literature that Permacol® is a promising material, but as we have demonstrated, it has the potential to evoke a foreign body reaction and rejection in certain subjects.

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Key words: Abdominal wound closure; Permacol rejection; Foreign body reaction; Biocompatibility

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Wotton FT, Akoh JA. Rejection of Permacol® mesh used in

INTRODUCTION

Abdominal wall closure in the presence of overt sepsis is associated with a high failure rate. Biological prostheses are often used to reduce the risk of sepsis and ensure a trouble-free recovery. This is a case report of an experience involving the use of Permacol® mesh in abdominal wound dehiscence following an emergency laparotomy for caecal perforation. This patient later exhibited a severe foreign body reaction to the implant requiring its removal. Below, we outline an overview of the case followed by a review of the literature (using PubMed and Medline keywords: Permacol; porcine dermis collagen, abdominal wall repair; hernia repair), and resulting conclusions.

CASE REPORT

This case describes a 72-year-old man who was admitted as an emergency with acute abdominal pain and vomiting. On examination he had a distended rigid abdomen with reduced bowel sounds. He underwent an emergency laparotomy and right hemicolectomy for a perforated caecum, with localised abscess and generalised peritonitis. The wound was closed with “0” loop Polydioxanone (PDS) single layer with staples to the skin. Postoperatively he was admitted to the High Dependency Unit (HDU) but discharged to the ward the next day as he was making good progress. He developed a wound infection, manifested as discharge, on the 7th postoperative day. Some skin staples were removed to allow wound drainage and a vacuum assisted closure (VAC) dressing was applied on the 14th postoperative day. Two days after this, he was taken back to theatre to deal with a full thickness dehiscence of the abdominal wound. Following a thorough lavage, a Permacol® mesh was used to close the abdominal wound, partly as a bridge prosthesis as the fascial edges could not be approximated. This second operation was complicated by superficial wound dehiscence of the wound seven days later. A VAC dressing was reapplied

and the patient was discharged into the community. After sometime, the infection was controlled and there was pink granulation tissue formation in the wound. However, there was no demonstrable wound contraction or attempt at skin cover. He had application of silver nitrate to areas of over-granulation without significant response. Four months later, he underwent an elective exploration of the wound. At the time of surgery there was macroscopic evidence of rejection of the Permacol® mesh with nodular foreign body reaction and no attempt at wound healing at the level of the skin. The Permacol® mesh was excised and replaced with a Surgipro® mesh, with an uneventful postoperative period. Review of the wound 11 wk post-exchange of Permacol® with Surgipro® revealed evidence of wound healing in the superior section, although there remained an area (about 3 cm × 2 cm) of non-healing in the inferior section with the suspicion of a small piece of Permacol® remaining in the wound. An elective excision of abdominal wound sinus was carried out. Histology revealed features of acute and chronic inflammation superficially and granulomatous inflammation in the deep layer consistent with a “stitch granuloma”.

DISCUSSION

Permacol® (Tissue Science Laboratory, Covington, USA) is a biomaterial that has been used across a variety of surgical specialties since the 1980s, for urological, plastic, and gynaecological procedures^[1,2]. It was reported to have encouraging results when used in the form of a mesh for the repair of abdominal wall defects, and parastomal and inguinal hernias^[3]. Permacol® is derived from porcine skin and undergoes the removal of cellular components and genetic material before cross-linking the remaining extracellular matrix. The aim of this process is to produce a material that induces minimal foreign body reaction in tissues and is resistant to biodegradation by native collagenases. This is in contrast to Surgipro® (Cook Surgical, USA) which comprises monofilament fibres of polypropylene polymers to form a strong non-absorbable mesh, inducing a fibrous reaction which is the mainstay for the current repair of abdominal wall herniae and fascial defects^[3].

The proposed advantages of using a biomaterial over non-biomaterials are reduced infection; reduced risk of adhesion and fistula formation; and less rejection and erosion^[2,3]. Also, it is claimed that Permacol® is more suitable for use in contaminated surgical fields, where the risk of infection with a non-absorbable prosthesis is high^[2,4]. Permacol® initially takes on a structural role before becoming vascularized, followed by the incorporation of host cells, leading to remodeled tissue similar to that of the host. However, it has been proposed that there is a higher associated risk of hernia recurrence with biomaterials when compared to synthetic materials^[2,4].

This is an unusual case of extensive tissue reaction leading to rejection of a bioprosthesis (Permacol®). Animal studies (in a rat model) have demonstrated only a

minor chronic inflammatory response, limited evidence of collagen deposition or vascular ingrowth, and no foreign body reaction^[5,6]. However, Petter-Puchner *et al*^[7] who studied tissue responses to porcine cross-linked collagen implants in 10 rats at 17 d and three months showed extensive signs of foreign body inflammatory reaction, with three rats requiring euthanasia due to the migration of implants transcutaneously, and concluded that porcine dermal collagen shows suboptimal biocompatibility. Human studies revealed conflicting evidence of biocompatibility, lack of fibroblast penetration into the graft due to cross-linking of the porcine collagen matrix, absent acute polymorph cellular reaction, and occasional chronic foreign body reaction^[8-10]. Although the prosthesis had to be removed in this case, several studies have reported the successful use of Permacol® in abdominal wall or hernia repair^[2,11]. Hsu *et al*^[11] successfully used Permacol® in the reconstruction of incisional hernias or open abdomens in 28 patients with none requiring the prosthesis to be removed.

The decision to use Permacol® in this case is supported by others^[12,13] who described successful repair of complicated incisional herniae involving contaminated or uncontaminated surgical fields, with no post operative complications, wound infections or recurrence of herniae. Furthermore, Jehle *et al*^[14] described a case of complete wound dehiscence post elective panproctocolectomy where Permacol® was used to reconstruct the abdominal wall defect, and combined it with topical negative pressure dressing to achieve wound healing at five months. In another case where an emergency Hartmann's procedure for a sigmoid stercoral perforation was complicated by wound dehiscence and polyglactin absorbable mesh reconstruction of the abdominal wall resulted in an enterocutaneous fistula, resection and abdominal wall closure was achieved with Permacol® mesh.

Permacol® was used to repair complex abdominal wall defects in nine patients with incisional hernias following the removal of infected mesh, excision of abdominal wall tumour, wound infections and strangulated hernia repair. Despite the contaminated surgical field, five out of the nine patients had no complications due to infection. Two reported cases of explantation of Permacol® involved a patient who developed an abdominal wall abscess seven months after surgery^[15]. A paediatric renal transplant patient required Permacol® insertion as an adjunct to abdominal wall closure following transplantation, but suffered skin dehiscence 23 d postoperatively^[16].

In conclusion, our report provides the third reported case of Permacol® removal but for a very different reason-rejection. There was no sign of infection but the wound would not heal. Histology showed a mixture of acute and chronic inflammation, and foreign body inflammation. We believe this is the first documented case of Permacol® rejection in humans. Review of the literature has revealed the proposed biocompatibility of Permacol®, which is substantiated by the reported successes of its use in the repair of

incisional hernia and abdominal wall repair, including those with a contaminated surgical field. Most common complications include seromas, wound dehiscence or infection with only two reported cases in the literature where Permacol® was required to be removed. It would appear that Permacol® is a promising biomaterial but, as we have reported, it has the potential to induce severe foreign body reaction or rejection in certain subjects.

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CASE REPORT

Resection of the uncinate process of the pancreas due to a ganglioneuroma

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Poves I, Burdío F, Iglesias M, Martínez-Serrano MÁ, Aguilar G, Grande L. Resection of the uncinate process of the pancreas due to a ganglioneuroma. *World J Gastroenterol* 2009; 15(34): 4334-4338 Available from: URL: <http://www.wjgnet.com/1007-9327/15/4334.asp> DOI: <http://dx.doi.org/10.3748/wjg.15.4334>

Abstract

A 33-year-old woman who presented with epigastric discomfort and diarrhea underwent an abdominal ultrasound (US). This investigation and subsequent contrast-enhanced computed tomography, magnetic resonance imaging and endoscopic US with fine needle aspiration (FNA) revealed a 40 mm well-circumscribed mass in the uncinate process of the pancreas. Findings were suggestive of a mucinous or solid-cystic pseudopapillary tumor of the pancreas, although other lesions such as a non-functioning neuroendocrine tumor could not be ruled out. FNA samples were negative for malignant cells, but of limited value due to poor cellularity. It was decided to surgically remove the tumor because malignancy could not be discounted. Multiple intraoperative biopsies were suggestive of mesenchymal tumor and consequently a conservative resection (uncinectomy) was performed. The postoperative course was uneventful. The definitive diagnosis was ganglioneuroma. Immunocytochemistry showed positive staining with vimentin, S-100 protein, neurofilament and neuron-specific enolase. Ganglioneuroma is a rare benign tumor that can also present as a pancreatic tumor. Uncinectomy is feasible, safe and a good surgical technique for the treatment of non-malignant tumors located in the uncinate process of the pancreas.

INTRODUCTION

Ganglioneuroma is a rare benign soft tissue tumor that arises from sympathetic nerve fibers. It is most frequently discovered during childhood or in young adults. The two most common presentations are in the mediastinum and retroperitoneum^[1,2]. Although often difficult and not always possible, a preoperative diagnosis can be obtained by fine-needle aspiration (FNA)^[1-3]. Clinical features, FNA and histological findings have been accurately described^[3]. Adequate treatment consists of radical resection of the whole tumor and definitive diagnosis is obtained after surgical removal by morphologic examination of the complete specimen.

Most solid pancreatic masses are malignant and extended radical surgery is usually the standard of care. Conservative surgery of the pancreas has been advocated mostly for benign or premalignant lesions, most of which are cystic or neuroendocrine tumors (NETs)^[4,5]. Uncinectomy is a relatively novel surgical technique recently described for the treatment of non-malignant lesions^[6].

We present the case of a young woman who had an apparent solid-cystic pancreatic mass in the uncinate process of the head of the pancreas and who was treated by conservative resection (uncinectomy), with a definitive diagnosis of ganglioneuroma. Informed consent for writing this article has been obtained from the patient.



Figure 1 A hypodense and non-infiltrative tumor is occupying the uncinate process of the pancreas (1); It is in close contact with the superior mesenteric vein (2) and superior mesenteric artery (3).



Figure 2 Hypointense-T1 and heterogeneous tumor that does not present any modifications following IV contrast administration with Gadolinium.

CASE REPORT

A 33-year-old woman was diagnosed with a well-circumscribed pancreatic mass measuring 40 mm in diameter in the head of the pancreas. The mass was discovered during the course of an abdominal ultrasound performed while the patient was being assessed for epigastric pain and diarrhea. The physical examination did not reveal any relevant findings. Serum analysis showed a discrete hypochromic microcytic anemia only. On initial abdominal ultrasound (US), the lesion appeared to have a solid consistency. An abdominal contrast-enhanced computed tomography (CT), contrast-enhanced magnetic resonance imaging (MRI) and endoscopic ultrasound (EUS) with FNA biopsy were all carried out to complete the patient's assessment. Tumoral (CA 19.9, CEA) and hormonal markers were normal. Following the guidelines of our centre, the study protocol for pancreatic nodules was followed.

CT (Figure 1)

This examination showed the presence of a hypodense, non-infiltrative tumor measuring 36 mm × 22 mm × 36 mm in both arterial and portal phases, with the appearance of having internal walls, located in the head of the pancreas and occupying the uncinate process in close contact with the superior mesenteric artery and vein, celiac trunk and hepatic artery. These findings were suggestive of a cystic or solid-cystic pseudopapillary tumor of the pancreas.

MRI (Figure 2)

This examination showed a hypointense-T1 lesion that did not present any modifications following IV contrast administration (Gadolinium) that would be suggestive of a mucinous pancreatic tumor.

EUS

This examination showed a 42 mm × 21 mm well-defined, non-infiltrative and heterogeneous mass in the uncinate process of the pancreas, in contact with the portal-mesenteric axis. There were no pathologic lymph nodes and the remaining pancreas looked normal. The

pancreatic duct was not dilated. Repeated direct FNA showed a tumor of extremely hard consistency, which made it difficult to obtain good quality samples. The ones obtained were negative for malignant cells, but of limited cellularity.

Although the diagnosis was not clear, it was decided to surgically remove the tumor because of a suspected solid-cystic pseudopapillary tumor or a non-functioning NET. Informed consent for conducting the protocol study and posterior operation was obtained from the patient. The operation was carried out through a right subcostal incision extended slightly to the left. During the exploration a well-delineated, non-infiltrative mass that occupied the uncinate process of the head of the pancreas was found. The mass was attached to the portal and superior mesenteric veins and to the superior mesenteric artery. Intraoperative multiple tumor biopsies (three tru-cut) showed fibrous tissue with some histiocytic cells suggestive of mesenchymal tumor. Intraoperative excisional biopsy of the intraaortic-cava, portal and hepatic lymph nodes showed no malignancy. Inferior pancreato-duodenal vessels were preserved. A complete resection of the uncinate process with preservation of the duodenum and the head of the pancreas was then performed (Figure 3A). Harmonic scalpel (Ethicon Endo-Surgery, Johnson & Johnson, Cincinnati, OH, USA) was used for cutting the pancreatic parenchyma. Cholecystectomy was carried out and a posterior cholangiography showed no leaking in the bile or in the pancreatic ducts. TachoSil® (Nycomed Pharma S.A.) sealant was applied, wrapping the head of the pancreas to prevent the formation of a pancreatic fistula (Figure 3B). An aspirative drain was established from the bed of the pancreatic resection site. The postoperative course was uneventful, and the patient was discharged on the sixth day.

The definitive diagnosis was a ganglioneuroma (Figures 4-6). The immunocytochemistry study showed positive staining with vimentin, S-100 protein, neurofilament and neuron-specific enolase (Figure 6).

DISCUSSION

Ganglioneuromas are very rare soft tissue tumors composed of gangliocytes and mature stroma^[1,3,7]. They usually

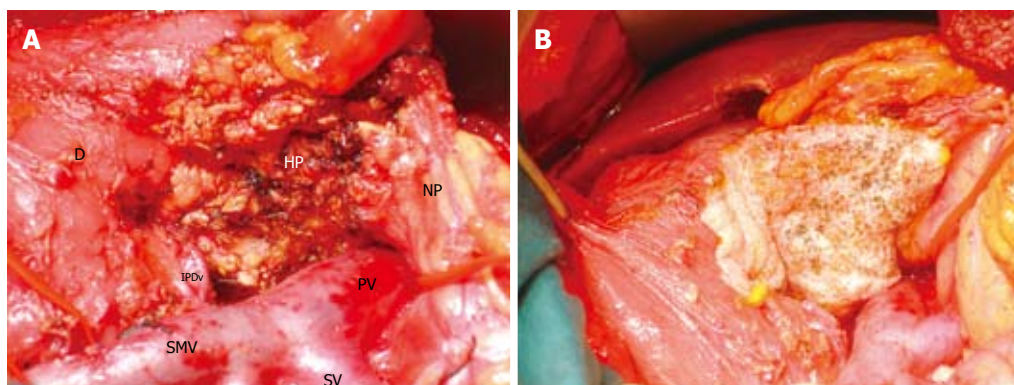


Figure 3 Appearance of surgical field with specimen resected. A: Uncinate process resected. Duodenum (D), neck of the pancreas (NP) and rest of the head of the pancreas (HP) are preserved. Superior mesenteric vein (SMV) is separated from uncinate process that is removed in the picture. Portal (PV) and splenic vein (SV) are also dissected. Inferior pancreaticoduodenal vessels (IPDV) are preserved; B: TachoSil® sealant is applied over the surface of the surgical bed for prevention of pancreatic fistula.

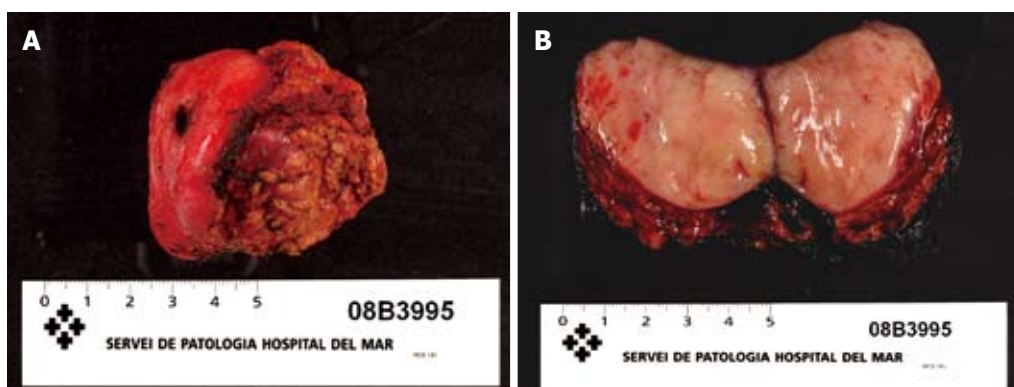


Figure 4 Macroscopic appearance of the surgical specimen. A: Nodular lesion with well-demarcated margins with normal residual pancreatic tissue adjoining; B: The tumor is well-circumscribed and firm. The inner surface is tan without evidence of necrosis or hemorrhage.

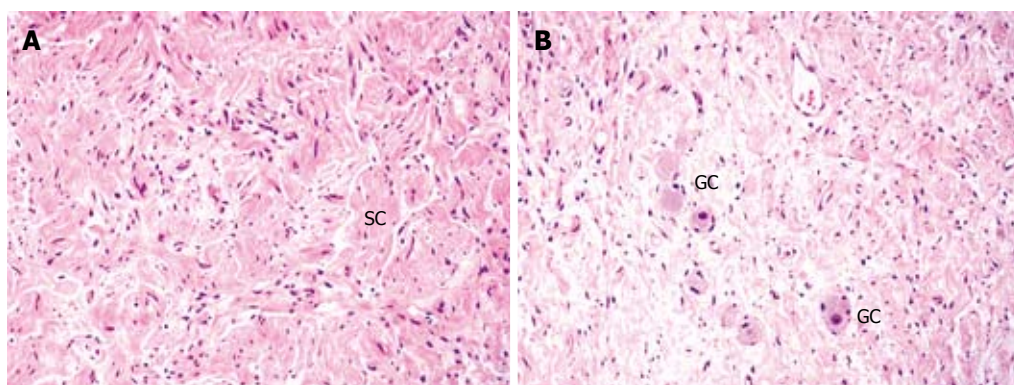


Figure 5 Microscopic images of the tumor (HE, × 10). A: The lesion is composed of a proliferation of spindle-shaped cells in a whorled or fascicular pattern [Schwann cells (SC) and nerve fibers]. The cells have elongated and wavy nuclei with eosinophilic cytoplasm; B: Scattered mature ganglion cells (GC) are another of the histopathologic components of the tumor.

consist of benign masses that produce symptoms caused by their growth and location. Ganglioneuromas are usually asymptomatic and are often discovered casually during the course of explorations when looking for other diseases. Although the most frequent presentations are in the mediastinum and in the retroperitoneum^[1-3], ganglioneuromas can also be found at any other location of the body such as in the neck or pelvis. In our review of the literature we have not been able to find any previous description of

a pancreatic localization. Diagnostic imaging is very difficult because ganglioneuromas present as a non-specific solid mass. MRI seems to be the most accurate diagnostic imaging method although not a definitive one^[7]. Unlike in other tumors, FNA is not always definitive, but its usefulness has been proven in some cases. A definitive diagnosis can only be reached after morphological examination of the removed specimen. Adequate treatment of ganglioneuromas is total surgical removal.

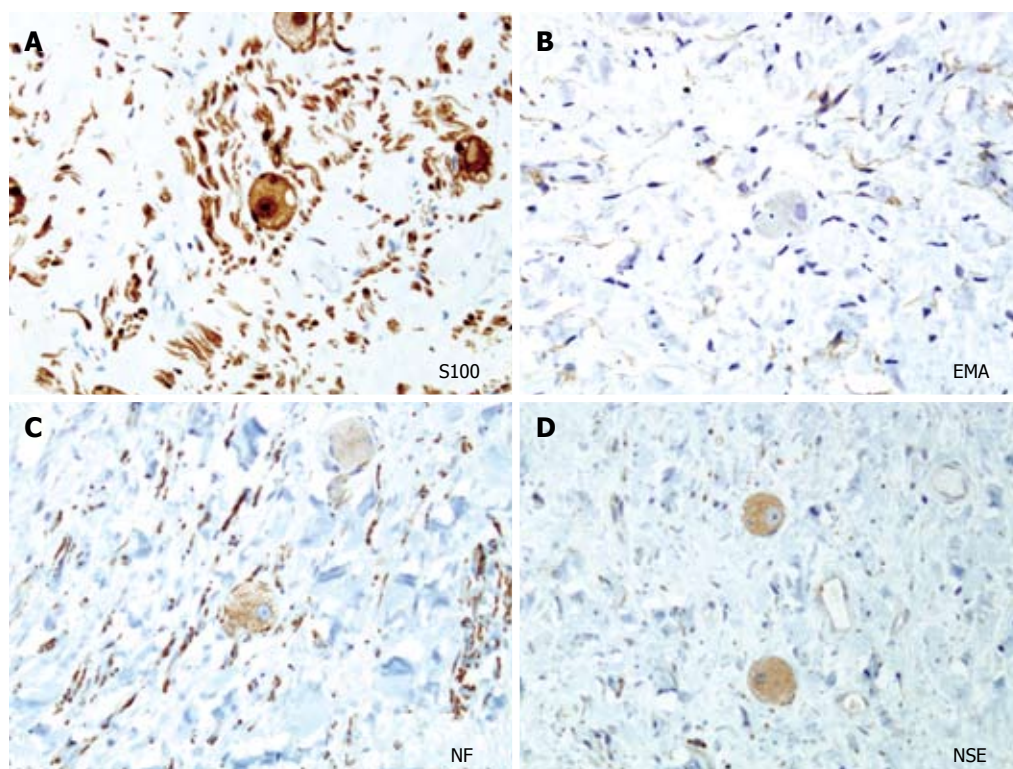


Figure 6 Representative immunocytochemistry samples of the typical ganglioneuroma ($\times 20$). A: S100 protein stain showing mostly Schwann cells and ganglion cells; B, C: Epithelial membrane antigen (EMA) and neurofilament (NF) stain showing the nerve fibers, highlighting the perineural cells and the axons of the nerve fibers; D: Ganglion cells are positive for neuronal-specific enolase (NSE).

Retroperitoneal ganglioneuromas usually present as sarcomatous soft tissue tumors. In the case we present here, the macroscopic appearance of the tumor was that of a pancreatic mass with limits that extended into the pancreas as confirmed by all the imaging studies performed. Although it can be hypothesized that these tumors usually originate from peripancreatic nerve fibers, they invade pancreatic tissue, as it is impossible to find a plane between the tumor and the pancreatic parenchyma. In fact, in this case the morphological macroscopic study showed that the mass was completely attached to the pancreas in a continuous manner (Figure 4A). The only way to obtain a definitive diagnosis and to provide total certainty of no malignancy was total excision of the tumor. A differential diagnosis, taking into account tumor characteristics and preoperative imaging results, was done to differentiate between mucinous cystadenoma, non-functioning NET and solid-cystic papillary tumor, all of which could have the potential to become malignant. NETs can also present as cystic lesions although this occurrence is rare^[8]. Somatostatin receptor scintigraphy with octreoscan has been recommended as the best imaging technique in NETs, being able to visualize more than 70% of these type of tumors^[9]. In our case, we decided not to perform this exploration because the decision for radical resection was proposed independently of this result. We preferred to carry out multiple tru-cut biopsies intraoperatively, prior to deciding what type of pancreatic resection to perform. Biopsy results were suggestive of a benign soft tissue tumor, although some malignant entities such as pseudoinflam-

matory tumor or primary malignant fibrous histiocytoma could not be completely ruled out before the tumor was removed in its entirety. Local resection without standard lymphadenectomy was performed.

Further knowledge gained over the last few years regarding the anatomy of the pancreas has helped to develop the concept of conservative surgery. Conservative surgery has gained popularity among surgeons and not only for lesions located in the left pancreas^[10]. Preserving the duodeno-pancreatic region is an issue for tumors that are also nested in the head of the pancreas. Resection of the uncinate process is an alternative to pancreato-duodenectomy (PD) for the treatment of non-malignant or pre-malignant lesions located in this area. Sharma *et al*^[6] described the technique used to perform an uncinectomy for the treatment of intraductal papillary mucinous neoplasms. Some other procedures such as resection of the head of the pancreas with preservation of the duodenum, median or central pancreatectomy, or enucleation, can be carried out depending on the type and precise location of the lesion. When these procedures have to be carried out, it is mandatory to obtain adequate preoperative or intraoperative confirmation of the benign nature of the samples. The postoperative course and long term sequelae are much better for conservative surgery than for PD.

As with enucleation, the main concern when performing an uncinectomy is the risk of leakage into the main pancreatic duct. To minimize the risk of this severe complication, direct intraoperative US exploration is highly recommended during resection. Nevertheless, in

many cases the pancreatic duct is narrow and is hard to identify. Intraoperative cholangiography and retrograde contrast back filling of the pancreatic duct can be an option for assessing the integrity of both the biliary and pancreatic ducts.

In conclusion, ganglioneuroma is a rare benign tumor that can also present as a pancreatic tumor. When a favorable location permits it, conservative resection of the pancreas is the treatment of choice. Uncinectomy is feasible, safe and a good surgical technique for the treatment of non-malignant tumors located in the uncinate process of the pancreas.

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Novel *ABCB11* mutations in a Thai infant with progressive familial intrahepatic cholestasis

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Abstract

Progressive familial intrahepatic cholestasis (PFIC) type 2 is caused by mutations in *ABCB11*, which encodes bile salt export pump (BSEP). We report a Thai female infant who presented with progressive cholestatic jaundice since 1 mo of age, with normal serum γ -glutamyltransferase. Immunohistochemical staining of the liver did not demonstrate BSEP along the canaliculi, while multidrug resistance protein 3 was expressed adequately. Novel mutations in *ABCB11*, a four-nucleotide deletion in exon 3, c.90_93delGAAA, and a single-nucleotide insertion in exon 5, c.249_250insT, were identified, with confirmation in her parents. These mutations were predicted to lead to synthesis of truncated forms of BSEP. Immunostaining and mutation analysis thus established the diagnosis of PFIC type 2.

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Key words: *ABCB11*; Bile salt export pump; Progressive familial intrahepatic cholestasis

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INTRODUCTION

Progressive familial intrahepatic cholestasis (PFIC) refers to a heterogeneous group of autosomal recessive liver disorders of childhood in which cholestasis of hepatocellular origin often presents in the neonatal period or first year of life, and leads to death from liver failure at ages ranging from infancy to adolescence^[1,2]. Three types of PFIC have been found; each is related to mutations in hepatocellular transport system genes involved in bile formation^[1-3]. PFIC type 1 (Byler disease) is caused by mutations in *ATP8B1* (chromosome 18q21-22), which encodes familial intrahepatic cholestasis 1 (FIC1). PFIC type 2 is caused by mutations in *ABCB11* (chromosome 2q24), which encodes bile salt export pump (BSEP). Mutations in *ABCB4* (chromosome 7q21), which encodes multidrug resistance protein 3 (MDR3), which is responsible for biliary secretion of phospholipids, cause PFIC type 3. In PFIC types 1 and 2, low or normal serum γ -glutamyltransferase (GGT) levels are found, whereas GGT levels are high in PFIC type 3^[1,2].

The diagnosis of PFIC can be difficult, especially where genetic testing is not readily available, as in Thailand. We report here a Thai infant diagnosed with PFIC type 2.

CASE REPORT

A 2-mo-old female infant presented with a 1-mo history of icteric sclerae associated with pale yellowish stools. She was a normal full-term infant (birth weight 2700 g). Hypothyroidism had been diagnosed at age 1 mo [free

Table 1 Evolution of clinical biochemistry test results with body weight and height

Age (mo)	ALP (U/L) ¹	AST (U/L) (15-37)	ALT (U/L) (30-65)	GGT (U/L) ²	Alb (g/L) (34-50)	TB (mg/dL) (0-1.5)	DB (mg/dL) (0-0.5)	Chol (mg/dL) (114-203)	BW (kg)	Ht (cm)
2	417	354	264	47	37.4	5.7	3.9	155	4.95	56.0
6	298	375	344	37	49.8	7.7	6.4	229	7.20	64.0
12	267	472	354	66	33.8	5.3	4.5	191	8.20	68.0
18	332	311	237	59	38.1	6.2	5.2	151	10.4	71.4
24	269	239	171	59	44.1	6.1	4.5	121	11.4	78.0
30	373	340	240	60	41.2	7.9	6.8	102	11.5	80.0

ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; Alb: Albumin; TB: Total bilirubin; DB: Direct bilirubin; Chol: Cholesterol; BW: Body weight; Ht: Height. Units and expected ranges for test-result values are given in parentheses. ¹Normal values for female children < 1 year, 185-555; 1-2 years, 185-520; 3 years, 185-425 U/L^[17]; ²Normal values for children 1-2 mo, 12-123; 2-4 mo, 8-90; 4 mo-10 years, 5-32 U/L^[18].

T4 0.94 ng/dL, thyroid stimulating hormone (TSH) 20.5 mIU/L], and thyroxine therapy begun. Growth and development were otherwise normal. No family history of liver disease was elicited. She had mildly icteric sclerae and hepatomegaly without splenomegaly or ascites. All other physical-examination findings were normal.

Conjugated-bilirubin and transaminase values were elevated but albumin, cholesterol, and GGT values were within expected ranges (Table 1). Prolonged coagulogram values were corrected by intravenous vitamin K administration. No IgM-class antibodies against cytomegalovirus were found, and VDRL testing was non-reactive. Plasma amino acid analysis found only mildly elevated methionine levels, interpreted as a nonspecific consequence of liver disease. Urine-reducing substances were absent. Other laboratory investigation results were normal, including free T4 and TSH, complete blood count, electrolytes, glucose, urea nitrogen, creatinine, ammonia and alpha-fetoprotein levels. Ultrasonography revealed a normal liver and bile duct. DISIDA scanning showed good hepatic function with demonstrable intraduodenal tracer at 3 h. Microscopy of a liver-biopsy specimen found changes interpreted as neonatal hepatitis. Ursodeoxycholic acid (UDCA) and fat-soluble vitamins were given.

Cholestasis persisted (Table 1), with development of severe pruritus and hepatosplenomegaly. On repeat liver biopsy aged 10 mo, hepatocellular swelling with multinucleation was found, as was canalicular and hepatocellular cholestasis (Figure 1A). Mild portal lymphocytic infiltration and fibrosis were also observed (Figure 1B). BSEP was not detected along the canaliculi on immunostaining (Figure 1C and D), while the homologous transport protein MDR3 was expressed adequately, which demonstrated that tissue fixation was adequate and permitted the inference that lack of BSEP expression was BSEP-specific. As these results were compatible with PFIC type 2, *ABCB11*, which encodes BSEP, was sequenced after parental consent was obtained.

Treatment with UDCA and fat-soluble vitamins was continued. At the time of writing, the patient is 30 mo old, with persistent jaundice and growth delay [Table 1; body weight 11.5 kg (P25) and height 80 cm (< P3)]. Serum alpha-fetoprotein concentrations are normal and sonographic monitoring has found no focal changes. She awaits liver transplantation.

Mutation analysis

ABCB11 was analyzed by direct sequencing of PCR products obtained from genomic DNA extracted from peripheral blood leukocytes. All exons, together with the adjacent parts of the intronic sequences, were amplified by PCR with intronic oligonucleotide primers as reported previously^[4]. The amplicons were gel-purified, extracted with QIAquick spin columns (Qiagen, Hilden, Germany), and used as templates for the sequencing reaction with Big Dye Terminator kit v3.1 (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The products were analyzed on a Genetic Analyzer 3130 (Applied Biosystems).

Two suspected sequence disturbances were predicted from the dual sequence readings at 20 bp from the potential mutation start site in both directions. The presence of both mutations was verified by molecular cloning into a plasmid vector pCR4.1-TOPO (Invitrogen, Carlsbad, CA), and sequencing the cloned wild-type and the mutated allele separately. In addition, the presence of suspected mutations was also examined by direct sequencing of appropriate amplicons obtained from the proband's parents. Two different heterozygous *ABCB11* mutations were found in the patient: a four-nucleotide deletion in exon 3 (protein coding exon 2), c.90_93delGAAA, inherited from the patient's mother, and a single-nucleotide insertion in exon 5 (protein coding exon 4), c.249_250insT, inherited from the patient's father (Figure 2). Both mutations are predicted to cause reading frame shift and premature termination of DNA translation, respectively p.Lys30AsnfsX31 and p.Gly84TrpfsX9, and therefore are considered to be pathogenic.

DISCUSSION

To the best of our knowledge, this is the first case report of PFIC type 2 in Thailand. The patient had a typical clinical presentation of PFIC type 2 with cholestasis of onset in early infancy, with development of hepatomegaly and severe pruritus^[1,2]. In PFIC type 2 serum bile acid concentrations are high, biliary bile-salt concentrations are very low, and GGT and cholesterol values are normal^[2,5]. While serum bile-acid and biliary bile-salt concentrations could not be determined, as such studies are unavailable in Thailand, her severe pruritus

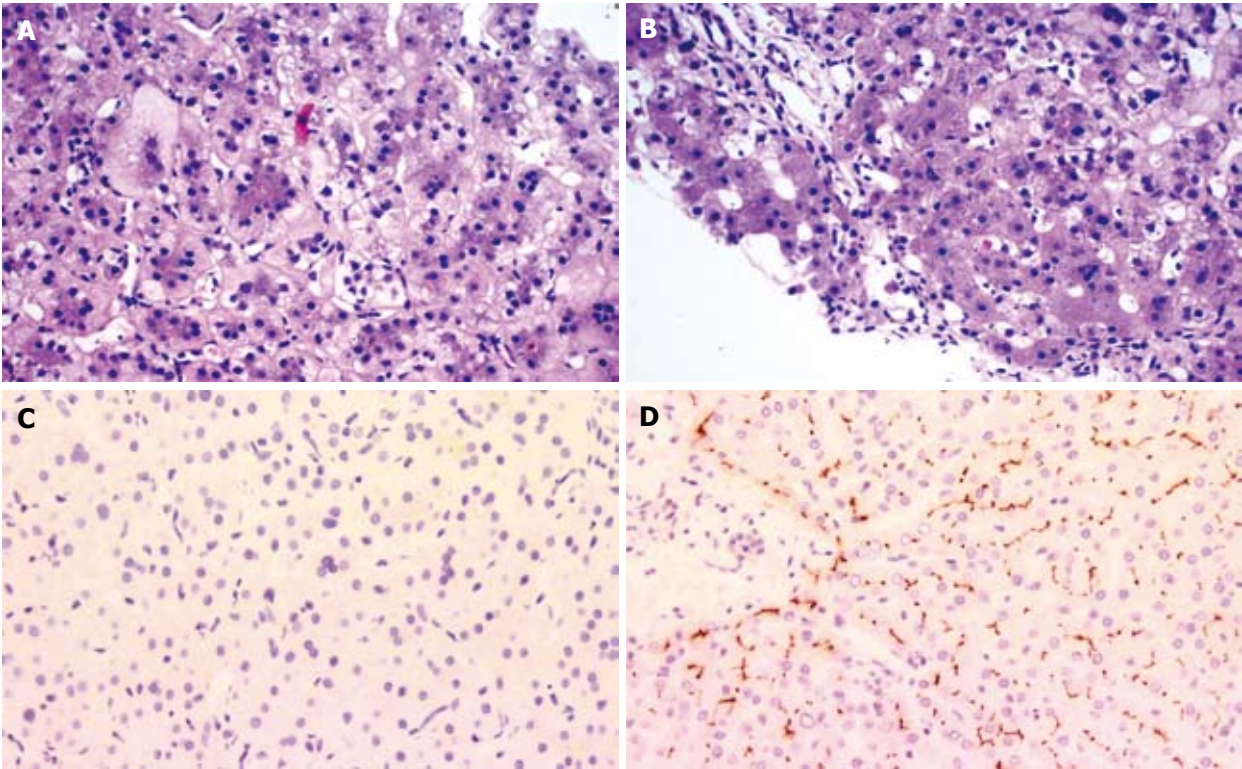


Figure 1 Liver, second biopsy (10 mo). Hepatocellular swelling and multinucleation, with intralobular cholestasis (A), accompanying mild portal-tract lymphocytic infiltration and fibrosis (B). On immunostaining, bile salt export pump (BSEP) expression was not detected (C); canaliculi in control liver stained normally for BSEP (D). [Hematoxylin/eosin, A and B; rabbit anti-BSEP polyclonal antibody (generous gift of Dr B Stieger)/hematoxylin, C and D; original magnification, all images, × 200].

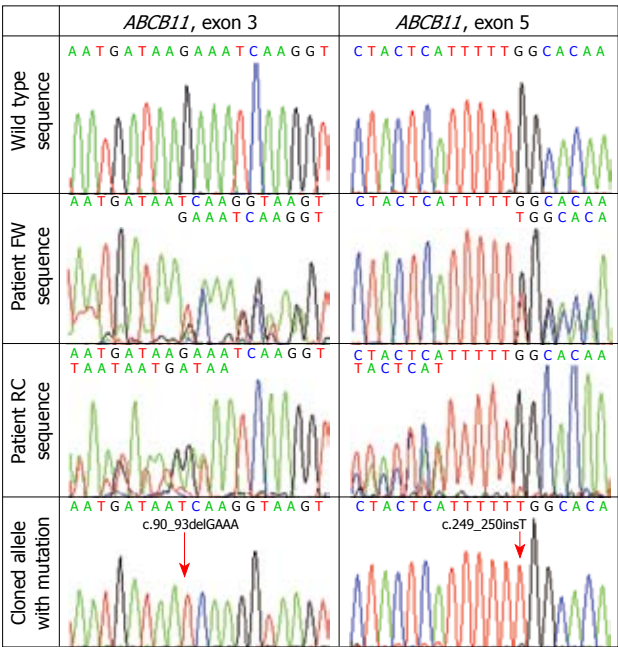


Figure 2 Mutations c.90_93delGAAA and c.249_250insT detected in exon 3 and 5 of *ABCB11*. FW: Forward reading (coding strand sequence); RC: Reversed and complemented reading (reversed and complemented sequence of the complementary strand).

indicated hypercholanemia. This feature and our patient’s GGT and cholesterol values were compatible with PFIC type 2. Although hypothyroidism in this patient might have caused cholestasis by delayed emptying of the biliary tract, and by changes in bile composition and

excretion rate^[6], normalization of free T4 and TSH levels after thyroxine therapy argued against this possibility. Since hypoglycemia was not present and initial growth was unremarkable, hypopituitarism was not pursued.

While PFIC type 1 has initial clinical and laboratory findings similar to those of PFIC type 2, histological features may differ. In PFIC type 1, bland canalicular cholestasis with variable fibrosis is found. In PFIC type 2, variable features include canalicular cholestasis and a neonatal hepatitis pattern, with hepatocellular swelling and giant cell transformation^[1,2]. Lobular and portal inflammation and fibrosis are more pronounced in PFIC type 2 than type 1^[2]. Immunostaining is a useful diagnostic tool for PFIC type 2 since most patients with *ABCB11* mutations and hepatobiliary disease of onset in infancy have no canalicular BSEP expression^[7]. At initial liver biopsy (2 mo) changes of neonatal hepatitis were found. At follow-up biopsy, these changes persisted, and BSEP was not detected along the canaliculi. The clinicopathological picture, felt to be compatible with PFIC type 2, thus prompted *ABCB11* analysis.

BSEP is an ATP-dependent transporter located on the canalicular membrane of hepatocytes^[8,9]. It is a major exporter of primary bile salts from hepatocyte cytoplasm into the bile-canalculus lumen, and works against an extreme concentration gradient^[2,9]. Mutations in *ABCB11* that lead to failure of BSEP expression, or to expression of functionally defective BSEP, in turn lead to accumulation of bile salts inside hepatocytes, with ongoing severe hepatocellular damage and diminished bile flow.

To date, more than 100 mutations in *ABCB11* have been identified^[7,10-14]; however, genotype-phenotype correlations are not wholly clear. Severe phenotypes are often associated with mutations that lead to premature protein truncation or failure of protein production. Insertion, deletion, nonsense, and splicing mutations result in damaging effects, and patients who have clinical PFIC associated with such mutations exhibit little or no BSEP expression in hepatocyte canaliculi^[2,7]. Missense mutations are also common. These can affect protein processing and trafficking or disrupt functional domains and protein structure^[2,7,15]. Detectable BSEP expression does not exclude functional BSEP deficiency^[2,7].

E297G and D482G are the two most common mutations in persons of European descent, and account for approximately 58% of BSEP mutations in European studies^[7]. In Asian patients, few reports of mutations in PFIC type 2 exist^[12-14]. Goto *et al*^[14] have reported four mutations in *ABCB11*, predicted to yield V330X, R487H, R575X and E636G, in two Japanese PFIC patients. Chen *et al*^[13] have reported seven BSEP mutations (M183V, V284L, R303K, R487H, W493X, G1004D and 1145delC) in four PFIC patients of Chinese descent; none of these mutations has been described in Caucasian patients. To the best of our knowledge, the mutations identified in our patient are novel. These mutations are predicted to lead to synthesis of truncated forms of BSEP.

Patients with PFIC type 2 are at risk for hepatobiliary malignancy^[7,16]. Hepatocellular carcinoma or cholangiocarcinoma developed in 19 of 128 patients (15%)^[7] and those who had two protein-truncating mutations were at particular risk^[7]. Close surveillance of BSEP-deficient patients who retain their native liver is therefore essential.

In conclusion, we report a Thai infant with clinical features of normal-GGT PFIC. Her liver did not express immunohistochemically demonstrable BSEP. Novel mutations in *ABCB11* were identified in the infant, with confirmation in her parents. These mutations were predicted to lead to synthesis of truncated forms of BSEP. Immunostaining and mutation analysis thus established the diagnosis of PFIC type 2.

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Primary squamous cell carcinoma of pancreas diagnosed by EUS-FNA: A case report

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Abstract

Squamous cell carcinoma of the pancreas has been sparsely described since the 1940s, and generally has a poor prognosis. Herein, we present a case of primary squamous cell carcinoma of the pancreas with liver metastasis, both confirmed by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA). To the best of our knowledge, this is the first case report in literature utilizing EUS-FNA for a cell-type specific diagnosis of primary pancreatic squamous cell carcinoma with a liver metastasis.

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INTRODUCTION

Primary squamous cell carcinoma is rare among all pancreatic neoplasms, constituting less than 1% of cases. Herein, we present a case of primary squamous cell carcinoma of the pancreas with liver metastasis, both confirmed by endoscopic ultrasound-guided fine needle aspiration (EUS-FNA).

CASE REPORT

A 76-year-old American African female was referred with a few weeks history of dull epigastric pain with radiation to the back, and weight loss of 10 pounds. Initial physical examination revealed only minimal tenderness over the epigastrium, and blood tests were all normal including serum lipase (29 IU/L, reference: 10-50 IU/L) and CA 19-9 (2 IU/mL, reference: 0-37 IU/mL). Upper endoscopy showed mild gastritis. Subsequent computed tomography (CT) of the thorax and abdomen revealed a 5 cm partial cystic mass in the tail of the pancreas and a 1 cm subtle hypodense non-enhanced lesion in the left lobe of the liver, with normal chest and mediastinum. CT-guided biopsy of the liver mass was performed, however, preliminary histology only revealed atypical cells. Endoscopic ultrasound (EUS) was performed for clarification of the pathology. A complex cystic mass with a large solid component was seen in the tail of the pancreas measuring up to 68 mm. Another 14 mm × 18 mm ill-defined, almost isoechoic lesion was also noted in the left lobe of the liver (Figure 1). Transgastric EUS-FNA, using a 25-gauge needle, of both the liver and tail of pancreas lesions was carried out, which revealed squamous cell carcinoma in both sites, although on-site interpretation was difficult due to the unusual cell type (Figure 2).

DISCUSSION

Squamous cell carcinoma of the pancreas has been

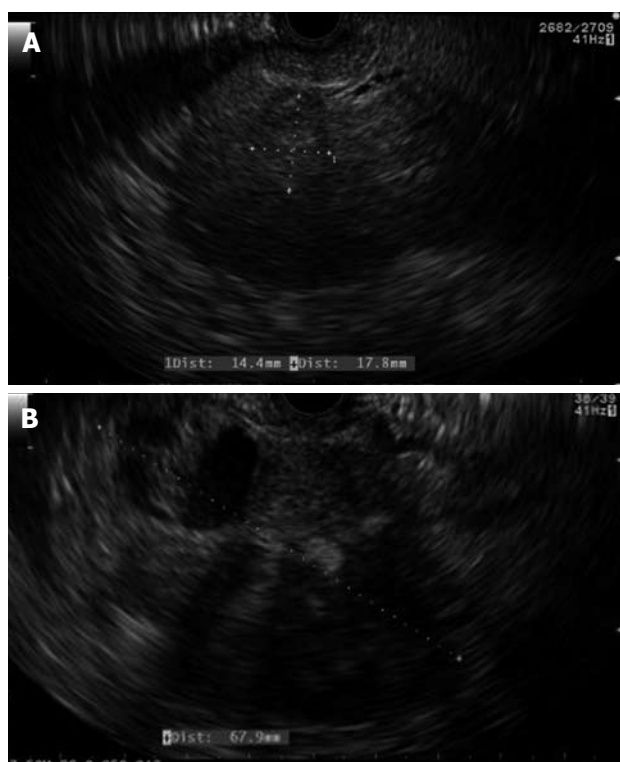


Figure 1 Endoscopic ultrasound image of the heterogenous, nearly isoechoic lesion in the left lobe of the liver (A) and the complex cystic mass in the tail of pancreas (B).

rarely described since the 1940s, and generally has a poor prognosis^[1]. It is hypothesized that squamous metaplasia of pancreatic ductal epithelium after chronic inflammation (e.g. chronic pancreatitis), could be one of the possible oncogenic mechanisms^[2]. A subset of pancreatic adenocarcinoma, adenosquamous carcinoma is occasionally found in surgical specimens after Whipple's operation. In one series, dual differentiation towards both adenocarcinoma and squamous cell carcinoma was seen in 25 pancreatic cancer patients^[3]. However, pure squamous cell carcinoma of the pancreas is extremely rare, and is often mistaken as either benign squamous cells from upper gastrointestinal contamination when it is well-differentiated, or metastasis from other sites (e.g. lung and upper aerodigestive tract) when it is obviously malignant. A MEDLINE search only identified 14 case reports in the English literature so far^[4-17], and all diagnoses were based on surgical specimens. Hypervascularity on contrast CT has been reported as a characteristic finding, but was not seen in this case^[15].

Since the introduction of EUS-FNA for investigating pancreatic cancer in the 1990s^[18], it has now become the standard procedure for pancreatic lesions. Cytopathological confirmation can be obtained with EUS-FNA, so as to avoid unnecessary pancreatic resection^[19]. From our knowledge, this is the first case report in the literature utilizing EUS-FNA for a cell-type specific diagnosis of primary pancreatic squamous cell carcinoma with a liver metastasis. The distinction of well-differentiated squamous cell carcinoma from benign disease such as lymphoepithelial cysts (LEC) of the

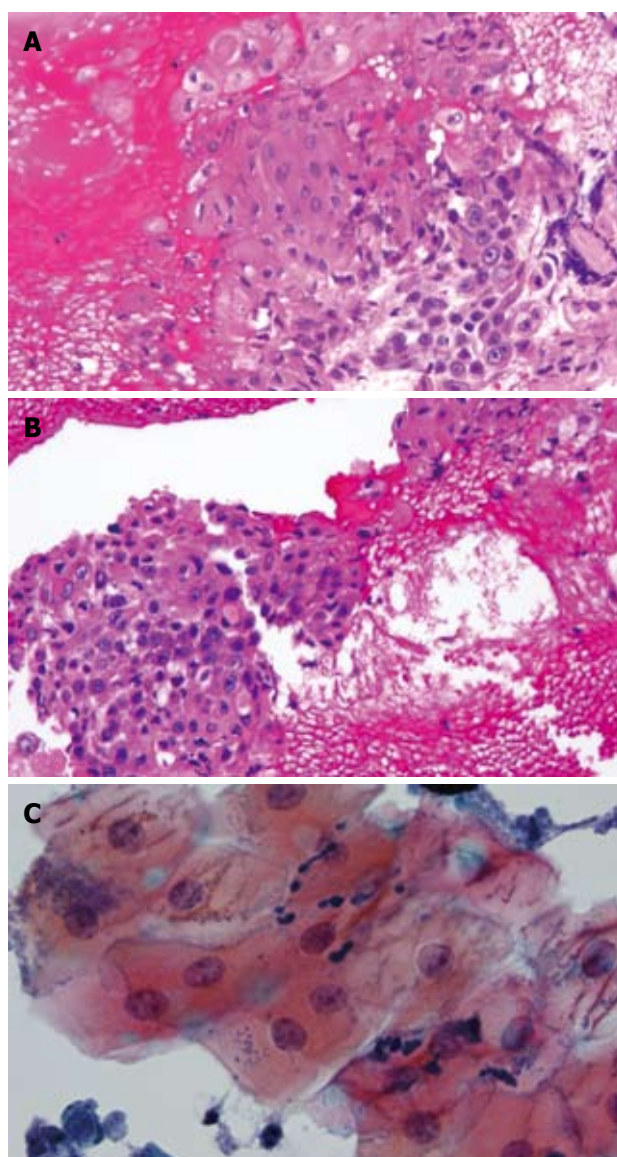


Figure 2 Fine-needle aspiration specimen. A: Liver mass (HE, × 100); B: Pancreatic mass (HE, × 100). The cell block shows atypical squamous cells consistent with keratinizing squamous cell carcinoma. C: Liver mass (ThinPrep, × 400). Squamous cell contamination from the esophagus as evidenced by the presence of bacteria and fungal organisms.

pancreas may be difficult. LEC is an infrequent benign condition and EUS-FNA typically shows squamous cells, lymphocytes, notched crystals and keratin debris^[20-22]. Because cystic degeneration can occur in pancreatic cancers, as in our case^[23], differentiation between benign and malignant squamous cell lesions could be difficult. If there is a solid component, it should be targeted during EUS-FNA rather than the cyst. In general, dense orangeophilic keratin debris, atypical parakeratosis, and nuclear atypia including hyperchromasia and nuclear membrane irregularities help us to differentiate cancer from reactive squamous metaplastic cells^[24]. A glandular component should also be sought when noting atypical squamous carcinoma cells, as adenosquamous carcinoma is more common than the pure squamous cell type^[3]. Squamous cell contaminants could also contribute to diagnostic uncertainty; although transgastric and

transduodenal routes for EUS-FNA should not produce many of these cells, however, they appear to have been noted in our case (Figure 2C)^[25]. In this patient, diagnosis of primary squamous cell carcinoma of the pancreas with liver metastasis was confirmed, as EUS-FNA obtained the same type of cancer cells from both pancreatic and liver lesions, and CT and upper endoscopy did not identify other possible primary squamous cell malignancy.

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LETTERS TO THE EDITOR

Biomarkers for noninvasive biochemical diagnosis of nonalcoholic steatohepatitis: Tools or decorations?

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Abstract

In light of the growing epidemics of nonalcoholic fatty liver disease (NAFLD), identification and validation of the novel biochemical surrogate markers for nonalcoholic steatohepatitis (NASH) are paramount to reduce the necessity for liver biopsy. The availability of such markers has tremendous potential to radically alter the management strategies of NAFLD patients and to monitor the disease activity. Although current biomarkers do not entirely fulfill the many requirements for the identification of patients with NASH, they should not discourage our quest, but remind us that we need to cognize the challenges ahead.

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Key words: Nonalcoholic fatty liver disease; Nonalcoholic steatohepatitis; Biomarkers; Liver biopsy

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TO THE EDITOR

In light of the dramatic increase in the prevalence of nonalcoholic fatty liver disease (NAFLD), noninvasive, simple, reproducible, and reliable biomarkers that can allow identifying patients with nonalcoholic steatohepatitis (NASH) among NAFLD patients are greatly needed^[1]. The availability of such biomarkers has tremendous potential to radically alter the diagnostic and monitoring strategies through the reduction in the need for liver biopsy^[2].

To be introduced in the clinical practice, the ideal biomarker for NASH must fulfill many requirements, such as ease of interpretation by clinicians, accurateness, reproducibility obtained in a standardized fashion, as well as high sensitivity and specificity. This latter point is both important and dependent on the design of study.

The recent report by Uslusoy *et al*^[3] published in the *World Journal of Gastroenterology* has provided evidence that certain noninvasive markers for liver injury, including aminotransferase levels and AST/ALT ratio, do not entirely reflect the histological aspects of liver biopsy in patients with NASH. Based on their results, the authors concluded that aminotransferase levels and AST/ALT ratio do not seem to be reliable predictors for NASH. Although numerous non-invasive biomarkers are available, all patients with fatty liver should undergo liver biopsy^[3]. It is feasible, however, that this radical conclusion may be too far to reach given the important caveats of this study. Firstly, the authors limited their analysis to aminotransferase levels. It has been previously shown, in this regard, that serum levels of caspase-cleaved cytokeratin 18 may be a potential biochemical marker for NASH in NAFLD patients with normal aminotransferase levels^[4]. Secondly, the statistical analysis of data, demonstrating the lack of an association of aminotransferase levels and AST/ALT ratio with NASH, is likely to be underpowered as the study enrolled too few participants to identify such differences. Underpowered studies are overly prone to making false-negative conclusions, or committing what epidemiologists call type II errors^[5]. Finally, appropriate use of biomarker results requires use of a Bayesian approach^[6], i.e. integrating pretest probabilities with biomarker test results (expressed as sensitivity and specificity) to estimate the posttest probability of disease.

Prerequisites for the clinical use of biomarkers for NASH include the elucidation of specific indications, the standardization of analytical methods, the characterization of analytical features, the assessment of performance characteristics, the incremental yield of different markers for given clinical indications, and the demonstration of cost-effectiveness. Although the development of NASH biomarkers fulfilling these features is challenging, it should not discourage our quest, but remind us that we need to cognize the challenges ahead. Technological advances will likely facilitate the use of multimarker profiling^[7] to identify patients with NASH in the near future.

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Events Calendar 2009

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Hyatt Regency San Francisco, San Francisco, CA
Mouse Models of Cancer

January 21-24, 2009
Westin San Diego Hotel, San Diego, CA
Advances in Prostate Cancer Research

February 3-6, 2009
Carefree Resort and Villas, Carefree, AZ (Greater Phoenix Area)
Second AACR Conference
The Science of Cancer Health Disparities in Racial/Ethnic Minorities and the Medically Underserved

February 7-10, 2009
Hyatt Regency Boston, Boston, MA
Translation of the Cancer Genome

February 8-11, 2009
Westin New Orleans Canal Place, New Orleans, LA
Chemistry in Cancer Research: A Vital Partnership in Cancer Drug Discovery and Development

February 13-16, 2009
Hong Kong Convention and Exhibition Centre, Hong Kong, China
19th Conference of the APASL
<http://www.apasl2009hongkong.org/en/home.aspx>

February 27-28, 2009
Orlando, Florida
AGAI/AASLD/ASGE/ACG Training Directors' Workshop

February 27-Mar 1, 2009
Vienna, Austria
EASL/AASLD Monothematic: Nuclear Receptors and Liver Disease
www.easl.ch/vienna2009

March 13-14, 2009
Phoenix, Arizona
AGAI/AASLD Academic Skills Workshop

March 20-24, 2009
Marriott Wardman Park Hotel
Washington, DC
13th International Symposium on Viral Hepatitis and Liver Disease

March 23-26, 2009
Glasgow, Scotland
British Society of Gastroenterology (BSG) Annual Meeting
Email: bsg@mailbox.ulcc.ac.uk

April 8-9, 2009
Silver Spring, Maryland
2009 Hepatotoxicity Special Interest Group Meeting

April 18-22, 2009
Colorado Convention Center, Denver, CO
AACR 100th Annual Meeting 2009

April 22-26, 2009
Copenhagen, Denmark
the 44th Annual Meeting of the European Association for the Study of the Liver (EASL)
<http://www.easl.ch/>

May 17-20, 2009
Denver, Colorado, USA
Digestive Disease Week 2009

May 29-June 2, 2009
Orange County Convention Center
Orlando, Florida
45th ASCO Annual Meeting
www.asco.org/annualmeeting

May 30, 2009
Chicago, Illinois
Endpoints Workshop: NASH

May 30-June 4, 2009
McCormick Place, Chicago, IL
DDW 2009
<http://www.ddw.org>

June 17-19, 2009
North Bethesda, MD
Accelerating Anticancer Agent Development

June 20-26, 2009
Flims, Switzerland
Methods in Clinical Cancer Research (Europe)

June 24-27, 2009
Barcelona, Spain
ESMO Conference: 11th World Congress on Gastrointestinal Cancer
www.worldgicancer.com

June 25-28, 2009
Beijing International Convention Center (BICC), Beijing, China
World Conference on Interventional Oncology
<http://www.chinamed.com.cn/wcio2009/>

July 5-12, 2009
Snowmass, CO, United States
Pathobiology of Cancer: The Edward A. Smuckler Memorial Workshop

July 17-24, 2009
Aspen, CO, United States
Molecular Biology in Clinical Oncology

August 1-7, 2009
Vail Marriott Mountain Resort, Vail, CO, United States
Methods in Clinical Cancer Research

August 14-16, 2009
Bell Harbor Conference Center, Seattle, Washington, United States
Practical Solutions for Successful Management
<http://www.asge.org/index.aspx?id=5040>

September 23-26, 2009
Beijing International Convention Center (BICC), Beijing, China
19th World Congress of the International Association of Surgeons, Gastroenterologists and Oncologists (IASGO)
<http://iasgo2009.org/en/index.shtml>

September 27-30, 2009
Taipei, China
Asian Pacific Digestive Week
<http://www.apdwcongress.org/2009/index.shtml>

October 7-11, 2009
Boston Park Plaza Hotel and Towers, Boston, MA, United States
Frontiers in Basic Cancer Research

October 13-16, 2009
Hyatt Regency Mission Bay Spa and Marina, San Diego, CA, United States
Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications

October 20-24, 2009
Versailles, France
Fifth International Conference on Tumor Microenvironment: Progression, Therapy, and Prevention

October 30-November 3, 2009
Boston, MA, United States
The Liver Meeting

November 15-19, 2009
John B. Hynes Veterans Memorial Convention Center, Boston, MA, United States
AACR-NCI-EORTC Molecular Targets and Cancer Therapeutics

November 21-25, 2009
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Gastro 2009 UEGW/World Congress of Gastroenterology
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Patent (list all authors)

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Statistical data

Write as mean \pm SD or mean \pm 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 *v* (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, *p* (CEA) = 8.6 ± 24.5 μ g/L; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; 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.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

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